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RESEARCH ARTICLE

# Headspace volatile markers of *Sandesh*, a *chhana*-based delicacy stored at elevated temperatures

**Karpurapu Uma[1], Narender Raju Panjagari[1] (✉), Rakesh Kumar Raman[1], Ashish Kumar Singh[1], Lal Chand Sharma[1], Sangita Ganguly[1], Rajan Sharma[2] and Vivek Sharma[2]**

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**Abstract:** The present study explores the headspace volatiles of *Sandesh* (dessert made from heat acid coagulated milk) that contribute to the product quality during storage. *Sandesh* is packaged in clear and dark-coloured glass containers and stored at 30 °C and 45 °C in an incubator. *Sandesh* quality during the storage was estimated by biochemical, microbiological and sensory analysis. The concentration of head-space volatiles was simultaneously determined by employing headspace solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). The identified volatiles were pertaining to various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. As a result, acetic acid, propanoic acid, valeric acid and butyric acid were suggested as headspace freshness markers, while spoilage markers were identified as 1-hexanol 2-ethyl-; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-; pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The obtained volatile markers are essential to develop the intelligent packaging systems for monitoring the product's quality

**Keywords:** *Sandesh*, Headspace, Volatile organic compounds, Solid-phase micro-extraction, Quality change, Spoilage markers

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## Introduction

India is a unique and traditionally rich country in terms of producing a variety of region-specific dairy products. Among several such region-specific dairy products, *Sandesh* is one of the most popular products of acid-coagulated hot milk (*Chhana*, which is similar to cottage cheese but contains sugar). It has a smooth texture with a firm body, which consists of a high amount of milk proteins, fat, sucrose, and fat-soluble vitamins (Aneja et al. 2002; Khamrui and Solanki, 2010). It is highly popular in the eastern and northeastern states of India but gaining huge popularity across India and overseas due to its flavour and palatability. Flavour is composed principally of the sensation of smell and taste and it is the most important factor, which governs our appreciation of the food that we consume. Volatile constituents of the food can determine its aroma and flavour. Previous reports indicated identification of nine volatile carbonyl compounds namely formaldehyde, acetaldehyde, propionaldehyde and /or acetone, butyraldehyde, pentan-2-one, heptan-2-one, benzaldehyde, octan-2-one, nonan-2-one in fresh samples of *chhana* using the Gas-Liquid Chromatography technique (Kumar and Srinivasan, 1984). The major volatiles found in Queso blanco cheese, which is similar to *Sandesh* (but contains salt instead of sugar) are acetone, acetaldehyde, butanol, isopropanol, formic acid, acetic acid, propionic acid and butyric acid, which contribute to the cheese's flavour and aroma (Torres and Chandan, 1981).

Volatile constituents of a product are not only responsible for determining its flavour and aroma, but also play a pivotal role in the estimation of its shelf life. The volatile compounds can generate during storage due to various microbial and biochemical reactions in the product (Cheng, 2010). The profiling of headspace volatiles of food products has been extensively studied for the determination of spoilage markers. In an earlier report (Li et al. 2022), potential volatile compounds associated with deteriorating raw milk were investigated and suggested monitoring 2-ethyl-5-methylpyrazine, 2-pentanone, pyridine, 2-butanone, n-butyraldehyde, and 2,3 butanedione as possible raw milk deterioration indicators. Similarly, Song et al. (2021) identified three headspace volatile compounds namely ethanol, 2,3-butanediol and 2-ethyl-1-hexanol as decay markers of minced

pork by characterizing headspace volatiles during storage. In this regard, several researchers (Anagnostopoulos et al. 2022; Martín-Gómez et al. 2022; Opara et al. 2022; Pavlidis et al. 2021; Sarnoski et al. 2010; Waghmode et al. 2021; Wierda et al. 2006; Yang et al. 2022) reported various spoilage and freshness markers in food products by using volatolomics approach.

The headspace solid-phase microextraction (HS-SPME) technique combined with Gas chromatography-mass spectrometry is an advanced and potential tool to evaluate the quantitative and qualitative headspace volatiles of foods. Pawliszy and co-workers developed the SPME method, which describes the preparation of samples without using solvents for chromatographic analysis. In the SPME method, volatiles are adsorbed on the reusable fibre, which is layered with a stationary phase (Arthur and Pawliszy, 1990). In recent studies, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibres have been reported to be most effective for extracting a wide range of analytes with different polarities and molecular weights. Further, identification and quantification of the compounds are achieved by injecting the fibre extracts into a gas chromatography-mass spectrometer (GC-MS). The combination of SPME and GC-MS has been employed to examine the flavour of various dairy products (Balasubramanian and Panigrahi, 2011). To the best of our knowledge, the investigation on determining headspace volatiles of most of the traditional Indian dairy products has not been conducted so far using advanced techniques, except for *ghee* (Duhan et al. 2020; Wadodkar, 2003). The main purpose of this study is to evaluate the range of volatiles produced during storage under various conditions using GC-MS technique coupled with headspace solid-phase microextraction (HS-SPME) to identify the key quality markers of *Sandesh*.

## Materials and methods

### Preparation of *Sandesh*

Cow's milk and cream were procured from the Experimental Dairy of ICAR-National Dairy Research Institute, Karnal, India. Milk was standardized to 4% milk fat and 8.2% milk solids-not-fat (MSNF) in the laboratory. Soft grade *(Narampak) Sandesh* was prepared by following the standard protocol given by Sen and Rajorhia, 1990. The standardized milk was subjected to heating at 90°C and coagulation with 1% citric acid solution until clear whey. The obtained coagulated mass (pH 5.2-5.4) strained using a muslin cloth for whey draining for 30 min. The obtained mass (*Chhana*) was kneaded to get fine consistency and sugar (30% by weight of *chhana*) was added to it. Later, the mixture was heated to 70°C for 15 min. and further heating continued to 60°C for about 10 min with continuous stirring. Then the mixture was moulded (22g / piece) in a mould and cooled (37°C) to attain *sandesh.*

### Proximate composition analysis of *Sandesh*

*Sandesh* samples were analysed for moisture and fat as per BIS (1981) procedure. The protein and ash content were estimated by using AOAC (2016) methods. The total carbohydrate percentage of the sample was calculated by the difference of the sum of the moisture, fat, protein and ash from the total weight (*Sandesh*).

### Packaging and storage of samples

A large portion of *Sandesh* sold in market in plastic containers. However, glass packaging materials were chosen to determine headspace volatiles. The reason was to avoid migration and scalping of headspace volatiles from the container. Freshly prepared *Sandesh* was packaged in two types of packaging materials such as clear glass vials (CG) and dark-coloured glass vials (DG) (M/s Labco, India, Cat No: 993/4). They were stored at 30 °C and 45 °C (Fig. 1a) in an incubator. Initially, 8 g of sample was weighed into each vial of 30 mL for headspace volatiles determination. Later, 150 g of sample was weighed in CG and DG containers (300 mL) (Yera Airtight Manufactures, India) to determine the biochemical, microbial and sensory changes during storage. The analysis was carried out at 24 h interval until visible mould growth (Fig. 1d).

### Extraction and measurement of headspace volatile compounds by HS-SPME-GC/MS

The head-space volatiles from the *Sandesh* samples were extracted by using a headspace solid-phase microextraction (HS-SPME) device consisting of a fibre (Supelco cat. No: 57348-U, Bellefonte, PA, USA) with a film thickness of 50/30µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and a manual holder (Supelco cat. No: 57330-U, Bellefonte, PA, USA). The SPME syringe was introduced into the vial's headspace, followed by the extension of the fibre (2 cm) into the vial (Fig. 1b). The gap between the fibre tip and the product surface in the bottle was 0.5 cm. The extraction time of samples stored at 30 °C and 45 °C for 40 and 45 min, respectively, were optimized by conducting experiments (Fig. A1& Fig. A2). After extraction of volatiles, the fibre was placed into the manual holder and inserted into the heated injection port of the GC-MS for thermal desorption of the samples for 15 min (Fig. 1c). GC-MS (TQ-8030, Shimadzu Corporation, Kyoto, Japan) instrument located at the National Referral Centre for Milk Quality and Safety, ICAR-NDRI, Karnal, India was employed in this study. Chromatographic separation was performed on an Equity-1 column (Capillary column; 30 m X 0.32 mm i.d. X1.0 µm film thickness, Supelco cat. No: 28057-U, Bellefonte, PA, USA). The injector, detector and interface were operated at 270 °C, 220 °C, and 260 °C, respectively. GC oven was initially set at 270 °C for 11 min, then ramped linearly at a rate of 10 °C/min to 300 °C and it was kept for 5 min at the same temperature. Flow rate of helium

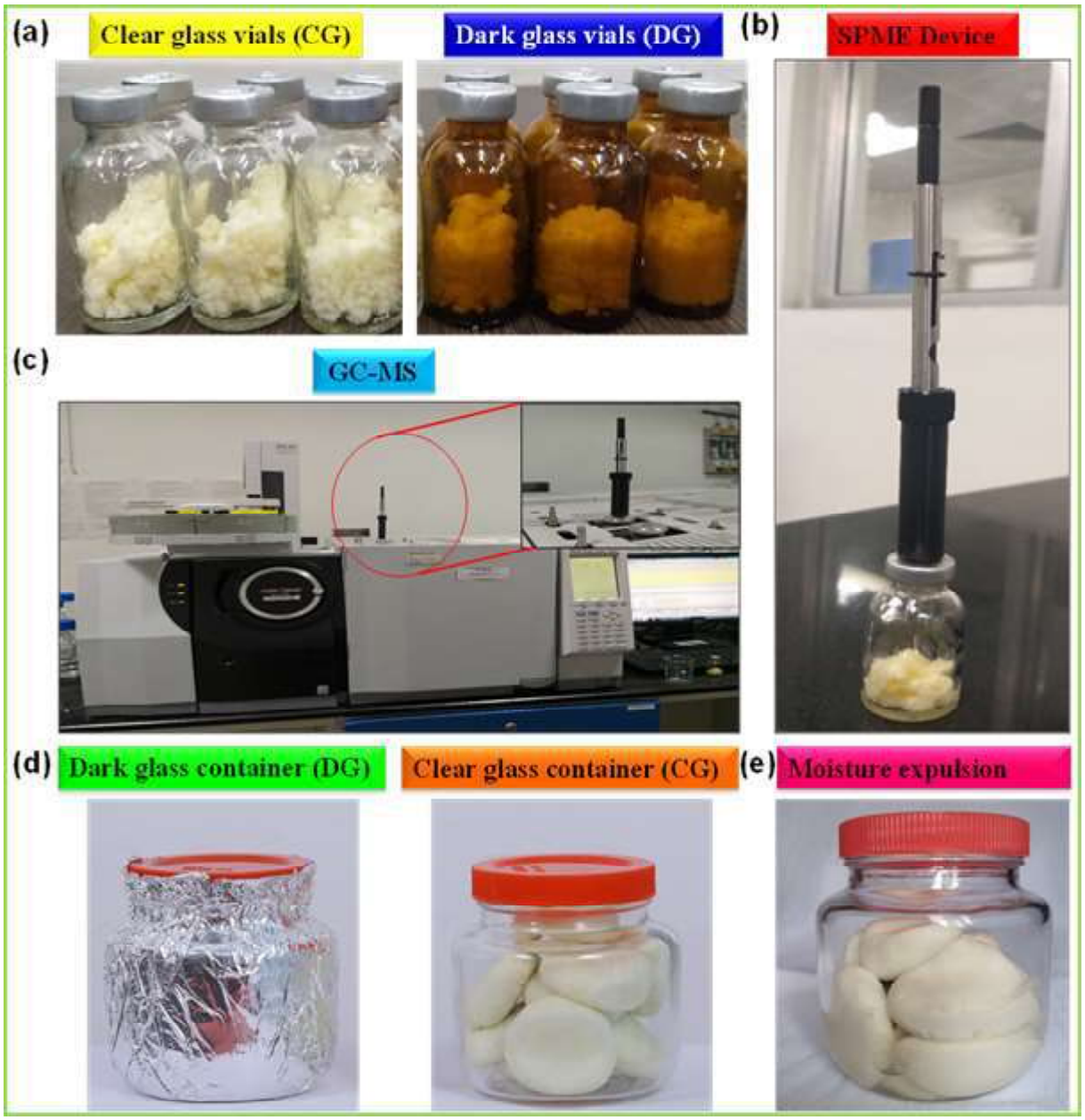


**Fig. 1** **(a)** Clear glass vials and dark coloured glass vials for headspace volatiles analysis, **(b)** Headspace volatiles collection using SPME device, **(c)** GC-MS coupled with SPME, **(d)** Clear and dark (aluminium wrapped) glass containers for quality analysis, **(e)** Moisture expulsion from samples during storage

carrier gas was maintained at 2.33 mL/min under splitless mode. The standard ionization energy of 70 eV was maintained in Electron Ionization (EI) under full scan mode in a range of 50-500m/z to analyse the volatiles. The identification of volatile compounds was accomplished by using NIST 17 and NIST 14S (National Institute of Standard and Technology, New York, USA) mass spectral library. The results from the headspace volatile analysis were expressed in terms of peak area (total ion concentration).

**Analysis of biochemical changes**

The titratable acidity of the sample was determined using the method described in BIS (1981). Sample pH was measured with the help of a calibrated pH analyser (Labndia, New Delhi, version 1). The free fatty acids content of *Sandesh* was estimated using Deeth et al. (1975) method. The extent of protein breakdown (proteolysis) was evaluated by employing the reported method of Jupfs (1973). The extent of oxidation of fat in terms of

thiobarbituric acid (TBA) value was analysed using a method described by Strange et al. (1977).

**Analysis of microbiological changes**

Standard plate count (SPC) and, yeast and mould count (Y&M) were analysed according to the BIS (1981) procedure. Coliform count, aerobic spore count, proteolytic count, and lipolytic count were performed by using the specific methods given by Marshall (1992).

**Analysis of sensory changes**

Samples of *Sandesh* were evaluated for attributes such as colour and appearance, flavour, texture, and overall acceptability by a panel of eight semi-trained judges. The panel members were chosen based on their experience in judging traditional Indian dairy products. The panellists assessed the sensory parameters based on a 9-point hedonic scale, wherein "9" denoted extremely desirable and "1" denoted extremely undesirable.

**Statistical analysis**

Variations in biochemical, microbial and sensory parameters were investigated by analysis of variance (ANOVA) using IBM SPSS software (ver. 20). The results are represented in terms of mean and standard deviations (SD). To compare mean values, Duncan's test (DMRT) was applied and the significant differences were identified. Principal component analysis (PCA) was performed using Origin pro software (ver. 2023) to determine significant volatile functional groups that affect the headspace of *Sandesh* during storage. Correlation analysis between headspace volatiles and quality attributes was expressed using polar heatmap (Origin pro software).

**Results and Discussion**

**Chemical composition of *Sandesh***

The proximate composition of *Sandesh* (soft grade) revealed 26.90 ± 0.56% moisture, 22.27± 1.00% (*db* w/w) fat, 22.80 ± 0.56% (*db* w/w) protein, 53.40 ± 1.66% total carbohydrates and 1.48 ± 0.08% (*db* w/w) ash. The obtained composition was in agreement with the earlier reports (Khamrui and Solanki, 2010; Sen and Rajorhia, 1991).

**Changes in headspace volatile of *Sandesh* stored under different conditions**

Detected and identified headspace volatiles of *Sandesh* stored in two packaging materials such as CG vial and DG vial at 30°C and 45°C at each interval are represented in a polar heatmap (Fig. 2). Compounds with a peak area (total ion concentration) of more than $10^4$ were considered for data analysis in the study. The peak area of detected compounds for all the samples is mentioned in Table A1 (supplementary materials). The identified volatiles are affirmatively confirmed to be acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. Approximately, 140 product-related headspace volatiles were successfully identified along with a small number (35-40) of fibre-related volatile contaminants, which include derivatives of hydrazine carboxamide, semicarbazide, silanediol, carbohydrazide and polysiloxane were noticed. However, these volatile contaminants were eliminated while processing the data as recommended in the earlier reports (Grimm and Champagne, 2001; Zhang et al. 2022).

***Major volatiles***

Acids, alcohols, alkanes, ketones and aldehydes have been noticed to be the largest functional group compounds that are present in the headspace of *Sandesh*. Forty acids were identified in *Sandesh* during the storage under various conditions. Among them, carboxylic acids with a chain length ranging from 1 to 20 ($C_1$-$C_{20}$) are the majority of compounds. The individual volatile acids possess a non-specific trend during storage due to the continuous degradation and production of acids. A similar non-specific trend of individual volatiles in fermented milk during storage was reported by Dan et al. (2017). However, the sum of the peak areas of all the acids (total peak area) has been considered to analyse the changes during storage. It can be observed from Fig. 3 that the total peak area of the volatile acids has decreased during the storage irrespective of various storage conditions. This could be due to the acid degradation by β-oxidation (saturated fatty acids) and auto-oxidation (unsaturated fatty acids) reactions (Cheng, 2010). The highest percentage of decrease (58.6%) in total peak area was observed in CG samples at both storage temperatures due to the susceptible property of packaging material (transparent) to auto-oxidation of fatty acids. It is believed that acetic acid, propanoic acid, valeric acid and butyric acid were responsible for the decrement in acids' total peak area. Hence, these acids could be considered while determining the product's freshness. In addition, we have identified a few more acids in *Sandesh* during storage such as cyacetacide, formic acid, hexanoic acid and stearic acid. The obtained acids have been earlier reported to be present in milk (Dursun et al. 2017). Despite this, carboxylic acids are essential aroma compounds which are also considered to be prominent precursors for methyl ketones, alcohols, aldehydes, alkanes, alkenes, and esters (Cheng, 2010).

In this study, twenty-two different alcohols are identified in *Sandesh* headspace. Ethanol and methanol were found to be the major alcohol compounds present in *Sandesh* (Table A1). Similar to earlier trend of non-specific peak area of acids, the alcohols have also followed the same trend at each storage interval among all the samples (Fig. 3). It can observe that the predominant total peak area has been recorded for alcohols at end of the shelf life in all the samples compared to fresh *Sandesh*. The observable non-

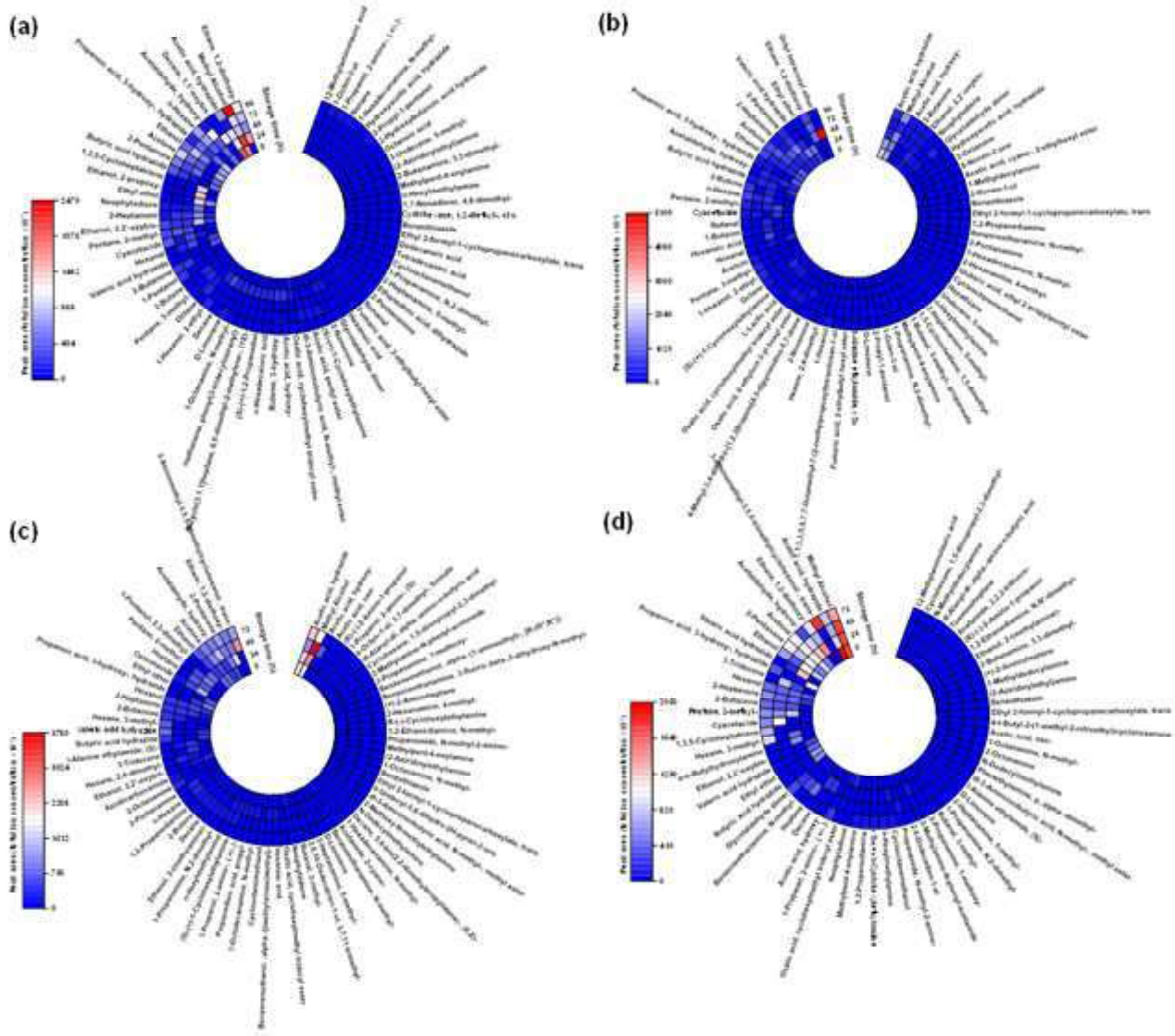


**Fig. 2** Polar heat map of headspace volatiles of *Sandesh* during storage, **(a)** Samples stored at 30°C in dark glass vials, **(b)** Samples stored at 30°C in clear glass vials, **(c)** Samples stored at 45°C in dark glass vials, **(d)** Samples stored at 45°C in clear glass vials.

specific trend variations could have been due to various reasons, which include the production of alcohols from aldehydes and ketones reduction, fermentation of lactose, amino acid metabolism, and also partial esterification of resulting alcohols with acids (Yue et al. 2015). It can be believed that these specific compounds of 1-hexanol, 2-ethyl-; 1-hexanol; 1,1,3,3,5,5,7,7-octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol; 2-propyl-1-pentanol in CG & 1-pentanol; 1-hexanol, 2-ethyl-; 2-propyl-1-pentanol; methyl alcohol in DG and, 3-aminomethyl-3,5,5-trimethylcyclohexanol, trans; 1-Propanol, 2-amino-, (.+/-.)-; Cyclooctanemethanol compounds in CG and 3-Aminomethyl-3,5,5-trimethylcyclohexanol , trans-; methanol compounds in DG are responsible for the peak area predominance in alcohols at 30°C and 45°C at the end of storage, respectively (Table A1). The observed specific alcohols of primary alcohols might have been originated from the oxidation of unsaturated fatty acids (Cheng, 2010). However, hexanol and pentanol are derived from the aldehydes such as hexanal and pentanal during lipid oxidation (Kilcawley et al. 2018). Rashid et al. 2019 findings revealed that 1-pentanol had appeared as the second-largest volatile compound in pasteurized milk at 10°C after 18 days of storage and 1-heptanol at the end of 16 days of storage. Urbach (1990) reported that ethanol, propan-2-ol and 3-methyl-butna-1-ol were major volatiles present in raw milk stored at 7 °C for 3 days. Therefore, the enhanced amount of alcohol compounds in stored samples of *Sandesh* in our present investigation could be attributed to auto-oxidation of unsaturated fatty acids.

Alkanes emerged as one of the important classes of volatiles in the headspace of *Sandesh* at the end of their shelf life, although they were absent in fresh *Sandesh*. However, aliphatic groups of alkanes with a chain length of $C_2$-$C_{11}$ have been identified along

with the detection of nineteen alkanes in the product . The total peak area of alkanes has been recognized to be enlarged with progressing storage time at 30°C irrespective of packaging materials till 48 h of storage and it has followed the non-specific trend further (Fig. 3). Furthermore, alcohol concentration was observed to be high in both the packaging materials at 45°C compared to *Sandesh* stored at 30°C till 24 h of storage. Eventually, the maximum total peak area was observed in samples stored in CG materials at 30 °C followed by samples in DG materials at the same temperature at the end of the shelf life. Usually, alkanes are secondary oxidation products that are formed from the decomposition of hydroperoxides during the auto-oxidation of unsaturated lipids (Kubow, 1992). However, an increasing trend of peak area has been conspicuously noticed, which was raised due to the presence of ethane, 1,2-diethoxy-; n-hexane; pentane, 3-methyl-; hexane, 3-methyl; pentane, 2-methyl-; heptane among all the alkanes. These results were substantiated by Frankel et al. (1982) who reported that the decomposition of 9- and 13-hydroperoxide from linoleic acid led to the production of pentane and ethane.

In addition, twelve different ketones were identified in *Sandesh* samples stored under different conditions and changes in their concentration are presented in Fig. 3. Among all the identified ketones, four compounds namely acetone, 2-pentanone, 2-heptanone and 2-butanone were shown highest peak area. These ketones were present in fresh and also formed during storage of *Sandesh* . The earlier studies (Clarke et al. 2020; Yue et al. 2015) demonstrated the presence of ketones in fresh milk and heated milk. Moreover, acetone, 2-heptanone and 2-pentanone have also been considered to be the major three ketones in microfiltered pasteurized milk (Yue et al. 2015). Kumar and Srinivasan (1984) reported that five ketones namely acetone, pentan-2-one, heptan-2-one, octan-2-one, nonan-2-one were identified in fresh samples of *chhana* prepared from cow's milk, buffalo's milk and samples procured from the market. In fresh *Sandesh,* these might have originated from milk and also due to the heat treatment during preparation. The total peak area of the ketones in both packaging materials was observed to be decreased during the first 24 h. Later on, it is increased gradually by the end of 48 h at 30 °C. The peak area of ketones increased slightly with progressing storage interval irrespective of packaging materials at 45 °C. Moreover, the maximum total peak area of the ketones was observed at end of the storage period among samples stored *Sandesh* at 30 °C, which indicates that the temperature played a significant role in ketones generation. Methyl ketones that are present in the product are typically liberated by decarboxylation of saturated fatty acids or by β-ketoacids decarboxylation (Cheng, 2010). Li et al. (2012) reported that 2-heptanone was one of the major compounds that enhanced with increasing storage time of milk powder. Li and Wang (2016) have also described 2-heptanone and 2-nonanone the typical volatiles of oxidized flavours in dairy products.

Aldehydes namely acetaldehyde, hydroxy-; butanal, 3-hydroxy-; glycolaldehyde dimer; hexanal; nonanal; butanal; butanal, 3-methyl-; 2,6-dodecadien-1-al have been determined in our present investigation. According to the earlier report (Yue et al. 2015), heated milk contains aldehydes significantly. The total peak area dynamics of aldehydes of *Sandesh* (Fig. 1e) revealed that the concentration of aldehyde presence has increased notably at the end of 72 h and 48 h of storage time in samples stored at 30 °C and 45 °C, respectively, in both the packaging materials. However, the maximum total peak area was estimated in the samples during the storage at 45 °C. Among all the aldehydes, we have observed the maximum total peak area of hexanal and acetaldehyde, which were present in both fresh and stored *Sandesh*. It is believed that the rapid production and spontaneous degradation of aldehydes into alcohols (Cheng, 2010) could be responsible for the total peak area dynamics of aldehydes. Earlier studies reported that aldehyde compounds can be emerged due to unsaturated fatty acids autoxidation, lactose fermentation and amino acid metabolism (Cadwallader and Singh, 2009; Cheng, 2010; Li et al. 2012).

***Minor volatiles***

Alkenes, amines, amides, esters and ethers were minor functional groups in the study of volatiles. However, alkene levels revealed that none of the samples exhibited any observable specific trend during storage except CG samples at 45 °C of storage. As alkanes, alkenes are also secondary oxidation products of milk fat during auto-oxidation (Kubow, 1992). From the earlier reports (van Beilen and Funhoff, 2007), the alkenes are preferably converted into alcohols, aldehydes and carboxylic acids due to microbial action. Amines were identified at low concentrations in fresh as well as stored *Sandesh* samples. Although, a maximum number of amines have been noticed in *Sandesh*, their concentration was less compared to acids, alcohols and alkanes (Fig. 2). The majority of amines present in *Sandesh* pertain to the aliphatic group. Usually, amines are familiar intermediate products of protein degradation and their further decomposition can lead to the formation of acids, aldehydes, alcohols, esters and sulfur compounds (Cadwallader and Singh, 2009). Like amines, amides were also generated from the protein degradation, but this class of compounds was present in negligible concentration among all the samples during storage. Also, we have observed eight esters in our present study. Esters corresponding to alcohols and acids in the product were identified to be the highest concentration at end of the shelf life among all the samples at 30 °C rather than the samples at 40 °C. Esters are commonly produced from alcohols and fatty acids through the enzymatic process. Ethers have been identified in the least concentration (Table A1).

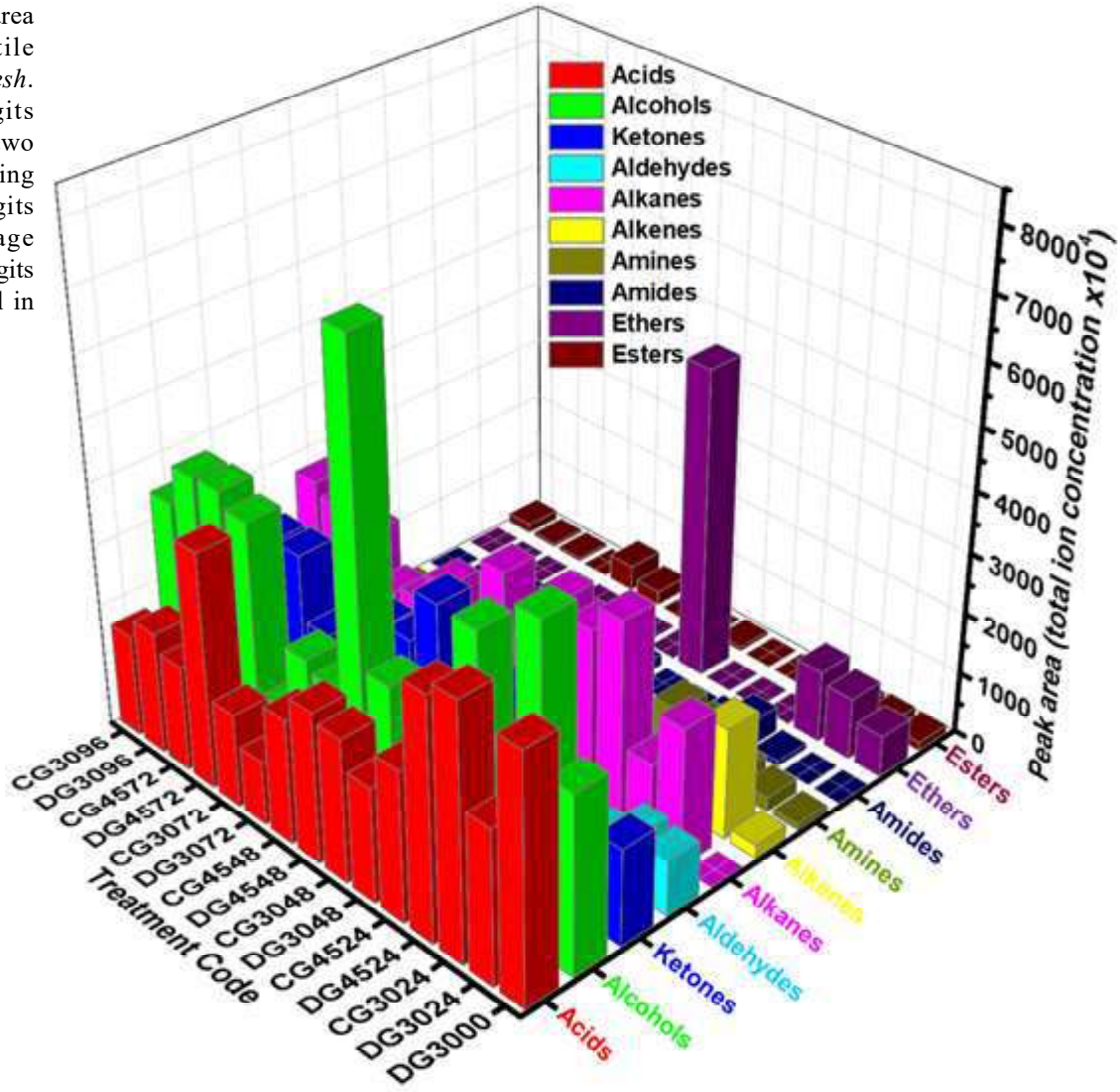


**Fig. 3** Changes in peak area of headspace volatile groups in stored *Sandesh*. Reference to six digits treatment code: first two digits indicate packaging material; next two digits indicate storage temperature; last two digits indicate storage interval in hours.

**Changes in quality attributes of *Sandesh* stored under different conditions**

We have carried out the analysis of quality changes in *Sandesh* stored under various conditions until satisfactory sensory scores have been recorded or visible mould growth

***Biochemical changes***

Biochemical changes of *Sandesh* samples stored under different conditions are presented in Table A2 (Supplementary materials). Present results displayed that the initial mean titratable acidity (% lactic acid) value of *Sandesh* significantly ($P<0.05$) increased from 0.46 to 0.56 and 0.58 at 30 °C; to 0.73 and 0.72 at 45 °C, respectively, in DG and CG during storage (Fig. 4a). Furthermore, ANOVA results revealed that the titratable acidity of *Sandesh* has been significantly ($P<0.05$) influenced by incubation temperature and non-significantly ($P>0.05$) by packaging materials. As evidenced, there was less improvement in the titratable acidity of *Sandesh* stored *at* 30 °C. The present observations were in good agreement with the earlier report (Yadu, 2014). After an initial pH of 5.89, *Sandesh* pH reduced to 5.69 and 5.76 at 30 °C; 5.73 and 5.71 at 45 °C, respectively in DG and CG during storage (Fig. 4b), which could be attributed to the production of lactic acid by spoilage microorganisms. Storage time shows its significant ($P<0.05$) impact on the pH of the product, whereas storage temperature and packaging materials had a non-significant ($P>0.05$) effect during storage. The decrease in pH of *Sandesh* samples has been earlier reported by Mandal (2019). In addition, the TBA value of all *Sandesh* samples enhanced significantly ($P<0.05$) with progressing storage time among all samples (Fig. 4c). The highest TBA value was observed in the CG container at 45 °C in the order of 0.229. The remarkable enhancement of TBA values could be attributed to the presence of a considerable amount of fat in *Sandesh*. This fat is highly susceptible to oxidation under higher temperature conditions.

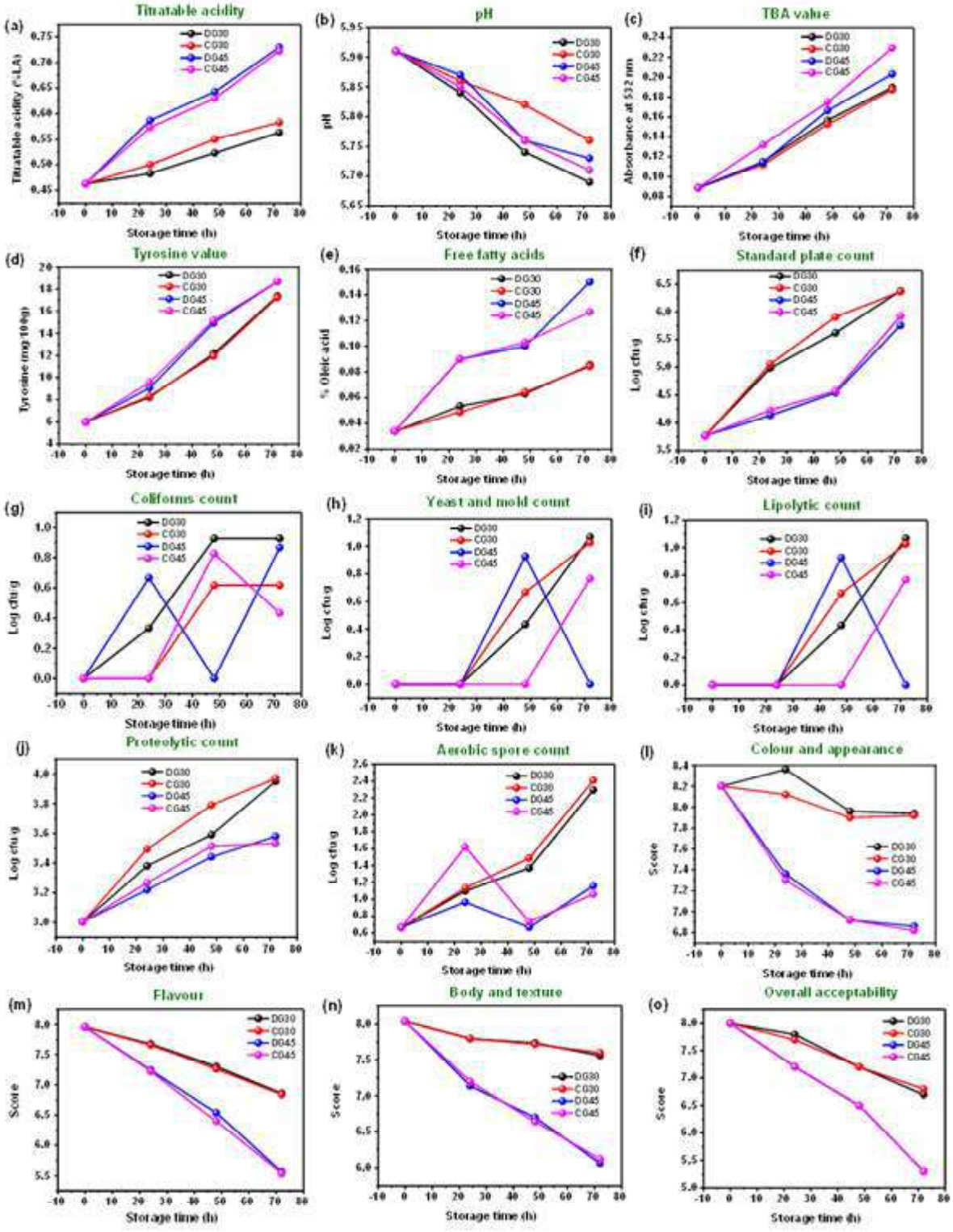


**Fig. 4.** Quality changes in *Sandesh* stored under various conditions **a)** Titratable acidity **b)** pH **c)** TBA value **d)** Tyrosine value **e)** Free fatty acids **f)** Standard plate count **g)** Coliforms count **h)** Yeast and mold count **i)** Lipolytic count **j)** Proteolytic count **k)** Aerobic spore count **l)** Colour and appearance **m)** Flavour **n)** Body and texture **o)** Overall acceptability. Reference to four digits treatment code: first two digits indicate packaging material; last two digits indicate storage temperature

Gargouri et al. (2015) reported that the rate of oxidation is influenced by many factors including temperature, oxygen and light. The significant increase in TBA values of samples stored in CG at 45 °C might be due to the combined effect of the storage temperature as well as light transmittance through the packaging material. Mandal (2019) has also observed a considerable enhancement in the TBA value (% malonaldehyde) of *Sandesh* during storage.

The tyrosine content (mg/100g) of *Sandesh* stored at 30 °C has been identified to be enhanced ($P<0.05$) from 5.98 to 17.38 and 17.24; to 18.66 and 18.60 in DG and CG, respectively at 45 °C. A significant amount of tyrosine content ($P<0.05$) in *Sandesh* is noticed at the end of the shelf life at 45 °C. However, non-significant ($P>0.05$) differences were observed in packaging materials. A similar increment in the tyrosine content of *Sandesh* during the shelf life has been mentioned in the earlier report (Yadu, 2014). The FFA values of *Sandesh* notably ($P<0.05$) varied from 0.034 to 0.15% oleic acid among all the samples (Fig. 4e). ANOVA revealed that the storage temperature substantially influenced the FFA value, but not packaging materials.

***Microbiological changes***

We have investigated the microbiological changes of *Sandesh* stored under different conditions. The standard plate count (SPC) (log cfu/g) of *Sandesh* exhibited a remarkable ($P<0.05$) increase from 3.76 to 6.37 when stored at 30 °C and to 5.92 at 45 °C. SPC of samples stored in DG container at 30 °C was estimated to be significantly ($P<0.05$) higher than samples stored at 45 °C, whereas non-significant ($P>0.05$) with samples stored in CG at 30 °C. However, the SPC of samples in both the packaging materials was found to be non-significantly different ($P>0.05$) at 72 h. The microbiological quality of all the samples stored under various conditions was found toexceed the prescribed standards (maximum limit 5.54 log cfu/g) recommended by FSSR (2011) after 48 hours.

It can be observed that coliforms were absent in fresh samples. Despite the presence of coliforms in the first dilution during storage, they did not exceed the standards set by FSSR (2011), indicating hygienic conditions were maintained during manufacturing and handling. Moreover, yeast and mould (Y&M) were not detected in the first dilution of the samples stored at 30 °C and 45 °C, respectively up to 48 h. On further storage, the count has been significantly ($P<0.05$) multiplied among all the samples (Fig. 4h). The Y & M count of *Sandesh* stored at 30 °C in both packaging materials exceeded the standards (2.17 cfu/g) prescribed by FSSR (2011). The count has been estimated to be substantially ($P<0.05$) greater than the samples stored at 45 °C in two packaging materials, which could be attributed to the favourable growth temperature and increased acidity of samples. The observations of enhanced Y&M count were also reported by the earlier worker, Yadu (2014). A similar trend was observed in the lipolytic bacteria count of *Sandesh* during the storage. However, the count was non-significantly ($P>0.05$) increased to 1.06 log cfu/g during storage (Fig. 4i). The proteolytic count of *Sandesh* samples has also been multiplied rapidly (Table A2) in storage time. In addition, aerobic spore count (log cfu/g) was found to substantially enhance from 0.66 to 2.40 among samples (Fig. 4k) but statistically non-significant ($P>0.05$) at the end of 72 h of storage. The increase in the count might be due to favourable temperature for the growth of aerobic spore count during prolonged storage.

***Sensory changes***

Sensory scores of *Sandesh* stored under different conditions for the stipulated period are displayed in Fig. 4. A non-significant ($P>0.05$) decrement in colour and appearance (C&A) scores of *Sandesh* was observed at 45 °C, whereas significant ($P<0.05$) decrement at 30°C in both the packaging materials. Further, it was observed that slight deformation of the shape of samples occurred due to the stacking of pieces in the container and the expulsion of moisture during storage, which might have contributed to reduced scores (Fig. 1e). However, no visible Y&M growth was observed even after a chemical or physical deterioration of samples when stored at 45 °C. Conversely, visible mould growth was observed in all the samples at 30 °C after 72 h of storage interval.

The flavour scores of *Sandesh* have been remarkably decreased in both the cases of DG and CG due to the production of off-flavours as a combined effect of deteriorative reactions. In this regard, storage conditions displayed a substantial effect on the flavour scores after 48 h of storage interval. The scores were noticed to be decreased rapidly in samples stored at 45 °C than 30 °C and also the formation of off-flavours was rapid at higher temperatures. Moreover, a drastic decline was observed in body and texture scores of *Sandesh* from 8.04 to 6.06 during storage at various conditions (Fig. 4n). Statistical analysis revealed that the samples stored at 30 °C exhibit substantial greater scores than those stored at 45 °C in both packaging materials, which could be due to the expulsion of moisture from the samples at 45 °C (Fig. 1e). The overall acceptability score decreased conspicuously ($P<0.05$) from 8.02 to 6.74 and 6.82 at 30 °C; to 5.32 and 5.30 at 45 °C DG and CG, respectively during the storage. The overall acceptability scores of *Sandesh* samples stored at 30 °C exhibited significantly higher values than the scores of samples at 45 °C (Fig. 4o). From biochemical, microbial and sensory results, the shelf life of *Sandesh* was estimated to be 72 h and 48 h at 30 °C and 45°C respectively, regardless of packaging materials.

**Principal component analysis**

The principal component analysis (PCA) was employed to determine the significant volatile functional groups that affected the headspace of *Sandesh during* storage and, also evaluate the

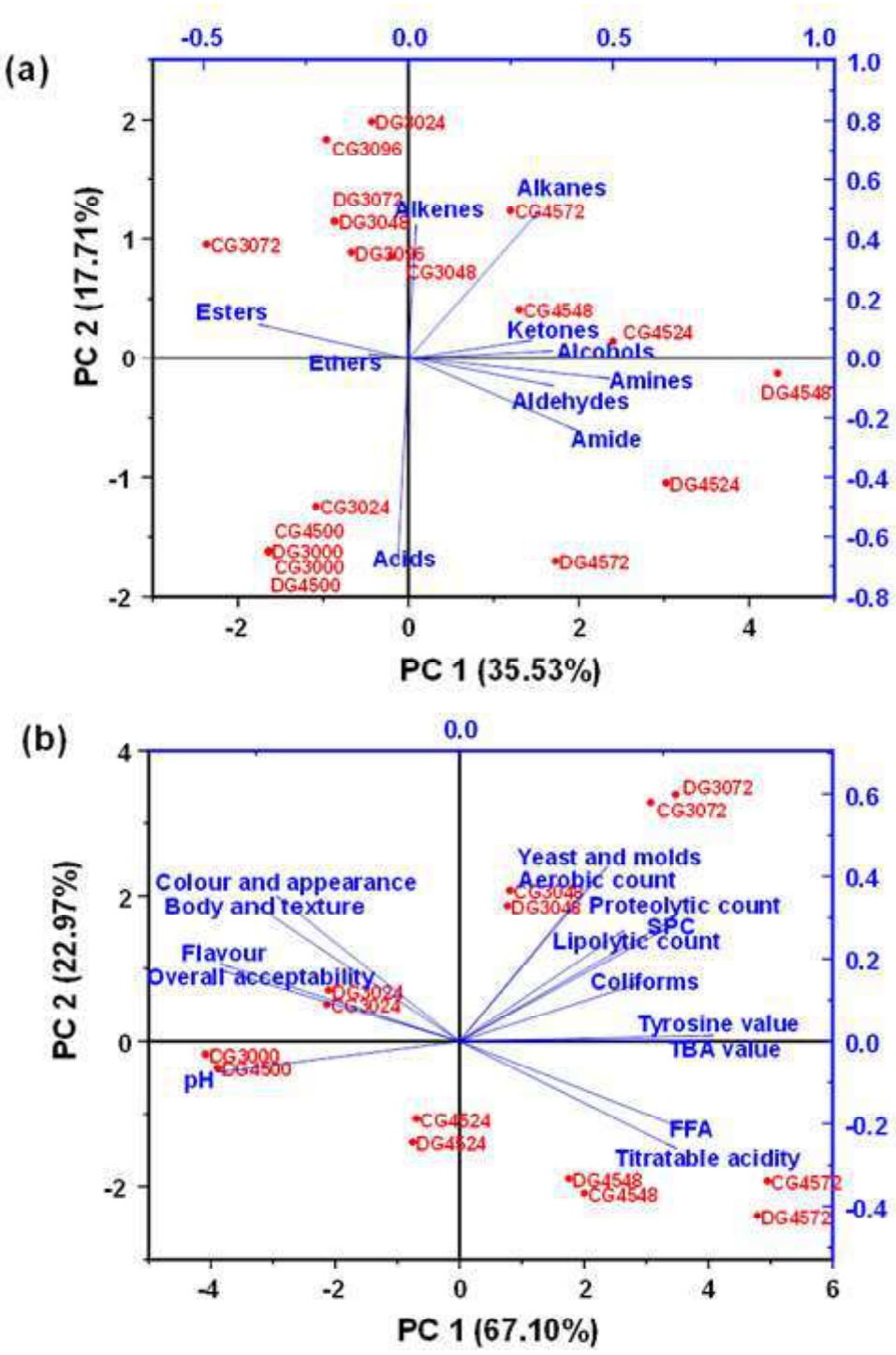


**Fig. 5.** Principal component analysis **(a)** Biplot for volatile components **(b)** Biplot for quality parameters. Reference to six digits treatment code: first two digits indicate packaging material; last two letters indicate storage temperature; last two digits indicate storage interval in hours.

considerable effect of various treatments of *Sandesh* on volatile functional groups. As a result of PCA, a biplot enabled us to visualize the interaction between the observations and variables (Fig. 5). In a biplot, the elongated line indicates greater variance in the variables, while the shorter line indicates less variance. The cosine angle between the lines in the biplot reflects the degree of correlation between their variables. The correlation between their variables decreases as the angle approaches 90 or 270°. However, a correlation of 1 or -1 is represented by an angle of 0 or 180 degrees, respectively (Kohler et al. 2005). We have observed that the first four principal components (PC) expressed approximately 78.21% of the variance between sample and headspace volatile functional groups. Of four principal components, PC1 is estimated to be 33.87% of the variance and is characterized by amines, amides and alcohols. PC2 was evaluated to be 17.98% of the variation and was defined by

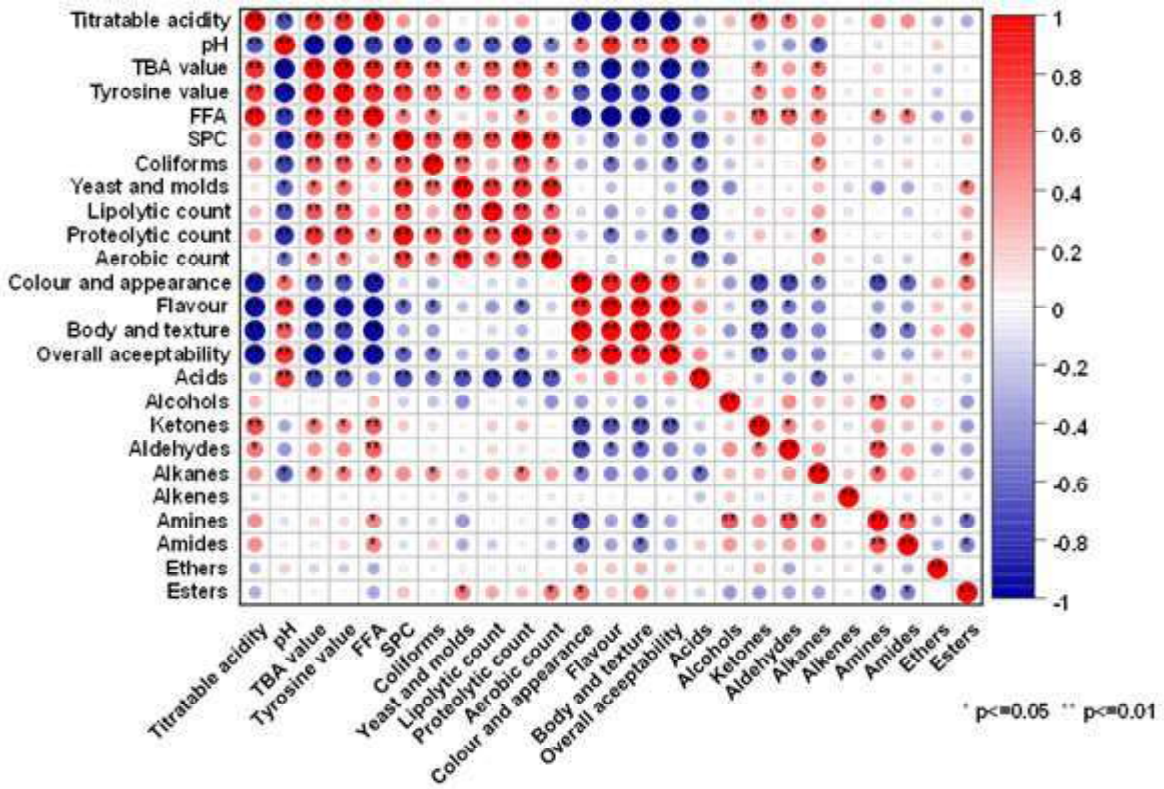


**Fig. 6.** Colour map of correlation analysis between headspace volatiles and quality attributes of *Sandesh* during storage

alkanes and opposed by acids. It revealed that amines, amides, alcohols and alkanes are increased with increasing shelf life and acids are inversely related. In the present study, loading with an absolute value of more than 0.7 indicates a strong influence on *Sandesh* quality during storage. From the results, fresh *Sandesh* has the highest number of acids. From the results, a greater total peak area of the acids is observed conspicuously in fresh *Sandesh* whereas the total peak area of alkanes, alcohols and ketones are recognized to be prominent in the treatments of DG4572, DG4548, and DG4524 samples. Moreover, amines, aldehydes and amides are noticed to be high in DG4572, DG4548 and DG4524 samples.

The major quality parameters (biochemical, microbial and sensory) of *Sandesh* during storage have been systematically evaluated from the PCA. According to the biplot of PCA (Fig. 2b), PC1 and PC2 were explained to be 67.10% and 22.97% of the total variance, respectively. Therefore, the total variance for the two factors can be explained as 90.07%. Furthermore, we have obtained meritorious levels of SPC, proteolytic count, aerobic count, lipolytic count and coliforms for DG3072, CG3072, DG3048 and DG3048 samples. Additionally, FFA and titratable acidity levels were observed to be more in CG4572, DG4572, DG4548 and CG4548 and also estimated the least sensory scores for CG4572, DG4572, DG4548 and CG4548.

**Correlation analysis between the headspace volatiles and quality changes**

The correlation analysis was systematically carried out to identify the association of the headspace volatile functional groups with quality attributes of *Sandesh* during storage under different conditions (Fig. 6). The correlation between volatile functional groups and quality changes revealed that the proteolytic count ($r=-0.774$, $P<0.01$), lipolytic count ($r=-0.765$, $P<0.01$), TBA value ($r=-0.726$, $P<0.01$), SPC ($r=-0.718$, $P<0.01$), tyrosine value ($r=-0.698$, $P<0.01$), Y&M count ($r=-0.696$, $P<0.01$), aerobic spore count ($r=-0.672$, $P<0.01$), coliform count ($r=-0.559$, $P<0.05$) were negatively correlated with acids, whereas pH ($r=+0.793$, $P<0.01$) was positively correlated with acids of *Sandesh* during storage. Moreover, titratable acidity ($r=+0.714$, $P<0.01$), FFA value ($r=+0.661$, $P<0.05$), TBA value ($r=+0.552$, $P<0.05$), tyrosine value ($r=+0.522$, $P<0.05$) were positively correlated with ketones, whereas colour and appearance ($r=-0.743$, $P<0.01$), body and texture ($r=-0.680$, $P<0.01$), flavour ($r=-0.657$, $P<0.01$), overall acceptability ($r=-0.649$, $P<0.01$) were negatively correlated with ketones of *Sandesh* during storage. FFA value ($r=+0.590$, $P<0.05$), proteolytic count ($r=+0.545$, $P<0.05$), TBA value ($r=+0.543$, $P<0.05$), tyrosine value ($r=+0.517$, $P<0.05$) and coliforms ($r=+0.500$, $P<0.05$) were positively correlated with alkanes, whereas pH ($r=-0.621$, $P<0.05$) and, colour and appearance ($r=-0.519$, $P<0.05$) were negatively correlated with alkanes of *Sandesh* during storage.

As we noticed, FFA value (r=+0.626, P<0.05) and titratable acidity (r=+0.563, P<0.05) were positively correlated with aldehydes since colour and appearance (r=-0.716, P<0.01), body and texture (r=-0.617, P<0.05) and flavour (r=-0.522, P<0.05) were negatively correlated with aldehydes of *Sandesh* during storage. It can be observed that FFA value (r=+0.514, P<0.05) was positively associated with amines, as colour and appearance (r=-0.701, P<0.01) and body and texture (r=-0.584, P<0.05) were negatively correlated with amines of *Sandesh* during storage. In amides, FFA value (r=+0.539, P<0.05) was positively correlated, whereas colour and appearance (r=-0.615, P<0.05) and, body and texture (r=-0.556, P<0.05) were negatively associated with amides of *Sandesh* during storage. We have also explored that colour and appearance (r=+0.562, P<0.05), Y&M (r=+0.543, P<0.05) and aerobic spore count (r=+0.541, P<0.05) were positively correlated with esters of *Sandesh* during storage.

## Conclusion

In summary, we have investigated 140 product-related headspace volatiles for determining the product's quality markers. The identified volatiles were of various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. However, interestingly, among all the volatiles of functional groups, acids, alcohols, alkanes and ketones were found to be the largest in terms of peak area during the storage. The dynamics of volatile compounds revealed continuous degradation of produced metabolite volatiles, which lead to the production of new volatiles. Notably, we have determined the predominant total peak area for the alcohols at end of the shelf life compared to the fresh product. Nevertheless, the peak area of alkanes and ketones enhanced gradually during storage in most of the treatments. Also, as expected significant changes in biochemical, microbial and sensory parameters of *Sandesh* packaged and stored at various conditions were observed. As a result of these quality changes, the shelf life of *Sandesh* has been assessed to be 72 h and 48 h at 30 °C and 45 °C, respectively, irrespective of the packaging materials. Of all the groups, amines, amides, alcohols, alkanes and acids are noticed to be the most significant functional groups among all the classes according to principal component analysis. The obtained *Sandesh* quality factors have been observed to be well correlated with acids, ketones, alkanes and aldehydes during storage from the correlation studies. From this study, headspace freshness marker of acid group was recognized along with headspace key spoilage markers such as alkanes, alcohols and ketones, which consists of 1-hexanol, 2-ethyl-; 1-pentanol; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-, (.+/-.); pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The presence of these markers can evaluate the quality of *Sandesh* in both the ways such as freshness and spoilage. The present investigation could be used for quality control aspects in dairy industry and also in the development of intelligent packaging systems for monitoring the real-time quality of the packaged product.

## Acknowledgement

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RESEARCH ARTICLE

# Quality and functional attributes of vacuum-packed yak milk *mozzarella* cheese as influenced by storage

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**Abstract:** The fresh m*ozzarella* cheese was developed from yak milk by using direct acidification method, packaged under vacuum atmosphere and stored at 4±1°C for 35 days. The effect of storage was evaluated on proximate composition, physicochemical, functional, microbiological and sensory properties of yak milk *mozzarella* cheese at defined interval. Among the proximate composition, the moisture content of cheese varied from 48.17% at day 0 to 47.00% at the end of storage. As the storage period progressed, a non-significant differences ($p>0.05$) was observed for the fat, protein and ash content. Storage time significantly ($p<0.05$) affected the physicochemical properties of cheese sample, including pH, titratable acidity [lactic acid (% by weight)], tyrosine value (µg/g) and total free fatty acids (µm/g). A significant increase ($p<0.05$) in functional properties (meltability, free oil formation and stretchablity) of cheese sample was recorded as the storage progressed. A significant increase in the standard plate count and, yeast and mould count was also observed during progress of storage. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage. Therefore, it was concluded that the vacuum-packed yak milk *mozzarella* cheese sample could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes.

**Keywords:** Yak milk *mozzarella* cheese; vacuum packaging; quality attributes; functional properties; storage stability

## Introduction

*Mozzarella* is the most widely available un-ripened cheeses in the market. It is an Italian, highly valued and fresh stretched curd cheese that be linked to the pasta *filata* group (El Owni and Osman, 2009). It involves skilfully stretching the curd in hot water to provide a smooth texture and lively surface (Kosikowski, 1982). It is regulated by law as well as a member of the European Protected Designation of Origin (European Communities, 1996). Traditionally, *mozzarella* cheese is made from milk of buffalo, which is premium and nutrient dense cheese world-wide (Sameen et al. 2008; Vogt et al. 2015). Other milks may also be utilized for the production of *mozzarella* cheese, such as cow's, goat's, and sheep's in many countries. Yak milk is superfood and produced in considerable amount especially in Himalayan region of Ladakh, Jammu & Kashmir, Arunachal Pradesh, Sikkim, Himachal Pradesh, Uttarakhand (Singh et al. 2023a). Yak milk cheese production can help highlanders in sustaining their nutrition and boosting their economic activities despite the region's difficult climatic conditions (Singh et al. 2023b). Commercial yak cheese production exist in the yak rearing countries like Tibet, China, Bhutan, Nepal and Russia having a huge demands in the market. However, in India this sector is not well developed due to dwindling yak population. Yak milk is a good source of cheese making owing to its high casein-fat ratio, total solids, larger fat globules and have special qualities as well as it is also beneficial to human health (Zhang et al. 2017; Zhang et al. 2020; Singh et al. 2023a, b). Recently, in India, cheddar style-yak milk cheese (Singh et al. 2023b), yak milk *paneer* (Singh et al. 2022a) and yak milk *ghee* (Singh et al. 2022b) was developed by using yak milk. Increasing demand for *mozzarella* cheese is being driven by the diversification of pizza parlours and fast food chains worldwide (Bhattarai and Acharya, 2010). Since it melts and stretches easily,

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and primarily used in the fast food industry as well as in cheese-based salad dressings (Vogt et al. 2015). It is therefore imperative to standardise the process that uses yak milk and improves the quality of local cheese. It can be a boost to the highland pastoral nomads and yak dairy industries. As far as the use of yak milk in *mozzarella* cheese production is concerned, no research has been carried out in India. In light of yak milk's compositional differences as compared to cow and buffalo milk, it would be interesting to investigate how it is processed and the quality of its *mozzarella* cheese. Therefore, in the present study, yak milk *mozzarella* cheese was developed and was evaluated the effect of storage on proximate composition, physicochemical, functional, microbiological and sensory properties of the vacuum-packed yak milk *mozzarella* cheese during refrigerated storage.

## Materials and Methods

### Materials

The fresh yak milk (dry matter 16–18%, fat 5.50–7.50%, solid-non-fat 10-11% and protein 3.75–4.25%) was obtained from the Nyukmadung farm of ICAR-National Research Centre on yak, Dirang situated at Nyukmadung, Arunachal Pradesh, India, between latitude of 27°25.948' North and longitude of 092°08.658' East at an altitude of 2750 meters above mean sea level. Immediately, milk was brought to the laboratory under refrigerated conditions for cheese preparation. The microbial rennet (Meito®) commercially produced in granular form from *Mucor pusillus* var. *Lindt* was obtained from M/s Meito Sangyo Co., Ltd., Tokyo, Japan for yak milk *mozzarella* cheese preparation. Analytical grade chemicals and reagents used for various laboratory analyses were procured from standard firms. The commercially available rectangular plastic packaging material of 127µm thickness for vacuum packaging was purchased from Swiss Pac Pvt. Ltd., Gujrat, India.

### Preparation of yak milk mozzarella cheese

The yak milk *mozzarella* cheese preparation was carried out according to Guinee et al. (2002) and Kosikowski (1982) with some modifications (Fig. 1). The fresh yak milk was at first filtered and then standardised to a casein-fat ratio of 0.80 by using skim milk. The standardized milk was pasteurised at 72±1°C for 15 sec in a stainless-steel vat and then cooled down to 4-8°C. The pH of yak milk (6.46) was adjusted to 5.2-5.4 by using 25% citric acid, then the temperature was raised to 30±1°C. This was followed by the addition of rennet @25mg/L milk, mixed effectively and then the milk was left undisturbed at 30±1°C and waited for 30-45 min or until a firm set was reached. After coagulation, set curd was cut into small cubes (1-1.5 $cm^3$ in size) using sterile cheese knives (horizontally and vertically). The curd was left undisturbed for healing for 5-10 min, after healing the cooking was done from 31-41°C with in 40-45 min under simultaneous stirring. After cooking, the whey was drained through strainer. And cheese *coagulum* was taken out and submerged in 82±1°C water and manual stretching was done until forming a shining, smooth and homogenous mass. Thereafter, it was moulded into balls of 200-250 g in size and placed in to chilled brine solution (7.5%w/v non-iodised salt) for 2 h and subsequently surface dried for 4 h under refrigerated conditions (7±1°C). The weight of the prepared

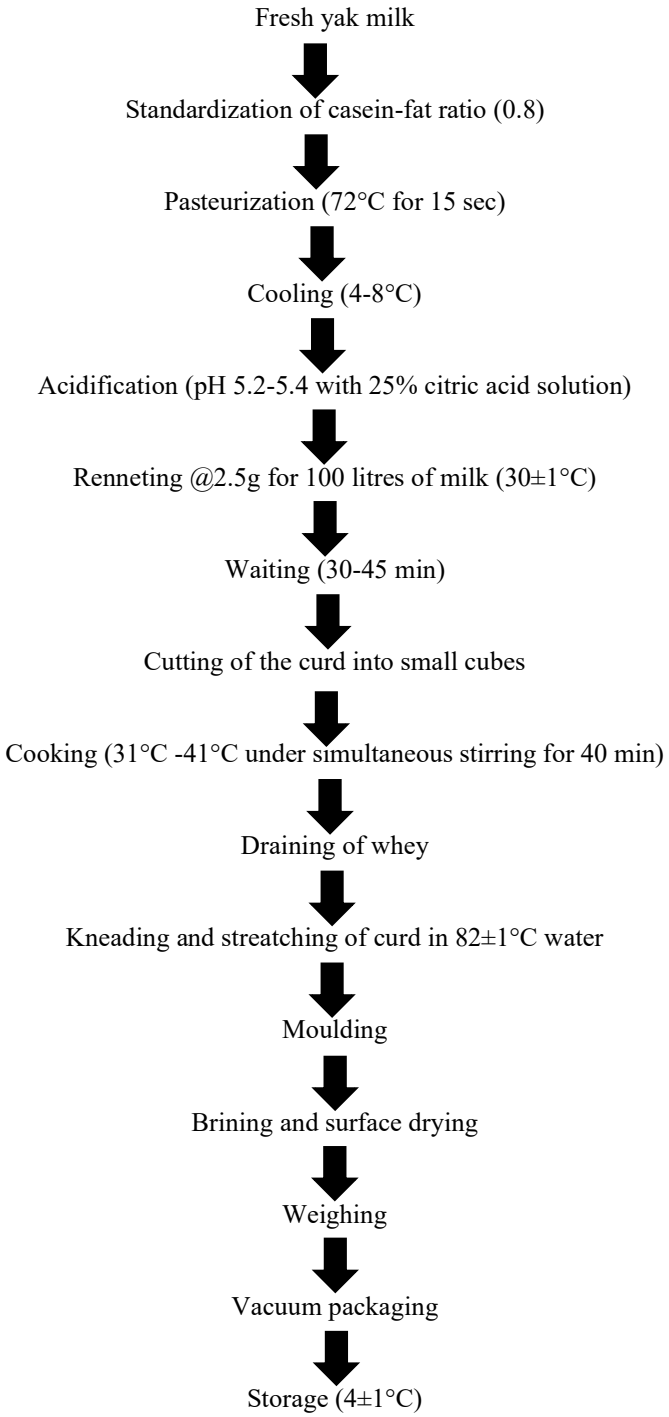


**Fig 1.** Process of preparation of yak milk *mozzarella* cheese by direct acidification

cheese was recorded and packed under vacuum nylon packs (127µm thickness) by using vacuum packaging machine (Model: QS500VSV4; Make: Sevana Electrical Appliances Pvt. Ltd., Kerala, India) and stored at 4±1°C for 35 days. Samples of cheese were drawn and evaluated for proximate composition, physicochemical, functional, microbiological, and sensory properties at defined intervals. The experiment was repeated twice, and the respective analysis were done in triplicate.

**Analytical procedures**

**Mozzarella cheese yield**

The *mozzarella* cheese yield (%) was calculated using the equation as given below:

$$Cheese\ yield\ (\%) = \frac{Weight\ of\ cheese\ (kg)}{Weight\ of\ milk\ taken\ (kg)} \times 100$$

**Proximate composition**

The moisture and ash content of cheese sample was estimated by following the Association of Official Analytical Chemists' approved methods (AOAC, 2005). whereas, the fat content of cheese sample was evaluated by using the Gerber method and total protein content was estimated by determining the total nitrogen using the Kjeldahl method (IDF, 1993) and converting it to protein content by multiplying by 6.38.

**Physicochemical properties**

The pH values (grated cheese sample and distilled water, 1:2) were recorded electrometrically using a digital pH meter (Make: PC 2700, Oakton®, India). The titratable acidity was determined by the titration method suggested by AOAC (2005) and results were recorded in lactic acid (% by weight). Juffs (1973) method was used to determine the tyrosine value and results were shown in µg/g. Deeth and Fitz-Gerald (1976) extraction-titration method was used to evaluate the total free fatty acids (TFFA) content, and results were shown in µm/g.

**Functional Properties**

The meltability of cheese sample was evaluated using a prescribed method from Muthukumarappan et al. (1999) with slight modifications. Samples (3.5 cm in diameter and 1 cm in height was tempered at room temperature for 30 min. Thereafter, sample was then heated at 100±1°C for 5 min in hot air oven. After the cooling of melted cheese at room temperature for 5 min, the meltability of cheese was recorded by measuring the final diameter (minimum and maximum) of the cheese discs at 5 different places and expressed as the mean values in cm. Stretchability of cheese sample was evaluated by following the method of Lemay et al. (1994). The average values in cm was measured as streatchbility of cheese. Free oil formation or oiling off property of cheese sample was evaluated by the method of Breen et al. (1964) with the following modification. Discs of 1.5 cm in diameter and 0.5 cm in thickness were cut, melted on filter paper in the oven at 100±1°C for 5 min. The average of free oil formation at four different angles was expressed in cm.

**Microbiological analysis**

Microbiological analysis of the sample was done using standard methods mentioned by the American public health association (APHA, 2005). In order to prepare the media, careful attention was paid to the manufacturer's instructions (Plate Count Agar for standard plate count, Potato Dextrose Agar for yeast and mould count, and Violet Red Bile Agar for coliform count). The plates showed 30–300 colonies were counted, and their number was multiplied by reciprocal of dilution. The microbial count was recorded as $\log_{10}$CFU/g of the sample.

**Sensory analysis**

A twenty four (n-24) semi-trained members (aged between 20 to 60 years) composed of scientists, administrative staff and other employees from ICAR-NRC on Yak, Dirang were selected as the sensory panellists for assessing the yak milk *mozzarella* cheese. The properties of the developed product were defined, and panellists were familiarised with the Sensory Performa before performing the analysis. The cheese was cut uniformly and pieces were tempered for 20-30 min at room temperature (20±2°C) then served individually to each panellists. The coded samples were evaluated for their sensory traits like colour & appearance, flavour, body & texture and overall acceptability using a hedonic scale of 9-point intensity varied from extreme dislike (score = 1) to extreme like (score = 9).

**Statistical analysis**

Statistical analysis was done using the one-way analysis of variance (ANOVA). The ANOVA was done by using Statistical Package for the Social Sciences (SPSS) software package trial version 22.0 (IBM SPSS Inc. Chicago IL, USA). The statistical significance at 5% level was considered significant. Post-hoc analysis was done using Duncan's multiple range test. The data were recorded in the form of average ± standard deviation.

**Results and discussion**

The obtained result showed the average yield of 15.09% for yak milk *mozzarella* cheese in the present study. The effect of the storage period on the proximate composition of cheese sample is summarized in Table 1. The moisture content (%) of cheese varied from 48.17±0.89 at day 0 to 47.00±0.61 at the end of storage. Various factors may contribute to the variation in moisture content in cheese, including preparation methods (El-Owni and Osman, 2009), cooking temperatures (McSweeney, 2007), and compositional differences (size of fat globules and casein micelles

characteristics) of yak milk (Zhang et al. 2020). Guinee et al. (2002) stated that having a low pH during storage causes the protein network to become unstable, which causes more moisture to be released. A non-significant differences ($p>0.05$) was recorded for the fat, protein and ash content during the progress of storage period. The fat content (%), protein content (%) and ash content (%) was 24.24±0.40, 22.64±0.55 and 2.96±0.22 on day 0 and 24.76±0.51, 23.27±0.94 and 3.14±0.19 on day 35, respectively. Smaller variations in the proximate composition of cheese might be due to variations during storage conditions. The compositional differences of yak milk influences the proximate composition of cheese (Singh et al. 2023a). Table 2 showed physicochemical properties of cheese sample during storage. Storage period ($p<0.05$) had a significant effect on pH and TA of cheese sample. The pH and TA value varied from 5.45±0.04 to 5.62±0.03 and 0.39±0.03 to 0.52±0.04, respectively, during the storage time. This would be due to biochemical changes occurred during storage and proximate composition of cheese (Sameen et al. 2008). Storage period had a significant effect on the tyrosine value of cheese sample ($p<0.05$). It was increased from 101.50 µg/g to 322.33 µg/g in the first 28 days of storage, and then began to decrease after the 28$^{th}$ day of storage period. In cheese sample, tyrosine level has been found to increase because enzymes and microorganisms hydrolysed proteins during storage (Singh et al. 2012). The results are in line with the study of Singh et al. (2022a) who also reported the increase in tyrosine value of yak milk *paneer* during storage. There was significant increase in free fatty acid (FFA) content of cheese sample during storage. At day 0 of storage, the FFA value was 1.69µm/g and at the end of storage period (day 35), it was increased to 2.45µm/g. It might be due to slower rate of lipolysis under mentioned storage conditions.

Functional characteristics such as meltability, free oil formation (FOF) and stretchablity are presented in Table 3. Meltability is the ability of cheese to melt uniformly, smoothly, and homogeneously (cheese shred should not be visible) without releasing oil or becoming watery (Johnson, 2000). The initial meltability of cheese sample on day 0 was 4.90 cm which significantly increased ($p<0.05$) to 7.53 after 35 days of storage. Similar observations were also observed by Imm et al. (2003) who observed that refrigerated storage of bovine and caprine *mozzarella* cheese (MC) influenced the meltability. The meltability of MC depended on various factors such as water partitioning, rearrangement of protein matrix (McMahon et al. 1999), displacement of the para-casein matrix (Guinee et al. 2001) and the amount as well as distribution of fat in the protein matrix (Imm et al. 2003). Free oil formation, also known as 'oiling off' or 'fat leakage', occurs when free oil separates from the melted cheese and accumulates in pockets or pools, particularly on its surface (Jana et al. 2017). A significant increase ($p<0.05$) in FOF of cheese sample was recorded as the storage progressed (Table 3). Free

**Table 1:** Effect of storage on proximate composition of yak milk *mozzarella* cheese (Mean±S.D.)

| Proximate composition (%) | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Moisture content | 48.17 ±0.89$^{b}$ | 48.32 ±0.32$^{b}$ | 47.93 ±0.56$^{b}$ | 47.46 ±0.66$^{ab}$ | 47.60 ±0.75$^{ab}$ | 47.00 ±0.61$^{a}$ |
| Fat | 24.24 ±0.40$^{a}$ | 24.20 ±0.49$^{a}$ | 24.35 ±0.54$^{a}$ | 24.56 ±0.55$^{a}$ | 24.44 ±0.57$^{a}$ | 24.76 ±0.51$^{a}$ |
| Protein | 22.64 ±0.55$^{a}$ | 22.59 ±0.56$^{a}$ | 22.71 ±0.56$^{a}$ | 23.02 ±0.45$^{a}$ | 22.97 ±0.42$^{a}$ | 23.27 ±0.94$^{a}$ |
| Ash | 2.96 ±0.22$^{a}$ | 2.92 ±0.20$^{a}$ | 2.98 ±0.19$^{a}$ | 3.07 ±0.12$^{a}$ | 3.04 ±0.18$^{a}$ | 3.14 ±0.19$^{a}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

**Table 2:** Effect of storage on physicochemical properties of yak milk *mozzarella* cheese (Mean±S.D.)

| Physicochemical properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| pH | 5.58 ±0.07$^{cd}$ | 5.62 ±0.03$^{d}$ | 5.60 ±0.02$^{cd}$ | 5.56 ±0.02$^{bc}$ | 5.52 ±0.05$^{b}$ | 5.45 ±0.04$^{a}$ |
| Titratable Acidity [TA; lactic acid (% by weight)] | 0.39 ±0.03$^{a}$ | 0.41 ±0.03$^{a}$ | 0.42 ±0.04$^{a}$ | 0.46 ±0.03$^{b}$ | 0.47 ±0.04$^{b}$ | 0.52 ±0.04$^{c}$ |
| Tyrosine value (µg/g) | 101.50 ±8.17$^{a}$ | 238.44 ±14.08$^{c}$ | 274.83 ±20.14$^{d}$ | 280.11 ±12.27$^{d}$ | 322.33 ±23.68$^{e}$ | 214.83 ±5.96$^{b}$ |
| Total free fatty acids (µm/g) | 1.69 ±0.12$^{a}$ | 1.81 ±0.16$^{ab}$ | 2.01 ±0.15$^{bc}$ | 2.13 ±0.27$^{cd}$ | 2.33 ±0.21$^{de}$ | 2.45 ±0.17$^{e}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

oil started increasing significantly ($p<0.05$) after 14 days of storage in cheese sample, and it continued to increase until 35 days of storage. There are several factors that affect the formation of free oil in *mozzarella* cheese, including fat content, size of fat globules, fatty acid profile, and proteolysis (Tunick, 1994). The stretchability of melted cheese refers to its ability to form fibrous strands that elongate without breaking under tension (Jana et al. 2017). The maximum stretch (35.92 cm) was observed on day 35 of storage which was significantly ($p<0.05$) higher than the value (26.25 cm) obtained on day 1 of storage. According to Rehman et al. (2008), a commercial pizza cheese has a stretch value of 25.27 cm. Whereas, a minimum stretch of 3.0 inches (7.62 cm) of unbroken string is specified for Pizza cheese in the United States (USDA, 2007).

It is important from the perspective of food safety to consider the microbiological quality of cheese, since it is an excellent growth medium for a variety of microorganisms (Dharaiya et al. 2021). Table 4 reports the changes in microbiological properties for cheese sample during storage. The initial SPC ($log_{10}$CFU/g) in cheese sample was 3.37 on day 0 of storage which was increased to 4.66 on day 35 of storage. The initial average value of YMC count ($log_{10}$CFU/g) of cheese sample increased from 1.13 to 2.74 after the end of storage period. It indicates storage had significant impact ($p<0.05$) on changes in SPC and YMC counts. The coliform count was absent in cheese sample throughout the storage. During the scalding process, high temperatures reduce the microbial flora associated with contamination and increase the safety of *mozzarella* cheese (Marth and Steele, 2005). According to Han et al. (2015), natural *mozzarella* cheese prepared by direct acidification had a viable count of 5.8 $log_{10}$CFU/g.

*Mozzarella* cheese made from yak milk had shown delicious milky flavour, homogenous texture, and a whitish-yellowish colour

**Table 3:** Effect of storage on functional properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Functional properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Meltability (cm) | 4.90 ±0.24$^{a}$ | 5.32 ±0.21$^{b}$ | 5.80 ±0.24$^{c}$ | 6.27 ±0.48$^{d}$ | 6.98 ±0.25$^{e}$ | 7.53 ±0.23$^{f}$ |
| Free oil formation (cm) | 2.45 ±0.10$^{a}$ | 2.65 ±0.10$^{a}$ | 3.00 ±0.14$^{b}$ | 3.30 ±0.23$^{c}$ | 3.77 ±0.23$^{d}$ | 4.35 ±0.22$^{e}$ |
| Stretchability (cm) | 26.25 ±1.57$^{a}$ | 28.67 ±2.04$^{b}$ | 29.33 ±1.08$^{bc}$ | 31.17 ±1.50$^{c}$ | 33.50 ±1.70$^{d}$ | 35.92 ±1.80$^{e}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

**Table 4:** Effect of storage on microbiological properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Microbiological properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Standard Plate Count ($Log_{10}$CFU/g) | 3.37 ±0.26$^{a}$ | 3.84 ±0.06$^{b}$ | 4.13 ±0.07$^{c}$ | 4.25 ±0.04$^{c}$ | 4.51 ±0.02$^{d}$ | 4.66 ±0.03$^{e}$ |
| Yeast & Mould Count ($Log_{10}$CFU/g) | 1.13 ±0.21$^{a}$ | 1.88 ±0.10$^{b}$ | 2.01 ±0.12$^{b}$ | 2.38 ±0.09$^{c}$ | 2.64 ±0.07$^{d}$ | 2.74 ±0.04$^{d}$ |
| Coliform count ($Log_{10}$CFU/g) | ND | ND | ND | ND | ND | ND |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$). ND-Not detected

**Table 5:** Effect of storage on sensory properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Sensory properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Colour & Appearance | 8.33 ±0.76$^{a}$ | 8.35 ±0.91$^{a}$ | 8.17 ±0.78$^{a}$ | 8.02 ±0.80$^{a}$ | 8.23 ±0.83$^{a}$ | NP |
| Flavour | 8.27 ±0.71$^{a}$ | 7.79 ±0.99$^{a}$ | 8.04 ±0.82$^{a}$ | 7.73 ±0.98$^{a}$ | 8.19 ±0.89$^{a}$ | NP |
| Body & Texture | 8.25 ±0.75$^{b}$ | 8.17 ±0.86$^{b}$ | 8.13 ±0.77$^{b}$ | 7.15 ±1.04$^{a}$ | 8.29 ±0.86$^{b}$ | NP |
| Overall Acceptability | 8.19 ±0.82$^{a}$ | 8.30 ±0.82$^{a}$ | 8.13 ±0.72$^{a}$ | 7.91 ±1.02$^{a}$ | 8.29 ±0.86$^{a}$ | NP |

n=24, *mean with different superscripts in a row differs significantly ($p<0.05$). NP-Not Performed

during sensory evaluation by the sensory panellists. Samples of cheese were evaluated periodically for various sensory properties including colour and appearance, flavour, body and texture, and overall acceptability. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage (Table 5). It was evident from Table 5, the sensory scores was higher at the beginning and it was lower in later part of storage period. It is because *mozzarella* cheese, unlike most other cheeses, is not ripened or aged, and its sensory properties reduce with the progress of storage time (Yazici et al. 2010; Sulieman et al. 2013). The sensory evaluation of cheese was discontinued on day 35 of storage period due to development of off flavour and mouldy surface on cheese sample. Changes in physicochemical, biochemical, and microbiological properties in cheese during storage might explain this trend.

## Conclusion

In the present study, *mozzarella* cheese was developed by using yak milk for the first time in India. In order to determine the shelf-life of vacuum-packed yak milk *mozzarella* cheese (YMMC), the cheese sample was analysed for proximate composition, physicochemical, functional, microbiological and sensory properties during storage. Based on the obtained results, it was found that storage significantly influenced the moisture content, meltability, free oil formation, stretchablity, physicochemical and microbiological properties of YMMC. The cheese was relished by the sensory panellists throughout the storage and could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes. Further research is also needed to fully understand the textural, rheological behaviour and other preparation methods for YMMC. Taking part in yak cheese business could be lucrative for highlanders and provide them with financial assistance.

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RESEARCH ARTICLE

# Prevalence of mastitis and antibiotic resistant *E. coli* and *S. aureus* in dairy animals

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**Abstract:** The prevalence of mastitis in milking animals and subsequent antibiotic usage is one of the major concerns in dairy sector. In this work, a baseline data was generated by collecting 675 raw milk samples from organized and individual dairy farms in Haryana. The milk samples were screened for mastitis, antibiotic residues and antimicrobial resistance (AMR) *E. coli* and *S. aureus* using rapid BD Phoenix M50 ID/ AST system and conventional methods. The study indicated 14.37% animals infected with sub-clinical mastitis and 11.25% with clinical mastitis. 79 milk samples from normal and infected animals were found contaminated with antibiotic residues with presence of enrofloxacin, streptomycin, tetracycline, sulfa drugs and multi drug residues. Out of 675 samples, 173 were infected with mastitis with involvement of *E. coli* in 18.49% and *S. aureus* in 38.72%. In *E. coli* isolates, the maximum resistance of 25% was observed against Ampicillin, Ciprofloxacin and Levofloxacin and Extended spectrum β-Lactamase (ESBL) productionwas observed in 15.65% infected samples.The maximum resistance of 32% was observed in *Staphylococcus aureus* against Ampicillin and Penicillin-G and Methicilin Resistant Staphylococcus aureus (MRSA) was found in 22.38% along with β-Lactamase resistance in 31.34% of infected samples.The main reason behind the increasing number of resistant pathogens may be due to the indiscriminate use of antibiotics by untrained veterinary professionals and lack of diagnostic facilities, hence regular screening of sub-clinical mastitis should be practiced to control the usage of antimicrobials and resistant development in dairy pathogens.

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## Introduction

Mastitis is a major problem affecting all milk producing animals worldwide and is one of the main reasons for impaired milk quality (Bradley, 2002; Le Roux et al. 2003). Mastitis is the inflammation of udder; the term comes from the Greek word i.e. Masto- referring to the mammary gland and its meaning "inflammation" (Blood and Studdert., 1999). Mastitis can be sub-clinical, clinical and chronic depending on severity of inflammation. Dairy farmers face a financial burden from bovine mastitis, and preventive mitigation strategies are essential for the long-term viability of any dairy production. Controlling the infection, minimizing the risk of persistent infections, and directing antimicrobial therapy all need the identification of etiological agents (Duarte et al. 2015). It is mainly caused by bacterial pathogens which include *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, and Mycoplasma sp.* and environmental pathogens involving *E. coli* and *Klebsiella spp*. Other common pathogens include *Corynebacterium spp., coagulase-negative staphylococci* and *Pseudomonas aeruginosa* (Motwani & Kishore., 2011). Despite extensive research into controlling bovine mastitis, the occurrence of mastitis remains high, resulting in massive losses for the dairy industry (Lee et al. 2008). The pathogens responsible for producing mastitis will determine the sort of mastitis treatment that should be offered. Greater than 200,000 somatic cells per milliliter (SCC) is a sign of inflammation and subclinical mastitis (Cobirka et al. 2020). During the infection caused by the micro-organisms, the host immune response is activated to eliminate the invading microorganisms lead to inflammation and damage milk-producing tissue of the mammary gland leading to decreased in milk yield (Egyedy and Ametaj, 2022) . Milk from animals with mastitis cannot be used for human consumption because it has altered chemical composition and organoleptic proprieties (Kobayashi et al. 2013). Moreover, milk from diseased animals negatively affects the milk processing and shelf-life of final dairy products. This disease is considered to be the major cause of economic loss to dairy farmers (Tommasoni et al. 2023). Mastitis can be reduced by some aspects of dairy farming such as feeding

practices, animal husbandry, hygiene and general health care. Increasing number of infection has led to the increased use of antibiotics for treatment but their indiscriminate use by untrained veterinary professionals or quacks has resulted in increased resistance of antibiotics among the dairy animals which is a growing concern and need to be monitored carefully (Eltholth et al. 2022). The present work is focused on detection of mastitis, antibiotic residues, bacterial pathogens (*E. coli* and *S. aureus*) and their resistance pattern in raw milk samples.

**Material and methods**

All the experiments required for present research were run in triplicate and results were interpreted all the study carried out at National Referral Centre for Milk Quality and Safety and Dairy Microbiology Division, NDRI, Karnal.

**Sample collection**

A total of 675 raw milk samples were collected from different districts of Haryana i.e. Karnal, Ambala and Sonipat during the year 2018-20. Collection of milk samples was done from healthy as well as infected cows/ buffaloes. Milk sampling was carried out following aseptic procedures as described by National Mastitis Council (NMC, 2004). The time chosen for milk sample collection was before milking. All the details of the animal along with the collection date were recorded. The hands were washed properly with soap and water and the gloves were worn while sampling. Any dirt or debris present on the teat of the animal was brushed and initial few streams of milk were discarded. The teat was pre-dipped with an effective teat dip (6 part of 0.5% iodine with 1 part of glycerin) and left for few seconds. Each teat was dried properly with paper or cloth towel. The teat end was scrubbed for 15-20 seconds with cotton or cloth gauze moistened with 70% to 80% alcohol or isopropyl alcohol. The sample container was opened and immediately the sample was taken preventing the teat touching the container. The sample container was kept in ice box until delivered to lab.

**Detection of Mastitis using CMT**

The California Mastitis Test (CMT) is a simple indicator of the Somatic Cell Count (SCC) of milk. The procedure of CMT was followed as per the instructions given in the kit manual. 3.0 ml milk was taken in four-compartment paddle and equal amount of CMT reagent was added. After addition of reagent and sample, CMT paddle was rotated 10 times in an anti-clockwise direction and graded based on gel formation, scores as Negative (N), Trace (T), 1, 2and 3 were given.

**Detection of Mastitis using Somatic cell counter**

Somatic cells are purely animal body cells present in small levels in normal milk. High levels of SCCs in milk indicate poor quality milk that is caused by an intra-mammary infection. The milk analyzer (Model- Ekomilk Scan) measures the flowing time of the milk through the sample mixer capillary and determines the number of somatic cells in accordance with time. The viscosity measurement is temperature sensitive and uses Ekoprim reagent as surfactant. The somatic cells were measured as per the instruction's manual. 10mL of milk sample and 5mL of Ekoprim reagent was added in the sample bulb and the bulb rotated for few seconds after pressing the run button and displayed somatic cell count on the screen along with the time based on viscosity

**Detection of antibiotics using Spore based kits**

Preliminary screening of antibiotics was done using Paper strip and DPA kits developed at ICAR-NDRI, Karnal as per the test procedure given by Swathi, 2017. Test kit is working on spore germination- inhibition principle and can detect antibiotics in milk at regulatory limits set by FSSAI/ CODEX. In the presence of antibiotics, spore germination is inhibited whereas in the absence of antibiotics spores germinate leading to release of DPA/or enzyme that react with the substrate functionalized on strip resulting in color change from purple to yellow in DPA kit and colorless to blue on strip test.

**Quantitative detection of antibiotic groups using AOAC approved CHARM/ ROSA**

The antibiotic contaminated milk samples from normal and infected animals were tested using Rapid One step Assay (ROSA) which works on the principle of lateral flow assay. The ROSA Test uses receptors with binding to drugs. The test was performed as per instructions in operator's manual. The incubator was set at desired temperature. 300µL of milk sample was added to the strip and incubated for 8 min and results were observed on the strip and quantified using ROSA reader.

**Isolation and identification of *E. coli and S.aureus***

Isolation and identification of *E. coli* was done using ISO procedure IS: 5887 Part-1:1976 (RA-2018). The milk samples were first enriched in McConkey broth and loopful was streaked on McConkey Agar and Eosin methylene blue (EMB) agar. The inoculated media was then incubated at 37°C overnight. If there was growth in McConkey broth along with fermentation of lactose, the loopful was streaked onto solid media and incubated overnight at 37°C. The suspected colonies were further identified using biochemical tests as mentioned in ISO protocol. The confirmed isolates were also tested using BD Phoenix M50. Isolation and identification of *S. aureus* was done using ISO procedure IS: 5887(Part-8/sec-1):2002 (RA-2018). The test sample was spread onto Baird Parker agar (BPA) plates and incubated for 24-48 hrs at 37°C. The suspected grey black colonies with opaque zone were identified using catalase and coagulase test. The confirmed isolates were also tested using BD Phoenix M50.

**Antibiotic Susceptibility Test (AST)**

The samples which were found positive for *E. coli* and *S. aureus* were further processed for their antibiotic sensitivity/ resistance using different antibiotic discs. Standard disk diffusion assay was conducted using Muller-Hinton agar and broth culture equivalent to 0.5 McFarland standards as recommended by the Clinical and Laboratory Standards Institute (2014). Antibiotic disks were chosen based on commonly used antibiotics for animal and human therapy in the study region. Antibiotics used for *E. coli* were Ceftriaxone, Ceftazidime, Cefotaxime and Cefepime, Imipenem, Meropenem, Ertapenem and Doripenem. Antibiotics used for *S. aureus* were Cefoxitin and Oxacillin. *E. coli* and *S. aureus* isolates were processed for AST using disc diffusion method for preliminary screening and further the resistance pattern was studied using confirmatory tests: double disc test for Extended spectrum β- lactamases (ESBL) detection, Modified Hodge test (MHT) and Modified carbapenamase inactivation method (mCIM)for Carbapenamase (KPC) detection and streaking on Methicillin resistant *S.aureus* (MeReSa) agar for MRSA detection. The AST pattern of positive isolates was studied using conventional method and random samples were further confirmed with BD Phoenix M50.

