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CENTRAL OFFICE : Indian Dairy Association, IDA House, Sector IV, R.K. Puram, New Delhi-110022. Phones: 011-26170781, 26165237, 26165355. Email: idahq@rediffmail.com /www.indairyasso.org

ZONAL BRANCHES & CHAPTERS : **South Zone:** Mr. C.P. Charles, Chairman, IDA House, NDRI Campus, Adugodi, Bangalore-560 030. Ph.: 080-25710661 Fax: 080-25710161. **West Zone:** Mr. Arun Patil, Chairman; A-501, Dynasty Business Park, Andheri-Kurla Road, Andheri (East), Mumbai 400059 Email: arunpatilida@gmail.com / secretary@idawz.org Ph.: 91 22 49784009 **North Zone :** Shri S.S. Mann, Chairman; c/o IDA House, Sector IV, R.K. Puram, New Delhi - 110 022 Phones: 011-26170781, 26165355. **East Zone:** Mr. Sudhir Kumar Singh, Chairman, c/o NDDB, Block-DK, Sector-II, Salt Lake City, Kolkata-700 091 Phones: 033-23591884-7. **Gujarat State Chapter:** Dr.J.B. Prajapati, Chairman; c/o SMC College of Dairy Science, Anand Agricultural University, Anand-388110 Gujarat. Email: idagscac@gmail.com/jbprajapati@gmail.com **Kerala State Chapter:** Dr. S.N. Rajakumar, Chairman; c/o Prof. and Head, KVASU Dairy Plant, Mannuthy, E mail: idakeralachapter@gmail.com **Rajasthan State Chapter:** Mr. Rahul Saxena, Chairman; Cabin no 1, Ground Floor, Manoram, #2, Ambeshwar Colony, New Sanganer Road, Near Shyam Nagar Metro Station, Jaipur-302019 E-mail: idarajchapter@yahoo.com **Punjab State Chapter:** Dr. B.M. Mahajan, Chairman; c/o Director, Dairy Development Deptt., Punjab Livestock Complex, 4th Floor, Near Army Institute of Law, Sec-68, Mohali. Ph.: 0172-5027285/2217020 Email: ida.pb@rediffmail.com **Bihar State Chapter:** Mr. D.K. Srivastava, Chairman; c/o Former Managing Director, Mithila Milk Union; House No. 16 Mangalam Enclave, Baily Road, Near Saguna SBI, Patna-814146 (Bihar). E-mail: idabihar2019@gmail.com **Haryana State Chapter:** Dr. S.K. Kanawjia, Chairman; c/o D.T. Division, NDRI, Karnal-132 001 (Haryana). Ph.: 09896782850 Email: skkanawjia@rediffmail.com. **Tamil Nadu State Chapter:** Mr. S. Ramamoorthy, Chairman; c/o Department of Dairy Science, Madras Veterinary College, Vepery, Chennai-600007. **Andhra Pradesh Local Chapter:** Prof. Ravi Kumar Sreebhashvam, Chairman; c/o College of Dairy Technology, Sri Venkateshwara Veterinary University, Thirupathi - 517502 Email: idaap2020@gmail.com **Eastern UP Local Chapter:** Prof. D.C. Rai, Chairman; c/o Prof. of Dairy Sci.& Tech., Head, Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 Ph.: 0542-2368009 Email: dcrai@bhu.ac.in **Western UP Local Chapter:** Mr. Vijendra Agarwal, Chairman; c/o Kailash Dairy Ltd., Rithani, Delhi Road, Meerut. Ph.: 9837019596 Email: vijendraagarwal2012@gmail.com **Jharkhand Local Chapter:** Mr. Pavan Kumar Marwah, Chairman; c/o Jharkhand Milk Federation, FTC Complex, Dhurwa Sector-2, Ranchi, Jharkhand-834004 Email: jharkhandida@gmail.com

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RESEARCH ARTICLE

Effect of storage temperature on quality characteristics of iron fortified milk chocolate

Manpreet Singh, Rekha Chawla and Venus Bansal

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Abstract: This study aimed to evaluate the storage stability of iron-fortified milk chocolate at two different temperatures viz., ambient (25±2°C) and refrigerated (4±1°C). The developed product was packed in aluminium foil (0.2 mm thick), and change in quality attributes in terms of sensory, physico-chemical, instrumental textural, and microbiological investigation were performed at an interval of 7 days. The keeping quality decreased with the progress of storage at both temperatures. However, the product stored at ambient temperature became unacceptable after 21 days of storage. An increase in HMF, TBA, peroxide, aw, FFA, hardness, cohesiveness, gumminess, SPC, and YM were observed in both the samples, whereas the changes were more pronounced at ambient temperature. Henceforth, samples stored at refrigerated temperature were acceptable till 91 days of storage and exhibited significantly ($p<0.05$) higher sensory scores from day 7. The study indicated that iron-fortified chocolates could be successfully stored for a longer period at refrigerated temperature and can serve as a potential substitute for conventional chocolate.

Keywords: Iron fortification, Milk chocolate, Quality, Shelf life, Temperature

Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, 141 004, Punjab, India

Rekha Chawla (✉)
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, 141 004, Punjab, India
Email: mails4rekha@gmail.com

Introduction

Iron deficiency is the most common mineral deficiency affecting an estimated 25% of the world's population suffering with anaemia (Warner and Kamran, 2021). It is more prevalent among children, reproductive women, and sportsmen, leading to the global disease burden and national economy. The particular deficiency is usually the result of insufficient dietary intake of iron or poor utilization of iron from ingested food or both in combination. Nutritional iron deficiency is a major health problem in developing countries. Therefore, fortification of iron into food products and iron supplements are used nowadays to increase the dietary iron intake among people. Alamen et al. (2016) and Rebellato et al. (2015) suggested that fortified foods with high levels of bioavailable iron may be used as tools to combat such deficiency. Direct addition of iron to edible products might be an effective means of increasing the dietary intake of iron to the general population. In this context, Iron fortiûcation of various cereal-based products like ûour, bread, cereals, and refined oils is often practiced to increase dietary iron consumption. However, there is a need to develop new products with enhanced iron content to reach the global population and combat the deficiencies occurring from the lesser intake of dietary iron. Chocolate, which is categorized under food confection and relished by all the age groups is a potential food product for iron fortification. Ieggli et al. (2011) reported that chocolate is a potential source of many essential nutrients and can contribute to a healthy diet with added micronutrients.

Chocolate is made either in the form of a liquid or paste, block, or used as a flavoring ingredient in other sweets. Chocolate is a range of products derived from cocoa beans (seeds of the Theobroma cacao tree). This cacao tree is native to South America, from where it was naturally spread into Central America (Bearden et al. 2000). Generally, it is a dispersion of around 70 percent of fine particles such as cocoa powder, sugar, and milk solids in a continuous phase, usually cocoa butter and milk fat, which depends on the specific formulation of the chocolate (Fernandez et al. 2013). Technically, it is the product that consists of non-fat particles (sugar and cocoa solids and, eventually, milk powder particles) dispersed in the fat phase (cocoa butter) (Beckett, 2009; Schantz and Rohm, 2005).

It is also one of the most craved foods, probably due to its uniquely attractive taste, positive contribution to human nutrition with antioxidants and potential to arouse positive emotions (Afoakwa et al. 2007; El-Kalyoubi et al. 2011). Various studies have demonstrated the beneficial health effect of chocolate on coronary vasculature, insulin secretion, and endothelial function (Richelle et al. 2001; Taubert et al. 2007; Davison et al. 2008). Therefore, the direct addition of iron to chocolate can contribute to a healthy diet with added micronutrients (Ieggli et al. 2011). However, iron fortification of chocolate can affect the quality in terms of the sensory, physico-chemical, and microbiological profile of the product in terms of acting as a prooxidant. Therefore, the present study was conducted to evaluate the effect of storage temperature on iron-fortified milk chocolate stored at refrigerated and ambient temperatures.

The preparation of control chocolate without any iron fortification at lab scale using response surface methodology has already been available (Manpreet et al. 2017) which was further made functional using iron supplementation. In the current study, milk chocolate was fortified with sodium-iron-EDTA (NaFe-EDTA) salt at the rate of 800 mg/Kg. The developed product was packed in 0.2 mm aluminium foil and was stored at two different temperatures, viz., refrigerated and ambient. The experimental samples were evaluated for physico-chemical, textural, microbiological, and sensory profiles during storage.

Materials and Methods

Raw materials

The cocoa butter (continuous fat phase ingredient) for preparing the chocolate was procured from 'chocoville' cocoa butter, Indore. Skim milk powder was procured from the Punjab state cooperative milk producers' federation limited available under the brand name 'Verka.' Cocoa powder used in milk chocolate preparation from Hershey's cocoa, Mumbai; and Icing sugar of good quality was procured from the local market of Ludhiana. Sodium iron EDTA (Food grade) salt and ascorbic acid were procured from Central Drug House (P) Ltd.

Sample preparation

Pre-weighed ingredients (SMP, sugar, cocoa powder, and iron salt along with ascorbic acid) were mixed adequately in a planetary mixer at 300-350 rpm (Orange foodstuff, Mumbai). After proper mixing, cocoa butter (38-40ºC) was added, and contents were mixed for 1.30-2 hours for proper mixing of the ingredients and flavour development. During the mixing of contents, a coil was wrapped around the planetary mixer, and hot water was circulated through the water bath to maintain the desired conching temperature (50-55 °C), which aided in process and temperature control. This step imitated the commercial conching process to prepare smoother and silkier chocolate. When ingredients were properly mixed and formed a paste-like structure, the mix was poured in mould of the desired shape and kept at refrigerated temperature (4±1 º C). After 30 minutes, the moulded product was kept out and packed in aluminium foil (0.2mm) to avoid contamination of chocolate.

Packaging and storage of product

After preparation, cubes of milk chocolate 3x3x0.5 cm dimensions were packed in 0.2mm aluminium foil. The samples were coded with number separately for two storage temperatures and stored at ambient temperature in a stability chamber (Make: REMI, SC-6 PLUS, Mumbai) maintained at 25±2°C and at a refrigerated temperature in a refrigerator maintained at 4±1°C. The samples were evaluated at a fixed interval of 7 days up to 3 months for sensory and physico-chemical properties. All the samples were evaluated in triplicates to remove the possible chance of error.

Sensory evaluation

A nine-point hedonic scale was employed to evaluate samples (Amerine et al. 1965) and a total of seven different attributes were generated pertaining to parameters that could fully describe the iron-fortified milk chocolate. On a specific day interval for both the temperatures, the sample was served to a fixed panel of 7 semi-trained panelists for sensory evaluation. Prior to sensory evaluation, the sensory panel was briefed about the desirable characteristics of the product.

Physico-Chemical Analysis

Water activity (a_w)

The samples were analysed using Rotronic Water Activity Meter (Series HP 23 Aw).

Peroxide value

Peroxide value (PV) was estimated by AOAC method (AOAC, 1995).

Hydroxymethyl furfural (HMF)

Total HMF samples was determined as method described by Keeney and Bassette (1959) with slight modifications. Three g of sample was thoroughly mixed with 7 ml distilled water. Then 5 ml of 0.3N oxalic acid was added and the tube was kept in boiling water bath for 60 minutes. The contents of the tube were cooled and subsequently 5 ml of 40% trichloroacetic acid solution was added and precipitation occurred. The precipitated mixture was filtered through Whatman No. 42 filter paper. Of the filtrate, 0.5 ml of the solution, 3.5 ml distilled water and 1 ml of 0.05 M Thiobarbituric acid solution (aq.) was added in 5 ml test tube and mixed well. The tubes were kept in water bath at 40°C for 50 minutes. After cooling to room temperature, absorbance was

measured at 443 nm. A blank test was carried out in the same manner as above substituting distilled water for milk chocolate.

Free fatty acid

The method prescribed by Yadav et al. (2011) was used to estimate the FFA content of iron fortified milk chocolate.

Thiobarbituric Acid

The thiobarbituric acid reactive substances (TBARS) in milk chocolate were determined by Bird and Draper (1984) method.

Textural Profile Analysis

Various textural properties such as Hardness, Adhesiveness, Cohesiveness, and Gumminess were measured using Texture Analyser TMS-Pro (Food Technology Corporation) equipped with 100 N load cell. A two-bite test force distance compression curve (Bourne 1978) was obtained from the resulting force-time curves. A 75 mm compression plate was used for texture profile analysis of the samples. The samples of cube shape of height 8mm and 8mm diameter were subjected to mono-axial compression of 25% of the initial sample height which were already tempered to 25°C.