**Statistical Analysis**

Data was analyzed statistically in three replicates according to Snedecor and Cochran (1980)

**Results and Discussion**

A total of 675 raw milk samples from both organized and individual dairy farmers were collected from three districts of Haryana state i.e. Karnal, Ambala and Sonipat. Selection of animals in organized dairy farms and un-organized sector was random. Milk samples were collected directly into the sterile containers from cow's teat and immediately transferred to lab after proper labeling. These samples were tested for Mastitis infection, presence of antibiotic residues, bacterial pathogens i.e. *E. coli* and *S. aureus* and their phenotypic resistance profile.

**Prevalence of mastitis**

The study showed prevalence of 11.25% clinical mastitis and 14.37% sub-clinical mastitis (Fig.1). Among Mastitis positive samples, S.aureus was detected in 40.78% of clinical mastitis and 37.11% in clinical cases; while in case of *E.coli* 17.10% was detected in clinical mastitis cases and 19.58% in sub-clinical cases, as shown in (Fig 2).

The presence of mastitis in raw milk was 28% using CMT and 25.62% using SCC indicating strong positive correlation as also reported by Badiuzzaman et al. 2015 and Bitew et al.2010. In a similar investigation, Bhat et al. 2017 reported 11.50% and 27.81% (Maheshwari et al. 2016) respectively. The variation in sub-clinical mastitis may be attributed to the selection of animals, season, breed and sanitary conditions. Higher prevalence of sub-clinical mastitis compared to clinical mastitis in present investigation was also supported by Sori et al. 2011. Sub-clinical mastitis give invisible and silent symptoms as reported by Karimuribo et al. 2017 which is usually given less attention by farmers when it comes to treatment unlike clinical mastitis which shows visible and prominent symptoms towards which treatment and control efforts are concentrated.

**Prevalence of Antibiotic Residues**

The presence of antibiotic residues in milk is a serious concern keeping in view of its processing implications in terms of starter failure in fermented products and public health implications through development of antimicrobial resistance (AMR). Accordingly, surveillance study on antibiotic residues in milk was carried out. The milk samples collected from normal and infected animals were initially screened for Qualitative analysis using spore based kits (DPA/ paper strips) developed at ICAR-NDRI, Karnal. Out of 675 milk samples, normal milk used for

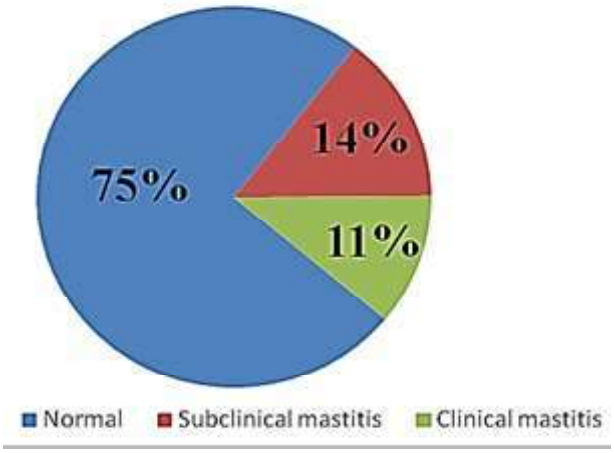


**Fig. 1** Incidence of mastitis in raw milk

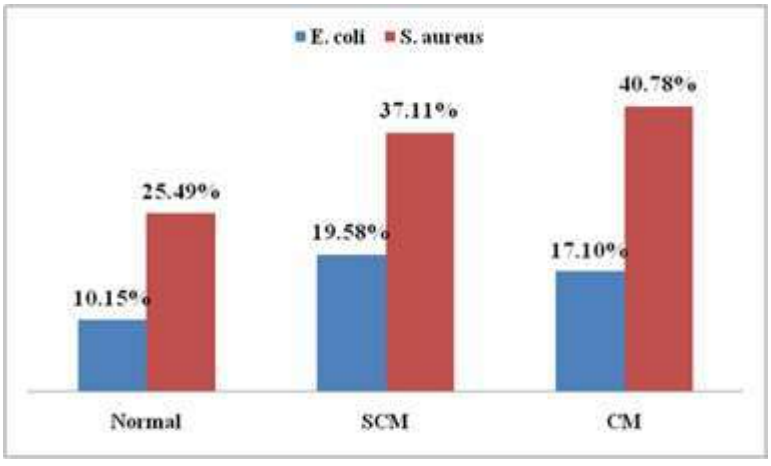


**Fig. 2** Incidence of E. coli and S. aureus in raw milk

processing purpose showed presence of antibiotics in 2.96%. Milk from treated animals which is unfit for processing with sub-clinical mastitis were positive with antibiotic in 4.1 % and clinical milk samples with 4.59%. Antibiotic residues like enrofloxacin, streptomycin, tetracycline, sulfa drugs and multidrug residues were detected. In a similar study, Moudgil et al. 2019 reported 11.30% milk samples from Punjab contaminated with antibiotic residues. In recent study, FSSAI (2018) has also reported the presence of antibiotic residues in milk however, the sample size was small and needs further surveillance work to support the findings reported in the current investigation.

**Isolation and Identification of *E. coli* & *S. aureus***

Out of 675 samples, 173 were infected with mastitis with involvement of *S. aureus* in 40.78% in clinical and 37.11% in sub-clinical cases. Similarly, *E. coli* was detected in 17.10 % and 19.58% respectively (Fig.2). Kumar et al. 2015 reported similar findings with incidence of 33.82%*S. aureus* and 14.91%*E. coli*. The non-infected milk samples also showed presence of *E. coli* in 10.15% and *S. aureus* in 25.49% .

**Antibiotic Susceptibility Test**

The AST pattern of positive isolates was studied using conventional method and random samples were further confirmed with BD Phoenix M50.

**AST pattern of *E.coli* and *S.aureus* isolates**

In the case of *E. coli* isolates, the highest level of resistance at 25% was observed against Ampicillin, Lowest level of resistance at 6.25% was observed against chloramphenical- and no resistance was observed against amikacin, amoxy-clav, Cefoparazone-sulbactam ,Cefoxitin, Gentamicin, Imipenem, Meropenem and Piperacillin- tazobactam(Fig. 3a). In case of *S. aureus*, the highest resistance of 32% was observed against Ampicillin and Penicillin- No resistance was observed against broad range of antibiotic (Fig. 3b).

In case of *S. aureus*, the maximum resistance of 32% was observed against Ampicillin and Penicillin-G followed by Cefazolin, Cefoxitin and Oxacillin (14%). The resistance against norfloxacin, gentamicin and tetracycline ranged between 2-8%. No resistance

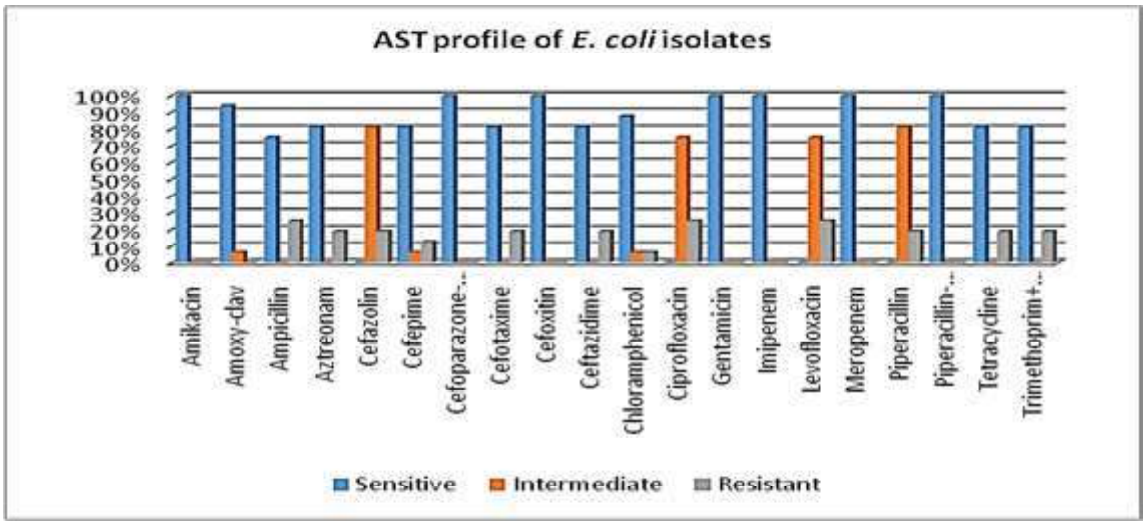


**Fig. 3 (a)** Graph showing AST profile of *E. coli* isolates

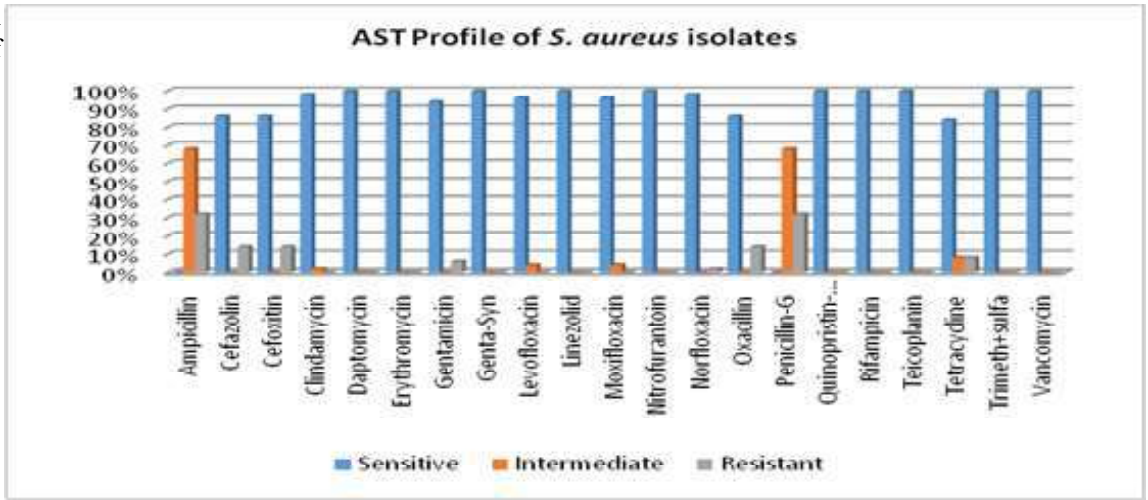


**Fig. 3 (b)** Graph showing AST profile of *S. aureus* isolates

was observed against Clindamycin, Daptomycin, Erythromycin, Gentamycin-Syn, Levofloxacin, Linozolid, Moxifloxacin, Nitrofurantoin, Quinopristin-dalfopristin, Rifampicin, Teicoplanin, Trimethoprim+ sulfamethaxole and Vancomycin (Fig. 3b)

The incidence of ESBL producing *E. coli was* 26.31% in sub-clinical mastitis . None of the clinical milk samples showed presence of ESBL E.coli . However , normal milk samples also showed presence of ESBL in 7.84% and carbapenase producing E.coli in 1.96 % which was considered as serious finding (Fig.4a). Our findings are in agreement with reports of Sharif et al.2017 who recorded average incidence of 20% ESBL in infected samples. Bhoomika et al. 2016 and Dewangan et al. 2017 also reported incidences of ESBL producing *E. coli* in raw milk samples as 8.22% and 7.69% in Chattisgarh.

The presence of *S. aureus* with resistance of MRSA was observed 25% in sub-clinical and 19.35% in clinical mastitis. In a similar study, Shah et al.2019 reported Methicillin resistance *Staphylococcus aureus* (MRSA) resistance in *S. aureus* with 25% in infected samples. β-lactamase resistance (BLACT) in *S. aureus* was also investigatedwith presence of 36.11% in sub-clinical and 25.80%in clinical mastitis (Fig.4b).The presence of MRSA and BLACT was also detected in normal samples wherein incidence of 9.37% and 13.28% was observed respectively. In a recent study carried out by Deepak et al. 2020 reported 9.3% presence of MRSA in bovine milk collected from healthy cattle in Chennai.

Normal milk samples showed ESBL presence at 7.84% and carbapenase producing E.coli at 1.96 % which was considered a notable finding Fig 4a. β-lactamase resistance (BLACT) in *S. aureus* was also probed at 36.11% in sub-clinical and 25.80%in clinical mastitis (Fig.4 b).

## Conclusion

The prevalence of mastitis in milking animals is one of the growing concerns in dairy sector. The current investigation was carried out in infected milk samples collected from organized as well as un-organized sector keeping in view of the fact that prevailing hygienic conditions are widely different across the country. For its understanding, a baseline data was generated by collecting 675 raw milk samples from organized and individual dairy farms in Haryana. The presence of mastitis in raw milk was 28% using CMT and 25.62% using SCC indicating significant correlation. The study showed prevalence of 11.25% clinical mastitis and 14.37% sub-clinical mastitis. The variation in sub-clinical mastitis may be attributed to the selection of animals, season, breed and sanitary conditions. Sub-clinical mastitis give invisible and silent symptoms which is usually given less attention by farmers when it comes to treatment unlike clinical mastitis which shows visible and prominent symptoms towards which treatment and control efforts are concentrated. 675 milk samples were analyzed for the presence of antibiotic residues and enrofloxacin, streptomycin, tetracycline, sulfa drugs and multidrug residues were detected. Study on ID profile indicated the presence of *E. coli* in 18.49% and *S. aureus* in 38.72% infected milk samples. The AST profile revealed that *E. coli* isolates showed maximum resistance of 25% against Ampicillin, Ciprofloxacin and Levofloxacin and Extended spectrum β-Lactamase (ESBL) production in 15.65% infected samples. The maximum resistance of 32% was observed in *Staphylococcus aureus* against Ampicillin and Penicillin-G and Methicilin Resistant *Staphylococcus aureus* (MRSA) was found in 22.38% along with β-Lactamase resistance in 31.34% of infected samples. *S. aureus* remains the major pathogen in infected samples which may pose a threat to public health. The main reason behind the increasing number of resistant pathogens may be due to the indiscriminate use of antibiotics by untrained veterinary professionals and lack of diagnostic facilities; hence regular screening of sub-clinical mastitis should be practiced to

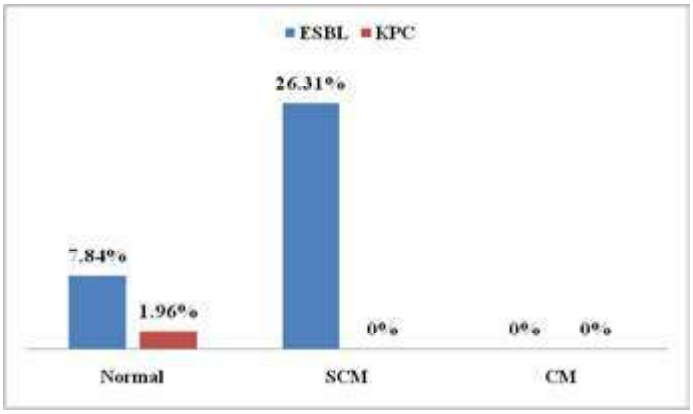


**Fig. (4a):** Resistance pattern of E. coli isolates at different stages of mastitis

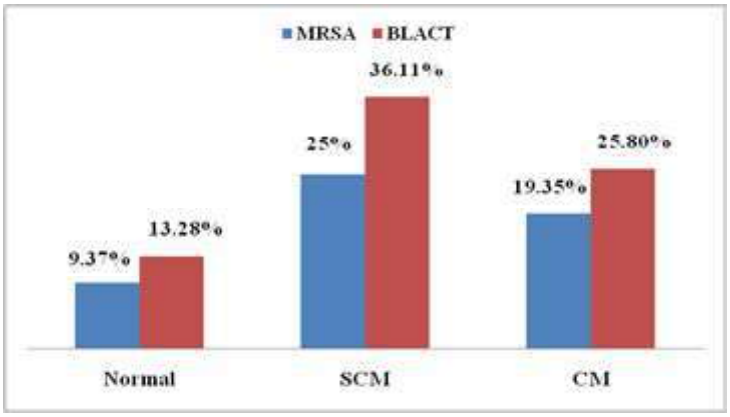


**Fig. (4b):** Resistance pattern of S. aureus isolates at different stages of mastitis

control the usage of antimicrobials and resistant development in dairy pathogens.

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Indian J Dairy Sci 77(2): 123-129
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RESEARCH ARTICLE

# Comparative antibiogram analysis of bacterial isolates from mastitic milk of cattle and buffalo in Haryana

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**Abstract:** Mastitis causes huge economic losses to dairy industries worldwide. It is caused by various microorganisms, of which bacterial etiology is primarily important. Both Gram-positive and Gram-negative bacteria are involved in causation of disease. In the present study, 8561 milk samples from both cattle and buffalo combined were tested and occurrence of mastitis was recorded in 72.73 % (n = 6227/8561) milk samples. Occurrence of mastitis was non–significantly ($p$>0.05) higher in cattle (86.31%) than buffaloes (66.97%). California Mastitis Test (CMT) showed significantly ($p$<0.05) lower prevalence of mastitis compared to the culture examination statistically. By CMT analysis, buffalo had a significantly ($p$<0.05) lower percentage of mastitis positivity than cattle. Whereas, culture examination revealed that both cattle and buffalo exhibited a high prevalence of mastitis, with 97.75% and 98.15% positive samples, respectively. Gram-positive bacteria were found as the predominating etiological agents causing mastitis in 62.62% samples followed by Gram-negative bacteria (24.54%) from milk samples of cattle and buffalo combined. Mixed infection of both Gram-positive and Gram-negative bacteria was found in 10.50% milk samples. Over all 2970 samples from cattle (n = 1071) and buffalo (n = 1899) were subjected for culture examination and antibiotic sensitivity assay. The findings from the present study revealed variations in antibiotic sensitivity across different districts. The district of Bhiwani consistently showed lower sensitivity rates for most antibiotics compared to the other districts. Overall, chloramphenicol, enrofloxacin, gentamicin, ciprofloxacin, cefoperazone and levofloxacin were most sensitive antibiotic across all districts. Amoxicillin + Clavulanic acid and ampicillin were most resistant antibiotics in all districts.

**Keywords:** Antibiotic resistance; Buffalo; Cattle; Mastitis

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## Introduction

Mastitis is characterised by an inflammation of the mammary gland in dairy animals such as cattle and buffalo. It causes significant economic losses to national economy and compromises animal welfare (El-Ashker et al. 2020; Yadav et al. 2020). Mastitis can be classified into sub-clinical, clinical and chronic mastitis, depending upon causative organisms, breed, age, immunity, and stage of lactation of the animal (Maity et al. 2020). It is caused by a variety of microorganisms; where bacterial infections are the primarily causative agents. Both Gram-positive and Gram-negative bacteria are involved in bovine mastitis (Algammal et al. 2020; Chhabra et al. 2020). Mastitis results in decreased quality and quantity of milk production. Such infected milk or milk products, usually enters into the food chain and humans can also acquire the infection through the consumption of contaminated milk (Krishnamoorthy et al. 2021). Antimicrobial resistance is also serious concern for both human and animal health. To effectively manage and treat mastitis, it is crucial to understand the bacterial pathogens involved and their antibiotic susceptibility patterns. Appropriate selection of antibiotics can be done on the basis of antibiotic susceptibility profiles of causative agents (Yadav et al. 2020; Ali et al. 2021). Furthermore, understanding the antibiotic resistance patterns of these bacteria is essential for implementing appropriate control measures and preventing the spread of multidrug-resistant strains among human and animal population (Yadav et al. 2021; Singh, 2022). Therefore, the present study seeks to bridge this knowledge gap and contribute to the current understanding of mastitis management in these important livestock species. The aim of present study is to investigate the antibiogram of bacterial isolates obtained from mastitis milk in cattle and buffalo by comprehensive approach, involving bacterial isolation and susceptibility testing, to generate a detailed antibiogram. This study may have practical implications for veterinarians, farmers, and dairy industry stakeholders. It may also contribute to develop the prevention

and control strategies for mastitis and generate awareness about the emergence of antimicrobial resistance.

## Material s and Methods

### Sample collection and detection of mastitis

Milk samples from cattle and buffalo were carefully collected using aseptic techniques at different districts of Haryana (Ambala, Bhiwani, Mahendergarh and Rohtak). Farmers were properly guided for aseptic collection of samples in sterilized container. Samples were processed for detection of mastitis during the period of July 2020 to June 2021 at various Disease Investigation Labs i.e Ambala, Bhiwani, Mahendergarh and Rohtak, Lala Lajpat Rai University of Veterinary and Animal Sciences, (LUVAS), Haryana, India. Mastitis detection was performed by California mastitis test (CMT) and culture examination. Severity of mastitis was detected in quarter milk samples (subclinical) by performing CMT and analysed by observing degree of gel formation graded as trace (t), mild (+), moderate (++) and severe (+++) as described by Belay et al. (2022).

### Bacterial isolation and identification

Detection of various bacterial isolates was carried out by culture examinations of the samples using standard procedure (Quinn et al. 2011). Briefly, milk samples were inoculated onto Nutrient agar and MacConkey agar. The plates were then incubated at the optimal temperature for bacterial growth, typically 37°C, for a period of 24 to 48 hours. Colony morphology, including shape, size, color, and other distinguishing characteristics, were observed and recorded. Gram staining was performed to differentiate the bacterial isolates as Gram-positive or Gram-negative.

### Antibiotic Susceptibility Testing

For antibiotic susceptibility testing, standard guidelines were followed as per the described by Bauer (1966) and (Quinn et al. 2011) for various antibiotics (Himedia). Briefly, bacterial suspension was prepared from pure cultures of the bacterial isolates. Mueller-Hinton agar (Himedia) plates were inoculated with the bacterial suspension using sterilized swab to obtain lawn culture (Bauer, 1966). Antibiotics discs were placed on the surface of agar and plates were then incubated at the appropriate temperature and duration as recommended for each antibiotic. Following incubation, the zones of inhibition around the antibiotic discs were measured and recorded. Interpretation of zone of inhibition was carried out according to the guidelines provided by the Clinical and laboratory standards institute (CLSI, 2015) and European committee on antimicrobial susceptibility testing (EUCAST, 2015). The collected data were compiled to create an antibiogram representing the antibiotic susceptibility patterns of the bacterial isolates.

### Statistical analysis

To analyze the data, the prevalence of antibiotic resistance among the bacterial isolates from mastitic milk in cattle and buffalo was determined. The frequency of resistance for each antibiotic was calculated in Microsoft Excel Version 2010, and statistical analysis, such as chi-square was done by Statistical Package for Social Sciences (SPSS) Version 26 Software (George and Mallery, 2019) to evaluate any significant differences in antibiotic resistance patterns in cattle and buffalo from different geographical areas.

## Results and Discussion

### Occurrence of mastitis

A total of 8561 milk samples from both species combined were tested for detection of mastitis. A comprehensive analysis of mastitis and related parameters among cattle and buffalo were shown in table 1. Overall, the occurrence of mastitis was recorded in 72.73% (n = 6227/8561) milk samples from both species combined. The species-wise analysis indicates that the occurrence of mastitis was non–significantly ($p$>0.05) higher in cattle (86.31%) than in buffaloes (66.97%). Contrary to our study, Dabele et al. (2021) found that the prevalence of mastitis in lactating Zebu cows of Ethiopia was found to be lesser than our findings 30.5% (95% CI: 26.0–35.2%). The slightly higher occurrence of mastitis in cattle compared to buffaloes (86.31% vs. 66.97%) could be attributed to factors such as anatomical differences between the udders of cattle and buffaloes, which might contribute to variations in mastitis susceptibility. Cattle have four quarters, while buffaloes generally have two larger lobes. The structural differences in mammary glands may influence the efficiency of milk let-down, milking procedures, and the ability to clear infections (Hughes and Watson, 2018). The non-significant difference in mastitis occurrence between cattle and buffaloes suggests that similar approaches can be used for mastitis prevention and control in both species. Implementing good husbandry practices, such as udder hygiene, proper milking techniques, and regular monitoring of udder health, can help reduce the incidence of mastitis in both cattle and buffaloes (Sah et al. 2020). Variation in management practices between cattle and buffalo farms might explain the observed differences in mastitis occurrence (Sharun et al. 2021). Al-Zurgani and Mohammed (2021) stated that (CMT) is a quick and distinguished field and laboratory test to detect mastitis in farm animals, including buffaloes. Hokmabad et al. (2011) and Ali and Dahl (2022) also found that, CMT has acceptable sensitivity and specificity in diagnosis of mastitis among buffaloes.

Of the 8561 milk samples, 5591 and 2970 milk samples were tested CMT and culture examination, respectively. CMT showed significantly ($p$<0.05) lower prevalence of mastitis compared to the culture examination with chi-square value (9.688) and the $p$-

value (0.002). By CMT analysis, buffalo had significantly ($p$<0.05) lower percentage of mastitis positivity than cattle. Whereas, culture examination revealed that both cattle and buffalo exhibited a high prevalence of mastitis, with 97.75% and 98.15% positive samples, respectively. The etiological agents causing mastitis were also investigated. Gram-positive bacteria were found as the predominating etiological agents causing mastitis in 62.62% samples followed by Gram-negative bacteria (24.54%) from milk samples of cattle and buffalo combined. Mixed infections of Gram-positive and Gram-negative bacteria were found in 10.50% samples from both species. Similar to our observation Verma et al. (2022) found that Gram-positive bacteria was found as the major cause of bovine mastitis 46.67% samples followed by Gram-negative bacteria (36.67%) and mixed infection of both (16.67 %). The high frequency of Gram-positive bacterial infections indicates unhygienic and poor management practices at farms. The contamination usually occurs from the external surface of the udder and teats, milker's hand and from the surface of the milking equipment and utensils etc. (Ali et al. 2021). While Gram-negative infections such as coliform mastitis were usually occurs due to poor environmental hygiene, contaminated water, fecal contamination, inadequate refrigeration etc (Deddefo et al. 2023).

The significant difference in detection of mastitis by CMT and culture examination showed variations in sensitivity and specificity between these diagnostic methods. Although, CMT being a quick and cost-effective screening tool, might underestimate the true prevalence of mastitis compared to culture examination, which provides a more accurate identification of causative agents (Mbindyo et al. 2020). The lower percentage of mastitis positivity observed in buffaloes compared to cattle by CMT analysis, although not statistically significant, could be attributed to differences in udder anatomy and physiological characteristics (Hughes and Watson, 2018; Diwakar et al. 2020). Further studies are needed to explore these factors in more detail and understand the potential implications for mastitis diagnosis in buffaloes. Gram-positive bacteria were identified as the major etiological agents, consistent with findings from Girma and Tamir (2022), who did a meta-analysis of mastitis data between year 2005-2022 in Ethiopia and concluded that Gram-positive bacteria (84.70%) were the most prevalent mastitis causing agents compared with Gram-negative bacteria (15.30%). Additionally, mixed infections (10-30%) of both Gram-positive and Gram-negative organisms were also reported by previous reporters, highlighting the complexity of mastitis and emphasising the need for targeted treatment approaches (Steele et al. 2020; Saleh et al. 2022).

Mastitis occurrence in different areas among cattle and buffalo populations was shown in Table 2. A notable variation in mastitis prevalence was observed only in Mahendergarh district. The occurrence of mastitis was non-significantly ($p$>0.05) higher in cattle (86.31%) than buffalo (66.97%). Both cattle and buffalo exhibited similar high percentages of positive samples in Ambala, Bhiwani and Rohtak districts. The chi-square test indicates no significant difference between the two species as the $p$-value (>0.05) was above the significance threshold. Looking at the individual areas, it is observed that mastitis prevalence varies across locations. In cattle, occurrence of mastitis was non-significantly ($p$>0.05) higher in Rohtak (100%) followed by

**Table 1:** Occurrence of mastitis in cattle and buffalo

| Parameters | Samples processed (n) | Mastitis | | Chi-square | *df* (degree of freedom) | *p* value |
|---|---|---|---|---|---|---|
| | | *+ve* | % | | | |
| Species wise (n = 8561) | | | | | | |
| *Cattle* | 2550 | 2201 | 86.31 | 2.359 | 1 | 0.125 |
| *Buffalo* | 6011 | 4026 | 66.97 | | | |
| Total | 8561 | 6227 | 72.73 | | | |
| *California mastitis test* (n = 5591) | | | | | | |
| *Cattle* | 1479 | 1154 | 78.02 | 4.771 | 1 | 0.029* |
| *Buffalo* | 4112 | 2162 | 52.57 | | | |
| Total | 5591 | 3316 | 59.30 | | | |
| *Culture examination* (n = 2970) | | | | | | |
| *Cattle* | 1071 | 1047 | 97.75 | 0.0 | 1 | 1.000 |
| *Buffalo* | 1899 | 1864 | 98.15 | | | |
| Total | 2970 | 2911 | 98.01 | | | |
| *Organisms isolated* | | | | | | |
| Gram-positive | | 1860 | 62.62 | | | |
| Gram-negative | | 729 | 24.54 | | | |
| *Candida* spp. | 2970 | 10 | 0.03 | 85.891 | 3 | 0.000* |
| No Growth | | 59 | 1.9 | | | |
| Mixed infection | | 312 | 10.50 | | | |

*Level of significance: $p$ value is significant at the 0.05 level or lesser.
$n$= no. of animals; *df*: degree of freedom

**Table 2:** Area wise occurrence of mastitis in cattle (n = 2550) & buffaloes (n = 6011)

| Area | Cattle | | | Buffalo | | | Statistical significance (between species) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | *+ve* | % | *n* | *+ve* | % | Chi square | *df* | *p value* |
| Ambala | 345 | 330 | 95.65 | 228 | 217 | 95.18 | 0.005 | 1 | 0.942 |
| Bhiwani | 225 | 223 | 99.11 | 737 | 728 | 98.78 | 0.000 | 1 | 1.000 |
| Mahendergarh | 1871 | 1539 | 82.25 | 4760 | 2799 | 58.80 | 3.752 | 1 | 0.053 |
| Rohtak | 109 | 109 | 100 | 286 | 282 | 98.60 | 0.005 | 1 | 0.943 |
| Grand Total | 2550 | 2201 | 86.31 | 6011 | 4026 | 66.97 | 2.359 | 1 | 0.125 |

Statistical significance in occurrence of mastitis between different areas

| | Cattle | Buffalo | Aggregate |
|---|---|---|---|
| Chi square | 2.215 | 12.864 | 5.978 |
| *df* | 3 | 3 | 3 |
| *p value* | 0.529 | 0.005* | 0.113 |

*Level of significance: *p value is significant at the 0.05 level or less*
*df:* degree of freedom

**Table 3:** Season wise occurrence of mastitis in cattle (n = 1979) & buffaloes (n = 5036)

| Season | Cattle | | | Buffalo | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total samples | Positive (*n*) | % | Total samples | Positive (*n*) | % | Chi square | *df* | *p value* |
| Rainy | 987 | 845 | 85.61 | 2011 | 1350 | 67.13 | 2.359 | 1 | 0.125 |
| Spring/ Autumn | 515 | 450 | 87.38 | 1514 | 1025 | 67.70 | 2.329 | 1 | 0.127 |
| Winter | 474 | 386 | 81.43 | 1855 | 1175 | 63.34 | 2.250 | 1 | 0.134 |
| Summer | 574 | 520 | 90.59 | 631 | 476 | 75.44 | 1.542 | 1 | 0.214 |
| Grand Total | 2550 | 2201 | 72.74 | 6011 | 4026 | 86.31 | 2.359 | 1 | 0.125 |

Statistical significance in occurrence of mastitis (per animals) between different seasons

| | Cattle | Buffalo | Aggregate |
|---|---|---|---|
| Chi square | 0.588 | 1.095 | 0.812 |
| *df* | 3 | 3 | 3 |
| *p value* | 0.899 | 0.778 | 0.847 |

*Level of significance: *p value is significant at the 0.05 level or less, df:* degree of freedom

Note: Rainy (*July, August, September*), Spring/Autumn (*October, November, March*), Winter (*December, January, February*), Summer (*April, May, June*)

Bhiwani (99.11%), Ambala (95.65%) and Mahendergarh (82.25%). In buffaloes, occurrence of mastitis was significantly ($p<0.05$) higher in Bhiwani (98.78%) followed by Rohtak (98.60%), Ambala (95.18%) and Mahendergarh (58.80%). The variation in the studies with respect to season and prevalence of mastitis is due to the varying agro-climatic conditions and geographical areas.

These include climate, weather conditions, environmental hygiene, management practices and breed characteristics (Easaw and Vijayakumar, 2022). Differences in management practices, including milking routines, udder hygiene, and housing conditions, also affect mastitis occurrence. Additionally, certain breeds may be more susceptible to mastitis, and variations in breed dominance across regions can lead to differences in mastitis prevalence. Mastitis was most common in Jersey breeds (78.6%), than in Holstein Friesian and indigenous zebu cow crossbreeds (51.9%), and least common in indigenous zebu breeds (16.7%). Moreover, the availability and accessibility of veterinary services play a role in mastitis control and delay in mastitis treatment could amplify the number of cases or complicate the mastitis (Caneschi et al. 2023). Also, the credibility/education status of veterinary practitioners reaching to the doorsteps of the owners (if the owners not visiting the veterinary clinics or if the villages do not have the veterinary dispensaries), misuse and overuse of antibiotics, underdosing of antibiotics, not following the proper treatment regimen by owners, over-reliance on home-recipes for mastitis controls are some other factors which affect the fate of mastitis affected glands.

Season wise occurrence of mastitis in cattle and buffalo was shown in table 3. It was observed that occurrence of mastitis varies with different seasons across all regions. However, the difference was not statistically significant ($p$>0.05) in between same species of different areas and different species of same area. Overall, occurrence of mastitis was non-significantly ($p$>0.05) higher in summer followed by spring, rainy and winter season in case of cattle and buffalo. Season to season variation was observed in the occurrence of mastitis due to variation in growth of pathogenic organism. For example, hot and humid climates can create favourable conditions for bacterial growth, while poor sanitation practices or limited access to clean water can also contribute to higher mastitis rates (Singh et al. 2021). Previous reports in India, showed highest prevalence of mastitis during summer and rainy season in as compared to winter season (Easaw and Vijayakumar, 2022). This could be associated with increased multiplication of organisms and environmental stress, which altered the immune system, thereby making animals prone of infection/mastitis. The high prevalence during monsoon season could be attributed to the temperature and humidity conditions. This was contradictory to the findings by Ranjan et al. (2011) who found least prevalence during raining season (7.37%). OldeRiekerink et al. (2007) found that clinical mastitis was found in high frequency (increasing somatic cell count) during winter season in USA. Therefore, the Understanding these agro climatic conditions in different geographical areas are crucial for implementing targeted control strategies and interventions to reduce mastitis incidence and improve udder health in specific areas (Chen et al. 2023).

**Antibiotic Susceptibility Testing**

Overall, 2970 samples from cattle (n = 1071) and buffalo (n = 1899) were subjected for culture examination and antibiotic sensitivity assay (Table 4). The findings from present study revealed variations in antibiotic sensitivity across different districts. The district of Bhiwani consistently showed lower sensitivity rates for most antibiotics compared to the other districts. In terms of individual antibiotics, amikacin showed relatively highest sensitivity in Mahendergarh (84.44%) and Rohtak (81.51%), while lower sensitivity rates in Ambala (57.49%) and Bhiwani (32.70%).

**Table 4** Antibiogram of organisms isolated from bovine mastitic milk samples

| Total | Sensitivity (%) | | | | Resistance (%) | | | |
|---|---|---|---|---|---|---|---|---|
| Antibiotics | Ambala | Bhiwani | Mahendergarh | Rohtak | Ambala | Bhiwani | Mahendergarh | Rohtak |
| Amikacin (30 mcg) | 57.49 | 32.70 | 84.44 | 81.51 | 42.52 | 67.30 | 13.50 | 18.50 |
| Amoxiclav 30 [Amoxicillin (20mcg)+ Clavulanic acid (10 mcg)] | 67.36 | 23.10 | 27.72 | 67.59 | 32.64 | 76.90 | 71.89 | 32.41 |
| Ampicillin (10 mcg) | - | - | 15.70 | 15.70 | - | - | 84.11 | 84.30 |
| Cefoperazone (75 mcg) | 62.85 | 28.70 | 75.69 | 62.79 | 37.16 | 71.30 | 20.96 | 37.21 |
| Ceftizoxime (30 mcg) | 57.62 | 13.30 | 43.66 | 86.35 | 42.38 | 86.70 | 55.85 | 13.65 |
| Ceftriaxone (30 mcg) | 65.51 | 24.00 | 42.93 | 63.26 | 34.49 | 76.00 | 56.23 | 36.74 |
| Chloramphenicol (30 mcg) | 58.85 | 33.80 | 83.79 | 77.19 | 41.15 | 66.20 | 15.91 | 22.81 |
| Ciprofloxacin (30 mcg) | - | 23.20 | 80.62 | 63.37 | - | 76.80 | 18.18 | 36.64 |
| Enrofloxacin (5 mcg) | 86.71 | 27.80 | 83.97 | 94.08 | 13.30 | 72.20 | 15.15 | 5.92 |
| Gentamicin (10 mcg) | 60.56 | 34.40 | 95.20 | 95.84 | 39.45 | 65.60 | 4.22 | 4.16 |
| Levofloxacin (05 mcg) | 75.82 | 24.40 | 70.74 | 84.41 | 24.19 | 75.60 | 28.28 | 15.59 |
| Moxifloxacin (05 mcg) | - | 23.10 | 64.35 | - | - | 76.90 | 33.59 | - |
| Oxytetracycline (30 mcg) | - | 20.10 | 55.38 | 80.01 | - | 79.90 | 43.84 | 20.00 |

Similarly, enrofloxacin was high sensitivity in Rohtak (94.08%) but relatively low sensitivity in Bhiwani (27.80%). Amoxicillin + Clavulanic acid have higher sensitivity in Rohtak (67.59%) and Ambala (67.36%) than Mahendergarh (27.72%) and Bhiwani (23.10%). Regarding antibiotic resistance, the combination of amoxiclav shows high resistance in Mahendergarh (71.89%) and Bhiwani (76.90%), while Rohtak has relatively lower resistance (32.41%). Additionally, gentamicin exhibited high resistance in Ambala (95.17%) and Rohtak (95.84%), but much lower resistance in Bhiwani (34.40%). Overall, chloramphenicol, enrofloxacin, gentamicin, ciprofloxacin, cefoperazone and levofloxacin were most sensitive antibiotic across all districts. Amoxiclav and ampicillin were most resistant antibiotics in all districts. Similar to our findings, Ranjan et al. (2011) found high sensitivity towards enrofloxacin (91.67%). Pankaj et al. (2012), studied the antibiogram of isolates of mastitis and revealed high (90.90-100%) sensitivity to cefoperazone, enrofloxacin and gentamicin. Serdal and Funda (2021) found that ampicillin and streptomycin were the least effective antimicrobial agents, while the most effective antibiotics were amikacin and kanamycin.

## Conclusion

In conclusion, findings of present study highlighted the importance of regional differences in antibiotic sensitivity and resistance patterns among mastitis-causing bacteria. Understanding these variations is crucial for the selection and proper use of antibiotics in mastitis treatment and control strategies. It is essential to consider local antibiotic sensitivity profiles and regularly monitor for changes in resistance patterns to ensure effective management of mastitis in cattle and buffaloes across different districts.

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RESEARCH ARTICLE

# Application of Image Analysis Technique in Coagulation of Milk for *Paneer* Manufacturing

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**Abstract:** This study was aimed to investigate the coagulation process of *Paneer* manufacturing and to determine the optimal conditions for its production using image analysis techniques. *Paneer*, a traditional coagulated dairy product, is widely recognized and valued for their nutrition and easy to use. The whey images were captured and analyzed at different levels of agitator speed (20, 30 and 40 RPM) and coagulation temperatures (70, 75 and 80°C), and the *L*, a*,* and *b** values of each sample were evaluated using Adobe Photoshop software. The *a** value was specifically used to assess the greenish colour of the sample image, which is an important indicator of complete coagulation of milk. The results revealed that the optimal coagulation conditions were 70°C coagulation temperature, 40 RPM agitator speed and 140 s coagulation time with *a** value of -5.39. These findings suggest that image processing may be an effective tool for monitoring and standardizing the coagulation process of milk for *Paneer* manufacturing. By using this technique, the quality and consistency of *Paneer* may be improved, and human intervention in the production process may also be minimized.

**Keywords:** *Paneer*; Coagulation; Image; Software; Temperature

## Introduction

In recent years, the food industry has been witnessing a significant growth in the use of image analysis techniques for quality control and inspection of various food products (Mollazade et al. 2012). The applications of these techniques have not only facilitated assessment of food quality and safety but also led to the development of novel and innovative food products (Brosnan and Sun, 2004). Among the many applications of image analysis techniques in the food industry, colour analysis is one of the most important and widely used techniques (Ogawa and Adachi, 2014). The colour of food products is an essential factor that influences consumer perception, acceptance, and purchasing decisions (Ares and Deliza, 2010). Therefore, the accurate and reliable measurement of food colour is crucial for ensuring the quality and consistency of food products.

In the dairy industry, colour analysis is of utmost importance, especially for products such as milk, *paneer, yogurt, ghee* and *butter* (Kamthania et al. 2014). In context to *Paneer*, colour is influenced by several factors viz. breed of the animal, the stage of lactation, the processing method, and the storage conditions (Chandan, 2007). The colour of *Paneer* can provide important information regarding product quality, freshness, and the presence of defects (Prajapati et al. 2021). Traditional methods for measuring the colour of dairy products involve visual assessment by human experts, which can be subjective and prone to error (Revilla et al. 2016). In recent years, the use of image analysis techniques for colour analysis of dairy products has become increasingly popular.

Image analysis techniques for colour analysis of dairy products can be broadly classified into two categories, namely, colorimetric and image processing techniques. Colorimetric techniques involve the measurement of the colour of dairy products using colorimeters or spectrophotometers (Minz and Saini, 2021). These instruments measure the intensity of light reflected from the surface of the dairy product and provide colour information in terms of colour space coordinates, such as CIELAB, CIELUV, and CIEXYZ. The colour information can then be used to calculate various colour parameters, such as hue, chroma, and lightness.

The image processing techniques involve the analysis of digital images of dairy products captured using digital cameras or scanners. Image processing techniques can provide more detailed and comprehensive colour information compared to colorimetric techniques (Cabaret et al. 2007). Image processing techniques involve several steps, including image acquisition, image

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segmentation, feature extraction, and classification. In the image acquisition step, digital-images of dairy products are captured using a digital camera or scanner. In the image segmentation step, the dairy product in the image is isolated from the background using various image processing algorithms (Poursaberi et al. 2010). In the final step, colour features such as *L*, a*,* and *b** values can be used to assess the product quality.

*L*, a*,* and *b** values are colour space coordinates that are commonly used in colorimetry for colour analysis of dairy products, including *Paneer* (Leon et al. 2006). *L** represents the lightness or brightness of the colour (0 being black and 100 being white), *a** axis represents the red-green axis (positive values indicating redness and negative values indicating greenness). The *b** axis represents the yellow-blue axis, with positive values indicating yellowness and negative values indicating blueness.

In the context of *Paneer* manufacturing, the coagulation of milk and whey separation is important step. The colour of whey is a decisive factor about completion of milk coagulation process. Generally the colour of whey is greenish after milk coagulation such as greenish color (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). This allows for a more objective assessment of the colour of whey, rather than relying on subjective assessments made by human experts (Yam and Papadakis, 2004). So, objective of the present investigation is determination of colour values of whey using image processing technique to provide a quantitative assessment of the colour of the whey for milk coagulation stage.

## Material and Methods

### Raw Material

Standardized buffalo milk (6% Fat and 9% SNF) was collected from the Experimental Dairy, ICAR-NDRI, Karnal, Haryana, India.

### Experimental setup and procedure

The experimental set up (Fig. 1) consists of cylindrical coagulation tank with paddle agitator, image acquisition system (lighting system, digital camera) and a computer with installed Adobe Photoshop software (version 7.0). The coagulation tank was insulated with glass-wool insulation to minimize heat loss from it.

### Image acquisition

#### *Lighting system*

To obtain accurate colour images of food samples, it is crucial to use appropriate lighting because the colour of the food samples depends on the spectrum of light reflected from it. To standardize the spectral power distribution of the light source, the CIE has established standard illuminants that are identified by their colour temperatures. In food research, the most commonly used standard illuminants are A (2856K), C (6774K), $D_{65}$ (6500K), and D (7500K), with C, $D_{65}$, and D being designed to imitate different variations of daylight (Sharma, 2018). To capture the colour accurately, the camera lens axis and the lighting source axis should be at an angle of about $45^0$, as this angle produces the diffuse reflection responsible for the colour. Additionally, the light intensity should be uniform across the food sample, which can be achieved by experimenting with lighting arrangements, such as altering the distance between the light source and the food sample, taking pictures in a dark room, and verifying the results with a light meter (Yam and Papadakis, 2004).

#### *Digital camera*

The images were captured using Ravtron web camera (Full HD 1920 × 1080 pixels) which was integrated to the coagulation tank (Fig.1).

### Colour image processing

Adobe Photoshop software (version 7.0) was employed to evaluate the values of *L*, a*,* and *b**. It has various tools that can be applied to analyse the colour of food samples. It has abundant features for editing images and its ability to analyse colour in comparison to more expensive colour analysis softwares. Additionally, it provides more advanced capabilities for managing and producing consistent colours than the other graphics software. A computer (Intel Core-i3, 4GB RAM, 1TB hard disk) was used to operate the software. This software is widely accessible in numerous laboratories and receives strong support from both the manufacturer and users (Yam and Papadakis, 2004).

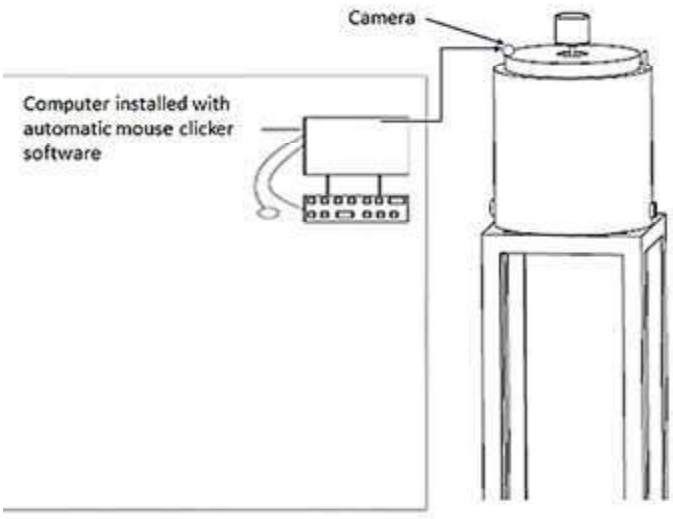


**Fig.1** Experimental set up for image capturing

For quantitative analysis, *L*, a*,* and *b** values were utilized because these values are not device-dependent and encompass a broader range than RGB and CMYK. The Adobe Photoshop software can exhibit *L*, a*,* and *b** values, as well as RGB and CMYK values, in the Info Palette and Histogram Window. The Histogram method was applied (Shahraki et al. 2014) to assess the *L**, *a**, and *b** distribution of the samples. The Histogram Window presents the data (average, standard deviation, median, percentage, etc.) of the colour value (*L*) for a chosen area in the coagulation image. The Histogram Window can also provide the data for two other colour values (*a* and *b*) by selecting them from the Channel drop-down menu. Obtaining the average color of a sample or its any part is effortless using the Histogram Window (Afshari-Jouybari and Farahnaky, 2011). The *L, a* and *b* values displayed in the Histogram Window are not standardized colour values. However, they can be transformed to *L**, *a**, and *b** values by using following standard formulaes (Yam and Papadakis, 2004).

$$L^* = \left[\frac{L}{255}\right] \times 100 \quad (1)$$

$$a^* = \left[\frac{240a}{255}\right] - 120 \quad (2)$$

$$b^* = \left[\frac{240b}{255}\right] - 120 \quad (3)$$

The chroma value and hue angle were calculated from the *L*, a** and *b** values (Barnwal et al. 2015; Pathare et al. 2013):

$$Chroma\ value = \sqrt{\left(a^{*2} + b^{*2}\right)} \quad (4)$$

$$Hue\ angle\ (^\circ) = \left(\frac{180}{\pi}\right) \times \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (5)$$

Whiteness index (WI) was calculated by using following standard relation (Barnwal et al. 2015; Pathare et al. 2013; Wasnik et al. 2017):

$$WI = 100 - \sqrt{\left(100 - L^{*2}\right) + a^{*2} + b^{*2}} \quad (6)$$

Yellowness Index was computed by following equation (Pathare et al. 2013; Wasnik et al. 2017):

$$YI = \frac{142.86 \times b^*}{L^*} \quad (7)$$

Equations (1) to (7) were used to describe the colour change of whey during coagulation of milk for *paneer* manufacturing.

**Experimentation and Analysis**

*Paneer* was prepared in the laboratory using the standard method (Aneja et al. 2002) for application of image analysis technique in coagulation of milk for *Paneer* Manufacturing. Initially, preliminary trials were conducted to determine the range of agitator speed that could be applied during the experiments. Three different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperatures (70, 75 and 80 °C) were selected after the preliminary trials. The various images were captured from dosing of coagulant to till complete separation of whey from coagulum. The images were captured at 5 s interval during milk coagulation for 2.5 minutes. The each sample image was analysed for *L*, a** and *b** values by importing the image in the Adobe Photoshop software.

The colour of whey, obtained during *paneer* manufacturing, is a crucial factor about end point of milk coagulation process. Normally, the colour of whey is greenish after milk coagulation such as greenish color (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). So, *a** values were used to assess the greenish colour of the whey during coagulation.

For comparison of the variation in several sets of data, it is generally desirable to use a measure of relative variation i.e. the coefficient of variation (CV, %) or relative standard deviation (RSD, %). The CV (%) or RSD (%) may be computed as (Johnson, 2005; Rao, 2018):

$$RSD\ (\%) = \frac{\sigma}{\bar{x}} \times 100 \quad (8)$$

The mean or arithmetic mean ($\bar{x}$), and standard deviation ($\sigma$) can be calculated as (Johnson, 2005):

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \quad (9)$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \quad (10)$$

Where, $x_i$ and n are i-th data number of data in respective colour attribute column.

The mean deviation (M.D.) may be computed as (Murthy 2013):

$$M.D. = \frac{\sum_{i=1}^{n} (x_i - \bar{x})}{n}$$

Microsoft Excel-2013 software was used for regression analysis and graphs preparation.

**Results and Discussion**

The various captured images at 5 second interval were analysed and processed in Adobe Photoshop software (version 7.0) software for *a** values. Table 1 shows the effect of agitation speed or agitator speed and the desirable coagulation temperature on *L**, *a**, and *b** values whereas Table 2 represents the effect of agitation speed and the desirable coagulation temperature on hue angle (°), chroma value, yellowness index (YI) and whiteness index (WI). Reliability reproducibility was obtained with a RSD from 0.063 to 0.933 % i.e. lower than 1 %. The mean deviation was ranged from 0.005 to 0.160. The mean deviation (0.005 to 0.160) and RSD (0.063 to 0.933 %) show that the precision of colour attributes (*L*, a*, b*,* hue angle, chroma value, yellowness index and whiteness index) of whey are favourable for various combination of process parameters i.e. coagulation temperature, agitation speed and coagulation time (Tables 1-2). The different coagulation time (range: 140-185 s) was observed for different combinations of coagulation temperature and agitator speed.

It was reported that the colour of whey is greenish after milk coagulation (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). Therefore, the second order (quadratic) was established through regression analysis (Table 3) which may be used for prediction of coagulation time for desired coagulation of milk for *paneer* manufacturing using desirable *a**-values (-5.393 to -5.289). Second order (quadratic) equations and $R^2$ values of *a** values in terms of coagulation time at three different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperature (70, 75 and 80 °C) were determined. It was observed that the $R^2$ values for the different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperatures (70, 75 and 80 °C) were higher and closer to 1.0, which indicates a good fit to the model (Anup et al. 2019).

**Table 1:** Effect of agitation speed and coagulation temperature on *L**, *a** and *b** values of whey

| Coagulation Temperature, °C | Agitation speed (RPM) | Coagulation time (seconds) | *L** value | *a** value | *b** value |
|---|---|---|---|---|---|
| | 40 | 140 | 62.584 | -5.393 | 9.585 |
| 70 | 30 | 160 | 62.596 | -5.384 | 9.618 |
| | 20 | 180 | 62.635 | -5.318 | 9.750 |
| | 40 | 150 | 62.603 | -5.384 | 9.592 |
| 75 | 30 | 170 | 62.568 | -5.374 | 9.675 |
| | 20 | 190 | 62.674 | -5.289 | 9.769 |
| | 40 | 145 | 62.670 | -5.355 | 9.878 |
| 80 | 30 | 165 | 62.580 | -5.342 | 9.675 |
| | 20 | 185 | 62.643 | -5.319 | 9.731 |
| M.D. | | | 0.034 | 0.030 | 0.076 |
| RSD (%) | | | 0.063 | 0.678 | 0.983 |

**Table 2:** Effect of agitation speed and coagulation temperature on hue angle (p ), chroma value, yellowness index and whiteness index of whey

| Coagulation Temperature, °C | Agitation speed (RPM) | Coagulation time (seconds) | Hue Angle (°) | Chroma Value | Yellowness index | Whiteness index |
|---|---|---|---|---|---|---|
| | 40 | 140 | -60.628 | 10.998 | 21.880 | 86.008 |
| 70 | 30 | 160 | -60.753 | 11.022 | 21.951 | 85.989 |
| | 20 | 180 | -61.382 | 11.106 | 22.238 | 85.926 |
| | 40 | 150 | -60.687 | 11.000 | 21.889 | 86.008 |
| 75 | 30 | 170 | -60.942 | 11.067 | 22.091 | 85.952 |
| | 20 | 190 | -61.561 | 11.109 | 22.268 | 85.927 |
| | 40 | 145 | -61.529 | 11.236 | 22.517 | 85.826 |
| 80 | 30 | 165 | -61.087 | 11.056 | 22.086 | 85.965 |
| | 20 | 185 | -61.331 | 11.090 | 22.192 | 85.939 |
| M.D. | | | 0.005 | 0.053 | 0.160 | 0.039 |
| RSD (%) | | | 0.598 | 0.664 | 0.933 | 0.065 |

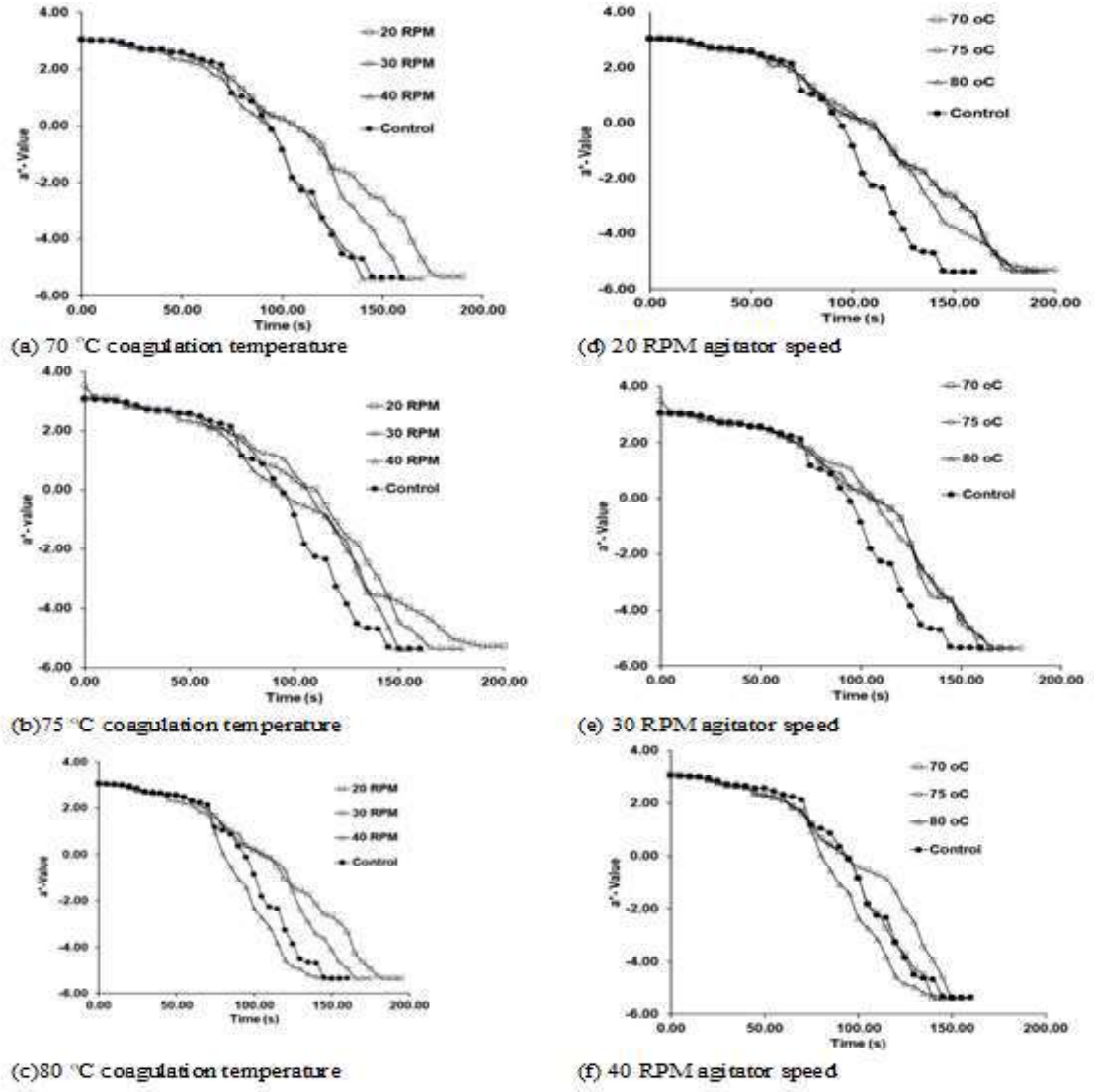


**Fig. 2** Changes in a*-values of whey with coagulation time at (a) 70 °C (b) 75 °C (c) 80 °C (d) 20 RPM (e) 30 RPM (f) 40 RPM

**Table 3:** Second order (quadratic) equations and $R^2$ – values of *a**-values of whey in terms of coagulation time at different coagulation temperatures and agitator speeds

| Coagulation temperature °C | Agitator speed, RPM | Regression equation | $R^2$ | RMSE (%) |
|---|---|---|---|---|
| 70 | 20 | $y = -0.0002x^2 - 0.0068x + 3.2031$ | 0.9937 | 0.2970 |
| | 30 | $y = -0.0004x^2 + 0.0103x + 2.906$ | 0.9920 | 0.5309 |
| | 40 | $y = -0.0005x^2 + 0.008x + 3.0307$ | 0.9888 | 0.3801 |
| 75 | 20 | $y = -0.0002x^2 - 0.0195x + 3.5449$ | 0.9758 | 1.9222 |
| | 30 | $y = -0.0003x^2 - 0.0026x + 3.3035$ | 0.9816 | 0.2296 |
| | 40 | $y = -0.0004x^2 + 0.0049x + 3.0091$ | 0.9917 | 0.1742 |
| 80 | 20 | $y = -0.0002x^2 - 0.0106x + 3.2844$ | 0.9917 | 0.1568 |
| | 30 | $y = -0.0003x^2 + 0.0055x + 3.0019$ | 0.9891 | 0.4005 |
| | 40 | $y = -0.0003x^2 - 0.0225x + 3.6332$ | 0.9563 | 0.2893 |

Note: y = *a**-value; x = time (s)

Figures 2 (a-c) demonstrate the influence of agitator speed (RPM) at different coagulation temperatures (70 °C, 75 °C and 80 °C) on *a** values of the samples. The results indicated that coagulation occurred more rapidly at 40 RPM (140 s) than at 30 RPM (160 s) and 20 RPM (180 s) at 70 °C. Figures 2 (d-f) represent the effect of coagulation temperatures (°C) at different agitator speeds (20 RPM, 30 RPM and 40 RPM) on *a** values of the samples. Similarly, at 75 °C and 80 °C, the coagulation process was more rapid at 40

RPM than at 30 and 20 RPM. In all three cases, there was a steady decline in *a** value during the first 60 seconds of coagulation, followed by a sudden drop in *a** value, indicating the formation and separation of whey. The *a** value was found to be related to the agitator speed, with a more negative *a** value indicating optimal greenish whey separation at higher agitation speeds. Overall, these findings suggest that the optimal conditions for coagulating milk for *Paneer* involve a careful balance of agitation speed and coagulation temperature to promote efficient coagulation and whey separation.

## Conclusion

From this study, it was found that the desirable *a**-values were -5.393 to -5.289 for whey separation for complete coagulation of milk for *paneer* manufacturing. The optimal coagulation conditions were achieved with a coagulation temperature of 70°C, an agitator speed of 40 RPM for 140 s, and *a** value of -5.39.

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RESEARCH ARTICLE

# Enhancing time availability for milk processing using thermal oil as solar heat reservoir

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**Abstract:** India is a world leader in milk production, with an annual production of 230.6 MT. Only 30 percent of milk is currently handled in the organized sector. A cheap source of reliable energy is needed to shift the unorganized sector to an organized sector, as dairy operations are energy-intensive. Solar energy is renewable, inexhaustible, promising, and abundantly available in India. In the literature, it was found that dairy operations such as sterilization of milk can be done using solar energy. However, the limited sunshine hours make it possible to handle only a limited quantity of milk day-to-day. This paper aims to discuss the performance evaluation of a solar heat reservoir to enhance the operational hours during the daytime when solar radiation is insufficient to provide the required energy to carry out the operations. It was found that by using the designed thermal reservoir, the working hours for milk processing were enhanced by 25 to 50 percent, depending on the ambient solar conditions.

**Keywords:** Farm processing; Heat exchanger; Milk; Renewable energy; Solar; Thermal

**Standard Abbreviations:** °C- degree Celsius; h- hour; MT- million tonnes; m- meter; cm- centimeter; Fig.- figure; s- seconds; L- liter.

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## Introduction

India is the largest milk-producing nation, with an annual production of 230.6 MT (NDDB, 2024). Milk processing is an energy-intensive activity; the use of solar energy can partly replace conventional sources of energy (Jaglan et al. 2018; Sharma et al. 2019; Sain et al. 2020; Hosouli et al. 2023; Zlaoui et al. 2023; Patel and Patel, 2024). It was reported that if solar energy is used, about 30,000 L of milk gets pasteurized, and there will be a saving of 80–100 L of furnace oil on a daily basis (Kedare et al. 2012). Operations such as electricity generation, water heating/cooling, drying, steam generation, pumping of dairy fluids, and others can be performed using solar energy (Chopde et al. 2016; Sharma et al. 2017; Sain et al. 2020). In contrast, thermal energy storage (TES) materials are gaining much attention because they enhance energy efficiency, facilitate renewable energy Integration, and offer economic benefits (Rohit et al. 2023; Masera et al. 2023). The processing of milk and other perishable agricultural products may benefit from the use of TES materials in conjunction with solar energy (Sain et al. 2019a; Munir et al. 2023).

Research efforts should be made to find the aforementioned combination as well as develop equipment for such activities. This paper deals with the performance evaluation of a system developed for entrapping solar heat via a thermal storage fluid, transferring heat from the thermal storage fluid to water for heating, and using the generated hot water for milk processing. The aim of the study was to enhance the working hours, beyond the availability of daily sunshine hours, for milk processing when only solar energy is used as a thermal heat source.

## Materials and Methods

The study was performed at the Department of Dairy Engineering, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, at 30.8929 latitude and 75.7981 longitude and an altitude of 245 m above mean sea level.

**Description of the heat reservoir**

A predesigned mild steel single cavity (Sharma et al. 2019) named configuration 1 (shown in Fig. 1a) was used for the selection of suitable TES material. Another mild steel two-cavity thermal reservoir named configuration 2 and configuration 3 (Figs. 1b and 1c, respectively) was developed in the study performed by Sain et al. (2019b). Referring to Figs. 1b, and 1c, Section 1 (oil side) had a volume of 6 L, and Section 2 (water side) had an 18 L volume. The scheme was to use the minimum amount of water in Section 2 so that it can be converted into steam using the least energy from the oil in Section 1; the rest of the energy stored in the oil should be available for temperature compensation in Section 3. This is due to the fact that there would be a temperature drop in the water present in Section 3 because it would be circulated for the heating of milk. So, the temperature drops of water need to be raised again to maintain the constant temperature of water for milk heating.

Basically, Configuration 3 was a modification of Configuration 2. In Configuration 2, the following method was used for hot water generation:

**Configuration 2:** A copper pipe of diameter 12 mm was coiled with a total length of 3 m; water passing through this coil gets heated up, taking energy from the steam in section 2 (referred to as C22 hereafter) (Fig. 1b).

After removing the copper pipe, the modified configuration was named Configuration 3, which is as follows:

**Configuration 3:** The copper pipe was removed from the cavity, and the cylindrical cavity of volume 9.5 L capacity, fully filled with water, was used for hot water generation via heat exchange between section 3 and steam of section 2 of configuration 3 (to be referred to as C23 hereafter) (Fig. 1c).

In configuration 2, the water circulation in the copper pipe was started when the temperature in the second cavity was above 100°C, i.e., steaming. The water was circulated using a centrifugal pump after recording the time taken to achieve the circulation temperature (90°C) in Section 3. Similarly, in C23, Section 3 was completely filled with water. Water present in section 3 received the heat from hot water present in section 2, which was simultaneously getting heat from section 1, which was the only medium for the transfer of heat from one layer or section to another. The heat source from where section 1 was getting heat and transferring it to the other sections was solar energy only, and the reservoir was placed on the focal point of the parabolic dish. The complete setup with the thermal reservoir mounted on the parabolic dish is shown in Fig. 2a (Sain et al. 2019b).

Temperature profiles in Section 1 and Section 2 of Configuration 2 After achieving the desired temperature in Section 3, the thermal reservoir was taken off from the focal point of the parabolic solar dish and kept in the laboratory under ambient conditions. The fall in temperature in various sections of the setup was recorded to observe the time period for which paraffin oil can supply heat to the water in Section 2 in order to maintain the temperature above 90°C.

**A tube-in-tube type arrangement for milk heating**

A tube-in-tube type arrangement for milk processing was developed to check the milk's heating by using the designed thermal reservoir. The vessel was made of stainless steel (SS-304) with a thickness of 1 mm. The length of the vessel was 59 cm, with an internal diameter of 3.81 cm and an outer diameter of 5.08 cm. The capacity of the milk side cavity was 500 ml, and the waterside cavity was 500 ml. The vessel was insulated with the help of cotton and aluminum foil to prevent losses. There were two nozzles for the inlet and outlet of hot water, as shown in Fig. 2b. The assumptions made for the development of the arrangement were:

1) Heat transfers under steady-state conditions, as the temperature of the outer wall of the milk holder is assumed to be constant.

2) Heat transfers by conduction as each ring of the cylinder is at the same temperature. The reason behind this assumption was that milk was held in a tube/pipe, so it was a batch process where the outer wall of the tube remained at a constant temperature. Also, looking into the diameter of the tube, the milk would be heated more radially than longitudinally. Thus, instead of convection, conduction was assumed.

3) Heat accumulation will occur as the milk side temperature rises from ambient to processing temperature.

4) There will be no heat losses to the environment as the water cavity is insulated.

**Methodology for heating milk using a tube-in-tube type heat exchanger**

A simulation study was conducted to assess the hot water temperature drop at 90°C and the milk temperature rise from 30°C. The drop in temperature was not compensated by heating. It was a batch process where milk was poured into the inner side cavity, and 9.5 L of hot water was circulated through the outer jacket with the help of a centrifugal pump. The heated water (around 90°C) was collected in an insulated container, as shown in Fig. 2b. A drop in the temperature of the circulating water and an increase in the temperature of the milk was noted with the help of a mercury thermometer.

Here, in this paper, the performance analysis of only configuration 2 (C22 and C23) is reported, as configuration 1 was used only for

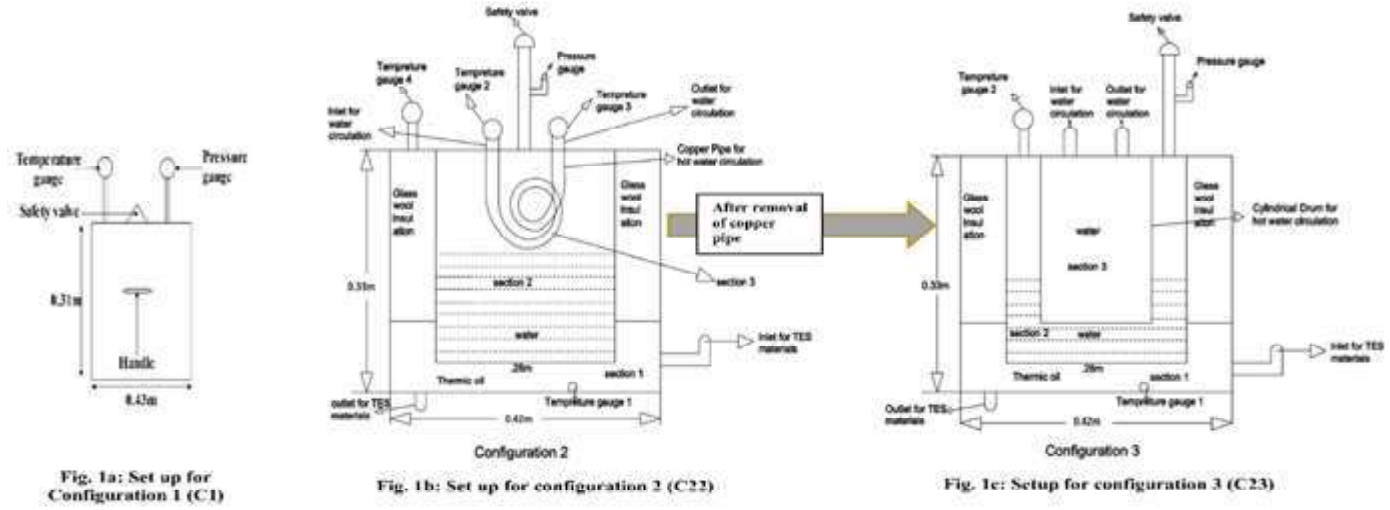


Fig. 1a: Set up for Configuration 1 (C1)

Fig. 1b: Set up for configuration 2 (C22)

Fig. 1c: Setup for configuration 3 (C23)

**Fig. 1** Schematic diagrams for all three configurations (Sain et al. 2019b)

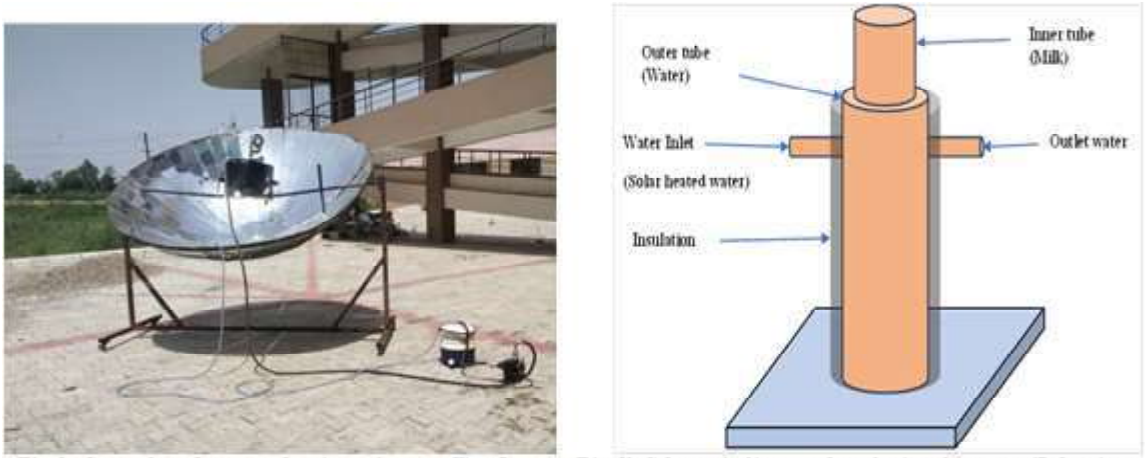


Fig. 2a Set up for solar water heating using paraffin oil

Fig. 2b Schematic diagram for tube-in-tube type milk heating equipment

**Fig. 2** Solar setup for water heating and schematic diagram for milk heating equipment

a preliminary study to ascertain the temperature attainment by the thermal fluid.

## Results and Discussion

### *Performance evaluation of the solar thermal reservoir*

### Configuration 2:C22

It was found that paraffin oil followed a logarithmic trend line, as shown in Fig. 3a, under various outside temperatures as compared to a straight-line trend under configuration 1 during the preliminary study (Sain et al. 2019a). It can be due to the simultaneous heat transfer from section 1 to section 2 (at the same time, section 1 was getting heated by the concentrated solar radiation as it was placed at the focal point of the concentrator, as shown in Fig. 2a) in comparison to the single cavity in configuration 1 (Sain et al. 2019a), where no simultaneous heat transfer occurred to any fluid.

When the temperature in section 2 exceeded 100°C, water circulation was started in section 3 through the copper pipe (Sain et al. 2019b). The temperature profile of water in Section 2 of C22 is shown in Fig. 3b. It shows that the water present in Section 2, which was receiving heat from paraffin oil (in Section 1), followed a linear trend line. The $R^2$ values under different weather conditions ranged from 0.68305 to 0.9266. Similarly, the $R^2$ values for paraffin oil ranged from 0.59759 to 0.90307 under various weather conditions during the study period.

From Fig. 3b, it can also be observed that the temperature of the water was raised to 90°C within 30 to 40 minutes, depending on the outside dry bulb temperature. The temperature differential was maintained between the paraffin oil and the water as the two curves became parallel to each other after attaining peak

**Fig. 3** Temperature profile curves in C22

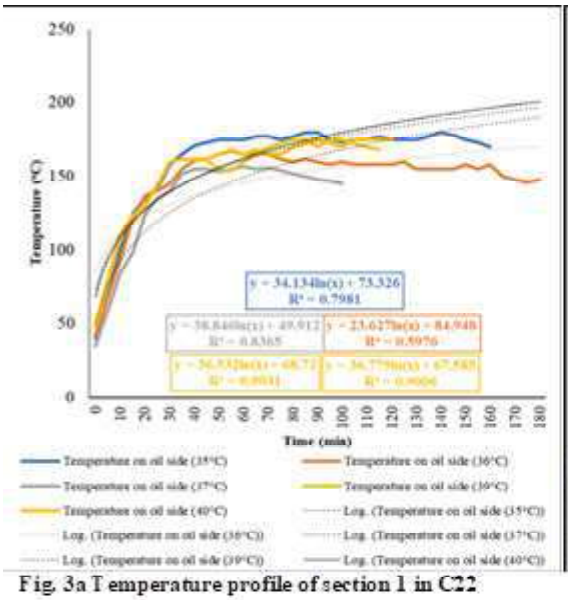


Fig. 3a Temperature profile of section 1 in C22

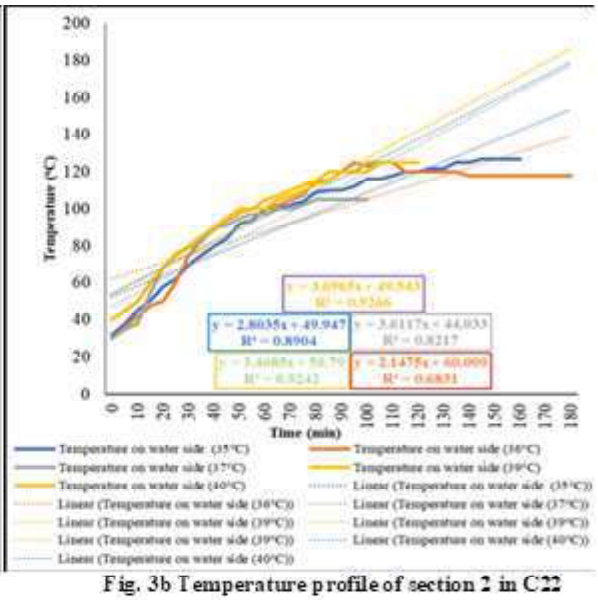


Fig. 3b Temperature profile of section 2 in C22

**Fig. 4** Temperature profile curves in C23

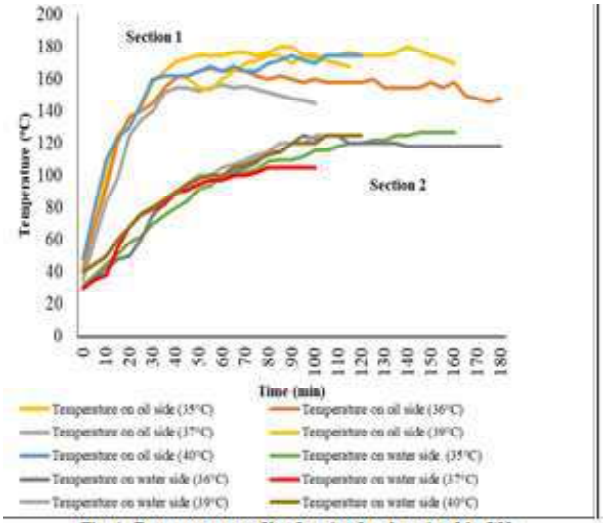


Fig. 4a Temperature profile of section 1 and section 2 in C22

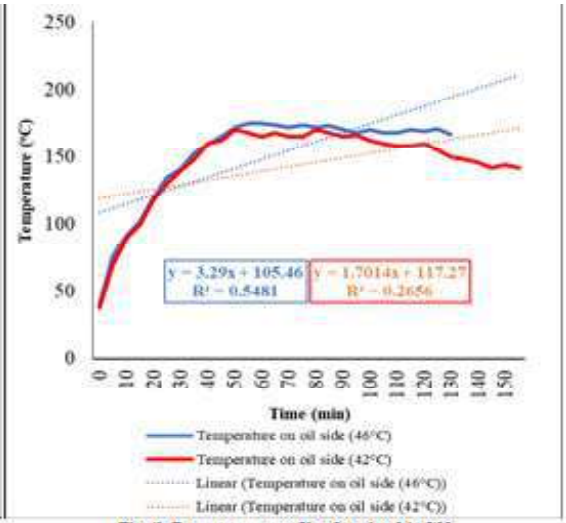


Fig. 4b Temperature profile of section 1 in C23

temperatures, as shown in Fig. 4a. The gradient varied from 20°C to 90°C under various weather conditions. It shows that paraffin oil holds enough energy to supply and maintain the water temperature in Section 2. The temperatures attained by paraffin oil during the study were 175, 170, 168, 148, and 145°C under different weather conditions, i.e., 40°C sunny, 35°C sunny, 39°C sunny, 36°C partly cloudy, and 37°C sunny with heavy wind, respectively. These mentioned temperatures were for the experiment period, which was on different days when plenty of sunshine was available to conduct the experiment.

**Configuration 2: C23**

When the temperature in Section 3 reached the desired temperature of 90°C, water circulation was started. It was observed that after 30 minutes of continuous circulation, there was a drop of 10°C of water in Section 3. The temperature profiles of Section 1 and Section 2 can be seen in Figs. 4b, 5a, and 5b.

**Time taken to attain the desired circulation temperature**

As shown in Fig. 6a, the time taken by water to achieve a temperature of 90°C in C22 was 220 minutes, whereas it was 115 minutes in the case of C23. There was a significant difference in the time taken to achieve the desired circulation temperature of 90°C in C23 compared to that in C22. This may be due to the fact that in C22, a small heat exchange surface area was available; less residence time inside the copper tube as the volume of the copper tube was very low (0.333 L). Whereas the surface area (0.231m$^2$) of the cylindrical drum (section 3) in contact with hot water and steam (section 2) in C23 was higher, so was the high residence time of water due to the larger volume of the cylindrical

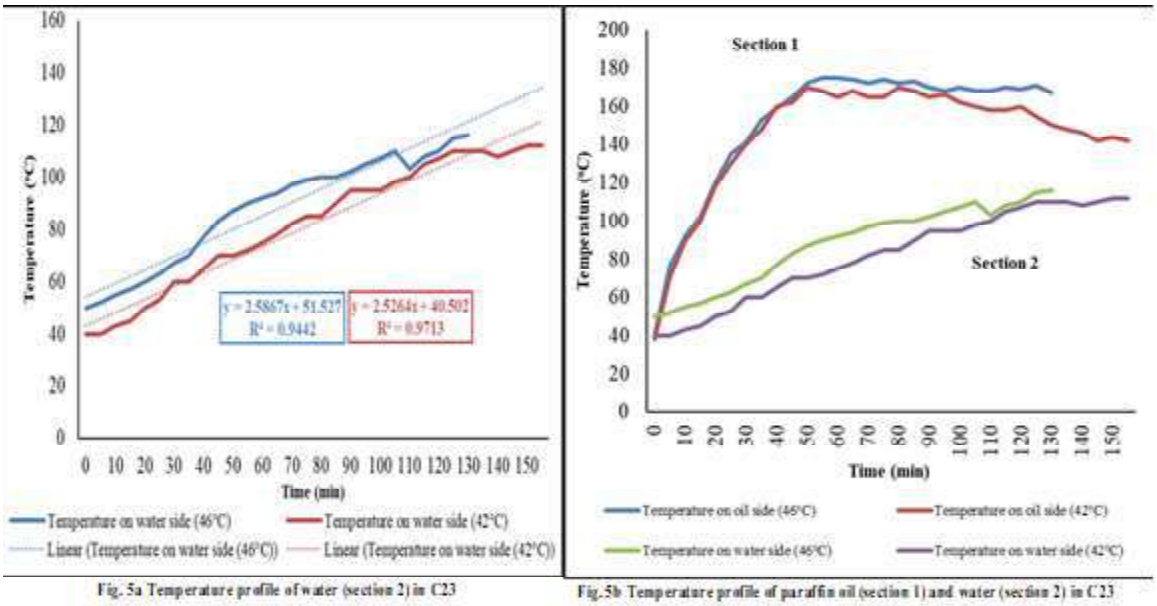


Fig. 5a Temperature profile of water (section 2) in C23

Fig. 5b Temperature profile of paraffin oil (section 1) and water (section 2) in C23

**Fig. 5** Temperature profile of water and paraffin oil in C23

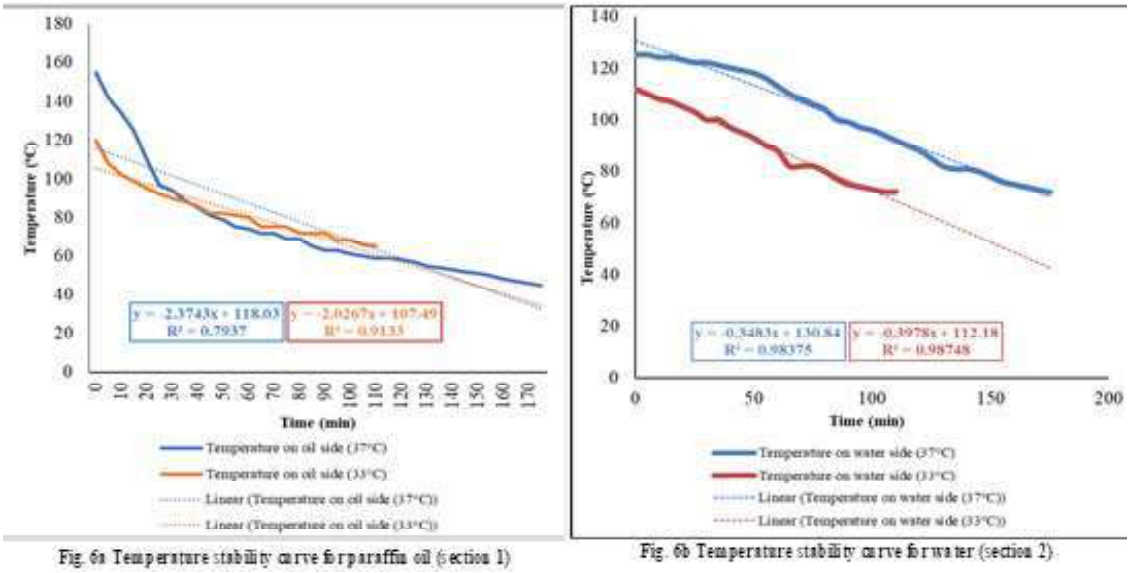


Fig. 6a Temperature stability curve for paraffin oil (section 1)

Fig. 6b Temperature stability curve for water (section 2)

**Fig. 6** Temperature stability curves for paraffin oil and water

drum (9.5 L), which gave better heat transfer. It may also be because, in C22, steam heated the water in a copper pipe, so the time water takes in Section 2 to get converted to steam contributes towards the longer duration of 220 minutes. Also, the copper pipe was not in contact with water in Section 2.

**Temperature stability of paraffin oil and water**

After the attainment of maximum temperatures by paraffin oil (section 1) and water (section 2), it was required to observe the stability of temperatures in both sections. A fall in temperature was recorded at an interval of 5 minutes to observe the temperature stability in both sections. The temperature stability of paraffin oil and water can be seen in Figs. 6a and 6b, respectively. The curve followed the linear trend with a negative slope. It was observed that the hot water temperature could be maintained for at least 1 to 2 h even when the setup was placed in the laboratory under ambient conditions. Preliminary trials of the present study and an earlier study by Sharma et al. (2019) found that the most effective time to process the milk using solar parabolic concentrator energy under available sunshine is about 4 h, whereas, with the use of thermal energy storage oil, the processing hours were increased to 5 to 6 h. So, the present study has shown the possibility of increasing processing time by at least 25 to 50 percent (4 h of efficient sunshine and an additional 1-2 h from the thermal energy stored by paraffin oil) depending upon the ambient conditions by using paraffin oil as a thermal energy storage material (Sain et al. 2019a).

**Table 1:** Performance evaluation of tube-in-tube milk processing equipment

| Time (seconds) | Temperature of) circulating water (90°C | Temperature of milk (°C) |
|---|---|---|
| 0 | 90 | 30 |
| 60 | 84 | 40 |
| 160 | 80 | 63 |
| 175 | 78 | 73 |
| 180 | 77 | 75 |

**Performance evaluation of developed tube-in-tube-type milk processing equipment**

Table 1 shows the fall in temperature of hot water from the initial temperature of 90°C and the rise in temperature of milk from the initial temperature of 30°C. It was recorded that the milk temperature rose from an initial 30 to 75°C, i.e., temperature above 72°C (milk pasteurization temperature), which is required for continuous pasteurization of milk. The temperature was attained in 180 s or 3 minutes. From the experiment, it was found that solar energy, in combination with paraffin oil as a thermal storage substance, can be effectively used to attain pasteurization temperatures for the continuous pasteurization of milk. It implies that milk can also be batch-pasteurized using the setup used in the present study.

## Conclusion

The present study has revealed that a solar parabolic dish, along with a three-cavity container having paraffin oil as a TES material, can attain sufficient temperature to process milk even during off-peak sun hours. The sole aim of the study was to extend working hours using solar energy, which was successfully attained by increasing the available energy time by 25 to 50 percent. C23 was found to be better than C22 as it took less time (115 minutes) to achieve circulation temperature (90°C) as compared to C22 (220 minutes). The whole study was based on a lab scale setup, but a scaled-up, modified setup can be tested for its application in milk processing operations at a small-scale level. Also, the setup can be tested for plate heat exchangers, as they are widely used in the milk processing industries due to their high thermal efficiency.

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RESEARCH ARTICLE

# Probiotic evaluation studies and elemental composition of iron-fortified sweet corn milk-based probiotic yoghurt

**P Geetha**

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**Abstract**: Yoghurt is one of the important fermented milk products with numerous nutrition and health benefits but has low iron content. To further improve the nutritional value sweet corn milk was incorporated at 30% along with cow milk and ferrous lactate for the development of iron-fortified probiotic sweet corn blend milk yoghurt. The premix was fermented with 2% probiotic cultures: *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* at 42°C for 4h. It was stored for 16 days under refrigerated conditions. Food grade ferrous lactate hydrate salt was used at different concentrations like 10mg and 20 mg per 100 ml of premix for fortification. It proved the addition of ferrous lactate had not affected yoghurt characteristics. The elemental composition analysis was done using an X-ray fluorescence spectrophotometer. 20 mg ferrous lactate fortified probiotic sweet corn blend milk yoghurt remained acceptable even at 16 days of storage. This iron-fortified probiotic yoghurt serves as a potential fermented milk product for commercial use.

**Keywords**: Cow milk (4.5%); Ferrous lactate, Fortification; *Lactobacillus acidophilus;* Sweet corn; *Streptococcus thermophilus,*

## Introduction

Yoghurt as a health food has attracted the attention of the middle class in India because of increased disposable income and better health-benefit awareness. Yoghurt prepared with probiotic cultures is being commercialized in the market with health promoting and gut health improving aspects. This has been proven to promote gut microbiota with numerous therapeutic benefits like cholesterol-lowering effect, increase in the bioavailability of minerals and treatment of gut-related aliments (Hadjimbei et al. 2022).

Daily iron requirement of human may vary based on age and gender. It could be summarized as 8 to 18 mg of iron may be required for humans. For example, females of age 18 and above may require 18 gms of iron whereas males of same age group may require 8 gms of iron. Since iron fortified yoghurt is having 20 gms of iron per 100 ml, it is recommended that females could take 100 ml of iron fortified yoghurt per day whereas males could consume 50 ml per day (NIH, 2023). As cow milk has lower iron content food grade ferrous hydrate can serve as a potential iron fortifier in yoghurt. Food fortification with iron has been recommended as one of the preferred approaches for preventing and eradicating iron deficiency. However, fortification with bioavailable iron sources often presents multiple challenges in product acceptance, product shelf life, and effectiveness in improving iron status. In developing an effective iron fortification technology, it is critical that the chemical property of iron that contributes to the development of undesirable organoleptic properties is taken into consideration (Banjare et al. 2019). It is well known that two major off-flavors may be associated with fortified dairy products: oxidized flavor resulting from catalysis of lipid oxidation by iron and metallic flavor contributed by iron salts. No oxidative rancidity had been detected in freshly prepared and stored samples of yoghurt whereas iron fortified yoghurt showed slight rancidity due to different sources of iron.

Further, sweet corn milk is being used as a supplement or replacer for many milk-based products. This would serve as alternative vegetable milk. Sweet Corn *(Zea mays L.* ssp. *saccharata)* is one of the largest vegetable crops grown. Primary interest has been directed to carbohydrates, since in the milky stage when the grain is harvested for food use, carbohydrates determine flavor and texture (Kokkinidou et al. 2019). Several workers have investigated animal milk or soy-milk yoghurts, but little work has been done on corn–milk yoghurt. Production of yoghurt from

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corn milk was aimed to combine the good sensory characteristics of the corn milk with the well-known yoghurt flavor.

It was reported that the sensory quality of iron-fortified dairy foods has been shown to be affected by the type of iron used, the amount of iron added and the properties of dairy products being fortified (Amira et al. 2011 and Hurrell, 2021). Hence this study was taken up to formulate iron-fortified sweet corn milk incorporated yoghurt with *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* and analyzed for viability and acid tolerance during storage.

## Materials and Methods

### Materials

Milk (4.5% fat), sweet corn and skim milk powder were obtained from the local market. *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were purchased from Centre for Food Technology, Anna University, Chennai. *Lactobacillus* MRS agar for *Lactobacillus acidophilus* and M17 media for *Streptococcus thermophilus* were obtained from Hi-Media and were used for microbial analysis. Food grade ferrous lactate hydrate was obtained from Sigma Aldrich and was used for fortification.

### Preparation of Corn milk

To prepare the corn milk, the corn cobs were firstly husked, the silks removed and washed with water. The seeds were then separated from the cleaned cobs using knives. The corn seeds were ground using a grinder. 50 ml of water was added to 100 g of corn seeds during grinding. The slurry was then filtered using a filter to produce a milky liquid. The corn milk was heated to 80°C for 10 min and stored at -18°C until use. Two types of corn were used in this study. Both corn milk and sweet corn milk were prepared by this method.

### Stock culture preparation

The Slant cultures of *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were grown by inoculating into M17 broth and MRS medium respectively for 18 h at 37 °C. One loop of each culture was transferred into 10 ml of litmus milk prepared by mixing 16% (w/v) skim milk powder (SMP) and 0.3% (w/v) yeast extract. The inoculated culture was incubated for 18 h at 37 °C and stored at 5 °C until use.

### Mother culture

An individual mother culture was freshly prepared before conducting the experiment by inoculating one loop of stock culture into 100 ml of sterilized milk medium containing 16% (w/v) SMP and 0.1% (w/v) yeast extract. The inoculated culture was incubated at 37°C for 18 h and kept at 5°C until use.

### Preparation of Iron-fortified Sweet corn milk supplemented yoghurt

In preparation of control yoghurt 100% cow milk and unfortified sweet corn blend milk yoghurt of 70% cow milk and 30% sweet corn milk were used using the below-mentioned method (Fig 1) as described in Geetha et al (2018). Ferrous lactate hydrate of food grade at 10, 20 and 30 mg was incorporated as an iron fortifier. The yoghurt containing 30 mg of ferrous lactate resulted in high whey syneresis, hence it was not considered for optimization. Control = 100 % cow milk, Sample 1 = cow milk: sweet corn milk (70:30) ml, Sample 2 = cow milk : sweet corn milk (70:30) ml + 10 mg Ferrous lactate, Sample 3 = cow milk: sweet corn milk (70:30) ml + 20 mg Ferrous lactate was taken for studies.

### Probiotic evaluation studies

Viable count and acid tolerance tests were done using methods stated in B.I.S.2002 and ICMR-DBT 2011. Analysis was carried out on 0,4,8,12 and 16$^{th}$ days of storage.

### Elemental composition analysis

The mineral composition of yoghurt samples was determined by using the X-ray fluorescence spectrophotometer (Model: Minipal 4 Benchtop XRF, Elemental range : Al…Y, Pd…U, Size : 300x550x450 mm$^3$, Fine focus X-ray tube with MO Target, Multilayer monochromator 17.5 Kev) . All the yoghurt samples were individually freeze-dried in a freeze-dryer (CRYO Technologies, Chennai) to obtain a fine powder. The powdered samples were placed in separate compartments in the XRF analyzer. The elemental analysis was done and results were obtained in graphical representation.

## Results and Discussion

### Probiotic evaluation analysis of yoghurt

#### *Viable count of probiotic organisms in yoghurt during 16 days of storage studies*

The viability of both the strains decreased as the days of storage increased in all the samples along with the control sample (Fig 2). The % viability of *Lactobacillus acidophilus* of control samples were 94.95 %, 87.90%, 84.01% and 78.13% on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 92.6%, 88.5%, 83.06% and 79.93% of viability on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Lactobacillus acidophilus* in yoghurt during 16$^{th}$ days of storage. The viable count showed colonies greater than 10$^9$ CFU/ml during 16 days of storage studies. However the values reported by Codex stated that lactobacilli in yoghurt should be in the range of 10$^7$ ( (Lopez, 2014 and CODEX STAN, 2011).

The % viability of *Streptococcus thermophilus* in control samples was 96.3%, 92.43%, 88.42% and 86.31% on 4th, 8th, 12th and end of 16th day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 93.33%, 88.32%, 86.22% and 81.65% on 4th, 8th, 12th and end of 16th day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Streptococcus thermophilus* in yoghurt during 16th days of storage. The viable count showed colonies greater than $10^9$ CFU/ml during 16 days of storage studies. Many studies reported that the iron fortification in yoghurt and dairy foods and their impact on sensory qualities and survivability of yoghurt bacteria (Azzam, 2009). There is a lack of studies about iron fortification in probiotic yoghurt and its effect on the viability of probiotics. Many of the studies reported that the viability of the starter microflora as well as probiotic was not affected by the iron fortification in yoghurt (Dabour et al. 2019).

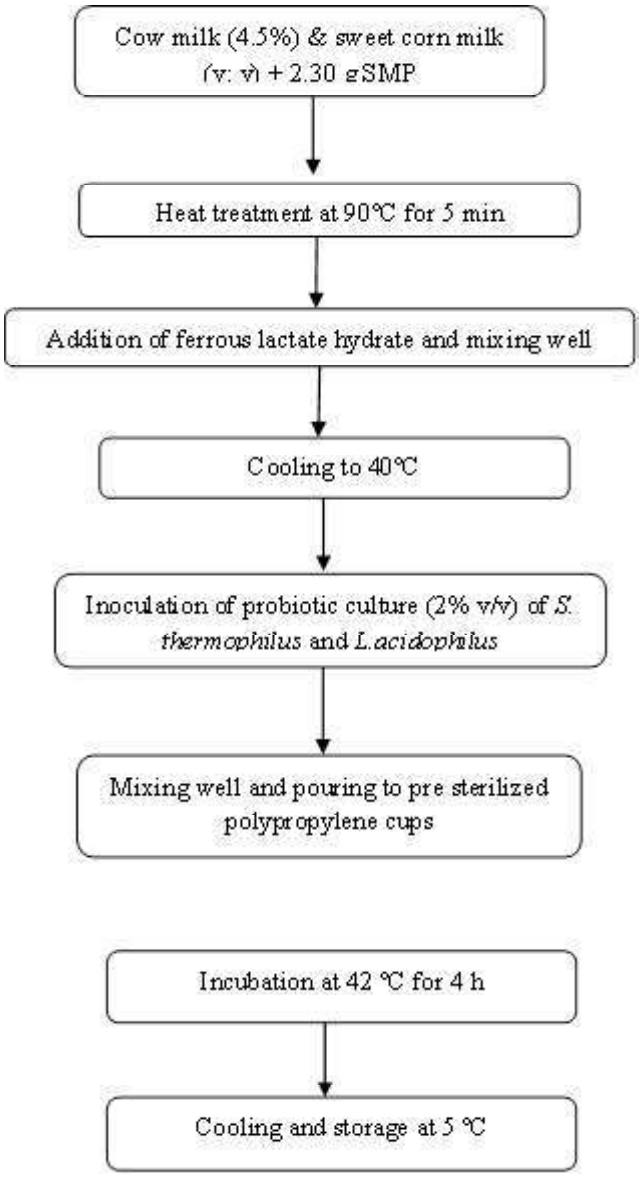


**Fig 1.** Optimized flow diagram for preparation of ferrous lactate fortified sweet corn blend milk yoghurt

**Acid tolerance test for probiotic organisms in yoghurt**

The number of cell counts remained significantly unchanged during all the interval transit at pH 3 and pH 7.2. Good acid tolerance properties exhibited by the bacteria are closely related to their strains specification as they are always strains dependent (Huang and Adams, 2004; Lin et al. 2006). The % viability of *Lactobacillus acidophilus* was 93.25 % and 97.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt respectively. Viability counts of the bacteria usually decline tremendously when exposed to simulated gastric juice of pH 1.5 after an incubation period of 3 hours. The threshold point to determine acid resistance was set at a pH value of 3.0 and an incubation period of 3 hours in the *in vitro* studies as it simulates the residence time in the stomach (Prasad et al. 1998; Haddadin et al. 2008). The developed 20 mg of iron fortified corn milk yoghurt has proven to be successful to meet the minimum requirement of $10^6$ viable probiotic cells per ml at pH 3 after exposure for 3 hours (Sahadeva et al. 2011) (Fig 3) FAO/WHO (2002). The % viability of *Streptococcus thermophilus* was 93.29% and 90.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt. From the graph, *S. thermophilus and L. acidophilus* strains showed acid tolerance at pH 7.2 and 3.0 after 3 hours of incubation. Many studies supported that bioavailability of iron was good in yoghurt (Drago and Valencia 2002).

**Elemental composition analysis of yoghurt**

The X-ray fluorescence analysis (XRF) of milk and dairy products has not yet become widespread in dairy industry, although the method has a great potential, as the dried samples can be analyzed directly without any chemical treatment and XRF equipment is rather accessible (Galina Pashkova, 2009).

An X-ray photon of sufficient energy strikes an atom; it dislodges an electron from one of its inner shells (K in this case). The atom fills the vacant K shell with an electron from the L shell; as the electron drops to the lower energy state, excess energy is released as a $K_a$ X-ray. The atom fills the vacant K shell with an electron from the M shell; as the electron drops to the lower energy state, excess energy is released as a $K_b$ X-ray. The emission of fluorescence is specific for individual elements. The graphical representation of this electron transition within the orbital is given by concentration cps vs. Kev (energy)

Based on the standard observations made on different elements (Van Grieken, & Markowicz, 2001),

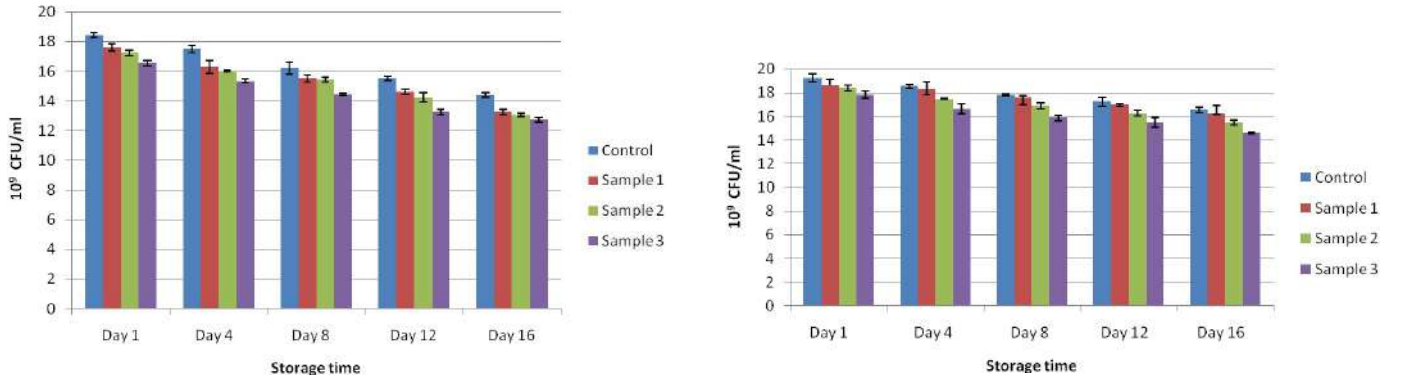


**Fig. 2** Viable count of *Lactobacillus acidophilus* and of *Streptococcus thermophilus* in yoghurt during 16 days of storage studies

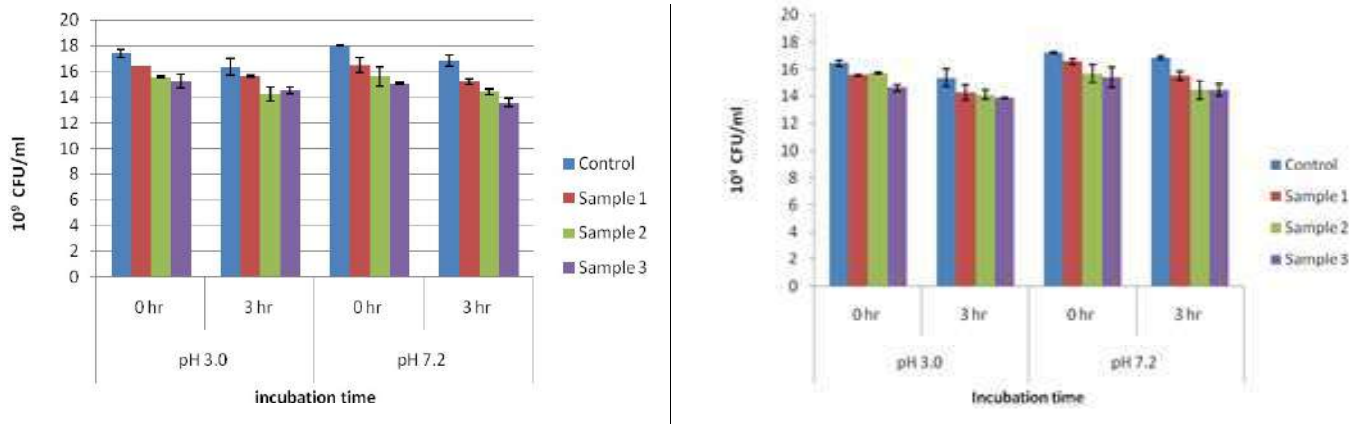


**Fig.3.** Acid tolerance results of *L. acidophilus* and *S. thermophilus* in yoghurt

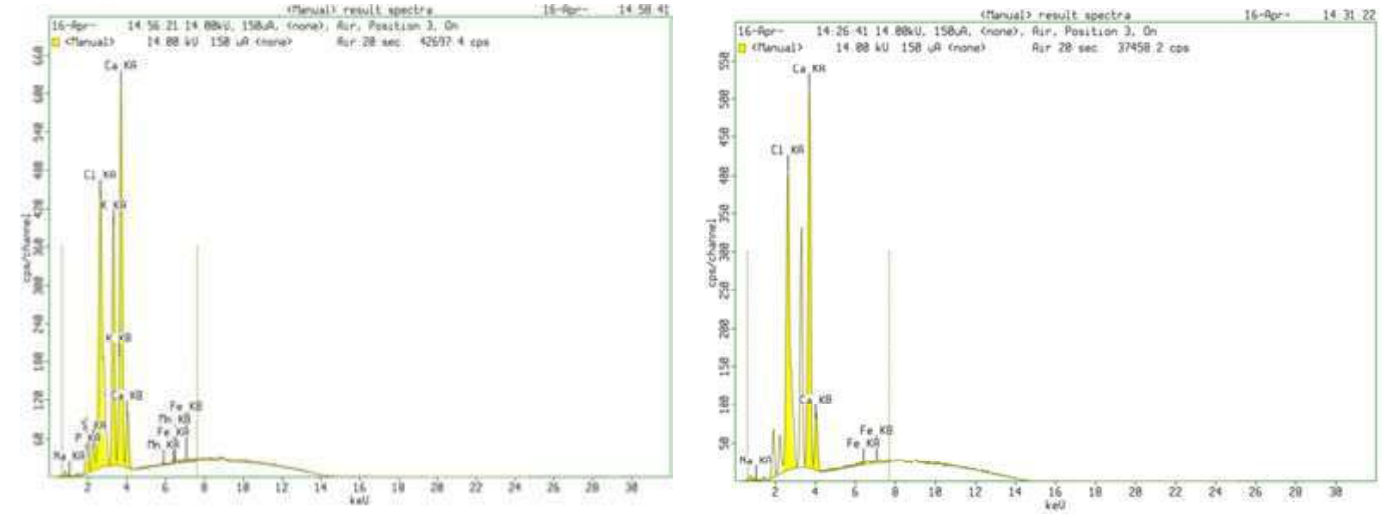

**Fig. 4** XRF analysis graph of control and probiotic sweet corn blend milk yoghurt

- Peak intensity >100 cps corresponds to concentrations >10,000 ppm (% levels)
- Peak intensity of 10-100 cps corresponds to concentrations of ~100-1000 ppm

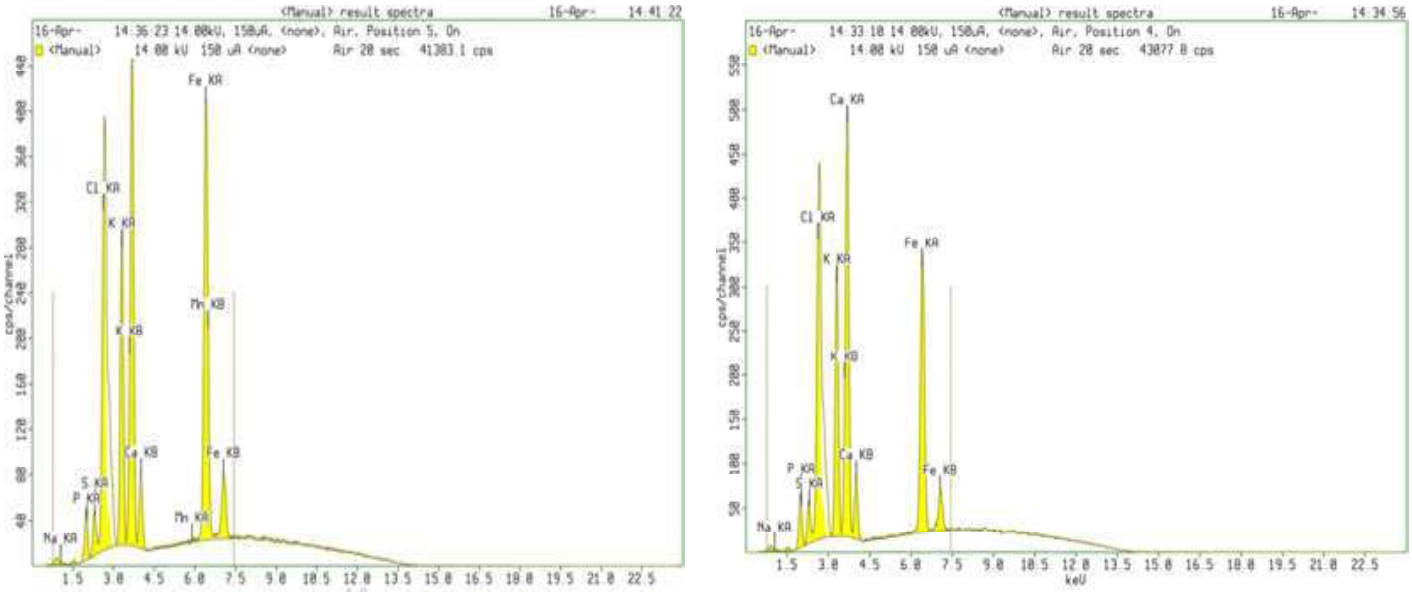


**Fig 5**. XRF analysis graph of probiotic sweet corn blend milk yoghurt fortified with 10 and 20 mg of ferrous lactate hydrate

- Peak intensity of 1-10 cps corresponds to concentrations ~10-100 ppm
- Peak intensity < 1 cps corresponds to concentrations ~1-10 ppm

The Cps (counts per second) versus the energy graphs, depicting the concentration of different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) for the yoghurt made from 100% cow milk are presented in Fig 4. The concentrations for Na, K, Ca, and Fe expressed as cps were 13.986 cps, 5098.625 cps, 8541.48 cps and 13.125 cps respectively. The approximate results show K and Ca are greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm. The concentration for Na, Ca and Fe was 23.986 cps, 7292.234 cps and 16.290 cps for yoghurt without elemental iron fortification. The approximate results show Ca is greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm.

The Cps (counts per second) Vs the energy, the concentration for different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) was found. The concentration for Na, K, Ca and Fe was 21.944 cps, 3963.33cps, 6597.43cps and 5119.99 cps for the probiotic yoghurt incorporated with 10 mg elemental iron. The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The concentration for Na, K, Ca and Fe was 26.639 cps, 3620.663 cps, 6090.110 cps and 6574.40 cps for probiotic yoghurt with 20 mg iron fortification (Fig 5). The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The peak values corresponding to iron showed a gradual increase from control yoghurt to 10 and 20 mg of iron-fortified corn milk yoghurt. From the results, it was concluded that qualitatively Fe concentration was present.

## Conclusion

Yoghurt is a fermented milk product that is popular around the world for its health benefits. It acts as a medium for accumulating various nutrients. Since milk is deficient in iron, many researchers around the world have experimented with different methods and iron sources to fortify yoghurt. Overall, the study indicates that the iron-fortified corn milk yogurt maintained the viability of Lactobacillus acidophilus and Streptococcus thermophilus during storage and demonstrated good acid tolerance properties, which are important traits for probiotic microorganisms. Elemental analysis showed increased iron concentrations in the 10 mg and 20 mg iron fortified corn milk yogurt samples. Thus, the developed product shows successful results in terms of its properties.

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RESEARCH ARTICLE

# Impact of brewery waste on the productive and reproductive traits in Jersey crossbred dairy cattle

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**Abstract:** The study was conducted on twenty four Jersey crossbred dairy cows for a period of one year with three treatments to see the impact of brewery waste on productive and reproductive traits. The control (T0), brewery waste (T1) treatment and balanced ration (T2) treatment was carried out in farmer's field with eight animals in each group. The control, brewery and balanced ration animals were fed as per traditional and standard feeding practices. Productive traits *viz*., milk yield, milk fat, solids not fat (SNF), body weight were assessed before and at the end of experiment. Productive traits *viz*., lactation period and dry period and reproductive trait like number of services per conception (SPC) were also assessed. The results for milk yield and body weight showed that T0 is at par with T1 and T1 at par with T2 ($P>0.05$), and only in T2 significant increase ($P<0.05$) compared to T0. Notable decrease of milk fat and SNF is evident for T1, compared to T2 although the loss deemed to be non significant ($P>0.05$). Among the reproductive traits, no significant difference ($P>0.05$) is evident in lactation period between the treatments, with the values being higher for T2 (302.39 days) and lower for T0. With regard to dry period and SPC, no significant difference ($P>0.05$) is evident between treatments with values being higher for T0 (70.60 days & 2.16) and lower for T2 (63.11 days & 1.83). To conclude, balanced ration significantly increased ($P<0.05$) the milk yield and body weight of the animals followed by brewery waste and control in the descending order of magnitude.

**Key words:** Balanced ration, Body weight, Brewery waste, Milk fat, Milk yield, Solids not fat, Reproductive traits

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## Introduction

India being an agricultural country, the role of livestock has its own significance in economy and socio- economic development. India is an agrarian economy and farmers are known as the backbone of the economy. For many years livestock plays an important role in generating income towards sustenance and for their livelihood. In the present scenario due to dwindling variations in the climate the expected crop returns could not be achieved and under such circumstances, dairying is considered as a major component in the era of Indian agriculture. In the recent past, dairy farming has turned up in to a vital component in alleviating the problem of unemployment for augmenting income generation and livelihood security. In dairy farming, feeding cost plays an important role which could affect the profit of the enterprise. The cost of feeding is the single most important factor affecting the profitability of a dairy enterprise. Due to the shortage of raw feed ingredients, feed cost increased day by day continuously. The scarcity of raw feed ingredients will compel to utilize the newer or non-conventional feed resources for feeding of livestock. To attain maximum profit through livestock, farmers can use agricultural and industrial by products which are cost effective.

During computation of cattle feed in lactating cows, it has been stated that after drying wet brewer's grain could be efficiently used (Dhiman et al. 2003). Wet brewery grain has 20 – 32% dry matter which is good source of rumen un-degradable or "bypass – protein" and the concentration of rumen degradable protein ranges from 28 – 43% (Thomas et al. 2016). In the recent past, wet brewery is used by most of the dairy farmers because of its affordable price. Hence, the present study was undertaken to see the comparative performance of brewery waste on the productive and reproductive traits in Jersey crossbred dairy cattle.

## Materials and Methods

### Experimental site and selection of animals

The study was carried out in two farmer's field at Melvenkatapuram village, Ranipet District of Tamil Nadu. A total of 24 dairy animals were selected and randomly distributed in to

three groups with eight animals in each group possessing uniform body weight and milk yield. The experiment was initiated in farmer's field wherein the first possess 16 animals and the second farmer had 8 animals. Selected dairy animals were of Jersey crossbred in 1st lactation of 3 – 4 years which were calved around 45 – 60 days with an average milk yield of 5 - 5.5 kgs/day and body weight of 250.02 kgs. The study was carried out in September 2019 with deworming being carried out as per standard schedule using fenbendazole. All the selected dairy animals were given an adaptation period of two weeks prior to the experimental study from continued from October 2019 to September 2020.

**Experimental diets**

The study was carried out with three treatments Viz., T0 (Control), brewery waste (T1) and balanced ration (T2) with T0 and T1 treatment being carried out in 1st farmers field and T2 treatment in 2nd farmers field. In T0 (Control), animals were fed with rice bran/ wheat bran, oil cakes as per their traditional feeding practices being followed in the field. Based on the dry matter requirement and milk production, T1 and T2 animals were fed during the trial period. During this period, green fodder @ 9 Kgs /animal/day and dry fodder (paddy straw) @ 5 Kgs/animal/day were fed to the experimental groups animals. The brewery waste was fed @ 1 Kg/kg of milk production in T1 group and the concentrate feed was provided @ 400 gms/Kg of milk production in T2 group dairy cattle. For 1 kg of milk production, approximately 1000 kcal of gross energy is required and the brewery waste (T1) contained 1931 kcal/ kg on dry matter basis. Hence, 1 kg of brewery waste, is needed for every 1 kg of milk production and for every 1 kg of milk production, 400 grams of concentrate feed is required which could equate with ICAR 2013 standard. During dry period, T1 and T2 were fed @ 4 kgs of brewery waste and 1.5 kgs per day per animal respectively as maintenance requirement.

Initially the experimental diet on control (T0 - control), brewery waste (T1) and balanced ration (T2) were analysed for proximate principles at Animal Feed Analytical and Quality Assurance Laboratory, Namakkal (AOAC, 1990) and are presented in Table 1.

**Body weight estimation**

The body weight of the dairy cattle was calculated before and final experimentation using Shaffer's Formula (Sastry et al. 1983) in all three treatment groups (T0, T1 and T2).

$$\text{Body weight (kgs)} = \frac{L \times G^2 \times 0.4536}{300}$$

L = Length from the point of shoulder to the point of pin bone (in inches)

G = Heart girth of the animals (in inches)

**Collection of milk for estimation of Milk fat and Solids not fat**

Milk samples were collected in wide mouthed plastic bottles from the cows prior to start of the experiment and on every month till the end of experiment for analysis of milk fat and solids not fat (SNF) by Gerber's method (Indian Standard, 1977).

**Recording of Milk production**

The milk production of the control (T0), brewery waste (T1) and balanced ration (T2) feeding was recorded daily from the start to the end of experiment.

**Collection of data for productive and reproductive traits**

The data pertinent to date of calving, number of services, date of conception, and date of drying were recorded. The lactation length, dry period and number of services/conception were also assessed.

**Statistical Analysis**

The data collected on productive traits (milk yield, fat, solids not fat, body weight, lactation period and dry period) and reproductive traits (number of services per conception)

from the Jersey animals were subjected to one way Analysis of Variance (ANOVA) using statistical software, IBM SPSS version 20.0. This analysis was performed to find out the significant difference between treatments and final interpretation was done as per procedure of Gomez and Gomez (1984).

**Table 1**: Proximate Principles (in %)

| Sr.No | Particulars | Control (T0) | Brewery waste (T1) | Balanced ration (T2) |
|---|---|---|---|---|
| 1. | Moisture | 9.15 | 73.17 | 12.22 |
| 2. | Crude protein | 7.36 | 13.90 | 19.18 |
| 3. | Crude Fibre | 5.95 | 6.40 | 9.02 |
| 4. | Ether Extract | 4.95 | 5.13 | 6.09 |
| 5. | Total Ash | 5.02 | 5.76 | 7.19 |
| 6. | Gross Energy (K.Cal/kg) | 1323 | 1931 | 3708 |

## Results and Discussion

### Productive traits

Milk Yield: The mean values/ gain in milk yield (kgs/animal/day) and total milk production / daily body weight gain of the Jersey cross bred cattle under various treatment regimens are presented in Table 2 and 3. It was evident that there was a marginal gain in milk yield for all the treatments after the end of experimentation. A significant difference ($P<0.05$) in gain of milk yield was observed between the treatments. There was maximum increase in gain of milk yield in balanced ration group (T2:1.56 kgs/animal/day) followed by brewery (T1: 0.73 kgs/animal/day) and control (T0: 0.13 kgs/animal/day) groups. The total milk production was higher for T2 followed by T1 and T0, but the difference was non-significant ($P>0.05$).

In control (T0) animals, there was a marginal increase in milk yield of 0.13 kgs/ animal/ day at the end of experimentation and they were actually fed with wheat/rice bran, rice gruel and ground nut oil cake (GNC) in an imbalanced proportion without meeting the dietary requirement of the animal. The concentrates fed to the control animals contained 1323 K.cal/kg energy, 7.36% protein and 5.95% crude fibre. This could be the probable reason for comparative less milk yield than brewery (T1) and balanced ration (T2) fed groups. Any animal if underfed or fed imbalanced ration without meeting the requirement, there will be definite decline in milk production. This corroborated with the findings of Garg et al. (2016) who observed 10.36 kgs/day milk production before experimentation and after ration balancing program, the milk yield significantly increased ($P<0.01$) to 11.67 kgs/day implying the importance of balanced feeding on milk production. Research also suggested that the increase in dietary crude protein (on a dry matter basis) from 17 % to 19 % for lactating dairy cows would definitely meet the nutritional requirements (Ibtisham et al. 2018). Protein sources provide specific amino acids to the dairy animals which are very essential for body maintenance, milk production and reproduction. Nutritional management during pre-parturient and early lactation is most important in dairy cattle in which the milk yield increases at a faster rate than energy intake in the first 4 to 6 weeks after parturition and hence the intake of balanced ration is very important to meet out the nutritional requirement.

In lactating dairy cows the protein deficiency may decrease appetite and dry matter intake resulting in low milk production (Ibtisham et al. 2018).

In case of brewery waste fed dairy cattle (T1), the gain in milk yield (0.73 Kgs/animal/day) at the end of experimentation were higher than the control (T0) animals, but marginally lower than the balanced ration (T2) fed animals. The higher milk yield for brewery treated animals could be attributed to the fact that the brewery waste had larger degradable fraction of protein, which is converted into microbial cell protein, digested and absorbed in the duodenum and increased the milk yield. This is in accordance with the findings of Senthil Murugan et al. (2015), who stated that feeding ration with 20% wet brewer's grain increased the milk yield than 30% inclusion level and control diets. The results also supported with the earlier findings of Imaizumi et al. (2015), who reported increased milk yield in lactating Holstein dairy cows fed with ration containing wet brewer's grain.

The higher milk yield of brewery treated animals in current study could be due to the presence of high amount of un-degradable protein in brewery waste, which is essential for body building and body reserves needed for milk synthesis during lactation. Moreover, the high amount of un-degradable protein makes them a good source of rumen by pass protein which remains intact and becomes available in the abomasum and small intestine where they are utilized by the animals for milk production. Further, Chiou et al. (1998) observed that the brewery grain had higher amount of un-degradable protein, making them a good source of rumen by pass protein and the use of increased amount of rumen un-degradable protein (by pass protein) from dietary concentrates increased the milk yield because of improved protein supply and improved intake of metabolisable energy from concentrates. The gain in milk yield (1.56 kgs/animal/day) and total milk production (2099.09 kgs) were higher in balanced ration fed animals (T2) than control (T0) and brewery waste (T1) fed animals. This increase in milk yield could be due to the supply of balanced nutrition which increased the rumen microbial protein synthesis to make more optimal rumen function for increased milk production (Garg et al. 2014). They also stated that feeding of balanced ration increased ($P<0.05$) the average daily milk yield by 6.7% than unbalanced feeding regimen. Energy and protein are the most important limiting factors for milk production and its supplementation in the diets of lactating ruminants would have increased milk yield (Manjunatha et al. 2018; Garg et al. 2016). Further the increase in milk yield could be due to balanced nutrients which would have improved the microbial protein synthesis and supplied essential nutrients (Garg et al. 2016). On feeding a balanced ration, dietary energy and protein can be utilized in a more efficient manner resulting in higher milk yield.

### Milk Fat and Solids Not Fat

The mean values and gain/ loss of milk fat and SNF (%) in Jersey crossbred cattle under different treatment regimens are presented in Table 2. Significant difference ($P<0.01$) is evident between treatment groups after the end of experimentation and the milk fat and solids not fat content was higher for balanced ration fed groups (T2) than control (T0) and brewery (T1) fed groups. The milk fat and SNF loss was higher for control groups (T0: - 0.05% & - 0.03%) followed by brewery (T1: - 0.35 % & - 0.12%) fed group animals. On the other hand there was a gain in milk fat for balanced ration animals (T2: 0.47%), but the difference was non-significant ($P>0.05$) among the groups.

**Table 2:** Average and gain in milk yield, fat, solids not fat and body weight (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr. No | Parameter | Before experimentation | | | F value | At the end of experimentation | | | F value | Gain/ loss | | | F value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | | T0 | T1 | T2 | | T0 | T1 | T2 | |
| 1. | Milk yield (kg/animal/ day) | 4.91 ±0.31 | 5.01 ±0.20 | 5.38 ±0.47 | 0.52$^{NS}$ | 5.04 ± 0.71$^{b}$ | 5.74 ±0.38$^{ab}$ | 6.94 ± 0.50$^{a}$ | 2.72$^{NS}$ | 0.13 ±0.02$^{b}$ | 0.73 ±0.07$^{ab}$ | 1.56 ±0.15$^{a}$ | 3.63$^{*}$ |
| 2. | Fat (%) | 4.43 ± 0.09 | 4.75 ± 0.51 | 4.65 ±0.62 | 0.12 NS | 4.38 ±0.07$^{b}$ | 4.40 ±0.14$^{b}$ | 5.12 ±0.28$^{a}$ | 14.13$^{**}$ | – 0.05 ± 0.09 | – 0.35 ± 0.50 | 0.47 ± 0.65 | 0.75 NS |
| 3. | Solids Not Fat (%) | 8.48 ±0.09 | 8.45 ±0.08 | 8.37 ±0.14 | 0.30 NS | 8.45 ± 0.06$^{b}$ | 8.33 ±0.04$^{b}$ | 8.62 ± 0.05$^{a}$ | 8.42$^{**}$ | – 0.03 ± 0.13 | – 0.12 ± 0.09 | 0.25 ± 0.11 | 3.03 NS |
| 4. | Body weight ( kg/animal) | 258.79 ± 10.87 | 234.78 ± 18.19 | 266.48 ± 17.40 | 1.01$^{NS}$ | 254.04 ± 13.03 | 254.31 ± 20.93 | 300.31 ± 30.78 | 2.12$^{NS}$ | –4.75 ± 1.78$^{b}$ | 19.52 ±2.53$^{ab}$ | 33.83 ± 3.42$^{a}$ | 5.32* |

*Means bearing same superscripts within rows do not differ significantly*

*$^{**}$ - Highly Significant (P<0.01)  $^{*}$ - Significant (P<0.05)  NS – Non Significant (P>0.05)*

**Table 3:** Average total milk production and daily body weight gain (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr.No | Parameters | Mean gain/ loss | | | F value |
|---|---|---|---|---|---|
| | | T0 | T1 | T2 | |
| 1. | Total milk production (kg/animal) | 1488.30 ± 36.28$^{b}$ | 1722.45 ± 62.67$^{ab}$ | 2099.09 ± 74.92$^{a}$ | 3.15$^{NS}$ |
| 2. | Average daily gain (gms/day/animal) | – 13.01 ± 0.35$^{b}$ | 53.48 ± 3.26$^{ab}$ | 92.68 ± 3.99$^{a}$ | 5.32* |

*Means bearing same superscripts within rows do not differ significantly*

*NS – Non Significant (P>0.05)  $^{*}$ - Significant (P<0.05)*

**Table 4 :** Average productive and reproductive traits (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr.No | Parameters | Mean values on reproductive parameters | | | F value |
|---|---|---|---|---|---|
| | | T0 | T1 | T2 | |
| 1. | Lactation period (days) | 295.20 ± 6.48 | 299.99 ± 12.96 | 302.39± 4.14 | 0.18$^{NS}$ |
| 2 | Dry period (days) | 70.60 ± 6.48 | 66.10 ± 12.96 | 63.11 ± 3.22 | 0.13$^{NS}$ |
| 3. | Number of Services Per Conception (SPC) | 2.16 ± 0.31 | 2.00 ± 0.26 | 1.83 ± 0.31 | 0.33$^{NS}$ |

*Means bearing same superscripts within rows do not differ significantly*

*NS – Non Significant (P>0.05)*

The control animals were fed with wheat/rice bran, rice gruel and ground nut oil cake (GNC) in an imbalanced proportion without meeting the dietary requirement of the animal. Feeding diet with low nutrient content may cause reduction of the milk fat and SNF percentage. Hence, ration balancing is most important to augment fat and SNF content in milk. Garg et al. (2016) stated that the milk fat and SNF significantly increased (P<0.01) from 3.98% to 4.35% and from 7.93% to 8.93%, respectively in cows maintained on ration balancing program compared to those on unbalanced ration.

In case of brewery waste fed animals (T1), the mean milk fat and SNF decreased from 4.75% to 4.40% and from 8.45% to 8.33%, respectively during the study period. A notable decrease of – 0.35% and – 0.12% of milk fat and SNF was observed in the animals after experimentation. The depression of milk fat and SNF after experimentation could be due to the presence of rich source of poly unsaturated fatty acid in brewery waste which would have promoted for its depression. This corroborated with the findings of Faccenda et al. (2017), who noted 0.04 g/kg of milk fat depression for every 1% soybean meal replacement with DBG. Moreover, the poly unsaturated fatty acids in brewery waste would have reduced the SNF content of milk after experimentation. This corroborated with the findings of Senthil Murugan et al. (2015). They observed that the SNF content of milk decreased from 7.775% to 7.674% at 20 and 30 percent inclusion level of WBG respectively. Brewery spent grain mainly consists of polyunsaturated fatty acids (67.46%), followed by saturated fatty acids (26.92%) and mono unsaturated fatty acids (10.62%) respectively (Arranz et al. 2008; Niemi et al. 2012). The decrease in milk fat might be due to decrease in dry matter intake (DMI) of the lactating animals. Davis et al. (1982) observed that the milk fat content decreased at 20 & 30% (3.3%) level due to depression in DMI with increasing levels of pressed brewer's grain. The decrease in milk fat could also be due to complete change of concentrate in terms of brewery waste which contained higher level of ether extract composition (5.13%) with higher amount of unsaturated fatty acids and the same fed to dairy cattle causes a bio hydrogenation process in rumen leading to depression in milk fat. Solomon (2007) stated that feeding of a diet containing 5 – 6% ether extract with large amounts of unsaturated fatty acids in dairy cattle depressed the milk fat. Mahnken (2010) reported that the short and medium chain fatty acids production decreased with increasing brewery spent grain inclusion. In other words, the total long chain fatty acids and total unsaturated fatty acids increased with increasing brewery spent grain feeding. Also increased amounts of long chain fatty acids supplied in the diet can inhibit de novo synthesis. As a consequence of decreased production of short and medium chain fatty acids, the fat percentage reduced in milk.

In case of balanced ration (T2) animals, the milk fat and SNF percentage increased to from 4.65% to 5.12% and from 8.37% to 8.62%. The reason for the increased milk fat and SNF (T2 group) may be due to feeding of balanced ration containing adequate amount of energy and protein which would have beneficial effects. This was in accordance with the findings of Garg et al. (2013) who stated that feeding balanced ration increased the milk fat by 0.2 – 1.5%. The improvement in milk fat may be due to balanced nutrients which would have improved rumen environment with maximum utilization of nutrients. Also in balanced ration the essential minerals fulfilled the requirement for better performance. On feeding a balanced ration, the dietary energy and protein could be utilized in a more efficient manner for lactating cows (Garg and Bhanderi, 2011). Moreover, it could be attributed to increased rumen microbial protein synthesis due to more optimal rumen function because of the more balanced nutrient supply (Garg et al. 2014). Similarly the increase in SNF content of milk in balanced ration fed (T2) animals could be due to feeding of balanced ration containing all essential amino acids which helps for synthesis of milk protein and SNF content. The optimum levels of energy, protein and minerals are essential for rumen fermentation functions and used for synthesis of milk components in mammary gland. Rumen microbes convert dietary protein into microbial protein, which is a primary source of essential amino acids for the dairy animals (Bailey et al. 2005) and these amino acids are used for the synthesize milk proteins in mammary gland. The increase in SNF content may be due to availability of energy, protein and minerals in appropriate quantity (Bhanderi et al. 2016).

**Body Weight**

The mean values of body weight (in kgs/animal) and the gain/loss of body weight in Jersey crossbred dairy cattle under different treatment regimens are presented in Table 2. There was a reasonable increase in body weight of the animals fed with brewery (T1) and balanced ration (T2) and a marginal decrease in body weight was noticed in control (T1) animals. The average daily gains for the crossbred animals are presented in Table 3. The total body weight gain and average daily gain was significantly (P<0.05) higher for balanced ration fed animals (T2: 33.83 kgs & 92.68 gms/day/animal) followed by brewery (T1: 19.52 Kgs & 53.48 gms/day/animal) and control (T0: - 4.75 Kgs & - 13.01 gms/day/animal) in the descending order of magnitude.

A marginal loss in total body weight (- 4.75 kgs/animal) and average daily weight loss (-13.01 gms/day/animal) was observed in control (T0) animals after the end of experimentation. This could be due to feeding of imbalanced ration containing wheat/rice bran, rice gruel and ground nut oil cake (GNC) which would not met the dietary requirement of the animal. The concentrates feed offered to the control animals contained only 1323 k.cal/kg energy, 7.36% protein and 5.95% crude fibre, which was not sufficient to meet out the nutrient requirement. When protein is lacking, microbial growth is depressed and as a result, microbial fermentation was reduced and less energy become available.

Moreover, cows would lose weight to compensate for the lack of dietary energy (John Moran, 2005).

In case of brewery (T1) fed animals a significant (P<0.05) increase in gain in body weight was observed. This might be due to the increased availability of undegradable protein (UDP) in the brewery waste which has a positive effect on body weight. Also the rate of degradable protein (RDP) in brewery waste was 47.5%, which was higher than the requirement of 35% RDP (NRC, 2001). Further, the presence of protein in the brewer's grain is a source of amino acids, which are absorbed from the intestines showing marked improvement of feed utilization efficiency (ARC, 1984). Davis et al. (1982) observed a significant improvement on dry matter consumption and weight gain in milking cows while feeding different levels of dried brewer's grains when compared to control group. The increase in body weight might be attributed to increased dry matter intake (DMI).

A significant (P<0.05) increase in gain in body weight (in kgs) and average daily gain (g/day) in balanced ration (T2) fed animals may be due to feeding of balanced ration containing sufficient quantity of energy, protein and mineral mixture which would have beneficial effects in boosting up the body weight of the animal. Provision of balanced ration to the dairy animal augments DMI which leads to increase in body weight. This is in accordance with the findings of Sherasia et al. (2016), who observed a highly significant (P<0.01) increase in body weight in early lactating cows. Significant (P<0.05) increase in dry matter intake was also observed on feeding a balanced ration in dairy cows which eventually reflected the increase in body weight of the animal (Garg et al. 2016). Further, Krishnamurthy et al. (2018) studied the effect of balanced ration supplementation on body weight gain and milk yield in different breeds of cattle (cross bred Jersey, HF & Ongole) and observed that the body weight increased by 13.9%, 9.32% and 16.3%, respectively than initial weights implying that the balanced ration improved the body weight in dairy cattle.

**Lactation Period and Dry Period**

The mean lactation period for different types of treatments was presented in Table 4. The number of days in lactation were higher for balanced ration (T2: 302.39 days) fed animals and marginally lower for brewery (T1: 299.99 days) and control (T0: 295.20 days) animals, but the difference among the groups was non-significant (P>0.05). The mean dry period for different types of treatments was presented in Table 4. Although the dry period was higher for control (T0: 70.60 days) and slightly lower (T1: 66.10 days & T2: 63.11 days) for brewery (T1) and balanced ration (T2) fed animals, the different was non-significant (P>0.05).

The lactation period and dry period observed in this study are more or less comparable with the normal lactation and dry periods for dairy cattle. The standard lactation period for dairy cattle is 305 days and recommended dry period for dairy cattle is 60 days. Slight reduction of lactation length in T0 than other groups might be due to the feeding of imbalanced ration to the animals in control group. Although it was non-significant, nutritional imbalance in ration of control group may have resulted 3 days and 7 days longer dry period as compared to T1 and T2 groups.

**Reproductive traits**

**Number of Services per Conception (SPC)**

The mean number of services per conception for different types of treatments was presented in Table 4**.** The number of SPC for control (T0), brewery (T1) and balanced ration (T2) fed animals were 2.16, 2.00 and 1.83. The mean SPC among different groups did not differ significantly (P>0.05). In case of control (T0) animals, the average number of SPC was marginally higher than other treatments (T1 & T2). The imbalanced feeding in control animals might be attributed to more number of SPC than other treatment groups. Further the concentrate feed of control animals contained lower level of nutrients (1323 K.cal/kg energy, 7.36% protein and 5.95% crude fibre) to meet out the dietary requirement of the animals which was below the recommended level and hence the number of SPC increased. Research also suggested that 17% to 19% dietary crude protein (on a dry matter basis) should be provided to the lactating cows to improve reproductive performance particularly SPC (Ibtisham et al. 2018). The low level of protein along with energy supply may be the possible reason on increasing the number of services per conception.

In case of brewery (T1) fed animals, the average number of SPC was 2.0 which was marginally higher than T2. The brewery fed animals contained lower level of nutrients (1931 K.cal/kg energy, 13.90% protein, 5.13% ether extract and 6.40% crude fibre) which was also not sufficient to meet out the dietary requirement of the animals as like control and hence the number of SPC was increased. Rochijan et al. (2016) observed less number of services per conception (1.17 and 1.5, respectively) with 32.78% and 27.47% rumen undegradable protein supplementation while studying the impact of high rumen un-degraded protein supplementation on reproductive performance in early lactation dairy cows. The brewery waste contained higher amount of rumen un-degradable protein which could be responsible for improving the number of services per conception. The brewery waste should not be used as a complete independent diet instead of concentrates, because they are low in fat and carbohydrate content which lead to lowering the availability of micro nutrients, in turn causes higher number of SPC. Hence this could be served as an additive with other cereals shots e.g., corn silage, green fodder and protein rich legumes for improving the productive performances of dairy cattle.

In case of balanced ration (T2) fed animals, the average number of SPC was 1.83 which was marginally lower than other treatment

groups (T0 & T1). The balanced ration contained sufficient nutrients (3708 K.cal/kg energy, 19.18% crude protein and 9.02% crude fibre) to meet the requirement of the animals which would have synergistic effect in conception rate of the animals. The number of services per conception depends on various factors such as quality of semen, state of reproductive system of the female, efficient heat detection, time of insemination, skill of the inseminator, management factors and agro-climatic conditions affects SPC in Jersey crossbreds (Vinothraj et al. 2016).

## Conclusion

The results of the study indicated that complete feeding of brewery waste to dairy cattle, increased the milk yield compared with control but lower than balanced ration animals. On the other hand, the milk fat and solids not fat decreased when compared to balanced ration animals. Hence complete feeding of brewery waste to lactating dairy animals is not recommended so as to avoid the decrease in milk fat and SNF which could affect the net returns of the livestock farmers.

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RESEARCH ARTICLE

# Socio-economic status and constraints faced by dairy farmers of Kangra District, Himachal Pradesh

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**Abstract:** Dairy farming is an imperative part of the rural economy in hilly areas. It helps in improving the socio-economic status of the farmers, provides nutrition and employment opportunities to the population. Milk production of Himachal Pradesh is low (1.531MT) and contributes only 0.7% to total milk production of India. Kangra district of Himachal Pradesh is second largest producer of milk in the state, therefore present study was undertaken to study the socio-economic status and constraints in dairy farming in the district. A comprehensive questionnaire was prepared to analyse the socio-economic status, cropping patterns, milk productivity and constraints in dairy farming. Analysis was done by Garret ranking technique. In most of the farmers were of small category and were rearing the animals for subsistence purpose. The large farmers of the study area were adopting dairy as a profession. High cost of construction of animal shed, lack of availability of green fodder, heat detection problem, low prices of dairy products and lack of emergency veterinary services are the major constraints faced by the farmers in the study area. In order to sustain and improve milk production, the focus need to be done on green fodder cultivation, milk marketing channels, low cost housing designs, improving AI coverage and establishing mobile veterinary clinics for attracting rural youth to adopt dairy as a profession.

**Keywords:** Constraints, Dairy farming, Farmers, Socio-economic

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## Introduction

The United Nations projects global population growth of almost 50% since 2000 to 9.5 billion by 2050 and it has been studied that approximately 1 out of 9 people in the world are undernourished. Under changing climatic scenario, the supply of quality food to the growing human population will remain a major challenge to the agriculture scientists and the governments. Global demand for milk-based proteins is increasing with increase in the human population. India is predominantly an agrarian society where animal husbandry forms the backbone of the agricultural economy and acts as an essential component of traditional agriculture. The livestock sector contributes 5.1% out of 19.9% of total GDP contributed by agriculture and allied sectors to the total GDP. The livestock sector contributes in several ways in enhancing livelihood and socioeconomic status of the farmers by generating continuous flow of income and acting as a cushion against income shocks in case of crop failures (Anonymous 2015). Inspite of being the largest milk producer in the world, India's productivity per animal is 987 kg/lactation which is very low in comparison to the world average of 2038 kg/lactation (Adhikari, 2020). Dairy farming is the most important economic activity in the rural areas of Himachal Pradesh because people have subsistence land holding and dairy farming is a major way to supplement their family income. Himachal government had also introduced many schemes to boost dairy farming in the state, i.e Dhoodh ganga yojana and Utam pashu purshkar yojana in recent years were introduced (Anonymous, 2017[a]). Statistically, the population of cattle in the state is 21.49 lakh, which contributes 1.14 percent to the country's population. Milk production of the state is 1.3 MT contributing only 0.7 percent to the Indian dairy industry (NDDB, 2019). The milk production trends shows that there is an increase in milk production from 772.47 thousand tonnes in 2003 to 1392 thousand tonnes in 2017, registering a total growth of 80% with a CAGR of 4.00% (Khalandar et al. 2022). Livestock rearing plays a crucial role in the economy of Himachal Pradesh because out of total share of agriculture and allied sector in 28-30% is contributed by livestock sector (Bishist et al. 2022). The cattle population trends in the

Himachal Pradesh showed that there is decrease in the population from 4.7 million in 1972 to 4.4 million in 2017 (NDDB, 2019). No, doubt, various public and private institutions are developing a number of technologies with huge investment but most of these technologies and practices are not reaching the large number of population (Nagrale 2015). There are various factors which affect the development of dairy sector including feeding practices, marketing and institutional factors. Therefore, the present study was conducted to examine the socioeconomic status and various constraints faced by the dairy farmers in Kangra district of Himachal Pradesh.

## Materials and methods

### Selection of study area

Present study was carried out in Kangra district, which falls in the mid hill region of Himachal Pradesh (Fig. 1). In the study, it was selected because it is the 2nd largest producer of milk in the state with annual production of 259.25 thousand tonnes. In the study, five blocks namely; Baijnath, Dehra, Kangra, Indora and Nagrota-Surian were randomly selected through multistage random sampling technique. Two villages were selected from each block and 10 dairy farmers from each village were selected randomly to constitute a sample size of 100.

Data was collected from these respondents with the help of a structured questionnaire and the participants were interviewed about socioeconomic status, dairy farming practices and different problems faced by the farmers pertaining to the dairy farming.

All the problems faced by participants were listed. A complete study of milk producer households in each of ten sample villages along with their herd size was carried out with the help of veterinary professionals and respondents. The list was arranged in ascending order of importance in terms of standard animal units with its cube -root frequencies were obtained and distributed into three different farm size groups of small, medium and large farms. Samples stratification was done by cumulative cube root frequency methods (Singh and Mangat, 1996) and standard animal unit method (Sirohi et al. 2019). After analysing the data according to standard animal unit and cube root frequency method households were divided into three categories namely small, medium, large in which 57 households in small category, 38 households in medium category and 5 were in large category.

Garret's ranking technique was adopted to rank the given set of constraints faced by dairy farmers in the study area. The order of merits that were given by the respondents converted into ranks by using the following formula (Garret and Woodworth, 1969)

$$\% \text{ position} = 100(Rij-0.05)/Nij$$

Where,

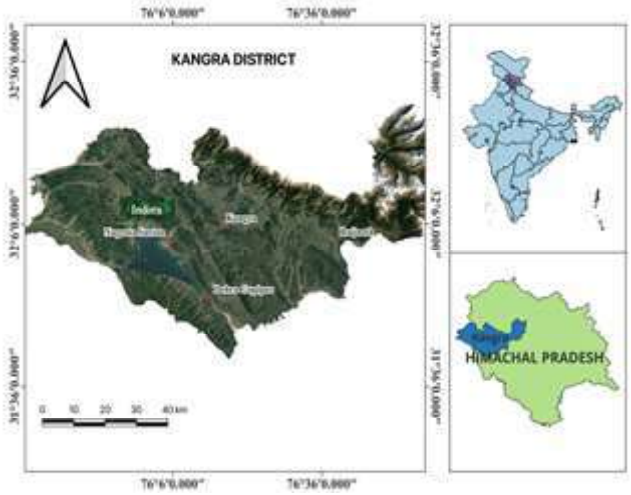


**Fig 1.** Map of the study area

Rij - Rank given for the ith factor by the jth individual.

Nij - Number of factors ranked by the jth individual.

The % position of each rank thus obtained was converted into scores by referring to table given by the Garret. For each factor or problem, the average score was worked out to arrive at mean scores and thus based on the mean scores, the ranks were given and the most important factor was ranked first and the least important problem was ranked as the last.

## Results and Discussion

### Socio-Economic Status of Dairy Farmers

In the study, socio-economic status of small, medium and large dairy farmers in the study area were studied which is presented in Table 1. It was found that in the study area the average size of family was 4.99 and overall number of males and females was 57.77 and 42.23 per cent, respectively. The study conducted by (Bishist et al. 2022) also reported that 52.58% and 47.42% livestock farmers were male and female which correlated with the present study. The majority of farmers belong to the nuclear family (70.82 %) followed by the joint family (29.18%). The education profile of dairy farmers revealed that the maximum respondents 22.35% studied up to secondary level followed by matriculate level (17.97%), middle (17.55%), primary (15.99%) and graduation level (14.80%). The overall literacy rate was found to be 88.65 per cent. The overall literacy index (2.51) showed the quality of education and revealed that quality is not up to the mark. Occupational status revealed that 71.08% of households are engaged in agriculture and allied activities followed by 9.6 % in services, 8.78% in private jobs and 8.56% in business, respectively. Feroze et al. (2016) also reported the similar results in hilly areas and found that most of the households were studied up to secondary level and most of the households engaged in the agriculture and dairy sector. The overall dependency ratio

**Fig. 2** Land utilization pattern of sampled households

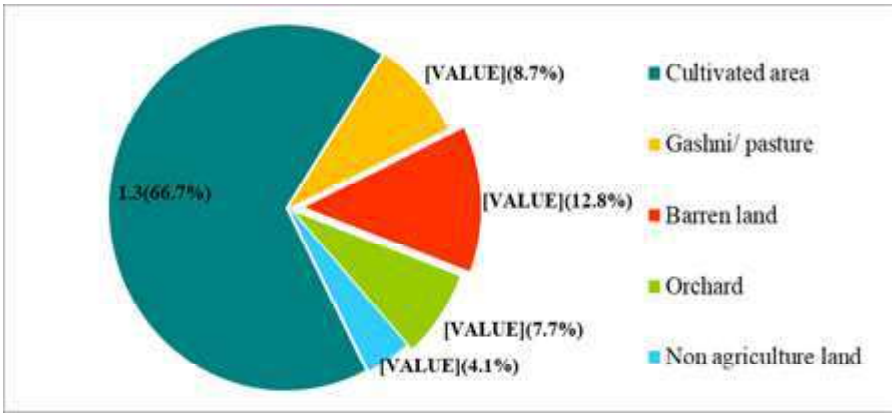


**Table 1:** Socio-Economic status of the sampled households in the study area

| Particulars | Herd size category | | | |
|---|---|---|---|---|
| | Small | Medium | Large | Overall |
| Average size of family (No) | 5 | 5.36 | 4.6 | 4.99 |
| Number of males (%) | 53.68 | 54.41 | 65.21 | 57.77 |
| Number of female (%) | 46.32 | 45.59 | 34.79 | 42.23 |
| Nuclear family (%) | 71.93 | 60.52 | 80 | 70.82 |
| Joint family (%) | 28.07 | 39.48 | 20 | 29.18 |
| Illiterate (%) | 10.94 | 9.44 | 13.66 | 11.35 |
| Primary (%) | 18.11 | 16.23 | 13.63 | 15.99 |
| Middle (%) | 15.09 | 19.37 | 18.18 | 17.55 |
| Matriculate (%) | 20.38 | 19.89 | 13.63 | 17.97 |
| Secondary (%) | 23.39 | 20.94 | 22.72 | 22.35 |
| Graduation (%) | 12.09 | 14.13 | 18.18 | 14.80 |
| Literacy Rate (%) | 89.06 | 90.56 | 86.34 | 88.65 |
| Literacy Index | 2.58 | 2.34 | 2.61 | 2.51 |
| Services (%) | 11.21 | 11.72 | 5.88 | 9.6 |
| Business (%) | 8.78 | 11.03 | 5.88 | 8.56 |
| Private Job (%) | 9.75 | 4.82 | 11.76 | 8.78 |
| Agriculture including dairying and allied services (%) | 70.24 | 72.41 | 70.58 | 71.08 |
| Average No. of workers | 3.58 | 3.8 | 3.6 | 3.66 |
| Average No. of dependents | 1.42 | 1.56 | 1 | 1.33 |
| Average No. of family | 5 | 5.36 | 4.6 | 4.99 |
| Dependency ratio w.r.t total workers | 0.39 | 0.41 | 0.27 | 0.35 |
| Dependency ratio w.r.t family size | 0.28 | 0.29 | 0.2 | 0.25 |

w.r.t total workers was recorded 0.35 which showed that on an average one worker is needed to assist one family member in all the farm size categories.

**Land Utilization Pattern of sampled households in the study area**

The data pertaining to the land utilization pattern of the sampled households is presented in Fig 2, which indicate that the average land holding varied between 1.50 to 2.33 hectares among different categories of farm with an average of 1.95 hectare. Similar finding were reported by Feroze et al. (2016) in East Khasi Hills and Ri-Bhoi District with the average land holding size is 1.67 and 1.85 out of which 1.3 hectare area is cultivated with sole crops and

mixed crops, followed by area under barren lands 0.33 hectare, ghasni or pasture 0.17 hectare, orchard 0.15 hectare and 0.08 hectare under non agriculture land, respectively.

The cropping pattern of sampled households is presented in Table 2. On the average farms, wheat was the most important crop in study area accounting 18.73 per cent of total cropped area. In the study area the net sown area was 1.45 hectare. The area under fodder crops is only 4.44% of total cropped area and found out to be more than national average which is only 4 per-

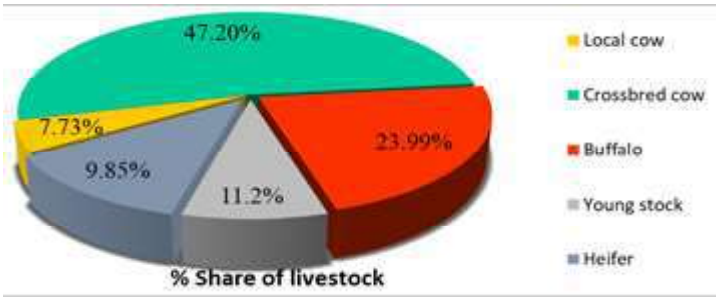


**Fig. 3** Share of livestock among sampled households

**Table 2:** Cropping pattern of sampled households in the study area

| Particulars | | Herd size category | | | |
|---|---|---|---|---|---|
| | | Small | Medium | Large | Overall |
| Kharif crops | | | | | |
| | Cereals | | | | |
| 1 | Maize | 0.33 (15.21) | 0.35 (12.46) | 0.48 (17.14) | 0.39 (14.94) |
| | Paddy | 0.30 (13.82) | 0.32 (11.39) | 0.32 (11.43) | 0.31 (12.21) |
| | Vegetables | | | | 0.00 |
| 2 | Brinjal | 0.07 (3.23) | 0.15 (5.34) | 0.10 (3.57) | 0.11 (4.05) |
| | Bottle gourd | 0.08 (3.69) | 0.18 (6.41) | 0.17 (6.07) | 0.14 (5.39) |
| | Okra | 0.12 (5.53) | 0.19 (6.76) | 0.15 (5.36) | 0.15 (5.88) |
| 3 | Fodder crop | 0.04 (1.84) | 0.06 (2.14) | 0.04 (1.43) | 0.05 (1.80) |
| Rabi crops | | | | | |
| | Cereals | | | | |
| 1 | Wheat | 0.43 (19.82) | 0.50 (17.79) | 0.52 (18.57) | 0.48 (18.73) |
| | Barley | 0.20 (9.22) | 0.25 (8.90) | 0.22 (7.86) | 0.22 (8.66) |
| | Oilseed | | | | |
| 2 | Mustard | 0.22 (10.14) | 0.26 (9.25) | 0.25 (8.93) | 0.24 (9.44) |
| | Vegetables | | | | |
| 3 | Beans | 0.07 (3.23) | 0.10 (3.56) | 0.12 (4.29) | 0.10 (3.69) |
| | Potato | 0.12 (5.53) | 0.20 (7.12) | 0.21 (7.50) | 0.18 (6.72) |
| 4 | Fodder crops | 0.04 (1.84) | 0.07 (2.49) | 0.10 (3.57) | 0.07 (2.64) |
| Orchard | | 0.15 (6.91) | 0.18 (6.41) | 0.12 (4.29) | 0.15 (5.87) |
| Gross cropped area | | 2.17 (100.00) | 2.81 (100.00) | 2.80 (100.00) | 2.59 (100.00) |
| Net sown area | | 1.24 | 1.57 | 1.54 | 1.45 |
| Cropping intensity | | 175.00 | 178.98 | 181.82 | 178.60 |

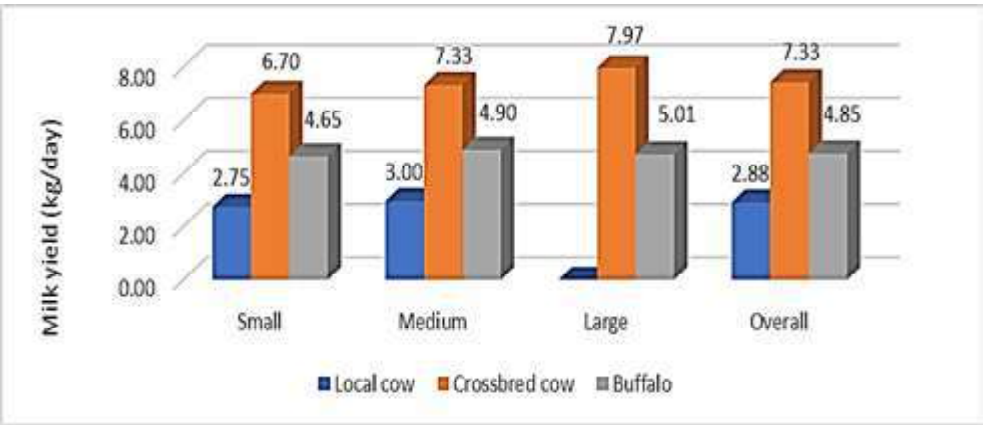


**Fig. 4** Average milk yield/ day of sampled households in the study area

cent of total cropped area in the country (Singh et al. 2022). Therefore, more emphasis on cultivating forage crops in the cropping systems will overcome the scarcity of the fodder problem. The cropping intensity indicates about the crop intensification in the study area and found 178.60 per cent in the area.

**Livestock holding among sampled households**

The results showed that average livestock holdings varied between 2.75 (small), 4.68 (medium) and 8.20 SAU in the large category of farmers. In the study area dominance of crossbred cows among different farm sizes was noticed with the highest share of (47.20%) followed by buffaloes (23.99%) and local cows (7.73%), respectively (Fig 3). Results of study indicated that the farmers in the study area are more inclined towards rearing crossbred cows mainly due to higher milk yield and easy availability of semen for breeding purposes. It was also observed that in the large farmers category, the farmers were rearing crossbred cows and buffaloes and none of the farmers in the study area were rearing local cattle.

**Average milk yield among sampled households**

Average milk yield is considered to be the main output for dairy enterprises. The milk yield of the animals depends upon various factors like breed, feed and fodder, health, climate and management practices. Average milk yield was worked out dividing total milk produced by total milking animals in a category. The highest average milk yield per day (Fig. 4) was noticed among the crossbred cow (7.33 litres/day) followed by buffalo 4.85 (litres/ day) and 2.88 (litres/ day) in case of local cows. It was also observed that the milk productivity of the crossbred cows and buffaloes increased with the farm size in the study area. The yield of crossbred cows in the study area is more than national level yield level (7.22 liters/day) (Anonymous 2019). Therefore, increasing the area under fodder crops and the number of crossbred cattles in the study area will boost the income of the farmers.

**Constraints in dairy farming in the study area**

Constraints means the problem faced by dairy farmers while conducting day to day animal husbandry practices in their dairy enterprise (Gamit 2020). In the study area, different constraints studied were divided into five categories i.e., Housing, feeding, breeding, marketing, and institutional constraints. The data on constraints in dairy farming ranked by famers in study areas were collected and analysed by using Garret ranking technique and results are presented in Table 3.

Among the housing constraints in dairy farming, high cost of construction of animal shed was the major constraint followed by provision of cooling, quality of roofing material, concrete non-grooved and slippery floor, less space availability i.e. open area, ranked first, second, third fourth and fifth, respectively by dairy farmers. in the study it was observed that majority of the farmers belonged to small and marginal category and they tend to make thatched houses in order to reduce the cost per cow for better returns. Similar results were reported by Rajadurai et al. (2018) and Balasubramanian (1995) also reported that in Tamil Nadu the majority of farmers had problems with animal shed and housing facilities because of their poor economic status.

The country faces scarcity of 35.6% green fodder, 10.5% dry fodder and 44% concentrates (Singh et al. 2022). In the study area the lack of green fodder availability was the major constraint noticed among feeding constraints followed by high cost of concentrates, low availability of dry fodder, lack of availability of concentrates, providing unbalanced feed, non-availability of land for fodder production, less storage space for dry fodder and concentrates, no sufficient water availability during the lean period, less availability of separate water troughs, poor quality of drinking water given to animals constraints ranked first, second, third, fourth, fifth, sixth, seventh, eight, nine and tenth respectively by dairy farmers. Similar findings were also reported by other studies like Tailor et al. (2012); Nagrale et al. (2015); Sharma et al. (2018) and Adhikari et al. (2020), where low availability of green fodder was the major constraint in adopting dairy farming as an enterprise.

Among the breeding constraints faced by dairy farmers, problems of heat detection, poor quality of bulls were the major constraints

**Table 3:** Various constraints faced by dairy farmers in mid hills of Himachal Pradesh

| S No. | | Mean | Ranks |
|---|---|---|---|
| | Housing Constraints | | |
| 1 | High cost of construction –shed | 75.7 | 1 |
| 2 | Less space availability – open area | 48.5 | 5 |
| 3 | Quality of roofing material | 60.56 | 3 |
| 4 | Concrete, non-grooved and slippery floor | 55.55 | 4 |
| 5 | Provision of cooling | 64.8 | 2 |
| | Feeding Constraints | | |
| 1 | Lack of availability of green fodder | 73.12 | 1 |
| 2 | Low availability of dry fodder | 70.14 | 3 |
| 3 | Lack of availability of concentrates | 66.65 | 4 |
| 4 | Giving unbalanced feed | 60.55 | 5 |
| 4 | Non availability of land for fodder production | 54.02 | 6 |
| 6 | Less storage space for dry fodder and concentrates | 50.38 | 7 |
| 7 | High cost of concentrates | 71.42 | 2 |
| 8 | No sufficient water availability all the time | 44.17 | 8 |
| 9 | Less availability of separate water troughs | 40.98 | 9 |
| 10 | Low quality of drinking water given to animals | 32.85 | 10 |
| | Breeding Constraints | | |
| 1. | Low conception rate | 58.18 | 4 |
| 2. | Poor quality of bulls | 66.2 | 2 |
| 3. | Problem of heat detection | 68.7 | 1 |
| 4. | Incidence of reproductive disorder | 42.86 | 6 |
| 5. | Less availability of improved germplasm | 60.12 | 3 |
| 6. | Unavailability of trained inseminator | 52.55 | 5 |
| | Marketing Constraints | | |
| 1. | Inadequate market information | 54.58 | 3 |
| 2. | Problem of transportation of products | 46.08 | 4 |
| 3. | Delay in payments | 33.12 | 5 |
| 4. | Low price offered for the products | 75.1 | 1 |
| 5. | Irregular demand for milk and other products (excluding dung) | 68.43 | 2 |
| | Institutional Constraints | | |
| 1. | Lack of emergency veterinary services. | 81.9 | 1 |
| 2. | High cost of medicines/ veterinary services | 76.65 | 2 |
| 3. | Lack of improved equipments | 48.7 | 6 |
| 4. | Irregular visits of veterinary staff | 64.98 | 3 |
| 5. | Unavailability of ambulance | 54.85 | 5 |
| 6. | Lack of awareness of new practices/ technologies | 58.5 | 4 |

faced by the farmers, due to the non - availability of breeding bulls in the study area. Other minor constraints faced were less availability of improved germplasm, low conception rate, unavailability of trained inseminator and incidence of reproductive disorder. Similar results were reported by Quddus (2012) and Lawrence et al. (2015) in Bangladesh and Kenya. The main reason for the inability of farmers to detect heat in animals is due to lack of awareness among the farmers and timely management of breeding at the village level result in poor conception rate.

Low prices offered of the products was the major constraint noticed among the dairy farmers. Other minor constraints were irregular demand for milk and other products, inadequate market information, problem of transportation of products and delay in payment by the buyers. Farmers faced the problem of irregular demand from the different vendors like sweet shop, restaurants and local chai-wala, due to lack of market demand occurred in different seasons of the year like during festival season, tourist season and also during lockdowns (Covid-19) which resulted into market disequilibrium and losses were suffered by dairy farmers. These findings were according to the study conducted by Anh et al. (2013) and Kishan and Ramachandran (2022).

In the study area farmers were also facing various constraints at institutional level, which included lack of emergency veterinary services, high cost of medicine and veterinary services, irregular visit of veterinary staff, lack of awareness of new practices and technologies, unavailability of ambulances, lack of improved equipment's constraints. It was noticed that in the study area the animal casualties were higher due to lack of emergency veterinary

services provided by the department of veterinary and animal husbandry. Therefore, it is suggested that emergency veterinary services and provision of mobile veterinary vans will help in better animal husbandry services to reduce animal casualties. A similar constraint was also reported by Bijla et al. (2019).

## Conclusion

From present study it can be concluded that the dairy farmers of the area are keeping good lactating animals with higher average milk yield as compared to the national average. However, the farmers face different constraints viz. high cost of construction cost of animal shed, lack of green fodder, problems in heat detection, low prices offered for the product and lack of emergency veterinary services, which are hindering the future prospects of the dairying as a profession in the study area. Therefore, it is suggested that the attention should be paid towards provision of subsidy for building animal sheds, advising farmers to grow leguminous rich, high quality fodder crop like berseem, oats, sorghum, bajra, makhan grass and fodder trees such as beul, kachnar, mulberry bamboo etc which will help in time of dearth period and reducing the cost of milk production in the area. Heat detection problems can be solved by providing scientific training and front-line demonstration to farmers with the help of aligned departments and universities. Marketing constraints can be overcome by fixing the price of milk and establishment of milk cooperative societies in the study area. Major institutional constraints can be overcome by establishing a network of mobile veterinary clinics for remote areas.

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RESEARCH ARTICLE

# Economics of livestock-based farming systems in saline and normal areas of West Bengal: A comparative analysis

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**Abstract:** Salinity has deleterious effects on both crops and livestock. The present study evaluated the impact of salinity and estimated the cost and returns of different livestock-based farming systems in saline and normal areas of West Bengal. The study suggested the farmers the best farming systems to be adopted based on output-input ratio. Saline areas are dominated by the indigenous cow and normal areas by the crossbred jerseys. This may be due to shortage of green fodder in saline areas. Cattle-goat-crop (N2) had highest crop area (1.80 acres) while Cattle-goat-crop-fish (S3) had highest fishing area (0.70 acres). Due to low maintenance cost, Sheep-poultry (S1) and Goat-poultry (S2) farming systems had output-input ratios close to 2. Whereas other farming systems in saline areas, the output-input ratios were close to 1.5. In case of normal areas, Cattle-goat-crop-fish had the highest output-input ratio of 1.58. Goat-poultry (S2) farming system had the highest net return of ₹ 53,967.68 per annum among landless farmers and cattle-goat-crop-fish (S3) with a net return of ₹ 1,01,686.50 per annum among landholders in saline areas. The farming system, Cattle-goat-poultry-crop (N1) had highest net return of ₹ 1,15,578 per annum) in normal areas. Share of labour was highest (56% to 58%) in total cost in crop enterprises and feed and fodder cost had a major share (53% to 72%) in total cost for livestock enterprises. Share of cattle enterprise in total cost and net return was highest. However, its share in net return was lower than its share in total cost. In case of goat and fish enterprises, their share in net return was higher than their share in total cost.

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**Key words:** Capital recovery cost, Livestock-based farming systems, Net return, Normal areas, Output-Input ratio, Saline areas, West Bengal.

## Introduction

Salinity condition of the soil is becoming more and more prominent each year, making it difficult for farmers to maintain their property (Wongsomsak, 1986). Due to salinity issues, agricultural potential is limited (Ladeiro, 2012). High salinity affects 20 per cent of total cultivated area and 33 per cent of irrigated agricultural land around the world (Shrivastava and Kumar, 2015). Degraded land covers around 147 million ha in India, with 23 million ha degraded due to salinity/alkalinity/acidification, the second most common source of soil degradation after water erosion (94 million ha) (Kumar and Sharma, 2020). Soil salinity, which is responsible for around 20 per cent yield reduction in these areas, puts a strain on rice yield, spikelet sterility, and thousand-grain weight in the coastal belt (Clermont et al. 2010). Due to salinity, there is a shortage of fodder crops in coastal saline areas, which affects cattle milk yield (Wistrand, 2003). Skin illnesses, liver fluke, diarrhoea, body weight loss, and immune system breakdown plague animals in salty locations due to ingestion of salinity-affected fodder crops (Alam et al. 2017). Due to the intake of salinity-affected agricultural goods, pregnant women in coastal areas experience greater gestational hypertension than pregnant women in non-coastal areas (Khan et al. 2008; WHO, 2003).

The Indo Gangetic Plains (IGP) are well recognised for providing approximately half of the country's total food consumption and feeding 40 per cent of the people (Pal et al. 2009). The plains are the agriculturally most fruitful region of the country, with almost 36 per cent of the country's bovine population. The bovine sector alone contributes 235 billion to the IGP GDP among the livestock sector (Singh et al. 2005). Every year, approximately 10 per cent of the extra land becomes salinized, and by 2050, nearly half of all arable land will be contaminated by salt (Kumar and Sharma, 2020). Salinity increases in the area beneath the Indo-Gangetic plains will jeopardise our country's food security. West Bengal controls 78.84 per cent (4,41,272 ha) of the total saline areas in the IGP region (5,59,719 ha) (Mandal et al. 2010). The coastal saline zone suffers from both soil and water salinity, as well as a

milk and livestock deficit (Wistrand, 2003). Hence, the West Bengal state was considered as an ideal location for a comparative study of livestock-based farming systems in saline and normal environments.

**Methodology**

**Sampling plan**

The major part of the coastal saline areas in West Bengal is in the Sundarban area of districts South 24 Parganas, parts of North 24 Parganas and Purba Midnapore (Bandyopadhyay *et al.* 2003). Sampling units were selected with the help of the multistage sampling technique. Within the selected districts where saline soil can be found, 17 blocks of South 24 Parganas, 6 blocks of North 24 Parganas and 10 blocks of Purba Midnapore is having saline areas. The rest of the blocks i.e., 12 blocks of South 24 Parganas, 16 blocks of North 24 Parganas and 15 blocks of Purba Midnapore are considered normal areas for the comparison of livestock-based farming systems in saline and normal areas (GoW, 2018). Finally, from the above-mentioned blocks in saline and normal areas, Basanti, Namkhana and Canning II from South 24 Parganas; Hingalganj from North 24 Parganas; Khejuri II and Nandigram I from Purba Midnapore were randomly selected for saline areas. For normal areas, Mograhat I and Mograhat II from South 24 Parganas; Barasat I and Bongaon from North 24 Parganas; Bhagwanpur I and Bhagwanpur II from Purba Midnapore blocks were selected randomly. Twenty households from each block were selected based on random sampling. A total of 120 households were selected from each of the saline and normal areas, thus total sample size constituting 240 households. Primary data were collected through the personal interview method on a structured interview schedule from the door-steps of the respondents on various aspects of livestock and crop enterprises from selected households for the year 2019-2020. Farmers who were having 50 per cent or more income from livestock were only considered as respondents for the present study.

Identification of different types of livestock-based farming systems was done based on the highest income contribution from livestock enterprises. For example; if the highest share of income earned by a household from livestock enterprises is through sheep rearing, then the system will be named sheep-based farming system and so on.

**Cost categories of different enterprises**

**Table 1.** Conversion factors of man equivalent days

| Particulars | Man-equivalent days |
|---|---|
| Child workers (<14 years) | 0.50 |
| Male (15-39 years) | 1 |
| Female (15-39 years) | 0.75 |

**Fixed costs:** Depreciation on farm machinery and farm building, land revenue cesses and other taxes and interest on working capital. Rent paid for leased in land, interest on the value of owned capital assets, the rental value of owned land and rent paid for leased in land were considered as fixed costs for the study.

**Variable costs:** Value of purchased material inputs such as seed, insecticide and pesticide, manure, fertilizer, etc., hired human labour, animal labour (hired or owned) hired farm machinery, irrigation charges, feed and fodder cost, veterinary and miscellaneous expenditure. In case of purchased feed and fodder, the cost was worked out as a product of the quantity fed to animals and the purchase price of respective feed. In case of home-grown feed and fodder, the relevant prices were the farm-harvest prices. When the concentrate feed was prepared at home, its cost was computed by taking the weighted prices of ingredients used in the concentrate, the weights being the share of each ingredient in the concentrate composition.

**Labour cost:** It included the cost of the family as well as paid labour (hired labour). The cost of hired labour was calculated considering the type of work allotted and wages paid whereas, family labour costs were determined based on the existing wage rate of permanent farm labour. Total time spent was converted to man-days by using conversion as presented in Table 1:

**Veterinary and miscellaneous costs:** The expenditure on breeding and health care of the animals was covered under the veterinary expenses. It included the cost of artificial insemination (AI), natural service, vaccination, medicines, the fee of veterinary doctors and other related expenses. The miscellaneous expenditure included expenses for repair of fixed assets, water and electricity charges, insurance premiums and any other incidental charges. These being joint costs, apportionment of the same was based on SAU.

**Standard Animal Units (SAUs):** Considering the differences in regional endowments of animal wealth and species, the livestock animals were converted into SAUs using factors suggested by Kumbhare *et al.* (1983) (Table .2);

**Table 2.** Standard animal units for different livestock animals

| Type of Animals | SAU |
|---|---|
| Local cow | 1.00 |
| Crossbreed cow | 1.40 |
| Buffalo | 1.30 |
| Bullock/he-buffalo | 1.00 |
| Local cow/ Buffalo heifer >2 years | 0.75 |
| Crossbreed heifer >1 year | 0.75 |
| Calf of buffalo & local cow >1 year | 0.50 |
| All calves <1 year | 0.33 |
| Goat & Sheep | 0.50 |
| Other animals | 1.00 |

**Depreciation cost:** Capital Recovery Cost (CRC) method was used to calculate depreciation cost which is defined as the annual payment that will repay the cost of fixed input over the useful life of input and provide an economic rate of return on investment. The interest on fixed capital does not need to be accounted for separately in the CRC approach. The formula for estimation of CRC is:

$$R = Z\left[\frac{(1+r)^n r}{(1+r)^n - 1}\right]$$

Where,

R = Capital recovery cost

Z = Initial value of the capital asset

r = Interest rate

n = Useful life of the assets

In case of practical difficulties in getting the information on initial outlay at the field level, the current value of the asset was considered. When the asset was purchased from borrowed capital the actual interest rate charged by the bank was taken as 'r', while useful life (n) of both fixed and livestock assets were considered as the value suggested by Rao, 1991 (Table 3). The total CRC was then apportioned to the individual animal by the Standard Animal Units (SAUs).

**Cost concepts:**

**Total cost:** It was obtained by adding all the cost components including fixed and variable costs.

Total cost = Total variable cost + Total fixed cost

**Table 3:** Useful life of farm assets

| Fixed assets | In years |
|---|---|
| Own fund (Term deposits) | 1-5 |
| Pucca cattle shed | 50 |
| Katcha cattle shed | 10 |
| Manual chaff cutter | 6 |
| Power-operated cutter | 10 |
| Livestock | |
| Local cow (in years) | 10 |
| Crossbreed cow (in years) | 8 |
| Buffaloes (in years) | 10 |
| Sheep (in months) | 6-8 |
| Goat (in months) | 3-5 |
| Pig (in months) | 5-6 |

**Gross returns:** Gross returns were obtained by multiplying the milk yield of an individual milch animal with respective prevailing prices in the study area

Gross returns = Quantity of milk × Market price of milk

**Net returns:** Net return was calculated by subtracting net cost from gross returns

Net returns = Gross returns -Total cost

Gross returns from farming systems: Gross returns were estimated by summing up the returns obtained from both main product and by-products of various farm enterprises undertaken on the farm, evaluated at their market prices.

**Output-Input Ratio:**

$$\text{Output} - \text{Input Ratio} = \frac{\text{Gross return of output}}{\text{Total cost of inputs}}$$

**Results and Discussion**

The farming systems identified in saline and normal areas of the study area in West Bengal are presented in Table 4.

**Composition of livestock and poultry and average operational area under different farming systems**

Due to increased salinity, there is a shortage of grazing land and fodder crop for livestock production. Saline areas were dominated by indigenous cows because these breeds can withstand low fodder availability. Cross breeding in cattle is a total failure due to the non-availability of feed, salinity, lack of availability of artificial insemination services coupled with the absence of a market for milk (Das, 2011). A study conducted in saline areas of Bangladesh by Sarker et al. (2018) also found that 17 per cent of the household kept crossbred cows and 62 per cent had indigenous cows. Although the performance of the indigenous or native stock is poor relative to highly selected commercial lines, they can survive in harsh and challenging environments (Crawford and Christman, 1992). The cattle-goat-crop-fish (S3)

**Table 4:** Identified farming systems in the study area

| Code | Type of farming systems identified |
|---|---|
| | Farming systems in saline areas |
| S1 | Sheep-Poultry |
| S2 | Goat-Poultry |
| S3 | Cattle-Goat-Crop-Fish |
| S4 | Cattle-Poultry-Crop-Fish |
| | Farming systems in normal areas |
| N1 | Cattle-Goat-Poultry-Crop |
| N2 | Cattle-Goat-Crop |
| N3 | Cattle-Poultry-Crop |

had the highest average Standard Animal Unit (SAU) of cattle under this system (5.16) followed by cattle-poultry-crop-fish (3.08) under saline areas (Table 5).

In normal areas, crossbreed jersey is a popular dairy animal. Under cattle-goat-poultry-crop (N1) on an average 6.03 SAU of cattle were available followed by cattle-poultry-crop (N3) (5.71 SAU) and cattle-goat-crop (N2) (3.08 SAU).

In saline areas, two types of farming systems were popular among the landless farmers, i.e., sheep-poultry (S1) and goat-poultry (S2). Near the Matla River of the Sundarban area few landless farmers engaged in Garole sheep farming. Salinity in the Matla increased by around 32 per cent between 1984 and 2013 (Trivedi et al. 2016). Garole breed is known for its bi-annual lambing, high prolificacy rate, high mothering instincts, adaptability to marsh saline as well as hot and humid climatic condition, grazing on aquatic weeds and grass in knee-dip water and resistance to some common diseases (Banerjee, 2008: Sahana et al. 2001). Interestingly this breed has naturally developed resistance against foot rot, FMD and reproductive disorders, etc. and is considerably more resistant to the dreaded roundworm *Haemonchus contortus* as well as to the tropical liver fluke (Nimbkar, 2002). Households engaged in sheep-poultry farming system hold on an average 7.50 SAU of Garole sheep.

Black Bengal goat, which is famous for its high-quality meat and skin, was common in both saline and normal areas. Landless farmers under the goat-poultry farming system depend heavily on Black Bengal goats, on average, they had 7.50 SAU followed by cattle-goat-crop-fish (S3) (5.30 SAU). In normal areas, average SAU under cattle-goat-poultry-crop (N1) and cattle-goat-crop (N2) was 5.25 and 5.50, respectively.

Poultry birds are mainly reared for egg purposes. Chicken breeds like Vanaraja, Rhode Island Red along with domestic duck breed Khaki Campbell were reared in the back yard of the household. In saline areas, the highest number of birds were found in the goat-poultry (S2) farming system (15 nos.) followed by sheep-poultry (13 nos.) and cattle-poultry-crop fish (12.50 nos.).

In normal areas, two types of farming systems such as cattle-goat-poultry-crop (N1) and cattle-poultry-crop (N3) include poultry enterprises with an average number of birds of 12 and 11.50, respectively.

In West Bengal, 82 per cent of the farmers are marginal with landholding of less than one hectare (Mandal, 2016). The findings of the current study were also in the same line as the previous studies (quote some references). In saline areas, only two farming systems, i.e., cattle-goat-crop-fish (S3) and cattle-poultry-crop-fish (S4), have crop and fish components. Under S3 on an average 1.10 acres was under crop cultivation, and 0.70 acres are under fish cultivation and in case of S4, it was 0.75 acres and 0.55 acres, respectively (Table 5). Rice and fish constitute the principal diet in the Bengali community. Households in this area are engaged in mono-cropping of rice both in the *kharif* and *rabi* seasons. The land use map showed that 80 per cent of the total agricultural land is under rice cultivation (Ghosh and Mistri, 2020). Salinization of coastal lands threatens the livelihood security of thousands of small rice farmers. Sea-level rise, storm surge, and coastal

**Table 5:** Average composition of livestock and poultry under different farming systems

| Animal Type | Farming systems | | | | | | |
|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | N1 | N2 | N3 |
| | | | | Cattle (in SAU) | | | |
| Milch | - | - | 3.00 | 2.00 | 4.20 | 2.80 | 2.80 |
| Heifer | - | - | 1.50 | 0.75 | 1.50 | 0.75 | 2.25 |
| Calf | - | - | 0.66 | 0.33 | 0.33 | 0.33 | 0.66 |
| Total | - | - | 5.16 | 3.08 | 6.03 | 3.88 | 5.71 |
| | | | | Goat (in SAU) | | | |
| Adult | - | 5.25 | 3.97 | - | 3.67 | 3.85 | - |
| Kid | - | 2.25 | 1.33 | - | 1.58 | 1.65 | - |
| Total | - | 7.50 | 5.30 | - | 5.25 | 5.50 | - |
| | | | | Sheep (in SAU) | | | |
| Adult | 6.00 | - | - | - | - | - | - |
| Kid | 1.50 | - | - | - | - | - | - |
| Total | 7.50 | - | - | - | - | - | - |
| | | | | Poultry (in no.) | | | |
| Adult | 7.80 | 9.00 | - | 7.50 | 7.20 | - | 6.90 |
| Grower | 3.90 | 1.50 | - | 3.75 | 1.20 | - | 3.45 |
| Chicks | 1.30 | 4.50 | - | 1.25 | 3.60 | - | 1.15 |
| Total | 13.00 | 15.00 | - | 12.50 | 12.00 | - | 11.50 |
| Crop area (in acre) | - | - | 1.10 | 0.75 | 1.60 | 1.80 | 1.50 |
| Fishing area (in acre) | - | - | 0.70 | 0.55 | - | - | - |

erosion increase the risk of salinity in this area. Thus, farmers are dependent on indigenous salt-tolerant varieties like Dudhersar, Lal Dhan, Rupsal, Patnai, etc. Fish is cultivated in the backyard pond.

In normal areas, all the sample households had a few acres of the cropped area. They mostly cultivate different types of high-yielding varieties (HYV) of rice such as Khitish, Swarna Mahsuri, Sada Swarna, etc. along with mustard and jute. The highest land under cultivation is under cattle-goat-crop (N2) farming system (1.80 acres), followed by cattle-goat-poultry-crop (N1) (1.60 acres) and cattle-poultry (N3) (1.50 acres).

**Cost and return of different enterprises across different farming systems:**

In saline areas, farmers mostly cultivated salt-tolerant indigenous rice varieties such as Dudhersar, Lal Dhan, Rupsal, Patnai etc. Labour cost had the major share of total cost. It's share under S3 and S4 farming systems were 58.74 and 57.87 per cent of total cost, respectively (Table 6). Rice was mainly cultivated for home consumption purposes. Rice bran and rice straw are used for cattle feed. For rice, market prices vary variety wise and at the end of the year net return obtained from crop enterprises under S3 and S4 farming systems were ₹ 6,771 per annum and ₹ 4,771 per annum, respectively.

Garole sheep rearing in S1 farming system is a unique feature in saline areas. Various authors (Ghalsasi and Nimbkar, 1993, Bose and Moitra, 1995, Singh and Bohra, 1996, Sharma et al. 1999 and Sahana et al. 2001) have reported that the breeding of Garole sheep is localized in the Sundarban regions of West Bengal and Bangladesh. Marginal and landless farmers from socially backward and underprivileged classes maintain this type of sheep. Total cost, gross return and net return from sheep rearing were ₹ 25,107.50 per annum, ₹ 51,900 per annum and ₹ 26,792 per annum, respectively. Sheep were sold in the market at 12 months of age with an average of 15 kg body weight at the rate of ₹ 300/kg of meat. Out of total cost, 75 per cent was variable cost and 25 per cent was fixed cost. Harvested grass, weeds, tree leaves, dry grass and rice straw are used as supplementary feed. The animals are even found to drink saline water for several days, as there are limited sources of fresh water on many islands (Mandal et al. 2017).

Under S2 farming system, the Black Bengal goat was reared by the landless farmers. This animal also required low inputs like Garole sheep but is more prone to diseases like diarrhea, parasitic infection, skin problem, anemia, etc. In saline zone, there is a scarcity of clean water and farmers used to provide pond water without any treatment. Farmers preferred their goats to be stall-fed as they fall sick due to consumption of poisonous weeds during grazing and also to protect them from stray dogs' attacks. Cereal by-products like mug chuni, bhusa of pulses, wheat husk, whole rice bean after harvesting, etc. are provided during stall-fed. The average gross and net return from goat rearing under S2 farming system were ₹ 69,200/annum and ₹ 36,486/annum, respectively, which was higher than the income received from sheep rearing. The higher return was due to the meat of the Black Bengal goat being delicate and highly demanded. Demand increases during festivals like Muharram and Durga Puja. Goats are sold in the market at 12 months of age with an average of 15-18 kg body weight at the rate of ₹ 400/kg of meat. Farmers prefer castrating male kids to be raised for meat purposes within one month of age. The average daily milk yield was around 150-200 g, which is used to feed the kids and not for selling purposes, however, goat manure is used in crop fields. Similar to sheep rearing, in case of goat rearing also variable cost and fixed costs were 75.83 and 24.17 per cent of total cost, respectively.

Landless farmers under S1 and S2 farming systems also engaged in non-farm activities like permanent labour in others' fields and MGNREGA labour. Net returns from non-farm activities were ₹ 9,735.55/annum and ₹ 11,512.68/annum for S1 and S2 farming systems, respectively.

Under S3 and S4 farming systems, the cattle were mostly indigenous cows. Share of total feed and fodder in livestock enterprise were 59.70 per cent and 62.00 per cent of total cost, respectively. The previous studies also indicated that about 60 to 80 per cent cost was estimated for feed and fodder (Lal and Chandel, 2016; Patil, 2010; Kumari, 2020). Most of the cattles depend on low-quality roughages like straw and locally available natural grasses to fill their stomach which cannot fulfil the actual nutrients requirement of the animals (Sarker et al. 2018). Farmers mostly depend on concentrate to provide sufficient nutrients to the cattle. Concentrates fed by the farmers were mostly bought from the market or prepared at home by mixing rice polish, wheat bran, pulse bran, broken rice and mustard oil cakes in the surveyed area. Share of variable and fixed costs for S3 farming system which includes both cattle and goats, were 86.72 per cent and 13.28 per cent of total cost, respectively. Net return for S3 farming system was ₹ 58,335.50 per annum. In S4 farming system, which includes only cattle generates a net return of ₹ 36,862 per annum. Due to less cooperative societies in the region, farmers mainly sell the milk either in sweet shops or sell them door to door, at a rate of ₹ 35-40/litre. Sandesh which is a popular Bengal sweet, is made from milk and has a high demand. Farmers usually get a higher rate for their milk if they sell it in sweet shops.