Microbiological Analysis

Standard plate counts, yeast and mould counts and coliform counts were recorded as per procedure by Wehr and Frank (2004) using the media nutrient agar for standard plate count, potato dextrose agar for yeast and mould and violet red bile agar for *coliform*.

Statistical Analysis

The data was analysed statistically using IBM SPSS statistics 23 software package and the mean comparison was done by Duncan Post Hoc test. Differences of $p<0.05$ were considered to be significant.

Results and Discussion

Effect of storage temperature on sensory scores

The effect of storage temperature on sensory scores has been presented in Fig. 1. The chocolate samples stored at 25 °C were not served after 21 days of storage as the mean scores were for all the parameters were less than 6.5. The significant difference ($p <0.05$) in sensory scores for samples stored at 4 and 25 °C was observed from day 7th, and scores gradually decreased for samples stored at 25 °C. On the other hand, the sensory scores (except texture and sweetness) of samples stored at 25 °C firstly decreased from day 0 and day 7 and then increased ($p>0.05$) till day 21, and a further increase in storage period showed a gradual decrease in scores. The faster decrease in sensory scores at 25 °C might be due to iron acting at catalyst, which might have increased the rate of change of appeal of the samples during storage.

The colour and appearance scores decreased significantly ($p<0.05$) at 25 and 4 °C from 7.93 to 6.43 at the end of 21 days and 7.93 to 6.74 after 91 days of storage, respectively. Fig. 1 showed that the decrease in colour scores was more rapid and pronounced at ambient temperature than product stored at refrigerated temperature. Similar findings were reported by Briones and Aguilera (2005) in chocolate, wherein authors noted yellowish colour in chocolate stored at a higher temperature compared to samples stored at lower temperature. The researchers suggested that this colour change at higher temperatures might be due to fat bloom at higher temperatures. Aguilera et al. (2004) stated that the bloom on chocolate is produced by action of high temperature and includes a gradual discolouration, loss of glossiness, and caused grey surface appearance of chocolate. A similar study was conducted by Bui and Coad (2014), in which colour changes were recorded among the experimental and control samples during storage, and an increase in lightness (L) was observed at a higher temperature.

Correspondingly, flavour scores decreased from 7.27 to 6.53 at the end of 91 days of storage for the samples stored at 4 °C, while it decreased to 6.47 at 25 °C on a concluding day. The flavour scores decreased significantly ($p<0.05$) after 7 days for samples stored at 25 °C. A non-significant ($p>0.05$) trend was observed at 25 °C, which continued to decrease throughout the storage period. The decrease in flavour scores might be due to fat oxidation during storage, which was more prominent at ambient temperature. Similar observations were recorded by Rathor et al. (2016) in banana chocolate, where authors observed a decrease in flavour scores with the progress of storage.

The decrease in mouthfeel scores might be due to a change in the product's flavor, which gave the panelists a less acceptable mouthfeel. There was a significant ($p<0.05$) decrease in mouthfeel score, though it was noticeable only after 49th days of storage at 4 °C, whereas samples stored at ambient temperature were significantly ($p<0.05$) different throughout 21 days of storage.

The melting score was found significantly differing ($p<0.05$) between the sample stored at different conditions of temperature on 7th day of storage. The melting scores decreased from 7.52 to 6.47 and 6.58 during ambient and refrigerated temperature storage after 21st and 91th day, respectively. This may be attributed to the drying of the product and loss of the requisite amount of moisture with the progress of storage. During storage, texture scores decreased gradually, which may be because of loss of moisture accompanied by an increase in hardness of the samples. These results are in relation to instrumental value for hardness which increased with the progress of storage (Fig. 3). The results

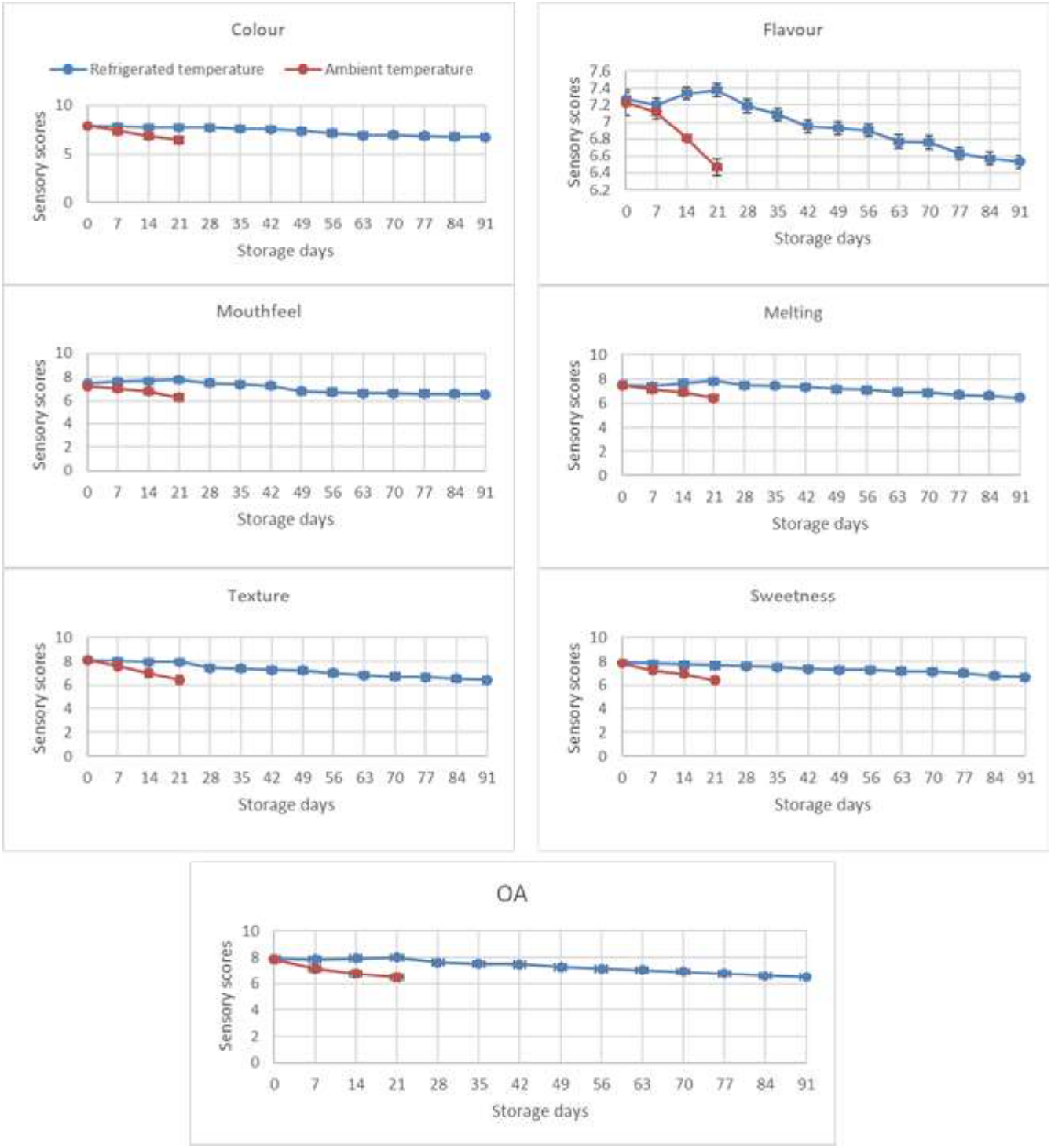

Fig. 1: Effect of storage on sensory attributes of milk chocolate

of texture scores are in congruence with the finding of Mexis et al. (2010), wherein the author suggested that changes in texture, accompanied by a change in colour might be due to fat bloom. Rathor et al. (2016) also found the decreased texture score of banana chocolate during the storage of the product.

On similar grounds, the sweetness scores of the chocolate decreased significantly ($p<0.05$), from an initial value of 7.88 to 6.66 and 6.41 at 5 and 25 °C, respectively. It might be due to a change in chocolate's flavour profile (slightly bitter) with time. Also, the perceivable intensity of the product declined

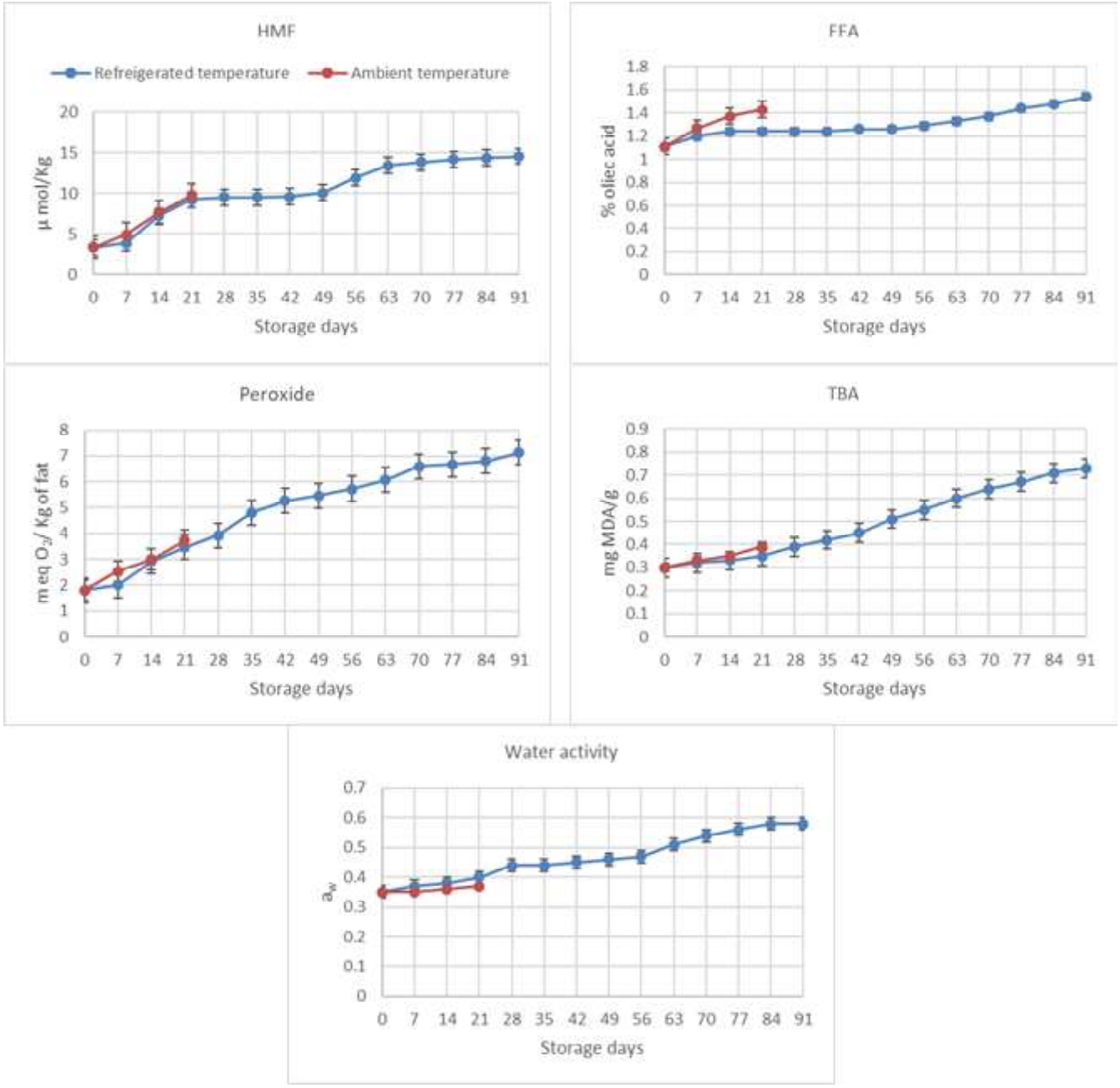

Fig. 2 Effect of storage on physico-chemical properties of milk chocolate

progressively and could be attributed to the personal sensory perception of an individual. There is no significant difference between the samples stored at different temperatures for the first 14 days.

Overall acceptability, average of various sensory parameters, which further depends on factors like proteolysis, lipolysis, and flavour changes; also decreased during storage (Fig. 1). Estimated means of sensory scores as computed by statistical analysis of the data indicated that storage period had a significant effect ($p<0.05$) on all the sensory attributes (Fig. 1). The product was sensorially acceptable till 21 days at ambient temperature and up to 91 days of storage study at refrigerated temperature. Similar results by Montes and Trindade (2010) indicated that the storage time significantly affected the overall quality of the products, resulting in a minor loss of acceptance of the product. The study conducted by Pandey and Singh (2011) also showed that the sensory scores of chocolate decreased during storage.