Chicken breeds like Vanaraja, Rhode Island Red along with domestic duck breed like Khaki Campbell were reared in the back yard of the household. Poultry birds were reared mainly for egg production, the net return from poultry for S1, S2 and S4 farming systems were ₹ 7,499/annum, ₹ 5,969/annum and ₹ 4,439/annum, respectively. The differences in net profit under different farming

**Table 6:** Cost and return of different enterprises across different farming systems under saline areas (₹ /hh/year)

| Components | S1(Sheep-Poultry) | S2 (Goat-Poultry) | S3 (Cattle-Goat-Crop-Fish) | S4 (Cattle-Poultry-Crop-Fish) |
|---|---|---|---|---|
| | | Crop | | |
| 1) Labour | - | - | 10,500.00 (58.74) | 8,000.00 (57.87) |
| 2) Seed | - | - | 950.00 (5.31) | 900.00 (6.43) |
| 3) Manure and fertilizer | - | - | 1,694.00 (9.48) | 1,270.00 (9.38) |
| 4) Plant protection chemicals | - | - | 400.00 (2.24) | 300.00 (2.23) |
| 5) Interest in working capital | - | - | 462.50 (2.58) | 347.00 (2.48) |
| 6) Total variable cost (TVC) (1+2+3+3+4+5) | - | - | 14,006.50 (78.35) | 10,817.00 (78.39) |
| 7) Depreciation | - | - | 244.50 (1.36) | 183.50 (1.37) |
| 8) Land rent | - | - | 3,225.00 (18.04) | 2,418.50 (17.53) |
| 9) Interest on fixed capital | - | - | 400.00 (2.24) | 380.00 (2.75) |
| 10) Total fixed cost (TFC) (7+8+9) | - | - | 3,869.50 (21.65) | 2,982.00 (21.61) |
| 11) Total cost (TC) (6+10) | - | - | 17,876.00 (100.00) | 13,799.00 (100.00) |
| 12) Gross return (GR) | - | - | 24,647.00 | 18,750.00 |
| 13) Net return (NR) (12-11) | - | - | 6,771.00 | 4,771.00 |
| | | Livestock | | |
| Total feed and fodder cost | 13,461.00 (53.60) | 17,811.00 (54.44) | 61,776.00 (59.70) | 50,300.00 (62.00) |
| 1.a) Fodder cost | 4,374.82 (17.42) | 6,055.74 (18.51) | 27,181.44 (26.26) | 21,880.50 (26.97) |
| 1.b) Feed/Concentrate cost | 9,086.18 (36.19) | 11,755.26 (35.93) | 34,594.56 (33.44) | 28,419.50 (35.03) |
| 2) Labour | 4,725.00 (18.80) | 6,300.00 (19.25) | 27,325.00 (24.48) | 20,225.00 (25.91) |
| 3) Miscellaneous cost | 655.00 (2.60) | 700.00 (2.14) | 2,625.00 (2.53) | 2,050.00 (2.52) |
| 4) Total variable cost (TVC) (1+2+3) | 18,841.00 (75.00) | 24,811.00 (75.83) | 89,726.00 (86.72) | 72,575.00 (89.00) |
| 5) Depreciation | 6,266.50 (25.00) | 7,903.00 (24.17) | 13,738.50 (13.28) | 8,563.00 (11.00) |
| 6) Total fixed cost (TFC) (5) | 6,266.00 (25.00) | 7,903.00 (24.17) | 13,738.50 (13.28) | 8,563.00 (11.00) |
| 7) Total cost (TC) (4+6) | 25,107.50 (100.00) | 32,714.00 (100.00) | 1,03,464.50 (100.00) | 81,138.00 (100.00) |
| 8) Gross return (GR) | 51,900.00 | 69,200.00 | 1,61,800.00 | 1,18,000.00 |
| 9) Net return (NR) (8-7) | 26,792.00 | 36,486.00 | 58335.50 | 36,862.00 |
| | | Poultry | | |
| 1) Total cost | 7,261.00 | 6,511.00 | - | 5,761.00 |
| 2) Gross return (GR) | 14,760.00 | 12,480.00 | - | 10,200.00 |
| 3) Net return (NR) (2-1) | 7,499.00 | 5,969.00 | - | 4,439.00 |
| | | Fish | | |
| 1) Total cost | - | - | 57,600.00 | 44,200.00 |
| 2) Gross return (GR) | - | - | 94,180.00 | 70,635.00 |
| 3) Net return (NR) (2-1) | - | - | 36,580.00 | 26,435.00 |
| | | Non-farm activity | | |
| 1) Total cost | 3,000.00 | 2,700.00 | - | - |
| 2) Gross return (GR) | 12,735.55 | 14,212.68 | - | - |
| 3) Net return (NR) (2-1) | 9,735.55 | 11,512.68 | - | - |

*Note:* Figures in parenthesis indicate percent to column total

systems were due to differences in average flock size. Eggs are sold at the rate of ₹ 6/egg and income from selling poultry birds for the income purpose was at the rate of ₹ 160/kg of meat in the local market.

Fish is an important component of S3 and S4 farming systems. Under S3 farming system total cost, gross return and net return were to the tune of ₹ 57,600/annum, ₹ 94,180/annum and ₹ 36,580/annum, respectively. Under S4 farming system, total cost (₹ 43,200/annum), gross return (₹ 70,635/annum) and net return

**Table 7:** Cost and return of different enterprises across different farming systems under normal areas (₹ /hh/year)

| Components | N1 (Cattle-Goat-Poultry-Crop) | N2 (Cattle-Goat-Crop) | N3 (Cattle-Poultry-Crop) |
|---|---|---|---|
| | Crop | | |
| 1) Labour | 14,000.00 | 15,000.00 | 13,500.00 |
| | (56.82) | (56.95) | (58.87) |
| 2) Seed | 1,750.00 | 1,800.00 | 1,500.00 |
| | (7.10) | (6.83) | (6.54) |
| 3) Manure and fertilizer | 2,350.00 | 2,541.00 | 2,117.50 |
| | (9.53) | (9.65) | (9.23) |
| 4) Plant protection chemicals | 560.00 | 600.00 | 450.00 |
| | (2.28) | (2.28) | (1.96) |
| 5) Interest in working capital | 630.00 | 694.00 | 578.50 |
| | (2.56) | (2.64) | (2.53) |
| 6) Total variable cost (TVC) (1+2+3+3+4+5) | 19,290.00 | 20,635.00 | 18,146.00 |
| | (78.29) | (78.35) | (79.13) |
| 7) Depreciation | 310.00 | 500.00 | 306.00 |
| | (1.26) | (1.90) | (1.33) |
| 8) Land rent | 4,560.00 | 4,837.00 | 4,031.00 |
| | (18.50) | (18.36) | (17.58) |
| 9) Interest on fixed capital | 480.00 | 500.00 | 450.00 |
| | (1.95) | (1.90) | (1.96) |
| 10) Total fixed cost (TFC) (7+8+9) | 5,350.00 | 5,704.00 | 4,787.00 |
| | (21.71) | (21.65) | (20.87) |
| 11) Total cost (TC) (6+10) | 24,640.00 | 26,339.00 | 22,933.00 |
| | (100.00) | (100.00) | (100.00) |
| 12) Gross return (GR) | 32,500.00 | 34,500.00 | 30,250.00 |
| 13) Net return (NR) (12-11) | 7,860.00 | 8,161.00 | 7,317.00 |
| | Livestock | | |
| Total feed and fodder cost | 1,13,680.00 | 1,11,276.00 | 99,697.29 |
| | (68.39) | (69.43) | (72.28) |
| 1.a) Fodder cost | 52,292.80 | 52,856.10 | 45,362.27 |
| | (31.46) | (32.98) | (32.89) |
| 1.b) Feed/Concentrate cost | 61,387.20 | 58,419.90 | 54,335.02 |
| | (36.93) | (36.45) | (39.39) |
| 2) Labour | 27,297.00 | 25,325.00 | 20,127.71 |
| | (16.42) | (15.80) | (14.59) |
| 3) Miscellaneous cost | 2,568.00 | 2,625.00 | 2,250.00 |
| | (1.54) | (1.64) | (1.63) |
| 4) Total variable cost (TVC) (1+2+3) | 1,43,545.00 | 1,39,226.00 | 1,22,075.00 |
| | (86.36) | (87.00) | (88.50) |
| 5) Depreciation | 22,672.00 | 21,035.50 | 15,860.00 |
| | (13.64) | (13.00) | (11.50) |
| 6) Total fixed cost (TFC) (5) | 22,672.00 | 21,035.50 | 15,860.00 |
| | (13.64) | (13.00) | (11.50) |
| 7) Total cost (TC) (4+6) | 1,66,217.00 | 1,60,261.50 | 1,37,935.00 |
| | (100.00) | (100.00) | (100.00) |
| 8) Gross return (GR) | 2,66,600.00 | 2,55,400.00 | 2,10,600.00 |
| 9) Net return (NR) (8-7) | 1,00,383.00 | 95,138.50 | 72,665.00 |
| | Poultry | | |
| 1)Total cost | 5,965.00 | - | 6,911.00 |
| 2) Gross return | 10,300.00 | - | 12,980.00 |
| 3) Net return (3-1) | 4,335.00 | - | 6,069.00 |

*Note:* Figures in parenthesis indicate percent to column total

(₹ 26,435/annum) were lower than S3 farming system due to the lower size of the pond area (Table 6). Fishing enterprise is mainly backyard fish cultivation where farm-made feed was prepared by mixing mustard oilcake and rice bran at 1:1 ratio. The commonly cultivated fish in the region were Catla, Rohu, Mrigel, Common Carp, Silver Carp, Grass Carp etc.In normal areas, farmers prefer to cultivate high yielding varieties of rice such as Khitish, Swarna Mahsuri, Sada Swarna etc. Net returns under N1, N2, and N3 were accounted for ₹ 7,860/annum, ₹ 8,161/annum and ₹ 7,317/annum, respectively, which were higher than the saline areas (Table 7). Labour costs under all the farming systems accounted for almost half of total cost, it was 56.82 per cent, 56.95 per cent and 58.87 per cent for N1, N2 and N3 farming systems, respectively.

Under livestock enterprise, net returns were much higher than in saline areas because of the presence of crossbred cows. However, across different farming systems net return was different due to differences in herd size. Net returns from livestock under N1, N2, and N3 farming systems were ₹ 1,00,383/annum, ₹ 95,138/annum and ₹ 72,665/annum, respectively. Milk price did not vary between saline and normal areas. Although, there were few cooperatives present in normal areas, the farmer gets low price (₹ 28/litre) for selling milk in cooperatives due to it's low-fat content. Due to this, the majority of farmers sold their milk in sweet shops or door to door at the rate of ₹ 35-40 per litre. Due to the presence of fodder farms in this area, green fodders like hybrid napier, para-grass, lathyrus were easily available. In the wet summer season natural grasses are more available but farmers supply more concentrates in the winter season due to the shortage of green fodders.

Poultry breeds like Vanaraja, Rhode Island Red and Khaki Campbell were reared in backyards like in saline areas. Net returns from poultry enterprise under N1 and N3 farming systems were ¹ 4,335/annum and ¹ 6,069/annum, respectively.

**Cost and return of different farming systems**

Cost and return from different farming systems are presented in Table 8, which helps us to understand which farming system is more profitable than the other in both saline and normal areas. Output input ratios were computed by dividing gross return by total cost. These ratios indicate return per unit of investment, higher the ratio better is the profitability for each household (HH).

In saline areas, households under S1 and S2 farming systems were the poorest of the poor earning net return of ₹ 38,905.35/HH/year and ₹ 53,967.68/HH/year, respectively. Although, net return under these farming systems were lower than other farming systems in this area, but their output-input ratios were 2.10 and 2.28, respectively, which was higher than all other farming systems. This was due to the low maintenance cost of goats and sheep, which leads to total cost being lower than other farming systems. The study conducted by Kumar et al. (2012) also found similar results of lower production cost and higher output-input ratio. S4 farming system has the lowest output-input ratio of 1.45 in saline areas. The highest gross and net return were obtained under S3 farming system i.e., ≠ 2,80,627.00/HH/year and ₹ 1,01,686.50/HH/year, respectively. However, due to higher maintenance cost of cattle, total cost was also the highest (₹ 1,78,940.50/HH/year) which leads to drop in output-input ratio to 1.57. This indicates that farmers will receive ₹ 1.57 by investing ₹ 1 in S3 farming system.

In normal areas, N1 farming system has the highest net return and output input ratio of ₹ 1,15,578/HH/year and 1.58, respectively. This farming system includes more activities and is more integrated than other existing systems in the area. Kumar et al. (2011) also reported increase in net income through an integrated farming system due to the use of recycled products within the system. Output input ratios for other farming systems were 1.52 and 1.51 for N2 and N3 farming systems, respectively.

Share of labour was highest (56% to 58%) in total cost in crop enterprises and feed and fodder cost had a major share (53% to 72%) in total cost for livestock enterprises. Due to low maintenance cost Sheep-poultry (S1) and Goat-poultry (S2) farming systems had output-input ratios close to 2. In case of other farming systems, the output-input ratios were close to 1.5 in saline areas. In case of normal areas, Cattle-goat-crop-fish (1.58) had the highest output-input ratio. Goat-poultry (S2) farming

**Table 8:** Cost and returns of different farming systems

| Code (1) | Farming systems (2) | Total cost (3) | Gross return (4) | Net return (NR) (5= 4-3) | Output input ratio (6=4/3) |
|---|---|---|---|---|---|
| S1 | Sheep-Poultry | 35,368.50 | 74,273.85 | 38,905.35 | 2.10 |
| S2 | Goat-Poultry | 41,925.00 | 95,892.68 | 53,967.68 | 2.28 |
| S3 | Cattle-Goat-Crop-Fish | 1,78,940.50 | 2,80,627.00 | 1,01,686.50 | 1.57 |
| S4 | Cattle-Poultry-Crop-Fish | 1,43,898.00 | 2,08,652.10 | 64,754.10 | 1.45 |
| N1 | Cattle-Goat-Poultry-Crop | 1,96,822.00 | 3,09,400.00 | 1,15,578.00 | 1.58 |
| N2 | Cattle-Goat-Crop | 1,86,600.50 | 2,83,632.76 | 97,032.26 | 1.52 |
| N3 | Cattle-Poultry-Crop | 1,67,779.00 | 2,53,830.00 | 86,051.00 | 1.51 |

system had the highest net return of ₹ 53,967.68/annum among landless farmers and cattle-goat-crop-fish (S3) with a net return of ₹ 1,01,686.50/annum among landholders in saline areas. Cattle-goat-poultry-crop (N1) farming system had highest net return of ₹ 1,15,578/annum) in normal areas.

## Conclusions

Goat-poultry (S2) farming system had a higher output-input ratio (2.28) than Sheep-poultry (S1) (2.10). So, S2 farming system should be suggested for resource-poor land-less farmers. For other landholders, Cattle-goat-crop-fish (S3) should be recommended in saline areas. Whereas in case of normal areas, Cattle-goat-poultry-crop (N1) should be recommended as it had the highest output-input ratio than other farming systems in that area. Irrespective of saline and normal areas, variable cost had a king's share in total costs for both crop and livestock management. During survey it was found that, presence of cooperative societies in both saline and normal areas was very meagre. If cooperative societies are promoted and farmers obtain these variable inputs from those cooperatives then variable costs and hence total cost will be reduced.

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RESEARCH ARTICLE

# Determinants of farmer's choice of milk marketing outlet in Jaipur District of Rajasthan

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**Abstract:** This investigation was undertaken in Jaipur district of Rajasthan with a view to identify the factors affecting the farmer's choice of specific dairy outlet to sell their marketable surplus. The data were collected from a sample of 80 sample respondents selected through multistage purposive sampling for the year 2021-22. A tabular analysis was done to list prevalent marketing channels and then a multinomial logit model was fitted to identify the factors affecting farmer's decision of choosing a particular milk-marketing outlet. The farmers used one of the three marketing channels to deliver milk to final consumers. Channel-I was direct marketing channel (Producer – Consumer) and channel II (Producer - Milk vendor- Consumer), channel III (Producer – Collection centre – Co-operative milk plant – Consumer) were indirect marketing channels of milk. The Channel-III was the most preferred channel of milk marketing, as it was opted by 47.50 per cent of the total farmers. Multinomial logit (MNL) regression's results indicated that herd size, marketable surplus, caste categories, access to institutional credit, BPL economic class, income from livestock and breed type of animals were the seven significant factors affecting farmer's decision of choosing a particular milk-marketing outlet out of three. Although direct marketing channels are highly efficient and fetch more prices to producers, still most of the farmer choose channel III for marketing of milk, may be because of easy disposal of marketable surplus. This trend might continue, therefore, farmers should be made aware about quality parameters like fat percentage in the milk, or even provided with affordable fat testing kits through *Pashu Vigyan Kendras/* Extension centres like KVKs, so that farmers can test it at their level and be assured about the prices they receive in the collection centres. There is need for the effective and improved spread of modern market outlets and dairy cooperatives, so that all the farmers irrespective of social and economic background may benefit from livestock farming.

**Keywords:** Milk marketable surplus, Farmer's Choice, Dairy Outlet, Marketing Channel, Multinomial logit regression.

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## Introduction

India is the world's largest milk producer, contributing about 24 per cent of the global milk production (FAO, 2023). The country has 56.7 per cent of buffalo, 12.5 per cent of cattle, and 20.4 per cent of small ruminant population of the world. During the last few decades, the livestock sector grew at an annual rate of 5.3 per cent during 1980s, 3.9 per cent during 1990s, 3.6 per cent during 2000s and 7.9 per cent during last five years (Economic Survey, 2021). Despite deceleration, livestock sector remained about 1.5 times larger than that of crop sector which implies its critical role in cushioning agricultural growth. It has been witnessed over the years that the stability in dairy income is far stronger than the income realized from agricultural activities (Kumar and Shah, 2016). Growing human population, increasing urbanization, changing lifestyles, increased health awareness and rising domestic incomes have led to increase in the demand of dairy products from consumer's end (Anita and John, 2001, Kharkwal *et. al* 2021). Dairy products are the most acceptable and affordable nutritious source of animal protein for large vegetarian segment of Indian population (Kundu and Banerjee, 2015). India is also the world's largest consumer of dairy products, consuming almost 100 per cent of its own milk production (Shree and Prabu, 2019).

About 90 per cent of the milk production comes from small farmers located in rural areas. Currently, 80 per cent of the milk produced in the country is marketed by the unorganized sector through local vendors and 20 per cent through organized sector including cooperative societies and private companies (Dept. of Animal

Husbandry, Dairying & Fisheries, Ministry of Agriculture and Farmers Welfare, GOI). Marketing of the majority of the milk through unorganized sectors is likely to dissuade small dairy farmers from expending production, which is absolutely necessary to keep up with the strong demand growth (Saran et al. 2022).

Rajasthan is predominantly an agricultural state with an excellent potential for milk production. The state ranks second in milk production and per capita availability of milk in India. In Rajasthan, livestock sector plays major role in improving socio-economic status and fulfilling nutritional needs of rural masses. It is not only a subsidiary occupation to agriculture but also a major economic activity, especially in the arid and semi-arid regions of the Rajasthan. Rajasthan has about only 11.27 per cent of the country's livestock and contributes about 12.6 per cent of the total milk production. Of the total milk produced, 53 per cent is buffalo milk, 36 per cent is cattle milk and 11 per cent is goat milk. Animal husbandry has great potential for rural self-employment and is contributing about 10% in the G.D.P. of the State (Dept. of Animal Husbandry, GOR, 2022).

Milk value chains can range from simple to the complex one's depending upon the scale of its production as well as type of market availability. Farmers may choose to dispose-off the marketable surplus of milk through numerous indirect and direct channels considering marketing cost and profit prospective. Their choice can be an economic indicator for the policy makers in order to set an effective linkage between the milk producers and consumers for fixing the price of milk rationally. Therefore, in this backdrop, this study was undertaken to identify the prevalent dairy marketing channels in Jaipur district of Rajasthan and determine the factors affecting the farmer's choice of a specific dairy outlet in the study area.

**Materials and methods**

A Multistage purposive sampling was used to select the sample. At first stage, Jaipur district from Rajasthan was chosen purposively as it is the largest milk producing district of Rajasthan. Then Amber and Chomu tehsils of Jaipur district were chosen purposively as these tehsils maintain the highest livestock population, accounting 12.04 per cent and 11.83 per cent of total cattle and buffalo of the district, respectively (Dept. of Animal Husbandry, GOR, 2019). In the next step, two villages, one from peri-urban area and one from rural area were chosen from each selected tehsil. Peri-urban area was the area located within 8-10 Kms from nearest town/ market and rural area was the area located at more than 8-10 Kms away from nearest town/ market. Thus, a total of 4 villages Dhand (rural) and KuKas (peri-urban) from Amber tehsil and Cheta ka bas (rural) and Jetpura (peri-urban) from Chomu tehsil were selected for the study**.** At final stage, a separate list of all farmers from each selected village along with herd size owned by them was prepared with the help of Patwaris and the concerned personnel of the selected villages. These dairy farmers were then categorized into the three standard size groups viz., small (1-3 SAUs), medium (4-6 SAUs) and large (e"7) as in Table 1. Finally, total 80 farmers were chosen from these village using probability proportion to size (PPS) method from each category of the herd size, which produced and sold milk in the market for detailed investigation. The primary data were collected for 2021-22 from the selected farmers through personal interview method with the help of pre- structured schedule.

**Table 1:** Category wise distribution of sample according to herd size

| Category | Herd size | Households (No.) |
|---|---|---|
| Small | 1-3 SAUs | 42 |
| Medium | 4-6 SAUs | 22 |
| Large | ≥7 SAUs | 16 |

SAUs = Standard animal units (Lal and Chandel 2016)

A number of econometric models such as probit model by Goetz (1992), structural model based on censoring by Key et al. (2000) and Bayesian double-hurdle model by Holloway et al. (2005) have been employed in identifying factors affecting farmer's choices in marketing their produce. However, if there are finite number of choices and dependent variable is qualitative, multinomial logit estimation is appropriate to analyze the effect of exogenous variables on choices. It is a simple extension of the binary choice model and is the most frequently used model for nominal outcomes that are often used when a dependent variable has more than two choices.

For this study at first a tabular analysis was done to list all direct and indirect milk marketing channels prevalent in the study region, then to study the factors affecting the farmer's choice of a specific dairy outlet, major socio-economic characteristics of the respondents in the study region were listed. The multinomial logistic regression was fitted to identify the factors affecting the producer's choice of selecting particular outlet with the following functional form:

$$M_{ij} = \beta_j X_{ij} + \varepsilon_{ij}$$

Where,

$M_{ij}$ = Vector of marketing choice.

ßj= Vector of channel specific characteristics.

εij= Random error estimation.

xij= Vector of producer characteristics that together might influence dairy farmer market channel decision,

Table 2 presents the list of explanatory variables which fall under six broad categories: (i) Physical capital (ii) Human capital (iii)

Demographic characteristics (iv) Institutional support (v) Economic factors and (vi) Animal characteristics.

The factors considered under physical capital were herd size and marketable surplus of milk. Human capital was proxied by the educational attainment of a farmer. A higher level of education enhances their capability for better management and, thus, makes them more likely to adopt modern marketing practices and select better-paying marketing channels (Marenya and Barret 2006; Gong et al. 2007). Four variables were included to capture the influence of demographic characteristics, namely; the age of household head, household size, gender of a household head and social group (ST, SC, OBC, GEN). Studies such as Morrison et al. 2007; Barham and Chitemi 2009; Vigneri and Holmes 2009; Aregu et al. 2011; Amani 2014; Eerdewijk and Danielsen 2015 suggest female-headed households are less successful than male-headed households at accessing new market opportunities due to lack of resources. Hence, the variable was taken to test the hypothesis that male headed households are more likely to market milk through modern marketing arrangements, while females headed households resort to the traditional one's. Similarly, hypothesis regarding social group was, the dairy farmers who come from the bottom of the social caste pyramid prefer informal system of milk marketing over the organized ones. To assess the effect of institutional support mechanisms such as access to institutional credit, as well as government sponsored schemes like rural employment guarantee programs (Mahatma Gandhi National Rural Employment Guarantee Act [MGNREGA]), below poverty line (BPL) and village location were taken. On the basis of share of livestock income in the total income, livestock occupation as principal or subsidiary was taken as a proxy of economic variable under the hypothesis that farmers with a higher share of income from livestock, may show specialization in livestock production with higher milk production, and hence may prefer to choose modern milk-marketing outlet. In animal characteristics animal type (local cow, crossbred, buffalo) and livestock age were taken as proxy explanatory variables.

**Table 2:** Vector of Explanatory variables

| S. No. | Explanatory variables | Indicators |
|---|---|---|
| 1. | Physical Capital | Herd size (No.), Marketable surplus (lt./day/hh) |
| 2. | Human Capital | Literate without formal education, below primary school, Primary school, Middle school, Secondary school, Secondary and above, Training (Yes/no) |
| 3. | Demographic Characteristics | Age of household head (yrs), Gender (Male/Females), Household size (No.), social group (ST, SC, OBC, Others) |
| 4. | Institutional Support | Access to institutional credit (Yes/No), Participation in MGNREGA (Yes/No), BPL cardholder (Yes/No), Village location (Rural/Peri-Urban) |
| 5. | Economic Factors | Principal occupation livestock (Yes/No) |
| 6. | Animal Characteristics | Age, animal type (cow, crossbred, buffalo, combination of any of these) |

It is also very important to consider the effect of various species/ breed of milch animals kept by farm households both separately and collectively by converting them into standard equivalent units. For this purpose, Standard Animal Units (SAU) of the bovine stock was derived for each farm household as per the specification given by Kumbhare et al. (1983) given in Table 3.

## Results and Discussion

This section is discussed under three subheads; first one is the distribution of farmers in the prevalent milk marketing channels, secondly a highlight of major socio-economic characteristics of the respondents and last subsection deals with the results of multinomial logistic regression.

### Distribution of Farmers in the milk marketing channel

Dairy farmers of the study area were observed to sell the milk in one of the three prevalent marketing channels as given in Table 4. A perusal of the table indicates that Channel-I was direct marketing channel (Producer – Consumer) and channel II (Producer - Milk vendor- Consumer), channel III (Producer – Collection centre – Co-operative milk plant – Consumer) were indirect marketing channels for milk disposal in study region. Table further reveals that Channel-III was the most preferred channel of milk marketing, as it was opted by 47.50 per cent of the total farmers. This may be due to organised system and easier disposal of their marketable surplus in this channel as compared to others. Channel-I was adopted by 28.75 per cent sample farmers, while 23.75 per cent farmers sold milk through Channel-II. The farmer's preference of similar milk marketing channels was also reported by Kashish et al. 2014 and Kumar et al. 2022.

### Socio-economic characteristics of the respondents

Socio-economic profile gives an understanding of social status and overall standard of living of people. Table 5 highlights the major socio-economic characteristics of sample farmers in the

study area. It is evident from the table that the average age of the household's head in the study area was 48.21 years. Of whom, 42.50 percent heads had attained average age of 46.88 years. The caste category wise distribution of sample households indicated that OBC formed the largest fraction of total respondents, i.e., around 58.75 per cent, followed by General (25.00%), ST (8.75%) and SC (7.50%) category. The average family size of the respondents was 6.30, of whom 63.75 per cent of the total families had more than 5 members.

The education of the head of the family is an important factor as family head is mainly responsible for making any decision in the household. The Table 5 further suggests that about 23.75 per cent families were headed by illiterate heads. Among the remaining 76.25 per cent literate heads, of whom, 42.50 per cent had education only up to primary level, 13.75 per cent had education up to secondary level, 12.50 per cent had education up to high secondary level and only 7.50 per cent were educated till graduation level.

Furthermore, on an average, one household owned 8.75 animals, out of which approximately half i.e. 4.38 were milch animals. Local cow, crossbred and buffalo accounted for 18.86, 16.28 and 64.86 per cent of these milch animals, respectively. Converting these

**Table 3:** Standard animal units (SAU) of milch animal

| S. No. | Milch Animal | Standard Equivalents |
|---|---|---|
| 1 | Buffalo | 1.30 |
| 2 | Crossbred cow | 1.40 |
| 3 | Local cow | 1.00 |

**Table 4:** Distribution of farmers in the milk marketing channel

| S. No. | Channels | Small (1-3 SAUs) | Medium (4-6 SAUs) | Large (≥7 SAU) | Total (N=80) |
|---|---|---|---|---|---|
| I. | Producer – Consumer | 20 (47.62) | 2 (9.09) | 1 (6.25) | 23 (28.75) |
| II. | Producer - Milk vendor-Consumer | 8 (19.05) | 5 (22.73) | 6 (37.5) | 19 (23.75) |
| III. | Producer – Collection centre – Co-operative milk plant – Consumer | 14 (33.33) | 15 (68.18) | 9 (56.25) | 38 (47.50) |
| | Total | 42 (100.00) | 22 (100.00) | 16 (100.00) | 80 (100.00) |

**Note**: Figures in Parentheses indicate per cent to total respondents.

**Table 5:** Socio-economic characteristics of the respondents

A. Age-wise distribution of household head

| Age(yrs) | No. of respondents | Average Age (Yrs) |
|---|---|---|
| 20-40 | 17 (21.25) | 37.88 |
| 41-50 | 34 (42.50) | 46.88 |
| >50 | 29 (36.25) | 55.83 |
| Total | 80 (100) | 48.21 |

B. Caste category wise distribution of sample households

| Caste | No. of respondents | Percentage |
|---|---|---|
| GEN | 20 | 25.00 |
| OBC | 47 | 58.75 |
| SC | 6 | 7.50 |
| ST | 7 | 8.75 |
| Total | 80 | 100.00 |

C. Distribution of sample households according to size of family

| Family size (No. of members) | No. of respondents | Average Size |
|---|---|---|
| 1 – 4 | 11 (13.75) | 3.72 |
| 5 – 7 | 51 (63.75) | 5.56 |
| 8 or more | 18 (22.50) | 9.77 |
| Total | 80 (100.00) | 6.26 |

D. Distribution of households depending upon level of educational of the household head

| Level of education of the head of the household | Respondents |
|---|---|
| A. Illiterate | 19 (23.75) |
| B. Literates | 61 (76.25) |
| i. Primary | 34 (42.50) |
| ii. Secondary | 11 (13.75) |
| iii. High Secondary | 10 (12.50) |
| Graduate | 6 (7.50) |
| Total | 80 (100.00) |

E. Distribution of average number of animals per household

| Category | Average |
|---|---|
| Total milch animal | 4.38 (100.00) |
| a. Local cow | 0.83 (18.86) |
| b. Cross bred | 0.71 (16.28) |
| c. Buffalo | 2.84 (64.86) |
| Total SAUs (milch) | 5.51 |
| Calves & heifer | 2.90 |
| Dry animal | 1.47 |
| Total animal | 8.75 |

Note: Figures in Parentheses indicate percentage figures

animals equivalent to a local cow indicated the presence of average 5.51 SAUs per household in the study area. Further, calves & heifer per family were 2.90, while on an average a family owned 1.47 dry animals.

**Factors affecting farmer's choice of a specific dairy outlet**

The choice of a milk-marketing channel can be either supplier or producer-driven (Vandeplas et al. 2013). It depends on a variety of factors and different milk-marketing outlets, as well as a number of social and economic factors. In this study, dairy farmers were observed to make a choice amongst three milk-marketing outlets for the disposal of their milk marketable surplus. These three outlets were: (1) Collection Centre, (2) Direct to Consumers, and (3) Milk vendor. All these milk-marketing outlets can be considered independent from each other and cannot be ordered in any logical way therefore, a multinomial logit (MNL) model was used to identify the factors affecting farmer's decision of choosing a particular milk-marketing outlet. The maximum likelihood of independent factors to influence farmers' choice of specific dairy outlet was estimated taking "direct to consumer" as base outlet category. Table6 presents the results of multinomial logistic regression.

An examination of table indicates that the estimated model was significant at 1% level, and demonstrated a good predictive capability as indicated by a pseudo-$R^2$ value of 0.57.

Among physical capital, the coefficient of herd size was found negative and significant for both the category of outlets *viz.,* collection center and milk vender, which points that as the herd-size increases, farmers will be more likely to sell the milk directly to the consumer. The marginal effects figure indicates that one per cent increase in herd size will decrease the probability of selling milk to collection center by 0.19 per cent and to milk vender by 02 per cent. It is in contrast to our expected hypothesis, as it was expected that larger herd size will translate into larger milk marketable surplus, which will be disposed off through organized marketing channel. Some studies suggest that herd size is a significant determinant in market channel participation for modern market channels (Tsougiannis et al. 2008 and Mutura et al. 2015 and Brar et al. 2018). The reverse situation in the study area may be due to the less productivity of milch animals, connoting milk production might not have been proportionately increased with the herd size. Kuma et al. (2013) also observed that number of milking cows owned by households negatively affected the farmer's choice of accessing cooperative milk market outlet.

The coefficient of marketable surplus was positive and significant at 1 per cent level of significance. The corresponding marginal effect values indicate that one per cent increase in the marketable surplus increased the probability of selling milk at collection center and to milk vender by 0.72 per cent and 1.89 per cent, respectively. Meena and Tiwari (2015) also endorsed the positive relationship of marketable surplus with farmer's choice of selling milk to milk and co-operatives.

The table further indicates that the negative and highly significant (at 1%) coefficient of education for the farmers who were educated to senior secondary level. They did not prefer to sell milk to milk venders, rather favoured selling it direct to the consumers, the results confirm this study's postulation. The findings are consistent with the fact that education levels considerably affect market information interpretation and hence, market participation levels of farmers by helping them analyze and exploit the best marketing strategies at their disposal (Jari, 2009; Park, 2009; Moturi et. al.2015).

The caste coefficient representing demographic characteristics of milk farmers showed that farmers belonging to ST category preferred selling directly to the consumers instead of going to collection center or milk vender. On the other hand, farmers belonging to SC category preferred selling milk to milk venders. Sarkar (2020), who conducted study with total of 35,200 agricultural households all over India using NSSO data observed that SC households lacked access to better marketing facilities for the disposition of milk. Only around 17 per cent of the agricultural households of the SC community could sell milk to a cooperative and government agency. Even SC households received a lower average price per litre of milk than all other social groups, which was further corroborated by Ahuja and Redmond (2004). Thorat (2009), based on the Action Aid study in 2001 covering 550 villages across 11 states in India observed exclusionary practices in the consumer markets particularly prominent in the case of milk and vegetables. In about 47 per cent of study villages, SCs were not allowed to sell milk to the

**Table 6:** Factor affecting farmer's choice of a specific dairy outlet

| Base category – Consumer (2) | | | | | | |
|---|---|---|---|---|---|---|
| | Collection Centre (1) | | | Milk Vendor (3) | | |
| Variables | Coefficient | Std. Error | Marginal effects dy/dx | Coefficient | Std. Error | Marginal effects dy/dx |
| i) Physical capital | | | | | | |
| Herd size (log) (no.) | -24.468** | 11.111 | -0.199 | -44.474** | 14.261 | -2.001 |
| Marketable surplus (log) (lt./day/hh) | 37.583* | 10.606 | 0.716 | 54.622* | 12.108 | 1.895 |
| ii) Human capital | | | | | | |
| Education (nominal) | | | | | | |
| (1) Primary | 1.482 | 1.914 | 0.111 | .9198 | 2.222 | 0.023 |
| (2) Secondary | 0.356 | 2.007 | 0.211 | -3.124 | 2.940 | -0.222 |
| (3) Sen. Secondary | 1.845 | 1.537 | 0.439 | -17.845* | 3.355 | -0.362 |
| (4) Intermediate | 0.666 | 1.912 | 0.319 | -5.145 | 3.833 | -0.319 |
| iii) Demographic characteristics | | | | | | |
| Age of Household (log) (yr.) | 3.996 | 8.343 | 2.324 | -24.293 | 18.251 | -2.390 |
| Gender (1=male, 0=female) | 0.951 | 1.909 | 0.162 | -0.548 | 2.008 | -0.118 |
| Caste (nominal) | | | | | | |
| (1) OBC | 1.321 | 1.512 | 0.145 | .227 | 1.815 | -0.080 |
| (2) SC | 1.811 | 2.843 | 0.156 | 5.401*** | 3.122 | 0.284 |
| (3) ST | -7.313 ** | 3.635 | -0.266 | -7.074*** | 3.820 | -0.078 |
| iv) Institutional support | | | | | | |
| Institutional credit (1=yes, 0=no) | 5.981 ** | 2.740 | 0.154 | 8.152* | 3.096 | 0.255 |
| MGNREGA (1=yes, 0=no) | 1.403 | 1.820 | 0.381 | -2.708 | 2.513 | -0.338 |
| BPL (1=yes, 0=no) | -2.741 | 1.894 | -0.034 | -4.225*** | 2.223 | -0.159 |
| Village Location (1=urban, 0= rural) | 1.277 | 1.554 | 0.119 | .587 | 1.686 | -0.044 |
| v) Economic factors | | | | | | |
| Principal occupation livestock (1=yes, 0=no) | 0.722 | 1.459 | 0.215 | 4.124** | 2.108 | 0.301 |
| vi) Animal Characteristics | | | | | | |
| Livestock Age (log) (yr.) | -3.017 | 7.019 | -0.020 | -7.846 | 7.291 | -0.450 |
| Animal Type (nominal) | | | | | | |
| (1) Buffalo | 2.586 | 2.427 | 0.325 | -0.577 | 2.997 | -0.215 |
| (2) Crossbred & Buffalo | 3.029 | 1.986 | 0.311 | 0.403 | 2.620 | -0.166 |
| (3) Cow, CB & Buffalo | 16.632 * | 3.729 | 0.356 | 15.544 | 4.407 | 0.007 |

Pseudo $R^2 = 0.5760$
Prob> $chi^2 = 0.0000$

* = Significant at 1% level of significance
** = Significant at 5% level of significance
*** = Significant at 10% level of significance

village cooperative and to private buyers. Singhal *et el.* 2020 also observed that most of the lower caste households sold milk to the informal channel while organized channel was dominated by general caste households in Punjab. Our results are in consonance with the finding of these studies.

Among the factors under institutional support, access to institutional credit was significant at 5 per cent level of significance for the collection center outlet category and at 1 per cent level of significance under milk vender category. The positive sign in each category shows that if the institutional access is available, farmers will prefer to sell milk in these outlets rather than selling it directly to the consumers. The corresponding marginal effect value shows that if access to institutional credit is improved by 1 per cent, the famer's probability of selling milk in collection center and to milk vender will increase by 0.15 per cent and 0.25 per cent, respectively. This meant that if a farmer had institutional support, then knowledge transmission amongst fellow farmers make them confident in opting suitable marketing channels. This corroborates with findings by Mburu et al. (2007), where group membership was taken as a proxy for social capital and had a positive effect toward farmer participation in the cooperative channel. Table also revealed that farmers belonging to BPL category, preferred to dispose-off the marketable surplus of milk directly to the consumers rather than selling it to the milk venders. The reason of choosing direct channel may be less marketable surplus availability as most of the milk produced might have been utilized for family requirements.

Furthermore, the farmers whose primary occupation was livestock, they favoured selling milk to milk venders over selling it directly to the consumers. The possible reason may be availability of higher milk surplus due to more focus on livestock enterprise, which in turn would have made it difficult to directly sell milk to consumers. Among the animal characteristics, farmers who owned all the types of animals *viz.,* cows, crossbreds and buffaloes preferred to sell milk in the collection center rather than selling it directly to the consumers. This may be due to unavailability of specific customers for separate kind of milk, which might have prompted producer to mix all kind of milk and dispose it at collection center. The value of marginal effects indicates one per cent increase in such unit, led to 0.36 per cent increase in the probability of selling milk in the collection center.

## Conclusion

It can be concluded that, that herd size, marketable surplus, caste categories, access to institutional credit, BPL economic class, income from livestock and breed type of animals were seven significant factors affecting farmer's decision of choosing a particular milk-marketing outlet out of three. Although direct marketing channels highly efficient and fetch more prices to producers, still most of the farmer choose channel III for marketing of milk, may be because of easy disposal of marketable surplus. This trend might continue; therefore, farmers should be made aware about quality parameters like fat percentage in the milk, or even provided with affordable fat testing kits through *Pashu Vigyan Kendras/* Extension centres like KVKs, so that farmers can test it at their level and be assured about the prices they receive in the collection centres. There is need for the effective and improved spread of modern market outlets and dairy cooperatives so that all the farmers irrespective of social and economic background may benefit from livestock farming.

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RESEARCH ARTICLE

# Impact of climate services on the operational decision of Murrah buffalo farmers in Haryana

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**Abstract:** Global warming and its concomitant changes in mean climate variables and climate variability have an impact on animal feed and fodder, animal health, production, and water availability. Buffaloes are the mainstay of the Indian dairy economy and the backbone of the rural economy and dairy industry in the Haryana state in particular. Murrah buffalo-based production system has to be imparted the ability to withstand the adversities associated with climate change as well as to maintain their productivity. Therefore, the present study was undertaken to develop climate services and analyze their impact on Murrah buffalo farmers' operational decision-making related to dairy farming. The study was conducted in the Hisar, Jind, and Rohtak districts of Haryana state. Two blocks were selected randomly from each district and from each block three experimental villages and one control village were selected, resulting in 18 experimental and 6 control villages in total. The three experimental villages of each block were randomly assigned to the intervention mode of either WhatsApp, Text SMS, and Mobile application which was exclusively developed for the present study thus resulting in 6 villages each receiving treatment through WhatsApp, Text SMS, and Mobile application. From each village, 15 farmers were selected randomly and provided with treatment i.e., weekly THI-based Murrah buffalo climate service module. The findings of the study revealed a positive treatment effect of the climate services on various practices like the adoption of improved varieties of fodder, and nutrition management through the inclusion of oilcake, miner mixture in animal diets. The adoption of rubber mats, providing chopped fodder, use of bedding materials and covering open spaces of the animal shed during winter, the practice of deworming the herd and maintenance of cattle shed hygiene, and others. Hence, the climate services for Murrah buffalo farmers were found to be a potential adaptation tool to enhance the resilience capacity of vulnerable dairy farmers to adapt to climate change.

**Keywords:** Climate change, Climate services, Weather, Impact, THI, Murrah buffalo

## Introduction

Livestock production systems all across the world are being directly impacted by the phenomenon of global climate change. The detrimental consequences of global warming affecting both productive and reproductive performance (Upadhyay et al. 2007) is due to the combination of genetic factors of the animal and climatic factors affecting livestock such as temperature, relative humidity, solar radiation, precipitation, and wind speed (Hahn et al. 2003). These changes will significantly influence livestock production due to reduced feed intake, milk production and productivity, livestock diseases, conception rates, animal growth, water availability, and feed and fodder production and availability (Rojas et al. 2017). The negative impact of temperature rise on total milk production for India has been estimated about more than 15 MT by 2050 (Upadhyaya et al. 2013). High heat load in lactating buffaloes reduces their milk production and shortens the duration of lactation length (Upadhyay et al. 2007). As a result, dairy farming in India is highly vulnerable to weather and climate risks, and advanced adaption strategies such as weather forecasting and forecast-based climate services assist in minimizing losses while sustaining production through suitable weather-related livestock management practices (Vashisth et al. 2013). Various amelioration strategies to adverse impacts of climate change on dairy animals include providing sprinklers aided with a fan under the shade during hot dry summers (Ahmad et al. 2019), wallowing which is highly efficient in reducing heat stress (Aggarwal and Singh 2008), loose housing with a shade (may be shed) and open area for night hours (Aggarwal and Singh, 2008), regular showers in addition to wallowing facilities during summer (Mishra 2021, Roy et al. 1968), nutritional

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adjustment strategies through balanced feeding, concentrates, etc (Pankaj et al. 2013).

Using weather information, agricultural producers may be able to make better decisions whose outcomes are influenced by the weather vagaries. In contrast to crop farmers (60%), only a small fraction of livestock farmers (32%), reported using weather data in their farming activities. For decisions regarding the movement of livestock, ranchers increasingly used weather forecasting data (Frisvold and Murugesan 2013). The digital iCow advisory services have quipped dairy farmers with basic, timely knowledge and solutions that improved their production. The advisories on health and disease, deworming, managing fodder, optimum feeding, reproduction management, and other dairy management practices have assisted dairy farmers to realize higher outputs and consequently improve their incomes (Marwa et al. 2020). Weather-based advisories had a positive treatment effect on operational decisions of dairy management, such as watering management, feeding management, shelter management, and vaccination scheduling (Manjusree et al. 2022).

Haryana is having largest Murrah buffalo density among states, with a population of 5.1 million Murrah buffalo out of a total 6.6 million, accounting for 77% of the total buffalo population (Breed survey 2013). Central Haryana is considered as the breeding tract of Murrah buffalo (Parmar and Sangwan 2016). This makes Murrah buffaloes the backbone of the state's rural economy and dairy industry, which contributes to more than 75% of the state's milk production (Balhara et al. 2017).

Haryana has a semi-arid, subtropical climate with scarce, excessive and untimely rains, heat waves, cold waves and hot winds during summer, dust storms, fog, frost, and hails, all of which have a negative impact on crop and livestock production (Singh et al. 2008). Extremely hot summers and very cold winters are common in Haryana. While summer's mean temperature ranges from 48 to 35 degrees Celsius, the winter's average ranges from 3 to 9 degrees Celsius. Climate projections for the state is quite alarming with both Mean maximum and Minimum temperature projected to increase by $1.3^0$C and $2.1^0$C towards mid-century, respectively and mean annual rainfall is projected to decrease marginally by about 63 mm (3%) by 2050s (HSAPCC 2011). Out of 22 districts in Haryana, 15 districts are in the range of medium to high vulnerability towards climate change (Rao et al. 2016).

Given the significance of Haryana's Murrah buffalo cropping system and the fact that it is very sensitive to climate change, timely and reliable climate services are essential for managing day to day livestock operations and minimising losses (Rathore and Chattopadhyay 2016) thereby building the resilience of the buffalo-based farming system to the changing climatic conditions. Thus, the present study was formulated to develop THI based climate services for the Murrah buffalo farmers of Haryana and to assess its impact on the operational decision making.

## Materials and Methods

### Sampling

The study was purposively conducted in the Hisar, Jind and Rohtak districts of central Haryana which is considered as breeding tract of Murrah buffalo having higher concentration of Murrah buffalo (Parmar and Sangwan 2016). Two blocks from each district thus 6 blocks in total were selected randomly i.e. Agroha and Barwala block from Hisar district, Pillukhera and Safindo block from Jind district and Meham and Rohtak block of Rohtak district. Four villages from each block were then randomly chosen, three of which were experimental villages which were randomly administered treatment through WhatsApp, text message, or a mobile application, and one of which was a control village. Thus, the study covered a total of 24 villages resulting in 18 experimental villages (6 each WhatsApp, Text SMS and Mobile Application) and 6 control villages. Finally, farmers who had been rearing Murrah buffalo for the last 10 years and had a minimum herd size of 4 Murrah buffalo and 15 such farmers from each village were randomly selected as respondents. Hence, the total sample size of the present study was 360. Farmers in experimental villages were provided with treatment i.e., a weekly module on the climate information and THI-based advisories on Murrah buffalo rearing. The experimental and control group has undergone a pre-test as well as a post-test before and after the treatment was administered. The collected primary and secondary data from the study area was tabulated and statistically analyzed using statistical tools, like mean, frequency, standard deviation, range, cumulative square root frequency method, regression coefficient, etc. to arrive at a conclusion.

## Results and Discussion

It is apparent from the Table1 that half of the respondents were middle aged farmers having medium farming experience of 18-32 years and around one third of them owing a land holding size of 2-4 ha. It is also clear that majority of the farmers were in medium to high knowledge level categories regarding climate change, its impacts and adoption practices related with concerned system. Most of respondents perceived weather based advisory services as highly useful in their farming activities. Nearly half of the respondents had possessed small herd size and had an annual income of 5.86-9.17 lakhs.

### Herd size and production profile of the dairy animal

Table 2 depicts the number of animals in milk and dry animals as well as heifer and calves maintained in the herd among all three dairy animal types. It is also observed from the same table 3 that the average productivity of buffalo was 8.20 liters and 9.73 liters per day during summer and winter, respectively. Results also show that the productivity of crossbred cattle was 13.34 liters and 15.29 liters per day and indigenous cattle of the region had productivity of only 3.64 liters/day and 4.11 liters/day during

summer and winter, respectively. All three species of dairy animal reach their peak yield after 2-4 weeks, with a lactation length of 282 days in buffalo, 254 days in indigenous cattle, and 291.25 days in crossbred cattle.

**Impact of climate services on feed and fodder management**

**A. Using improved/multicut varieties of fodder crops**

Results from Table 4 show that there was a considerable increase in the number of farmers who have adopted the improved multicut varieties of fodder crops as a result of climate services in all three modes of intervention i.e., Text SMS, WhatsApp, and Mobile App. Dairy is the major contributor to the livelihood of farmers in the region and most of them were stall feeding with almost no grazing, demand for a continuous regular supply of green fodder might be a reason behind the already significant majority of farmers using these improved varieties. Ghosh et al. (2016) in their study have also stressed that the development of improved varieties of perennial grasses, fodder crops and legumes and fodder trees has a role to address the fodder issues like supply-demand gap, silage preparation and etc.

In order to increase fodder productivity and meet fodder demand, Singh et al. (2022) advocated raising awareness about the necessity of using high quality seed of improved fodder varieties and increasing the seed replacement rate from the current 2%-3% to at least 10%.

**B. Use of Oil cakes in the animal feed**

It's obvious from the results of the Table 4, that the climate services had a positive treatment effect on the use of oil seed cakes on the animals in terms of an increase in the number of farmers adopting the practice. Oilseed cakes due to their rich protein content, they are used as animal feed, especially for ruminants and fish (Ramachandran et al. 2007), they are highly

**Table 1:** Socio-economic profile of the respondents (n=360)

| Variable | Categories | % of farmers |
|---|---|---|
| Age | Young (<35 years) | 20.83 |
| | Middle (35-55 years) | 48.06 |
| | Elder (>55 years) | 31.11 |
| Farming experience | 10-18 years | 22.50 |
| | 18-32 years | 44.44 |
| | 32-50 years | 33.06 |
| Operational land holding | < 1 ha | 20.55 |
| | 1-2 ha | 27.50 |
| | 2-4 ha | 32.80 |
| | 4-10 ha | 18.05 |
| | >10 ha | 1.11 |
| Annual income | 2-5.85 lakhs | 37.50 |
| | 5.86-9.17 lakhs | 48.33 |
| | 9.18-19 lakhs | 14.17 |
| Knowledge on climate change & its impact on livestock (Range: 6-20) | Low (6-11.23) | 26.39 |
| | Medium (11.24-14.18) | 39.44 |
| | High (14.19-20) | 34.17 |
| Perception regarding weather based advisory services (Range: 47-77) | Least useful (47-60.01) | 23.89 |
| | Moderately useful (60.02-66.87) | 34.72 |
| | Highly useful (66.88-77) | 41.39 |
| Herd size (Standard Animal Unit) | Small (3.9-7.84) | 47.22 |
| | Medium (7.85-12.06) | 36.67 |
| | Large (12.07-35.95) | 16.11 |

**Table 2:** Average household holding of different types of dairy animals (n=360)

| Category | Buffalo (n=360) | Indigenous cattle (n=86) | Cross-bred cattle (n=74) |
|---|---|---|---|
| In Milk | 4.37 | 1.27 | 1.59 |
| Dry | 1.30 | 0.37 | 0.41 |
| Heifer | 0.88 | 0.50 | 0.54 |
| Calves | 1.11 | 0.47 | 0.54 |
| Total | 7.66 | 2.62 | 3.10 |

nutritive and make a significant contribution to the energy content (Rakita et al. 2023) of the animal diet as part of a balanced ration and help in maintaining milk production. Mustard, cotton, groundnut, and soybean were the most commonly used oil cakes, which were fed to the animals twice a day, in the morning and late at night.

**C. Use of mineral mixture to maintain productivity and health**

In terms of an increase in farmers adopting the use of mineral mixture, climate services had a positive treatment impact (Table 4) in all three modes of intervention i.e., text SMS, WhatsApp, and Mobile App. An increase in milk production and a significant difference in first postpartum estrus and conception rate were observed in animals supplemented with the mineral mixture (Kumar et al. 2020). Cariappa et al. (2022) in their study have also reported that the Anionic mineral mixture prevents milk fever and improves farmer income.

**D. Providing chopped fodder to avoid wastage**

Table 4 depicts the slight increase in the adoption of the practice of providing chopped fodder. The majority of the respondents were already using the chopped fodder for their animals for better digestion, to avoid fodder wastage also the chopping of fodder enables the better mixing of different feed and fodders like dry and green fodder, wheat husk, paddy husk, etc. Manohar et al. (2014) from their study have found that, all the respondents in the study region used to chop dry fodder before feeding while 70 per cent of respondent chopped green fodder. Abilzhanuly (2019) in his study found that, feeding cattle chopped hay results in a weight gain of 35% when compared to feeding cattle non chopped hay.

**Impact of climate services on the health, hygiene and housing practices**

**A. Calcium supplementation**

Results from Table 5 depict the positive treatment effect of climate services on the use of calcium supplementation in the number of farmers adopting the use of calcium supplementation. A study by KVK Jabalpur (MP) has found that balance feeding with feed

**Table 3:** Production profile of the livestock in the sampled households (n=360)

| Productive Parameters | Buffalo (n=360) | Indigenous cattle (n=86) | Cross-bred cattle (n=74) |
|---|---|---|---|
| | Mean±SD | | |
| Milk yield in Summer (lit/day) | 8.20±1.31 | 3.64±1.13 | 13.34±1.29 |
| Milk yield in Winter (lit/day) | 9.73±1.48 | 4.11±1.28 | 15.29±1.32 |
| Lactation Length (Days) | 282.44±13.15 | 254.65±11.31 | 291.25±7.18 |
| Peak Yield (Kg) | 15.09±1.37 | 5.74±1.58 | 18.00±1.33 |
| Dry period (days) | 146.15±14.15 | 155.89 ± 3.18 | 101.05 ± 4.76 |

**Table 4:** Impact of climate services on feed and fodder management (n=360)

| | Improved fodder varieties | | Oil seedcakes | | mineral mixture | | chopped fodder | |
|---|---|---|---|---|---|---|---|---|
| Treatment group | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Control (n=90) | 77.78 | 80.00 | 86.66 | 80.00 | 12.22 | 8.88 | 90.00 | 90.00 |
| Text SMS (n=90) | 81.11 | 88.88 | 83.33 | 93.33 | 14.44 | 24.44 | 93.33 | 96.66 |
| WhatsApp (n=90) | 76.66 | 85.55 | 76.66 | 87.77 | 10.00 | 21.11 | 94.44 | 97.77 |
| Mobile App (n=90) | 82.22 | 95.50 | 77.77 | 92.22 | 16.66 | 30.00 | 88.88 | 94.44 |

**Table 5:** Impact of climate services on animal health, hygiene and housing practices (n=360)

| | Calcium supplementation | | Deworming | | Use of rubber mats | | Shed hygiene | | Mosquito nets | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment group | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Control (n=90) | 42.22 | 40.00 | 36.66 | 38.88 | 64.44 | 65.55 | 73.33 | 68.88 | 70.00 | 71.11 |
| Text SMS (n=90) | 44.44 | 47.77 | 47.77 | 57.77 | 58.88 | 63.33 | 75.55 | 87.77 | 75.55 | 81.11 |
| WhatsApp (n=90) | 33.33 | 38.88 | 41.11 | 55.55 | 60.00 | 67.77 | 67.77 | 84.44 | 68.88 | 75.55 |
| Mobile App (n=90) | 38.88 | 42.22 | 44.44 | 61.11 | 63.33 | 72.22 | 77.77 | 91.11 | 62.22 | 68.88 |

supplements like mineral mixture @ 50g and calcium supplementation @100ml per day per animal during last two months of pregnancy in buffaloes has resulted in reduction of post-partum problems by 40%, increased in milk yield by 25.80% and 40.31% increase in net returns (Annual Report 2015-16 ICAR-ATARI, Jabalpur). In dairy cows, low blood calcium levels after calving can be troublesome, especially for older cows. Acidogenic salts in the diet prior to calving and oral calcium supplements after calving reduce postpartum health and production-related problems (Vagnoni et al. 2021).

**B. Deworming of animals at optimum intervals**

Results from Table 5 reveal that there was a positive treatment effect of the climate services on the number of farmers adopting the practice of deworming of their animals. Findings of Thapa Shrestha *et al.* (2020) have reported that milk production in cows and buffaloes increased steadily in the first month after administering the deworming. The study revealed the importance of deworming and management practises in controlling the prevalence of parasitic diseases. And it also recommended that, in order to achieve the intended goals in deworming activities, sensible use of anthelminthic medications, and effective farm management, periodic monitoring of the incidence of Gastrointestinal parasites among farm animals is required (Gunathilaka et al. 2018).

**C. Use of rubber mats for animals**

Climate services had a positive treatment effect on the adaption of rubber mats for animals as revealed in Table 5. The gap still existing might be due to the cost element involved in the purchase of cow mats specially for small and marginal farmers. Use of cow mats provides a non-slippery surface, reducing injuries to their feet and knees, and are easy to clean and disinfect thereby reducing chances of infection or udder diseases. On the concrete floor, the average minimum slippage amounted to 4.4 occurrences, whereas the rubber mat floor saw only 2.6 instances. Housing cows on the rubber mat floor resulted in a notable 30.4% boost in milk production when compared to the concrete floor, primarily due to the increased comfort it offered (Jain et al. 2013).

**D. Proper disposal of dung, urine, drainage facility, and hygiene maintenance in the animal shed**

Results from Table 5 show that there was a positive treatment effect of the climate services on the adaption of the practice of "proper disposal of dung, urine, drainage facility, and hygiene maintenance in the animal shed, etc." Since the practice is simple and doesn't involve any extra cost, at the same time shed hygiene has a positive effect on animal health by controlling disease-causing pathogens and their vectors, so the practice was adapted by the farmers. A study by Rathod et al. (2017) has disclosed that the incidence of subclinical mastitis in dairy animals was more in case of the animal sheds that were less hygienic, which ultimately affects the milk yield and economic returns, highlighting proper shed hygiene's underlying contribution to animals health and production.

**E. Use of mosquito nets around the shelter to prevent flies, mosquitoes, and other vectors**

Results from Table 5 display that there was a positive treatment effect of the climate services on the adaption of mosquito nets around the shelter to prevent flies, mosquitoes, and other vectors. Since there was a high mosquito and flies problem in the region and almost two-thirds of them were using mosquito nets, The lack of an appropriate shed to install the net and the cost were cited as constraints, while few claimed that alternative methods, such as fogging and the use of mosquito coils and liquid, were sufficient for control. Haque et al. (2021) in their study have reported that, despite the fact that mosquito nets help prevent mosquitoes, flies etc which are the vectors of many diseases including lumpy skin disease, most farmers (91.17 %) did not use one in their cattle barn at night.

**Conclusion**

The changing climatic conditions pose a serious threat to dairy animals in general and Murrah buffaloes in particular, which are highly sensitive to heat stress. Reliable climate information and related advisory services recommending timely weather-related management practices can make dairy farming climate resilient. The exclusive climate services developed for the Murrah had a positive treatment effect on all the operational decision-making of the herd management and hence should be given utmost priority in making available these services to farmers on a regular basis. Climate Services which link the climatic information with available climate resilient dairy farming practices is an important adaptation strategy assisting vulnerable dairy farming populations in coping with the climate of today and of the future. Extension agents' role is imperative in creating climate literacy among farmers, convincing them of the importance of these climate services, interpreting these scientific advisories, and further assistance at all stages of implementation for enhanced uptake and utilization of climate services.

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SHORT COMMUNICATION

# Standardisation and quality evaluation of betel leaf based yoghurt

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**Abstract:** Yoghurts are those milk products which are fermented and have a good impact on the human health. Incorporation of herbs or medicinal plants into the yoghurt, can improve the variety as well as the nutritional and therapeutic benefit of the yoghurt. The present study aimed at developing yoghurt incorporated with betel leaf extract and was successful. The incorporation of 15% betel leaf extract had better organoleptic qualities when compared to the other treatments. The selected yoghurt was stored for 15 days in refrigerated condition and was subjected to organoleptic and nutritional evaluation. The organoleptic scores gradually decreased during storage. Physico-chemical parameters such as moisture, water holding capacity, and syneresis were significantly decreased as the storage period increased. The nutritional components such as energy and carbohydrate were also significantly different as the storage period was extended. Further studies have to be carried out to understand the shelf stability and medicinal properties of the prepared yoghurt.

**Keywords:** Yoghurt, Betel leaf, Medicinal plants, Herbs

Yoghurt has a good impact on human health because they include a variety of bioactive proteins, hydrolyzed carbohydrates, vitamins, and minerals with enhanced bioavailability. *Streptococcus salivarius* subsp. *thermophilus* (SST) and *Lactobacillus delbrueckii* strain. *bulgaricus* (LDB) are used in cooperation to develop yoghurt (Deshwal et al. 2021). Nutritional value and therapeutic potential of yoghurt can both be enhanced by including herbs or medicinal plants such as betel leaves. Betel leaves (*Piper betle L.)* are rich sources of flavonoids, terpenoids, tannins, alkaloids and many bioactive compounds which make it a suitable choice for several therapeutic preparations (Chauhan et al. 2016). In line with the rising demand for such herbal foods, the present research is being done to develop betel leaves based yoghurt.

Betel leaf based yoghurt was prepared by adding the fresh leaf juice to a mixture of preheated milk, skimmed milk powder (1%) and sugar (8%). The mixture was pasteurised, cooled to 55°C and yoghurt culture (2%) was added. The yoghurt was incubated at 42°C for 8 hours and then refrigerated at 4°C. Various treatments were used to standardise the percentage incorporation of betel leaf by modifying the milk and juice ratio. A best treatment was selected through organoleptic evaluation and the selected treatment underwent further evaluations.

Organoleptic evaluations preferred plain yoghurt to betel leaf yoghurts, although the most palatable betel leaf yoghurt was produced by combining 15% betel leaf extract with 85% homogenized milk. Even though, the organoleptic scores decreased during storage, the yoghurt was still acceptable till 15 days. According to a study by Mazumder (2019), dahi was well-accepted when betel leaf extract was added at a rate of 2%. The physic-chemical and nutritional constituents of the prepared yoghurt were studied initially and at five days intervals. Table 1 details the physic-chemical and nutritional constituents recorded during the storage period. The initial moisture content of the betel leaf yoghurt was 76.42%, and it gradually increased during storage. Betel leaf dahi (Mazumder, 2019) had a moisture content that was higher than that of the current study (81.56%). Similar to betel leaf dahi, acidity of the yoghurt in the present also rose from 0.68% to 0.89% during storage. The phenolic compounds in the betel leaf may have prevented the development of acidity

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**Table 1:** Physico-chemical and nutritional constituents of the betel leaf based yoghurt during storage

| Components | Day 1 | Day 5 | Day 10 | Day 15 | C.D. Value |
|---|---|---|---|---|---|
| Moisture (%) | 76.42[c] | 77.02[bc] | 78.54[ab] | 79.14[a] | 1.809 |
| Acidity (%) | 0.68 | 0.75 | 0.81 | 0.89 | NS |
| pH | 4.43 | 4.21 | 3.84 | 3.49 | NS |
| Water holding capacity (%) | 57.32[a] | 56.85[a] | 54.25[b] | 51.05[c] | 2.788 |
| Syneresis (%) | 1.00[b] | 1.70[ab] | 2.30[a] | 2.70[a] | 1.031 |
| Energy (Kcal) | 80.84a | 76.48b | 73.11c | 66.49d | 2.146 |
| Carbohydrate (g) | 7.97a | 7.52ab | 6.98b | 6.23c | 0.960 |
| Protein(g) | 9.54 | 9.35 | 9.16 | 8.93 | NS |
| Fat (g) | 1.20 | 1.00 | 0.95 | 0.65 | NS |
| Total sugar(g) | 9.27 | 9.14 | 8.68 | 8.21 | NS |
| Reducing sugar (g) | 5.17 | 5.10 | 5.03 | 4.95 | NS |
| Vitamin A (IU) | 2.10 | 1.98 | 1.87 | 1.74 | NS |
| Vitamin C (mg) | 0.89 | 0.88 | 0.86 | 0.83 | NS |

DMRT row wise comparison, NS – Non Significant

(Kriangkrai and Penkhae, 2009). The pH ranged from 4.43, which falls within the recommended pH range of 4.6 for yoghurt. Sugar fermentation and lactic acid generation by microbial activities may be the cause for the pH decrease. Starting at 57.32%, water holding capacity (WHC) decreased to 51.05% on day 15, indicating a less robust gel network. The consistency and hardness of dahi may be affected by betel leaf extract. Syneresis in betel leaf yoghurt ranged from 1% to 2.70%. Syneresis, which happens when the gel network loses its ability to maintain the serum phase, results in whey separation during yoghurt storage and can affect customer acceptability Joon et al. (2017).

Essential nutrients like carbohydrates, proteins, lipids, vitamin A, and vitamin C was enhanced by the addition of betel leaf extract to yoghurt. By the fifteenth day of storage, the amounts of reducing sugar and total sugar had fallen from 5.17g and 9.27g to 4.95g and 8.21g, respectively. 7.97g/100g of carbohydrates were present. While in storage, the protein content drastically dropped but stayed at 9.54g/100g. Due to the enzymatic activity of lipase and lipoxidase produced by the microorganisms, the fat content gradually decreased (Mao et al. 2022). The amount of vitamin A ranged from 2.10IU to 1.74IU, and the amount of vitamin C increased with the addition of betel leaves, falling from 0.89 mg to 0.83mg over the course of storage.

## Conclusion

The study aimed at developing a betel leaf based yoghurt, wherein the incorporation of 15 per cent of betel leaf extract was found to have better organoleptic qualities compared to other treatments. As the storage period increased, there were decline in the sensory qualities and also variations in the nutritional constituents. The study should be further continued to understand the shelf stability of the yoghurt. Also the evaluation of the medicinal properties of the prepared yoghurt can thus make us understand its therapeutic properties. It can thus be understood that betel leaf incorporation can bring a variety to the yoghurt flavour, however, further studies can help to reveal its nutritional and medicinal properties

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SHORT COMMUNICATION

# Effect of inulin addition on the sensory attributes of dairy beverage (*Rab*)

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**Abstract:** Being a traditional part of Rajasthani cuisine, *Rab* is widely consumed and relished for its taste. Inulin (prebiotic) has been proven beneficial for gut health. The addition of inulin in *Rab* results in a superfood; containing characteristics of traditional as well as functional beverages. The investigation was performed to prepare and standardize pearl millet (PM) and horse gram dal (HGD) *Rab* with the inulin. The inulin was added at 0%, 1%, 3%, and 5% rates and let ferment for 6-7h, at 35$^0$C. All the fermented treatments were stored at 4$^0$C. All treatments were evaluated for sensory characteristics. All treatments were tested for sensory characteristics including color, consistency, taste and flavor, mouthfeel, aftertaste, and overall acceptability on the Nine-point Hedonic scale and obtained scores above 7 points. It was concluded that the inclusion of inulin significantly improved the sensory characteristics of the functional beverage.

**Keywords**: Functional *Rab, Rabadi* inulin, Prebiotic drink, Pearl millet beverage, Buttermilk

From ancient times, native people of dry and semi-arid areas of western India consumed a cereal and dairy-based beverage called *Rab*. It is made up of the region's staple cereal and buttermilk. Mostly, dry mint, salt, cumin, or even ghee is added as per their preferences. Pearl millet has a large content of phosphorus which is good for bone health. Pearl millet is high in iron and zinc and is a gluten-free grain, a great alternative food for patients with celiac disease (Satnakar and Kumar, 2020). Ramachandra et al. (2021) concluded that *Lactobacillus* sp. obtained from domestic *dahi* samples showed a probiotic nature and sensitivity against various antibiotics. Prebiotics are the fiber that stimulates the growth and metabolism of the human microbiota. Inulin and FOS are mainly used as natural sources of prebiotics. In a review article, Shams and Wadhawan (2021) mentioned that inulin improves the mouthfeel and texture of processed foods and can be used as an important functional ingredient in food processing. In 2022, an article published by Cuamatzin-García et al. stated consumption of fermented foods and beverages improves human health by positively working on immunity, gastrointestinal tract, metabolic disorders, lipid levels, and body fat accumulation. Fornelli et al. 2014 studied the effect of oligofructose and inulin on the sensory characteristics of symbiotic dairy beverages. The investigation mentioned that the addition of inulin and oligofructose did not adversely affect the overall acceptance and marketability of the beverages. Similarly, Moghadam et al. 2019 also stated that Inulin fortification improved yogurt's probiotic viability and textural and flavor characteristics. Therefore, in the present study, an attempt was made to prepare and study the sensory properties of an Inulin-fortified pearl-millet-based fermented beverage (*Rab*)

Buttermilk was procured from the local dairy. Good quality pearl millet (PM) flour, horse gram dal (HGD) flour, salt, and roasted cumin powder were procured from the local market. Inulin powder (brand name- Urban Platter) was procured from the online retailer (Amazon. in).

For the preparation of *Rab*, 80 g PM flour and 20 g HGD powder were cooked with 500 mL water for 15 minutes. The mixture was cooled and then 1500 mL of buttermilk (BM), 0.5% salt, and 0.4% RCP were added, mixed, and divided into 4 treatments. Inulin was added as 0% (controlled), 1% ($PMT_2$), 3% ($PMT_3$) and 5% ($PMT_4$). The mixture was blended with an electrical blender, sieved (18-mesh size strainer), and sat to ferment for 7 h. The final product was packed in pre-sterilized polypropylene cups (200 mL capacity) and stored at 4$^0$C for further analysis.

Sensory evaluation of samples was carried out under laboratory conditions by 30 semi-trained panel members who were scientists,

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**Table 1:** Sensory test result of inulin-incorporated pearl millet Rab

| Property | $PMT_1$ | $PMT_2$ | $PMT_3$ | $PMT_4$ | F-Ratio | p-value | Result |
|---|---|---|---|---|---|---|---|
| Color | 7.33 ± 0.48 | 8.17 ± 0.38 | 7.83 ± 0.38 | 8.00 ± 0.59 | 18.04 | 0.000 | *** |
| Consistency | 8.17 ± 0.70 | 7.83 ± 0.38 | 8.17 ± 0.38 | 8.00 ± 0.00 | 3.94 | 0.010 | * |
| Taste & Flavor | 7.00 ± 0.83 | 7.83 ± 0.38 | 7.17 ± 0.38 | 7.83 ± 0.38 | 20.57 | 0.000 | *** |
| Mouth Feel | 7.50 ± 0.97 | 8.17 ± 0.91 | 7.17 ± 0.38 | 7.00 ± 0.59 | 14.07 | 0.000 | *** |
| After Taste | 6.83 ± 0.70 | 7.83 ± 1.09 | 7.50 ± 0.97 | 7.33 ± 0.76 | 6.53 | 0.000 | *** |
| Overall Acceptability | 8.00 ± 0.59 | 7.83 ± 0.91 | 7.50 ± 0.51 | 7.83 ± 0.70 | 2.74 | 0.046 | * |

Mean +SD, n = 30
*** (P<0.001)
* (P<0.05)

and students of the College of Community and Applied Sciences, MPUAT, Udaipur. Each panelist was asked to taste the given samples and rate the sensory properties (Rangana, 2010) including color, consistency, flavor & taste, mouthfeel, after-taste, and overall acceptability; on a 9-point hedonic scale (Jones, Peryam, and Thurstone, 1995).

Mean values and standard deviation (SD) of triplicate determinations were calculated with the help of Microsoft Excel (Microsoft Office, 2010). All statistical analyses were conducted on SPSS 16 software. One-way analysis of variance was used to determine the existence of any differences among treatment means.

As shown in Table 1, sensory scores of PM- *Rab* (without inulin) for color, consistency, taste & flavor, mouthfeel, after-taste, and overall acceptability were 7.33 ± 0.48, 8.17 ± 0.70, 7.00 ± 0.83, 7.50 ± 0.97, 6.83 ± 0.70, and 8.00 ± 0.59; whereas $PMT_2$ obtained 8.17 ± 0.38, 7.83 ± 0.38, 7.83 ± 0.38, 8.17 ± 0.91, 7.83 ± 1.09, and 7.83 ± 0.91; respectively. For color, *Rab* with 3% inulin scored 7.83 ± 0.38 and Rab with 5% inulin scored 8.00 ± 0.59. Scores obtained for taste & flavor, mouthfeel, after-taste, and overall acceptability by $PMT_3$ were 7.17 ± 0.38, 7.17 ± 0.38, 7.50 ± 0.97, 7.50 ± 0.51; and for $PMT_4$ were 7.83 ± 0.38, 7.00 ± 0.59, 7.33 ± 0.76, and 7.83 ± 0.70. Differences among treatments for color, taste & flavor, mouthfeel, and after-taste properties were found to be significant ((P<0.001), whereas for consistency and overall acceptability, differences were found to be significant (P<0.05).

## Conclusion

Being a tropical country India has a variety of drinks to quench the thirst of people as per the weather conditions. The assortment of drinks is according to the different regions and the availability of raw ingredients including local spices, herbs, taste, and abundance of the main ingredient. One such traditional drink in Rajasthani culture is *Rab*, which combines cereal and buttermilk. The inclusion of prebiotics (inulin) resulted in the enhancement of the sensory properties of *Rab*. All developed treatments scored above 7 points on a 9-point hedonic scale concluding that all treatments were well-liked by the judges and can be introduced in the upcoming beverage market. It has proved to be an effective carrier to provide the required hydration and prebiotics.

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SHORT COMMUNICATION

# Influence of nano zinc supplementation on digestibilty and rumen fermentation parameters under in vitro conditions

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**Abstract:** In vitro experiments were conducted to find out the effects of supplementation of nano zinc (nZnO) @ 0, 1, 5, 10, 20 and 40 ppm to substrate on digestibility and rumen fermentation parameters. The substrate consisted of concentrate mixture and maize fodder in the ratio of 40:60. The results showed that supplementation of Zn in the form of ZnO nanoparticle at 10 and 20 ppm level increased gas production, digestibility, ME values, acetate production and cellulose digestion under ***in vitro*** conditions.

**Keywords**: Nano Zn supplementation, in vitro digestibility, rumen fermentation

Among different essential trace minerals, zinc (Zn) is very important for all forms of life due to its role in gene expression, replication and part of many enzymes (Suttle, 2010). Bonhomme (1990) suggested that Zn is bound to the cell surface of rumen bacteria. Thus, Zn might be affecting the adhesion of microbial cells to cellulose particles. Martinez and Church (1970) showed Zn increased *in vitro* cellulose digestion. Woods (1965) suggested that the Zn requirement of rumen microorganisms for optimum cellulose digestion was less than 1 ppm. Protozoal growth (*Entodinium* sp.) was stimulated at Zn level of 5-10 ppm. Therefore, protozoa incorporated Zn easily and were intolerant to high Zn level, however, Zn did not penetrate bacterial cell readily and reduced metabolic activity. Eryavuz and Dehority (2009) found that 50 ppm level of Zn supplementation reduced cellulose digestion at 24 hours. Gupta (2016) reported that the bioaccessibility of Zn was highest in mustard seed cake (58.94 ppm) followed by cotton seed cake (38.93 ppm) and wheat straw (16.67 ppm) under *in vitro* conditions. In vitro DM digestibilty (IVDMD), *in vitro* OM digestibility (IVOMD), gas volume, metabolizable energy (ME) and short chain fatty acids (SCFA) contents were higher ($P<0.01$) in Zn supplemented diets (Parshuramalu et al. 2013). Aliarabi (2006) reported that Zn supplementation upto 120 ppm, either inorganic or chelated form, did not show significant effect on rumen fermentation parameters, however, negative effect was seen on IVDMD and IVOMD at 160 ppm level. Though study on different forms and sources of Zn are available but very few studies have been conducted regarding effect of nano Zn supplementation on digestibilty and rumen fermentation parameters in ruminants.

Samples of concentrate mixture and maize fodder were collected from Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana. The samples were dried in hot air oven at 65°C for 2 days and a constant weight was attained. The dried samples were ground through 1 mm sieve using electrically operated Willey mill. The basal substrate was prepared using concentrate mixture (% parts: maize 36, groundnut cake 10, full fat soya 15, wheat bran 18, de-oiled rice bran 18, mineral mixture 2 common salt 1) and maize fodder in 40: 60 ratios on DM basis. The proximate principles (DM, OM, CP, EE and total ash) in feeds were determined (AOAC, 2005) while cell wall constituents (NDF and ADF) were analysed as per Van Soest et al. (1991). The Zn contents in feeds were estimated using atomic absorption spectrophotometer (ZEEnit-700P) at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana.

In vitro trials were conducted to estimate gas production (IVGP), true dry matter digestibility (TDMD), true organic matter digestibility (TOMD), microbial biomass production (MBP), pH,

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ammonia nitrogen ($NH_3$-N), individual volatile fatty acids (IVFAs) and cellulose digestion. The basal substrate used in this experiment consisted of dried ground maize fodder and concentrate mixture (% parts: maize 36, groundnut cake 10, full fat soya 15, wheat bran 18, de-oiled rice bran 18, mineral mixture 2 common salt 1) in the ratio of 60: 40. The substrate was supplemented with nano Zn (nZnO) @ 0, 1, 5, 10, 20 and 40 ppm in treatments $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$, respectively.

For in vitro studies, rumen liquor was collected from 3 adult male Murrah buffalos maintained to meet the nutrient requirement (ICAR, 2013) before morning feeding and watering into a pre-warmed thermos flask and brought to the laboratory. Total gas production (Menke and Steingass 1988), true DM and OM digestibility (TDMD and TOMD) were estimated (Van Soest et al. 1991). Metabolizable energy (ME) of feedstuff was calculated using the prediction equation of Menke and Steingass (1988). The pH of strained rumen liquor was estimated (HANNA Instruments, USA).

Microbial biomass production (MBP) was calculated using data of TDOM and net gas volume (Blummel et al. 1997; Blummel and Lebzien 2001). For estimation of $NH_3$-N, 5 mL of acidified supernatant was mixed with 10 mL of NaOH (1 N) and immediately steam distilled using KEL PLUS® - N analyzer (Pelican, India). The $NH_3$ evolved was collected in boric acid solution (20% w/v) having mixed indicator and titrated against N/100 $H_2SO_4$ (AOAC, 2005).

For analysis of individual fatty acids (IVFA), the *in vitro* rumen fermentation was arrested by chilling at 4°C and the syringe contents were then centrifuged at 3000 rpm for 10 min. A portion of 5 mL of supernatant was added to 1 mL of 25% metaphosphoric acid and kept overnight at 4ºC (Patra et al. 2006). The mixture was centrifuged at 3000 rpm for 15 min. and 2 mL of supernatant was taken and stored at -20ºC for VFA analysis. The individual VFA in the samples were determined using Gas Chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless-steel column packed with chromosorb 101 mesh 80-100 (length 1.5 m; o.d 3.175 mm; i.d. 2 mm). Analytical conditions for fractionation of VFA were as follows: Injection port temperature 210ºC, column temperature 180ºC and detector temperature 230ºC. The flow rate of the carrier gas $N_2$ was 40 mL/min). Individual volatile fatty acids (Acetate, propionate and butyrate) in the samples were determined on the basis of retention time and their concentration was calculated by comparing the retention time as well as the peak area of the standard after blank correction.

*In vitro* cellulose digestion was done using a basal purified cellulose medium contained the following ingredients per 100 mL:

1. 15 mL each of mineral solutions I and II (Bryant and Burkey, 1953)
2. 0.1 mL of 0.1g/100 mL resazurin solution
3. 25 mL of a 3 g/100 mL suspension of cellulose (Sigma, St. Louis, MO, USA)
4. 40 mL of strained rumen fluid
5. 3.33 mL of 12 g/100 mL $Na_2CO_3$
6. 1.67 mL of 3 g/100 mL cysteine hydrocholoride

An aliquote of 8 mL was tubed under $O_2$-free $CO_2$ into 16 × 150 mm culture tubes closed with rubber stopper and autoclaved in racks at 121°C for 20 min. (Dehority, 1969). A solution of 1 mL of either sterile distilled water or different concentration of nano zinc solution was added at the time of inoculation to make the final volume to 10 mL. The cellulose concentration in the final medium was 0.075 g/mL. The mixture was agitated for 3 min. under a vigorous stream of $O_2$-free $CO_2$.

After incubation, the entire contents of the culture tubes were transferred to a previously weighed test tube and centrifuged at 1000×g for 10 min. at room temperature (20-23°C). The supernatant was decanted and 5 mL of acid detergent solution were added (Van Soest, 1963). The tubes were mixed and heated on hot plate for 1 h at 100°C. The insoluble residue was centrifuged as stated earlier and supernatant was discarded. The sediment was washed twice with boiled distilled water. The tubes were dried overnight in an oven at 100°C, placed in a desiccator and weighed (Hiltner and Dehority, 1983). The cellulose digestion was based on the difference between the weight of cellulose measured in the blank tubes (0 h) and other tubes after 24 h incubation time.

Statistical analysis of experimental data was analysed by one way analysis of variance (ANOVA) model as per Snedecor and Cochran (1994). This statistical ANOVA model was incorporated with General Linear Models procedure (SPSS, 2012, version: 20).

The contents of OM, CP, EE, NDF and ADF in concentrate mixture were 93.95, 20.45, 5.02, 30.42 and 12.27% (DM basis) with the corresponding values of 89.13, 11.02, 1.65, 56.43 and 30.53% for maize fodder. The Zn content in concentrate mixture and maize fodder were found to be 24.40 and 21.78 ppm, respectively. The effects of nano Zn supplementation on values of in vitro gas production (IVGP), true DM digestibility (TDMD), true OM digestibility (TOMD), partitioning factor (PF), short chain fatty acid (SCFA), microbial biomass production (MBP) and metabolizable energy (ME) in different treatments have been presented in Table 1. The IVGP was higher ($P<0.05$) in treatments $T_4$ and $T_5$ and the lowest value was observed in treatment $T_1$. In contrary to our findings, nZnO with 20 or 40 ppm (Zaboli and Aliarabi, 2013) had no significant effect on gas production. The highest ($P<0.05$) values of TDMD (%) were recorded in treatments $T_4$ and $T_5$ and the lowest in treatment $T_1$. Similar results were obtained by Ahmed et al. (2022) where maximum digestibility was obtained at supplementary level of 30 ppm Zn. Zinc supplementation in form of proteinate, propionate (Nagalakshmi

et al. 2013) and Zn peptide (Mallaki et al. 2015) resulted in higher *in vitro* digestibility compared to $ZnSO_4$ addition (Arelovich et al. 2000) which might be due to more Zn and amino acid availability for rumen microbes. The TOMD values were lower ($P<0.05$) in treatments $T_1$, $T_2$ and $T_3$ compared to treatments $T_4$ and $T_5$. The enhancement in digestibility was reflected from increased total gas production. Similar findings were reported by Juncai et al. (2011) using nZnO upto 400 ppm, A significant increase in PF value was seen in treatment $T_1$ lower in treatments $T_4$ and $T_5$. The lower PF values indicate more gas production from the feed which signifies less DM intake by the animal with better performance (Blummel et al. 1997). The level of SCFA was significantly ($P<0.05$) higher in treatments $T_4$ and $T_5$ and lowest in treatment $T_1$. Blummel and Orskov (1993) observed a high significant correlation between SCFA and gas production. SCFA is directly related with gas production. Addition of inorganic Zn (Parshuramalu et al. 2013) and Zn peptide (Mallaki et al. 2015) increased short chain fatty acid (SCFA) level. The MBP value was lower ($P<0.05$) in treatments $T_4$ and $T_5$ compared to treatments $T_1$ and $T_2$. Microbial biomass is the major source of protein for the ruminant animals which is a source of truly available protein post ruminaly. *In vitro* gas production reflects primarily SCFA production and an inverse relationship exist between SCFA and microbial efficiency (Blummel et al. 1997). The ME value was the lowest in treatment $T_1$ and the highest in treatments $T_4$ and $T_5$. Supplementation of inorganic Zn (Parsurammalu et al. 2013), organic Zn (Nagalakshmi et al. 2013), Zn peptide (Mallaki et al. 2015) enhanced the ME value of the feeds.

The average values for pH, ammonia nitrogen ($NH_3$-N), individual fatty acids (IVFA; acetate, propionate and butyrate), IVFA (mol/100 mol) and A: P ratios have been presented in Table 2. The range of pH was found to be 6.70 to 6.83 in different treatments and the values were similar. Juncai et al. (2011) and Hassan et al. (2019) reported that supplementation of nano Zn had no effect on rumrn pH. The value of $NH_3$-N ranged from 23.38 to 24.47 mg/

**Table 1**: In vitro gas production, digestibility, microbial biomass production and ME values as affected by different levels of nano Zn supplementation

| Parameter | Level of nano Zn (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| $IVGP_{24h}$(mL/g) | $149.40^{c}\pm0.10$ | $169.59^{b}\pm0.65$ | $170.07^{b}\pm0.93$ | $177.11^{a}\pm1.47$ | $179.68^{a}\pm2.01$ | $167.90^{b}\pm1.65$ |
| TDMD (%) | $66.41^{c}\pm0.47$ | $67.91^{b}\pm0.38$ | $68.41^{b}\pm0.36$ | $70.67^{a}\pm0.22$ | $70.47^{a}\pm0.46$ | $69.52^{ab}\pm0.49$ |
| TOMD (%) | $69.24^{c}\pm0.49$ | $69.49^{c}\pm0.10$ | $69.94^{bc}\pm0.12$ | $71.95^{a}\pm0.07$ | $72.49^{a}\pm0.18$ | $71.41^{ab}\pm0.19$ |
| PF | $4.01^{a}\pm0.09$ | $3.86^{ab}\pm0.05$ | $3.75^{ab}\pm0.07$ | $3.61^{b}\pm0.07$ | $3.64^{b}\pm0.08$ | $3.80^{ab}\pm0.02$ |
| SCFA (mmol) | $0.70^{b}\pm0.02$ | $0.75^{ab}\pm0.01$ | $0.76^{ab}\pm0.02$ | $0.80^{a}\pm0.02$ | $0.79^{a}\pm0.02$ | $0.76^{ab}\pm0.01$ |
| MBP (mg/g) | $283.23^{a}\pm7.90$ | $281.48^{a}\pm4.65$ | $264.39^{ab}\pm6.47$ | $255.07^{b}\pm8.03$ | $255.40^{b}\pm8.69$ | $273.93^{ab}\pm2.56$ |
| ME (MJ/kg) | $7.53^{c}\pm0.12$ | $7.86^{b}\pm0.06$ | $7.91^{b}\pm0.10$ | $8.17^{a}\pm0.09$ | $8.24^{a}\pm0.08$ | $7.81^{b}\pm0.03$ |

[a,b,c]Values bearing different superscripts in a row differ significantly ($P<0.05$)

**Table 2:** The effect of nano Zn supplementation on rumen pH, ammonia-N and volatile fatty acids under in vitro conditions

| Parameter | Level of nano Zn (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| pH | 6.70±0.03 | 6.73±0.06 | 6.77±0.03 | 6.77±0.02 | 6.83±0.04 | 6.67±0.02 |
| $NH_3$-N (mg/dL) | 23.43±0.42 | 23.86±0.25 | 23.38±0.35 | 23.52±0.13 | 23.89±0.42 | 24.47±0.23 |
| | | | IVFA (mM) | | | |
| Acetate | $39.13^{c}\pm1.06$ | $45.65^{b}\pm1.20$ | $54.47^{a}\pm0.76$ | $57.57^{a}\pm1.14$ | $57.90^{a}\pm1.24$ | $56.24^{a}\pm0.99$ |
| Propionate | 15.93±0.55 | 16.68±0.72 | 17.00±0.70 | 17.20±0.64 | 17.92±0.62 | 18.35±0.65 |
| Butyrate | 6.60±0.40 | 6.61±0.42 | 7.77±0.32 | 7.37±0.19 | 7.52±0.87 | 7.72±0.37 |
| A: P ratio | $2.46^{c}\pm0.07$ | $2.75^{bc}\pm0.08$ | $3.23^{ab}\pm0.13$ | $3.37^{a}\pm0.17$ | $3.25^{ab}\pm0.13$ | $3.08^{ab}\pm0.14$ |
| | | | IVFA (mol/100mol) | | | |
| Acetate | $63.44^{c}\pm0.70$ | $66.24^{bc}\pm0.42$ | $68.78^{ab}\pm0.61$ | $70.08^{a}\pm0.96$ | $69.48^{ab}\pm1.32$ | $68.32^{ab}\pm0.85$ |
| Propionate | $25.81^{a}\pm0.56$ | $24.18^{ab}\pm0.65$ | $21.43^{bc}\pm0.70$ | $20.95^{c}\pm0.79$ | $21.48^{bc}\pm0.58$ | $22.29^{bc}\pm0.75$ |
| Butyrate | 10.75±0.75 | 9.58±0.50 | 9.79±0.29 | 8.97±0.22 | 9.04±1.08 | 9.39±0.49 |

[a,b,c,d] Values bearing different superscripts in a row differ significantly ($P<0.05$)

dL in different treatments. Similar report (Hassan et al. 2019) exist using sheep rumen liquor under in vitro. Juncai et al. (2011) found that the concentration of $NH_3$-N decreased ($P<0.05$) with the supplementation of 100 ppm nano ZnO. In contrary to this, addition of Zn decreased the $NH_3$-N released in vitro (Arelovich et al. 2000) and rumen fluid of sheep (Rodriguez et al. 1995). At the 6 and 12 h of incubation in vitro, the supplementation levels of 100 and 200 mg/kg of nano-zinc oxide considerably ($P<0.05$) reduced the concentration of $NH_3$-N and the ratio of acetate to propionate (Chen et al. 2011)

The values of acetate production increased in treatments $T_3$, $T_4$, $T_5$ and $T_6$. Juncai et al. (2011) also reported that supplementation of nano ZnO under in vitro rumen conditions increased VFA production. Chen et al. (2011) showed that VFA production increased ($P<0.05$) with the supplementation levels of 100 and 200 mg/kg of nano-zinc oxide at the 6 and 12 h of incubation in vitro. In contrast, Aliarabi (2006) and Hassan et al. (2019) reported that Zn supplementation either in inorganic or chelated and Nano Zn form, respectively did not show significant effect on rumen fermentation parameters. Propionate production ranged from 15.93 to 18.35 mM and value of butyrate production varied from 6.60 to 7.72 mM in different treatments. Spear et al. (2004) reported that propionate was higher ($P<0.05$) and butyrate was lower ($P<0.05$) in steers fed Zn-Met compared to $ZnSO_4$ diets. A significant ($P<0.05$) increase in the A: P value was seen in treatment $T_4$ and lower value in treatment $T_1$. However, Juncai et al. (2011) reported that at 50 ppm and Hassan et al. (2019) reported that at dose from 20 to 60 ppm of nano ZnO supplementation A: P ratio was same but higher than control group.

In vitro cellulose digested was lower ($P<0.05$) in treatment $T_1$ and higher at 10 and 20 ppm level of supplementation. Similar value of pH was seen in different treatments. Addition of Zn upto 10 ppm increased *in vitro* cellulose digestion (Martinez and Church 1970; Little et al. 1958). Further supplementation of 20 and 30 ppm of added Zn resulted in a decrease ($P<0.05$) in cellulose digestion. Eryavuz and Dehority (2009) found that 50 ppm Zn supplementation to the cellulose media reduced cellulose digestion. The adhesion of cellulolytic bacteria to cellulose is a critical early step in cellulose fermentation. Bonhomme (1990) suggested that Zn is bound to the cell surface of bacteria. Thus, Zn might be affecting the adhesion of microbial cells to cellulose particles.

## Conclusion

Therefore, inclusion of Zn in form of ZnO nanoparticle @10 and 20 ppm of basal substrate showed enhancement ($p<0.05$) in acetate production digestibility and ME contents.

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RESEARCH ARTICLE

# Headspace volatile markers of *Sandesh*, a *chhana*-based delicacy stored at elevated temperatures

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**Abstract:** The present study explores the headspace volatiles of *Sandesh* (dessert made from heat acid coagulated milk) that contribute to the product quality during storage. *Sandesh* is packaged in clear and dark-coloured glass containers and stored at 30 °C and 45 °C in an incubator. *Sandesh* quality during the storage was estimated by biochemical, microbiological and sensory analysis. The concentration of head-space volatiles was simultaneously determined by employing headspace solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). The identified volatiles were pertaining to various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. As a result, acetic acid, propanoic acid, valeric acid and butyric acid were suggested as headspace freshness markers, while spoilage markers were identified as 1-hexanol 2-ethyl-; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-; pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The obtained volatile markers are essential to develop the intelligent packaging systems for monitoring the product's quality

**Keywords:** *Sandesh*, Headspace, Volatile organic compounds, Solid-phase micro-extraction, Quality change, Spoilage markers

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## Introduction

India is a unique and traditionally rich country in terms of producing a variety of region-specific dairy products. Among several such region-specific dairy products, *Sandesh* is one of the most popular products of acid-coagulated hot milk (*Chhana*, which is similar to cottage cheese but contains sugar). It has a smooth texture with a firm body, which consists of a high amount of milk proteins, fat, sucrose, and fat-soluble vitamins (Aneja et al. 2002; Khamrui and Solanki, 2010). It is highly popular in the eastern and northeastern states of India but gaining huge popularity across India and overseas due to its flavour and palatability. Flavour is composed principally of the sensation of smell and taste and it is the most important factor, which governs our appreciation of the food that we consume. Volatile constituents of the food can determine its aroma and flavour. Previous reports indicated identification of nine volatile carbonyl compounds namely formaldehyde, acetaldehyde, propionaldehyde and /or acetone, butyraldehyde, pentan-2-one, heptan-2-one, benzaldehyde, octan-2-one, nonan-2-one in fresh samples of *chhana* using the Gas-Liquid Chromatography technique (Kumar and Srinivasan, 1984). The major volatiles found in Queso blanco cheese, which is similar to *Sandesh* (but contains salt instead of sugar) are acetone, acetaldehyde, butanol, isopropanol, formic acid, acetic acid, propionic acid and butyric acid, which contribute to the cheese's flavour and aroma (Torres and Chandan, 1981).

Volatile constituents of a product are not only responsible for determining its flavour and aroma, but also play a pivotal role in the estimation of its shelf life. The volatile compounds can generate during storage due to various microbial and biochemical reactions in the product (Cheng, 2010). The profiling of headspace volatiles of food products has been extensively studied for the determination of spoilage markers. In an earlier report (Li et al. 2022), potential volatile compounds associated with deteriorating raw milk were investigated and suggested monitoring 2-ethyl-5-methylpyrazine, 2-pentanone, pyridine, 2-butanone, n-butyraldehyde, and 2,3 butanedione as possible raw milk deterioration indicators. Similarly, Song et al. (2021) identified three headspace volatile compounds namely ethanol, 2,3-butanediol and 2-ethyl-1-hexanol as decay markers of minced

pork by characterizing headspace volatiles during storage. In this regard, several researchers (Anagnostopoulos et al. 2022; Martín-Gómez et al. 2022; Opara et al. 2022; Pavlidis et al. 2021; Sarnoski et al. 2010; Waghmode et al. 2021; Wierda et al. 2006; Yang et al. 2022) reported various spoilage and freshness markers in food products by using volatolomics approach.

The headspace solid-phase microextraction (HS-SPME) technique combined with Gas chromatography-mass spectrometry is an advanced and potential tool to evaluate the quantitative and qualitative headspace volatiles of foods. Pawliszy and co-workers developed the SPME method, which describes the preparation of samples without using solvents for chromatographic analysis. In the SPME method, volatiles are adsorbed on the reusable fibre, which is layered with a stationary phase (Arthur and Pawliszy, 1990). In recent studies, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibres have been reported to be most effective for extracting a wide range of analytes with different polarities and molecular weights. Further, identification and quantification of the compounds are achieved by injecting the fibre extracts into a gas chromatography-mass spectrometer (GC-MS). The combination of SPME and GC-MS has been employed to examine the flavour of various dairy products (Balasubramanian and Panigrahi, 2011). To the best of our knowledge, the investigation on determining headspace volatiles of most of the traditional Indian dairy products has not been conducted so far using advanced techniques, except for *ghee* (Duhan et al. 2020; Wadodkar, 2003). The main purpose of this study is to evaluate the range of volatiles produced during storage under various conditions using GC-MS technique coupled with headspace solid-phase microextraction (HS-SPME) to identify the key quality markers of *Sandesh*.

## Materials and methods

### Preparation of *Sandesh*

Cow's milk and cream were procured from the Experimental Dairy of ICAR-National Dairy Research Institute, Karnal, India. Milk was standardized to 4% milk fat and 8.2% milk solids-not-fat (MSNF) in the laboratory. Soft grade *(Narampak) Sandesh* was prepared by following the standard protocol given by Sen and Rajorhia, 1990. The standardized milk was subjected to heating at 90°C and coagulation with 1% citric acid solution until clear whey. The obtained coagulated mass (pH 5.2-5.4) strained using a muslin cloth for whey draining for 30 min. The obtained mass (*Chhana*) was kneaded to get fine consistency and sugar (30% by weight of *chhana*) was added to it. Later, the mixture was heated to 70°C for 15 min. and further heating continued to 60°C for about 10 min with continuous stirring. Then the mixture was moulded (22g / piece) in a mould and cooled (37°C) to attain *sandesh.*

### Proximate composition analysis of *Sandesh*

*Sandesh* samples were analysed for moisture and fat as per BIS (1981) procedure. The protein and ash content were estimated by using AOAC (2016) methods. The total carbohydrate percentage of the sample was calculated by the difference of the sum of the moisture, fat, protein and ash from the total weight (*Sandesh*).

### Packaging and storage of samples

A large portion of *Sandesh* sold in market in plastic containers. However, glass packaging materials were chosen to determine headspace volatiles. The reason was to avoid migration and scalping of headspace volatiles from the container. Freshly prepared *Sandesh* was packaged in two types of packaging materials such as clear glass vials (CG) and dark-coloured glass vials (DG) (M/s Labco, India, Cat No: 993/4). They were stored at 30 °C and 45 °C (Fig. 1a) in an incubator. Initially, 8 g of sample was weighed into each vial of 30 mL for headspace volatiles determination. Later, 150 g of sample was weighed in CG and DG containers (300 mL) (Yera Airtight Manufactures, India) to determine the biochemical, microbial and sensory changes during storage. The analysis was carried out at 24 h interval until visible mould growth (Fig. 1d).

### Extraction and measurement of headspace volatile compounds by HS-SPME-GC/MS

The head-space volatiles from the *Sandesh* samples were extracted by using a headspace solid-phase microextraction (HS-SPME) device consisting of a fibre (Supelco cat. No: 57348-U, Bellefonte, PA, USA) with a film thickness of 50/30µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and a manual holder (Supelco cat. No: 57330-U, Bellefonte, PA, USA). The SPME syringe was introduced into the vial's headspace, followed by the extension of the fibre (2 cm) into the vial (Fig. 1b). The gap between the fibre tip and the product surface in the bottle was 0.5 cm. The extraction time of samples stored at 30 °C and 45 °C for 40 and 45 min, respectively, were optimized by conducting experiments (Fig. A1& Fig. A2). After extraction of volatiles, the fibre was placed into the manual holder and inserted into the heated injection port of the GC-MS for thermal desorption of the samples for 15 min (Fig. 1c). GC-MS (TQ-8030, Shimadzu Corporation, Kyoto, Japan) instrument located at the National Referral Centre for Milk Quality and Safety, ICAR-NDRI, Karnal, India was employed in this study. Chromatographic separation was performed on an Equity-1 column (Capillary column; 30 m X 0.32 mm i.d. X1.0 µm film thickness, Supelco cat. No: 28057-U, Bellefonte, PA, USA). The injector, detector and interface were operated at 270 °C, 220 °C, and 260 °C, respectively. GC oven was initially set at 270 °C for 11 min, then ramped linearly at a rate of 10 °C/min to 300 °C and it was kept for 5 min at the same temperature. Flow rate of helium

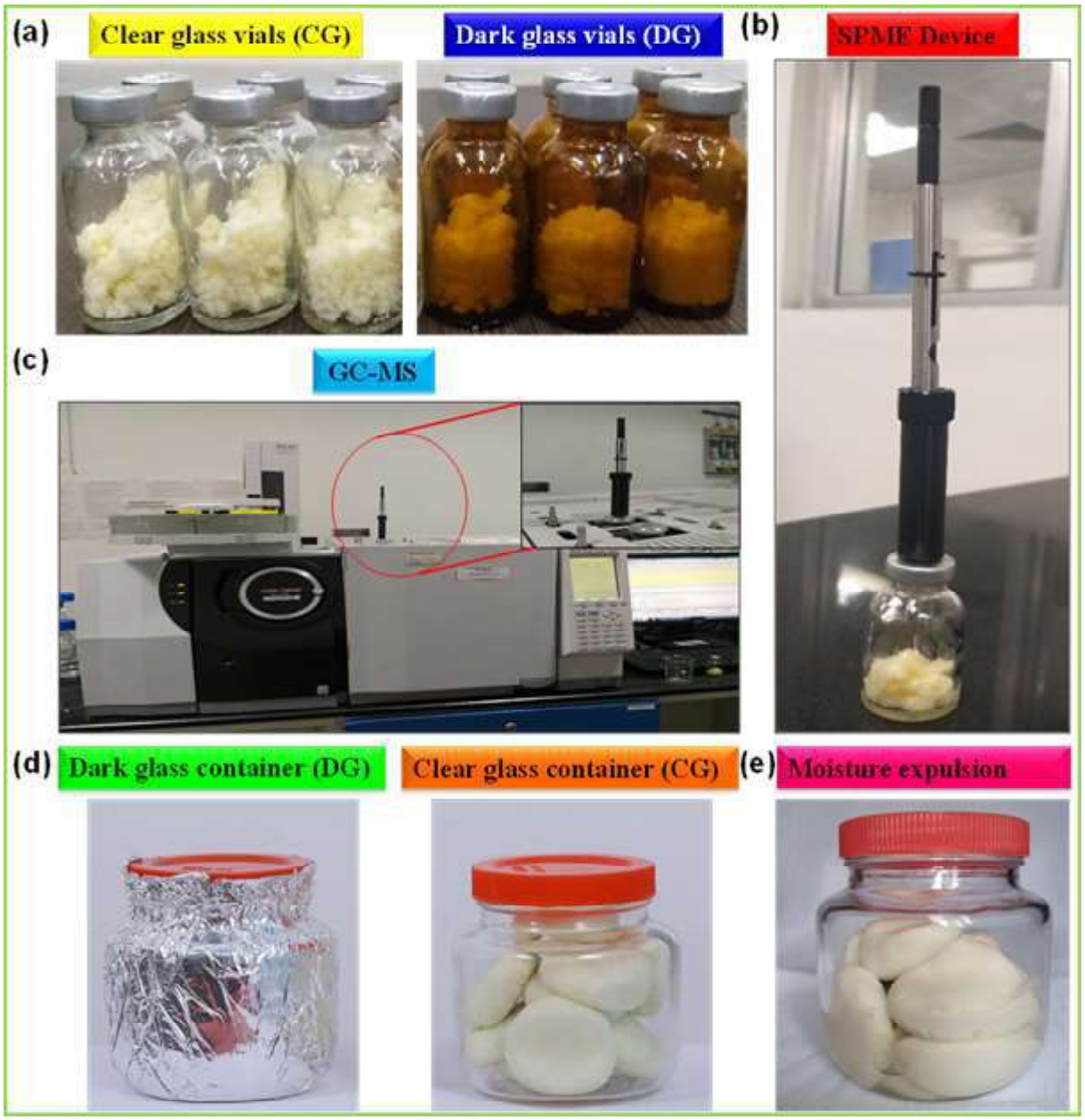


**Fig. 1** **(a)** Clear glass vials and dark coloured glass vials for headspace volatiles analysis, **(b)** Headspace volatiles collection using SPME device, **(c)** GC-MS coupled with SPME, **(d)** Clear and dark (aluminium wrapped) glass containers for quality analysis, **(e)** Moisture expulsion from samples during storage

carrier gas was maintained at 2.33 mL/min under splitless mode. The standard ionization energy of 70 eV was maintained in Electron Ionization (EI) under full scan mode in a range of 50-500m/z to analyse the volatiles. The identification of volatile compounds was accomplished by using NIST 17 and NIST 14S (National Institute of Standard and Technology, New York, USA) mass spectral library. The results from the headspace volatile analysis were expressed in terms of peak area (total ion concentration).

**Analysis of biochemical changes**

The titratable acidity of the sample was determined using the method described in BIS (1981). Sample pH was measured with the help of a calibrated pH analyser (Labndia, New Delhi, version 1). The free fatty acids content of *Sandesh* was estimated using Deeth et al. (1975) method. The extent of protein breakdown (proteolysis) was evaluated by employing the reported method of Jupfs (1973). The extent of oxidation of fat in terms of

thiobarbituric acid (TBA) value was analysed using a method described by Strange et al. (1977).

**Analysis of microbiological changes**

Standard plate count (SPC) and, yeast and mould count (Y&M) were analysed according to the BIS (1981) procedure. Coliform count, aerobic spore count, proteolytic count, and lipolytic count were performed by using the specific methods given by Marshall (1992).

**Analysis of sensory changes**

Samples of *Sandesh* were evaluated for attributes such as colour and appearance, flavour, texture, and overall acceptability by a panel of eight semi-trained judges. The panel members were chosen based on their experience in judging traditional Indian dairy products. The panellists assessed the sensory parameters based on a 9-point hedonic scale, wherein "9" denoted extremely desirable and "1" denoted extremely undesirable.

**Statistical analysis**

Variations in biochemical, microbial and sensory parameters were investigated by analysis of variance (ANOVA) using IBM SPSS software (ver. 20). The results are represented in terms of mean and standard deviations (SD). To compare mean values, Duncan's test (DMRT) was applied and the significant differences were identified. Principal component analysis (PCA) was performed using Origin pro software (ver. 2023) to determine significant volatile functional groups that affect the headspace of *Sandesh* during storage. Correlation analysis between headspace volatiles and quality attributes was expressed using polar heatmap (Origin pro software).

**Results and Discussion**

**Chemical composition of *Sandesh***

The proximate composition of *Sandesh* (soft grade) revealed 26.90 ± 0.56% moisture, 22.27± 1.00% (*db* w/w) fat, 22.80 ± 0.56% (*db* w/w) protein, 53.40 ± 1.66% total carbohydrates and 1.48 ± 0.08% (*db* w/w) ash. The obtained composition was in agreement with the earlier reports (Khamrui and Solanki, 2010; Sen and Rajorhia, 1991).

**Changes in headspace volatile of *Sandesh* stored under different conditions**

Detected and identified headspace volatiles of *Sandesh* stored in two packaging materials such as CG vial and DG vial at 30°C and 45°C at each interval are represented in a polar heatmap (Fig. 2). Compounds with a peak area (total ion concentration) of more than $10^4$ were considered for data analysis in the study. The peak area of detected compounds for all the samples is mentioned in Table A1 (supplementary materials). The identified volatiles are affirmatively confirmed to be acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. Approximately, 140 product-related headspace volatiles were successfully identified along with a small number (35-40) of fibre-related volatile contaminants, which include derivatives of hydrazine carboxamide, semicarbazide, silanediol, carbohydrazide and polysiloxane were noticed. However, these volatile contaminants were eliminated while processing the data as recommended in the earlier reports (Grimm and Champagne, 2001; Zhang et al. 2022).

***Major volatiles***

Acids, alcohols, alkanes, ketones and aldehydes have been noticed to be the largest functional group compounds that are present in the headspace of *Sandesh*. Forty acids were identified in *Sandesh* during the storage under various conditions. Among them, carboxylic acids with a chain length ranging from 1 to 20 ($C_1$-$C_{20}$) are the majority of compounds. The individual volatile acids possess a non-specific trend during storage due to the continuous degradation and production of acids. A similar non-specific trend of individual volatiles in fermented milk during storage was reported by Dan et al. (2017). However, the sum of the peak areas of all the acids (total peak area) has been considered to analyse the changes during storage. It can be observed from Fig. 3 that the total peak area of the volatile acids has decreased during the storage irrespective of various storage conditions. This could be due to the acid degradation by β-oxidation (saturated fatty acids) and auto-oxidation (unsaturated fatty acids) reactions (Cheng, 2010). The highest percentage of decrease (58.6%) in total peak area was observed in CG samples at both storage temperatures due to the susceptible property of packaging material (transparent) to auto-oxidation of fatty acids. It is believed that acetic acid, propanoic acid, valeric acid and butyric acid were responsible for the decrement in acids' total peak area. Hence, these acids could be considered while determining the product's freshness. In addition, we have identified a few more acids in *Sandesh* during storage such as cyacetacide, formic acid, hexanoic acid and stearic acid. The obtained acids have been earlier reported to be present in milk (Dursun et al. 2017). Despite this, carboxylic acids are essential aroma compounds which are also considered to be prominent precursors for methyl ketones, alcohols, aldehydes, alkanes, alkenes, and esters (Cheng, 2010).

In this study, twenty-two different alcohols are identified in *Sandesh* headspace. Ethanol and methanol were found to be the major alcohol compounds present in *Sandesh* (Table A1). Similar to earlier trend of non-specific peak area of acids, the alcohols have also followed the same trend at each storage interval among all the samples (Fig. 3). It can observe that the predominant total peak area has been recorded for alcohols at end of the shelf life in all the samples compared to fresh *Sandesh*. The observable non-

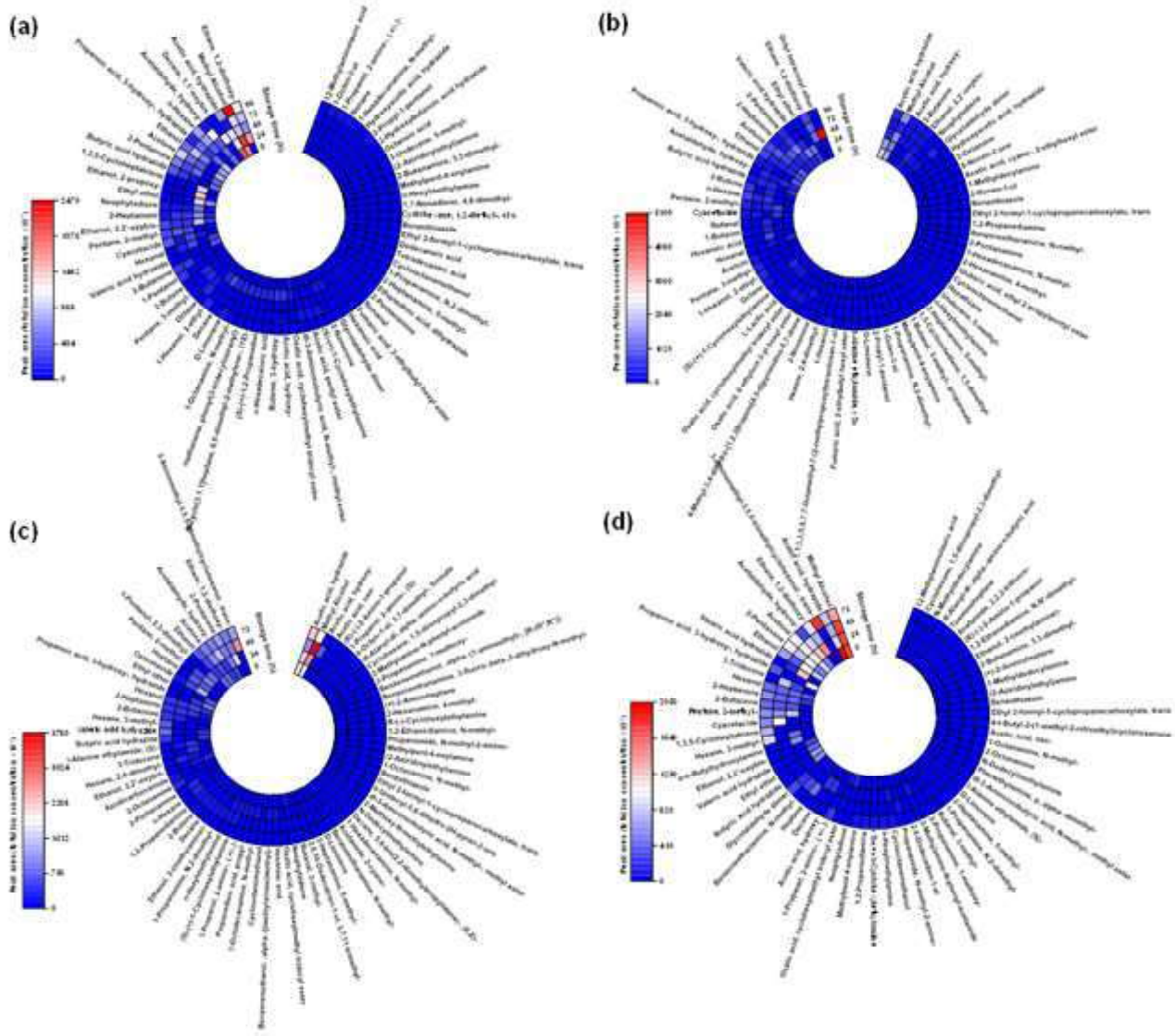


**Fig. 2** Polar heat map of headspace volatiles of *Sandesh* during storage, **(a)** Samples stored at 30°C in dark glass vials, **(b)** Samples stored at 30°C in clear glass vials, **(c)** Samples stored at 45°C in dark glass vials, **(d)** Samples stored at 45°C in clear glass vials.

specific trend variations could have been due to various reasons, which include the production of alcohols from aldehydes and ketones reduction, fermentation of lactose, amino acid metabolism, and also partial esterification of resulting alcohols with acids (Yue et al. 2015). It can be believed that these specific compounds of 1-hexanol, 2-ethyl-; 1-hexanol; 1,1,3,3,5,5,7,7-octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol; 2-propyl-1-pentanol in CG & 1-pentanol; 1-hexanol, 2-ethyl-; 2-propyl-1-pentanol; methyl alcohol in DG and, 3-aminomethyl-3,5,5-trimethylcyclohexanol, trans; 1-Propanol, 2-amino-, (.+/-.)-; Cyclooctanemethanol compounds in CG and 3-Aminomethyl-3,5,5-trimethylcyclohexanol , trans-; methanol compounds in DG are responsible for the peak area predominance in alcohols at 30°C and 45°C at the end of storage, respectively (Table A1). The observed specific alcohols of primary alcohols might have been originated from the oxidation of unsaturated fatty acids (Cheng, 2010). However, hexanol and pentanol are derived from the aldehydes such as hexanal and pentanal during lipid oxidation (Kilcawley et al. 2018). Rashid et al. 2019 findings revealed that 1-pentanol had appeared as the second-largest volatile compound in pasteurized milk at 10°C after 18 days of storage and 1-heptanol at the end of 16 days of storage. Urbach (1990) reported that ethanol, propan-2-ol and 3-methyl-butna-1-ol were major volatiles present in raw milk stored at 7 °C for 3 days. Therefore, the enhanced amount of alcohol compounds in stored samples of *Sandesh* in our present investigation could be attributed to auto-oxidation of unsaturated fatty acids.

Alkanes emerged as one of the important classes of volatiles in the headspace of *Sandesh* at the end of their shelf life, although they were absent in fresh *Sandesh*. However, aliphatic groups of alkanes with a chain length of $C_2$-$C_{11}$ have been identified along

with the detection of nineteen alkanes in the product . The total peak area of alkanes has been recognized to be enlarged with progressing storage time at 30°C irrespective of packaging materials till 48 h of storage and it has followed the non-specific trend further (Fig. 3). Furthermore, alcohol concentration was observed to be high in both the packaging materials at 45°C compared to *Sandesh* stored at 30°C till 24 h of storage. Eventually, the maximum total peak area was observed in samples stored in CG materials at 30 °C followed by samples in DG materials at the same temperature at the end of the shelf life. Usually, alkanes are secondary oxidation products that are formed from the decomposition of hydroperoxides during the auto-oxidation of unsaturated lipids (Kubow, 1992). However, an increasing trend of peak area has been conspicuously noticed, which was raised due to the presence of ethane, 1,2-diethoxy-; n-hexane; pentane, 3-methyl-; hexane, 3-methyl; pentane, 2-methyl-; heptane among all the alkanes. These results were substantiated by Frankel et al. (1982) who reported that the decomposition of 9- and 13-hydroperoxide from linoleic acid led to the production of pentane and ethane.

In addition, twelve different ketones were identified in *Sandesh* samples stored under different conditions and changes in their concentration are presented in Fig. 3. Among all the identified ketones, four compounds namely acetone, 2-pentanone, 2-heptanone and 2-butanone were shown highest peak area. These ketones were present in fresh and also formed during storage of *Sandesh* . The earlier studies (Clarke et al. 2020; Yue et al. 2015) demonstrated the presence of ketones in fresh milk and heated milk. Moreover, acetone, 2-heptanone and 2-pentanone have also been considered to be the major three ketones in microfiltered pasteurized milk (Yue et al. 2015). Kumar and Srinivasan (1984) reported that five ketones namely acetone, pentan-2-one, heptan-2-one, octan-2-one, nonan-2-one were identified in fresh samples of *chhana* prepared from cow's milk, buffalo's milk and samples procured from the market. In fresh *Sandesh,* these might have originated from milk and also due to the heat treatment during preparation. The total peak area of the ketones in both packaging materials was observed to be decreased during the first 24 h. Later on, it is increased gradually by the end of 48 h at 30 °C. The peak area of ketones increased slightly with progressing storage interval irrespective of packaging materials at 45 °C. Moreover, the maximum total peak area of the ketones was observed at end of the storage period among samples stored *Sandesh* at 30 °C, which indicates that the temperature played a significant role in ketones generation. Methyl ketones that are present in the product are typically liberated by decarboxylation of saturated fatty acids or by β-ketoacids decarboxylation (Cheng, 2010). Li et al. (2012) reported that 2-heptanone was one of the major compounds that enhanced with increasing storage time of milk powder. Li and Wang (2016) have also described 2-heptanone and 2-nonanone the typical volatiles of oxidized flavours in dairy products.

Aldehydes namely acetaldehyde, hydroxy-; butanal, 3-hydroxy-; glycolaldehyde dimer; hexanal; nonanal; butanal; butanal, 3-methyl-; 2,6-dodecadien-1-al have been determined in our present investigation. According to the earlier report (Yue et al. 2015), heated milk contains aldehydes significantly. The total peak area dynamics of aldehydes of *Sandesh* (Fig. 1e) revealed that the concentration of aldehyde presence has increased notably at the end of 72 h and 48 h of storage time in samples stored at 30 °C and 45 °C, respectively, in both the packaging materials. However, the maximum total peak area was estimated in the samples during the storage at 45 °C. Among all the aldehydes, we have observed the maximum total peak area of hexanal and acetaldehyde, which were present in both fresh and stored *Sandesh*. It is believed that the rapid production and spontaneous degradation of aldehydes into alcohols (Cheng, 2010) could be responsible for the total peak area dynamics of aldehydes. Earlier studies reported that aldehyde compounds can be emerged due to unsaturated fatty acids autoxidation, lactose fermentation and amino acid metabolism (Cadwallader and Singh, 2009; Cheng, 2010; Li et al. 2012).

***Minor volatiles***

Alkenes, amines, amides, esters and ethers were minor functional groups in the study of volatiles. However, alkene levels revealed that none of the samples exhibited any observable specific trend during storage except CG samples at 45 °C of storage. As alkanes, alkenes are also secondary oxidation products of milk fat during auto-oxidation (Kubow, 1992). From the earlier reports (van Beilen and Funhoff, 2007), the alkenes are preferably converted into alcohols, aldehydes and carboxylic acids due to microbial action. Amines were identified at low concentrations in fresh as well as stored *Sandesh* samples. Although, a maximum number of amines have been noticed in *Sandesh*, their concentration was less compared to acids, alcohols and alkanes (Fig. 2). The majority of amines present in *Sandesh* pertain to the aliphatic group. Usually, amines are familiar intermediate products of protein degradation and their further decomposition can lead to the formation of acids, aldehydes, alcohols, esters and sulfur compounds (Cadwallader and Singh, 2009). Like amines, amides were also generated from the protein degradation, but this class of compounds was present in negligible concentration among all the samples during storage. Also, we have observed eight esters in our present study. Esters corresponding to alcohols and acids in the product were identified to be the highest concentration at end of the shelf life among all the samples at 30 °C rather than the samples at 40 °C. Esters are commonly produced from alcohols and fatty acids through the enzymatic process. Ethers have been identified in the least concentration (Table A1).

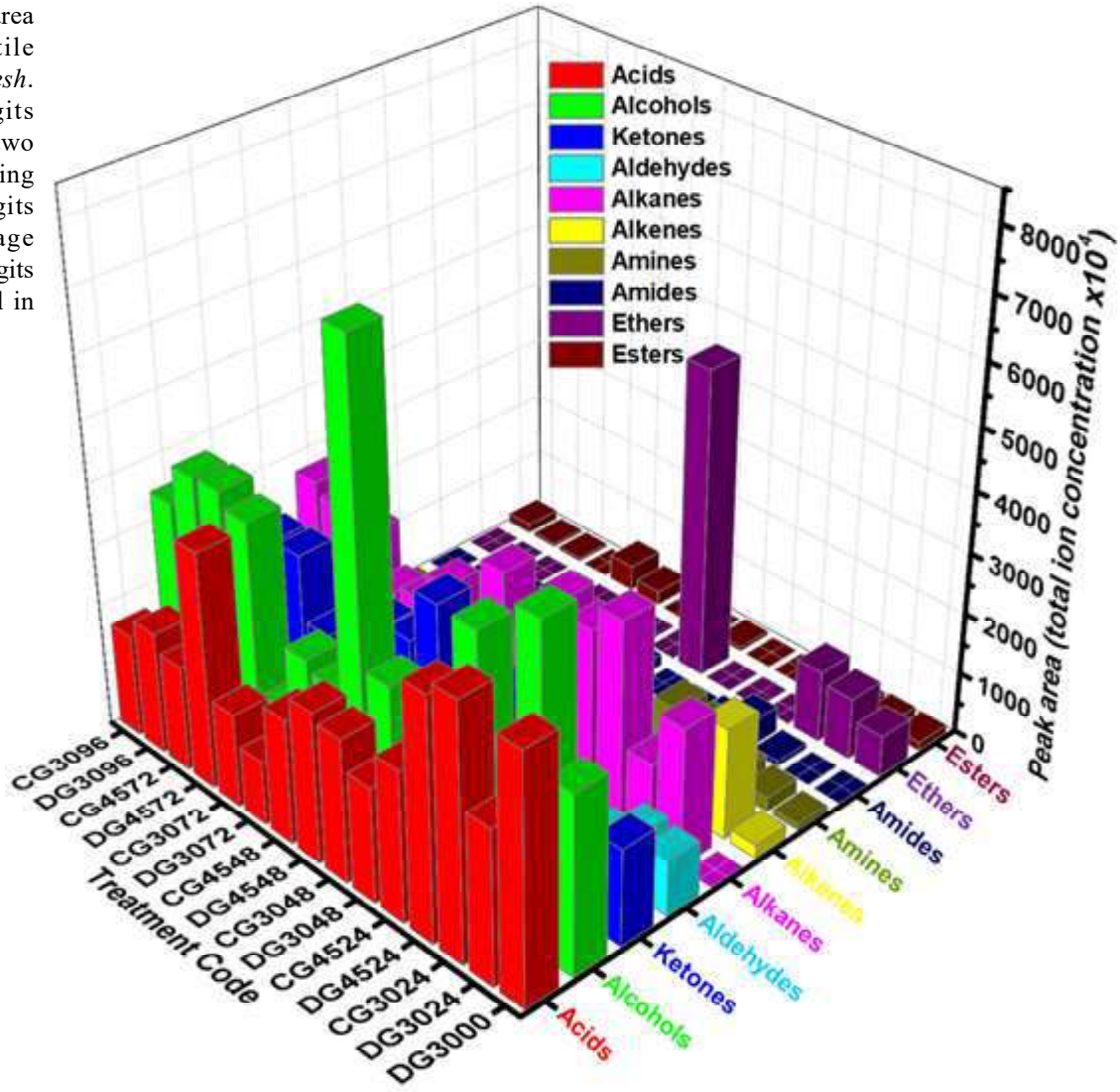


**Fig. 3** Changes in peak area of headspace volatile groups in stored *Sandesh*. Reference to six digits treatment code: first two digits indicate packaging material; next two digits indicate storage temperature; last two digits indicate storage interval in hours.

**Changes in quality attributes of *Sandesh* stored under different conditions**

We have carried out the analysis of quality changes in *Sandesh* stored under various conditions until satisfactory sensory scores have been recorded or visible mould growth

***Biochemical changes***

Biochemical changes of *Sandesh* samples stored under different conditions are presented in Table A2 (Supplementary materials). Present results displayed that the initial mean titratable acidity (% lactic acid) value of *Sandesh* significantly ($P<0.05$) increased from 0.46 to 0.56 and 0.58 at 30 °C; to 0.73 and 0.72 at 45 °C, respectively, in DG and CG during storage (Fig. 4a). Furthermore, ANOVA results revealed that the titratable acidity of *Sandesh* has been significantly ($P<0.05$) influenced by incubation temperature and non-significantly ($P>0.05$) by packaging materials. As evidenced, there was less improvement in the titratable acidity of *Sandesh* stored *at* 30 °C. The present observations were in good agreement with the earlier report (Yadu, 2014). After an initial pH of 5.89, *Sandesh* pH reduced to 5.69 and 5.76 at 30 °C; 5.73 and 5.71 at 45 °C, respectively in DG and CG during storage (Fig. 4b), which could be attributed to the production of lactic acid by spoilage microorganisms. Storage time shows its significant ($P<0.05$) impact on the pH of the product, whereas storage temperature and packaging materials had a non-significant ($P>0.05$) effect during storage. The decrease in pH of *Sandesh* samples has been earlier reported by Mandal (2019). In addition, the TBA value of all *Sandesh* samples enhanced significantly ($P<0.05$) with progressing storage time among all samples (Fig. 4c). The highest TBA value was observed in the CG container at 45 °C in the order of 0.229. The remarkable enhancement of TBA values could be attributed to the presence of a considerable amount of fat in *Sandesh*. This fat is highly susceptible to oxidation under higher temperature conditions.

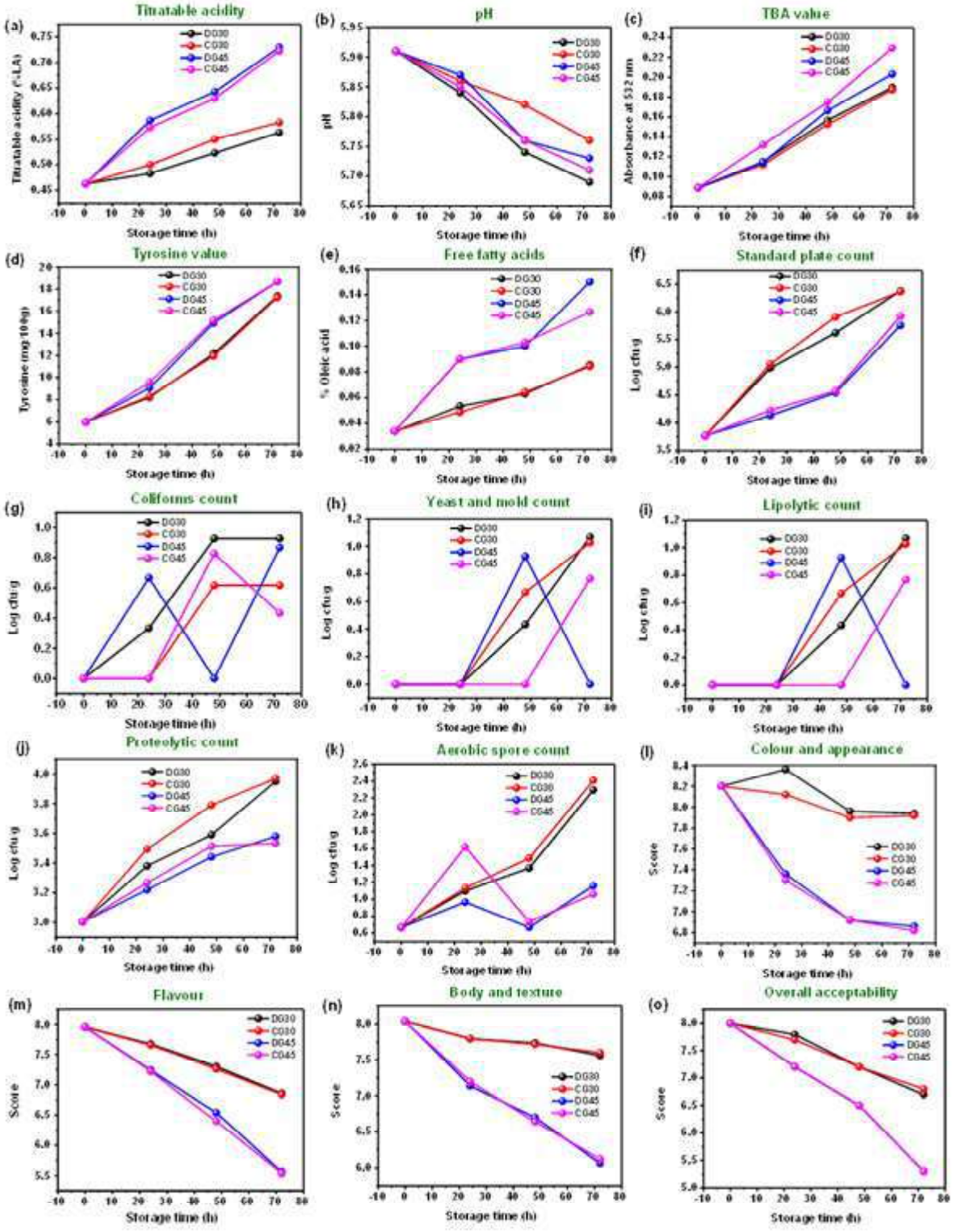


**Fig. 4.** Quality changes in *Sandesh* stored under various conditions **a)** Titratable acidity **b)** pH **c)** TBA value **d)** Tyrosine value **e)** Free fatty acids **f)** Standard plate count **g)** Coliforms count **h)** Yeast and mold count **i)** Lipolytic count **j)** Proteolytic count **k)** Aerobic spore count **l)** Colour and appearance **m)** Flavour **n)** Body and texture **o)** Overall acceptability. Reference to four digits treatment code: first two digits indicate packaging material; last two digits indicate storage temperature

Gargouri et al. (2015) reported that the rate of oxidation is influenced by many factors including temperature, oxygen and light. The significant increase in TBA values of samples stored in CG at 45 °C might be due to the combined effect of the storage temperature as well as light transmittance through the packaging material. Mandal (2019) has also observed a considerable enhancement in the TBA value (% malonaldehyde) of *Sandesh* during storage.

The tyrosine content (mg/100g) of *Sandesh* stored at 30 °C has been identified to be enhanced ($P<0.05$) from 5.98 to 17.38 and 17.24; to 18.66 and 18.60 in DG and CG, respectively at 45 °C. A significant amount of tyrosine content ($P<0.05$) in *Sandesh* is noticed at the end of the shelf life at 45 °C. However, non-significant ($P>0.05$) differences were observed in packaging materials. A similar increment in the tyrosine content of *Sandesh* during the shelf life has been mentioned in the earlier report (Yadu, 2014). The FFA values of *Sandesh* notably ($P<0.05$) varied from 0.034 to 0.15% oleic acid among all the samples (Fig. 4e). ANOVA revealed that the storage temperature substantially influenced the FFA value, but not packaging materials.

***Microbiological changes***

We have investigated the microbiological changes of *Sandesh* stored under different conditions. The standard plate count (SPC) (log cfu/g) of *Sandesh* exhibited a remarkable ($P<0.05$) increase from 3.76 to 6.37 when stored at 30 °C and to 5.92 at 45 °C. SPC of samples stored in DG container at 30 °C was estimated to be significantly ($P<0.05$) higher than samples stored at 45 °C, whereas non-significant ($P>0.05$) with samples stored in CG at 30 °C. However, the SPC of samples in both the packaging materials was found to be non-significantly different ($P>0.05$) at 72 h. The microbiological quality of all the samples stored under various conditions was found toexceed the prescribed standards (maximum limit 5.54 log cfu/g) recommended by FSSR (2011) after 48 hours.

It can be observed that coliforms were absent in fresh samples. Despite the presence of coliforms in the first dilution during storage, they did not exceed the standards set by FSSR (2011), indicating hygienic conditions were maintained during manufacturing and handling. Moreover, yeast and mould (Y&M) were not detected in the first dilution of the samples stored at 30 °C and 45 °C, respectively up to 48 h. On further storage, the count has been significantly ($P<0.05$) multiplied among all the samples (Fig. 4h). The Y & M count of *Sandesh* stored at 30 °C in both packaging materials exceeded the standards (2.17 cfu/g) prescribed by FSSR (2011). The count has been estimated to be substantially ($P<0.05$) greater than the samples stored at 45 °C in two packaging materials, which could be attributed to the favourable growth temperature and increased acidity of samples. The observations of enhanced Y&M count were also reported by the earlier worker, Yadu (2014). A similar trend was observed in the lipolytic bacteria count of *Sandesh* during the storage. However, the count was non-significantly ($P>0.05$) increased to 1.06 log cfu/g during storage (Fig. 4i). The proteolytic count of *Sandesh* samples has also been multiplied rapidly (Table A2) in storage time. In addition, aerobic spore count (log cfu/g) was found to substantially enhance from 0.66 to 2.40 among samples (Fig. 4k) but statistically non-significant ($P>0.05$) at the end of 72 h of storage. The increase in the count might be due to favourable temperature for the growth of aerobic spore count during prolonged storage.

***Sensory changes***

Sensory scores of *Sandesh* stored under different conditions for the stipulated period are displayed in Fig. 4. A non-significant ($P>0.05$) decrement in colour and appearance (C&A) scores of *Sandesh* was observed at 45 °C, whereas significant ($P<0.05$) decrement at 30°C in both the packaging materials. Further, it was observed that slight deformation of the shape of samples occurred due to the stacking of pieces in the container and the expulsion of moisture during storage, which might have contributed to reduced scores (Fig. 1e). However, no visible Y&M growth was observed even after a chemical or physical deterioration of samples when stored at 45 °C. Conversely, visible mould growth was observed in all the samples at 30 °C after 72 h of storage interval.

The flavour scores of *Sandesh* have been remarkably decreased in both the cases of DG and CG due to the production of off-flavours as a combined effect of deteriorative reactions. In this regard, storage conditions displayed a substantial effect on the flavour scores after 48 h of storage interval. The scores were noticed to be decreased rapidly in samples stored at 45 °C than 30 °C and also the formation of off-flavours was rapid at higher temperatures. Moreover, a drastic decline was observed in body and texture scores of *Sandesh* from 8.04 to 6.06 during storage at various conditions (Fig. 4n). Statistical analysis revealed that the samples stored at 30 °C exhibit substantial greater scores than those stored at 45 °C in both packaging materials, which could be due to the expulsion of moisture from the samples at 45 °C (Fig. 1e). The overall acceptability score decreased conspicuously ($P<0.05$) from 8.02 to 6.74 and 6.82 at 30 °C; to 5.32 and 5.30 at 45 °C DG and CG, respectively during the storage. The overall acceptability scores of *Sandesh* samples stored at 30 °C exhibited significantly higher values than the scores of samples at 45 °C (Fig. 4o). From biochemical, microbial and sensory results, the shelf life of *Sandesh* was estimated to be 72 h and 48 h at 30 °C and 45°C respectively, regardless of packaging materials.

**Principal component analysis**

The principal component analysis (PCA) was employed to determine the significant volatile functional groups that affected the headspace of *Sandesh during* storage and, also evaluate the

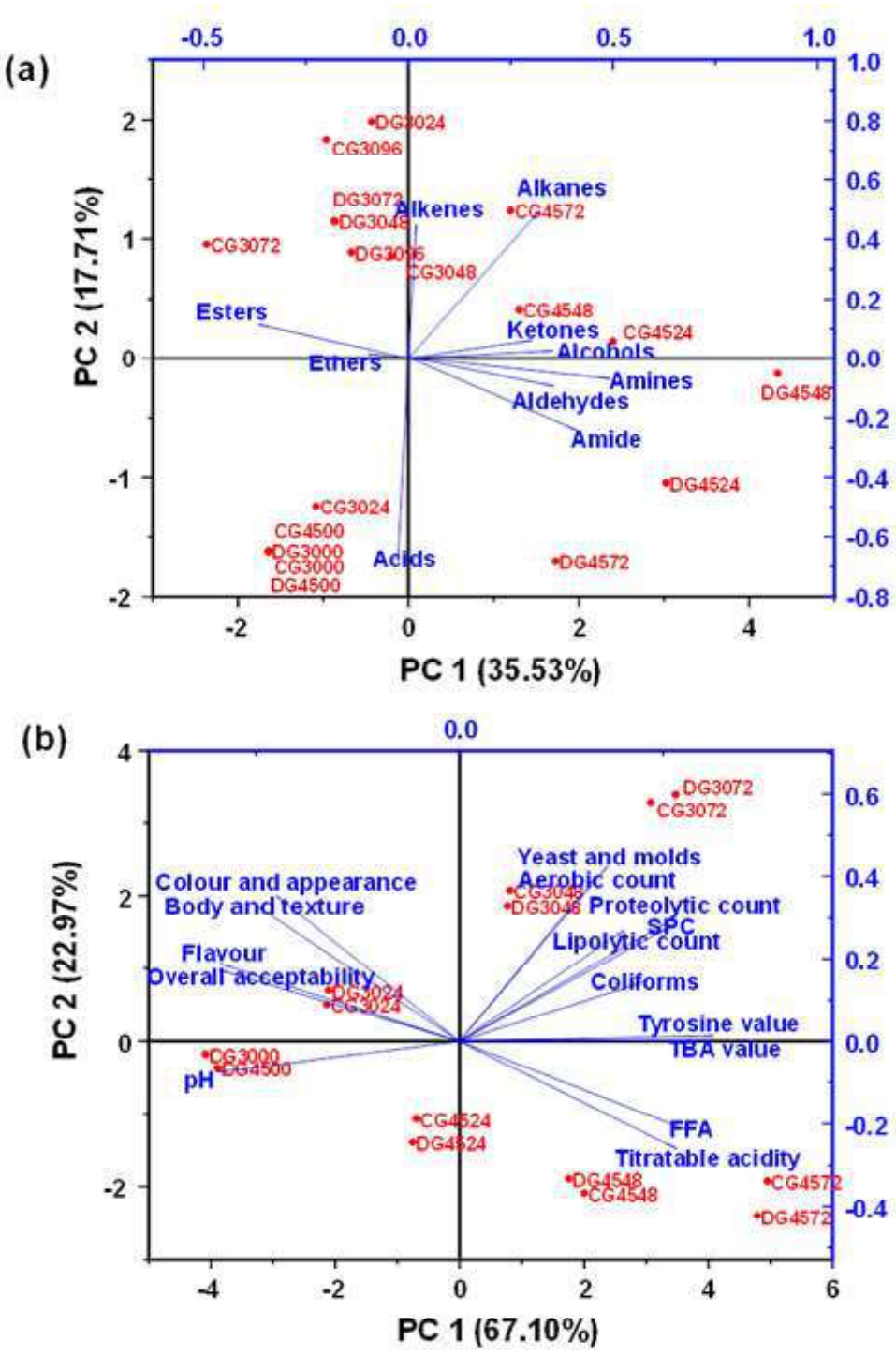


**Fig. 5.** Principal component analysis **(a)** Biplot for volatile components **(b)** Biplot for quality parameters. Reference to six digits treatment code: first two digits indicate packaging material; last two letters indicate storage temperature; last two digits indicate storage interval in hours.

considerable effect of various treatments of *Sandesh* on volatile functional groups. As a result of PCA, a biplot enabled us to visualize the interaction between the observations and variables (Fig. 5). In a biplot, the elongated line indicates greater variance in the variables, while the shorter line indicates less variance. The cosine angle between the lines in the biplot reflects the degree of correlation between their variables. The correlation between their variables decreases as the angle approaches 90 or 270°. However, a correlation of 1 or -1 is represented by an angle of 0 or 180 degrees, respectively (Kohler et al. 2005). We have observed that the first four principal components (PC) expressed approximately 78.21% of the variance between sample and headspace volatile functional groups. Of four principal components, PC1 is estimated to be 33.87% of the variance and is characterized by amines, amides and alcohols. PC2 was evaluated to be 17.98% of the variation and was defined by

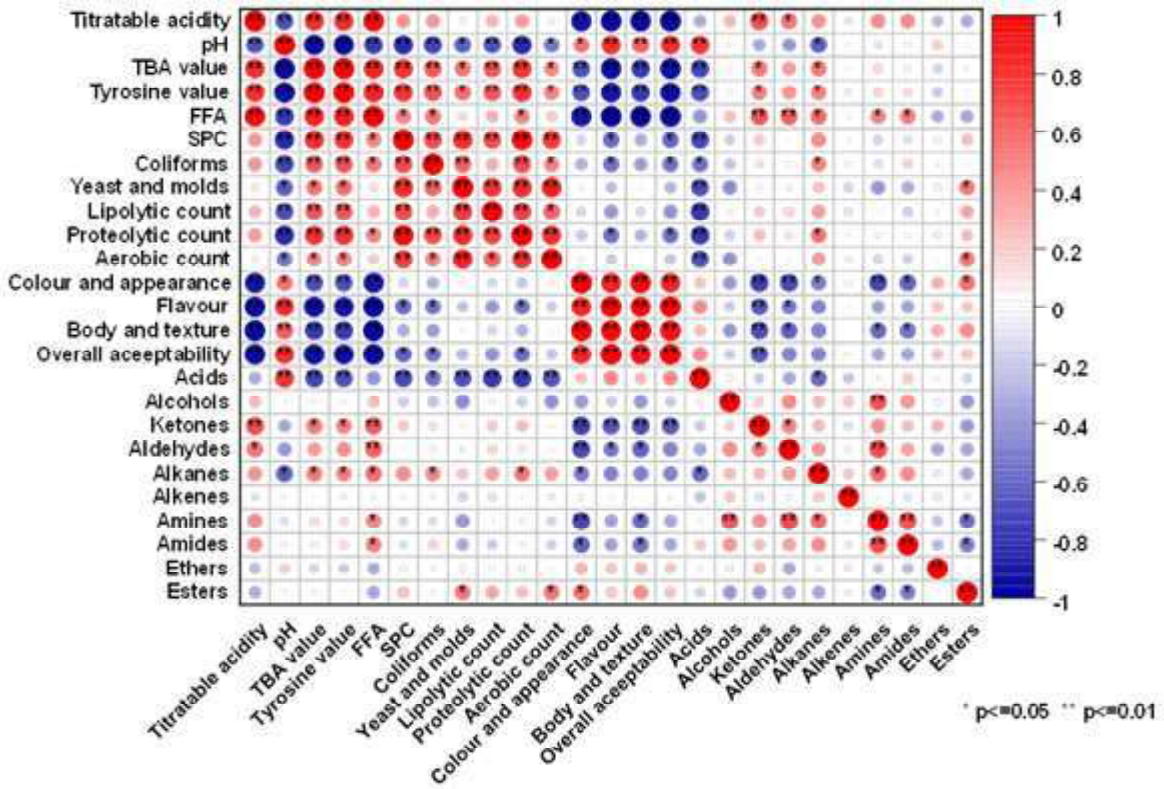


**Fig. 6.** Colour map of correlation analysis between headspace volatiles and quality attributes of *Sandesh* during storage

alkanes and opposed by acids. It revealed that amines, amides, alcohols and alkanes are increased with increasing shelf life and acids are inversely related. In the present study, loading with an absolute value of more than 0.7 indicates a strong influence on *Sandesh* quality during storage. From the results, fresh *Sandesh* has the highest number of acids. From the results, a greater total peak area of the acids is observed conspicuously in fresh *Sandesh* whereas the total peak area of alkanes, alcohols and ketones are recognized to be prominent in the treatments of DG4572, DG4548, and DG4524 samples. Moreover, amines, aldehydes and amides are noticed to be high in DG4572, DG4548 and DG4524 samples.

The major quality parameters (biochemical, microbial and sensory) of *Sandesh* during storage have been systematically evaluated from the PCA. According to the biplot of PCA (Fig. 2b), PC1 and PC2 were explained to be 67.10% and 22.97% of the total variance, respectively. Therefore, the total variance for the two factors can be explained as 90.07%. Furthermore, we have obtained meritorious levels of SPC, proteolytic count, aerobic count, lipolytic count and coliforms for DG3072, CG3072, DG3048 and DG3048 samples. Additionally, FFA and titratable acidity levels were observed to be more in CG4572, DG4572, DG4548 and CG4548 and also estimated the least sensory scores for CG4572, DG4572, DG4548 and CG4548.

**Correlation analysis between the headspace volatiles and quality changes**

The correlation analysis was systematically carried out to identify the association of the headspace volatile functional groups with quality attributes of *Sandesh* during storage under different conditions (Fig. 6). The correlation between volatile functional groups and quality changes revealed that the proteolytic count ($r=-0.774$, $P<0.01$), lipolytic count ($r=-0.765$, $P<0.01$), TBA value ($r=-0.726$, $P<0.01$), SPC ($r=-0.718$, $P<0.01$), tyrosine value ($r=-0.698$, $P<0.01$), Y&M count ($r=-0.696$, $P<0.01$), aerobic spore count ($r=-0.672$, $P<0.01$), coliform count ($r=-0.559$, $P<0.05$) were negatively correlated with acids, whereas pH ($r=+0.793$, $P<0.01$) was positively correlated with acids of *Sandesh* during storage. Moreover, titratable acidity ($r=+0.714$, $P<0.01$), FFA value ($r=+0.661$, $P<0.05$), TBA value ($r=+0.552$, $P<0.05$), tyrosine value ($r=+0.522$, $P<0.05$) were positively correlated with ketones, whereas colour and appearance ($r=-0.743$, $P<0.01$), body and texture ($r=-0.680$, $P<0.01$), flavour ($r=-0.657$, $P<0.01$), overall acceptability ($r=-0.649$, $P<0.01$) were negatively correlated with ketones of *Sandesh* during storage. FFA value ($r=+0.590$, $P<0.05$), proteolytic count ($r=+0.545$, $P<0.05$), TBA value ($r=+0.543$, $P<0.05$), tyrosine value ($r=+0.517$, $P<0.05$) and coliforms ($r=+0.500$, $P<0.05$) were positively correlated with alkanes, whereas pH ($r=-0.621$, $P<0.05$) and, colour and appearance ($r=-0.519$, $P<0.05$) were negatively correlated with alkanes of *Sandesh* during storage.

As we noticed, FFA value (r=+0.626, P<0.05) and titratable acidity (r=+0.563, P<0.05) were positively correlated with aldehydes since colour and appearance (r=-0.716, P<0.01), body and texture (r=-0.617, P<0.05) and flavour (r=-0.522, P<0.05) were negatively correlated with aldehydes of *Sandesh* during storage. It can be observed that FFA value (r=+0.514, P<0.05) was positively associated with amines, as colour and appearance (r=-0.701, P<0.01) and body and texture (r=-0.584, P<0.05) were negatively correlated with amines of *Sandesh* during storage. In amides, FFA value (r=+0.539, P<0.05) was positively correlated, whereas colour and appearance (r=-0.615, P<0.05) and, body and texture (r=-0.556, P<0.05) were negatively associated with amides of *Sandesh* during storage. We have also explored that colour and appearance (r=+0.562, P<0.05), Y&M (r=+0.543, P<0.05) and aerobic spore count (r=+0.541, P<0.05) were positively correlated with esters of *Sandesh* during storage.

## Conclusion

In summary, we have investigated 140 product-related headspace volatiles for determining the product's quality markers. The identified volatiles were of various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. However, interestingly, among all the volatiles of functional groups, acids, alcohols, alkanes and ketones were found to be the largest in terms of peak area during the storage. The dynamics of volatile compounds revealed continuous degradation of produced metabolite volatiles, which lead to the production of new volatiles. Notably, we have determined the predominant total peak area for the alcohols at end of the shelf life compared to the fresh product. Nevertheless, the peak area of alkanes and ketones enhanced gradually during storage in most of the treatments. Also, as expected significant changes in biochemical, microbial and sensory parameters of *Sandesh* packaged and stored at various conditions were observed. As a result of these quality changes, the shelf life of *Sandesh* has been assessed to be 72 h and 48 h at 30 °C and 45 °C, respectively, irrespective of the packaging materials. Of all the groups, amines, amides, alcohols, alkanes and acids are noticed to be the most significant functional groups among all the classes according to principal component analysis. The obtained *Sandesh* quality factors have been observed to be well correlated with acids, ketones, alkanes and aldehydes during storage from the correlation studies. From this study, headspace freshness marker of acid group was recognized along with headspace key spoilage markers such as alkanes, alcohols and ketones, which consists of 1-hexanol, 2-ethyl-; 1-pentanol; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-, (.+/-.); pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The presence of these markers can evaluate the quality of *Sandesh* in both the ways such as freshness and spoilage. The present investigation could be used for quality control aspects in dairy industry and also in the development of intelligent packaging systems for monitoring the real-time quality of the packaged product.

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RESEARCH ARTICLE

# Quality and functional attributes of vacuum-packed yak milk *mozzarella* cheese as influenced by storage

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**Abstract:** The fresh m*ozzarella* cheese was developed from yak milk by using direct acidification method, packaged under vacuum atmosphere and stored at 4±1°C for 35 days. The effect of storage was evaluated on proximate composition, physicochemical, functional, microbiological and sensory properties of yak milk *mozzarella* cheese at defined interval. Among the proximate composition, the moisture content of cheese varied from 48.17% at day 0 to 47.00% at the end of storage. As the storage period progressed, a non-significant differences ($p>0.05$) was observed for the fat, protein and ash content. Storage time significantly ($p<0.05$) affected the physicochemical properties of cheese sample, including pH, titratable acidity [lactic acid (% by weight)], tyrosine value (µg/g) and total free fatty acids (µm/g). A significant increase ($p<0.05$) in functional properties (meltability, free oil formation and stretchablity) of cheese sample was recorded as the storage progressed. A significant increase in the standard plate count and, yeast and mould count was also observed during progress of storage. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage. Therefore, it was concluded that the vacuum-packed yak milk *mozzarella* cheese sample could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes.

**Keywords:** Yak milk *mozzarella* cheese; vacuum packaging; quality attributes; functional properties; storage stability

## Introduction

*Mozzarella* is the most widely available un-ripened cheeses in the market. It is an Italian, highly valued and fresh stretched curd cheese that be linked to the pasta *filata* group (El Owni and Osman, 2009). It involves skilfully stretching the curd in hot water to provide a smooth texture and lively surface (Kosikowski, 1982). It is regulated by law as well as a member of the European Protected Designation of Origin (European Communities, 1996). Traditionally, *mozzarella* cheese is made from milk of buffalo, which is premium and nutrient dense cheese world-wide (Sameen et al. 2008; Vogt et al. 2015). Other milks may also be utilized for the production of *mozzarella* cheese, such as cow's, goat's, and sheep's in many countries. Yak milk is superfood and produced in considerable amount especially in Himalayan region of Ladakh, Jammu & Kashmir, Arunachal Pradesh, Sikkim, Himachal Pradesh, Uttarakhand (Singh et al. 2023a). Yak milk cheese production can help highlanders in sustaining their nutrition and boosting their economic activities despite the region's difficult climatic conditions (Singh et al. 2023b). Commercial yak cheese production exist in the yak rearing countries like Tibet, China, Bhutan, Nepal and Russia having a huge demands in the market. However, in India this sector is not well developed due to dwindling yak population. Yak milk is a good source of cheese making owing to its high casein-fat ratio, total solids, larger fat globules and have special qualities as well as it is also beneficial to human health (Zhang et al. 2017; Zhang et al. 2020; Singh et al. 2023a, b). Recently, in India, cheddar style-yak milk cheese (Singh et al. 2023b), yak milk *paneer* (Singh et al. 2022a) and yak milk *ghee* (Singh et al. 2022b) was developed by using yak milk. Increasing demand for *mozzarella* cheese is being driven by the diversification of pizza parlours and fast food chains worldwide (Bhattarai and Acharya, 2010). Since it melts and stretches easily,

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and primarily used in the fast food industry as well as in cheese-based salad dressings (Vogt et al. 2015). It is therefore imperative to standardise the process that uses yak milk and improves the quality of local cheese. It can be a boost to the highland pastoral nomads and yak dairy industries. As far as the use of yak milk in *mozzarella* cheese production is concerned, no research has been carried out in India. In light of yak milk's compositional differences as compared to cow and buffalo milk, it would be interesting to investigate how it is processed and the quality of its *mozzarella* cheese. Therefore, in the present study, yak milk *mozzarella* cheese was developed and was evaluated the effect of storage on proximate composition, physicochemical, functional, microbiological and sensory properties of the vacuum-packed yak milk *mozzarella* cheese during refrigerated storage.

## Materials and Methods

### Materials

The fresh yak milk (dry matter 16–18%, fat 5.50–7.50%, solid-non-fat 10-11% and protein 3.75–4.25%) was obtained from the Nyukmadung farm of ICAR-National Research Centre on yak, Dirang situated at Nyukmadung, Arunachal Pradesh, India, between latitude of 27°25.948' North and longitude of 092°08.658' East at an altitude of 2750 meters above mean sea level. Immediately, milk was brought to the laboratory under refrigerated conditions for cheese preparation. The microbial rennet (Meito®) commercially produced in granular form from *Mucor pusillus* var. *Lindt* was obtained from M/s Meito Sangyo Co., Ltd., Tokyo, Japan for yak milk *mozzarella* cheese preparation. Analytical grade chemicals and reagents used for various laboratory analyses were procured from standard firms. The commercially available rectangular plastic packaging material of 127µm thickness for vacuum packaging was purchased from Swiss Pac Pvt. Ltd., Gujrat, India.

### Preparation of yak milk mozzarella cheese

The yak milk *mozzarella* cheese preparation was carried out according to Guinee et al. (2002) and Kosikowski (1982) with some modifications (Fig. 1). The fresh yak milk was at first filtered and then standardised to a casein-fat ratio of 0.80 by using skim milk. The standardized milk was pasteurised at 72±1°C for 15 sec in a stainless-steel vat and then cooled down to 4-8°C. The pH of yak milk (6.46) was adjusted to 5.2-5.4 by using 25% citric acid, then the temperature was raised to 30±1°C. This was followed by the addition of rennet @25mg/L milk, mixed effectively and then the milk was left undisturbed at 30±1°C and waited for 30-45 min or until a firm set was reached. After coagulation, set curd was cut into small cubes (1-1.5 $cm^3$ in size) using sterile cheese knives (horizontally and vertically). The curd was left undisturbed for healing for 5-10 min, after healing the cooking was done from 31-41°C with in 40-45 min under simultaneous stirring. After cooking, the whey was drained through strainer. And cheese *coagulum* was taken out and submerged in 82±1°C water and manual stretching was done until forming a shining, smooth and homogenous mass. Thereafter, it was moulded into balls of 200-250 g in size and placed in to chilled brine solution (7.5%w/v non-iodised salt) for 2 h and subsequently surface dried for 4 h under refrigerated conditions (7±1°C). The weight of the prepared

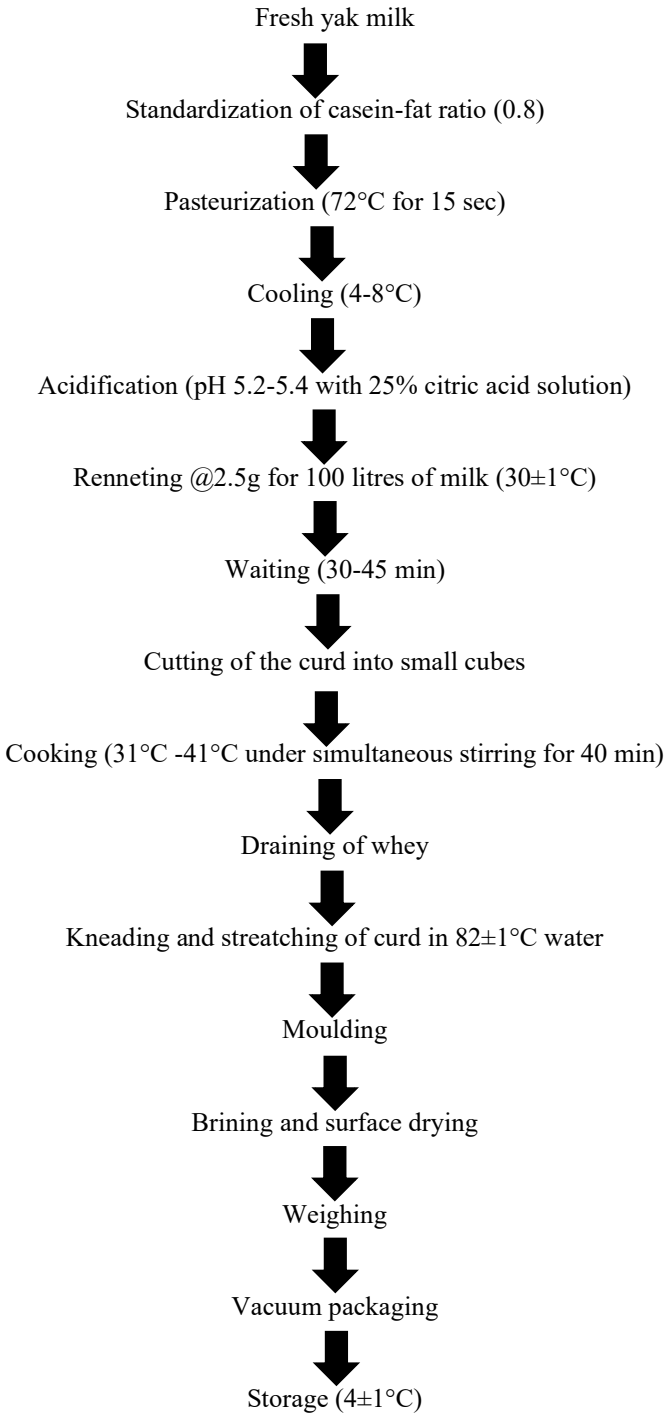


**Fig 1.** Process of preparation of yak milk *mozzarella* cheese by direct acidification

cheese was recorded and packed under vacuum nylon packs (127µm thickness) by using vacuum packaging machine (Model: QS500VSV4; Make: Sevana Electrical Appliances Pvt. Ltd., Kerala, India) and stored at 4±1°C for 35 days. Samples of cheese were drawn and evaluated for proximate composition, physicochemical, functional, microbiological, and sensory properties at defined intervals. The experiment was repeated twice, and the respective analysis were done in triplicate.

**Analytical procedures**

**Mozzarella cheese yield**

The *mozzarella* cheese yield (%) was calculated using the equation as given below:

$$Cheese\ yield\ (\%) = \frac{Weight\ of\ cheese\ (kg)}{Weight\ of\ milk\ taken\ (kg)} \times 100$$

**Proximate composition**

The moisture and ash content of cheese sample was estimated by following the Association of Official Analytical Chemists' approved methods (AOAC, 2005). whereas, the fat content of cheese sample was evaluated by using the Gerber method and total protein content was estimated by determining the total nitrogen using the Kjeldahl method (IDF, 1993) and converting it to protein content by multiplying by 6.38.

**Physicochemical properties**

The pH values (grated cheese sample and distilled water, 1:2) were recorded electrometrically using a digital pH meter (Make: PC 2700, Oakton®, India). The titratable acidity was determined by the titration method suggested by AOAC (2005) and results were recorded in lactic acid (% by weight). Juffs (1973) method was used to determine the tyrosine value and results were shown in µg/g. Deeth and Fitz-Gerald (1976) extraction-titration method was used to evaluate the total free fatty acids (TFFA) content, and results were shown in µm/g.

**Functional Properties**

The meltability of cheese sample was evaluated using a prescribed method from Muthukumarappan et al. (1999) with slight modifications. Samples (3.5 cm in diameter and 1 cm in height was tempered at room temperature for 30 min. Thereafter, sample was then heated at 100±1°C for 5 min in hot air oven. After the cooling of melted cheese at room temperature for 5 min, the meltability of cheese was recorded by measuring the final diameter (minimum and maximum) of the cheese discs at 5 different places and expressed as the mean values in cm. Stretchability of cheese sample was evaluated by following the method of Lemay et al. (1994). The average values in cm was measured as streatchbility of cheese. Free oil formation or oiling off property of cheese sample was evaluated by the method of Breen et al. (1964) with the following modification. Discs of 1.5 cm in diameter and 0.5 cm in thickness were cut, melted on filter paper in the oven at 100±1°C for 5 min. The average of free oil formation at four different angles was expressed in cm.

**Microbiological analysis**

Microbiological analysis of the sample was done using standard methods mentioned by the American public health association (APHA, 2005). In order to prepare the media, careful attention was paid to the manufacturer's instructions (Plate Count Agar for standard plate count, Potato Dextrose Agar for yeast and mould count, and Violet Red Bile Agar for coliform count). The plates showed 30–300 colonies were counted, and their number was multiplied by reciprocal of dilution. The microbial count was recorded as $\log_{10}$CFU/g of the sample.

**Sensory analysis**

A twenty four (n-24) semi-trained members (aged between 20 to 60 years) composed of scientists, administrative staff and other employees from ICAR-NRC on Yak, Dirang were selected as the sensory panellists for assessing the yak milk *mozzarella* cheese. The properties of the developed product were defined, and panellists were familiarised with the Sensory Performa before performing the analysis. The cheese was cut uniformly and pieces were tempered for 20-30 min at room temperature (20±2°C) then served individually to each panellists. The coded samples were evaluated for their sensory traits like colour & appearance, flavour, body & texture and overall acceptability using a hedonic scale of 9-point intensity varied from extreme dislike (score = 1) to extreme like (score = 9).

**Statistical analysis**

Statistical analysis was done using the one-way analysis of variance (ANOVA). The ANOVA was done by using Statistical Package for the Social Sciences (SPSS) software package trial version 22.0 (IBM SPSS Inc. Chicago IL, USA). The statistical significance at 5% level was considered significant. Post-hoc analysis was done using Duncan's multiple range test. The data were recorded in the form of average ± standard deviation.

**Results and discussion**

The obtained result showed the average yield of 15.09% for yak milk *mozzarella* cheese in the present study. The effect of the storage period on the proximate composition of cheese sample is summarized in Table 1. The moisture content (%) of cheese varied from 48.17±0.89 at day 0 to 47.00±0.61 at the end of storage. Various factors may contribute to the variation in moisture content in cheese, including preparation methods (El-Owni and Osman, 2009), cooking temperatures (McSweeney, 2007), and compositional differences (size of fat globules and casein micelles

characteristics) of yak milk (Zhang et al. 2020). Guinee et al. (2002) stated that having a low pH during storage causes the protein network to become unstable, which causes more moisture to be released. A non-significant differences ($p>0.05$) was recorded for the fat, protein and ash content during the progress of storage period. The fat content (%), protein content (%) and ash content (%) was 24.24±0.40, 22.64±0.55 and 2.96±0.22 on day 0 and 24.76±0.51, 23.27±0.94 and 3.14±0.19 on day 35, respectively. Smaller variations in the proximate composition of cheese might be due to variations during storage conditions. The compositional differences of yak milk influences the proximate composition of cheese (Singh et al. 2023a). Table 2 showed physicochemical properties of cheese sample during storage. Storage period ($p<0.05$) had a significant effect on pH and TA of cheese sample. The pH and TA value varied from 5.45±0.04 to 5.62±0.03 and 0.39±0.03 to 0.52±0.04, respectively, during the storage time. This would be due to biochemical changes occurred during storage and proximate composition of cheese (Sameen et al. 2008). Storage period had a significant effect on the tyrosine value of cheese sample ($p<0.05$). It was increased from 101.50 µg/g to 322.33 µg/g in the first 28 days of storage, and then began to decrease after the 28$^{th}$ day of storage period. In cheese sample, tyrosine level has been found to increase because enzymes and microorganisms hydrolysed proteins during storage (Singh et al. 2012). The results are in line with the study of Singh et al. (2022a) who also reported the increase in tyrosine value of yak milk *paneer* during storage. There was significant increase in free fatty acid (FFA) content of cheese sample during storage. At day 0 of storage, the FFA value was 1.69µm/g and at the end of storage period (day 35), it was increased to 2.45µm/g. It might be due to slower rate of lipolysis under mentioned storage conditions.

Functional characteristics such as meltability, free oil formation (FOF) and stretchablity are presented in Table 3. Meltability is the ability of cheese to melt uniformly, smoothly, and homogeneously (cheese shred should not be visible) without releasing oil or becoming watery (Johnson, 2000). The initial meltability of cheese sample on day 0 was 4.90 cm which significantly increased ($p<0.05$) to 7.53 after 35 days of storage. Similar observations were also observed by Imm et al. (2003) who observed that refrigerated storage of bovine and caprine *mozzarella* cheese (MC) influenced the meltability. The meltability of MC depended on various factors such as water partitioning, rearrangement of protein matrix (McMahon et al. 1999), displacement of the para-casein matrix (Guinee et al. 2001) and the amount as well as distribution of fat in the protein matrix (Imm et al. 2003). Free oil formation, also known as 'oiling off' or 'fat leakage', occurs when free oil separates from the melted cheese and accumulates in pockets or pools, particularly on its surface (Jana et al. 2017). A significant increase ($p<0.05$) in FOF of cheese sample was recorded as the storage progressed (Table 3). Free

**Table 1:** Effect of storage on proximate composition of yak milk *mozzarella* cheese (Mean±S.D.)

| Proximate composition (%) | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Moisture content | 48.17 ±0.89$^{b}$ | 48.32 ±0.32$^{b}$ | 47.93 ±0.56$^{b}$ | 47.46 ±0.66$^{ab}$ | 47.60 ±0.75$^{ab}$ | 47.00 ±0.61$^{a}$ |
| Fat | 24.24 ±0.40$^{a}$ | 24.20 ±0.49$^{a}$ | 24.35 ±0.54$^{a}$ | 24.56 ±0.55$^{a}$ | 24.44 ±0.57$^{a}$ | 24.76 ±0.51$^{a}$ |
| Protein | 22.64 ±0.55$^{a}$ | 22.59 ±0.56$^{a}$ | 22.71 ±0.56$^{a}$ | 23.02 ±0.45$^{a}$ | 22.97 ±0.42$^{a}$ | 23.27 ±0.94$^{a}$ |
| Ash | 2.96 ±0.22$^{a}$ | 2.92 ±0.20$^{a}$ | 2.98 ±0.19$^{a}$ | 3.07 ±0.12$^{a}$ | 3.04 ±0.18$^{a}$ | 3.14 ±0.19$^{a}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

**Table 2:** Effect of storage on physicochemical properties of yak milk *mozzarella* cheese (Mean±S.D.)

| Physicochemical properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| pH | 5.58 ±0.07$^{cd}$ | 5.62 ±0.03$^{d}$ | 5.60 ±0.02$^{cd}$ | 5.56 ±0.02$^{bc}$ | 5.52 ±0.05$^{b}$ | 5.45 ±0.04$^{a}$ |
| Titratable Acidity [TA; lactic acid (% by weight)] | 0.39 ±0.03$^{a}$ | 0.41 ±0.03$^{a}$ | 0.42 ±0.04$^{a}$ | 0.46 ±0.03$^{b}$ | 0.47 ±0.04$^{b}$ | 0.52 ±0.04$^{c}$ |
| Tyrosine value (µg/g) | 101.50 ±8.17$^{a}$ | 238.44 ±14.08$^{c}$ | 274.83 ±20.14$^{d}$ | 280.11 ±12.27$^{d}$ | 322.33 ±23.68$^{e}$ | 214.83 ±5.96$^{b}$ |
| Total free fatty acids (µm/g) | 1.69 ±0.12$^{a}$ | 1.81 ±0.16$^{ab}$ | 2.01 ±0.15$^{bc}$ | 2.13 ±0.27$^{cd}$ | 2.33 ±0.21$^{de}$ | 2.45 ±0.17$^{e}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

oil started increasing significantly ($p<0.05$) after 14 days of storage in cheese sample, and it continued to increase until 35 days of storage. There are several factors that affect the formation of free oil in *mozzarella* cheese, including fat content, size of fat globules, fatty acid profile, and proteolysis (Tunick, 1994). The stretchability of melted cheese refers to its ability to form fibrous strands that elongate without breaking under tension (Jana et al. 2017). The maximum stretch (35.92 cm) was observed on day 35 of storage which was significantly ($p<0.05$) higher than the value (26.25 cm) obtained on day 1 of storage. According to Rehman et al. (2008), a commercial pizza cheese has a stretch value of 25.27 cm. Whereas, a minimum stretch of 3.0 inches (7.62 cm) of unbroken string is specified for Pizza cheese in the United States (USDA, 2007).

It is important from the perspective of food safety to consider the microbiological quality of cheese, since it is an excellent growth medium for a variety of microorganisms (Dharaiya et al. 2021). Table 4 reports the changes in microbiological properties for cheese sample during storage. The initial SPC ($log_{10}$CFU/g) in cheese sample was 3.37 on day 0 of storage which was increased to 4.66 on day 35 of storage. The initial average value of YMC count ($log_{10}$CFU/g) of cheese sample increased from 1.13 to 2.74 after the end of storage period. It indicates storage had significant impact ($p<0.05$) on changes in SPC and YMC counts. The coliform count was absent in cheese sample throughout the storage. During the scalding process, high temperatures reduce the microbial flora associated with contamination and increase the safety of *mozzarella* cheese (Marth and Steele, 2005). According to Han et al. (2015), natural *mozzarella* cheese prepared by direct acidification had a viable count of 5.8 $log_{10}$CFU/g.

*Mozzarella* cheese made from yak milk had shown delicious milky flavour, homogenous texture, and a whitish-yellowish colour

**Table 3:** Effect of storage on functional properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Functional properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Meltability (cm) | 4.90 ±0.24$^{a}$ | 5.32 ±0.21$^{b}$ | 5.80 ±0.24$^{c}$ | 6.27 ±0.48$^{d}$ | 6.98 ±0.25$^{e}$ | 7.53 ±0.23$^{f}$ |
| Free oil formation (cm) | 2.45 ±0.10$^{a}$ | 2.65 ±0.10$^{a}$ | 3.00 ±0.14$^{b}$ | 3.30 ±0.23$^{c}$ | 3.77 ±0.23$^{d}$ | 4.35 ±0.22$^{e}$ |
| Stretchability (cm) | 26.25 ±1.57$^{a}$ | 28.67 ±2.04$^{b}$ | 29.33 ±1.08$^{bc}$ | 31.17 ±1.50$^{c}$ | 33.50 ±1.70$^{d}$ | 35.92 ±1.80$^{e}$ |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

**Table 4:** Effect of storage on microbiological properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Microbiological properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Standard Plate Count ($Log_{10}$CFU/g) | 3.37 ±0.26$^{a}$ | 3.84 ±0.06$^{b}$ | 4.13 ±0.07$^{c}$ | 4.25 ±0.04$^{c}$ | 4.51 ±0.02$^{d}$ | 4.66 ±0.03$^{e}$ |
| Yeast & Mould Count ($Log_{10}$CFU/g) | 1.13 ±0.21$^{a}$ | 1.88 ±0.10$^{b}$ | 2.01 ±0.12$^{b}$ | 2.38 ±0.09$^{c}$ | 2.64 ±0.07$^{d}$ | 2.74 ±0.04$^{d}$ |
| Coliform count ($Log_{10}$CFU/g) | ND | ND | ND | ND | ND | ND |

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$). ND-Not detected

**Table 5:** Effect of storage on sensory properties of yak milk *mozzarella* cheese (Mean±S.D.).

| Sensory properties | Storage days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Colour & Appearance | 8.33 ±0.76$^{a}$ | 8.35 ±0.91$^{a}$ | 8.17 ±0.78$^{a}$ | 8.02 ±0.80$^{a}$ | 8.23 ±0.83$^{a}$ | NP |
| Flavour | 8.27 ±0.71$^{a}$ | 7.79 ±0.99$^{a}$ | 8.04 ±0.82$^{a}$ | 7.73 ±0.98$^{a}$ | 8.19 ±0.89$^{a}$ | NP |
| Body & Texture | 8.25 ±0.75$^{b}$ | 8.17 ±0.86$^{b}$ | 8.13 ±0.77$^{b}$ | 7.15 ±1.04$^{a}$ | 8.29 ±0.86$^{b}$ | NP |
| Overall Acceptability | 8.19 ±0.82$^{a}$ | 8.30 ±0.82$^{a}$ | 8.13 ±0.72$^{a}$ | 7.91 ±1.02$^{a}$ | 8.29 ±0.86$^{a}$ | NP |

n=24, *mean with different superscripts in a row differs significantly ($p<0.05$). NP-Not Performed

during sensory evaluation by the sensory panellists. Samples of cheese were evaluated periodically for various sensory properties including colour and appearance, flavour, body and texture, and overall acceptability. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage (Table 5). It was evident from Table 5, the sensory scores was higher at the beginning and it was lower in later part of storage period. It is because *mozzarella* cheese, unlike most other cheeses, is not ripened or aged, and its sensory properties reduce with the progress of storage time (Yazici et al. 2010; Sulieman et al. 2013). The sensory evaluation of cheese was discontinued on day 35 of storage period due to development of off flavour and mouldy surface on cheese sample. Changes in physicochemical, biochemical, and microbiological properties in cheese during storage might explain this trend.

## Conclusion

In the present study, *mozzarella* cheese was developed by using yak milk for the first time in India. In order to determine the shelf-life of vacuum-packed yak milk *mozzarella* cheese (YMMC), the cheese sample was analysed for proximate composition, physicochemical, functional, microbiological and sensory properties during storage. Based on the obtained results, it was found that storage significantly influenced the moisture content, meltability, free oil formation, stretchablity, physicochemical and microbiological properties of YMMC. The cheese was relished by the sensory panellists throughout the storage and could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes. Further research is also needed to fully understand the textural, rheological behaviour and other preparation methods for YMMC. Taking part in yak cheese business could be lucrative for highlanders and provide them with financial assistance.

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RESEARCH ARTICLE

# Prevalence of mastitis and antibiotic resistant *E. coli* and *S. aureus* in dairy animals

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**Abstract:** The prevalence of mastitis in milking animals and subsequent antibiotic usage is one of the major concerns in dairy sector. In this work, a baseline data was generated by collecting 675 raw milk samples from organized and individual dairy farms in Haryana. The milk samples were screened for mastitis, antibiotic residues and antimicrobial resistance (AMR) *E. coli* and *S. aureus* using rapid BD Phoenix M50 ID/ AST system and conventional methods. The study indicated 14.37% animals infected with sub-clinical mastitis and 11.25% with clinical mastitis. 79 milk samples from normal and infected animals were found contaminated with antibiotic residues with presence of enrofloxacin, streptomycin, tetracycline, sulfa drugs and multi drug residues. Out of 675 samples, 173 were infected with mastitis with involvement of *E. coli* in 18.49% and *S. aureus* in 38.72%. In *E. coli* isolates, the maximum resistance of 25% was observed against Ampicillin, Ciprofloxacin and Levofloxacin and Extended spectrum β-Lactamase (ESBL) productionwas observed in 15.65% infected samples.The maximum resistance of 32% was observed in *Staphylococcus aureus* against Ampicillin and Penicillin-G and Methicilin Resistant Staphylococcus aureus (MRSA) was found in 22.38% along with β-Lactamase resistance in 31.34% of infected samples.The main reason behind the increasing number of resistant pathogens may be due to the indiscriminate use of antibiotics by untrained veterinary professionals and lack of diagnostic facilities, hence regular screening of sub-clinical mastitis should be practiced to control the usage of antimicrobials and resistant development in dairy pathogens.

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## Introduction

Mastitis is a major problem affecting all milk producing animals worldwide and is one of the main reasons for impaired milk quality (Bradley, 2002; Le Roux et al. 2003). Mastitis is the inflammation of udder; the term comes from the Greek word i.e. Masto- referring to the mammary gland and its meaning "inflammation" (Blood and Studdert., 1999). Mastitis can be sub-clinical, clinical and chronic depending on severity of inflammation. Dairy farmers face a financial burden from bovine mastitis, and preventive mitigation strategies are essential for the long-term viability of any dairy production. Controlling the infection, minimizing the risk of persistent infections, and directing antimicrobial therapy all need the identification of etiological agents (Duarte et al. 2015). It is mainly caused by bacterial pathogens which include *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, and Mycoplasma sp.* and environmental pathogens involving *E. coli* and *Klebsiella spp*. Other common pathogens include *Corynebacterium spp., coagulase-negative staphylococci* and *Pseudomonas aeruginosa* (Motwani & Kishore., 2011). Despite extensive research into controlling bovine mastitis, the occurrence of mastitis remains high, resulting in massive losses for the dairy industry (Lee et al. 2008). The pathogens responsible for producing mastitis will determine the sort of mastitis treatment that should be offered. Greater than 200,000 somatic cells per milliliter (SCC) is a sign of inflammation and subclinical mastitis (Cobirka et al. 2020). During the infection caused by the micro-organisms, the host immune response is activated to eliminate the invading microorganisms lead to inflammation and damage milk-producing tissue of the mammary gland leading to decreased in milk yield (Egyedy and Ametaj, 2022) . Milk from animals with mastitis cannot be used for human consumption because it has altered chemical composition and organoleptic proprieties (Kobayashi et al. 2013). Moreover, milk from diseased animals negatively affects the milk processing and shelf-life of final dairy products. This disease is considered to be the major cause of economic loss to dairy farmers (Tommasoni et al. 2023). Mastitis can be reduced by some aspects of dairy farming such as feeding

practices, animal husbandry, hygiene and general health care. Increasing number of infection has led to the increased use of antibiotics for treatment but their indiscriminate use by untrained veterinary professionals or quacks has resulted in increased resistance of antibiotics among the dairy animals which is a growing concern and need to be monitored carefully (Eltholth et al. 2022). The present work is focused on detection of mastitis, antibiotic residues, bacterial pathogens (*E. coli* and *S. aureus*) and their resistance pattern in raw milk samples.

**Material and methods**

All the experiments required for present research were run in triplicate and results were interpreted all the study carried out at National Referral Centre for Milk Quality and Safety and Dairy Microbiology Division, NDRI, Karnal.

**Sample collection**

A total of 675 raw milk samples were collected from different districts of Haryana i.e. Karnal, Ambala and Sonipat during the year 2018-20. Collection of milk samples was done from healthy as well as infected cows/ buffaloes. Milk sampling was carried out following aseptic procedures as described by National Mastitis Council (NMC, 2004). The time chosen for milk sample collection was before milking. All the details of the animal along with the collection date were recorded. The hands were washed properly with soap and water and the gloves were worn while sampling. Any dirt or debris present on the teat of the animal was brushed and initial few streams of milk were discarded. The teat was pre-dipped with an effective teat dip (6 part of 0.5% iodine with 1 part of glycerin) and left for few seconds. Each teat was dried properly with paper or cloth towel. The teat end was scrubbed for 15-20 seconds with cotton or cloth gauze moistened with 70% to 80% alcohol or isopropyl alcohol. The sample container was opened and immediately the sample was taken preventing the teat touching the container. The sample container was kept in ice box until delivered to lab.

**Detection of Mastitis using CMT**

The California Mastitis Test (CMT) is a simple indicator of the Somatic Cell Count (SCC) of milk. The procedure of CMT was followed as per the instructions given in the kit manual. 3.0 ml milk was taken in four-compartment paddle and equal amount of CMT reagent was added. After addition of reagent and sample, CMT paddle was rotated 10 times in an anti-clockwise direction and graded based on gel formation, scores as Negative (N), Trace (T), 1, 2and 3 were given.

**Detection of Mastitis using Somatic cell counter**

Somatic cells are purely animal body cells present in small levels in normal milk. High levels of SCCs in milk indicate poor quality milk that is caused by an intra-mammary infection. The milk analyzer (Model- Ekomilk Scan) measures the flowing time of the milk through the sample mixer capillary and determines the number of somatic cells in accordance with time. The viscosity measurement is temperature sensitive and uses Ekoprim reagent as surfactant. The somatic cells were measured as per the instruction's manual. 10mL of milk sample and 5mL of Ekoprim reagent was added in the sample bulb and the bulb rotated for few seconds after pressing the run button and displayed somatic cell count on the screen along with the time based on viscosity

**Detection of antibiotics using Spore based kits**

Preliminary screening of antibiotics was done using Paper strip and DPA kits developed at ICAR-NDRI, Karnal as per the test procedure given by Swathi, 2017. Test kit is working on spore germination- inhibition principle and can detect antibiotics in milk at regulatory limits set by FSSAI/ CODEX. In the presence of antibiotics, spore germination is inhibited whereas in the absence of antibiotics spores germinate leading to release of DPA/or enzyme that react with the substrate functionalized on strip resulting in color change from purple to yellow in DPA kit and colorless to blue on strip test.

**Quantitative detection of antibiotic groups using AOAC approved CHARM/ ROSA**

The antibiotic contaminated milk samples from normal and infected animals were tested using Rapid One step Assay (ROSA) which works on the principle of lateral flow assay. The ROSA Test uses receptors with binding to drugs. The test was performed as per instructions in operator's manual. The incubator was set at desired temperature. 300µL of milk sample was added to the strip and incubated for 8 min and results were observed on the strip and quantified using ROSA reader.

**Isolation and identification of *E. coli and S.aureus***

Isolation and identification of *E. coli* was done using ISO procedure IS: 5887 Part-1:1976 (RA-2018). The milk samples were first enriched in McConkey broth and loopful was streaked on McConkey Agar and Eosin methylene blue (EMB) agar. The inoculated media was then incubated at 37°C overnight. If there was growth in McConkey broth along with fermentation of lactose, the loopful was streaked onto solid media and incubated overnight at 37°C. The suspected colonies were further identified using biochemical tests as mentioned in ISO protocol. The confirmed isolates were also tested using BD Phoenix M50. Isolation and identification of *S. aureus* was done using ISO procedure IS: 5887(Part-8/sec-1):2002 (RA-2018). The test sample was spread onto Baird Parker agar (BPA) plates and incubated for 24-48 hrs at 37°C. The suspected grey black colonies with opaque zone were identified using catalase and coagulase test. The confirmed isolates were also tested using BD Phoenix M50.

**Antibiotic Susceptibility Test (AST)**

The samples which were found positive for *E. coli* and *S. aureus* were further processed for their antibiotic sensitivity/ resistance using different antibiotic discs. Standard disk diffusion assay was conducted using Muller-Hinton agar and broth culture equivalent to 0.5 McFarland standards as recommended by the Clinical and Laboratory Standards Institute (2014). Antibiotic disks were chosen based on commonly used antibiotics for animal and human therapy in the study region. Antibiotics used for *E. coli* were Ceftriaxone, Ceftazidime, Cefotaxime and Cefepime, Imipenem, Meropenem, Ertapenem and Doripenem. Antibiotics used for *S. aureus* were Cefoxitin and Oxacillin. *E. coli* and *S. aureus* isolates were processed for AST using disc diffusion method for preliminary screening and further the resistance pattern was studied using confirmatory tests: double disc test for Extended spectrum β- lactamases (ESBL) detection, Modified Hodge test (MHT) and Modified carbapenamase inactivation method (mCIM)for Carbapenamase (KPC) detection and streaking on Methicillin resistant *S.aureus* (MeReSa) agar for MRSA detection. The AST pattern of positive isolates was studied using conventional method and random samples were further confirmed with BD Phoenix M50.

**Statistical Analysis**

Data was analyzed statistically in three replicates according to Snedecor and Cochran (1980)

**Results and Discussion**

A total of 675 raw milk samples from both organized and individual dairy farmers were collected from three districts of Haryana state i.e. Karnal, Ambala and Sonipat. Selection of animals in organized dairy farms and un-organized sector was random. Milk samples were collected directly into the sterile containers from cow's teat and immediately transferred to lab after proper labeling. These samples were tested for Mastitis infection, presence of antibiotic residues, bacterial pathogens i.e. *E. coli* and *S. aureus* and their phenotypic resistance profile.