Physico-chemical changes in chocolate during storage

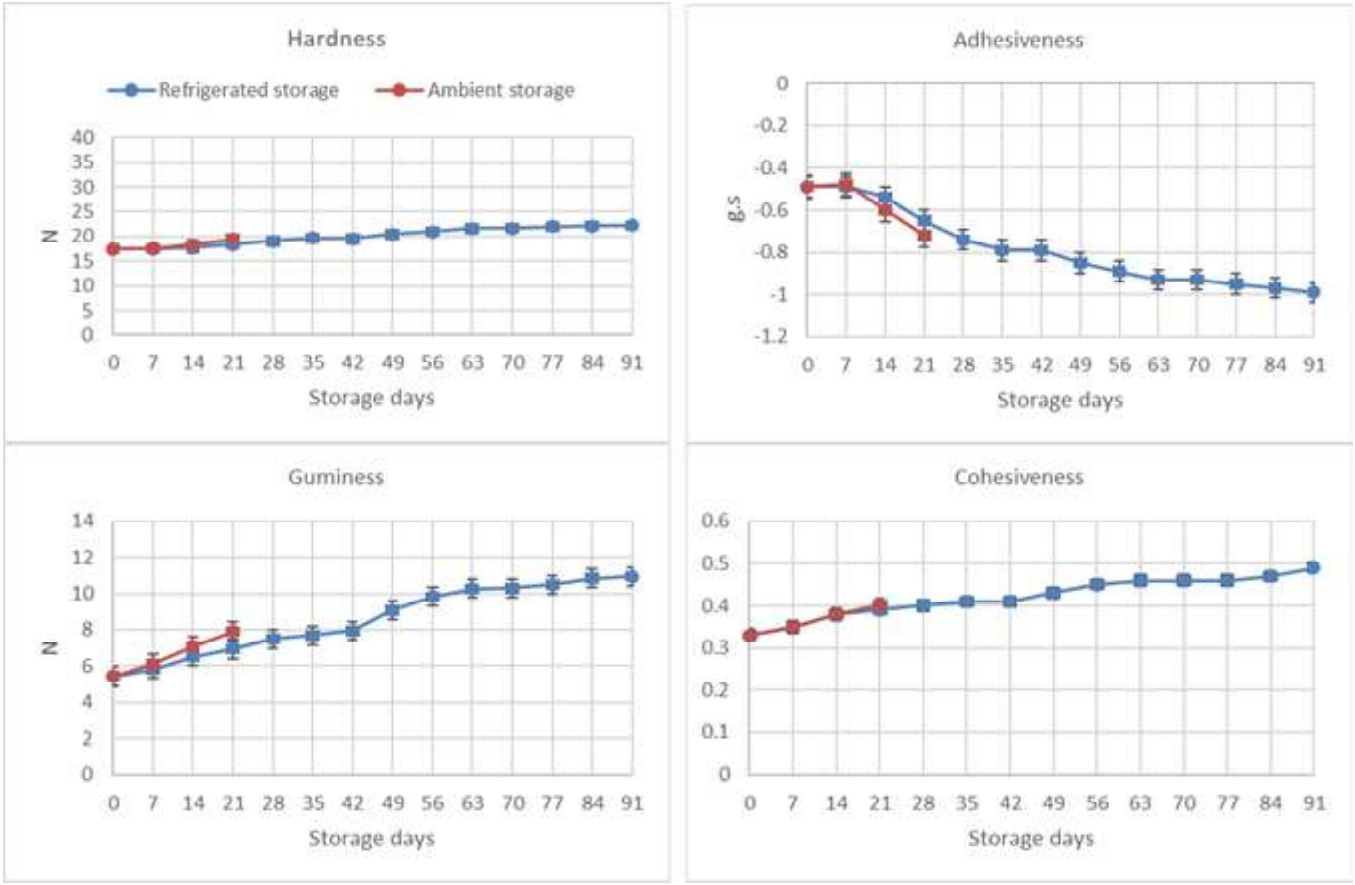

Fig. 3 Effect of storage temperature on instrumental texture properties

The stored samples were analysed for physico-chemical properties and results are shown in Fig. 2. There was significant increase in all the physico-chemical parameters except tyrosine value, which indicated that there is little or negligible proteolysis in the sample during storage at both the storage conditions. During storage, significant ($p<0.05$) increase in a_w of milk chocolate was noticed at the both temperatures and significant difference was found between both the samples stored at different temperatures, immediately after 7 days of storage. At ambient temperature, a_w increased from an initial value of 0.35 to 0.37 whilst, at refrigerated temperature the increase was recorded to 0.58. The increase in a_w at lower temperature was significantly ($p<0.05$) higher compared to samples stored at 25 °C. Similar trend of increase in a_w was found by Rossini et al. (2011) in white chocolate during storage of 10 months.

Peroxide value of milk chocolate increased significantly during storage at both the temperatures (Fig. 2). There was retardation of oxidative rancidity of chocolate due to presence of natural antioxidants of cocoa butter (Becker, 1951). However, in the present study the significant differences were observed only on 21st day for the peroxide value between the samples stored at refrigerated and ambient temperatures. Similar result of increased peroxide value of white chocolate during storage was found by Vercet (2003). The results of peroxide are in congruence with earlier finding by Rossini et al. (2011). Yadav et al. (2011) also found the similar results for peroxide value of chocolate during storage.

The fat hydrolysis is one of the major challenges that deteriorates the quality of chocolates. It could be observed from Fig. 2 that FFA content increased for both the samples with the progression of storage period. The significant increase (pd"0.05) in FFA content was observed during storage within the sample at both the temperatures. The extent of fat hydrolysis was expected to be higher at high temperature of storage, whereas comparatively less rate of growth was observed in samples stored at lower temperature. At zero-day, the FFA value was 1.11 µeq/g which rose to 1.43 µeq/g and 1.54 µeq/g after storage of 21 and 91 days, respectively. Increase in FFA content during storage of butter paper packaged chocolate was observed by Ali et al. (2001) and Yadav et al. (2009). The increase in FFA might be due to the proliferation of yeast and molds as storage progress.