**Prevalence of mastitis**

The study showed prevalence of 11.25% clinical mastitis and 14.37% sub-clinical mastitis (Fig.1). Among Mastitis positive samples, S.aureus was detected in 40.78% of clinical mastitis and 37.11% in clinical cases; while in case of *E.coli* 17.10% was detected in clinical mastitis cases and 19.58% in sub-clinical cases, as shown in (Fig 2).

The presence of mastitis in raw milk was 28% using CMT and 25.62% using SCC indicating strong positive correlation as also reported by Badiuzzaman et al. 2015 and Bitew et al.2010. In a similar investigation, Bhat et al. 2017 reported 11.50% and 27.81% (Maheshwari et al. 2016) respectively. The variation in sub-clinical mastitis may be attributed to the selection of animals, season, breed and sanitary conditions. Higher prevalence of sub-clinical mastitis compared to clinical mastitis in present investigation was also supported by Sori et al. 2011. Sub-clinical mastitis give invisible and silent symptoms as reported by Karimuribo et al. 2017 which is usually given less attention by farmers when it comes to treatment unlike clinical mastitis which shows visible and prominent symptoms towards which treatment and control efforts are concentrated.

**Prevalence of Antibiotic Residues**

The presence of antibiotic residues in milk is a serious concern keeping in view of its processing implications in terms of starter failure in fermented products and public health implications through development of antimicrobial resistance (AMR). Accordingly, surveillance study on antibiotic residues in milk was carried out. The milk samples collected from normal and infected animals were initially screened for Qualitative analysis using spore based kits (DPA/ paper strips) developed at ICAR-NDRI, Karnal. Out of 675 milk samples, normal milk used for

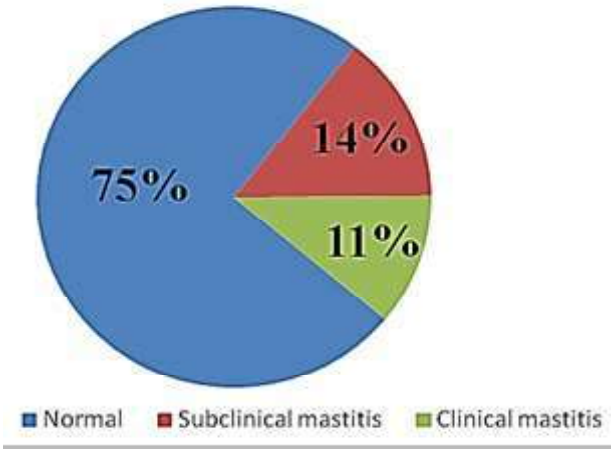


**Fig. 1** Incidence of mastitis in raw milk

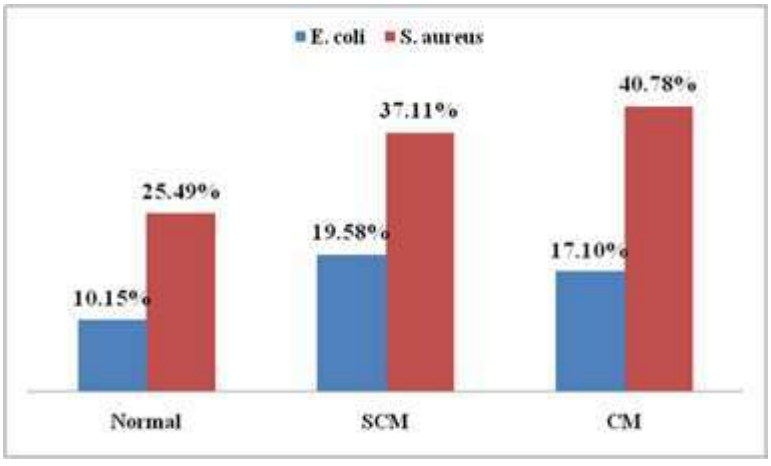


**Fig. 2** Incidence of E. coli and S. aureus in raw milk

processing purpose showed presence of antibiotics in 2.96%. Milk from treated animals which is unfit for processing with sub-clinical mastitis were positive with antibiotic in 4.1 % and clinical milk samples with 4.59%. Antibiotic residues like enrofloxacin, streptomycin, tetracycline, sulfa drugs and multidrug residues were detected. In a similar study, Moudgil et al. 2019 reported 11.30% milk samples from Punjab contaminated with antibiotic residues. In recent study, FSSAI (2018) has also reported the presence of antibiotic residues in milk however, the sample size was small and needs further surveillance work to support the findings reported in the current investigation.

**Isolation and Identification of *E. coli* & *S. aureus***

Out of 675 samples, 173 were infected with mastitis with involvement of *S. aureus* in 40.78% in clinical and 37.11% in sub-clinical cases. Similarly, *E. coli* was detected in 17.10 % and 19.58% respectively (Fig.2). Kumar et al. 2015 reported similar findings with incidence of 33.82%*S. aureus* and 14.91%*E. coli*. The non-infected milk samples also showed presence of *E. coli* in 10.15% and *S. aureus* in 25.49% .

**Antibiotic Susceptibility Test**

The AST pattern of positive isolates was studied using conventional method and random samples were further confirmed with BD Phoenix M50.

**AST pattern of *E.coli* and *S.aureus* isolates**

In the case of *E. coli* isolates, the highest level of resistance at 25% was observed against Ampicillin, Lowest level of resistance at 6.25% was observed against chloramphenical- and no resistance was observed against amikacin, amoxy-clav, Cefoparazone-sulbactam ,Cefoxitin, Gentamicin, Imipenem, Meropenem and Piperacillin- tazobactam(Fig. 3a). In case of *S. aureus*, the highest resistance of 32% was observed against Ampicillin and Penicillin- No resistance was observed against broad range of antibiotic (Fig. 3b).

In case of *S. aureus*, the maximum resistance of 32% was observed against Ampicillin and Penicillin-G followed by Cefazolin, Cefoxitin and Oxacillin (14%). The resistance against norfloxacin, gentamicin and tetracycline ranged between 2-8%. No resistance

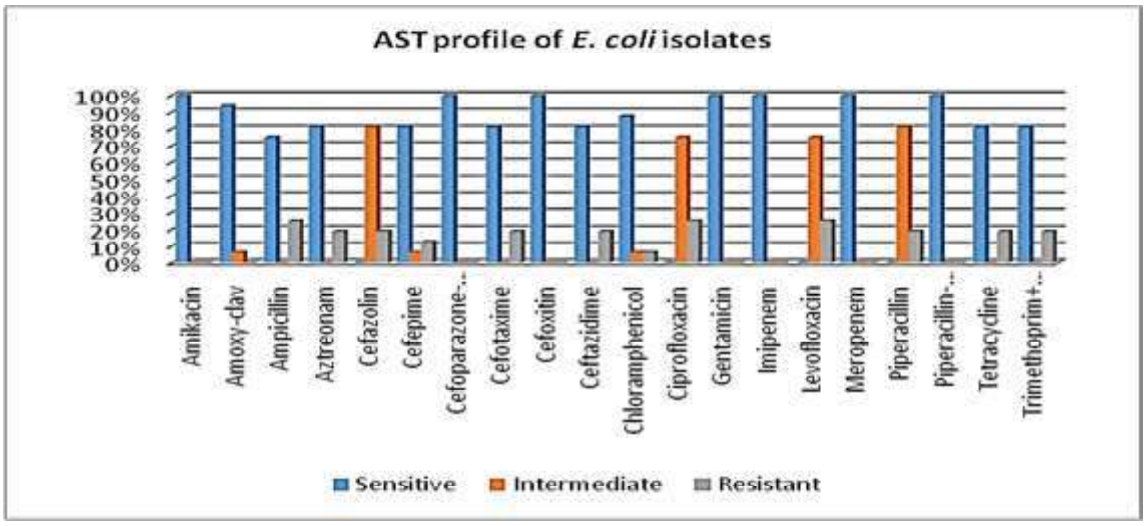


**Fig. 3 (a)** Graph showing AST profile of *E. coli* isolates

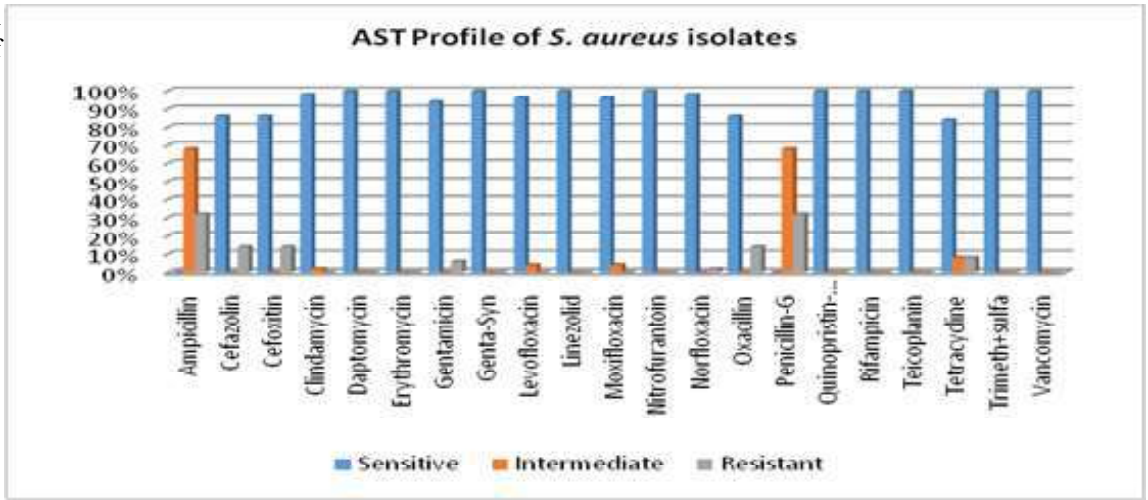


**Fig. 3 (b)** Graph showing AST profile of *S. aureus* isolates

was observed against Clindamycin, Daptomycin, Erythromycin, Gentamycin-Syn, Levofloxacin, Linozolid, Moxifloxacin, Nitrofurantoin, Quinopristin-dalfopristin, Rifampicin, Teicoplanin, Trimethoprim+ sulfamethaxole and Vancomycin (Fig. 3b)

The incidence of ESBL producing *E. coli was* 26.31% in sub-clinical mastitis . None of the clinical milk samples showed presence of ESBL E.coli . However , normal milk samples also showed presence of ESBL in 7.84% and carbapenase producing E.coli in 1.96 % which was considered as serious finding (Fig.4a). Our findings are in agreement with reports of Sharif et al.2017 who recorded average incidence of 20% ESBL in infected samples. Bhoomika et al. 2016 and Dewangan et al. 2017 also reported incidences of ESBL producing *E. coli* in raw milk samples as 8.22% and 7.69% in Chattisgarh.

The presence of *S. aureus* with resistance of MRSA was observed 25% in sub-clinical and 19.35% in clinical mastitis. In a similar study, Shah et al.2019 reported Methicillin resistance *Staphylococcus aureus* (MRSA) resistance in *S. aureus* with 25% in infected samples. β-lactamase resistance (BLACT) in *S. aureus* was also investigatedwith presence of 36.11% in sub-clinical and 25.80%in clinical mastitis (Fig.4b).The presence of MRSA and BLACT was also detected in normal samples wherein incidence of 9.37% and 13.28% was observed respectively. In a recent study carried out by Deepak et al. 2020 reported 9.3% presence of MRSA in bovine milk collected from healthy cattle in Chennai.

Normal milk samples showed ESBL presence at 7.84% and carbapenase producing E.coli at 1.96 % which was considered a notable finding Fig 4a. β-lactamase resistance (BLACT) in *S. aureus* was also probed at 36.11% in sub-clinical and 25.80%in clinical mastitis (Fig.4 b).

## Conclusion

The prevalence of mastitis in milking animals is one of the growing concerns in dairy sector. The current investigation was carried out in infected milk samples collected from organized as well as un-organized sector keeping in view of the fact that prevailing hygienic conditions are widely different across the country. For its understanding, a baseline data was generated by collecting 675 raw milk samples from organized and individual dairy farms in Haryana. The presence of mastitis in raw milk was 28% using CMT and 25.62% using SCC indicating significant correlation. The study showed prevalence of 11.25% clinical mastitis and 14.37% sub-clinical mastitis. The variation in sub-clinical mastitis may be attributed to the selection of animals, season, breed and sanitary conditions. Sub-clinical mastitis give invisible and silent symptoms which is usually given less attention by farmers when it comes to treatment unlike clinical mastitis which shows visible and prominent symptoms towards which treatment and control efforts are concentrated. 675 milk samples were analyzed for the presence of antibiotic residues and enrofloxacin, streptomycin, tetracycline, sulfa drugs and multidrug residues were detected. Study on ID profile indicated the presence of *E. coli* in 18.49% and *S. aureus* in 38.72% infected milk samples. The AST profile revealed that *E. coli* isolates showed maximum resistance of 25% against Ampicillin, Ciprofloxacin and Levofloxacin and Extended spectrum β-Lactamase (ESBL) production in 15.65% infected samples. The maximum resistance of 32% was observed in *Staphylococcus aureus* against Ampicillin and Penicillin-G and Methicilin Resistant *Staphylococcus aureus* (MRSA) was found in 22.38% along with β-Lactamase resistance in 31.34% of infected samples. *S. aureus* remains the major pathogen in infected samples which may pose a threat to public health. The main reason behind the increasing number of resistant pathogens may be due to the indiscriminate use of antibiotics by untrained veterinary professionals and lack of diagnostic facilities; hence regular screening of sub-clinical mastitis should be practiced to

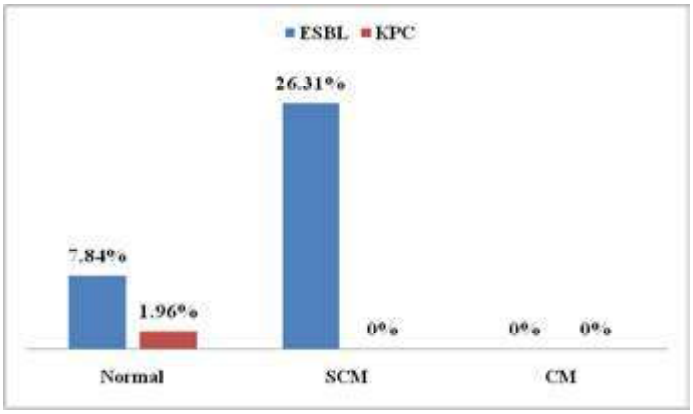


**Fig. (4a):** Resistance pattern of E. coli isolates at different stages of mastitis

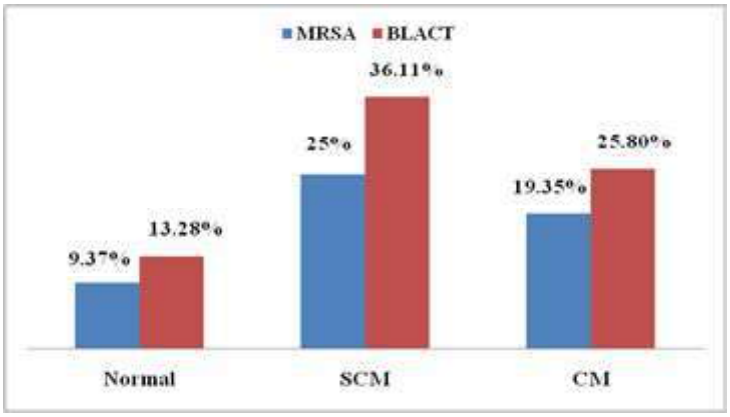


**Fig. (4b):** Resistance pattern of S. aureus isolates at different stages of mastitis

control the usage of antimicrobials and resistant development in dairy pathogens.

**Acknowledgement**

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Indian J Dairy Sci 77(2): 123-129
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RESEARCH ARTICLE

# Comparative antibiogram analysis of bacterial isolates from mastitic milk of cattle and buffalo in Haryana

**Rahul Yadav[1], Pankaj Kumar[2], Anand Prakash[3] and Vandna Bhanot[4](✉)**

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**Abstract:** Mastitis causes huge economic losses to dairy industries worldwide. It is caused by various microorganisms, of which bacterial etiology is primarily important. Both Gram-positive and Gram-negative bacteria are involved in causation of disease. In the present study, 8561 milk samples from both cattle and buffalo combined were tested and occurrence of mastitis was recorded in 72.73 % (n = 6227/8561) milk samples. Occurrence of mastitis was non–significantly ($p$>0.05) higher in cattle (86.31%) than buffaloes (66.97%). California Mastitis Test (CMT) showed significantly ($p$<0.05) lower prevalence of mastitis compared to the culture examination statistically. By CMT analysis, buffalo had a significantly ($p$<0.05) lower percentage of mastitis positivity than cattle. Whereas, culture examination revealed that both cattle and buffalo exhibited a high prevalence of mastitis, with 97.75% and 98.15% positive samples, respectively. Gram-positive bacteria were found as the predominating etiological agents causing mastitis in 62.62% samples followed by Gram-negative bacteria (24.54%) from milk samples of cattle and buffalo combined. Mixed infection of both Gram-positive and Gram-negative bacteria was found in 10.50% milk samples. Over all 2970 samples from cattle (n = 1071) and buffalo (n = 1899) were subjected for culture examination and antibiotic sensitivity assay. The findings from the present study revealed variations in antibiotic sensitivity across different districts. The district of Bhiwani consistently showed lower sensitivity rates for most antibiotics compared to the other districts. Overall, chloramphenicol, enrofloxacin, gentamicin, ciprofloxacin, cefoperazone and levofloxacin were most sensitive antibiotic across all districts. Amoxicillin + Clavulanic acid and ampicillin were most resistant antibiotics in all districts.

**Keywords:** Antibiotic resistance; Buffalo; Cattle; Mastitis

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## Introduction

Mastitis is characterised by an inflammation of the mammary gland in dairy animals such as cattle and buffalo. It causes significant economic losses to national economy and compromises animal welfare (El-Ashker et al. 2020; Yadav et al. 2020). Mastitis can be classified into sub-clinical, clinical and chronic mastitis, depending upon causative organisms, breed, age, immunity, and stage of lactation of the animal (Maity et al. 2020). It is caused by a variety of microorganisms; where bacterial infections are the primarily causative agents. Both Gram-positive and Gram-negative bacteria are involved in bovine mastitis (Algammal et al. 2020; Chhabra et al. 2020). Mastitis results in decreased quality and quantity of milk production. Such infected milk or milk products, usually enters into the food chain and humans can also acquire the infection through the consumption of contaminated milk (Krishnamoorthy et al. 2021). Antimicrobial resistance is also serious concern for both human and animal health. To effectively manage and treat mastitis, it is crucial to understand the bacterial pathogens involved and their antibiotic susceptibility patterns. Appropriate selection of antibiotics can be done on the basis of antibiotic susceptibility profiles of causative agents (Yadav et al. 2020; Ali et al. 2021). Furthermore, understanding the antibiotic resistance patterns of these bacteria is essential for implementing appropriate control measures and preventing the spread of multidrug-resistant strains among human and animal population (Yadav et al. 2021; Singh, 2022). Therefore, the present study seeks to bridge this knowledge gap and contribute to the current understanding of mastitis management in these important livestock species. The aim of present study is to investigate the antibiogram of bacterial isolates obtained from mastitis milk in cattle and buffalo by comprehensive approach, involving bacterial isolation and susceptibility testing, to generate a detailed antibiogram. This study may have practical implications for veterinarians, farmers, and dairy industry stakeholders. It may also contribute to develop the prevention

and control strategies for mastitis and generate awareness about the emergence of antimicrobial resistance.

## Material s and Methods

### Sample collection and detection of mastitis

Milk samples from cattle and buffalo were carefully collected using aseptic techniques at different districts of Haryana (Ambala, Bhiwani, Mahendergarh and Rohtak). Farmers were properly guided for aseptic collection of samples in sterilized container. Samples were processed for detection of mastitis during the period of July 2020 to June 2021 at various Disease Investigation Labs i.e Ambala, Bhiwani, Mahendergarh and Rohtak, Lala Lajpat Rai University of Veterinary and Animal Sciences, (LUVAS), Haryana, India. Mastitis detection was performed by California mastitis test (CMT) and culture examination. Severity of mastitis was detected in quarter milk samples (subclinical) by performing CMT and analysed by observing degree of gel formation graded as trace (t), mild (+), moderate (++) and severe (+++) as described by Belay et al. (2022).

### Bacterial isolation and identification

Detection of various bacterial isolates was carried out by culture examinations of the samples using standard procedure (Quinn et al. 2011). Briefly, milk samples were inoculated onto Nutrient agar and MacConkey agar. The plates were then incubated at the optimal temperature for bacterial growth, typically 37°C, for a period of 24 to 48 hours. Colony morphology, including shape, size, color, and other distinguishing characteristics, were observed and recorded. Gram staining was performed to differentiate the bacterial isolates as Gram-positive or Gram-negative.

### Antibiotic Susceptibility Testing

For antibiotic susceptibility testing, standard guidelines were followed as per the described by Bauer (1966) and (Quinn et al. 2011) for various antibiotics (Himedia). Briefly, bacterial suspension was prepared from pure cultures of the bacterial isolates. Mueller-Hinton agar (Himedia) plates were inoculated with the bacterial suspension using sterilized swab to obtain lawn culture (Bauer, 1966). Antibiotics discs were placed on the surface of agar and plates were then incubated at the appropriate temperature and duration as recommended for each antibiotic. Following incubation, the zones of inhibition around the antibiotic discs were measured and recorded. Interpretation of zone of inhibition was carried out according to the guidelines provided by the Clinical and laboratory standards institute (CLSI, 2015) and European committee on antimicrobial susceptibility testing (EUCAST, 2015). The collected data were compiled to create an antibiogram representing the antibiotic susceptibility patterns of the bacterial isolates.

### Statistical analysis

To analyze the data, the prevalence of antibiotic resistance among the bacterial isolates from mastitic milk in cattle and buffalo was determined. The frequency of resistance for each antibiotic was calculated in Microsoft Excel Version 2010, and statistical analysis, such as chi-square was done by Statistical Package for Social Sciences (SPSS) Version 26 Software (George and Mallery, 2019) to evaluate any significant differences in antibiotic resistance patterns in cattle and buffalo from different geographical areas.

## Results and Discussion

### Occurrence of mastitis

A total of 8561 milk samples from both species combined were tested for detection of mastitis. A comprehensive analysis of mastitis and related parameters among cattle and buffalo were shown in table 1. Overall, the occurrence of mastitis was recorded in 72.73% (n = 6227/8561) milk samples from both species combined. The species-wise analysis indicates that the occurrence of mastitis was non–significantly ($p$>0.05) higher in cattle (86.31%) than in buffaloes (66.97%). Contrary to our study, Dabele et al. (2021) found that the prevalence of mastitis in lactating Zebu cows of Ethiopia was found to be lesser than our findings 30.5% (95% CI: 26.0–35.2%). The slightly higher occurrence of mastitis in cattle compared to buffaloes (86.31% vs. 66.97%) could be attributed to factors such as anatomical differences between the udders of cattle and buffaloes, which might contribute to variations in mastitis susceptibility. Cattle have four quarters, while buffaloes generally have two larger lobes. The structural differences in mammary glands may influence the efficiency of milk let-down, milking procedures, and the ability to clear infections (Hughes and Watson, 2018). The non-significant difference in mastitis occurrence between cattle and buffaloes suggests that similar approaches can be used for mastitis prevention and control in both species. Implementing good husbandry practices, such as udder hygiene, proper milking techniques, and regular monitoring of udder health, can help reduce the incidence of mastitis in both cattle and buffaloes (Sah et al. 2020). Variation in management practices between cattle and buffalo farms might explain the observed differences in mastitis occurrence (Sharun et al. 2021). Al-Zurgani and Mohammed (2021) stated that (CMT) is a quick and distinguished field and laboratory test to detect mastitis in farm animals, including buffaloes. Hokmabad et al. (2011) and Ali and Dahl (2022) also found that, CMT has acceptable sensitivity and specificity in diagnosis of mastitis among buffaloes.

Of the 8561 milk samples, 5591 and 2970 milk samples were tested CMT and culture examination, respectively. CMT showed significantly ($p$<0.05) lower prevalence of mastitis compared to the culture examination with chi-square value (9.688) and the $p$-

value (0.002). By CMT analysis, buffalo had significantly ($p$<0.05) lower percentage of mastitis positivity than cattle. Whereas, culture examination revealed that both cattle and buffalo exhibited a high prevalence of mastitis, with 97.75% and 98.15% positive samples, respectively. The etiological agents causing mastitis were also investigated. Gram-positive bacteria were found as the predominating etiological agents causing mastitis in 62.62% samples followed by Gram-negative bacteria (24.54%) from milk samples of cattle and buffalo combined. Mixed infections of Gram-positive and Gram-negative bacteria were found in 10.50% samples from both species. Similar to our observation Verma et al. (2022) found that Gram-positive bacteria was found as the major cause of bovine mastitis 46.67% samples followed by Gram-negative bacteria (36.67%) and mixed infection of both (16.67 %). The high frequency of Gram-positive bacterial infections indicates unhygienic and poor management practices at farms. The contamination usually occurs from the external surface of the udder and teats, milker's hand and from the surface of the milking equipment and utensils etc. (Ali et al. 2021). While Gram-negative infections such as coliform mastitis were usually occurs due to poor environmental hygiene, contaminated water, fecal contamination, inadequate refrigeration etc (Deddefo et al. 2023).

The significant difference in detection of mastitis by CMT and culture examination showed variations in sensitivity and specificity between these diagnostic methods. Although, CMT being a quick and cost-effective screening tool, might underestimate the true prevalence of mastitis compared to culture examination, which provides a more accurate identification of causative agents (Mbindyo et al. 2020). The lower percentage of mastitis positivity observed in buffaloes compared to cattle by CMT analysis, although not statistically significant, could be attributed to differences in udder anatomy and physiological characteristics (Hughes and Watson, 2018; Diwakar et al. 2020). Further studies are needed to explore these factors in more detail and understand the potential implications for mastitis diagnosis in buffaloes. Gram-positive bacteria were identified as the major etiological agents, consistent with findings from Girma and Tamir (2022), who did a meta-analysis of mastitis data between year 2005-2022 in Ethiopia and concluded that Gram-positive bacteria (84.70%) were the most prevalent mastitis causing agents compared with Gram-negative bacteria (15.30%). Additionally, mixed infections (10-30%) of both Gram-positive and Gram-negative organisms were also reported by previous reporters, highlighting the complexity of mastitis and emphasising the need for targeted treatment approaches (Steele et al. 2020; Saleh et al. 2022).

Mastitis occurrence in different areas among cattle and buffalo populations was shown in Table 2. A notable variation in mastitis prevalence was observed only in Mahendergarh district. The occurrence of mastitis was non-significantly ($p$>0.05) higher in cattle (86.31%) than buffalo (66.97%). Both cattle and buffalo exhibited similar high percentages of positive samples in Ambala, Bhiwani and Rohtak districts. The chi-square test indicates no significant difference between the two species as the $p$-value (>0.05) was above the significance threshold. Looking at the individual areas, it is observed that mastitis prevalence varies across locations. In cattle, occurrence of mastitis was non-significantly ($p$>0.05) higher in Rohtak (100%) followed by

**Table 1:** Occurrence of mastitis in cattle and buffalo

| Parameters | Samples processed (n) | Mastitis | | Chi-square | *df* (degree of freedom) | *p* value |
|---|---|---|---|---|---|---|
| | | *+ve* | % | | | |
| Species wise (n = 8561) | | | | | | |
| *Cattle* | 2550 | 2201 | 86.31 | 2.359 | 1 | 0.125 |
| *Buffalo* | 6011 | 4026 | 66.97 | | | |
| Total | 8561 | 6227 | 72.73 | | | |
| *California mastitis test* (n = 5591) | | | | | | |
| *Cattle* | 1479 | 1154 | 78.02 | 4.771 | 1 | 0.029* |
| *Buffalo* | 4112 | 2162 | 52.57 | | | |
| Total | 5591 | 3316 | 59.30 | | | |
| *Culture examination* (n = 2970) | | | | | | |
| *Cattle* | 1071 | 1047 | 97.75 | 0.0 | 1 | 1.000 |
| *Buffalo* | 1899 | 1864 | 98.15 | | | |
| Total | 2970 | 2911 | 98.01 | | | |
| *Organisms isolated* | | | | | | |
| Gram-positive | | 1860 | 62.62 | | | |
| Gram-negative | | 729 | 24.54 | | | |
| *Candida* spp. | 2970 | 10 | 0.03 | 85.891 | 3 | 0.000* |
| No Growth | | 59 | 1.9 | | | |
| Mixed infection | | 312 | 10.50 | | | |

*Level of significance: $p$ value is significant at the 0.05 level or lesser.
$n$= no. of animals; *df*: degree of freedom

**Table 2:** Area wise occurrence of mastitis in cattle (n = 2550) & buffaloes (n = 6011)

| Area | Cattle | | | Buffalo | | | Statistical significance (between species) | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | *+ve* | % | *n* | *+ve* | % | Chi square | *df* | *p value* |
| Ambala | 345 | 330 | 95.65 | 228 | 217 | 95.18 | 0.005 | 1 | 0.942 |
| Bhiwani | 225 | 223 | 99.11 | 737 | 728 | 98.78 | 0.000 | 1 | 1.000 |
| Mahendergarh | 1871 | 1539 | 82.25 | 4760 | 2799 | 58.80 | 3.752 | 1 | 0.053 |
| Rohtak | 109 | 109 | 100 | 286 | 282 | 98.60 | 0.005 | 1 | 0.943 |
| Grand Total | 2550 | 2201 | 86.31 | 6011 | 4026 | 66.97 | 2.359 | 1 | 0.125 |

Statistical significance in occurrence of mastitis between different areas

| | Cattle | Buffalo | Aggregate |
|---|---|---|---|
| Chi square | 2.215 | 12.864 | 5.978 |
| *df* | 3 | 3 | 3 |
| *p value* | 0.529 | 0.005* | 0.113 |

*Level of significance: *p value is significant at the 0.05 level or less*
*df:* degree of freedom

**Table 3:** Season wise occurrence of mastitis in cattle (n = 1979) & buffaloes (n = 5036)

| Season | Cattle | | | Buffalo | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total samples | Positive (*n*) | % | Total samples | Positive (*n*) | % | Chi square | *df* | *p value* |
| Rainy | 987 | 845 | 85.61 | 2011 | 1350 | 67.13 | 2.359 | 1 | 0.125 |
| Spring/ Autumn | 515 | 450 | 87.38 | 1514 | 1025 | 67.70 | 2.329 | 1 | 0.127 |
| Winter | 474 | 386 | 81.43 | 1855 | 1175 | 63.34 | 2.250 | 1 | 0.134 |
| Summer | 574 | 520 | 90.59 | 631 | 476 | 75.44 | 1.542 | 1 | 0.214 |
| Grand Total | 2550 | 2201 | 72.74 | 6011 | 4026 | 86.31 | 2.359 | 1 | 0.125 |

Statistical significance in occurrence of mastitis (per animals) between different seasons

| | Cattle | Buffalo | Aggregate |
|---|---|---|---|
| Chi square | 0.588 | 1.095 | 0.812 |
| *df* | 3 | 3 | 3 |
| *p value* | 0.899 | 0.778 | 0.847 |

*Level of significance: *p value is significant at the 0.05 level or less, df:* degree of freedom

Note: Rainy (*July, August, September*), Spring/Autumn (*October, November, March*), Winter (*December, January, February*), Summer (*April, May, June*)

Bhiwani (99.11%), Ambala (95.65%) and Mahendergarh (82.25%). In buffaloes, occurrence of mastitis was significantly ($p<0.05$) higher in Bhiwani (98.78%) followed by Rohtak (98.60%), Ambala (95.18%) and Mahendergarh (58.80%). The variation in the studies with respect to season and prevalence of mastitis is due to the varying agro-climatic conditions and geographical areas.

These include climate, weather conditions, environmental hygiene, management practices and breed characteristics (Easaw and Vijayakumar, 2022). Differences in management practices, including milking routines, udder hygiene, and housing conditions, also affect mastitis occurrence. Additionally, certain breeds may be more susceptible to mastitis, and variations in breed dominance across regions can lead to differences in mastitis prevalence. Mastitis was most common in Jersey breeds (78.6%), than in Holstein Friesian and indigenous zebu cow crossbreeds (51.9%), and least common in indigenous zebu breeds (16.7%). Moreover, the availability and accessibility of veterinary services play a role in mastitis control and delay in mastitis treatment could amplify the number of cases or complicate the mastitis (Caneschi et al. 2023). Also, the credibility/education status of veterinary practitioners reaching to the doorsteps of the owners (if the owners not visiting the veterinary clinics or if the villages do not have the veterinary dispensaries), misuse and overuse of antibiotics, underdosing of antibiotics, not following the proper treatment regimen by owners, over-reliance on home-recipes for mastitis controls are some other factors which affect the fate of mastitis affected glands.

Season wise occurrence of mastitis in cattle and buffalo was shown in table 3. It was observed that occurrence of mastitis varies with different seasons across all regions. However, the difference was not statistically significant ($p$>0.05) in between same species of different areas and different species of same area. Overall, occurrence of mastitis was non-significantly ($p$>0.05) higher in summer followed by spring, rainy and winter season in case of cattle and buffalo. Season to season variation was observed in the occurrence of mastitis due to variation in growth of pathogenic organism. For example, hot and humid climates can create favourable conditions for bacterial growth, while poor sanitation practices or limited access to clean water can also contribute to higher mastitis rates (Singh et al. 2021). Previous reports in India, showed highest prevalence of mastitis during summer and rainy season in as compared to winter season (Easaw and Vijayakumar, 2022). This could be associated with increased multiplication of organisms and environmental stress, which altered the immune system, thereby making animals prone of infection/mastitis. The high prevalence during monsoon season could be attributed to the temperature and humidity conditions. This was contradictory to the findings by Ranjan et al. (2011) who found least prevalence during raining season (7.37%). OldeRiekerink et al. (2007) found that clinical mastitis was found in high frequency (increasing somatic cell count) during winter season in USA. Therefore, the Understanding these agro climatic conditions in different geographical areas are crucial for implementing targeted control strategies and interventions to reduce mastitis incidence and improve udder health in specific areas (Chen et al. 2023).

**Antibiotic Susceptibility Testing**

Overall, 2970 samples from cattle (n = 1071) and buffalo (n = 1899) were subjected for culture examination and antibiotic sensitivity assay (Table 4). The findings from present study revealed variations in antibiotic sensitivity across different districts. The district of Bhiwani consistently showed lower sensitivity rates for most antibiotics compared to the other districts. In terms of individual antibiotics, amikacin showed relatively highest sensitivity in Mahendergarh (84.44%) and Rohtak (81.51%), while lower sensitivity rates in Ambala (57.49%) and Bhiwani (32.70%).

**Table 4** Antibiogram of organisms isolated from bovine mastitic milk samples

| Total | Sensitivity (%) | | | | Resistance (%) | | | |
|---|---|---|---|---|---|---|---|---|
| Antibiotics | Ambala | Bhiwani | Mahendergarh | Rohtak | Ambala | Bhiwani | Mahendergarh | Rohtak |
| Amikacin (30 mcg) | 57.49 | 32.70 | 84.44 | 81.51 | 42.52 | 67.30 | 13.50 | 18.50 |
| Amoxiclav 30 [Amoxicillin (20mcg)+ Clavulanic acid (10 mcg)] | 67.36 | 23.10 | 27.72 | 67.59 | 32.64 | 76.90 | 71.89 | 32.41 |
| Ampicillin (10 mcg) | - | - | 15.70 | 15.70 | - | - | 84.11 | 84.30 |
| Cefoperazone (75 mcg) | 62.85 | 28.70 | 75.69 | 62.79 | 37.16 | 71.30 | 20.96 | 37.21 |
| Ceftizoxime (30 mcg) | 57.62 | 13.30 | 43.66 | 86.35 | 42.38 | 86.70 | 55.85 | 13.65 |
| Ceftriaxone (30 mcg) | 65.51 | 24.00 | 42.93 | 63.26 | 34.49 | 76.00 | 56.23 | 36.74 |
| Chloramphenicol (30 mcg) | 58.85 | 33.80 | 83.79 | 77.19 | 41.15 | 66.20 | 15.91 | 22.81 |
| Ciprofloxacin (30 mcg) | - | 23.20 | 80.62 | 63.37 | - | 76.80 | 18.18 | 36.64 |
| Enrofloxacin (5 mcg) | 86.71 | 27.80 | 83.97 | 94.08 | 13.30 | 72.20 | 15.15 | 5.92 |
| Gentamicin (10 mcg) | 60.56 | 34.40 | 95.20 | 95.84 | 39.45 | 65.60 | 4.22 | 4.16 |
| Levofloxacin (05 mcg) | 75.82 | 24.40 | 70.74 | 84.41 | 24.19 | 75.60 | 28.28 | 15.59 |
| Moxifloxacin (05 mcg) | - | 23.10 | 64.35 | - | - | 76.90 | 33.59 | - |
| Oxytetracycline (30 mcg) | - | 20.10 | 55.38 | 80.01 | - | 79.90 | 43.84 | 20.00 |

Similarly, enrofloxacin was high sensitivity in Rohtak (94.08%) but relatively low sensitivity in Bhiwani (27.80%). Amoxicillin + Clavulanic acid have higher sensitivity in Rohtak (67.59%) and Ambala (67.36%) than Mahendergarh (27.72%) and Bhiwani (23.10%). Regarding antibiotic resistance, the combination of amoxiclav shows high resistance in Mahendergarh (71.89%) and Bhiwani (76.90%), while Rohtak has relatively lower resistance (32.41%). Additionally, gentamicin exhibited high resistance in Ambala (95.17%) and Rohtak (95.84%), but much lower resistance in Bhiwani (34.40%). Overall, chloramphenicol, enrofloxacin, gentamicin, ciprofloxacin, cefoperazone and levofloxacin were most sensitive antibiotic across all districts. Amoxiclav and ampicillin were most resistant antibiotics in all districts. Similar to our findings, Ranjan et al. (2011) found high sensitivity towards enrofloxacin (91.67%). Pankaj et al. (2012), studied the antibiogram of isolates of mastitis and revealed high (90.90-100%) sensitivity to cefoperazone, enrofloxacin and gentamicin. Serdal and Funda (2021) found that ampicillin and streptomycin were the least effective antimicrobial agents, while the most effective antibiotics were amikacin and kanamycin.

## Conclusion

In conclusion, findings of present study highlighted the importance of regional differences in antibiotic sensitivity and resistance patterns among mastitis-causing bacteria. Understanding these variations is crucial for the selection and proper use of antibiotics in mastitis treatment and control strategies. It is essential to consider local antibiotic sensitivity profiles and regularly monitor for changes in resistance patterns to ensure effective management of mastitis in cattle and buffaloes across different districts.

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RESEARCH ARTICLE

# Application of Image Analysis Technique in Coagulation of Milk for *Paneer* Manufacturing

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**Abstract:** This study was aimed to investigate the coagulation process of *Paneer* manufacturing and to determine the optimal conditions for its production using image analysis techniques. *Paneer*, a traditional coagulated dairy product, is widely recognized and valued for their nutrition and easy to use. The whey images were captured and analyzed at different levels of agitator speed (20, 30 and 40 RPM) and coagulation temperatures (70, 75 and 80°C), and the *L*, a*,* and *b** values of each sample were evaluated using Adobe Photoshop software. The *a** value was specifically used to assess the greenish colour of the sample image, which is an important indicator of complete coagulation of milk. The results revealed that the optimal coagulation conditions were 70°C coagulation temperature, 40 RPM agitator speed and 140 s coagulation time with *a** value of -5.39. These findings suggest that image processing may be an effective tool for monitoring and standardizing the coagulation process of milk for *Paneer* manufacturing. By using this technique, the quality and consistency of *Paneer* may be improved, and human intervention in the production process may also be minimized.

**Keywords:** *Paneer*; Coagulation; Image; Software; Temperature

## Introduction

In recent years, the food industry has been witnessing a significant growth in the use of image analysis techniques for quality control and inspection of various food products (Mollazade et al. 2012). The applications of these techniques have not only facilitated assessment of food quality and safety but also led to the development of novel and innovative food products (Brosnan and Sun, 2004). Among the many applications of image analysis techniques in the food industry, colour analysis is one of the most important and widely used techniques (Ogawa and Adachi, 2014). The colour of food products is an essential factor that influences consumer perception, acceptance, and purchasing decisions (Ares and Deliza, 2010). Therefore, the accurate and reliable measurement of food colour is crucial for ensuring the quality and consistency of food products.

In the dairy industry, colour analysis is of utmost importance, especially for products such as milk, *paneer, yogurt, ghee* and *butter* (Kamthania et al. 2014). In context to *Paneer*, colour is influenced by several factors viz. breed of the animal, the stage of lactation, the processing method, and the storage conditions (Chandan, 2007). The colour of *Paneer* can provide important information regarding product quality, freshness, and the presence of defects (Prajapati et al. 2021). Traditional methods for measuring the colour of dairy products involve visual assessment by human experts, which can be subjective and prone to error (Revilla et al. 2016). In recent years, the use of image analysis techniques for colour analysis of dairy products has become increasingly popular.

Image analysis techniques for colour analysis of dairy products can be broadly classified into two categories, namely, colorimetric and image processing techniques. Colorimetric techniques involve the measurement of the colour of dairy products using colorimeters or spectrophotometers (Minz and Saini, 2021). These instruments measure the intensity of light reflected from the surface of the dairy product and provide colour information in terms of colour space coordinates, such as CIELAB, CIELUV, and CIEXYZ. The colour information can then be used to calculate various colour parameters, such as hue, chroma, and lightness.

The image processing techniques involve the analysis of digital images of dairy products captured using digital cameras or scanners. Image processing techniques can provide more detailed and comprehensive colour information compared to colorimetric techniques (Cabaret et al. 2007). Image processing techniques involve several steps, including image acquisition, image

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segmentation, feature extraction, and classification. In the image acquisition step, digital-images of dairy products are captured using a digital camera or scanner. In the image segmentation step, the dairy product in the image is isolated from the background using various image processing algorithms (Poursaberi et al. 2010). In the final step, colour features such as *L*, a*,* and *b** values can be used to assess the product quality.

*L*, a*,* and *b** values are colour space coordinates that are commonly used in colorimetry for colour analysis of dairy products, including *Paneer* (Leon et al. 2006). *L** represents the lightness or brightness of the colour (0 being black and 100 being white), *a** axis represents the red-green axis (positive values indicating redness and negative values indicating greenness). The *b** axis represents the yellow-blue axis, with positive values indicating yellowness and negative values indicating blueness.

In the context of *Paneer* manufacturing, the coagulation of milk and whey separation is important step. The colour of whey is a decisive factor about completion of milk coagulation process. Generally the colour of whey is greenish after milk coagulation such as greenish color (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). This allows for a more objective assessment of the colour of whey, rather than relying on subjective assessments made by human experts (Yam and Papadakis, 2004). So, objective of the present investigation is determination of colour values of whey using image processing technique to provide a quantitative assessment of the colour of the whey for milk coagulation stage.

## Material and Methods

### Raw Material

Standardized buffalo milk (6% Fat and 9% SNF) was collected from the Experimental Dairy, ICAR-NDRI, Karnal, Haryana, India.

### Experimental setup and procedure

The experimental set up (Fig. 1) consists of cylindrical coagulation tank with paddle agitator, image acquisition system (lighting system, digital camera) and a computer with installed Adobe Photoshop software (version 7.0). The coagulation tank was insulated with glass-wool insulation to minimize heat loss from it.

### Image acquisition

#### *Lighting system*

To obtain accurate colour images of food samples, it is crucial to use appropriate lighting because the colour of the food samples depends on the spectrum of light reflected from it. To standardize the spectral power distribution of the light source, the CIE has established standard illuminants that are identified by their colour temperatures. In food research, the most commonly used standard illuminants are A (2856K), C (6774K), $D_{65}$ (6500K), and D (7500K), with C, $D_{65}$, and D being designed to imitate different variations of daylight (Sharma, 2018). To capture the colour accurately, the camera lens axis and the lighting source axis should be at an angle of about $45^0$, as this angle produces the diffuse reflection responsible for the colour. Additionally, the light intensity should be uniform across the food sample, which can be achieved by experimenting with lighting arrangements, such as altering the distance between the light source and the food sample, taking pictures in a dark room, and verifying the results with a light meter (Yam and Papadakis, 2004).

#### *Digital camera*

The images were captured using Ravtron web camera (Full HD 1920 × 1080 pixels) which was integrated to the coagulation tank (Fig.1).

### Colour image processing

Adobe Photoshop software (version 7.0) was employed to evaluate the values of *L*, a*,* and *b**. It has various tools that can be applied to analyse the colour of food samples. It has abundant features for editing images and its ability to analyse colour in comparison to more expensive colour analysis softwares. Additionally, it provides more advanced capabilities for managing and producing consistent colours than the other graphics software. A computer (Intel Core-i3, 4GB RAM, 1TB hard disk) was used to operate the software. This software is widely accessible in numerous laboratories and receives strong support from both the manufacturer and users (Yam and Papadakis, 2004).

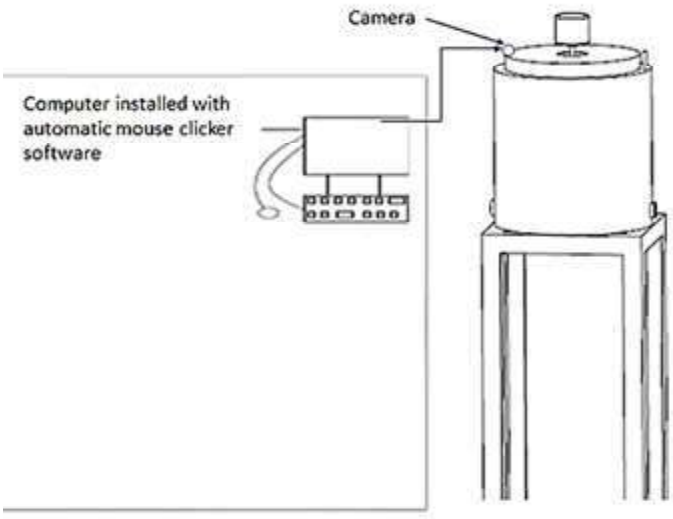


**Fig.1** Experimental set up for image capturing

For quantitative analysis, *L*, a*,* and *b** values were utilized because these values are not device-dependent and encompass a broader range than RGB and CMYK. The Adobe Photoshop software can exhibit *L*, a*,* and *b** values, as well as RGB and CMYK values, in the Info Palette and Histogram Window. The Histogram method was applied (Shahraki et al. 2014) to assess the *L**, *a**, and *b** distribution of the samples. The Histogram Window presents the data (average, standard deviation, median, percentage, etc.) of the colour value (*L*) for a chosen area in the coagulation image. The Histogram Window can also provide the data for two other colour values (*a* and *b*) by selecting them from the Channel drop-down menu. Obtaining the average color of a sample or its any part is effortless using the Histogram Window (Afshari-Jouybari and Farahnaky, 2011). The *L, a* and *b* values displayed in the Histogram Window are not standardized colour values. However, they can be transformed to *L**, *a**, and *b** values by using following standard formulaes (Yam and Papadakis, 2004).

$$L^* = \left[\frac{L}{255}\right] \times 100 \quad (1)$$

$$a^* = \left[\frac{240a}{255}\right] - 120 \quad (2)$$

$$b^* = \left[\frac{240b}{255}\right] - 120 \quad (3)$$

The chroma value and hue angle were calculated from the *L*, a** and *b** values (Barnwal et al. 2015; Pathare et al. 2013):

$$Chroma\ value = \sqrt{\left(a^{*2} + b^{*2}\right)} \quad (4)$$

$$Hue\ angle\ (^\circ) = \left(\frac{180}{\pi}\right) \times \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (5)$$

Whiteness index (WI) was calculated by using following standard relation (Barnwal et al. 2015; Pathare et al. 2013; Wasnik et al. 2017):

$$WI = 100 - \sqrt{\left(100 - L^{*2}\right) + a^{*2} + b^{*2}} \quad (6)$$

Yellowness Index was computed by following equation (Pathare et al. 2013; Wasnik et al. 2017):

$$YI = \frac{142.86 \times b^*}{L^*} \quad (7)$$

Equations (1) to (7) were used to describe the colour change of whey during coagulation of milk for *paneer* manufacturing.

**Experimentation and Analysis**

*Paneer* was prepared in the laboratory using the standard method (Aneja et al. 2002) for application of image analysis technique in coagulation of milk for *Paneer* Manufacturing. Initially, preliminary trials were conducted to determine the range of agitator speed that could be applied during the experiments. Three different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperatures (70, 75 and 80 °C) were selected after the preliminary trials. The various images were captured from dosing of coagulant to till complete separation of whey from coagulum. The images were captured at 5 s interval during milk coagulation for 2.5 minutes. The each sample image was analysed for *L*, a** and *b** values by importing the image in the Adobe Photoshop software.

The colour of whey, obtained during *paneer* manufacturing, is a crucial factor about end point of milk coagulation process. Normally, the colour of whey is greenish after milk coagulation such as greenish color (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). So, *a** values were used to assess the greenish colour of the whey during coagulation.

For comparison of the variation in several sets of data, it is generally desirable to use a measure of relative variation i.e. the coefficient of variation (CV, %) or relative standard deviation (RSD, %). The CV (%) or RSD (%) may be computed as (Johnson, 2005; Rao, 2018):

$$RSD\ (\%) = \frac{\sigma}{\bar{x}} \times 100 \quad (8)$$

The mean or arithmetic mean ($\bar{x}$), and standard deviation ($\sigma$) can be calculated as (Johnson, 2005):

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \quad (9)$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \quad (10)$$

Where, $x_i$ and n are i-th data number of data in respective colour attribute column.

The mean deviation (M.D.) may be computed as (Murthy 2013):

$$M.D. = \frac{\sum_{i=1}^{n} (x_i - \bar{x})}{n}$$

Microsoft Excel-2013 software was used for regression analysis and graphs preparation.

**Results and Discussion**

The various captured images at 5 second interval were analysed and processed in Adobe Photoshop software (version 7.0) software for *a** values. Table 1 shows the effect of agitation speed or agitator speed and the desirable coagulation temperature on *L**, *a**, and *b** values whereas Table 2 represents the effect of agitation speed and the desirable coagulation temperature on hue angle (°), chroma value, yellowness index (YI) and whiteness index (WI). Reliability reproducibility was obtained with a RSD from 0.063 to 0.933 % i.e. lower than 1 %. The mean deviation was ranged from 0.005 to 0.160. The mean deviation (0.005 to 0.160) and RSD (0.063 to 0.933 %) show that the precision of colour attributes (*L*, a*, b*,* hue angle, chroma value, yellowness index and whiteness index) of whey are favourable for various combination of process parameters i.e. coagulation temperature, agitation speed and coagulation time (Tables 1-2). The different coagulation time (range: 140-185 s) was observed for different combinations of coagulation temperature and agitator speed.

It was reported that the colour of whey is greenish after milk coagulation (Pranita et al. 2015; Baba et al. 2016; Chaudhari et al. 2022), greenish whey from cow's cheese (El Zubeir and Jabreel, 2008) and greenish whey from bovine milk cheese (Baig et al. 2022). Therefore, the second order (quadratic) was established through regression analysis (Table 3) which may be used for prediction of coagulation time for desired coagulation of milk for *paneer* manufacturing using desirable *a**-values (-5.393 to -5.289). Second order (quadratic) equations and $R^2$ values of *a** values in terms of coagulation time at three different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperature (70, 75 and 80 °C) were determined. It was observed that the $R^2$ values for the different agitator speeds (20, 30 and 40 RPM) and three different coagulation temperatures (70, 75 and 80 °C) were higher and closer to 1.0, which indicates a good fit to the model (Anup et al. 2019).

**Table 1:** Effect of agitation speed and coagulation temperature on *L**, *a** and *b** values of whey

| Coagulation Temperature, °C | Agitation speed (RPM) | Coagulation time (seconds) | *L** value | *a** value | *b** value |
|---|---|---|---|---|---|
| | 40 | 140 | 62.584 | -5.393 | 9.585 |
| 70 | 30 | 160 | 62.596 | -5.384 | 9.618 |
| | 20 | 180 | 62.635 | -5.318 | 9.750 |
| | 40 | 150 | 62.603 | -5.384 | 9.592 |
| 75 | 30 | 170 | 62.568 | -5.374 | 9.675 |
| | 20 | 190 | 62.674 | -5.289 | 9.769 |
| | 40 | 145 | 62.670 | -5.355 | 9.878 |
| 80 | 30 | 165 | 62.580 | -5.342 | 9.675 |
| | 20 | 185 | 62.643 | -5.319 | 9.731 |
| M.D. | | | 0.034 | 0.030 | 0.076 |
| RSD (%) | | | 0.063 | 0.678 | 0.983 |

**Table 2:** Effect of agitation speed and coagulation temperature on hue angle (p ), chroma value, yellowness index and whiteness index of whey

| Coagulation Temperature, °C | Agitation speed (RPM) | Coagulation time (seconds) | Hue Angle (°) | Chroma Value | Yellowness index | Whiteness index |
|---|---|---|---|---|---|---|
| | 40 | 140 | -60.628 | 10.998 | 21.880 | 86.008 |
| 70 | 30 | 160 | -60.753 | 11.022 | 21.951 | 85.989 |
| | 20 | 180 | -61.382 | 11.106 | 22.238 | 85.926 |
| | 40 | 150 | -60.687 | 11.000 | 21.889 | 86.008 |
| 75 | 30 | 170 | -60.942 | 11.067 | 22.091 | 85.952 |
| | 20 | 190 | -61.561 | 11.109 | 22.268 | 85.927 |
| | 40 | 145 | -61.529 | 11.236 | 22.517 | 85.826 |
| 80 | 30 | 165 | -61.087 | 11.056 | 22.086 | 85.965 |
| | 20 | 185 | -61.331 | 11.090 | 22.192 | 85.939 |
| M.D. | | | 0.005 | 0.053 | 0.160 | 0.039 |
| RSD (%) | | | 0.598 | 0.664 | 0.933 | 0.065 |

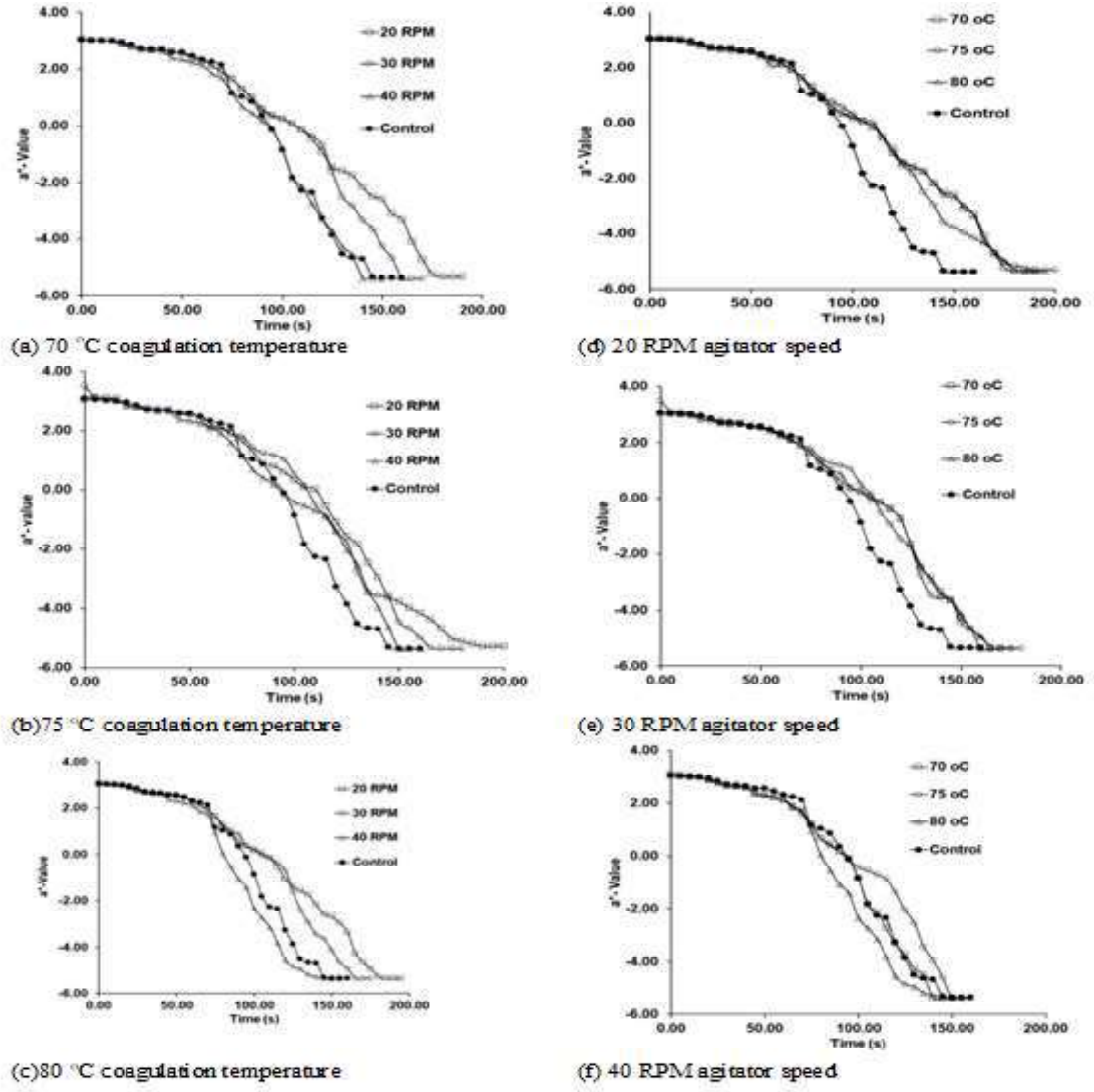


**Fig. 2** Changes in a*-values of whey with coagulation time at (a) 70 °C (b) 75 °C (c) 80 °C (d) 20 RPM (e) 30 RPM (f) 40 RPM

**Table 3:** Second order (quadratic) equations and $R^2$ – values of *a**-values of whey in terms of coagulation time at different coagulation temperatures and agitator speeds

| Coagulation temperature °C | Agitator speed, RPM | Regression equation | $R^2$ | RMSE (%) |
|---|---|---|---|---|
| 70 | 20 | $y = -0.0002x^2 - 0.0068x + 3.2031$ | 0.9937 | 0.2970 |
| | 30 | $y = -0.0004x^2 + 0.0103x + 2.906$ | 0.9920 | 0.5309 |
| | 40 | $y = -0.0005x^2 + 0.008x + 3.0307$ | 0.9888 | 0.3801 |
| 75 | 20 | $y = -0.0002x^2 - 0.0195x + 3.5449$ | 0.9758 | 1.9222 |
| | 30 | $y = -0.0003x^2 - 0.0026x + 3.3035$ | 0.9816 | 0.2296 |
| | 40 | $y = -0.0004x^2 + 0.0049x + 3.0091$ | 0.9917 | 0.1742 |
| 80 | 20 | $y = -0.0002x^2 - 0.0106x + 3.2844$ | 0.9917 | 0.1568 |
| | 30 | $y = -0.0003x^2 + 0.0055x + 3.0019$ | 0.9891 | 0.4005 |
| | 40 | $y = -0.0003x^2 - 0.0225x + 3.6332$ | 0.9563 | 0.2893 |

Note: y = *a**-value; x = time (s)

Figures 2 (a-c) demonstrate the influence of agitator speed (RPM) at different coagulation temperatures (70 °C, 75 °C and 80 °C) on *a** values of the samples. The results indicated that coagulation occurred more rapidly at 40 RPM (140 s) than at 30 RPM (160 s) and 20 RPM (180 s) at 70 °C. Figures 2 (d-f) represent the effect of coagulation temperatures (°C) at different agitator speeds (20 RPM, 30 RPM and 40 RPM) on *a** values of the samples. Similarly, at 75 °C and 80 °C, the coagulation process was more rapid at 40

RPM than at 30 and 20 RPM. In all three cases, there was a steady decline in *a** value during the first 60 seconds of coagulation, followed by a sudden drop in *a** value, indicating the formation and separation of whey. The *a** value was found to be related to the agitator speed, with a more negative *a** value indicating optimal greenish whey separation at higher agitation speeds. Overall, these findings suggest that the optimal conditions for coagulating milk for *Paneer* involve a careful balance of agitation speed and coagulation temperature to promote efficient coagulation and whey separation.

## Conclusion

From this study, it was found that the desirable *a**-values were -5.393 to -5.289 for whey separation for complete coagulation of milk for *paneer* manufacturing. The optimal coagulation conditions were achieved with a coagulation temperature of 70°C, an agitator speed of 40 RPM for 140 s, and *a** value of -5.39.

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RESEARCH ARTICLE

# Enhancing time availability for milk processing using thermal oil as solar heat reservoir

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**Abstract:** India is a world leader in milk production, with an annual production of 230.6 MT. Only 30 percent of milk is currently handled in the organized sector. A cheap source of reliable energy is needed to shift the unorganized sector to an organized sector, as dairy operations are energy-intensive. Solar energy is renewable, inexhaustible, promising, and abundantly available in India. In the literature, it was found that dairy operations such as sterilization of milk can be done using solar energy. However, the limited sunshine hours make it possible to handle only a limited quantity of milk day-to-day. This paper aims to discuss the performance evaluation of a solar heat reservoir to enhance the operational hours during the daytime when solar radiation is insufficient to provide the required energy to carry out the operations. It was found that by using the designed thermal reservoir, the working hours for milk processing were enhanced by 25 to 50 percent, depending on the ambient solar conditions.

**Keywords:** Farm processing; Heat exchanger; Milk; Renewable energy; Solar; Thermal

**Standard Abbreviations:** °C- degree Celsius; h- hour; MT- million tonnes; m- meter; cm- centimeter; Fig.- figure; s- seconds; L- liter.

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## Introduction

India is the largest milk-producing nation, with an annual production of 230.6 MT (NDDB, 2024). Milk processing is an energy-intensive activity; the use of solar energy can partly replace conventional sources of energy (Jaglan et al. 2018; Sharma et al. 2019; Sain et al. 2020; Hosouli et al. 2023; Zlaoui et al. 2023; Patel and Patel, 2024). It was reported that if solar energy is used, about 30,000 L of milk gets pasteurized, and there will be a saving of 80–100 L of furnace oil on a daily basis (Kedare et al. 2012). Operations such as electricity generation, water heating/cooling, drying, steam generation, pumping of dairy fluids, and others can be performed using solar energy (Chopde et al. 2016; Sharma et al. 2017; Sain et al. 2020). In contrast, thermal energy storage (TES) materials are gaining much attention because they enhance energy efficiency, facilitate renewable energy Integration, and offer economic benefits (Rohit et al. 2023; Masera et al. 2023). The processing of milk and other perishable agricultural products may benefit from the use of TES materials in conjunction with solar energy (Sain et al. 2019a; Munir et al. 2023).

Research efforts should be made to find the aforementioned combination as well as develop equipment for such activities. This paper deals with the performance evaluation of a system developed for entrapping solar heat via a thermal storage fluid, transferring heat from the thermal storage fluid to water for heating, and using the generated hot water for milk processing. The aim of the study was to enhance the working hours, beyond the availability of daily sunshine hours, for milk processing when only solar energy is used as a thermal heat source.

## Materials and Methods

The study was performed at the Department of Dairy Engineering, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, at 30.8929 latitude and 75.7981 longitude and an altitude of 245 m above mean sea level.

**Description of the heat reservoir**

A predesigned mild steel single cavity (Sharma et al. 2019) named configuration 1 (shown in Fig. 1a) was used for the selection of suitable TES material. Another mild steel two-cavity thermal reservoir named configuration 2 and configuration 3 (Figs. 1b and 1c, respectively) was developed in the study performed by Sain et al. (2019b). Referring to Figs. 1b, and 1c, Section 1 (oil side) had a volume of 6 L, and Section 2 (water side) had an 18 L volume. The scheme was to use the minimum amount of water in Section 2 so that it can be converted into steam using the least energy from the oil in Section 1; the rest of the energy stored in the oil should be available for temperature compensation in Section 3. This is due to the fact that there would be a temperature drop in the water present in Section 3 because it would be circulated for the heating of milk. So, the temperature drops of water need to be raised again to maintain the constant temperature of water for milk heating.

Basically, Configuration 3 was a modification of Configuration 2. In Configuration 2, the following method was used for hot water generation:

**Configuration 2:** A copper pipe of diameter 12 mm was coiled with a total length of 3 m; water passing through this coil gets heated up, taking energy from the steam in section 2 (referred to as C22 hereafter) (Fig. 1b).

After removing the copper pipe, the modified configuration was named Configuration 3, which is as follows:

**Configuration 3:** The copper pipe was removed from the cavity, and the cylindrical cavity of volume 9.5 L capacity, fully filled with water, was used for hot water generation via heat exchange between section 3 and steam of section 2 of configuration 3 (to be referred to as C23 hereafter) (Fig. 1c).

In configuration 2, the water circulation in the copper pipe was started when the temperature in the second cavity was above 100°C, i.e., steaming. The water was circulated using a centrifugal pump after recording the time taken to achieve the circulation temperature (90°C) in Section 3. Similarly, in C23, Section 3 was completely filled with water. Water present in section 3 received the heat from hot water present in section 2, which was simultaneously getting heat from section 1, which was the only medium for the transfer of heat from one layer or section to another. The heat source from where section 1 was getting heat and transferring it to the other sections was solar energy only, and the reservoir was placed on the focal point of the parabolic dish. The complete setup with the thermal reservoir mounted on the parabolic dish is shown in Fig. 2a (Sain et al. 2019b).

Temperature profiles in Section 1 and Section 2 of Configuration 2 After achieving the desired temperature in Section 3, the thermal reservoir was taken off from the focal point of the parabolic solar dish and kept in the laboratory under ambient conditions. The fall in temperature in various sections of the setup was recorded to observe the time period for which paraffin oil can supply heat to the water in Section 2 in order to maintain the temperature above 90°C.

**A tube-in-tube type arrangement for milk heating**

A tube-in-tube type arrangement for milk processing was developed to check the milk's heating by using the designed thermal reservoir. The vessel was made of stainless steel (SS-304) with a thickness of 1 mm. The length of the vessel was 59 cm, with an internal diameter of 3.81 cm and an outer diameter of 5.08 cm. The capacity of the milk side cavity was 500 ml, and the waterside cavity was 500 ml. The vessel was insulated with the help of cotton and aluminum foil to prevent losses. There were two nozzles for the inlet and outlet of hot water, as shown in Fig. 2b. The assumptions made for the development of the arrangement were:

1) Heat transfers under steady-state conditions, as the temperature of the outer wall of the milk holder is assumed to be constant.

2) Heat transfers by conduction as each ring of the cylinder is at the same temperature. The reason behind this assumption was that milk was held in a tube/pipe, so it was a batch process where the outer wall of the tube remained at a constant temperature. Also, looking into the diameter of the tube, the milk would be heated more radially than longitudinally. Thus, instead of convection, conduction was assumed.

3) Heat accumulation will occur as the milk side temperature rises from ambient to processing temperature.

4) There will be no heat losses to the environment as the water cavity is insulated.

**Methodology for heating milk using a tube-in-tube type heat exchanger**

A simulation study was conducted to assess the hot water temperature drop at 90°C and the milk temperature rise from 30°C. The drop in temperature was not compensated by heating. It was a batch process where milk was poured into the inner side cavity, and 9.5 L of hot water was circulated through the outer jacket with the help of a centrifugal pump. The heated water (around 90°C) was collected in an insulated container, as shown in Fig. 2b. A drop in the temperature of the circulating water and an increase in the temperature of the milk was noted with the help of a mercury thermometer.

Here, in this paper, the performance analysis of only configuration 2 (C22 and C23) is reported, as configuration 1 was used only for

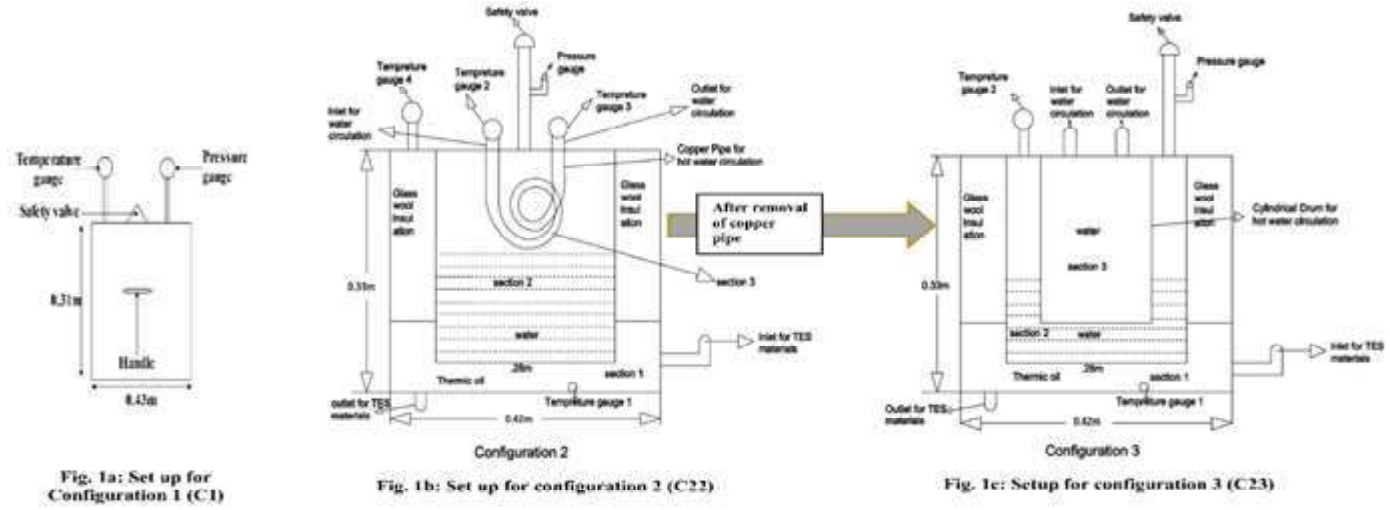


Fig. 1a: Set up for Configuration 1 (C1)

Fig. 1b: Set up for configuration 2 (C22)

Fig. 1c: Setup for configuration 3 (C23)

**Fig. 1** Schematic diagrams for all three configurations (Sain et al. 2019b)

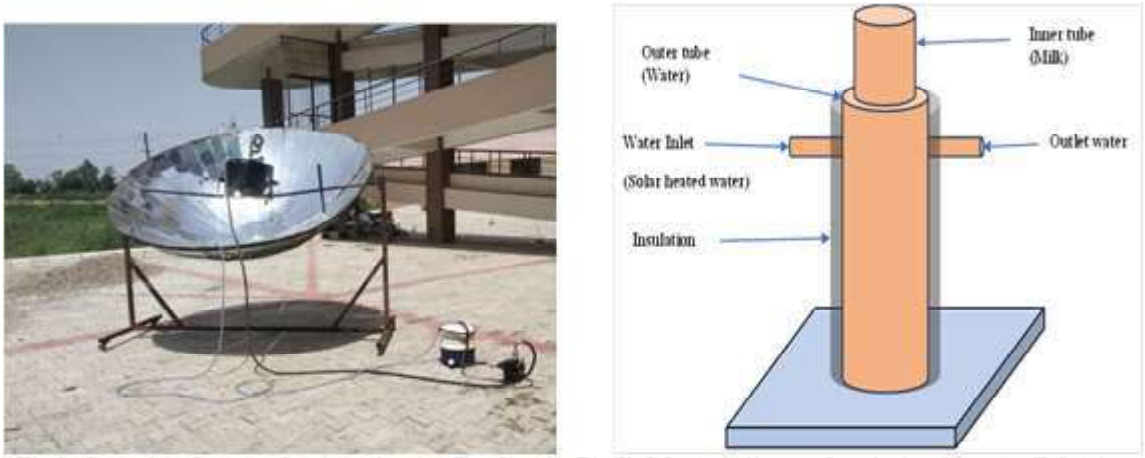


Fig. 2a Set up for solar water heating using paraffin oil

Fig. 2b Schematic diagram for tube-in-tube type milk heating equipment

**Fig. 2** Solar setup for water heating and schematic diagram for milk heating equipment

a preliminary study to ascertain the temperature attainment by the thermal fluid.

## Results and Discussion

### *Performance evaluation of the solar thermal reservoir*

### Configuration 2:C22

It was found that paraffin oil followed a logarithmic trend line, as shown in Fig. 3a, under various outside temperatures as compared to a straight-line trend under configuration 1 during the preliminary study (Sain et al. 2019a). It can be due to the simultaneous heat transfer from section 1 to section 2 (at the same time, section 1 was getting heated by the concentrated solar radiation as it was placed at the focal point of the concentrator, as shown in Fig. 2a) in comparison to the single cavity in configuration 1 (Sain et al. 2019a), where no simultaneous heat transfer occurred to any fluid.

When the temperature in section 2 exceeded 100°C, water circulation was started in section 3 through the copper pipe (Sain et al. 2019b). The temperature profile of water in Section 2 of C22 is shown in Fig. 3b. It shows that the water present in Section 2, which was receiving heat from paraffin oil (in Section 1), followed a linear trend line. The $R^2$ values under different weather conditions ranged from 0.68305 to 0.9266. Similarly, the $R^2$ values for paraffin oil ranged from 0.59759 to 0.90307 under various weather conditions during the study period.

From Fig. 3b, it can also be observed that the temperature of the water was raised to 90°C within 30 to 40 minutes, depending on the outside dry bulb temperature. The temperature differential was maintained between the paraffin oil and the water as the two curves became parallel to each other after attaining peak

**Fig. 3** Temperature profile curves in C22

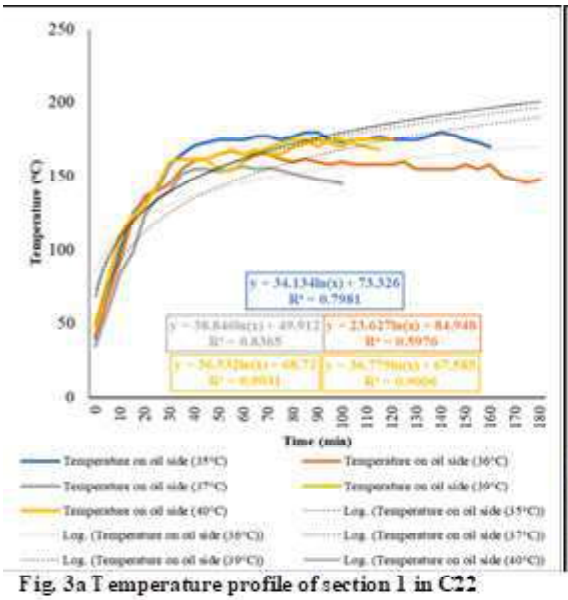


Fig. 3a Temperature profile of section 1 in C22

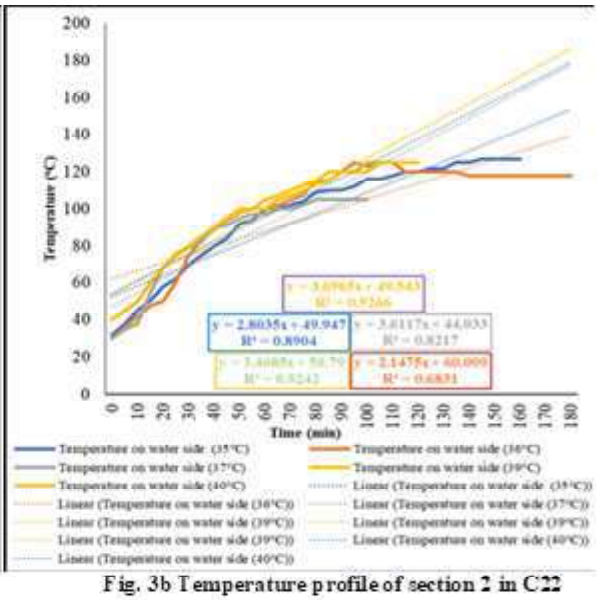


Fig. 3b Temperature profile of section 2 in C22

**Fig. 4** Temperature profile curves in C23

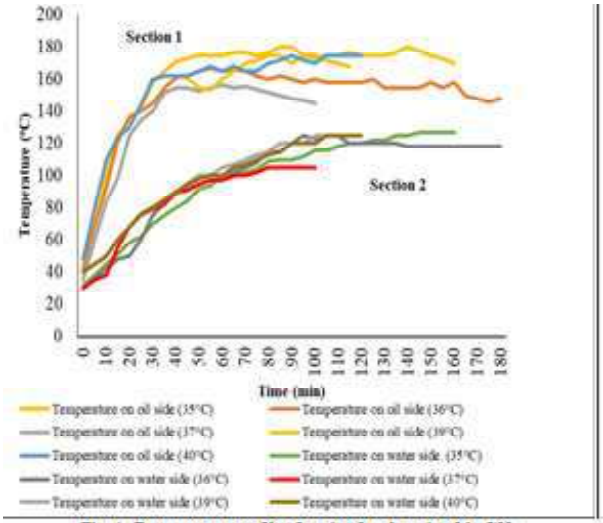


Fig. 4a Temperature profile of section 1 and section 2 in C22

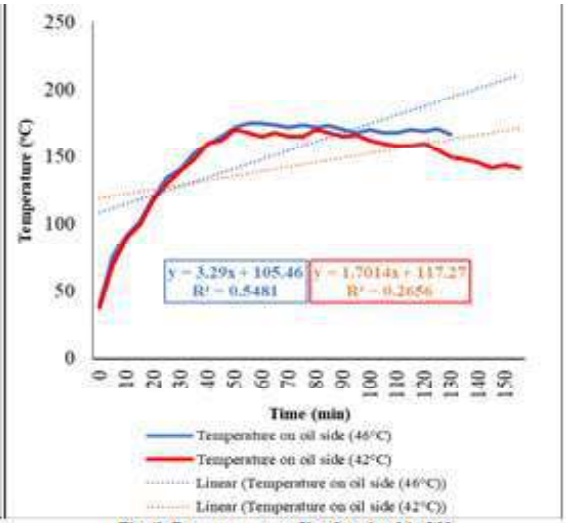


Fig. 4b Temperature profile of section 1 in C23

temperatures, as shown in Fig. 4a. The gradient varied from 20°C to 90°C under various weather conditions. It shows that paraffin oil holds enough energy to supply and maintain the water temperature in Section 2. The temperatures attained by paraffin oil during the study were 175, 170, 168, 148, and 145°C under different weather conditions, i.e., 40°C sunny, 35°C sunny, 39°C sunny, 36°C partly cloudy, and 37°C sunny with heavy wind, respectively. These mentioned temperatures were for the experiment period, which was on different days when plenty of sunshine was available to conduct the experiment.

**Configuration 2: C23**

When the temperature in Section 3 reached the desired temperature of 90°C, water circulation was started. It was observed that after 30 minutes of continuous circulation, there was a drop of 10°C of water in Section 3. The temperature profiles of Section 1 and Section 2 can be seen in Figs. 4b, 5a, and 5b.

**Time taken to attain the desired circulation temperature**

As shown in Fig. 6a, the time taken by water to achieve a temperature of 90°C in C22 was 220 minutes, whereas it was 115 minutes in the case of C23. There was a significant difference in the time taken to achieve the desired circulation temperature of 90°C in C23 compared to that in C22. This may be due to the fact that in C22, a small heat exchange surface area was available; less residence time inside the copper tube as the volume of the copper tube was very low (0.333 L). Whereas the surface area (0.231m$^2$) of the cylindrical drum (section 3) in contact with hot water and steam (section 2) in C23 was higher, so was the high residence time of water due to the larger volume of the cylindrical

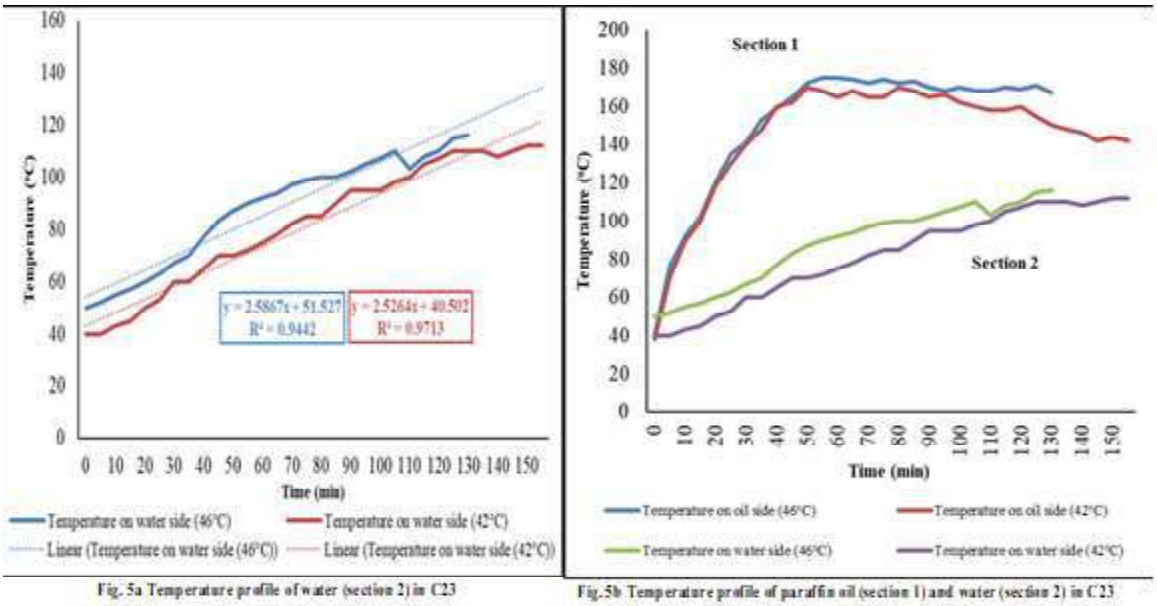


Fig. 5a Temperature profile of water (section 2) in C23

Fig. 5b Temperature profile of paraffin oil (section 1) and water (section 2) in C23

**Fig. 5** Temperature profile of water and paraffin oil in C23

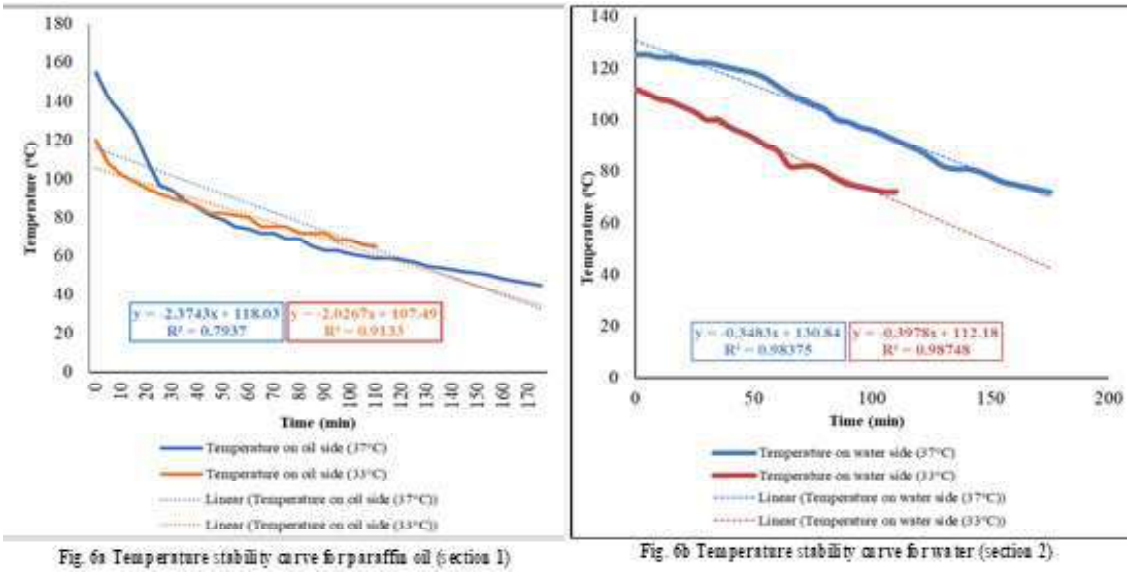


Fig. 6a Temperature stability curve for paraffin oil (section 1)

Fig. 6b Temperature stability curve for water (section 2)

**Fig. 6** Temperature stability curves for paraffin oil and water

drum (9.5 L), which gave better heat transfer. It may also be because, in C22, steam heated the water in a copper pipe, so the time water takes in Section 2 to get converted to steam contributes towards the longer duration of 220 minutes. Also, the copper pipe was not in contact with water in Section 2.

**Temperature stability of paraffin oil and water**

After the attainment of maximum temperatures by paraffin oil (section 1) and water (section 2), it was required to observe the stability of temperatures in both sections. A fall in temperature was recorded at an interval of 5 minutes to observe the temperature stability in both sections. The temperature stability of paraffin oil and water can be seen in Figs. 6a and 6b, respectively. The curve followed the linear trend with a negative slope. It was observed that the hot water temperature could be maintained for at least 1 to 2 h even when the setup was placed in the laboratory under ambient conditions. Preliminary trials of the present study and an earlier study by Sharma et al. (2019) found that the most effective time to process the milk using solar parabolic concentrator energy under available sunshine is about 4 h, whereas, with the use of thermal energy storage oil, the processing hours were increased to 5 to 6 h. So, the present study has shown the possibility of increasing processing time by at least 25 to 50 percent (4 h of efficient sunshine and an additional 1-2 h from the thermal energy stored by paraffin oil) depending upon the ambient conditions by using paraffin oil as a thermal energy storage material (Sain et al. 2019a).

**Table 1:** Performance evaluation of tube-in-tube milk processing equipment

| Time (seconds) | Temperature of) circulating water (90°C | Temperature of milk (°C) |
|---|---|---|
| 0 | 90 | 30 |
| 60 | 84 | 40 |
| 160 | 80 | 63 |
| 175 | 78 | 73 |
| 180 | 77 | 75 |

**Performance evaluation of developed tube-in-tube-type milk processing equipment**

Table 1 shows the fall in temperature of hot water from the initial temperature of 90°C and the rise in temperature of milk from the initial temperature of 30°C. It was recorded that the milk temperature rose from an initial 30 to 75°C, i.e., temperature above 72°C (milk pasteurization temperature), which is required for continuous pasteurization of milk. The temperature was attained in 180 s or 3 minutes. From the experiment, it was found that solar energy, in combination with paraffin oil as a thermal storage substance, can be effectively used to attain pasteurization temperatures for the continuous pasteurization of milk. It implies that milk can also be batch-pasteurized using the setup used in the present study.

## Conclusion

The present study has revealed that a solar parabolic dish, along with a three-cavity container having paraffin oil as a TES material, can attain sufficient temperature to process milk even during off-peak sun hours. The sole aim of the study was to extend working hours using solar energy, which was successfully attained by increasing the available energy time by 25 to 50 percent. C23 was found to be better than C22 as it took less time (115 minutes) to achieve circulation temperature (90°C) as compared to C22 (220 minutes). The whole study was based on a lab scale setup, but a scaled-up, modified setup can be tested for its application in milk processing operations at a small-scale level. Also, the setup can be tested for plate heat exchangers, as they are widely used in the milk processing industries due to their high thermal efficiency.

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RESEARCH ARTICLE

# Probiotic evaluation studies and elemental composition of iron-fortified sweet corn milk-based probiotic yoghurt

**P Geetha**

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**Abstract**: Yoghurt is one of the important fermented milk products with numerous nutrition and health benefits but has low iron content. To further improve the nutritional value sweet corn milk was incorporated at 30% along with cow milk and ferrous lactate for the development of iron-fortified probiotic sweet corn blend milk yoghurt. The premix was fermented with 2% probiotic cultures: *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* at 42°C for 4h. It was stored for 16 days under refrigerated conditions. Food grade ferrous lactate hydrate salt was used at different concentrations like 10mg and 20 mg per 100 ml of premix for fortification. It proved the addition of ferrous lactate had not affected yoghurt characteristics. The elemental composition analysis was done using an X-ray fluorescence spectrophotometer. 20 mg ferrous lactate fortified probiotic sweet corn blend milk yoghurt remained acceptable even at 16 days of storage. This iron-fortified probiotic yoghurt serves as a potential fermented milk product for commercial use.

**Keywords**: Cow milk (4.5%); Ferrous lactate, Fortification; *Lactobacillus acidophilus;* Sweet corn; *Streptococcus thermophilus,*

## Introduction

Yoghurt as a health food has attracted the attention of the middle class in India because of increased disposable income and better health-benefit awareness. Yoghurt prepared with probiotic cultures is being commercialized in the market with health promoting and gut health improving aspects. This has been proven to promote gut microbiota with numerous therapeutic benefits like cholesterol-lowering effect, increase in the bioavailability of minerals and treatment of gut-related aliments (Hadjimbei et al. 2022).

Daily iron requirement of human may vary based on age and gender. It could be summarized as 8 to 18 mg of iron may be required for humans. For example, females of age 18 and above may require 18 gms of iron whereas males of same age group may require 8 gms of iron. Since iron fortified yoghurt is having 20 gms of iron per 100 ml, it is recommended that females could take 100 ml of iron fortified yoghurt per day whereas males could consume 50 ml per day (NIH, 2023). As cow milk has lower iron content food grade ferrous hydrate can serve as a potential iron fortifier in yoghurt. Food fortification with iron has been recommended as one of the preferred approaches for preventing and eradicating iron deficiency. However, fortification with bioavailable iron sources often presents multiple challenges in product acceptance, product shelf life, and effectiveness in improving iron status. In developing an effective iron fortification technology, it is critical that the chemical property of iron that contributes to the development of undesirable organoleptic properties is taken into consideration (Banjare et al. 2019). It is well known that two major off-flavors may be associated with fortified dairy products: oxidized flavor resulting from catalysis of lipid oxidation by iron and metallic flavor contributed by iron salts. No oxidative rancidity had been detected in freshly prepared and stored samples of yoghurt whereas iron fortified yoghurt showed slight rancidity due to different sources of iron.

Further, sweet corn milk is being used as a supplement or replacer for many milk-based products. This would serve as alternative vegetable milk. Sweet Corn *(Zea mays L.* ssp. *saccharata)* is one of the largest vegetable crops grown. Primary interest has been directed to carbohydrates, since in the milky stage when the grain is harvested for food use, carbohydrates determine flavor and texture (Kokkinidou et al. 2019). Several workers have investigated animal milk or soy-milk yoghurts, but little work has been done on corn–milk yoghurt. Production of yoghurt from

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corn milk was aimed to combine the good sensory characteristics of the corn milk with the well-known yoghurt flavor.

It was reported that the sensory quality of iron-fortified dairy foods has been shown to be affected by the type of iron used, the amount of iron added and the properties of dairy products being fortified (Amira et al. 2011 and Hurrell, 2021). Hence this study was taken up to formulate iron-fortified sweet corn milk incorporated yoghurt with *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* and analyzed for viability and acid tolerance during storage.

## Materials and Methods

### Materials

Milk (4.5% fat), sweet corn and skim milk powder were obtained from the local market. *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were purchased from Centre for Food Technology, Anna University, Chennai. *Lactobacillus* MRS agar for *Lactobacillus acidophilus* and M17 media for *Streptococcus thermophilus* were obtained from Hi-Media and were used for microbial analysis. Food grade ferrous lactate hydrate was obtained from Sigma Aldrich and was used for fortification.

### Preparation of Corn milk

To prepare the corn milk, the corn cobs were firstly husked, the silks removed and washed with water. The seeds were then separated from the cleaned cobs using knives. The corn seeds were ground using a grinder. 50 ml of water was added to 100 g of corn seeds during grinding. The slurry was then filtered using a filter to produce a milky liquid. The corn milk was heated to 80°C for 10 min and stored at -18°C until use. Two types of corn were used in this study. Both corn milk and sweet corn milk were prepared by this method.