The extent of lipid oxidation in terms of TBA value showed a significant increase throughout storage time. There was a significant difference found in the samples stored at different temperature after 14 days of storage period. The results of TBA

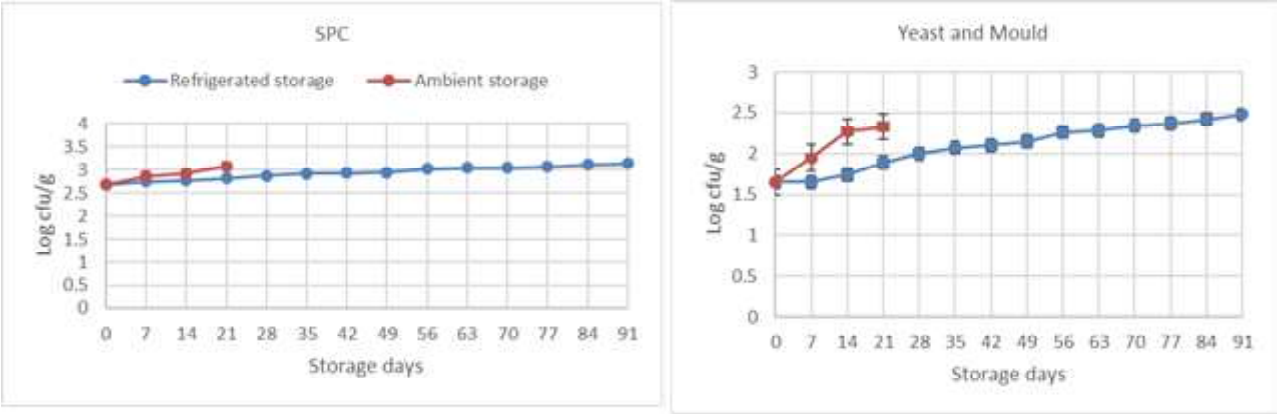

Fig. 4 Effect of storage temperature on microbiological profile

values are in agreement with Rossini et al. (2011) and Sawale et al. (2017) who worked on storage of white chocolate and chocolate vanilla dairy drink, respectively.

Formation of Hydroxymethyl furfural compound is linked directly with heat intensity applied during preparation of foods. This compound is not naturally present in fresh foods, and is considered as an index of deterioration of carbohydrate rich foods during storage. A significant ($p<0.05$) increase in HMF content of all samples was recorded during storage at both the temperatures (Fig. 2). The HMF value increased from an initial content of 3.37 µmoles/litre to 9.74 and 14.55 µmoles/litre after 21 and 90 days of storage at ambient and refrigerated temperatures, respectively. Similarly increase in HMF values for white chocolate during storage has been reported (Vercet, 2003). The results of HMF were well collaborated with earlier work by Sawale et al. (2017) as well, in stored sample of chocolate vanilla flavoured Drink.

Instrumental texture properties

The changes in various textural attributes (Hardness, Adhesiveness, Cohesiveness, and Gumminess) of milk chocolate are presented in Fig. 3 for refrigerated and ambient temperature. The texture of the product deteriorated rapidly at ambient temperature compared to refrigerated temperature during storage. A non-significant difference ($p>0.05$) was observed for adhesiveness and cohesiveness between samples stored at both temperatures.

Hardness may be defined as the maximum height of the curve during the first compression. The hardness of milk chocolate increased significantly ($p<0.05$) during storage at ambient (from 17.44 to 19.46) in 21 days and for refrigerated (from 17.44 to 22.25 N) temperature in 91 days and after 7 days significant difference for hardness value between the sample stored at different conditioned have been observed. The results correspond with the results of Afoakwa et al. (2009) for dark chocolate, Pandey and Singh (2011) for reduced sugar soy-containing compound chocolate. Whereas adhesion is measured as the negative work between the two cycles and is defined as the energy required for overcoming attractive forces between the food and any surface it is in contact with. The adhesiveness of the samples decreased significantly ($p<0.05$) with the extension of the storage period at ambient and refrigerated temperatures (Fig. 3). The decrease in adhesiveness was sharper at ambient temperature compared to refrigerated temperature and could be linked to more increase in free moisture content at ambient temperature.

Cohesiveness may be defined as how well the product withstands a second deformation relative to its behavior under the first deformation. It is measured as the work area during the second compression divided by the work area during the first compression. The increase in cohesiveness was significant ($p>0.05$) during storage at both ambient and refrigerated temperature conditions. Gumminess, a secondary attribute can be obtained by multiplying hardness with cohesiveness, showed a significantly ($p<0.05$) increasing trend at ambient and refrigerated temperature conditions. This might be due to a decrease in total solids due to gain of moisture. Gumminess was increased from an initial value of 5.44 N to 7.91 and 10.94 N after 21 and 91 days of storage at ambient and refrigerated temperatures, respectively. There was a significant ($p<0.05$) increase in gumminess values for both the sample. The increase in gumminess values for milk chocolate at both temperatures during storage may be attributed to increased hardness values. There is no significant difference found for gumminess value between the sample store at 5 ºC and 25 ºC during the first 7 days. However, thereafter significant difference was observed between these samples. The results of rheological properties (Hardness, cohesiveness, and gumminess) are in agreement with the findings of Andrae et al. (2009) for milk chocolate.

Microbiological profile

As expected in any food product, the standard plate count of iron-fortified milk chocolate increased during storage at both temperature conditions (Fig. 4). In fresh milk chocolate, the SPC

was 2.85 log10 cfu/g and might be due to post-processing contamination, handling of the products (utensils, equipment, air, etc.). The values of SPC at ambient for 21 days and refrigerated temperature for 91 days were in the range from 2.85 to 3.13 and 2.85 to 3.33 log10 cfu/g, respectively. The increased rate at ambient temperature could be attributed to a high growth rate at a higher temperature, whereas, at low temperature, psychrophilic microorganisms might have predominated for their growth and multiplication. There was a significant change in the SPC count during the storage study, and after 21 days, a significant difference was found between the two samples stored at different storage conditions.

The yeast and mould count of fresh milk chocolate was 1.64 log10 cfu/g. With the progress of the storage period, yeast and mould count increased; however, this increase was not statistically significant ($p>0.05$). The count increased from 1.6 to 2.38 and 1.64 to 2.42 log10 cfu/g in 21 days storage at ambient temperature and 91 days storage at refrigerated temperature. The slight increase in count could be due to improper packaging handling and direct aerial contamination. The increase in acidity and water activity during storage led to a favourable environment for the growth and multiplication of yeast & mould. The reason behind this could be temperature abuse and post contamination of the product during storage. It should be noted that the product was not served to panelists after the stipulated time due to the surface drying effect.

Conclusion

It can be concluded from this study that the storage temperature and period had a significant effect on the sensory, physico-chemical, instrumental textural, and microbiological properties of iron-fortified milk chocolate. The changes in these properties were rapid at ambient temperature. Sensory scores declined significantly while water activity increased with the progression of the storage period. A significant increase in biochemical parameters viz., HMF, TBA, FFA, and peroxide value and microbiologically – YMC and SPC values decided the product's ultimate shelf life. The product was sensorically unacceptable after 21 days at ambient storage; however, it was acceptable beyond 91 days at refrigerated temperature.

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RESEARCH ARTICLE

Whey removal characteristics during conventional production of *chhana*

Ammu VK, AD Vairat, PS Minz, AK Singh, Chitranayak, Amit Kumar Juneja and Dharinkumar Jayswal

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Abstract: Whey removal phenomena during *chhana* production process was studied in terms of whey removal rate, *chhana* weight and moisture content. Among the above factors, moisture content of *chhana* may be considered to be the most important one to maintain its soft texture and suitability for manufacturing of diversified products. The present study was undertaken to identify best mathematical model to express whey draining process so as to predict the relevant characteristics such as moisture ratio, moisture content and moisture removal rate during *chhana* production. Whey removal rate was recorded for different cow milk quantities (5, 10, 15 and 20 kg) using delayed straining technique for gravimetric removal of whey from the *chhana* mass. Among the mathematical models, Logarithmic model was found best in describing the whey draining characteristics and predicting the moisture ratio of *chhana*. Whey removal characteristics provides insights helpful for the development of equipments for *chhana* production, downstream whey processing and online sensors for monitoring the process that regulates the moisture content and final quality of *chhana* and *chhana* based products.

Keywords: *Chhana*, Moisture content, Mathematical models, Whey removal

Dairy Engineering Division, ICAR - National Dairy Research Institute, Karnal, Haryana, India

Ammu VK (✉)
Dairy Engineering Division,
ICAR-National Dairy Research Institute
Karnal, 132001, Haryana, India
E-mail-ammuvkishor@gmail.com, Mobile: 9741465047

Introduction

Chhana is a heat acid coagulated milk product and is Indian counter part of soft cottage cheese. It serves as the intermediate base product for a variety of milk products like rasagolla, sandesh, *paneer*, chamcham, *chhana podo*, *chhana kheer*, *kheer mohan* etc (Sahu, 2010). *Chhana* is the curd mass obtained from cow, buffalo or mixed milk by heat acid coagulation using sour milk, lactic acid or citric acid. According to the standards, fat content shall not be less than 50.0 per cent on dry matter basis and the moisture content in *chhana* shall not be more than 65.0 per cent (FSSAI, 2020). Organic acids are commonly used as coagulants, such as dilute lactic and citric acid. Calcium lactate is also suitable for production of cow milk *chhana*. In conventional method, lemon juice, sour whey and citric acid are widely used. The type of coagulant used for milk coagulation and its concentration plays a major role in *chhana* quality, as it regulates the moisture content of *chhana* (Kumar et al. 2015). *Chhana* comprises of fat, proteins and minerals such as calcium and phosphorus. High nutritional content of *chhana* is due to the presence of whey proteins. The average composition of cow milk *chhana* is 53.4 % moisture, 17.4 % protein, 24.8 % fat, 2.2 % lactose and 2.1 % ash whereas for buffalo milk *chhana,* it is 51.7 % moisture, 14.4 % protein, 29.7 % fat, 2.3 % lactose and 1.9 % ash. Whey proteins in *chhana* are known source of essential amino acids (Aneja et al. 2002). A number of *chhana* based products have been formulated like functional *chhana* murki (Gurditta et al. 2015), low fat *chhana* spread (Kumar et al. 2016), low calorie fiber enriched *chhana* balls (Singh et al. 2019), *chhana* podo (Franklin et al. 2019) and rasogolla (Kaur and Goswami, 2020).

Number of efforts have been made for process mechanization of *paneer* for small capacity applications (Halder et al. 2012; Chitranayak et al. 2017). In similar lines, systematic efforts have been made to mechanize the *chhana* production process (Ammu et al. 2020). But before developing any equipment for *chhana*, it is essential to study the traditional production process. There is a need to develop mathematical models to study the whey removal characteristics for better understanding of the draining process.

Technologists foreseeing the potential of *chhana* and its products have extensively studied characterization of quality attributes

and effect of coagulants on texture of *chhana*. Prevalent method for production is by hanging coagulum in muslin cloth. It requires time as the whey removal is by gravity. The delayed straining method results in *chhana* having a soft body, smooth texture, and desirable for manufacturing rasagolla. The product retains a high amount of moisture and has a good milk solids recovery along with lower hardness value in *chhana*. Variations in *chhana* manufacturing procedures is mostly related to use of different methods of controlling syneresis. It helps to obtain the desired properties in *chhana.* Syneresis is considered one of the most important process step. Two basic methods commercially used for *chhana* production are immediate and delayed method of whey removal.

Chhana production always aim at producing a product of defined moisture content, soft texture and suitable for manufacturing sweets. The moisture content of *chhana*, a heat acid coagulated product is a critical factor in determining the quality and texture of diversified products made from it. Draining characteristics of whey from *chhana* plays a pivotal role in this aspect. No work has been done to model the draining process of *chhana*. Characterizing the crucial whey drainage step during manufacture of *chhana* will help in building a descriptive mathematical model that can describe the process and predict the whey drainage kinetics. The objective of proposed study was to understand the whey removal behavior and pattern during the traditional *chhana* production process. The conventional mathematical models used for moisture removal during drying of food products were applied to determine whey draining characteristics during the *chhana* production process. The novelty of the work lay on the incorporation of mathematical modeling to the whey removal during *chhana* production process. The research work was undertaken to identify best mathematical model to express whey draining process so as to predict the relevant characteristics such as moisture ratio, moisture content and moisture removal rate.

Materials and Methods

Materials

Cow milk was obtained from Experimental Dairy, ICAR-National Dairy Research Institute, Karnal, India. Glasswares, petriplates and citric acid used during this study were procured from HiMedia Laboratories.