### Stock culture preparation

The Slant cultures of *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were grown by inoculating into M17 broth and MRS medium respectively for 18 h at 37 °C. One loop of each culture was transferred into 10 ml of litmus milk prepared by mixing 16% (w/v) skim milk powder (SMP) and 0.3% (w/v) yeast extract. The inoculated culture was incubated for 18 h at 37 °C and stored at 5 °C until use.

### Mother culture

An individual mother culture was freshly prepared before conducting the experiment by inoculating one loop of stock culture into 100 ml of sterilized milk medium containing 16% (w/v) SMP and 0.1% (w/v) yeast extract. The inoculated culture was incubated at 37°C for 18 h and kept at 5°C until use.

### Preparation of Iron-fortified Sweet corn milk supplemented yoghurt

In preparation of control yoghurt 100% cow milk and unfortified sweet corn blend milk yoghurt of 70% cow milk and 30% sweet corn milk were used using the below-mentioned method (Fig 1) as described in Geetha et al (2018). Ferrous lactate hydrate of food grade at 10, 20 and 30 mg was incorporated as an iron fortifier. The yoghurt containing 30 mg of ferrous lactate resulted in high whey syneresis, hence it was not considered for optimization. Control = 100 % cow milk, Sample 1 = cow milk: sweet corn milk (70:30) ml, Sample 2 = cow milk : sweet corn milk (70:30) ml + 10 mg Ferrous lactate, Sample 3 = cow milk: sweet corn milk (70:30) ml + 20 mg Ferrous lactate was taken for studies.

### Probiotic evaluation studies

Viable count and acid tolerance tests were done using methods stated in B.I.S.2002 and ICMR-DBT 2011. Analysis was carried out on 0,4,8,12 and 16$^{th}$ days of storage.

### Elemental composition analysis

The mineral composition of yoghurt samples was determined by using the X-ray fluorescence spectrophotometer (Model: Minipal 4 Benchtop XRF, Elemental range : Al…Y, Pd…U, Size : 300x550x450 mm$^3$, Fine focus X-ray tube with MO Target, Multilayer monochromator 17.5 Kev) . All the yoghurt samples were individually freeze-dried in a freeze-dryer (CRYO Technologies, Chennai) to obtain a fine powder. The powdered samples were placed in separate compartments in the XRF analyzer. The elemental analysis was done and results were obtained in graphical representation.

## Results and Discussion

### Probiotic evaluation analysis of yoghurt

#### *Viable count of probiotic organisms in yoghurt during 16 days of storage studies*

The viability of both the strains decreased as the days of storage increased in all the samples along with the control sample (Fig 2). The % viability of *Lactobacillus acidophilus* of control samples were 94.95 %, 87.90%, 84.01% and 78.13% on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 92.6%, 88.5%, 83.06% and 79.93% of viability on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Lactobacillus acidophilus* in yoghurt during 16$^{th}$ days of storage. The viable count showed colonies greater than 10$^9$ CFU/ml during 16 days of storage studies. However the values reported by Codex stated that lactobacilli in yoghurt should be in the range of 10$^7$ ( (Lopez, 2014 and CODEX STAN, 2011).

The % viability of *Streptococcus thermophilus* in control samples was 96.3%, 92.43%, 88.42% and 86.31% on 4th, 8th, 12th and end of 16th day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 93.33%, 88.32%, 86.22% and 81.65% on 4th, 8th, 12th and end of 16th day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Streptococcus thermophilus* in yoghurt during 16th days of storage. The viable count showed colonies greater than $10^9$ CFU/ml during 16 days of storage studies. Many studies reported that the iron fortification in yoghurt and dairy foods and their impact on sensory qualities and survivability of yoghurt bacteria (Azzam, 2009). There is a lack of studies about iron fortification in probiotic yoghurt and its effect on the viability of probiotics. Many of the studies reported that the viability of the starter microflora as well as probiotic was not affected by the iron fortification in yoghurt (Dabour et al. 2019).

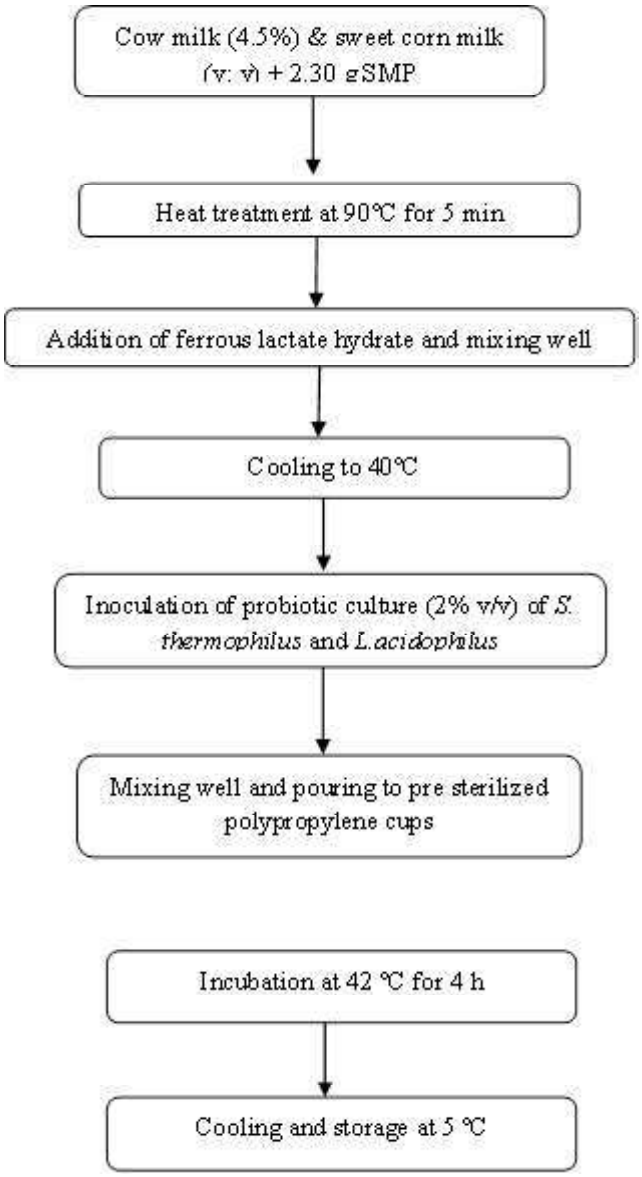


**Fig 1.** Optimized flow diagram for preparation of ferrous lactate fortified sweet corn blend milk yoghurt

**Acid tolerance test for probiotic organisms in yoghurt**

The number of cell counts remained significantly unchanged during all the interval transit at pH 3 and pH 7.2. Good acid tolerance properties exhibited by the bacteria are closely related to their strains specification as they are always strains dependent (Huang and Adams, 2004; Lin et al. 2006). The % viability of *Lactobacillus acidophilus* was 93.25 % and 97.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt respectively. Viability counts of the bacteria usually decline tremendously when exposed to simulated gastric juice of pH 1.5 after an incubation period of 3 hours. The threshold point to determine acid resistance was set at a pH value of 3.0 and an incubation period of 3 hours in the *in vitro* studies as it simulates the residence time in the stomach (Prasad et al. 1998; Haddadin et al. 2008). The developed 20 mg of iron fortified corn milk yoghurt has proven to be successful to meet the minimum requirement of $10^6$ viable probiotic cells per ml at pH 3 after exposure for 3 hours (Sahadeva et al. 2011) (Fig 3) FAO/WHO (2002). The % viability of *Streptococcus thermophilus* was 93.29% and 90.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt. From the graph, *S. thermophilus and L. acidophilus* strains showed acid tolerance at pH 7.2 and 3.0 after 3 hours of incubation. Many studies supported that bioavailability of iron was good in yoghurt (Drago and Valencia 2002).

**Elemental composition analysis of yoghurt**

The X-ray fluorescence analysis (XRF) of milk and dairy products has not yet become widespread in dairy industry, although the method has a great potential, as the dried samples can be analyzed directly without any chemical treatment and XRF equipment is rather accessible (Galina Pashkova, 2009).

An X-ray photon of sufficient energy strikes an atom; it dislodges an electron from one of its inner shells (K in this case). The atom fills the vacant K shell with an electron from the L shell; as the electron drops to the lower energy state, excess energy is released as a $K_a$ X-ray. The atom fills the vacant K shell with an electron from the M shell; as the electron drops to the lower energy state, excess energy is released as a $K_b$ X-ray. The emission of fluorescence is specific for individual elements. The graphical representation of this electron transition within the orbital is given by concentration cps vs. Kev (energy)

Based on the standard observations made on different elements (Van Grieken, & Markowicz, 2001),

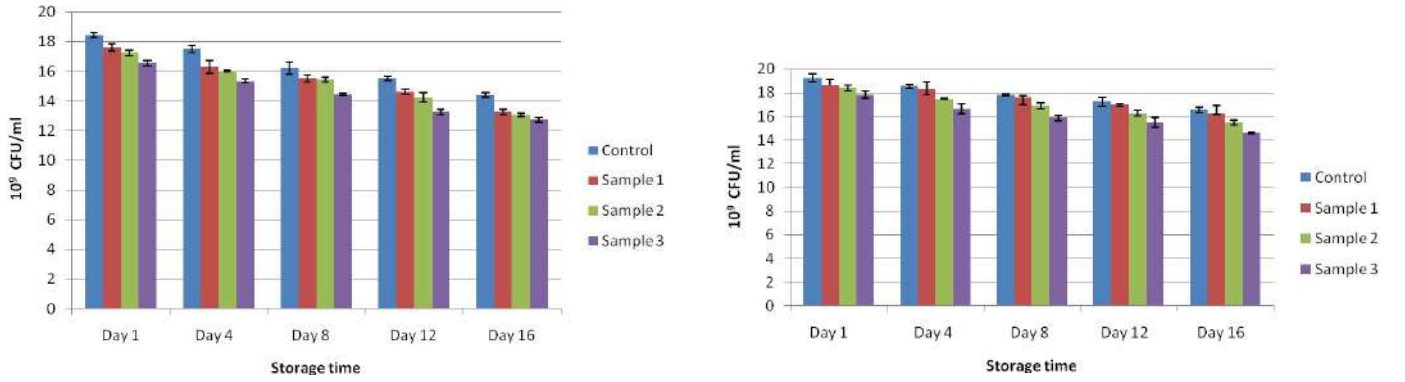


**Fig. 2** Viable count of *Lactobacillus acidophilus* and of *Streptococcus thermophilus* in yoghurt during 16 days of storage studies

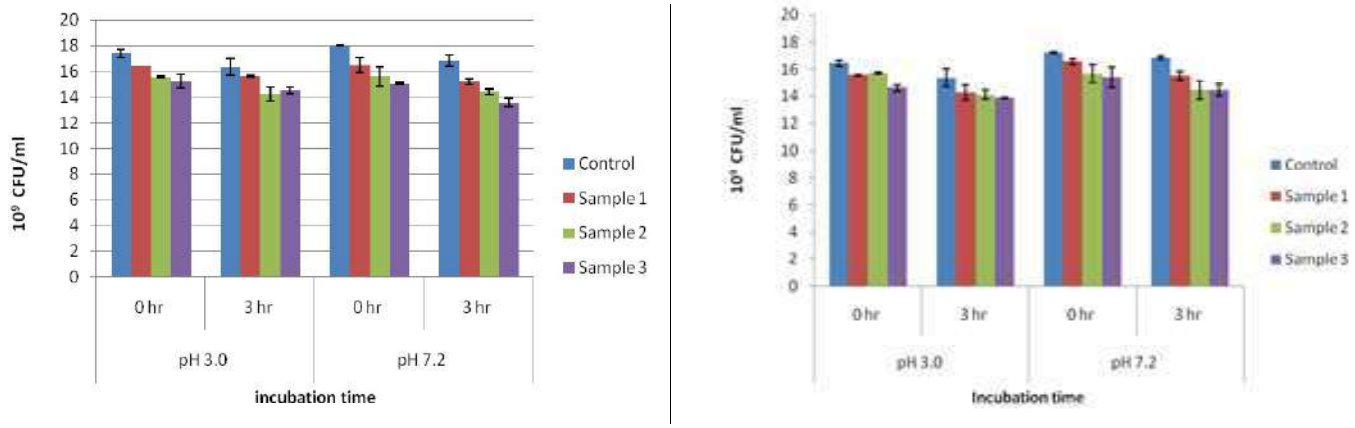


**Fig.3.** Acid tolerance results of *L. acidophilus* and *S. thermophilus* in yoghurt

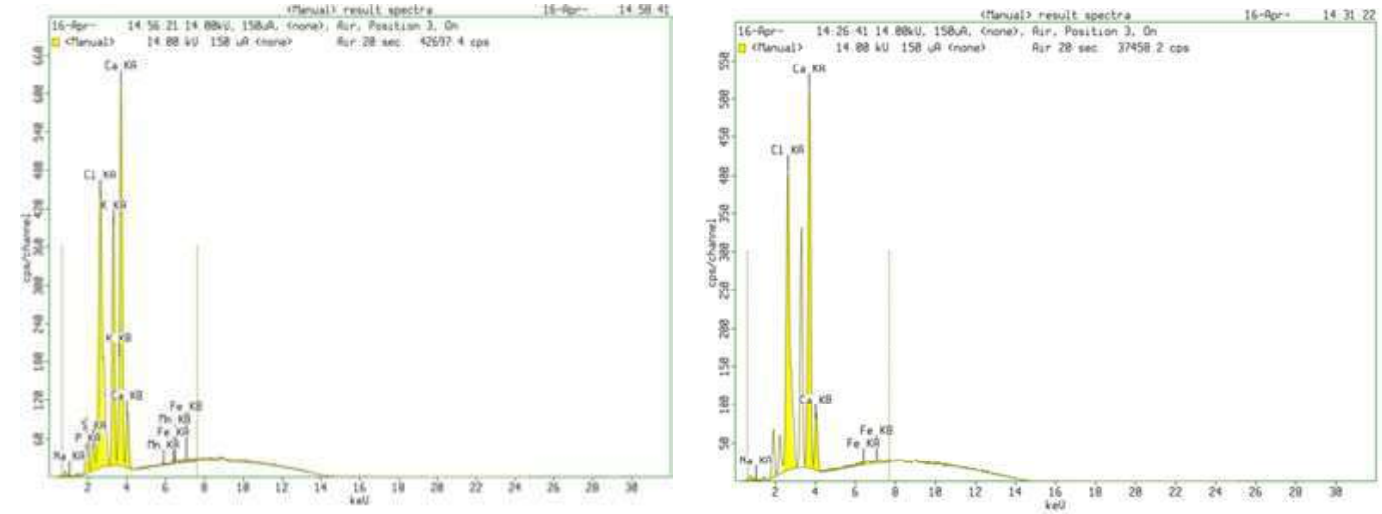

**Fig. 4** XRF analysis graph of control and probiotic sweet corn blend milk yoghurt

- Peak intensity >100 cps corresponds to concentrations >10,000 ppm (% levels)
- Peak intensity of 10-100 cps corresponds to concentrations of ~100-1000 ppm

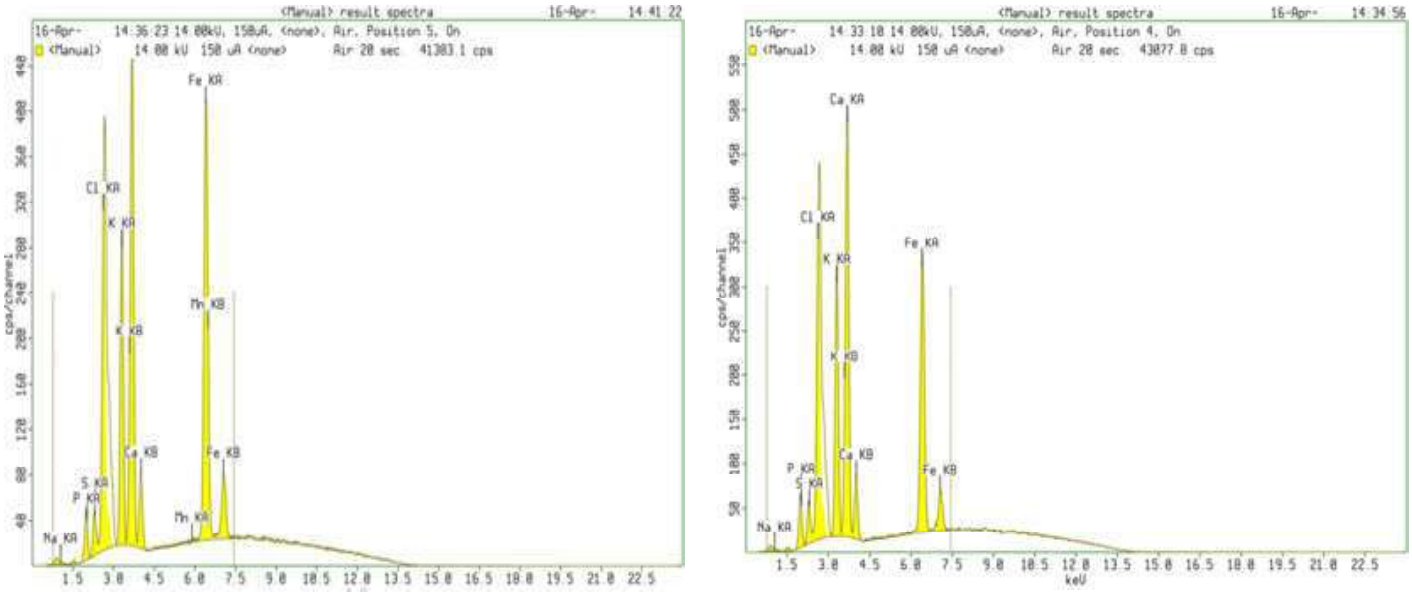


**Fig 5**. XRF analysis graph of probiotic sweet corn blend milk yoghurt fortified with 10 and 20 mg of ferrous lactate hydrate

- Peak intensity of 1-10 cps corresponds to concentrations ~10-100 ppm
- Peak intensity < 1 cps corresponds to concentrations ~1-10 ppm

The Cps (counts per second) versus the energy graphs, depicting the concentration of different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) for the yoghurt made from 100% cow milk are presented in Fig 4. The concentrations for Na, K, Ca, and Fe expressed as cps were 13.986 cps, 5098.625 cps, 8541.48 cps and 13.125 cps respectively. The approximate results show K and Ca are greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm. The concentration for Na, Ca and Fe was 23.986 cps, 7292.234 cps and 16.290 cps for yoghurt without elemental iron fortification. The approximate results show Ca is greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm.

The Cps (counts per second) Vs the energy, the concentration for different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) was found. The concentration for Na, K, Ca and Fe was 21.944 cps, 3963.33cps, 6597.43cps and 5119.99 cps for the probiotic yoghurt incorporated with 10 mg elemental iron. The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The concentration for Na, K, Ca and Fe was 26.639 cps, 3620.663 cps, 6090.110 cps and 6574.40 cps for probiotic yoghurt with 20 mg iron fortification (Fig 5). The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The peak values corresponding to iron showed a gradual increase from control yoghurt to 10 and 20 mg of iron-fortified corn milk yoghurt. From the results, it was concluded that qualitatively Fe concentration was present.

## Conclusion

Yoghurt is a fermented milk product that is popular around the world for its health benefits. It acts as a medium for accumulating various nutrients. Since milk is deficient in iron, many researchers around the world have experimented with different methods and iron sources to fortify yoghurt. Overall, the study indicates that the iron-fortified corn milk yogurt maintained the viability of Lactobacillus acidophilus and Streptococcus thermophilus during storage and demonstrated good acid tolerance properties, which are important traits for probiotic microorganisms. Elemental analysis showed increased iron concentrations in the 10 mg and 20 mg iron fortified corn milk yogurt samples. Thus, the developed product shows successful results in terms of its properties.

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RESEARCH ARTICLE

# Impact of brewery waste on the productive and reproductive traits in Jersey crossbred dairy cattle

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**Abstract:** The study was conducted on twenty four Jersey crossbred dairy cows for a period of one year with three treatments to see the impact of brewery waste on productive and reproductive traits. The control (T0), brewery waste (T1) treatment and balanced ration (T2) treatment was carried out in farmer's field with eight animals in each group. The control, brewery and balanced ration animals were fed as per traditional and standard feeding practices. Productive traits *viz*., milk yield, milk fat, solids not fat (SNF), body weight were assessed before and at the end of experiment. Productive traits *viz*., lactation period and dry period and reproductive trait like number of services per conception (SPC) were also assessed. The results for milk yield and body weight showed that T0 is at par with T1 and T1 at par with T2 ($P>0.05$), and only in T2 significant increase ($P<0.05$) compared to T0. Notable decrease of milk fat and SNF is evident for T1, compared to T2 although the loss deemed to be non significant ($P>0.05$). Among the reproductive traits, no significant difference ($P>0.05$) is evident in lactation period between the treatments, with the values being higher for T2 (302.39 days) and lower for T0. With regard to dry period and SPC, no significant difference ($P>0.05$) is evident between treatments with values being higher for T0 (70.60 days & 2.16) and lower for T2 (63.11 days & 1.83). To conclude, balanced ration significantly increased ($P<0.05$) the milk yield and body weight of the animals followed by brewery waste and control in the descending order of magnitude.

**Key words:** Balanced ration, Body weight, Brewery waste, Milk fat, Milk yield, Solids not fat, Reproductive traits

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## Introduction

India being an agricultural country, the role of livestock has its own significance in economy and socio- economic development. India is an agrarian economy and farmers are known as the backbone of the economy. For many years livestock plays an important role in generating income towards sustenance and for their livelihood. In the present scenario due to dwindling variations in the climate the expected crop returns could not be achieved and under such circumstances, dairying is considered as a major component in the era of Indian agriculture. In the recent past, dairy farming has turned up in to a vital component in alleviating the problem of unemployment for augmenting income generation and livelihood security. In dairy farming, feeding cost plays an important role which could affect the profit of the enterprise. The cost of feeding is the single most important factor affecting the profitability of a dairy enterprise. Due to the shortage of raw feed ingredients, feed cost increased day by day continuously. The scarcity of raw feed ingredients will compel to utilize the newer or non-conventional feed resources for feeding of livestock. To attain maximum profit through livestock, farmers can use agricultural and industrial by products which are cost effective.

During computation of cattle feed in lactating cows, it has been stated that after drying wet brewer's grain could be efficiently used (Dhiman et al. 2003). Wet brewery grain has 20 – 32% dry matter which is good source of rumen un-degradable or "bypass – protein" and the concentration of rumen degradable protein ranges from 28 – 43% (Thomas et al. 2016). In the recent past, wet brewery is used by most of the dairy farmers because of its affordable price. Hence, the present study was undertaken to see the comparative performance of brewery waste on the productive and reproductive traits in Jersey crossbred dairy cattle.

## Materials and Methods

### Experimental site and selection of animals

The study was carried out in two farmer's field at Melvenkatapuram village, Ranipet District of Tamil Nadu. A total of 24 dairy animals were selected and randomly distributed in to

three groups with eight animals in each group possessing uniform body weight and milk yield. The experiment was initiated in farmer's field wherein the first possess 16 animals and the second farmer had 8 animals. Selected dairy animals were of Jersey crossbred in 1st lactation of 3 – 4 years which were calved around 45 – 60 days with an average milk yield of 5 - 5.5 kgs/day and body weight of 250.02 kgs. The study was carried out in September 2019 with deworming being carried out as per standard schedule using fenbendazole. All the selected dairy animals were given an adaptation period of two weeks prior to the experimental study from continued from October 2019 to September 2020.

**Experimental diets**

The study was carried out with three treatments Viz., T0 (Control), brewery waste (T1) and balanced ration (T2) with T0 and T1 treatment being carried out in 1st farmers field and T2 treatment in 2nd farmers field. In T0 (Control), animals were fed with rice bran/ wheat bran, oil cakes as per their traditional feeding practices being followed in the field. Based on the dry matter requirement and milk production, T1 and T2 animals were fed during the trial period. During this period, green fodder @ 9 Kgs /animal/day and dry fodder (paddy straw) @ 5 Kgs/animal/day were fed to the experimental groups animals. The brewery waste was fed @ 1 Kg/kg of milk production in T1 group and the concentrate feed was provided @ 400 gms/Kg of milk production in T2 group dairy cattle. For 1 kg of milk production, approximately 1000 kcal of gross energy is required and the brewery waste (T1) contained 1931 kcal/ kg on dry matter basis. Hence, 1 kg of brewery waste, is needed for every 1 kg of milk production and for every 1 kg of milk production, 400 grams of concentrate feed is required which could equate with ICAR 2013 standard. During dry period, T1 and T2 were fed @ 4 kgs of brewery waste and 1.5 kgs per day per animal respectively as maintenance requirement.

Initially the experimental diet on control (T0 - control), brewery waste (T1) and balanced ration (T2) were analysed for proximate principles at Animal Feed Analytical and Quality Assurance Laboratory, Namakkal (AOAC, 1990) and are presented in Table 1.

**Body weight estimation**

The body weight of the dairy cattle was calculated before and final experimentation using Shaffer's Formula (Sastry et al. 1983) in all three treatment groups (T0, T1 and T2).

$$\text{Body weight (kgs)} = \frac{L \times G^2 \times 0.4536}{300}$$

L = Length from the point of shoulder to the point of pin bone (in inches)

G = Heart girth of the animals (in inches)

**Collection of milk for estimation of Milk fat and Solids not fat**

Milk samples were collected in wide mouthed plastic bottles from the cows prior to start of the experiment and on every month till the end of experiment for analysis of milk fat and solids not fat (SNF) by Gerber's method (Indian Standard, 1977).

**Recording of Milk production**

The milk production of the control (T0), brewery waste (T1) and balanced ration (T2) feeding was recorded daily from the start to the end of experiment.

**Collection of data for productive and reproductive traits**

The data pertinent to date of calving, number of services, date of conception, and date of drying were recorded. The lactation length, dry period and number of services/conception were also assessed.

**Statistical Analysis**

The data collected on productive traits (milk yield, fat, solids not fat, body weight, lactation period and dry period) and reproductive traits (number of services per conception)

from the Jersey animals were subjected to one way Analysis of Variance (ANOVA) using statistical software, IBM SPSS version 20.0. This analysis was performed to find out the significant difference between treatments and final interpretation was done as per procedure of Gomez and Gomez (1984).

**Table 1**: Proximate Principles (in %)

| Sr.No | Particulars | Control (T0) | Brewery waste (T1) | Balanced ration (T2) |
|---|---|---|---|---|
| 1. | Moisture | 9.15 | 73.17 | 12.22 |
| 2. | Crude protein | 7.36 | 13.90 | 19.18 |
| 3. | Crude Fibre | 5.95 | 6.40 | 9.02 |
| 4. | Ether Extract | 4.95 | 5.13 | 6.09 |
| 5. | Total Ash | 5.02 | 5.76 | 7.19 |
| 6. | Gross Energy (K.Cal/kg) | 1323 | 1931 | 3708 |

## Results and Discussion

### Productive traits

Milk Yield: The mean values/ gain in milk yield (kgs/animal/day) and total milk production / daily body weight gain of the Jersey cross bred cattle under various treatment regimens are presented in Table 2 and 3. It was evident that there was a marginal gain in milk yield for all the treatments after the end of experimentation. A significant difference ($P<0.05$) in gain of milk yield was observed between the treatments. There was maximum increase in gain of milk yield in balanced ration group (T2:1.56 kgs/animal/day) followed by brewery (T1: 0.73 kgs/animal/day) and control (T0: 0.13 kgs/animal/day) groups. The total milk production was higher for T2 followed by T1 and T0, but the difference was non-significant ($P>0.05$).

In control (T0) animals, there was a marginal increase in milk yield of 0.13 kgs/ animal/ day at the end of experimentation and they were actually fed with wheat/rice bran, rice gruel and ground nut oil cake (GNC) in an imbalanced proportion without meeting the dietary requirement of the animal. The concentrates fed to the control animals contained 1323 K.cal/kg energy, 7.36% protein and 5.95% crude fibre. This could be the probable reason for comparative less milk yield than brewery (T1) and balanced ration (T2) fed groups. Any animal if underfed or fed imbalanced ration without meeting the requirement, there will be definite decline in milk production. This corroborated with the findings of Garg et al. (2016) who observed 10.36 kgs/day milk production before experimentation and after ration balancing program, the milk yield significantly increased ($P<0.01$) to 11.67 kgs/day implying the importance of balanced feeding on milk production. Research also suggested that the increase in dietary crude protein (on a dry matter basis) from 17 % to 19 % for lactating dairy cows would definitely meet the nutritional requirements (Ibtisham et al. 2018). Protein sources provide specific amino acids to the dairy animals which are very essential for body maintenance, milk production and reproduction. Nutritional management during pre-parturient and early lactation is most important in dairy cattle in which the milk yield increases at a faster rate than energy intake in the first 4 to 6 weeks after parturition and hence the intake of balanced ration is very important to meet out the nutritional requirement.

In lactating dairy cows the protein deficiency may decrease appetite and dry matter intake resulting in low milk production (Ibtisham et al. 2018).

In case of brewery waste fed dairy cattle (T1), the gain in milk yield (0.73 Kgs/animal/day) at the end of experimentation were higher than the control (T0) animals, but marginally lower than the balanced ration (T2) fed animals. The higher milk yield for brewery treated animals could be attributed to the fact that the brewery waste had larger degradable fraction of protein, which is converted into microbial cell protein, digested and absorbed in the duodenum and increased the milk yield. This is in accordance with the findings of Senthil Murugan et al. (2015), who stated that feeding ration with 20% wet brewer's grain increased the milk yield than 30% inclusion level and control diets. The results also supported with the earlier findings of Imaizumi et al. (2015), who reported increased milk yield in lactating Holstein dairy cows fed with ration containing wet brewer's grain.

The higher milk yield of brewery treated animals in current study could be due to the presence of high amount of un-degradable protein in brewery waste, which is essential for body building and body reserves needed for milk synthesis during lactation. Moreover, the high amount of un-degradable protein makes them a good source of rumen by pass protein which remains intact and becomes available in the abomasum and small intestine where they are utilized by the animals for milk production. Further, Chiou et al. (1998) observed that the brewery grain had higher amount of un-degradable protein, making them a good source of rumen by pass protein and the use of increased amount of rumen un-degradable protein (by pass protein) from dietary concentrates increased the milk yield because of improved protein supply and improved intake of metabolisable energy from concentrates. The gain in milk yield (1.56 kgs/animal/day) and total milk production (2099.09 kgs) were higher in balanced ration fed animals (T2) than control (T0) and brewery waste (T1) fed animals. This increase in milk yield could be due to the supply of balanced nutrition which increased the rumen microbial protein synthesis to make more optimal rumen function for increased milk production (Garg et al. 2014). They also stated that feeding of balanced ration increased ($P<0.05$) the average daily milk yield by 6.7% than unbalanced feeding regimen. Energy and protein are the most important limiting factors for milk production and its supplementation in the diets of lactating ruminants would have increased milk yield (Manjunatha et al. 2018; Garg et al. 2016). Further the increase in milk yield could be due to balanced nutrients which would have improved the microbial protein synthesis and supplied essential nutrients (Garg et al. 2016). On feeding a balanced ration, dietary energy and protein can be utilized in a more efficient manner resulting in higher milk yield.

### Milk Fat and Solids Not Fat

The mean values and gain/ loss of milk fat and SNF (%) in Jersey crossbred cattle under different treatment regimens are presented in Table 2. Significant difference ($P<0.01$) is evident between treatment groups after the end of experimentation and the milk fat and solids not fat content was higher for balanced ration fed groups (T2) than control (T0) and brewery (T1) fed groups. The milk fat and SNF loss was higher for control groups (T0: - 0.05% & - 0.03%) followed by brewery (T1: - 0.35 % & - 0.12%) fed group animals. On the other hand there was a gain in milk fat for balanced ration animals (T2: 0.47%), but the difference was non-significant ($P>0.05$) among the groups.

**Table 2:** Average and gain in milk yield, fat, solids not fat and body weight (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr. No | Parameter | Before experimentation | | | F value | At the end of experimentation | | | F value | Gain/ loss | | | F value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | | T0 | T1 | T2 | | T0 | T1 | T2 | |
| 1. | Milk yield (kg/animal/ day) | 4.91 ±0.31 | 5.01 ±0.20 | 5.38 ±0.47 | 0.52$^{NS}$ | 5.04 ± 0.71$^{b}$ | 5.74 ±0.38$^{ab}$ | 6.94 ± 0.50$^{a}$ | 2.72$^{NS}$ | 0.13 ±0.02$^{b}$ | 0.73 ±0.07$^{ab}$ | 1.56 ±0.15$^{a}$ | 3.63$^{*}$ |
| 2. | Fat (%) | 4.43 ± 0.09 | 4.75 ± 0.51 | 4.65 ±0.62 | 0.12 NS | 4.38 ±0.07$^{b}$ | 4.40 ±0.14$^{b}$ | 5.12 ±0.28$^{a}$ | 14.13$^{**}$ | – 0.05 ± 0.09 | – 0.35 ± 0.50 | 0.47 ± 0.65 | 0.75 NS |
| 3. | Solids Not Fat (%) | 8.48 ±0.09 | 8.45 ±0.08 | 8.37 ±0.14 | 0.30 NS | 8.45 ± 0.06$^{b}$ | 8.33 ±0.04$^{b}$ | 8.62 ± 0.05$^{a}$ | 8.42$^{**}$ | – 0.03 ± 0.13 | – 0.12 ± 0.09 | 0.25 ± 0.11 | 3.03 NS |
| 4. | Body weight ( kg/animal) | 258.79 ± 10.87 | 234.78 ± 18.19 | 266.48 ± 17.40 | 1.01$^{NS}$ | 254.04 ± 13.03 | 254.31 ± 20.93 | 300.31 ± 30.78 | 2.12$^{NS}$ | –4.75 ± 1.78$^{b}$ | 19.52 ±2.53$^{ab}$ | 33.83 ± 3.42$^{a}$ | 5.32* |

*Means bearing same superscripts within rows do not differ significantly*

*$^{**}$ - Highly Significant (P<0.01)  $^{*}$ - Significant (P<0.05)  NS – Non Significant (P>0.05)*

**Table 3:** Average total milk production and daily body weight gain (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr.No | Parameters | Mean gain/ loss | | | F value |
|---|---|---|---|---|---|
| | | T0 | T1 | T2 | |
| 1. | Total milk production (kg/animal) | 1488.30 ± 36.28$^{b}$ | 1722.45 ± 62.67$^{ab}$ | 2099.09 ± 74.92$^{a}$ | 3.15$^{NS}$ |
| 2. | Average daily gain (gms/day/animal) | – 13.01 ± 0.35$^{b}$ | 53.48 ± 3.26$^{ab}$ | 92.68 ± 3.99$^{a}$ | 5.32* |

*Means bearing same superscripts within rows do not differ significantly*

*NS – Non Significant (P>0.05)  $^{*}$ - Significant (P<0.05)*

**Table 4 :** Average productive and reproductive traits (mean ± S.E) of the Jersey crossbred cattle under different treatment regimens

| Sr.No | Parameters | Mean values on reproductive parameters | | | F value |
|---|---|---|---|---|---|
| | | T0 | T1 | T2 | |
| 1. | Lactation period (days) | 295.20 ± 6.48 | 299.99 ± 12.96 | 302.39± 4.14 | 0.18$^{NS}$ |
| 2 | Dry period (days) | 70.60 ± 6.48 | 66.10 ± 12.96 | 63.11 ± 3.22 | 0.13$^{NS}$ |
| 3. | Number of Services Per Conception (SPC) | 2.16 ± 0.31 | 2.00 ± 0.26 | 1.83 ± 0.31 | 0.33$^{NS}$ |

*Means bearing same superscripts within rows do not differ significantly*

*NS – Non Significant (P>0.05)*

The control animals were fed with wheat/rice bran, rice gruel and ground nut oil cake (GNC) in an imbalanced proportion without meeting the dietary requirement of the animal. Feeding diet with low nutrient content may cause reduction of the milk fat and SNF percentage. Hence, ration balancing is most important to augment fat and SNF content in milk. Garg et al. (2016) stated that the milk fat and SNF significantly increased (P<0.01) from 3.98% to 4.35% and from 7.93% to 8.93%, respectively in cows maintained on ration balancing program compared to those on unbalanced ration.

In case of brewery waste fed animals (T1), the mean milk fat and SNF decreased from 4.75% to 4.40% and from 8.45% to 8.33%, respectively during the study period. A notable decrease of – 0.35% and – 0.12% of milk fat and SNF was observed in the animals after experimentation. The depression of milk fat and SNF after experimentation could be due to the presence of rich source of poly unsaturated fatty acid in brewery waste which would have promoted for its depression. This corroborated with the findings of Faccenda et al. (2017), who noted 0.04 g/kg of milk fat depression for every 1% soybean meal replacement with DBG. Moreover, the poly unsaturated fatty acids in brewery waste would have reduced the SNF content of milk after experimentation. This corroborated with the findings of Senthil Murugan et al. (2015). They observed that the SNF content of milk decreased from 7.775% to 7.674% at 20 and 30 percent inclusion level of WBG respectively. Brewery spent grain mainly consists of polyunsaturated fatty acids (67.46%), followed by saturated fatty acids (26.92%) and mono unsaturated fatty acids (10.62%) respectively (Arranz et al. 2008; Niemi et al. 2012). The decrease in milk fat might be due to decrease in dry matter intake (DMI) of the lactating animals. Davis et al. (1982) observed that the milk fat content decreased at 20 & 30% (3.3%) level due to depression in DMI with increasing levels of pressed brewer's grain. The decrease in milk fat could also be due to complete change of concentrate in terms of brewery waste which contained higher level of ether extract composition (5.13%) with higher amount of unsaturated fatty acids and the same fed to dairy cattle causes a bio hydrogenation process in rumen leading to depression in milk fat. Solomon (2007) stated that feeding of a diet containing 5 – 6% ether extract with large amounts of unsaturated fatty acids in dairy cattle depressed the milk fat. Mahnken (2010) reported that the short and medium chain fatty acids production decreased with increasing brewery spent grain inclusion. In other words, the total long chain fatty acids and total unsaturated fatty acids increased with increasing brewery spent grain feeding. Also increased amounts of long chain fatty acids supplied in the diet can inhibit de novo synthesis. As a consequence of decreased production of short and medium chain fatty acids, the fat percentage reduced in milk.

In case of balanced ration (T2) animals, the milk fat and SNF percentage increased to from 4.65% to 5.12% and from 8.37% to 8.62%. The reason for the increased milk fat and SNF (T2 group) may be due to feeding of balanced ration containing adequate amount of energy and protein which would have beneficial effects. This was in accordance with the findings of Garg et al. (2013) who stated that feeding balanced ration increased the milk fat by 0.2 – 1.5%. The improvement in milk fat may be due to balanced nutrients which would have improved rumen environment with maximum utilization of nutrients. Also in balanced ration the essential minerals fulfilled the requirement for better performance. On feeding a balanced ration, the dietary energy and protein could be utilized in a more efficient manner for lactating cows (Garg and Bhanderi, 2011). Moreover, it could be attributed to increased rumen microbial protein synthesis due to more optimal rumen function because of the more balanced nutrient supply (Garg et al. 2014). Similarly the increase in SNF content of milk in balanced ration fed (T2) animals could be due to feeding of balanced ration containing all essential amino acids which helps for synthesis of milk protein and SNF content. The optimum levels of energy, protein and minerals are essential for rumen fermentation functions and used for synthesis of milk components in mammary gland. Rumen microbes convert dietary protein into microbial protein, which is a primary source of essential amino acids for the dairy animals (Bailey et al. 2005) and these amino acids are used for the synthesize milk proteins in mammary gland. The increase in SNF content may be due to availability of energy, protein and minerals in appropriate quantity (Bhanderi et al. 2016).

**Body Weight**

The mean values of body weight (in kgs/animal) and the gain/loss of body weight in Jersey crossbred dairy cattle under different treatment regimens are presented in Table 2. There was a reasonable increase in body weight of the animals fed with brewery (T1) and balanced ration (T2) and a marginal decrease in body weight was noticed in control (T1) animals. The average daily gains for the crossbred animals are presented in Table 3. The total body weight gain and average daily gain was significantly (P<0.05) higher for balanced ration fed animals (T2: 33.83 kgs & 92.68 gms/day/animal) followed by brewery (T1: 19.52 Kgs & 53.48 gms/day/animal) and control (T0: - 4.75 Kgs & - 13.01 gms/day/animal) in the descending order of magnitude.

A marginal loss in total body weight (- 4.75 kgs/animal) and average daily weight loss (-13.01 gms/day/animal) was observed in control (T0) animals after the end of experimentation. This could be due to feeding of imbalanced ration containing wheat/rice bran, rice gruel and ground nut oil cake (GNC) which would not met the dietary requirement of the animal. The concentrates feed offered to the control animals contained only 1323 k.cal/kg energy, 7.36% protein and 5.95% crude fibre, which was not sufficient to meet out the nutrient requirement. When protein is lacking, microbial growth is depressed and as a result, microbial fermentation was reduced and less energy become available.

Moreover, cows would lose weight to compensate for the lack of dietary energy (John Moran, 2005).

In case of brewery (T1) fed animals a significant (P<0.05) increase in gain in body weight was observed. This might be due to the increased availability of undegradable protein (UDP) in the brewery waste which has a positive effect on body weight. Also the rate of degradable protein (RDP) in brewery waste was 47.5%, which was higher than the requirement of 35% RDP (NRC, 2001). Further, the presence of protein in the brewer's grain is a source of amino acids, which are absorbed from the intestines showing marked improvement of feed utilization efficiency (ARC, 1984). Davis et al. (1982) observed a significant improvement on dry matter consumption and weight gain in milking cows while feeding different levels of dried brewer's grains when compared to control group. The increase in body weight might be attributed to increased dry matter intake (DMI).

A significant (P<0.05) increase in gain in body weight (in kgs) and average daily gain (g/day) in balanced ration (T2) fed animals may be due to feeding of balanced ration containing sufficient quantity of energy, protein and mineral mixture which would have beneficial effects in boosting up the body weight of the animal. Provision of balanced ration to the dairy animal augments DMI which leads to increase in body weight. This is in accordance with the findings of Sherasia et al. (2016), who observed a highly significant (P<0.01) increase in body weight in early lactating cows. Significant (P<0.05) increase in dry matter intake was also observed on feeding a balanced ration in dairy cows which eventually reflected the increase in body weight of the animal (Garg et al. 2016). Further, Krishnamurthy et al. (2018) studied the effect of balanced ration supplementation on body weight gain and milk yield in different breeds of cattle (cross bred Jersey, HF & Ongole) and observed that the body weight increased by 13.9%, 9.32% and 16.3%, respectively than initial weights implying that the balanced ration improved the body weight in dairy cattle.

**Lactation Period and Dry Period**

The mean lactation period for different types of treatments was presented in Table 4. The number of days in lactation were higher for balanced ration (T2: 302.39 days) fed animals and marginally lower for brewery (T1: 299.99 days) and control (T0: 295.20 days) animals, but the difference among the groups was non-significant (P>0.05). The mean dry period for different types of treatments was presented in Table 4. Although the dry period was higher for control (T0: 70.60 days) and slightly lower (T1: 66.10 days & T2: 63.11 days) for brewery (T1) and balanced ration (T2) fed animals, the different was non-significant (P>0.05).

The lactation period and dry period observed in this study are more or less comparable with the normal lactation and dry periods for dairy cattle. The standard lactation period for dairy cattle is 305 days and recommended dry period for dairy cattle is 60 days. Slight reduction of lactation length in T0 than other groups might be due to the feeding of imbalanced ration to the animals in control group. Although it was non-significant, nutritional imbalance in ration of control group may have resulted 3 days and 7 days longer dry period as compared to T1 and T2 groups.

**Reproductive traits**

**Number of Services per Conception (SPC)**

The mean number of services per conception for different types of treatments was presented in Table 4**.** The number of SPC for control (T0), brewery (T1) and balanced ration (T2) fed animals were 2.16, 2.00 and 1.83. The mean SPC among different groups did not differ significantly (P>0.05). In case of control (T0) animals, the average number of SPC was marginally higher than other treatments (T1 & T2). The imbalanced feeding in control animals might be attributed to more number of SPC than other treatment groups. Further the concentrate feed of control animals contained lower level of nutrients (1323 K.cal/kg energy, 7.36% protein and 5.95% crude fibre) to meet out the dietary requirement of the animals which was below the recommended level and hence the number of SPC increased. Research also suggested that 17% to 19% dietary crude protein (on a dry matter basis) should be provided to the lactating cows to improve reproductive performance particularly SPC (Ibtisham et al. 2018). The low level of protein along with energy supply may be the possible reason on increasing the number of services per conception.

In case of brewery (T1) fed animals, the average number of SPC was 2.0 which was marginally higher than T2. The brewery fed animals contained lower level of nutrients (1931 K.cal/kg energy, 13.90% protein, 5.13% ether extract and 6.40% crude fibre) which was also not sufficient to meet out the dietary requirement of the animals as like control and hence the number of SPC was increased. Rochijan et al. (2016) observed less number of services per conception (1.17 and 1.5, respectively) with 32.78% and 27.47% rumen undegradable protein supplementation while studying the impact of high rumen un-degraded protein supplementation on reproductive performance in early lactation dairy cows. The brewery waste contained higher amount of rumen un-degradable protein which could be responsible for improving the number of services per conception. The brewery waste should not be used as a complete independent diet instead of concentrates, because they are low in fat and carbohydrate content which lead to lowering the availability of micro nutrients, in turn causes higher number of SPC. Hence this could be served as an additive with other cereals shots e.g., corn silage, green fodder and protein rich legumes for improving the productive performances of dairy cattle.

In case of balanced ration (T2) fed animals, the average number of SPC was 1.83 which was marginally lower than other treatment

groups (T0 & T1). The balanced ration contained sufficient nutrients (3708 K.cal/kg energy, 19.18% crude protein and 9.02% crude fibre) to meet the requirement of the animals which would have synergistic effect in conception rate of the animals. The number of services per conception depends on various factors such as quality of semen, state of reproductive system of the female, efficient heat detection, time of insemination, skill of the inseminator, management factors and agro-climatic conditions affects SPC in Jersey crossbreds (Vinothraj et al. 2016).

## Conclusion

The results of the study indicated that complete feeding of brewery waste to dairy cattle, increased the milk yield compared with control but lower than balanced ration animals. On the other hand, the milk fat and solids not fat decreased when compared to balanced ration animals. Hence complete feeding of brewery waste to lactating dairy animals is not recommended so as to avoid the decrease in milk fat and SNF which could affect the net returns of the livestock farmers.

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RESEARCH ARTICLE

# Socio-economic status and constraints faced by dairy farmers of Kangra District, Himachal Pradesh

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**Abstract:** Dairy farming is an imperative part of the rural economy in hilly areas. It helps in improving the socio-economic status of the farmers, provides nutrition and employment opportunities to the population. Milk production of Himachal Pradesh is low (1.531MT) and contributes only 0.7% to total milk production of India. Kangra district of Himachal Pradesh is second largest producer of milk in the state, therefore present study was undertaken to study the socio-economic status and constraints in dairy farming in the district. A comprehensive questionnaire was prepared to analyse the socio-economic status, cropping patterns, milk productivity and constraints in dairy farming. Analysis was done by Garret ranking technique. In most of the farmers were of small category and were rearing the animals for subsistence purpose. The large farmers of the study area were adopting dairy as a profession. High cost of construction of animal shed, lack of availability of green fodder, heat detection problem, low prices of dairy products and lack of emergency veterinary services are the major constraints faced by the farmers in the study area. In order to sustain and improve milk production, the focus need to be done on green fodder cultivation, milk marketing channels, low cost housing designs, improving AI coverage and establishing mobile veterinary clinics for attracting rural youth to adopt dairy as a profession.

**Keywords:** Constraints, Dairy farming, Farmers, Socio-economic

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## Introduction

The United Nations projects global population growth of almost 50% since 2000 to 9.5 billion by 2050 and it has been studied that approximately 1 out of 9 people in the world are undernourished. Under changing climatic scenario, the supply of quality food to the growing human population will remain a major challenge to the agriculture scientists and the governments. Global demand for milk-based proteins is increasing with increase in the human population. India is predominantly an agrarian society where animal husbandry forms the backbone of the agricultural economy and acts as an essential component of traditional agriculture. The livestock sector contributes 5.1% out of 19.9% of total GDP contributed by agriculture and allied sectors to the total GDP. The livestock sector contributes in several ways in enhancing livelihood and socioeconomic status of the farmers by generating continuous flow of income and acting as a cushion against income shocks in case of crop failures (Anonymous 2015). Inspite of being the largest milk producer in the world, India's productivity per animal is 987 kg/lactation which is very low in comparison to the world average of 2038 kg/lactation (Adhikari, 2020). Dairy farming is the most important economic activity in the rural areas of Himachal Pradesh because people have subsistence land holding and dairy farming is a major way to supplement their family income. Himachal government had also introduced many schemes to boost dairy farming in the state, i.e Dhoodh ganga yojana and Utam pashu purshkar yojana in recent years were introduced (Anonymous, 2017[a]). Statistically, the population of cattle in the state is 21.49 lakh, which contributes 1.14 percent to the country's population. Milk production of the state is 1.3 MT contributing only 0.7 percent to the Indian dairy industry (NDDB, 2019). The milk production trends shows that there is an increase in milk production from 772.47 thousand tonnes in 2003 to 1392 thousand tonnes in 2017, registering a total growth of 80% with a CAGR of 4.00% (Khalandar et al. 2022). Livestock rearing plays a crucial role in the economy of Himachal Pradesh because out of total share of agriculture and allied sector in 28-30% is contributed by livestock sector (Bishist et al. 2022). The cattle population trends in the

Himachal Pradesh showed that there is decrease in the population from 4.7 million in 1972 to 4.4 million in 2017 (NDDB, 2019). No, doubt, various public and private institutions are developing a number of technologies with huge investment but most of these technologies and practices are not reaching the large number of population (Nagrale 2015). There are various factors which affect the development of dairy sector including feeding practices, marketing and institutional factors. Therefore, the present study was conducted to examine the socioeconomic status and various constraints faced by the dairy farmers in Kangra district of Himachal Pradesh.

## Materials and methods

### Selection of study area

Present study was carried out in Kangra district, which falls in the mid hill region of Himachal Pradesh (Fig. 1). In the study, it was selected because it is the 2nd largest producer of milk in the state with annual production of 259.25 thousand tonnes. In the study, five blocks namely; Baijnath, Dehra, Kangra, Indora and Nagrota-Surian were randomly selected through multistage random sampling technique. Two villages were selected from each block and 10 dairy farmers from each village were selected randomly to constitute a sample size of 100.

Data was collected from these respondents with the help of a structured questionnaire and the participants were interviewed about socioeconomic status, dairy farming practices and different problems faced by the farmers pertaining to the dairy farming.

All the problems faced by participants were listed. A complete study of milk producer households in each of ten sample villages along with their herd size was carried out with the help of veterinary professionals and respondents. The list was arranged in ascending order of importance in terms of standard animal units with its cube -root frequencies were obtained and distributed into three different farm size groups of small, medium and large farms. Samples stratification was done by cumulative cube root frequency methods (Singh and Mangat, 1996) and standard animal unit method (Sirohi et al. 2019). After analysing the data according to standard animal unit and cube root frequency method households were divided into three categories namely small, medium, large in which 57 households in small category, 38 households in medium category and 5 were in large category.

Garret's ranking technique was adopted to rank the given set of constraints faced by dairy farmers in the study area. The order of merits that were given by the respondents converted into ranks by using the following formula (Garret and Woodworth, 1969)

$$\% \text{ position} = 100(Rij-0.05)/Nij$$

Where,

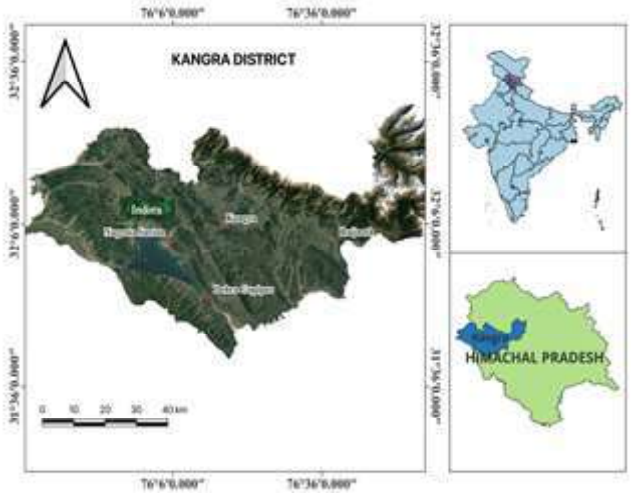


**Fig 1.** Map of the study area

Rij - Rank given for the ith factor by the jth individual.

Nij - Number of factors ranked by the jth individual.

The % position of each rank thus obtained was converted into scores by referring to table given by the Garret. For each factor or problem, the average score was worked out to arrive at mean scores and thus based on the mean scores, the ranks were given and the most important factor was ranked first and the least important problem was ranked as the last.

## Results and Discussion

### Socio-Economic Status of Dairy Farmers

In the study, socio-economic status of small, medium and large dairy farmers in the study area were studied which is presented in Table 1. It was found that in the study area the average size of family was 4.99 and overall number of males and females was 57.77 and 42.23 per cent, respectively. The study conducted by (Bishist et al. 2022) also reported that 52.58% and 47.42% livestock farmers were male and female which correlated with the present study. The majority of farmers belong to the nuclear family (70.82 %) followed by the joint family (29.18%). The education profile of dairy farmers revealed that the maximum respondents 22.35% studied up to secondary level followed by matriculate level (17.97%), middle (17.55%), primary (15.99%) and graduation level (14.80%). The overall literacy rate was found to be 88.65 per cent. The overall literacy index (2.51) showed the quality of education and revealed that quality is not up to the mark. Occupational status revealed that 71.08% of households are engaged in agriculture and allied activities followed by 9.6 % in services, 8.78% in private jobs and 8.56% in business, respectively. Feroze et al. (2016) also reported the similar results in hilly areas and found that most of the households were studied up to secondary level and most of the households engaged in the agriculture and dairy sector. The overall dependency ratio

**Fig. 2** Land utilization pattern of sampled households

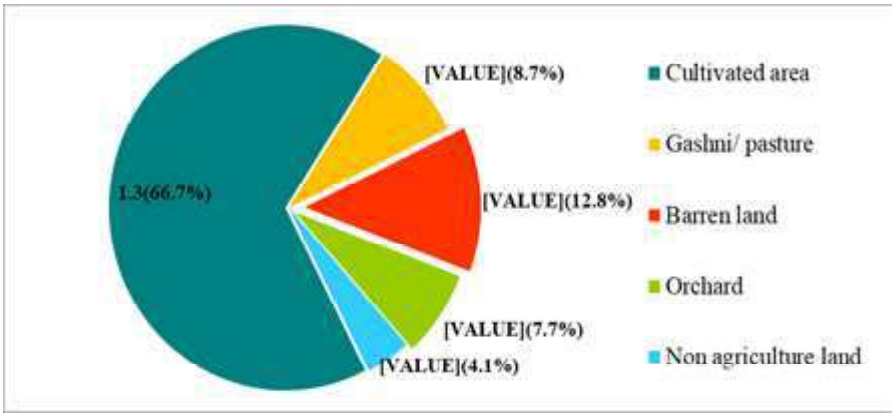


**Table 1:** Socio-Economic status of the sampled households in the study area

| Particulars | Herd size category | | | |
|---|---|---|---|---|
| | Small | Medium | Large | Overall |
| Average size of family (No) | 5 | 5.36 | 4.6 | 4.99 |
| Number of males (%) | 53.68 | 54.41 | 65.21 | 57.77 |
| Number of female (%) | 46.32 | 45.59 | 34.79 | 42.23 |
| Nuclear family (%) | 71.93 | 60.52 | 80 | 70.82 |
| Joint family (%) | 28.07 | 39.48 | 20 | 29.18 |
| Illiterate (%) | 10.94 | 9.44 | 13.66 | 11.35 |
| Primary (%) | 18.11 | 16.23 | 13.63 | 15.99 |
| Middle (%) | 15.09 | 19.37 | 18.18 | 17.55 |
| Matriculate (%) | 20.38 | 19.89 | 13.63 | 17.97 |
| Secondary (%) | 23.39 | 20.94 | 22.72 | 22.35 |
| Graduation (%) | 12.09 | 14.13 | 18.18 | 14.80 |
| Literacy Rate (%) | 89.06 | 90.56 | 86.34 | 88.65 |
| Literacy Index | 2.58 | 2.34 | 2.61 | 2.51 |
| Services (%) | 11.21 | 11.72 | 5.88 | 9.6 |
| Business (%) | 8.78 | 11.03 | 5.88 | 8.56 |
| Private Job (%) | 9.75 | 4.82 | 11.76 | 8.78 |
| Agriculture including dairying and allied services (%) | 70.24 | 72.41 | 70.58 | 71.08 |
| Average No. of workers | 3.58 | 3.8 | 3.6 | 3.66 |
| Average No. of dependents | 1.42 | 1.56 | 1 | 1.33 |
| Average No. of family | 5 | 5.36 | 4.6 | 4.99 |
| Dependency ratio w.r.t total workers | 0.39 | 0.41 | 0.27 | 0.35 |
| Dependency ratio w.r.t family size | 0.28 | 0.29 | 0.2 | 0.25 |

w.r.t total workers was recorded 0.35 which showed that on an average one worker is needed to assist one family member in all the farm size categories.

**Land Utilization Pattern of sampled households in the study area**

The data pertaining to the land utilization pattern of the sampled households is presented in Fig 2, which indicate that the average land holding varied between 1.50 to 2.33 hectares among different categories of farm with an average of 1.95 hectare. Similar finding were reported by Feroze et al. (2016) in East Khasi Hills and Ri-Bhoi District with the average land holding size is 1.67 and 1.85 out of which 1.3 hectare area is cultivated with sole crops and

mixed crops, followed by area under barren lands 0.33 hectare, ghasni or pasture 0.17 hectare, orchard 0.15 hectare and 0.08 hectare under non agriculture land, respectively.

The cropping pattern of sampled households is presented in Table 2. On the average farms, wheat was the most important crop in study area accounting 18.73 per cent of total cropped area. In the study area the net sown area was 1.45 hectare. The area under fodder crops is only 4.44% of total cropped area and found out to be more than national average which is only 4 per-

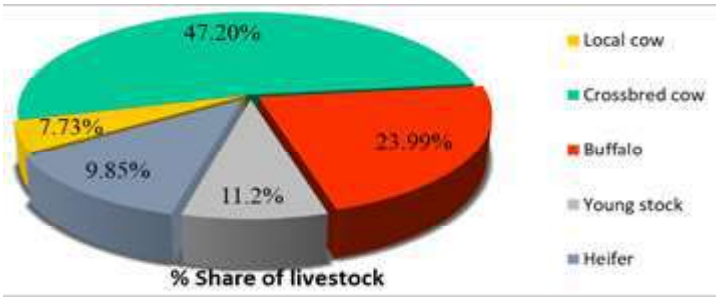


**Fig. 3** Share of livestock among sampled households

**Table 2:** Cropping pattern of sampled households in the study area

| Particulars | | Herd size category | | | |
|---|---|---|---|---|---|
| | | Small | Medium | Large | Overall |
| Kharif crops | | | | | |
| | Cereals | | | | |
| 1 | Maize | 0.33 (15.21) | 0.35 (12.46) | 0.48 (17.14) | 0.39 (14.94) |
| | Paddy | 0.30 (13.82) | 0.32 (11.39) | 0.32 (11.43) | 0.31 (12.21) |
| | Vegetables | | | | 0.00 |
| 2 | Brinjal | 0.07 (3.23) | 0.15 (5.34) | 0.10 (3.57) | 0.11 (4.05) |
| | Bottle gourd | 0.08 (3.69) | 0.18 (6.41) | 0.17 (6.07) | 0.14 (5.39) |
| | Okra | 0.12 (5.53) | 0.19 (6.76) | 0.15 (5.36) | 0.15 (5.88) |
| 3 | Fodder crop | 0.04 (1.84) | 0.06 (2.14) | 0.04 (1.43) | 0.05 (1.80) |
| Rabi crops | | | | | |
| | Cereals | | | | |
| 1 | Wheat | 0.43 (19.82) | 0.50 (17.79) | 0.52 (18.57) | 0.48 (18.73) |
| | Barley | 0.20 (9.22) | 0.25 (8.90) | 0.22 (7.86) | 0.22 (8.66) |
| | Oilseed | | | | |
| 2 | Mustard | 0.22 (10.14) | 0.26 (9.25) | 0.25 (8.93) | 0.24 (9.44) |
| | Vegetables | | | | |
| 3 | Beans | 0.07 (3.23) | 0.10 (3.56) | 0.12 (4.29) | 0.10 (3.69) |
| | Potato | 0.12 (5.53) | 0.20 (7.12) | 0.21 (7.50) | 0.18 (6.72) |
| 4 | Fodder crops | 0.04 (1.84) | 0.07 (2.49) | 0.10 (3.57) | 0.07 (2.64) |
| Orchard | | 0.15 (6.91) | 0.18 (6.41) | 0.12 (4.29) | 0.15 (5.87) |
| Gross cropped area | | 2.17 (100.00) | 2.81 (100.00) | 2.80 (100.00) | 2.59 (100.00) |
| Net sown area | | 1.24 | 1.57 | 1.54 | 1.45 |
| Cropping intensity | | 175.00 | 178.98 | 181.82 | 178.60 |

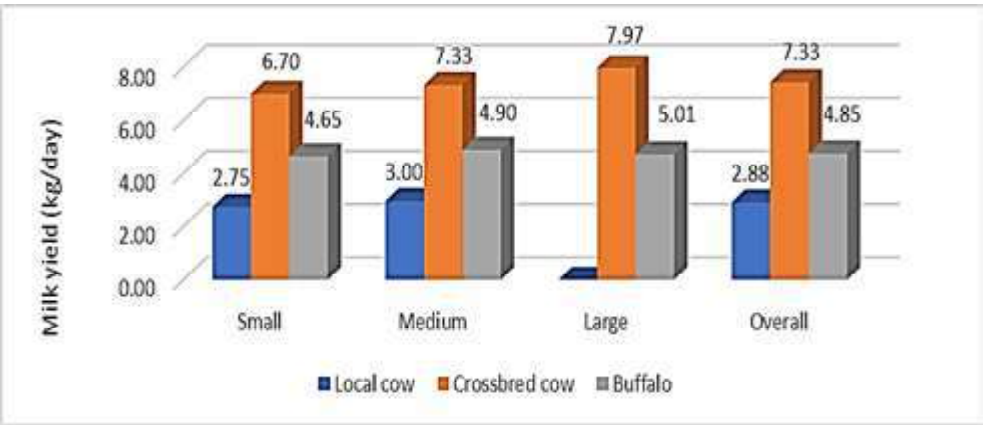


**Fig. 4** Average milk yield/ day of sampled households in the study area

cent of total cropped area in the country (Singh et al. 2022). Therefore, more emphasis on cultivating forage crops in the cropping systems will overcome the scarcity of the fodder problem. The cropping intensity indicates about the crop intensification in the study area and found 178.60 per cent in the area.

**Livestock holding among sampled households**

The results showed that average livestock holdings varied between 2.75 (small), 4.68 (medium) and 8.20 SAU in the large category of farmers. In the study area dominance of crossbred cows among different farm sizes was noticed with the highest share of (47.20%) followed by buffaloes (23.99%) and local cows (7.73%), respectively (Fig 3). Results of study indicated that the farmers in the study area are more inclined towards rearing crossbred cows mainly due to higher milk yield and easy availability of semen for breeding purposes. It was also observed that in the large farmers category, the farmers were rearing crossbred cows and buffaloes and none of the farmers in the study area were rearing local cattle.

**Average milk yield among sampled households**

Average milk yield is considered to be the main output for dairy enterprises. The milk yield of the animals depends upon various factors like breed, feed and fodder, health, climate and management practices. Average milk yield was worked out dividing total milk produced by total milking animals in a category. The highest average milk yield per day (Fig. 4) was noticed among the crossbred cow (7.33 litres/day) followed by buffalo 4.85 (litres/ day) and 2.88 (litres/ day) in case of local cows. It was also observed that the milk productivity of the crossbred cows and buffaloes increased with the farm size in the study area. The yield of crossbred cows in the study area is more than national level yield level (7.22 liters/day) (Anonymous 2019). Therefore, increasing the area under fodder crops and the number of crossbred cattles in the study area will boost the income of the farmers.

**Constraints in dairy farming in the study area**

Constraints means the problem faced by dairy farmers while conducting day to day animal husbandry practices in their dairy enterprise (Gamit 2020). In the study area, different constraints studied were divided into five categories i.e., Housing, feeding, breeding, marketing, and institutional constraints. The data on constraints in dairy farming ranked by famers in study areas were collected and analysed by using Garret ranking technique and results are presented in Table 3.

Among the housing constraints in dairy farming, high cost of construction of animal shed was the major constraint followed by provision of cooling, quality of roofing material, concrete non-grooved and slippery floor, less space availability i.e. open area, ranked first, second, third fourth and fifth, respectively by dairy farmers. in the study it was observed that majority of the farmers belonged to small and marginal category and they tend to make thatched houses in order to reduce the cost per cow for better returns. Similar results were reported by Rajadurai et al. (2018) and Balasubramanian (1995) also reported that in Tamil Nadu the majority of farmers had problems with animal shed and housing facilities because of their poor economic status.

The country faces scarcity of 35.6% green fodder, 10.5% dry fodder and 44% concentrates (Singh et al. 2022). In the study area the lack of green fodder availability was the major constraint noticed among feeding constraints followed by high cost of concentrates, low availability of dry fodder, lack of availability of concentrates, providing unbalanced feed, non-availability of land for fodder production, less storage space for dry fodder and concentrates, no sufficient water availability during the lean period, less availability of separate water troughs, poor quality of drinking water given to animals constraints ranked first, second, third, fourth, fifth, sixth, seventh, eight, nine and tenth respectively by dairy farmers. Similar findings were also reported by other studies like Tailor et al. (2012); Nagrale et al. (2015); Sharma et al. (2018) and Adhikari et al. (2020), where low availability of green fodder was the major constraint in adopting dairy farming as an enterprise.

Among the breeding constraints faced by dairy farmers, problems of heat detection, poor quality of bulls were the major constraints

**Table 3:** Various constraints faced by dairy farmers in mid hills of Himachal Pradesh

| S No. | | Mean | Ranks |
|---|---|---|---|
| | Housing Constraints | | |
| 1 | High cost of construction –shed | 75.7 | 1 |
| 2 | Less space availability – open area | 48.5 | 5 |
| 3 | Quality of roofing material | 60.56 | 3 |
| 4 | Concrete, non-grooved and slippery floor | 55.55 | 4 |
| 5 | Provision of cooling | 64.8 | 2 |
| | Feeding Constraints | | |
| 1 | Lack of availability of green fodder | 73.12 | 1 |
| 2 | Low availability of dry fodder | 70.14 | 3 |
| 3 | Lack of availability of concentrates | 66.65 | 4 |
| 4 | Giving unbalanced feed | 60.55 | 5 |
| 4 | Non availability of land for fodder production | 54.02 | 6 |
| 6 | Less storage space for dry fodder and concentrates | 50.38 | 7 |
| 7 | High cost of concentrates | 71.42 | 2 |
| 8 | No sufficient water availability all the time | 44.17 | 8 |
| 9 | Less availability of separate water troughs | 40.98 | 9 |
| 10 | Low quality of drinking water given to animals | 32.85 | 10 |
| | Breeding Constraints | | |
| 1. | Low conception rate | 58.18 | 4 |
| 2. | Poor quality of bulls | 66.2 | 2 |
| 3. | Problem of heat detection | 68.7 | 1 |
| 4. | Incidence of reproductive disorder | 42.86 | 6 |
| 5. | Less availability of improved germplasm | 60.12 | 3 |
| 6. | Unavailability of trained inseminator | 52.55 | 5 |
| | Marketing Constraints | | |
| 1. | Inadequate market information | 54.58 | 3 |
| 2. | Problem of transportation of products | 46.08 | 4 |
| 3. | Delay in payments | 33.12 | 5 |
| 4. | Low price offered for the products | 75.1 | 1 |
| 5. | Irregular demand for milk and other products (excluding dung) | 68.43 | 2 |
| | Institutional Constraints | | |
| 1. | Lack of emergency veterinary services. | 81.9 | 1 |
| 2. | High cost of medicines/ veterinary services | 76.65 | 2 |
| 3. | Lack of improved equipments | 48.7 | 6 |
| 4. | Irregular visits of veterinary staff | 64.98 | 3 |
| 5. | Unavailability of ambulance | 54.85 | 5 |
| 6. | Lack of awareness of new practices/ technologies | 58.5 | 4 |

faced by the farmers, due to the non - availability of breeding bulls in the study area. Other minor constraints faced were less availability of improved germplasm, low conception rate, unavailability of trained inseminator and incidence of reproductive disorder. Similar results were reported by Quddus (2012) and Lawrence et al. (2015) in Bangladesh and Kenya. The main reason for the inability of farmers to detect heat in animals is due to lack of awareness among the farmers and timely management of breeding at the village level result in poor conception rate.

Low prices offered of the products was the major constraint noticed among the dairy farmers. Other minor constraints were irregular demand for milk and other products, inadequate market information, problem of transportation of products and delay in payment by the buyers. Farmers faced the problem of irregular demand from the different vendors like sweet shop, restaurants and local chai-wala, due to lack of market demand occurred in different seasons of the year like during festival season, tourist season and also during lockdowns (Covid-19) which resulted into market disequilibrium and losses were suffered by dairy farmers. These findings were according to the study conducted by Anh et al. (2013) and Kishan and Ramachandran (2022).

In the study area farmers were also facing various constraints at institutional level, which included lack of emergency veterinary services, high cost of medicine and veterinary services, irregular visit of veterinary staff, lack of awareness of new practices and technologies, unavailability of ambulances, lack of improved equipment's constraints. It was noticed that in the study area the animal casualties were higher due to lack of emergency veterinary

services provided by the department of veterinary and animal husbandry. Therefore, it is suggested that emergency veterinary services and provision of mobile veterinary vans will help in better animal husbandry services to reduce animal casualties. A similar constraint was also reported by Bijla et al. (2019).

## Conclusion

From present study it can be concluded that the dairy farmers of the area are keeping good lactating animals with higher average milk yield as compared to the national average. However, the farmers face different constraints viz. high cost of construction cost of animal shed, lack of green fodder, problems in heat detection, low prices offered for the product and lack of emergency veterinary services, which are hindering the future prospects of the dairying as a profession in the study area. Therefore, it is suggested that the attention should be paid towards provision of subsidy for building animal sheds, advising farmers to grow leguminous rich, high quality fodder crop like berseem, oats, sorghum, bajra, makhan grass and fodder trees such as beul, kachnar, mulberry bamboo etc which will help in time of dearth period and reducing the cost of milk production in the area. Heat detection problems can be solved by providing scientific training and front-line demonstration to farmers with the help of aligned departments and universities. Marketing constraints can be overcome by fixing the price of milk and establishment of milk cooperative societies in the study area. Major institutional constraints can be overcome by establishing a network of mobile veterinary clinics for remote areas.

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RESEARCH ARTICLE

# Economics of livestock-based farming systems in saline and normal areas of West Bengal: A comparative analysis

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**Abstract:** Salinity has deleterious effects on both crops and livestock. The present study evaluated the impact of salinity and estimated the cost and returns of different livestock-based farming systems in saline and normal areas of West Bengal. The study suggested the farmers the best farming systems to be adopted based on output-input ratio. Saline areas are dominated by the indigenous cow and normal areas by the crossbred jerseys. This may be due to shortage of green fodder in saline areas. Cattle-goat-crop (N2) had highest crop area (1.80 acres) while Cattle-goat-crop-fish (S3) had highest fishing area (0.70 acres). Due to low maintenance cost, Sheep-poultry (S1) and Goat-poultry (S2) farming systems had output-input ratios close to 2. Whereas other farming systems in saline areas, the output-input ratios were close to 1.5. In case of normal areas, Cattle-goat-crop-fish had the highest output-input ratio of 1.58. Goat-poultry (S2) farming system had the highest net return of ₹ 53,967.68 per annum among landless farmers and cattle-goat-crop-fish (S3) with a net return of ₹ 1,01,686.50 per annum among landholders in saline areas. The farming system, Cattle-goat-poultry-crop (N1) had highest net return of ₹ 1,15,578 per annum) in normal areas. Share of labour was highest (56% to 58%) in total cost in crop enterprises and feed and fodder cost had a major share (53% to 72%) in total cost for livestock enterprises. Share of cattle enterprise in total cost and net return was highest. However, its share in net return was lower than its share in total cost. In case of goat and fish enterprises, their share in net return was higher than their share in total cost.

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**Key words:** Capital recovery cost, Livestock-based farming systems, Net return, Normal areas, Output-Input ratio, Saline areas, West Bengal.

## Introduction

Salinity condition of the soil is becoming more and more prominent each year, making it difficult for farmers to maintain their property (Wongsomsak, 1986). Due to salinity issues, agricultural potential is limited (Ladeiro, 2012). High salinity affects 20 per cent of total cultivated area and 33 per cent of irrigated agricultural land around the world (Shrivastava and Kumar, 2015). Degraded land covers around 147 million ha in India, with 23 million ha degraded due to salinity/alkalinity/acidification, the second most common source of soil degradation after water erosion (94 million ha) (Kumar and Sharma, 2020). Soil salinity, which is responsible for around 20 per cent yield reduction in these areas, puts a strain on rice yield, spikelet sterility, and thousand-grain weight in the coastal belt (Clermont et al. 2010). Due to salinity, there is a shortage of fodder crops in coastal saline areas, which affects cattle milk yield (Wistrand, 2003). Skin illnesses, liver fluke, diarrhoea, body weight loss, and immune system breakdown plague animals in salty locations due to ingestion of salinity-affected fodder crops (Alam et al. 2017). Due to the intake of salinity-affected agricultural goods, pregnant women in coastal areas experience greater gestational hypertension than pregnant women in non-coastal areas (Khan et al. 2008; WHO, 2003).

The Indo Gangetic Plains (IGP) are well recognised for providing approximately half of the country's total food consumption and feeding 40 per cent of the people (Pal et al. 2009). The plains are the agriculturally most fruitful region of the country, with almost 36 per cent of the country's bovine population. The bovine sector alone contributes 235 billion to the IGP GDP among the livestock sector (Singh et al. 2005). Every year, approximately 10 per cent of the extra land becomes salinized, and by 2050, nearly half of all arable land will be contaminated by salt (Kumar and Sharma, 2020). Salinity increases in the area beneath the Indo-Gangetic plains will jeopardise our country's food security. West Bengal controls 78.84 per cent (4,41,272 ha) of the total saline areas in the IGP region (5,59,719 ha) (Mandal et al. 2010). The coastal saline zone suffers from both soil and water salinity, as well as a

milk and livestock deficit (Wistrand, 2003). Hence, the West Bengal state was considered as an ideal location for a comparative study of livestock-based farming systems in saline and normal environments.

**Methodology**

**Sampling plan**

The major part of the coastal saline areas in West Bengal is in the Sundarban area of districts South 24 Parganas, parts of North 24 Parganas and Purba Midnapore (Bandyopadhyay *et al.* 2003). Sampling units were selected with the help of the multistage sampling technique. Within the selected districts where saline soil can be found, 17 blocks of South 24 Parganas, 6 blocks of North 24 Parganas and 10 blocks of Purba Midnapore is having saline areas. The rest of the blocks i.e., 12 blocks of South 24 Parganas, 16 blocks of North 24 Parganas and 15 blocks of Purba Midnapore are considered normal areas for the comparison of livestock-based farming systems in saline and normal areas (GoW, 2018). Finally, from the above-mentioned blocks in saline and normal areas, Basanti, Namkhana and Canning II from South 24 Parganas; Hingalganj from North 24 Parganas; Khejuri II and Nandigram I from Purba Midnapore were randomly selected for saline areas. For normal areas, Mograhat I and Mograhat II from South 24 Parganas; Barasat I and Bongaon from North 24 Parganas; Bhagwanpur I and Bhagwanpur II from Purba Midnapore blocks were selected randomly. Twenty households from each block were selected based on random sampling. A total of 120 households were selected from each of the saline and normal areas, thus total sample size constituting 240 households. Primary data were collected through the personal interview method on a structured interview schedule from the door-steps of the respondents on various aspects of livestock and crop enterprises from selected households for the year 2019-2020. Farmers who were having 50 per cent or more income from livestock were only considered as respondents for the present study.

Identification of different types of livestock-based farming systems was done based on the highest income contribution from livestock enterprises. For example; if the highest share of income earned by a household from livestock enterprises is through sheep rearing, then the system will be named sheep-based farming system and so on.

**Cost categories of different enterprises**

**Table 1.** Conversion factors of man equivalent days

| Particulars | Man-equivalent days |
|---|---|
| Child workers (<14 years) | 0.50 |
| Male (15-39 years) | 1 |
| Female (15-39 years) | 0.75 |

**Fixed costs:** Depreciation on farm machinery and farm building, land revenue cesses and other taxes and interest on working capital. Rent paid for leased in land, interest on the value of owned capital assets, the rental value of owned land and rent paid for leased in land were considered as fixed costs for the study.

**Variable costs:** Value of purchased material inputs such as seed, insecticide and pesticide, manure, fertilizer, etc., hired human labour, animal labour (hired or owned) hired farm machinery, irrigation charges, feed and fodder cost, veterinary and miscellaneous expenditure. In case of purchased feed and fodder, the cost was worked out as a product of the quantity fed to animals and the purchase price of respective feed. In case of home-grown feed and fodder, the relevant prices were the farm-harvest prices. When the concentrate feed was prepared at home, its cost was computed by taking the weighted prices of ingredients used in the concentrate, the weights being the share of each ingredient in the concentrate composition.

**Labour cost:** It included the cost of the family as well as paid labour (hired labour). The cost of hired labour was calculated considering the type of work allotted and wages paid whereas, family labour costs were determined based on the existing wage rate of permanent farm labour. Total time spent was converted to man-days by using conversion as presented in Table 1:

**Veterinary and miscellaneous costs:** The expenditure on breeding and health care of the animals was covered under the veterinary expenses. It included the cost of artificial insemination (AI), natural service, vaccination, medicines, the fee of veterinary doctors and other related expenses. The miscellaneous expenditure included expenses for repair of fixed assets, water and electricity charges, insurance premiums and any other incidental charges. These being joint costs, apportionment of the same was based on SAU.

**Standard Animal Units (SAUs):** Considering the differences in regional endowments of animal wealth and species, the livestock animals were converted into SAUs using factors suggested by Kumbhare *et al.* (1983) (Table .2);

**Table 2.** Standard animal units for different livestock animals

| Type of Animals | SAU |
|---|---|
| Local cow | 1.00 |
| Crossbreed cow | 1.40 |
| Buffalo | 1.30 |
| Bullock/he-buffalo | 1.00 |
| Local cow/ Buffalo heifer >2 years | 0.75 |
| Crossbreed heifer >1 year | 0.75 |
| Calf of buffalo & local cow >1 year | 0.50 |
| All calves <1 year | 0.33 |
| Goat & Sheep | 0.50 |
| Other animals | 1.00 |

**Depreciation cost:** Capital Recovery Cost (CRC) method was used to calculate depreciation cost which is defined as the annual payment that will repay the cost of fixed input over the useful life of input and provide an economic rate of return on investment. The interest on fixed capital does not need to be accounted for separately in the CRC approach. The formula for estimation of CRC is:

$$R = Z\left[\frac{(1+r)^n r}{(1+r)^n - 1}\right]$$

Where,

R = Capital recovery cost

Z = Initial value of the capital asset

r = Interest rate

n = Useful life of the assets

In case of practical difficulties in getting the information on initial outlay at the field level, the current value of the asset was considered. When the asset was purchased from borrowed capital the actual interest rate charged by the bank was taken as 'r', while useful life (n) of both fixed and livestock assets were considered as the value suggested by Rao, 1991 (Table 3). The total CRC was then apportioned to the individual animal by the Standard Animal Units (SAUs).

**Cost concepts:**

**Total cost:** It was obtained by adding all the cost components including fixed and variable costs.

Total cost = Total variable cost + Total fixed cost

**Table 3:** Useful life of farm assets

| Fixed assets | In years |
|---|---|
| Own fund (Term deposits) | 1-5 |
| Pucca cattle shed | 50 |
| Katcha cattle shed | 10 |
| Manual chaff cutter | 6 |
| Power-operated cutter | 10 |
| Livestock | |
| Local cow (in years) | 10 |
| Crossbreed cow (in years) | 8 |
| Buffaloes (in years) | 10 |
| Sheep (in months) | 6-8 |
| Goat (in months) | 3-5 |
| Pig (in months) | 5-6 |

**Gross returns:** Gross returns were obtained by multiplying the milk yield of an individual milch animal with respective prevailing prices in the study area

Gross returns = Quantity of milk × Market price of milk

**Net returns:** Net return was calculated by subtracting net cost from gross returns

Net returns = Gross returns -Total cost

Gross returns from farming systems: Gross returns were estimated by summing up the returns obtained from both main product and by-products of various farm enterprises undertaken on the farm, evaluated at their market prices.

**Output-Input Ratio:**

$$\text{Output} - \text{Input Ratio} = \frac{\text{Gross return of output}}{\text{Total cost of inputs}}$$

**Results and Discussion**

The farming systems identified in saline and normal areas of the study area in West Bengal are presented in Table 4.

**Composition of livestock and poultry and average operational area under different farming systems**

Due to increased salinity, there is a shortage of grazing land and fodder crop for livestock production. Saline areas were dominated by indigenous cows because these breeds can withstand low fodder availability. Cross breeding in cattle is a total failure due to the non-availability of feed, salinity, lack of availability of artificial insemination services coupled with the absence of a market for milk (Das, 2011). A study conducted in saline areas of Bangladesh by Sarker et al. (2018) also found that 17 per cent of the household kept crossbred cows and 62 per cent had indigenous cows. Although the performance of the indigenous or native stock is poor relative to highly selected commercial lines, they can survive in harsh and challenging environments (Crawford and Christman, 1992). The cattle-goat-crop-fish (S3)

**Table 4:** Identified farming systems in the study area

| Code | Type of farming systems identified |
|---|---|
| | Farming systems in saline areas |
| S1 | Sheep-Poultry |
| S2 | Goat-Poultry |
| S3 | Cattle-Goat-Crop-Fish |
| S4 | Cattle-Poultry-Crop-Fish |
| | Farming systems in normal areas |
| N1 | Cattle-Goat-Poultry-Crop |
| N2 | Cattle-Goat-Crop |
| N3 | Cattle-Poultry-Crop |

had the highest average Standard Animal Unit (SAU) of cattle under this system (5.16) followed by cattle-poultry-crop-fish (3.08) under saline areas (Table 5).

In normal areas, crossbreed jersey is a popular dairy animal. Under cattle-goat-poultry-crop (N1) on an average 6.03 SAU of cattle were available followed by cattle-poultry-crop (N3) (5.71 SAU) and cattle-goat-crop (N2) (3.08 SAU).

In saline areas, two types of farming systems were popular among the landless farmers, i.e., sheep-poultry (S1) and goat-poultry (S2). Near the Matla River of the Sundarban area few landless farmers engaged in Garole sheep farming. Salinity in the Matla increased by around 32 per cent between 1984 and 2013 (Trivedi et al. 2016). Garole breed is known for its bi-annual lambing, high prolificacy rate, high mothering instincts, adaptability to marsh saline as well as hot and humid climatic condition, grazing on aquatic weeds and grass in knee-dip water and resistance to some common diseases (Banerjee, 2008: Sahana et al. 2001). Interestingly this breed has naturally developed resistance against foot rot, FMD and reproductive disorders, etc. and is considerably more resistant to the dreaded roundworm *Haemonchus contortus* as well as to the tropical liver fluke (Nimbkar, 2002). Households engaged in sheep-poultry farming system hold on an average 7.50 SAU of Garole sheep.

Black Bengal goat, which is famous for its high-quality meat and skin, was common in both saline and normal areas. Landless farmers under the goat-poultry farming system depend heavily on Black Bengal goats, on average, they had 7.50 SAU followed by cattle-goat-crop-fish (S3) (5.30 SAU). In normal areas, average SAU under cattle-goat-poultry-crop (N1) and cattle-goat-crop (N2) was 5.25 and 5.50, respectively.

Poultry birds are mainly reared for egg purposes. Chicken breeds like Vanaraja, Rhode Island Red along with domestic duck breed Khaki Campbell were reared in the back yard of the household. In saline areas, the highest number of birds were found in the goat-poultry (S2) farming system (15 nos.) followed by sheep-poultry (13 nos.) and cattle-poultry-crop fish (12.50 nos.).

In normal areas, two types of farming systems such as cattle-goat-poultry-crop (N1) and cattle-poultry-crop (N3) include poultry enterprises with an average number of birds of 12 and 11.50, respectively.

In West Bengal, 82 per cent of the farmers are marginal with landholding of less than one hectare (Mandal, 2016). The findings of the current study were also in the same line as the previous studies (quote some references). In saline areas, only two farming systems, i.e., cattle-goat-crop-fish (S3) and cattle-poultry-crop-fish (S4), have crop and fish components. Under S3 on an average 1.10 acres was under crop cultivation, and 0.70 acres are under fish cultivation and in case of S4, it was 0.75 acres and 0.55 acres, respectively (Table 5). Rice and fish constitute the principal diet in the Bengali community. Households in this area are engaged in mono-cropping of rice both in the *kharif* and *rabi* seasons. The land use map showed that 80 per cent of the total agricultural land is under rice cultivation (Ghosh and Mistri, 2020). Salinization of coastal lands threatens the livelihood security of thousands of small rice farmers. Sea-level rise, storm surge, and coastal

**Table 5:** Average composition of livestock and poultry under different farming systems

| Animal Type | Farming systems | | | | | | |
|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | N1 | N2 | N3 |
| | | | | Cattle (in SAU) | | | |
| Milch | - | - | 3.00 | 2.00 | 4.20 | 2.80 | 2.80 |
| Heifer | - | - | 1.50 | 0.75 | 1.50 | 0.75 | 2.25 |
| Calf | - | - | 0.66 | 0.33 | 0.33 | 0.33 | 0.66 |
| Total | - | - | 5.16 | 3.08 | 6.03 | 3.88 | 5.71 |
| | | | | Goat (in SAU) | | | |
| Adult | - | 5.25 | 3.97 | - | 3.67 | 3.85 | - |
| Kid | - | 2.25 | 1.33 | - | 1.58 | 1.65 | - |
| Total | - | 7.50 | 5.30 | - | 5.25 | 5.50 | - |
| | | | | Sheep (in SAU) | | | |
| Adult | 6.00 | - | - | - | - | - | - |
| Kid | 1.50 | - | - | - | - | - | - |
| Total | 7.50 | - | - | - | - | - | - |
| | | | | Poultry (in no.) | | | |
| Adult | 7.80 | 9.00 | - | 7.50 | 7.20 | - | 6.90 |
| Grower | 3.90 | 1.50 | - | 3.75 | 1.20 | - | 3.45 |
| Chicks | 1.30 | 4.50 | - | 1.25 | 3.60 | - | 1.15 |
| Total | 13.00 | 15.00 | - | 12.50 | 12.00 | - | 11.50 |
| Crop area (in acre) | - | - | 1.10 | 0.75 | 1.60 | 1.80 | 1.50 |
| Fishing area (in acre) | - | - | 0.70 | 0.55 | - | - | - |

erosion increase the risk of salinity in this area. Thus, farmers are dependent on indigenous salt-tolerant varieties like Dudhersar, Lal Dhan, Rupsal, Patnai, etc. Fish is cultivated in the backyard pond.

In normal areas, all the sample households had a few acres of the cropped area. They mostly cultivate different types of high-yielding varieties (HYV) of rice such as Khitish, Swarna Mahsuri, Sada Swarna, etc. along with mustard and jute. The highest land under cultivation is under cattle-goat-crop (N2) farming system (1.80 acres), followed by cattle-goat-poultry-crop (N1) (1.60 acres) and cattle-poultry (N3) (1.50 acres).

**Cost and return of different enterprises across different farming systems:**

In saline areas, farmers mostly cultivated salt-tolerant indigenous rice varieties such as Dudhersar, Lal Dhan, Rupsal, Patnai etc. Labour cost had the major share of total cost. It's share under S3 and S4 farming systems were 58.74 and 57.87 per cent of total cost, respectively (Table 6). Rice was mainly cultivated for home consumption purposes. Rice bran and rice straw are used for cattle feed. For rice, market prices vary variety wise and at the end of the year net return obtained from crop enterprises under S3 and S4 farming systems were ₹ 6,771 per annum and ₹ 4,771 per annum, respectively.

Garole sheep rearing in S1 farming system is a unique feature in saline areas. Various authors (Ghalsasi and Nimbkar, 1993, Bose and Moitra, 1995, Singh and Bohra, 1996, Sharma et al. 1999 and Sahana et al. 2001) have reported that the breeding of Garole sheep is localized in the Sundarban regions of West Bengal and Bangladesh. Marginal and landless farmers from socially backward and underprivileged classes maintain this type of sheep. Total cost, gross return and net return from sheep rearing were ₹ 25,107.50 per annum, ₹ 51,900 per annum and ₹ 26,792 per annum, respectively. Sheep were sold in the market at 12 months of age with an average of 15 kg body weight at the rate of ₹ 300/kg of meat. Out of total cost, 75 per cent was variable cost and 25 per cent was fixed cost. Harvested grass, weeds, tree leaves, dry grass and rice straw are used as supplementary feed. The animals are even found to drink saline water for several days, as there are limited sources of fresh water on many islands (Mandal et al. 2017).

Under S2 farming system, the Black Bengal goat was reared by the landless farmers. This animal also required low inputs like Garole sheep but is more prone to diseases like diarrhea, parasitic infection, skin problem, anemia, etc. In saline zone, there is a scarcity of clean water and farmers used to provide pond water without any treatment. Farmers preferred their goats to be stall-fed as they fall sick due to consumption of poisonous weeds during grazing and also to protect them from stray dogs' attacks. Cereal by-products like mug chuni, bhusa of pulses, wheat husk, whole rice bean after harvesting, etc. are provided during stall-fed. The average gross and net return from goat rearing under S2 farming system were ₹ 69,200/annum and ₹ 36,486/annum, respectively, which was higher than the income received from sheep rearing. The higher return was due to the meat of the Black Bengal goat being delicate and highly demanded. Demand increases during festivals like Muharram and Durga Puja. Goats are sold in the market at 12 months of age with an average of 15-18 kg body weight at the rate of ₹ 400/kg of meat. Farmers prefer castrating male kids to be raised for meat purposes within one month of age. The average daily milk yield was around 150-200 g, which is used to feed the kids and not for selling purposes, however, goat manure is used in crop fields. Similar to sheep rearing, in case of goat rearing also variable cost and fixed costs were 75.83 and 24.17 per cent of total cost, respectively.

Landless farmers under S1 and S2 farming systems also engaged in non-farm activities like permanent labour in others' fields and MGNREGA labour. Net returns from non-farm activities were ₹ 9,735.55/annum and ₹ 11,512.68/annum for S1 and S2 farming systems, respectively.

Under S3 and S4 farming systems, the cattle were mostly indigenous cows. Share of total feed and fodder in livestock enterprise were 59.70 per cent and 62.00 per cent of total cost, respectively. The previous studies also indicated that about 60 to 80 per cent cost was estimated for feed and fodder (Lal and Chandel, 2016; Patil, 2010; Kumari, 2020). Most of the cattles depend on low-quality roughages like straw and locally available natural grasses to fill their stomach which cannot fulfil the actual nutrients requirement of the animals (Sarker et al. 2018). Farmers mostly depend on concentrate to provide sufficient nutrients to the cattle. Concentrates fed by the farmers were mostly bought from the market or prepared at home by mixing rice polish, wheat bran, pulse bran, broken rice and mustard oil cakes in the surveyed area. Share of variable and fixed costs for S3 farming system which includes both cattle and goats, were 86.72 per cent and 13.28 per cent of total cost, respectively. Net return for S3 farming system was ₹ 58,335.50 per annum. In S4 farming system, which includes only cattle generates a net return of ₹ 36,862 per annum. Due to less cooperative societies in the region, farmers mainly sell the milk either in sweet shops or sell them door to door, at a rate of ₹ 35-40/litre. Sandesh which is a popular Bengal sweet, is made from milk and has a high demand. Farmers usually get a higher rate for their milk if they sell it in sweet shops.

Chicken breeds like Vanaraja, Rhode Island Red along with domestic duck breed like Khaki Campbell were reared in the back yard of the household. Poultry birds were reared mainly for egg production, the net return from poultry for S1, S2 and S4 farming systems were ₹ 7,499/annum, ₹ 5,969/annum and ₹ 4,439/annum, respectively. The differences in net profit under different farming

**Table 6:** Cost and return of different enterprises across different farming systems under saline areas (₹ /hh/year)

| Components | S1(Sheep-Poultry) | S2 (Goat-Poultry) | S3 (Cattle-Goat-Crop-Fish) | S4 (Cattle-Poultry-Crop-Fish) |
|---|---|---|---|---|
| | | Crop | | |
| 1) Labour | - | - | 10,500.00 (58.74) | 8,000.00 (57.87) |
| 2) Seed | - | - | 950.00 (5.31) | 900.00 (6.43) |
| 3) Manure and fertilizer | - | - | 1,694.00 (9.48) | 1,270.00 (9.38) |
| 4) Plant protection chemicals | - | - | 400.00 (2.24) | 300.00 (2.23) |
| 5) Interest in working capital | - | - | 462.50 (2.58) | 347.00 (2.48) |
| 6) Total variable cost (TVC) (1+2+3+3+4+5) | - | - | 14,006.50 (78.35) | 10,817.00 (78.39) |
| 7) Depreciation | - | - | 244.50 (1.36) | 183.50 (1.37) |
| 8) Land rent | - | - | 3,225.00 (18.04) | 2,418.50 (17.53) |
| 9) Interest on fixed capital | - | - | 400.00 (2.24) | 380.00 (2.75) |
| 10) Total fixed cost (TFC) (7+8+9) | - | - | 3,869.50 (21.65) | 2,982.00 (21.61) |
| 11) Total cost (TC) (6+10) | - | - | 17,876.00 (100.00) | 13,799.00 (100.00) |
| 12) Gross return (GR) | - | - | 24,647.00 | 18,750.00 |
| 13) Net return (NR) (12-11) | - | - | 6,771.00 | 4,771.00 |
| | | Livestock | | |
| Total feed and fodder cost | 13,461.00 (53.60) | 17,811.00 (54.44) | 61,776.00 (59.70) | 50,300.00 (62.00) |
| 1.a) Fodder cost | 4,374.82 (17.42) | 6,055.74 (18.51) | 27,181.44 (26.26) | 21,880.50 (26.97) |
| 1.b) Feed/Concentrate cost | 9,086.18 (36.19) | 11,755.26 (35.93) | 34,594.56 (33.44) | 28,419.50 (35.03) |
| 2) Labour | 4,725.00 (18.80) | 6,300.00 (19.25) | 27,325.00 (24.48) | 20,225.00 (25.91) |
| 3) Miscellaneous cost | 655.00 (2.60) | 700.00 (2.14) | 2,625.00 (2.53) | 2,050.00 (2.52) |
| 4) Total variable cost (TVC) (1+2+3) | 18,841.00 (75.00) | 24,811.00 (75.83) | 89,726.00 (86.72) | 72,575.00 (89.00) |
| 5) Depreciation | 6,266.50 (25.00) | 7,903.00 (24.17) | 13,738.50 (13.28) | 8,563.00 (11.00) |
| 6) Total fixed cost (TFC) (5) | 6,266.00 (25.00) | 7,903.00 (24.17) | 13,738.50 (13.28) | 8,563.00 (11.00) |
| 7) Total cost (TC) (4+6) | 25,107.50 (100.00) | 32,714.00 (100.00) | 1,03,464.50 (100.00) | 81,138.00 (100.00) |
| 8) Gross return (GR) | 51,900.00 | 69,200.00 | 1,61,800.00 | 1,18,000.00 |
| 9) Net return (NR) (8-7) | 26,792.00 | 36,486.00 | 58335.50 | 36,862.00 |
| | | Poultry | | |
| 1) Total cost | 7,261.00 | 6,511.00 | - | 5,761.00 |
| 2) Gross return (GR) | 14,760.00 | 12,480.00 | - | 10,200.00 |
| 3) Net return (NR) (2-1) | 7,499.00 | 5,969.00 | - | 4,439.00 |
| | | Fish | | |
| 1) Total cost | - | - | 57,600.00 | 44,200.00 |
| 2) Gross return (GR) | - | - | 94,180.00 | 70,635.00 |
| 3) Net return (NR) (2-1) | - | - | 36,580.00 | 26,435.00 |
| | | Non-farm activity | | |
| 1) Total cost | 3,000.00 | 2,700.00 | - | - |
| 2) Gross return (GR) | 12,735.55 | 14,212.68 | - | - |
| 3) Net return (NR) (2-1) | 9,735.55 | 11,512.68 | - | - |

*Note:* Figures in parenthesis indicate percent to column total

systems were due to differences in average flock size. Eggs are sold at the rate of ₹ 6/egg and income from selling poultry birds for the income purpose was at the rate of ₹ 160/kg of meat in the local market.

Fish is an important component of S3 and S4 farming systems. Under S3 farming system total cost, gross return and net return were to the tune of ₹ 57,600/annum, ₹ 94,180/annum and ₹ 36,580/annum, respectively. Under S4 farming system, total cost (₹ 43,200/annum), gross return (₹ 70,635/annum) and net return

**Table 7:** Cost and return of different enterprises across different farming systems under normal areas (₹ /hh/year)

| Components | N1 (Cattle-Goat-Poultry-Crop) | N2 (Cattle-Goat-Crop) | N3 (Cattle-Poultry-Crop) |
|---|---|---|---|
| | Crop | | |
| 1) Labour | 14,000.00 | 15,000.00 | 13,500.00 |
| | (56.82) | (56.95) | (58.87) |
| 2) Seed | 1,750.00 | 1,800.00 | 1,500.00 |
| | (7.10) | (6.83) | (6.54) |
| 3) Manure and fertilizer | 2,350.00 | 2,541.00 | 2,117.50 |
| | (9.53) | (9.65) | (9.23) |
| 4) Plant protection chemicals | 560.00 | 600.00 | 450.00 |
| | (2.28) | (2.28) | (1.96) |
| 5) Interest in working capital | 630.00 | 694.00 | 578.50 |
| | (2.56) | (2.64) | (2.53) |
| 6) Total variable cost (TVC) (1+2+3+3+4+5) | 19,290.00 | 20,635.00 | 18,146.00 |
| | (78.29) | (78.35) | (79.13) |
| 7) Depreciation | 310.00 | 500.00 | 306.00 |
| | (1.26) | (1.90) | (1.33) |
| 8) Land rent | 4,560.00 | 4,837.00 | 4,031.00 |
| | (18.50) | (18.36) | (17.58) |
| 9) Interest on fixed capital | 480.00 | 500.00 | 450.00 |
| | (1.95) | (1.90) | (1.96) |
| 10) Total fixed cost (TFC) (7+8+9) | 5,350.00 | 5,704.00 | 4,787.00 |
| | (21.71) | (21.65) | (20.87) |
| 11) Total cost (TC) (6+10) | 24,640.00 | 26,339.00 | 22,933.00 |
| | (100.00) | (100.00) | (100.00) |
| 12) Gross return (GR) | 32,500.00 | 34,500.00 | 30,250.00 |
| 13) Net return (NR) (12-11) | 7,860.00 | 8,161.00 | 7,317.00 |
| | Livestock | | |
| Total feed and fodder cost | 1,13,680.00 | 1,11,276.00 | 99,697.29 |
| | (68.39) | (69.43) | (72.28) |
| 1.a) Fodder cost | 52,292.80 | 52,856.10 | 45,362.27 |
| | (31.46) | (32.98) | (32.89) |
| 1.b) Feed/Concentrate cost | 61,387.20 | 58,419.90 | 54,335.02 |
| | (36.93) | (36.45) | (39.39) |
| 2) Labour | 27,297.00 | 25,325.00 | 20,127.71 |
| | (16.42) | (15.80) | (14.59) |
| 3) Miscellaneous cost | 2,568.00 | 2,625.00 | 2,250.00 |
| | (1.54) | (1.64) | (1.63) |
| 4) Total variable cost (TVC) (1+2+3) | 1,43,545.00 | 1,39,226.00 | 1,22,075.00 |
| | (86.36) | (87.00) | (88.50) |
| 5) Depreciation | 22,672.00 | 21,035.50 | 15,860.00 |
| | (13.64) | (13.00) | (11.50) |
| 6) Total fixed cost (TFC) (5) | 22,672.00 | 21,035.50 | 15,860.00 |
| | (13.64) | (13.00) | (11.50) |
| 7) Total cost (TC) (4+6) | 1,66,217.00 | 1,60,261.50 | 1,37,935.00 |
| | (100.00) | (100.00) | (100.00) |
| 8) Gross return (GR) | 2,66,600.00 | 2,55,400.00 | 2,10,600.00 |
| 9) Net return (NR) (8-7) | 1,00,383.00 | 95,138.50 | 72,665.00 |
| | Poultry | | |
| 1)Total cost | 5,965.00 | - | 6,911.00 |
| 2) Gross return | 10,300.00 | - | 12,980.00 |
| 3) Net return (3-1) | 4,335.00 | - | 6,069.00 |

*Note:* Figures in parenthesis indicate percent to column total

(₹ 26,435/annum) were lower than S3 farming system due to the lower size of the pond area (Table 6). Fishing enterprise is mainly backyard fish cultivation where farm-made feed was prepared by mixing mustard oilcake and rice bran at 1:1 ratio. The commonly cultivated fish in the region were Catla, Rohu, Mrigel, Common Carp, Silver Carp, Grass Carp etc.In normal areas, farmers prefer to cultivate high yielding varieties of rice such as Khitish, Swarna Mahsuri, Sada Swarna etc. Net returns under N1, N2, and N3 were accounted for ₹ 7,860/annum, ₹ 8,161/annum and ₹ 7,317/annum, respectively, which were higher than the saline areas (Table 7). Labour costs under all the farming systems accounted for almost half of total cost, it was 56.82 per cent, 56.95 per cent and 58.87 per cent for N1, N2 and N3 farming systems, respectively.

Under livestock enterprise, net returns were much higher than in saline areas because of the presence of crossbred cows. However, across different farming systems net return was different due to differences in herd size. Net returns from livestock under N1, N2, and N3 farming systems were ₹ 1,00,383/annum, ₹ 95,138/annum and ₹ 72,665/annum, respectively. Milk price did not vary between saline and normal areas. Although, there were few cooperatives present in normal areas, the farmer gets low price (₹ 28/litre) for selling milk in cooperatives due to it's low-fat content. Due to this, the majority of farmers sold their milk in sweet shops or door to door at the rate of ₹ 35-40 per litre. Due to the presence of fodder farms in this area, green fodders like hybrid napier, para-grass, lathyrus were easily available. In the wet summer season natural grasses are more available but farmers supply more concentrates in the winter season due to the shortage of green fodders.

Poultry breeds like Vanaraja, Rhode Island Red and Khaki Campbell were reared in backyards like in saline areas. Net returns from poultry enterprise under N1 and N3 farming systems were ¹ 4,335/annum and ¹ 6,069/annum, respectively.

**Cost and return of different farming systems**

Cost and return from different farming systems are presented in Table 8, which helps us to understand which farming system is more profitable than the other in both saline and normal areas. Output input ratios were computed by dividing gross return by total cost. These ratios indicate return per unit of investment, higher the ratio better is the profitability for each household (HH).

In saline areas, households under S1 and S2 farming systems were the poorest of the poor earning net return of ₹ 38,905.35/HH/year and ₹ 53,967.68/HH/year, respectively. Although, net return under these farming systems were lower than other farming systems in this area, but their output-input ratios were 2.10 and 2.28, respectively, which was higher than all other farming systems. This was due to the low maintenance cost of goats and sheep, which leads to total cost being lower than other farming systems. The study conducted by Kumar et al. (2012) also found similar results of lower production cost and higher output-input ratio. S4 farming system has the lowest output-input ratio of 1.45 in saline areas. The highest gross and net return were obtained under S3 farming system i.e., ≠ 2,80,627.00/HH/year and ₹ 1,01,686.50/HH/year, respectively. However, due to higher maintenance cost of cattle, total cost was also the highest (₹ 1,78,940.50/HH/year) which leads to drop in output-input ratio to 1.57. This indicates that farmers will receive ₹ 1.57 by investing ₹ 1 in S3 farming system.

In normal areas, N1 farming system has the highest net return and output input ratio of ₹ 1,15,578/HH/year and 1.58, respectively. This farming system includes more activities and is more integrated than other existing systems in the area. Kumar et al. (2011) also reported increase in net income through an integrated farming system due to the use of recycled products within the system. Output input ratios for other farming systems were 1.52 and 1.51 for N2 and N3 farming systems, respectively.

Share of labour was highest (56% to 58%) in total cost in crop enterprises and feed and fodder cost had a major share (53% to 72%) in total cost for livestock enterprises. Due to low maintenance cost Sheep-poultry (S1) and Goat-poultry (S2) farming systems had output-input ratios close to 2. In case of other farming systems, the output-input ratios were close to 1.5 in saline areas. In case of normal areas, Cattle-goat-crop-fish (1.58) had the highest output-input ratio. Goat-poultry (S2) farming

**Table 8:** Cost and returns of different farming systems

| Code (1) | Farming systems (2) | Total cost (3) | Gross return (4) | Net return (NR) (5= 4-3) | Output input ratio (6=4/3) |
|---|---|---|---|---|---|
| S1 | Sheep-Poultry | 35,368.50 | 74,273.85 | 38,905.35 | 2.10 |
| S2 | Goat-Poultry | 41,925.00 | 95,892.68 | 53,967.68 | 2.28 |
| S3 | Cattle-Goat-Crop-Fish | 1,78,940.50 | 2,80,627.00 | 1,01,686.50 | 1.57 |
| S4 | Cattle-Poultry-Crop-Fish | 1,43,898.00 | 2,08,652.10 | 64,754.10 | 1.45 |
| N1 | Cattle-Goat-Poultry-Crop | 1,96,822.00 | 3,09,400.00 | 1,15,578.00 | 1.58 |
| N2 | Cattle-Goat-Crop | 1,86,600.50 | 2,83,632.76 | 97,032.26 | 1.52 |
| N3 | Cattle-Poultry-Crop | 1,67,779.00 | 2,53,830.00 | 86,051.00 | 1.51 |

system had the highest net return of ₹ 53,967.68/annum among landless farmers and cattle-goat-crop-fish (S3) with a net return of ₹ 1,01,686.50/annum among landholders in saline areas. Cattle-goat-poultry-crop (N1) farming system had highest net return of ₹ 1,15,578/annum) in normal areas.

## Conclusions

Goat-poultry (S2) farming system had a higher output-input ratio (2.28) than Sheep-poultry (S1) (2.10). So, S2 farming system should be suggested for resource-poor land-less farmers. For other landholders, Cattle-goat-crop-fish (S3) should be recommended in saline areas. Whereas in case of normal areas, Cattle-goat-poultry-crop (N1) should be recommended as it had the highest output-input ratio than other farming systems in that area. Irrespective of saline and normal areas, variable cost had a king's share in total costs for both crop and livestock management. During survey it was found that, presence of cooperative societies in both saline and normal areas was very meagre. If cooperative societies are promoted and farmers obtain these variable inputs from those cooperatives then variable costs and hence total cost will be reduced.

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RESEARCH ARTICLE

# Determinants of farmer's choice of milk marketing outlet in Jaipur District of Rajasthan

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**Abstract:** This investigation was undertaken in Jaipur district of Rajasthan with a view to identify the factors affecting the farmer's choice of specific dairy outlet to sell their marketable surplus. The data were collected from a sample of 80 sample respondents selected through multistage purposive sampling for the year 2021-22. A tabular analysis was done to list prevalent marketing channels and then a multinomial logit model was fitted to identify the factors affecting farmer's decision of choosing a particular milk-marketing outlet. The farmers used one of the three marketing channels to deliver milk to final consumers. Channel-I was direct marketing channel (Producer – Consumer) and channel II (Producer - Milk vendor- Consumer), channel III (Producer – Collection centre – Co-operative milk plant – Consumer) were indirect marketing channels of milk. The Channel-III was the most preferred channel of milk marketing, as it was opted by 47.50 per cent of the total farmers. Multinomial logit (MNL) regression's results indicated that herd size, marketable surplus, caste categories, access to institutional credit, BPL economic class, income from livestock and breed type of animals were the seven significant factors affecting farmer's decision of choosing a particular milk-marketing outlet out of three. Although direct marketing channels are highly efficient and fetch more prices to producers, still most of the farmer choose channel III for marketing of milk, may be because of easy disposal of marketable surplus. This trend might continue, therefore, farmers should be made aware about quality parameters like fat percentage in the milk, or even provided with affordable fat testing kits through *Pashu Vigyan Kendras/* Extension centres like KVKs, so that farmers can test it at their level and be assured about the prices they receive in the collection centres. There is need for the effective and improved spread of modern market outlets and dairy cooperatives, so that all the farmers irrespective of social and economic background may benefit from livestock farming.

**Keywords:** Milk marketable surplus, Farmer's Choice, Dairy Outlet, Marketing Channel, Multinomial logit regression.

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## Introduction

India is the world's largest milk producer, contributing about 24 per cent of the global milk production (FAO, 2023). The country has 56.7 per cent of buffalo, 12.5 per cent of cattle, and 20.4 per cent of small ruminant population of the world. During the last few decades, the livestock sector grew at an annual rate of 5.3 per cent during 1980s, 3.9 per cent during 1990s, 3.6 per cent during 2000s and 7.9 per cent during last five years (Economic Survey, 2021). Despite deceleration, livestock sector remained about 1.5 times larger than that of crop sector which implies its critical role in cushioning agricultural growth. It has been witnessed over the years that the stability in dairy income is far stronger than the income realized from agricultural activities (Kumar and Shah, 2016). Growing human population, increasing urbanization, changing lifestyles, increased health awareness and rising domestic incomes have led to increase in the demand of dairy products from consumer's end (Anita and John, 2001, Kharkwal *et. al* 2021). Dairy products are the most acceptable and affordable nutritious source of animal protein for large vegetarian segment of Indian population (Kundu and Banerjee, 2015). India is also the world's largest consumer of dairy products, consuming almost 100 per cent of its own milk production (Shree and Prabu, 2019).

About 90 per cent of the milk production comes from small farmers located in rural areas. Currently, 80 per cent of the milk produced in the country is marketed by the unorganized sector through local vendors and 20 per cent through organized sector including cooperative societies and private companies (Dept. of Animal

Husbandry, Dairying & Fisheries, Ministry of Agriculture and Farmers Welfare, GOI). Marketing of the majority of the milk through unorganized sectors is likely to dissuade small dairy farmers from expending production, which is absolutely necessary to keep up with the strong demand growth (Saran et al. 2022).

Rajasthan is predominantly an agricultural state with an excellent potential for milk production. The state ranks second in milk production and per capita availability of milk in India. In Rajasthan, livestock sector plays major role in improving socio-economic status and fulfilling nutritional needs of rural masses. It is not only a subsidiary occupation to agriculture but also a major economic activity, especially in the arid and semi-arid regions of the Rajasthan. Rajasthan has about only 11.27 per cent of the country's livestock and contributes about 12.6 per cent of the total milk production. Of the total milk produced, 53 per cent is buffalo milk, 36 per cent is cattle milk and 11 per cent is goat milk. Animal husbandry has great potential for rural self-employment and is contributing about 10% in the G.D.P. of the State (Dept. of Animal Husbandry, GOR, 2022).

Milk value chains can range from simple to the complex one's depending upon the scale of its production as well as type of market availability. Farmers may choose to dispose-off the marketable surplus of milk through numerous indirect and direct channels considering marketing cost and profit prospective. Their choice can be an economic indicator for the policy makers in order to set an effective linkage between the milk producers and consumers for fixing the price of milk rationally. Therefore, in this backdrop, this study was undertaken to identify the prevalent dairy marketing channels in Jaipur district of Rajasthan and determine the factors affecting the farmer's choice of a specific dairy outlet in the study area.

**Materials and methods**

A Multistage purposive sampling was used to select the sample. At first stage, Jaipur district from Rajasthan was chosen purposively as it is the largest milk producing district of Rajasthan. Then Amber and Chomu tehsils of Jaipur district were chosen purposively as these tehsils maintain the highest livestock population, accounting 12.04 per cent and 11.83 per cent of total cattle and buffalo of the district, respectively (Dept. of Animal Husbandry, GOR, 2019). In the next step, two villages, one from peri-urban area and one from rural area were chosen from each selected tehsil. Peri-urban area was the area located within 8-10 Kms from nearest town/ market and rural area was the area located at more than 8-10 Kms away from nearest town/ market. Thus, a total of 4 villages Dhand (rural) and KuKas (peri-urban) from Amber tehsil and Cheta ka bas (rural) and Jetpura (peri-urban) from Chomu tehsil were selected for the study**.** At final stage, a separate list of all farmers from each selected village along with herd size owned by them was prepared with the help of Patwaris and the concerned personnel of the selected villages. These dairy farmers were then categorized into the three standard size groups viz., small (1-3 SAUs), medium (4-6 SAUs) and large (e"7) as in Table 1. Finally, total 80 farmers were chosen from these village using probability proportion to size (PPS) method from each category of the herd size, which produced and sold milk in the market for detailed investigation. The primary data were collected for 2021-22 from the selected farmers through personal interview method with the help of pre- structured schedule.

**Table 1:** Category wise distribution of sample according to herd size

| Category | Herd size | Households (No.) |
|---|---|---|
| Small | 1-3 SAUs | 42 |
| Medium | 4-6 SAUs | 22 |
| Large | ≥7 SAUs | 16 |

SAUs = Standard animal units (Lal and Chandel 2016)

A number of econometric models such as probit model by Goetz (1992), structural model based on censoring by Key et al. (2000) and Bayesian double-hurdle model by Holloway et al. (2005) have been employed in identifying factors affecting farmer's choices in marketing their produce. However, if there are finite number of choices and dependent variable is qualitative, multinomial logit estimation is appropriate to analyze the effect of exogenous variables on choices. It is a simple extension of the binary choice model and is the most frequently used model for nominal outcomes that are often used when a dependent variable has more than two choices.

For this study at first a tabular analysis was done to list all direct and indirect milk marketing channels prevalent in the study region, then to study the factors affecting the farmer's choice of a specific dairy outlet, major socio-economic characteristics of the respondents in the study region were listed. The multinomial logistic regression was fitted to identify the factors affecting the producer's choice of selecting particular outlet with the following functional form:

$$M_{ij} = \beta_j X_{ij} + \varepsilon_{ij}$$

Where,

$M_{ij}$ = Vector of marketing choice.

ßj= Vector of channel specific characteristics.

εij= Random error estimation.

xij= Vector of producer characteristics that together might influence dairy farmer market channel decision,

Table 2 presents the list of explanatory variables which fall under six broad categories: (i) Physical capital (ii) Human capital (iii)

Demographic characteristics (iv) Institutional support (v) Economic factors and (vi) Animal characteristics.

The factors considered under physical capital were herd size and marketable surplus of milk. Human capital was proxied by the educational attainment of a farmer. A higher level of education enhances their capability for better management and, thus, makes them more likely to adopt modern marketing practices and select better-paying marketing channels (Marenya and Barret 2006; Gong et al. 2007). Four variables were included to capture the influence of demographic characteristics, namely; the age of household head, household size, gender of a household head and social group (ST, SC, OBC, GEN). Studies such as Morrison et al. 2007; Barham and Chitemi 2009; Vigneri and Holmes 2009; Aregu et al. 2011; Amani 2014; Eerdewijk and Danielsen 2015 suggest female-headed households are less successful than male-headed households at accessing new market opportunities due to lack of resources. Hence, the variable was taken to test the hypothesis that male headed households are more likely to market milk through modern marketing arrangements, while females headed households resort to the traditional one's. Similarly, hypothesis regarding social group was, the dairy farmers who come from the bottom of the social caste pyramid prefer informal system of milk marketing over the organized ones. To assess the effect of institutional support mechanisms such as access to institutional credit, as well as government sponsored schemes like rural employment guarantee programs (Mahatma Gandhi National Rural Employment Guarantee Act [MGNREGA]), below poverty line (BPL) and village location were taken. On the basis of share of livestock income in the total income, livestock occupation as principal or subsidiary was taken as a proxy of economic variable under the hypothesis that farmers with a higher share of income from livestock, may show specialization in livestock production with higher milk production, and hence may prefer to choose modern milk-marketing outlet. In animal characteristics animal type (local cow, crossbred, buffalo) and livestock age were taken as proxy explanatory variables.

**Table 2:** Vector of Explanatory variables

| S. No. | Explanatory variables | Indicators |
|---|---|---|
| 1. | Physical Capital | Herd size (No.), Marketable surplus (lt./day/hh) |
| 2. | Human Capital | Literate without formal education, below primary school, Primary school, Middle school, Secondary school, Secondary and above, Training (Yes/no) |
| 3. | Demographic Characteristics | Age of household head (yrs), Gender (Male/Females), Household size (No.), social group (ST, SC, OBC, Others) |
| 4. | Institutional Support | Access to institutional credit (Yes/No), Participation in MGNREGA (Yes/No), BPL cardholder (Yes/No), Village location (Rural/Peri-Urban) |
| 5. | Economic Factors | Principal occupation livestock (Yes/No) |
| 6. | Animal Characteristics | Age, animal type (cow, crossbred, buffalo, combination of any of these) |

It is also very important to consider the effect of various species/ breed of milch animals kept by farm households both separately and collectively by converting them into standard equivalent units. For this purpose, Standard Animal Units (SAU) of the bovine stock was derived for each farm household as per the specification given by Kumbhare et al. (1983) given in Table 3.

## Results and Discussion

This section is discussed under three subheads; first one is the distribution of farmers in the prevalent milk marketing channels, secondly a highlight of major socio-economic characteristics of the respondents and last subsection deals with the results of multinomial logistic regression.

### Distribution of Farmers in the milk marketing channel

Dairy farmers of the study area were observed to sell the milk in one of the three prevalent marketing channels as given in Table 4. A perusal of the table indicates that Channel-I was direct marketing channel (Producer – Consumer) and channel II (Producer - Milk vendor- Consumer), channel III (Producer – Collection centre – Co-operative milk plant – Consumer) were indirect marketing channels for milk disposal in study region. Table further reveals that Channel-III was the most preferred channel of milk marketing, as it was opted by 47.50 per cent of the total farmers. This may be due to organised system and easier disposal of their marketable surplus in this channel as compared to others. Channel-I was adopted by 28.75 per cent sample farmers, while 23.75 per cent farmers sold milk through Channel-II. The farmer's preference of similar milk marketing channels was also reported by Kashish et al. 2014 and Kumar et al. 2022.

### Socio-economic characteristics of the respondents

Socio-economic profile gives an understanding of social status and overall standard of living of people. Table 5 highlights the major socio-economic characteristics of sample farmers in the

study area. It is evident from the table that the average age of the household's head in the study area was 48.21 years. Of whom, 42.50 percent heads had attained average age of 46.88 years. The caste category wise distribution of sample households indicated that OBC formed the largest fraction of total respondents, i.e., around 58.75 per cent, followed by General (25.00%), ST (8.75%) and SC (7.50%) category. The average family size of the respondents was 6.30, of whom 63.75 per cent of the total families had more than 5 members.

The education of the head of the family is an important factor as family head is mainly responsible for making any decision in the household. The Table 5 further suggests that about 23.75 per cent families were headed by illiterate heads. Among the remaining 76.25 per cent literate heads, of whom, 42.50 per cent had education only up to primary level, 13.75 per cent had education up to secondary level, 12.50 per cent had education up to high secondary level and only 7.50 per cent were educated till graduation level.

Furthermore, on an average, one household owned 8.75 animals, out of which approximately half i.e. 4.38 were milch animals. Local cow, crossbred and buffalo accounted for 18.86, 16.28 and 64.86 per cent of these milch animals, respectively. Converting these

**Table 3:** Standard animal units (SAU) of milch animal

| S. No. | Milch Animal | Standard Equivalents |
|---|---|---|
| 1 | Buffalo | 1.30 |
| 2 | Crossbred cow | 1.40 |
| 3 | Local cow | 1.00 |

**Table 4:** Distribution of farmers in the milk marketing channel

| S. No. | Channels | Small (1-3 SAUs) | Medium (4-6 SAUs) | Large (≥7 SAU) | Total (N=80) |
|---|---|---|---|---|---|
| I. | Producer – Consumer | 20 (47.62) | 2 (9.09) | 1 (6.25) | 23 (28.75) |
| II. | Producer - Milk vendor-Consumer | 8 (19.05) | 5 (22.73) | 6 (37.5) | 19 (23.75) |
| III. | Producer – Collection centre – Co-operative milk plant – Consumer | 14 (33.33) | 15 (68.18) | 9 (56.25) | 38 (47.50) |
| | Total | 42 (100.00) | 22 (100.00) | 16 (100.00) | 80 (100.00) |

**Note**: Figures in Parentheses indicate per cent to total respondents.

**Table 5:** Socio-economic characteristics of the respondents

A. Age-wise distribution of household head

| Age(yrs) | No. of respondents | Average Age (Yrs) |
|---|---|---|
| 20-40 | 17 (21.25) | 37.88 |
| 41-50 | 34 (42.50) | 46.88 |
| >50 | 29 (36.25) | 55.83 |
| Total | 80 (100) | 48.21 |

B. Caste category wise distribution of sample households

| Caste | No. of respondents | Percentage |
|---|---|---|
| GEN | 20 | 25.00 |
| OBC | 47 | 58.75 |
| SC | 6 | 7.50 |
| ST | 7 | 8.75 |
| Total | 80 | 100.00 |

C. Distribution of sample households according to size of family

| Family size (No. of members) | No. of respondents | Average Size |
|---|---|---|
| 1 – 4 | 11 (13.75) | 3.72 |
| 5 – 7 | 51 (63.75) | 5.56 |
| 8 or more | 18 (22.50) | 9.77 |
| Total | 80 (100.00) | 6.26 |

D. Distribution of households depending upon level of educational of the household head

| Level of education of the head of the household | Respondents |
|---|---|
| A. Illiterate | 19 (23.75) |
| B. Literates | 61 (76.25) |
| i. Primary | 34 (42.50) |
| ii. Secondary | 11 (13.75) |
| iii. High Secondary | 10 (12.50) |
| Graduate | 6 (7.50) |
| Total | 80 (100.00) |

E. Distribution of average number of animals per household

| Category | Average |
|---|---|
| Total milch animal | 4.38 (100.00) |
| a. Local cow | 0.83 (18.86) |
| b. Cross bred | 0.71 (16.28) |
| c. Buffalo | 2.84 (64.86) |
| Total SAUs (milch) | 5.51 |
| Calves & heifer | 2.90 |
| Dry animal | 1.47 |
| Total animal | 8.75 |

Note: Figures in Parentheses indicate percentage figures

animals equivalent to a local cow indicated the presence of average 5.51 SAUs per household in the study area. Further, calves & heifer per family were 2.90, while on an average a family owned 1.47 dry animals.

**Factors affecting farmer's choice of a specific dairy outlet**

The choice of a milk-marketing channel can be either supplier or producer-driven (Vandeplas et al. 2013). It depends on a variety of factors and different milk-marketing outlets, as well as a number of social and economic factors. In this study, dairy farmers were observed to make a choice amongst three milk-marketing outlets for the disposal of their milk marketable surplus. These three outlets were: (1) Collection Centre, (2) Direct to Consumers, and (3) Milk vendor. All these milk-marketing outlets can be considered independent from each other and cannot be ordered in any logical way therefore, a multinomial logit (MNL) model was used to identify the factors affecting farmer's decision of choosing a particular milk-marketing outlet. The maximum likelihood of independent factors to influence farmers' choice of specific dairy outlet was estimated taking "direct to consumer" as base outlet category. Table6 presents the results of multinomial logistic regression.

An examination of table indicates that the estimated model was significant at 1% level, and demonstrated a good predictive capability as indicated by a pseudo-$R^2$ value of 0.57.

Among physical capital, the coefficient of herd size was found negative and significant for both the category of outlets *viz.,* collection center and milk vender, which points that as the herd-size increases, farmers will be more likely to sell the milk directly to the consumer. The marginal effects figure indicates that one per cent increase in herd size will decrease the probability of selling milk to collection center by 0.19 per cent and to milk vender by 02 per cent. It is in contrast to our expected hypothesis, as it was expected that larger herd size will translate into larger milk marketable surplus, which will be disposed off through organized marketing channel. Some studies suggest that herd size is a significant determinant in market channel participation for modern market channels (Tsougiannis et al. 2008 and Mutura et al. 2015 and Brar et al. 2018). The reverse situation in the study area may be due to the less productivity of milch animals, connoting milk production might not have been proportionately increased with the herd size. Kuma et al. (2013) also observed that number of milking cows owned by households negatively affected the farmer's choice of accessing cooperative milk market outlet.

The coefficient of marketable surplus was positive and significant at 1 per cent level of significance. The corresponding marginal effect values indicate that one per cent increase in the marketable surplus increased the probability of selling milk at collection center and to milk vender by 0.72 per cent and 1.89 per cent, respectively. Meena and Tiwari (2015) also endorsed the positive relationship of marketable surplus with farmer's choice of selling milk to milk and co-operatives.

The table further indicates that the negative and highly significant (at 1%) coefficient of education for the farmers who were educated to senior secondary level. They did not prefer to sell milk to milk venders, rather favoured selling it direct to the consumers, the results confirm this study's postulation. The findings are consistent with the fact that education levels considerably affect market information interpretation and hence, market participation levels of farmers by helping them analyze and exploit the best marketing strategies at their disposal (Jari, 2009; Park, 2009; Moturi et. al.2015).

The caste coefficient representing demographic characteristics of milk farmers showed that farmers belonging to ST category preferred selling directly to the consumers instead of going to collection center or milk vender. On the other hand, farmers belonging to SC category preferred selling milk to milk venders. Sarkar (2020), who conducted study with total of 35,200 agricultural households all over India using NSSO data observed that SC households lacked access to better marketing facilities for the disposition of milk. Only around 17 per cent of the agricultural households of the SC community could sell milk to a cooperative and government agency. Even SC households received a lower average price per litre of milk than all other social groups, which was further corroborated by Ahuja and Redmond (2004). Thorat (2009), based on the Action Aid study in 2001 covering 550 villages across 11 states in India observed exclusionary practices in the consumer markets particularly prominent in the case of milk and vegetables. In about 47 per cent of study villages, SCs were not allowed to sell milk to the

**Table 6:** Factor affecting farmer's choice of a specific dairy outlet

| Base category – Consumer (2) | | | | | | |
|---|---|---|---|---|---|---|
| | Collection Centre (1) | | | Milk Vendor (3) | | |
| Variables | Coefficient | Std. Error | Marginal effects dy/dx | Coefficient | Std. Error | Marginal effects dy/dx |
| i) Physical capital | | | | | | |
| Herd size (log) (no.) | -24.468** | 11.111 | -0.199 | -44.474** | 14.261 | -2.001 |
| Marketable surplus (log) (lt./day/hh) | 37.583* | 10.606 | 0.716 | 54.622* | 12.108 | 1.895 |
| ii) Human capital | | | | | | |
| Education (nominal) | | | | | | |
| (1) Primary | 1.482 | 1.914 | 0.111 | .9198 | 2.222 | 0.023 |
| (2) Secondary | 0.356 | 2.007 | 0.211 | -3.124 | 2.940 | -0.222 |
| (3) Sen. Secondary | 1.845 | 1.537 | 0.439 | -17.845* | 3.355 | -0.362 |
| (4) Intermediate | 0.666 | 1.912 | 0.319 | -5.145 | 3.833 | -0.319 |
| iii) Demographic characteristics | | | | | | |
| Age of Household (log) (yr.) | 3.996 | 8.343 | 2.324 | -24.293 | 18.251 | -2.390 |
| Gender (1=male, 0=female) | 0.951 | 1.909 | 0.162 | -0.548 | 2.008 | -0.118 |
| Caste (nominal) | | | | | | |
| (1) OBC | 1.321 | 1.512 | 0.145 | .227 | 1.815 | -0.080 |
| (2) SC | 1.811 | 2.843 | 0.156 | 5.401*** | 3.122 | 0.284 |
| (3) ST | -7.313 ** | 3.635 | -0.266 | -7.074*** | 3.820 | -0.078 |
| iv) Institutional support | | | | | | |
| Institutional credit (1=yes, 0=no) | 5.981 ** | 2.740 | 0.154 | 8.152* | 3.096 | 0.255 |
| MGNREGA (1=yes, 0=no) | 1.403 | 1.820 | 0.381 | -2.708 | 2.513 | -0.338 |
| BPL (1=yes, 0=no) | -2.741 | 1.894 | -0.034 | -4.225*** | 2.223 | -0.159 |
| Village Location (1=urban, 0= rural) | 1.277 | 1.554 | 0.119 | .587 | 1.686 | -0.044 |
| v) Economic factors | | | | | | |
| Principal occupation livestock (1=yes, 0=no) | 0.722 | 1.459 | 0.215 | 4.124** | 2.108 | 0.301 |
| vi) Animal Characteristics | | | | | | |
| Livestock Age (log) (yr.) | -3.017 | 7.019 | -0.020 | -7.846 | 7.291 | -0.450 |
| Animal Type (nominal) | | | | | | |
| (1) Buffalo | 2.586 | 2.427 | 0.325 | -0.577 | 2.997 | -0.215 |
| (2) Crossbred & Buffalo | 3.029 | 1.986 | 0.311 | 0.403 | 2.620 | -0.166 |
| (3) Cow, CB & Buffalo | 16.632 * | 3.729 | 0.356 | 15.544 | 4.407 | 0.007 |

Pseudo $R^2 = 0.5760$
Prob> $chi^2 = 0.0000$

* = Significant at 1% level of significance
** = Significant at 5% level of significance
*** = Significant at 10% level of significance

village cooperative and to private buyers. Singhal *et el.* 2020 also observed that most of the lower caste households sold milk to the informal channel while organized channel was dominated by general caste households in Punjab. Our results are in consonance with the finding of these studies.

Among the factors under institutional support, access to institutional credit was significant at 5 per cent level of significance for the collection center outlet category and at 1 per cent level of significance under milk vender category. The positive sign in each category shows that if the institutional access is available, farmers will prefer to sell milk in these outlets rather than selling it directly to the consumers. The corresponding marginal effect value shows that if access to institutional credit is improved by 1 per cent, the famer's probability of selling milk in collection center and to milk vender will increase by 0.15 per cent and 0.25 per cent, respectively. This meant that if a farmer had institutional support, then knowledge transmission amongst fellow farmers make them confident in opting suitable marketing channels. This corroborates with findings by Mburu et al. (2007), where group membership was taken as a proxy for social capital and had a positive effect toward farmer participation in the cooperative channel. Table also revealed that farmers belonging to BPL category, preferred to dispose-off the marketable surplus of milk directly to the consumers rather than selling it to the milk venders. The reason of choosing direct channel may be less marketable surplus availability as most of the milk produced might have been utilized for family requirements.

Furthermore, the farmers whose primary occupation was livestock, they favoured selling milk to milk venders over selling it directly to the consumers. The possible reason may be availability of higher milk surplus due to more focus on livestock enterprise, which in turn would have made it difficult to directly sell milk to consumers. Among the animal characteristics, farmers who owned all the types of animals *viz.,* cows, crossbreds and buffaloes preferred to sell milk in the collection center rather than selling it directly to the consumers. This may be due to unavailability of specific customers for separate kind of milk, which might have prompted producer to mix all kind of milk and dispose it at collection center. The value of marginal effects indicates one per cent increase in such unit, led to 0.36 per cent increase in the probability of selling milk in the collection center.

## Conclusion

It can be concluded that, that herd size, marketable surplus, caste categories, access to institutional credit, BPL economic class, income from livestock and breed type of animals were seven significant factors affecting farmer's decision of choosing a particular milk-marketing outlet out of three. Although direct marketing channels highly efficient and fetch more prices to producers, still most of the farmer choose channel III for marketing of milk, may be because of easy disposal of marketable surplus. This trend might continue; therefore, farmers should be made aware about quality parameters like fat percentage in the milk, or even provided with affordable fat testing kits through *Pashu Vigyan Kendras/* Extension centres like KVKs, so that farmers can test it at their level and be assured about the prices they receive in the collection centres. There is need for the effective and improved spread of modern market outlets and dairy cooperatives so that all the farmers irrespective of social and economic background may benefit from livestock farming.

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RESEARCH ARTICLE

# Impact of climate services on the operational decision of Murrah buffalo farmers in Haryana

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**Abstract:** Global warming and its concomitant changes in mean climate variables and climate variability have an impact on animal feed and fodder, animal health, production, and water availability. Buffaloes are the mainstay of the Indian dairy economy and the backbone of the rural economy and dairy industry in the Haryana state in particular. Murrah buffalo-based production system has to be imparted the ability to withstand the adversities associated with climate change as well as to maintain their productivity. Therefore, the present study was undertaken to develop climate services and analyze their impact on Murrah buffalo farmers' operational decision-making related to dairy farming. The study was conducted in the Hisar, Jind, and Rohtak districts of Haryana state. Two blocks were selected randomly from each district and from each block three experimental villages and one control village were selected, resulting in 18 experimental and 6 control villages in total. The three experimental villages of each block were randomly assigned to the intervention mode of either WhatsApp, Text SMS, and Mobile application which was exclusively developed for the present study thus resulting in 6 villages each receiving treatment through WhatsApp, Text SMS, and Mobile application. From each village, 15 farmers were selected randomly and provided with treatment i.e., weekly THI-based Murrah buffalo climate service module. The findings of the study revealed a positive treatment effect of the climate services on various practices like the adoption of improved varieties of fodder, and nutrition management through the inclusion of oilcake, miner mixture in animal diets. The adoption of rubber mats, providing chopped fodder, use of bedding materials and covering open spaces of the animal shed during winter, the practice of deworming the herd and maintenance of cattle shed hygiene, and others. Hence, the climate services for Murrah buffalo farmers were found to be a potential adaptation tool to enhance the resilience capacity of vulnerable dairy farmers to adapt to climate change.

**Keywords:** Climate change, Climate services, Weather, Impact, THI, Murrah buffalo

## Introduction

Livestock production systems all across the world are being directly impacted by the phenomenon of global climate change. The detrimental consequences of global warming affecting both productive and reproductive performance (Upadhyay et al. 2007) is due to the combination of genetic factors of the animal and climatic factors affecting livestock such as temperature, relative humidity, solar radiation, precipitation, and wind speed (Hahn et al. 2003). These changes will significantly influence livestock production due to reduced feed intake, milk production and productivity, livestock diseases, conception rates, animal growth, water availability, and feed and fodder production and availability (Rojas et al. 2017). The negative impact of temperature rise on total milk production for India has been estimated about more than 15 MT by 2050 (Upadhyaya et al. 2013). High heat load in lactating buffaloes reduces their milk production and shortens the duration of lactation length (Upadhyay et al. 2007). As a result, dairy farming in India is highly vulnerable to weather and climate risks, and advanced adaption strategies such as weather forecasting and forecast-based climate services assist in minimizing losses while sustaining production through suitable weather-related livestock management practices (Vashisth et al. 2013). Various amelioration strategies to adverse impacts of climate change on dairy animals include providing sprinklers aided with a fan under the shade during hot dry summers (Ahmad et al. 2019), wallowing which is highly efficient in reducing heat stress (Aggarwal and Singh 2008), loose housing with a shade (may be shed) and open area for night hours (Aggarwal and Singh, 2008), regular showers in addition to wallowing facilities during summer (Mishra 2021, Roy et al. 1968), nutritional

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adjustment strategies through balanced feeding, concentrates, etc (Pankaj et al. 2013).

Using weather information, agricultural producers may be able to make better decisions whose outcomes are influenced by the weather vagaries. In contrast to crop farmers (60%), only a small fraction of livestock farmers (32%), reported using weather data in their farming activities. For decisions regarding the movement of livestock, ranchers increasingly used weather forecasting data (Frisvold and Murugesan 2013). The digital iCow advisory services have quipped dairy farmers with basic, timely knowledge and solutions that improved their production. The advisories on health and disease, deworming, managing fodder, optimum feeding, reproduction management, and other dairy management practices have assisted dairy farmers to realize higher outputs and consequently improve their incomes (Marwa et al. 2020). Weather-based advisories had a positive treatment effect on operational decisions of dairy management, such as watering management, feeding management, shelter management, and vaccination scheduling (Manjusree et al. 2022).

Haryana is having largest Murrah buffalo density among states, with a population of 5.1 million Murrah buffalo out of a total 6.6 million, accounting for 77% of the total buffalo population (Breed survey 2013). Central Haryana is considered as the breeding tract of Murrah buffalo (Parmar and Sangwan 2016). This makes Murrah buffaloes the backbone of the state's rural economy and dairy industry, which contributes to more than 75% of the state's milk production (Balhara et al. 2017).

Haryana has a semi-arid, subtropical climate with scarce, excessive and untimely rains, heat waves, cold waves and hot winds during summer, dust storms, fog, frost, and hails, all of which have a negative impact on crop and livestock production (Singh et al. 2008). Extremely hot summers and very cold winters are common in Haryana. While summer's mean temperature ranges from 48 to 35 degrees Celsius, the winter's average ranges from 3 to 9 degrees Celsius. Climate projections for the state is quite alarming with both Mean maximum and Minimum temperature projected to increase by $1.3^0$C and $2.1^0$C towards mid-century, respectively and mean annual rainfall is projected to decrease marginally by about 63 mm (3%) by 2050s (HSAPCC 2011). Out of 22 districts in Haryana, 15 districts are in the range of medium to high vulnerability towards climate change (Rao et al. 2016).

Given the significance of Haryana's Murrah buffalo cropping system and the fact that it is very sensitive to climate change, timely and reliable climate services are essential for managing day to day livestock operations and minimising losses (Rathore and Chattopadhyay 2016) thereby building the resilience of the buffalo-based farming system to the changing climatic conditions. Thus, the present study was formulated to develop THI based climate services for the Murrah buffalo farmers of Haryana and to assess its impact on the operational decision making.

## Materials and Methods

### Sampling

The study was purposively conducted in the Hisar, Jind and Rohtak districts of central Haryana which is considered as breeding tract of Murrah buffalo having higher concentration of Murrah buffalo (Parmar and Sangwan 2016). Two blocks from each district thus 6 blocks in total were selected randomly i.e. Agroha and Barwala block from Hisar district, Pillukhera and Safindo block from Jind district and Meham and Rohtak block of Rohtak district. Four villages from each block were then randomly chosen, three of which were experimental villages which were randomly administered treatment through WhatsApp, text message, or a mobile application, and one of which was a control village. Thus, the study covered a total of 24 villages resulting in 18 experimental villages (6 each WhatsApp, Text SMS and Mobile Application) and 6 control villages. Finally, farmers who had been rearing Murrah buffalo for the last 10 years and had a minimum herd size of 4 Murrah buffalo and 15 such farmers from each village were randomly selected as respondents. Hence, the total sample size of the present study was 360. Farmers in experimental villages were provided with treatment i.e., a weekly module on the climate information and THI-based advisories on Murrah buffalo rearing. The experimental and control group has undergone a pre-test as well as a post-test before and after the treatment was administered. The collected primary and secondary data from the study area was tabulated and statistically analyzed using statistical tools, like mean, frequency, standard deviation, range, cumulative square root frequency method, regression coefficient, etc. to arrive at a conclusion.

## Results and Discussion

It is apparent from the Table1 that half of the respondents were middle aged farmers having medium farming experience of 18-32 years and around one third of them owing a land holding size of 2-4 ha. It is also clear that majority of the farmers were in medium to high knowledge level categories regarding climate change, its impacts and adoption practices related with concerned system. Most of respondents perceived weather based advisory services as highly useful in their farming activities. Nearly half of the respondents had possessed small herd size and had an annual income of 5.86-9.17 lakhs.

### Herd size and production profile of the dairy animal

Table 2 depicts the number of animals in milk and dry animals as well as heifer and calves maintained in the herd among all three dairy animal types. It is also observed from the same table 3 that the average productivity of buffalo was 8.20 liters and 9.73 liters per day during summer and winter, respectively. Results also show that the productivity of crossbred cattle was 13.34 liters and 15.29 liters per day and indigenous cattle of the region had productivity of only 3.64 liters/day and 4.11 liters/day during

summer and winter, respectively. All three species of dairy animal reach their peak yield after 2-4 weeks, with a lactation length of 282 days in buffalo, 254 days in indigenous cattle, and 291.25 days in crossbred cattle.

**Impact of climate services on feed and fodder management**

**A. Using improved/multicut varieties of fodder crops**

Results from Table 4 show that there was a considerable increase in the number of farmers who have adopted the improved multicut varieties of fodder crops as a result of climate services in all three modes of intervention i.e., Text SMS, WhatsApp, and Mobile App. Dairy is the major contributor to the livelihood of farmers in the region and most of them were stall feeding with almost no grazing, demand for a continuous regular supply of green fodder might be a reason behind the already significant majority of farmers using these improved varieties. Ghosh et al. (2016) in their study have also stressed that the development of improved varieties of perennial grasses, fodder crops and legumes and fodder trees has a role to address the fodder issues like supply-demand gap, silage preparation and etc.

In order to increase fodder productivity and meet fodder demand, Singh et al. (2022) advocated raising awareness about the necessity of using high quality seed of improved fodder varieties and increasing the seed replacement rate from the current 2%-3% to at least 10%.

**B. Use of Oil cakes in the animal feed**

It's obvious from the results of the Table 4, that the climate services had a positive treatment effect on the use of oil seed cakes on the animals in terms of an increase in the number of farmers adopting the practice. Oilseed cakes due to their rich protein content, they are used as animal feed, especially for ruminants and fish (Ramachandran et al. 2007), they are highly

**Table 1:** Socio-economic profile of the respondents (n=360)

| Variable | Categories | % of farmers |
|---|---|---|
| Age | Young (<35 years) | 20.83 |
| | Middle (35-55 years) | 48.06 |
| | Elder (>55 years) | 31.11 |
| Farming experience | 10-18 years | 22.50 |
| | 18-32 years | 44.44 |
| | 32-50 years | 33.06 |
| Operational land holding | < 1 ha | 20.55 |
| | 1-2 ha | 27.50 |
| | 2-4 ha | 32.80 |
| | 4-10 ha | 18.05 |
| | >10 ha | 1.11 |
| Annual income | 2-5.85 lakhs | 37.50 |
| | 5.86-9.17 lakhs | 48.33 |
| | 9.18-19 lakhs | 14.17 |
| Knowledge on climate change & its impact on livestock (Range: 6-20) | Low (6-11.23) | 26.39 |
| | Medium (11.24-14.18) | 39.44 |
| | High (14.19-20) | 34.17 |
| Perception regarding weather based advisory services (Range: 47-77) | Least useful (47-60.01) | 23.89 |
| | Moderately useful (60.02-66.87) | 34.72 |
| | Highly useful (66.88-77) | 41.39 |
| Herd size (Standard Animal Unit) | Small (3.9-7.84) | 47.22 |
| | Medium (7.85-12.06) | 36.67 |
| | Large (12.07-35.95) | 16.11 |

**Table 2:** Average household holding of different types of dairy animals (n=360)

| Category | Buffalo (n=360) | Indigenous cattle (n=86) | Cross-bred cattle (n=74) |
|---|---|---|---|
| In Milk | 4.37 | 1.27 | 1.59 |
| Dry | 1.30 | 0.37 | 0.41 |
| Heifer | 0.88 | 0.50 | 0.54 |
| Calves | 1.11 | 0.47 | 0.54 |
| Total | 7.66 | 2.62 | 3.10 |

nutritive and make a significant contribution to the energy content (Rakita et al. 2023) of the animal diet as part of a balanced ration and help in maintaining milk production. Mustard, cotton, groundnut, and soybean were the most commonly used oil cakes, which were fed to the animals twice a day, in the morning and late at night.

**C. Use of mineral mixture to maintain productivity and health**

In terms of an increase in farmers adopting the use of mineral mixture, climate services had a positive treatment impact (Table 4) in all three modes of intervention i.e., text SMS, WhatsApp, and Mobile App. An increase in milk production and a significant difference in first postpartum estrus and conception rate were observed in animals supplemented with the mineral mixture (Kumar et al. 2020). Cariappa et al. (2022) in their study have also reported that the Anionic mineral mixture prevents milk fever and improves farmer income.

**D. Providing chopped fodder to avoid wastage**

Table 4 depicts the slight increase in the adoption of the practice of providing chopped fodder. The majority of the respondents were already using the chopped fodder for their animals for better digestion, to avoid fodder wastage also the chopping of fodder enables the better mixing of different feed and fodders like dry and green fodder, wheat husk, paddy husk, etc. Manohar et al. (2014) from their study have found that, all the respondents in the study region used to chop dry fodder before feeding while 70 per cent of respondent chopped green fodder. Abilzhanuly (2019) in his study found that, feeding cattle chopped hay results in a weight gain of 35% when compared to feeding cattle non chopped hay.

**Impact of climate services on the health, hygiene and housing practices**

**A. Calcium supplementation**

Results from Table 5 depict the positive treatment effect of climate services on the use of calcium supplementation in the number of farmers adopting the use of calcium supplementation. A study by KVK Jabalpur (MP) has found that balance feeding with feed

**Table 3:** Production profile of the livestock in the sampled households (n=360)

| Productive Parameters | Buffalo (n=360) | Indigenous cattle (n=86) | Cross-bred cattle (n=74) |
|---|---|---|---|
| | Mean±SD | | |
| Milk yield in Summer (lit/day) | 8.20±1.31 | 3.64±1.13 | 13.34±1.29 |
| Milk yield in Winter (lit/day) | 9.73±1.48 | 4.11±1.28 | 15.29±1.32 |
| Lactation Length (Days) | 282.44±13.15 | 254.65±11.31 | 291.25±7.18 |
| Peak Yield (Kg) | 15.09±1.37 | 5.74±1.58 | 18.00±1.33 |
| Dry period (days) | 146.15±14.15 | 155.89 ± 3.18 | 101.05 ± 4.76 |

**Table 4:** Impact of climate services on feed and fodder management (n=360)

| | Improved fodder varieties | | Oil seedcakes | | mineral mixture | | chopped fodder | |
|---|---|---|---|---|---|---|---|---|
| Treatment group | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Control (n=90) | 77.78 | 80.00 | 86.66 | 80.00 | 12.22 | 8.88 | 90.00 | 90.00 |
| Text SMS (n=90) | 81.11 | 88.88 | 83.33 | 93.33 | 14.44 | 24.44 | 93.33 | 96.66 |
| WhatsApp (n=90) | 76.66 | 85.55 | 76.66 | 87.77 | 10.00 | 21.11 | 94.44 | 97.77 |
| Mobile App (n=90) | 82.22 | 95.50 | 77.77 | 92.22 | 16.66 | 30.00 | 88.88 | 94.44 |

**Table 5:** Impact of climate services on animal health, hygiene and housing practices (n=360)

| | Calcium supplementation | | Deworming | | Use of rubber mats | | Shed hygiene | | Mosquito nets | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment group | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Control (n=90) | 42.22 | 40.00 | 36.66 | 38.88 | 64.44 | 65.55 | 73.33 | 68.88 | 70.00 | 71.11 |
| Text SMS (n=90) | 44.44 | 47.77 | 47.77 | 57.77 | 58.88 | 63.33 | 75.55 | 87.77 | 75.55 | 81.11 |
| WhatsApp (n=90) | 33.33 | 38.88 | 41.11 | 55.55 | 60.00 | 67.77 | 67.77 | 84.44 | 68.88 | 75.55 |
| Mobile App (n=90) | 38.88 | 42.22 | 44.44 | 61.11 | 63.33 | 72.22 | 77.77 | 91.11 | 62.22 | 68.88 |

supplements like mineral mixture @ 50g and calcium supplementation @100ml per day per animal during last two months of pregnancy in buffaloes has resulted in reduction of post-partum problems by 40%, increased in milk yield by 25.80% and 40.31% increase in net returns (Annual Report 2015-16 ICAR-ATARI, Jabalpur). In dairy cows, low blood calcium levels after calving can be troublesome, especially for older cows. Acidogenic salts in the diet prior to calving and oral calcium supplements after calving reduce postpartum health and production-related problems (Vagnoni et al. 2021).

**B. Deworming of animals at optimum intervals**

Results from Table 5 reveal that there was a positive treatment effect of the climate services on the number of farmers adopting the practice of deworming of their animals. Findings of Thapa Shrestha *et al.* (2020) have reported that milk production in cows and buffaloes increased steadily in the first month after administering the deworming. The study revealed the importance of deworming and management practises in controlling the prevalence of parasitic diseases. And it also recommended that, in order to achieve the intended goals in deworming activities, sensible use of anthelminthic medications, and effective farm management, periodic monitoring of the incidence of Gastrointestinal parasites among farm animals is required (Gunathilaka et al. 2018).

**C. Use of rubber mats for animals**

Climate services had a positive treatment effect on the adaption of rubber mats for animals as revealed in Table 5. The gap still existing might be due to the cost element involved in the purchase of cow mats specially for small and marginal farmers. Use of cow mats provides a non-slippery surface, reducing injuries to their feet and knees, and are easy to clean and disinfect thereby reducing chances of infection or udder diseases. On the concrete floor, the average minimum slippage amounted to 4.4 occurrences, whereas the rubber mat floor saw only 2.6 instances. Housing cows on the rubber mat floor resulted in a notable 30.4% boost in milk production when compared to the concrete floor, primarily due to the increased comfort it offered (Jain et al. 2013).

**D. Proper disposal of dung, urine, drainage facility, and hygiene maintenance in the animal shed**

Results from Table 5 show that there was a positive treatment effect of the climate services on the adaption of the practice of "proper disposal of dung, urine, drainage facility, and hygiene maintenance in the animal shed, etc." Since the practice is simple and doesn't involve any extra cost, at the same time shed hygiene has a positive effect on animal health by controlling disease-causing pathogens and their vectors, so the practice was adapted by the farmers. A study by Rathod et al. (2017) has disclosed that the incidence of subclinical mastitis in dairy animals was more in case of the animal sheds that were less hygienic, which ultimately affects the milk yield and economic returns, highlighting proper shed hygiene's underlying contribution to animals health and production.

**E. Use of mosquito nets around the shelter to prevent flies, mosquitoes, and other vectors**

Results from Table 5 display that there was a positive treatment effect of the climate services on the adaption of mosquito nets around the shelter to prevent flies, mosquitoes, and other vectors. Since there was a high mosquito and flies problem in the region and almost two-thirds of them were using mosquito nets, The lack of an appropriate shed to install the net and the cost were cited as constraints, while few claimed that alternative methods, such as fogging and the use of mosquito coils and liquid, were sufficient for control. Haque et al. (2021) in their study have reported that, despite the fact that mosquito nets help prevent mosquitoes, flies etc which are the vectors of many diseases including lumpy skin disease, most farmers (91.17 %) did not use one in their cattle barn at night.

**Conclusion**

The changing climatic conditions pose a serious threat to dairy animals in general and Murrah buffaloes in particular, which are highly sensitive to heat stress. Reliable climate information and related advisory services recommending timely weather-related management practices can make dairy farming climate resilient. The exclusive climate services developed for the Murrah had a positive treatment effect on all the operational decision-making of the herd management and hence should be given utmost priority in making available these services to farmers on a regular basis. Climate Services which link the climatic information with available climate resilient dairy farming practices is an important adaptation strategy assisting vulnerable dairy farming populations in coping with the climate of today and of the future. Extension agents' role is imperative in creating climate literacy among farmers, convincing them of the importance of these climate services, interpreting these scientific advisories, and further assistance at all stages of implementation for enhanced uptake and utilization of climate services.

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SHORT COMMUNICATION

# Standardisation and quality evaluation of betel leaf based yoghurt

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**Abstract:** Yoghurts are those milk products which are fermented and have a good impact on the human health. Incorporation of herbs or medicinal plants into the yoghurt, can improve the variety as well as the nutritional and therapeutic benefit of the yoghurt. The present study aimed at developing yoghurt incorporated with betel leaf extract and was successful. The incorporation of 15% betel leaf extract had better organoleptic qualities when compared to the other treatments. The selected yoghurt was stored for 15 days in refrigerated condition and was subjected to organoleptic and nutritional evaluation. The organoleptic scores gradually decreased during storage. Physico-chemical parameters such as moisture, water holding capacity, and syneresis were significantly decreased as the storage period increased. The nutritional components such as energy and carbohydrate were also significantly different as the storage period was extended. Further studies have to be carried out to understand the shelf stability and medicinal properties of the prepared yoghurt.

**Keywords:** Yoghurt, Betel leaf, Medicinal plants, Herbs

Yoghurt has a good impact on human health because they include a variety of bioactive proteins, hydrolyzed carbohydrates, vitamins, and minerals with enhanced bioavailability. *Streptococcus salivarius* subsp. *thermophilus* (SST) and *Lactobacillus delbrueckii* strain. *bulgaricus* (LDB) are used in cooperation to develop yoghurt (Deshwal et al. 2021). Nutritional value and therapeutic potential of yoghurt can both be enhanced by including herbs or medicinal plants such as betel leaves. Betel leaves (*Piper betle L.)* are rich sources of flavonoids, terpenoids, tannins, alkaloids and many bioactive compounds which make it a suitable choice for several therapeutic preparations (Chauhan et al. 2016). In line with the rising demand for such herbal foods, the present research is being done to develop betel leaves based yoghurt.

Betel leaf based yoghurt was prepared by adding the fresh leaf juice to a mixture of preheated milk, skimmed milk powder (1%) and sugar (8%). The mixture was pasteurised, cooled to 55°C and yoghurt culture (2%) was added. The yoghurt was incubated at 42°C for 8 hours and then refrigerated at 4°C. Various treatments were used to standardise the percentage incorporation of betel leaf by modifying the milk and juice ratio. A best treatment was selected through organoleptic evaluation and the selected treatment underwent further evaluations.

Organoleptic evaluations preferred plain yoghurt to betel leaf yoghurts, although the most palatable betel leaf yoghurt was produced by combining 15% betel leaf extract with 85% homogenized milk. Even though, the organoleptic scores decreased during storage, the yoghurt was still acceptable till 15 days. According to a study by Mazumder (2019), dahi was well-accepted when betel leaf extract was added at a rate of 2%. The physic-chemical and nutritional constituents of the prepared yoghurt were studied initially and at five days intervals. Table 1 details the physic-chemical and nutritional constituents recorded during the storage period. The initial moisture content of the betel leaf yoghurt was 76.42%, and it gradually increased during storage. Betel leaf dahi (Mazumder, 2019) had a moisture content that was higher than that of the current study (81.56%). Similar to betel leaf dahi, acidity of the yoghurt in the present also rose from 0.68% to 0.89% during storage. The phenolic compounds in the betel leaf may have prevented the development of acidity

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**Table 1:** Physico-chemical and nutritional constituents of the betel leaf based yoghurt during storage

| Components | Day 1 | Day 5 | Day 10 | Day 15 | C.D. Value |
|---|---|---|---|---|---|
| Moisture (%) | 76.42[c] | 77.02[bc] | 78.54[ab] | 79.14[a] | 1.809 |
| Acidity (%) | 0.68 | 0.75 | 0.81 | 0.89 | NS |
| pH | 4.43 | 4.21 | 3.84 | 3.49 | NS |
| Water holding capacity (%) | 57.32[a] | 56.85[a] | 54.25[b] | 51.05[c] | 2.788 |
| Syneresis (%) | 1.00[b] | 1.70[ab] | 2.30[a] | 2.70[a] | 1.031 |
| Energy (Kcal) | 80.84a | 76.48b | 73.11c | 66.49d | 2.146 |
| Carbohydrate (g) | 7.97a | 7.52ab | 6.98b | 6.23c | 0.960 |
| Protein(g) | 9.54 | 9.35 | 9.16 | 8.93 | NS |
| Fat (g) | 1.20 | 1.00 | 0.95 | 0.65 | NS |
| Total sugar(g) | 9.27 | 9.14 | 8.68 | 8.21 | NS |
| Reducing sugar (g) | 5.17 | 5.10 | 5.03 | 4.95 | NS |
| Vitamin A (IU) | 2.10 | 1.98 | 1.87 | 1.74 | NS |
| Vitamin C (mg) | 0.89 | 0.88 | 0.86 | 0.83 | NS |

DMRT row wise comparison, NS – Non Significant

(Kriangkrai and Penkhae, 2009). The pH ranged from 4.43, which falls within the recommended pH range of 4.6 for yoghurt. Sugar fermentation and lactic acid generation by microbial activities may be the cause for the pH decrease. Starting at 57.32%, water holding capacity (WHC) decreased to 51.05% on day 15, indicating a less robust gel network. The consistency and hardness of dahi may be affected by betel leaf extract. Syneresis in betel leaf yoghurt ranged from 1% to 2.70%. Syneresis, which happens when the gel network loses its ability to maintain the serum phase, results in whey separation during yoghurt storage and can affect customer acceptability Joon et al. (2017).

Essential nutrients like carbohydrates, proteins, lipids, vitamin A, and vitamin C was enhanced by the addition of betel leaf extract to yoghurt. By the fifteenth day of storage, the amounts of reducing sugar and total sugar had fallen from 5.17g and 9.27g to 4.95g and 8.21g, respectively. 7.97g/100g of carbohydrates were present. While in storage, the protein content drastically dropped but stayed at 9.54g/100g. Due to the enzymatic activity of lipase and lipoxidase produced by the microorganisms, the fat content gradually decreased (Mao et al. 2022). The amount of vitamin A ranged from 2.10IU to 1.74IU, and the amount of vitamin C increased with the addition of betel leaves, falling from 0.89 mg to 0.83mg over the course of storage.

## Conclusion

The study aimed at developing a betel leaf based yoghurt, wherein the incorporation of 15 per cent of betel leaf extract was found to have better organoleptic qualities compared to other treatments. As the storage period increased, there were decline in the sensory qualities and also variations in the nutritional constituents. The study should be further continued to understand the shelf stability of the yoghurt. Also the evaluation of the medicinal properties of the prepared yoghurt can thus make us understand its therapeutic properties. It can thus be understood that betel leaf incorporation can bring a variety to the yoghurt flavour, however, further studies can help to reveal its nutritional and medicinal properties

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SHORT COMMUNICATION

# Effect of inulin addition on the sensory attributes of dairy beverage (*Rab*)

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**Abstract:** Being a traditional part of Rajasthani cuisine, *Rab* is widely consumed and relished for its taste. Inulin (prebiotic) has been proven beneficial for gut health. The addition of inulin in *Rab* results in a superfood; containing characteristics of traditional as well as functional beverages. The investigation was performed to prepare and standardize pearl millet (PM) and horse gram dal (HGD) *Rab* with the inulin. The inulin was added at 0%, 1%, 3%, and 5% rates and let ferment for 6-7h, at 35$^0$C. All the fermented treatments were stored at 4$^0$C. All treatments were evaluated for sensory characteristics. All treatments were tested for sensory characteristics including color, consistency, taste and flavor, mouthfeel, aftertaste, and overall acceptability on the Nine-point Hedonic scale and obtained scores above 7 points. It was concluded that the inclusion of inulin significantly improved the sensory characteristics of the functional beverage.

**Keywords**: Functional *Rab, Rabadi* inulin, Prebiotic drink, Pearl millet beverage, Buttermilk

From ancient times, native people of dry and semi-arid areas of western India consumed a cereal and dairy-based beverage called *Rab*. It is made up of the region's staple cereal and buttermilk. Mostly, dry mint, salt, cumin, or even ghee is added as per their preferences. Pearl millet has a large content of phosphorus which is good for bone health. Pearl millet is high in iron and zinc and is a gluten-free grain, a great alternative food for patients with celiac disease (Satnakar and Kumar, 2020). Ramachandra et al. (2021) concluded that *Lactobacillus* sp. obtained from domestic *dahi* samples showed a probiotic nature and sensitivity against various antibiotics. Prebiotics are the fiber that stimulates the growth and metabolism of the human microbiota. Inulin and FOS are mainly used as natural sources of prebiotics. In a review article, Shams and Wadhawan (2021) mentioned that inulin improves the mouthfeel and texture of processed foods and can be used as an important functional ingredient in food processing. In 2022, an article published by Cuamatzin-García et al. stated consumption of fermented foods and beverages improves human health by positively working on immunity, gastrointestinal tract, metabolic disorders, lipid levels, and body fat accumulation. Fornelli et al. 2014 studied the effect of oligofructose and inulin on the sensory characteristics of symbiotic dairy beverages. The investigation mentioned that the addition of inulin and oligofructose did not adversely affect the overall acceptance and marketability of the beverages. Similarly, Moghadam et al. 2019 also stated that Inulin fortification improved yogurt's probiotic viability and textural and flavor characteristics. Therefore, in the present study, an attempt was made to prepare and study the sensory properties of an Inulin-fortified pearl-millet-based fermented beverage (*Rab*)

Buttermilk was procured from the local dairy. Good quality pearl millet (PM) flour, horse gram dal (HGD) flour, salt, and roasted cumin powder were procured from the local market. Inulin powder (brand name- Urban Platter) was procured from the online retailer (Amazon. in).

For the preparation of *Rab*, 80 g PM flour and 20 g HGD powder were cooked with 500 mL water for 15 minutes. The mixture was cooled and then 1500 mL of buttermilk (BM), 0.5% salt, and 0.4% RCP were added, mixed, and divided into 4 treatments. Inulin was added as 0% (controlled), 1% ($PMT_2$), 3% ($PMT_3$) and 5% ($PMT_4$). The mixture was blended with an electrical blender, sieved (18-mesh size strainer), and sat to ferment for 7 h. The final product was packed in pre-sterilized polypropylene cups (200 mL capacity) and stored at 4$^0$C for further analysis.

Sensory evaluation of samples was carried out under laboratory conditions by 30 semi-trained panel members who were scientists,

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**Table 1:** Sensory test result of inulin-incorporated pearl millet Rab

| Property | $PMT_1$ | $PMT_2$ | $PMT_3$ | $PMT_4$ | F-Ratio | p-value | Result |
|---|---|---|---|---|---|---|---|
| Color | 7.33 ± 0.48 | 8.17 ± 0.38 | 7.83 ± 0.38 | 8.00 ± 0.59 | 18.04 | 0.000 | *** |
| Consistency | 8.17 ± 0.70 | 7.83 ± 0.38 | 8.17 ± 0.38 | 8.00 ± 0.00 | 3.94 | 0.010 | * |
| Taste & Flavor | 7.00 ± 0.83 | 7.83 ± 0.38 | 7.17 ± 0.38 | 7.83 ± 0.38 | 20.57 | 0.000 | *** |
| Mouth Feel | 7.50 ± 0.97 | 8.17 ± 0.91 | 7.17 ± 0.38 | 7.00 ± 0.59 | 14.07 | 0.000 | *** |
| After Taste | 6.83 ± 0.70 | 7.83 ± 1.09 | 7.50 ± 0.97 | 7.33 ± 0.76 | 6.53 | 0.000 | *** |
| Overall Acceptability | 8.00 ± 0.59 | 7.83 ± 0.91 | 7.50 ± 0.51 | 7.83 ± 0.70 | 2.74 | 0.046 | * |

Mean +SD, n = 30
*** (P<0.001)
* (P<0.05)

and students of the College of Community and Applied Sciences, MPUAT, Udaipur. Each panelist was asked to taste the given samples and rate the sensory properties (Rangana, 2010) including color, consistency, flavor & taste, mouthfeel, after-taste, and overall acceptability; on a 9-point hedonic scale (Jones, Peryam, and Thurstone, 1995).

Mean values and standard deviation (SD) of triplicate determinations were calculated with the help of Microsoft Excel (Microsoft Office, 2010). All statistical analyses were conducted on SPSS 16 software. One-way analysis of variance was used to determine the existence of any differences among treatment means.

As shown in Table 1, sensory scores of PM- *Rab* (without inulin) for color, consistency, taste & flavor, mouthfeel, after-taste, and overall acceptability were 7.33 ± 0.48, 8.17 ± 0.70, 7.00 ± 0.83, 7.50 ± 0.97, 6.83 ± 0.70, and 8.00 ± 0.59; whereas $PMT_2$ obtained 8.17 ± 0.38, 7.83 ± 0.38, 7.83 ± 0.38, 8.17 ± 0.91, 7.83 ± 1.09, and 7.83 ± 0.91; respectively. For color, *Rab* with 3% inulin scored 7.83 ± 0.38 and Rab with 5% inulin scored 8.00 ± 0.59. Scores obtained for taste & flavor, mouthfeel, after-taste, and overall acceptability by $PMT_3$ were 7.17 ± 0.38, 7.17 ± 0.38, 7.50 ± 0.97, 7.50 ± 0.51; and for $PMT_4$ were 7.83 ± 0.38, 7.00 ± 0.59, 7.33 ± 0.76, and 7.83 ± 0.70. Differences among treatments for color, taste & flavor, mouthfeel, and after-taste properties were found to be significant ((P<0.001), whereas for consistency and overall acceptability, differences were found to be significant (P<0.05).

## Conclusion

Being a tropical country India has a variety of drinks to quench the thirst of people as per the weather conditions. The assortment of drinks is according to the different regions and the availability of raw ingredients including local spices, herbs, taste, and abundance of the main ingredient. One such traditional drink in Rajasthani culture is *Rab*, which combines cereal and buttermilk. The inclusion of prebiotics (inulin) resulted in the enhancement of the sensory properties of *Rab*. All developed treatments scored above 7 points on a 9-point hedonic scale concluding that all treatments were well-liked by the judges and can be introduced in the upcoming beverage market. It has proved to be an effective carrier to provide the required hydration and prebiotics.

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SHORT COMMUNICATION

# Influence of nano zinc supplementation on digestibilty and rumen fermentation parameters under in vitro conditions

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**Abstract:** In vitro experiments were conducted to find out the effects of supplementation of nano zinc (nZnO) @ 0, 1, 5, 10, 20 and 40 ppm to substrate on digestibility and rumen fermentation parameters. The substrate consisted of concentrate mixture and maize fodder in the ratio of 40:60. The results showed that supplementation of Zn in the form of ZnO nanoparticle at 10 and 20 ppm level increased gas production, digestibility, ME values, acetate production and cellulose digestion under ***in vitro*** conditions.

**Keywords**: Nano Zn supplementation, in vitro digestibility, rumen fermentation

Among different essential trace minerals, zinc (Zn) is very important for all forms of life due to its role in gene expression, replication and part of many enzymes (Suttle, 2010). Bonhomme (1990) suggested that Zn is bound to the cell surface of rumen bacteria. Thus, Zn might be affecting the adhesion of microbial cells to cellulose particles. Martinez and Church (1970) showed Zn increased *in vitro* cellulose digestion. Woods (1965) suggested that the Zn requirement of rumen microorganisms for optimum cellulose digestion was less than 1 ppm. Protozoal growth (*Entodinium* sp.) was stimulated at Zn level of 5-10 ppm. Therefore, protozoa incorporated Zn easily and were intolerant to high Zn level, however, Zn did not penetrate bacterial cell readily and reduced metabolic activity. Eryavuz and Dehority (2009) found that 50 ppm level of Zn supplementation reduced cellulose digestion at 24 hours. Gupta (2016) reported that the bioaccessibility of Zn was highest in mustard seed cake (58.94 ppm) followed by cotton seed cake (38.93 ppm) and wheat straw (16.67 ppm) under *in vitro* conditions. In vitro DM digestibilty (IVDMD), *in vitro* OM digestibility (IVOMD), gas volume, metabolizable energy (ME) and short chain fatty acids (SCFA) contents were higher ($P<0.01$) in Zn supplemented diets (Parshuramalu et al. 2013). Aliarabi (2006) reported that Zn supplementation upto 120 ppm, either inorganic or chelated form, did not show significant effect on rumen fermentation parameters, however, negative effect was seen on IVDMD and IVOMD at 160 ppm level. Though study on different forms and sources of Zn are available but very few studies have been conducted regarding effect of nano Zn supplementation on digestibilty and rumen fermentation parameters in ruminants.

Samples of concentrate mixture and maize fodder were collected from Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana. The samples were dried in hot air oven at 65°C for 2 days and a constant weight was attained. The dried samples were ground through 1 mm sieve using electrically operated Willey mill. The basal substrate was prepared using concentrate mixture (% parts: maize 36, groundnut cake 10, full fat soya 15, wheat bran 18, de-oiled rice bran 18, mineral mixture 2 common salt 1) and maize fodder in 40: 60 ratios on DM basis. The proximate principles (DM, OM, CP, EE and total ash) in feeds were determined (AOAC, 2005) while cell wall constituents (NDF and ADF) were analysed as per Van Soest et al. (1991). The Zn contents in feeds were estimated using atomic absorption spectrophotometer (ZEEnit-700P) at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana.

In vitro trials were conducted to estimate gas production (IVGP), true dry matter digestibility (TDMD), true organic matter digestibility (TOMD), microbial biomass production (MBP), pH,

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ammonia nitrogen ($NH_3$-N), individual volatile fatty acids (IVFAs) and cellulose digestion. The basal substrate used in this experiment consisted of dried ground maize fodder and concentrate mixture (% parts: maize 36, groundnut cake 10, full fat soya 15, wheat bran 18, de-oiled rice bran 18, mineral mixture 2 common salt 1) in the ratio of 60: 40. The substrate was supplemented with nano Zn (nZnO) @ 0, 1, 5, 10, 20 and 40 ppm in treatments $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$, respectively.

For in vitro studies, rumen liquor was collected from 3 adult male Murrah buffalos maintained to meet the nutrient requirement (ICAR, 2013) before morning feeding and watering into a pre-warmed thermos flask and brought to the laboratory. Total gas production (Menke and Steingass 1988), true DM and OM digestibility (TDMD and TOMD) were estimated (Van Soest et al. 1991). Metabolizable energy (ME) of feedstuff was calculated using the prediction equation of Menke and Steingass (1988). The pH of strained rumen liquor was estimated (HANNA Instruments, USA).

Microbial biomass production (MBP) was calculated using data of TDOM and net gas volume (Blummel et al. 1997; Blummel and Lebzien 2001). For estimation of $NH_3$-N, 5 mL of acidified supernatant was mixed with 10 mL of NaOH (1 N) and immediately steam distilled using KEL PLUS® - N analyzer (Pelican, India). The $NH_3$ evolved was collected in boric acid solution (20% w/v) having mixed indicator and titrated against N/100 $H_2SO_4$ (AOAC, 2005).

For analysis of individual fatty acids (IVFA), the *in vitro* rumen fermentation was arrested by chilling at 4°C and the syringe contents were then centrifuged at 3000 rpm for 10 min. A portion of 5 mL of supernatant was added to 1 mL of 25% metaphosphoric acid and kept overnight at 4ºC (Patra et al. 2006). The mixture was centrifuged at 3000 rpm for 15 min. and 2 mL of supernatant was taken and stored at -20ºC for VFA analysis. The individual VFA in the samples were determined using Gas Chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless-steel column packed with chromosorb 101 mesh 80-100 (length 1.5 m; o.d 3.175 mm; i.d. 2 mm). Analytical conditions for fractionation of VFA were as follows: Injection port temperature 210ºC, column temperature 180ºC and detector temperature 230ºC. The flow rate of the carrier gas $N_2$ was 40 mL/min). Individual volatile fatty acids (Acetate, propionate and butyrate) in the samples were determined on the basis of retention time and their concentration was calculated by comparing the retention time as well as the peak area of the standard after blank correction.

*In vitro* cellulose digestion was done using a basal purified cellulose medium contained the following ingredients per 100 mL:

1. 15 mL each of mineral solutions I and II (Bryant and Burkey, 1953)
2. 0.1 mL of 0.1g/100 mL resazurin solution
3. 25 mL of a 3 g/100 mL suspension of cellulose (Sigma, St. Louis, MO, USA)
4. 40 mL of strained rumen fluid
5. 3.33 mL of 12 g/100 mL $Na_2CO_3$
6. 1.67 mL of 3 g/100 mL cysteine hydrocholoride

An aliquote of 8 mL was tubed under $O_2$-free $CO_2$ into 16 × 150 mm culture tubes closed with rubber stopper and autoclaved in racks at 121°C for 20 min. (Dehority, 1969). A solution of 1 mL of either sterile distilled water or different concentration of nano zinc solution was added at the time of inoculation to make the final volume to 10 mL. The cellulose concentration in the final medium was 0.075 g/mL. The mixture was agitated for 3 min. under a vigorous stream of $O_2$-free $CO_2$.

After incubation, the entire contents of the culture tubes were transferred to a previously weighed test tube and centrifuged at 1000×g for 10 min. at room temperature (20-23°C). The supernatant was decanted and 5 mL of acid detergent solution were added (Van Soest, 1963). The tubes were mixed and heated on hot plate for 1 h at 100°C. The insoluble residue was centrifuged as stated earlier and supernatant was discarded. The sediment was washed twice with boiled distilled water. The tubes were dried overnight in an oven at 100°C, placed in a desiccator and weighed (Hiltner and Dehority, 1983). The cellulose digestion was based on the difference between the weight of cellulose measured in the blank tubes (0 h) and other tubes after 24 h incubation time.

Statistical analysis of experimental data was analysed by one way analysis of variance (ANOVA) model as per Snedecor and Cochran (1994). This statistical ANOVA model was incorporated with General Linear Models procedure (SPSS, 2012, version: 20).

The contents of OM, CP, EE, NDF and ADF in concentrate mixture were 93.95, 20.45, 5.02, 30.42 and 12.27% (DM basis) with the corresponding values of 89.13, 11.02, 1.65, 56.43 and 30.53% for maize fodder. The Zn content in concentrate mixture and maize fodder were found to be 24.40 and 21.78 ppm, respectively. The effects of nano Zn supplementation on values of in vitro gas production (IVGP), true DM digestibility (TDMD), true OM digestibility (TOMD), partitioning factor (PF), short chain fatty acid (SCFA), microbial biomass production (MBP) and metabolizable energy (ME) in different treatments have been presented in Table 1. The IVGP was higher ($P<0.05$) in treatments $T_4$ and $T_5$ and the lowest value was observed in treatment $T_1$. In contrary to our findings, nZnO with 20 or 40 ppm (Zaboli and Aliarabi, 2013) had no significant effect on gas production. The highest ($P<0.05$) values of TDMD (%) were recorded in treatments $T_4$ and $T_5$ and the lowest in treatment $T_1$. Similar results were obtained by Ahmed et al. (2022) where maximum digestibility was obtained at supplementary level of 30 ppm Zn. Zinc supplementation in form of proteinate, propionate (Nagalakshmi

et al. 2013) and Zn peptide (Mallaki et al. 2015) resulted in higher *in vitro* digestibility compared to $ZnSO_4$ addition (Arelovich et al. 2000) which might be due to more Zn and amino acid availability for rumen microbes. The TOMD values were lower ($P<0.05$) in treatments $T_1$, $T_2$ and $T_3$ compared to treatments $T_4$ and $T_5$. The enhancement in digestibility was reflected from increased total gas production. Similar findings were reported by Juncai et al. (2011) using nZnO upto 400 ppm, A significant increase in PF value was seen in treatment $T_1$ lower in treatments $T_4$ and $T_5$. The lower PF values indicate more gas production from the feed which signifies less DM intake by the animal with better performance (Blummel et al. 1997). The level of SCFA was significantly ($P<0.05$) higher in treatments $T_4$ and $T_5$ and lowest in treatment $T_1$. Blummel and Orskov (1993) observed a high significant correlation between SCFA and gas production. SCFA is directly related with gas production. Addition of inorganic Zn (Parshuramalu et al. 2013) and Zn peptide (Mallaki et al. 2015) increased short chain fatty acid (SCFA) level. The MBP value was lower ($P<0.05$) in treatments $T_4$ and $T_5$ compared to treatments $T_1$ and $T_2$. Microbial biomass is the major source of protein for the ruminant animals which is a source of truly available protein post ruminaly. *In vitro* gas production reflects primarily SCFA production and an inverse relationship exist between SCFA and microbial efficiency (Blummel et al. 1997). The ME value was the lowest in treatment $T_1$ and the highest in treatments $T_4$ and $T_5$. Supplementation of inorganic Zn (Parsurammalu et al. 2013), organic Zn (Nagalakshmi et al. 2013), Zn peptide (Mallaki et al. 2015) enhanced the ME value of the feeds.

The average values for pH, ammonia nitrogen ($NH_3$-N), individual fatty acids (IVFA; acetate, propionate and butyrate), IVFA (mol/100 mol) and A: P ratios have been presented in Table 2. The range of pH was found to be 6.70 to 6.83 in different treatments and the values were similar. Juncai et al. (2011) and Hassan et al. (2019) reported that supplementation of nano Zn had no effect on rumrn pH. The value of $NH_3$-N ranged from 23.38 to 24.47 mg/

**Table 1**: In vitro gas production, digestibility, microbial biomass production and ME values as affected by different levels of nano Zn supplementation

| Parameter | Level of nano Zn (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| $IVGP_{24h}$(mL/g) | $149.40^{c}\pm0.10$ | $169.59^{b}\pm0.65$ | $170.07^{b}\pm0.93$ | $177.11^{a}\pm1.47$ | $179.68^{a}\pm2.01$ | $167.90^{b}\pm1.65$ |
| TDMD (%) | $66.41^{c}\pm0.47$ | $67.91^{b}\pm0.38$ | $68.41^{b}\pm0.36$ | $70.67^{a}\pm0.22$ | $70.47^{a}\pm0.46$ | $69.52^{ab}\pm0.49$ |
| TOMD (%) | $69.24^{c}\pm0.49$ | $69.49^{c}\pm0.10$ | $69.94^{bc}\pm0.12$ | $71.95^{a}\pm0.07$ | $72.49^{a}\pm0.18$ | $71.41^{ab}\pm0.19$ |
| PF | $4.01^{a}\pm0.09$ | $3.86^{ab}\pm0.05$ | $3.75^{ab}\pm0.07$ | $3.61^{b}\pm0.07$ | $3.64^{b}\pm0.08$ | $3.80^{ab}\pm0.02$ |
| SCFA (mmol) | $0.70^{b}\pm0.02$ | $0.75^{ab}\pm0.01$ | $0.76^{ab}\pm0.02$ | $0.80^{a}\pm0.02$ | $0.79^{a}\pm0.02$ | $0.76^{ab}\pm0.01$ |
| MBP (mg/g) | $283.23^{a}\pm7.90$ | $281.48^{a}\pm4.65$ | $264.39^{ab}\pm6.47$ | $255.07^{b}\pm8.03$ | $255.40^{b}\pm8.69$ | $273.93^{ab}\pm2.56$ |
| ME (MJ/kg) | $7.53^{c}\pm0.12$ | $7.86^{b}\pm0.06$ | $7.91^{b}\pm0.10$ | $8.17^{a}\pm0.09$ | $8.24^{a}\pm0.08$ | $7.81^{b}\pm0.03$ |

[a,b,c]Values bearing different superscripts in a row differ significantly ($P<0.05$)

**Table 2:** The effect of nano Zn supplementation on rumen pH, ammonia-N and volatile fatty acids under in vitro conditions

| Parameter | Level of nano Zn (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| pH | 6.70±0.03 | 6.73±0.06 | 6.77±0.03 | 6.77±0.02 | 6.83±0.04 | 6.67±0.02 |
| $NH_3$-N (mg/dL) | 23.43±0.42 | 23.86±0.25 | 23.38±0.35 | 23.52±0.13 | 23.89±0.42 | 24.47±0.23 |
| | | | IVFA (mM) | | | |
| Acetate | $39.13^{c}\pm1.06$ | $45.65^{b}\pm1.20$ | $54.47^{a}\pm0.76$ | $57.57^{a}\pm1.14$ | $57.90^{a}\pm1.24$ | $56.24^{a}\pm0.99$ |
| Propionate | 15.93±0.55 | 16.68±0.72 | 17.00±0.70 | 17.20±0.64 | 17.92±0.62 | 18.35±0.65 |
| Butyrate | 6.60±0.40 | 6.61±0.42 | 7.77±0.32 | 7.37±0.19 | 7.52±0.87 | 7.72±0.37 |
| A: P ratio | $2.46^{c}\pm0.07$ | $2.75^{bc}\pm0.08$ | $3.23^{ab}\pm0.13$ | $3.37^{a}\pm0.17$ | $3.25^{ab}\pm0.13$ | $3.08^{ab}\pm0.14$ |
| | | | IVFA (mol/100mol) | | | |
| Acetate | $63.44^{c}\pm0.70$ | $66.24^{bc}\pm0.42$ | $68.78^{ab}\pm0.61$ | $70.08^{a}\pm0.96$ | $69.48^{ab}\pm1.32$ | $68.32^{ab}\pm0.85$ |
| Propionate | $25.81^{a}\pm0.56$ | $24.18^{ab}\pm0.65$ | $21.43^{bc}\pm0.70$ | $20.95^{c}\pm0.79$ | $21.48^{bc}\pm0.58$ | $22.29^{bc}\pm0.75$ |
| Butyrate | 10.75±0.75 | 9.58±0.50 | 9.79±0.29 | 8.97±0.22 | 9.04±1.08 | 9.39±0.49 |

[a,b,c,d] Values bearing different superscripts in a row differ significantly ($P<0.05$)

dL in different treatments. Similar report (Hassan et al. 2019) exist using sheep rumen liquor under in vitro. Juncai et al. (2011) found that the concentration of $NH_3$-N decreased ($P<0.05$) with the supplementation of 100 ppm nano ZnO. In contrary to this, addition of Zn decreased the $NH_3$-N released in vitro (Arelovich et al. 2000) and rumen fluid of sheep (Rodriguez et al. 1995). At the 6 and 12 h of incubation in vitro, the supplementation levels of 100 and 200 mg/kg of nano-zinc oxide considerably ($P<0.05$) reduced the concentration of $NH_3$-N and the ratio of acetate to propionate (Chen et al. 2011)

The values of acetate production increased in treatments $T_3$, $T_4$, $T_5$ and $T_6$. Juncai et al. (2011) also reported that supplementation of nano ZnO under in vitro rumen conditions increased VFA production. Chen et al. (2011) showed that VFA production increased ($P<0.05$) with the supplementation levels of 100 and 200 mg/kg of nano-zinc oxide at the 6 and 12 h of incubation in vitro. In contrast, Aliarabi (2006) and Hassan et al. (2019) reported that Zn supplementation either in inorganic or chelated and Nano Zn form, respectively did not show significant effect on rumen fermentation parameters. Propionate production ranged from 15.93 to 18.35 mM and value of butyrate production varied from 6.60 to 7.72 mM in different treatments. Spear et al. (2004) reported that propionate was higher ($P<0.05$) and butyrate was lower ($P<0.05$) in steers fed Zn-Met compared to $ZnSO_4$ diets. A significant ($P<0.05$) increase in the A: P value was seen in treatment $T_4$ and lower value in treatment $T_1$. However, Juncai et al. (2011) reported that at 50 ppm and Hassan et al. (2019) reported that at dose from 20 to 60 ppm of nano ZnO supplementation A: P ratio was same but higher than control group.

In vitro cellulose digested was lower ($P<0.05$) in treatment $T_1$ and higher at 10 and 20 ppm level of supplementation. Similar value of pH was seen in different treatments. Addition of Zn upto 10 ppm increased *in vitro* cellulose digestion (Martinez and Church 1970; Little et al. 1958). Further supplementation of 20 and 30 ppm of added Zn resulted in a decrease ($P<0.05$) in cellulose digestion. Eryavuz and Dehority (2009) found that 50 ppm Zn supplementation to the cellulose media reduced cellulose digestion. The adhesion of cellulolytic bacteria to cellulose is a critical early step in cellulose fermentation. Bonhomme (1990) suggested that Zn is bound to the cell surface of bacteria. Thus, Zn might be affecting the adhesion of microbial cells to cellulose particles.

## Conclusion

Therefore, inclusion of Zn in form of ZnO nanoparticle @10 and 20 ppm of basal substrate showed enhancement ($p<0.05$) in acetate production digestibility and ME contents.

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