Preparation of *chhana*

Chhana was prepared from cow milk (4% fat, 8.5% SNF). Milk was heated up to boiling and allowed to cool to 80°C. The fundamental theory is that whey protein after heat treatment becomes susceptible to acid coagulation. For production of soft cheese like *ricotta cheese*, *paneer* and *chhana,* technology of heat acid coagulation is used. The critical step for production of good quality *chhana,* is to cool down the milk to 80°C. The temperature is maintained and acid is gradually added to milk with gentle stirring. A coagulating solution of 1% citric acid heated to same temperature of milk i.e. 80°C was used to coagulate the milk. Coagulation results in destabilization of casein micelles. Large aggregates of casein micelles are formed in which fat, denatured whey, colloidal and soluble solids are trapped along with whey. The agitation helps in mixing and uniform distribution of acid in milk. As a result, caseins and whey proteins coagulate together and form aggregates (Will, 2011).

Researches carried out have also revealed that the *chhana* prepared from 1% citric acid recorded the highest yield (Bankar et al. 2016) and hence 1% citric acid solution was used. The coagulum mass was filtered using a muslin cloth. In the entire process, *chhana* mass was gently handled without applying any pressure over it. Application of stress or pressure on the *chhana* mass results in compaction and development of *paneer* like hard texture. Whey was collected in a separate vessel during the solid-liquid separation process. *Chhana* mass held in the muslin cloth was washed by dipping in potable water. The *chhana* mass was hung to expel free whey by gravity. Delayed method of draining whey by hanging in muslin cloth was used in preparation of *chhana* conventionally. To study the effect of prolonged draining, *chhana* mass was subjected to whey removal by gravity for a period of 135 min.

Experimental setup

A small experimental setup was developed for hanging of *chhana* in muslin cloth using a digital spring balance. A cylindrical vessel was kept below the *chhana* mass to collect the whey draining out due to gravity. The whey collector was placed on a digital weighing balance. Thus by using two digital balances it was possible to record weight of *chhana* and whey simultaneously on real time basis.

Mathematical modeling to predict moisture in *chhana*

For process mechanization of conventional *chhana* production process, it is necessary to have knowledge of unit operations involved in the production of *chhana*, and whey removal kinetics. One of the most critical steps is whey removal from *chhana* mass by gravimetric separation. Studies have been carried out in various food materials to determine best model that will describe moisture removal kinetics during drying operations. For better understanding of whey removal phenomena, need was felt to explore analogous food systems and evaluate conventional mathematical models which are already being used to moisture removal unit operations like drying.

A number of mathematical models based on semi-theoretical and analytical frameworks are being used to for the drying process. Several researches have been applied for the mathematical modeling to characterize the drying process (Issis et al. 2019;

Fernandes et al. 2019; Compaoré et al. 2019; Zura-Bravo et al. 2019). Lumped parameter type is the most commonly used model for thin layer drying which consists of Newton equation (exponential) equation and the Page equation (Kingsly et al. 2007).

The time dependent dynamics in whey draining during conventional production of *chhana* was studied for 5, 10, 15 and 20 kg of milk with each trial in replicates of three and responses such as quantity of whey, weight of *chhana*, and whey draining rate were noted. The whey drainage data obtained was recorded as function of time. Moisture content in *chhana* was determined by AOAC (2000). The moisture ratio curves are better in explaining moisture removal behavior than moisture content curves. Hence, apart from studying the whey draining characteristics of *chhana* from moisture content curves, moisture ratio was also plotted. Moisture ratio data were fitted into Page's, logarithmic, exponential models and regression technique was used to select the best model for describing the process of whey removal. Three mathematical models (Page, Logarithmic and Newton models) were considered to predict moisture ratio of *chhana*:

(i) Page model (Diamante and Munro, 1993)

$$MR = \frac{M - Me}{M_0 - Me} = e^{(-Kt^N)} \quad (1)$$

Where, t = Drying time (min), k & n = constants of page's equation.

$$-\ln MR = \ln k + n(\ln t) \quad (2)$$

(ii) Logarithmic model (Yaldýz and Ertekin, 2001)

$$MR = \frac{M - Me}{M_0 - Me} = a + b \times \ln(t) \quad (3)$$

Where, a & b are constants of logarithmic model

(iii) Newton model (Ayensu, 1997)

$$MR = \frac{M - Me}{M_0 - Me} = e^{(-kt)} \quad (4)$$

Where k, A-constants of exponential model

The linearization was as follows,

$$(-\ln MR) = kt \quad (5)$$

The mathematical modeling was done by linearizing the moisture ratio equations of models and experimental data obtained were fitted into models. The mathematical modeling was done using Microsoft Excel.

Determination of moisture ratio

Initial moisture content was determined using AOAC (2000) method. At every fifteen minutes interval i.e. at time t, moisture content during draining process was calculated using the following equation:

$$M_t = M_i - (w_t/w_d) \quad (6)$$

Where,

M_t = moisture content at any time of draining (kg water/kg dry matter)

Mi = initial moisture content (kg water/kg dry matter)

w_t = loss of weight at time t (g)

w_d = dry matter weight (g)

The moisture ratio during draining was calculated from moisture content values using the following equation.

$$MR = (Mt - Me) / (Mi - Me) \quad (7)$$

Where,

MR = moisture ratio (dimensionless)

Mt = moisture content at any time of drying (kg water/kg drymatter)

Mi = initial moisture content (kg water/kg dry matter)

Me = equilibrium moisture content (kg water/kg dry matter)

Determination of whey draining rate

The draining rate was determined using the following equation

$$DR = (Mt' - Mt) / dt \quad (8)$$

Where,

DR = whey draining rate (kg/min)

Mt = moisture content at any time of draining (kg water/kg dry matter)

Mt' = moisture content after t minutes

t = time (min)

Statistical analysis

Moisture ratio data of *chhana* at different time intervals were fitted to various mathematical models. Three widely used empirical models Page, exponential and logarithmic model were taken into consideration in the present study. Selection of the best fit model was made on the basis of model having highest R square, lowest Chi and SSE square value. The model with high coefficient of determination, low value of chi square, and root mean square error indicates goodness of fit (Goyal et al. 2006). Values of chi square and root mean square error (RMSE) were calculated as follows:

$$\chi^2 = \frac{\sum_{i=1}^{N} (MR_{exp,i} - MR_{pre,i})^2}{N - z} \quad (9)$$

$$RMSE = \left[\frac{1}{N} \sum_{i=1}^{N} \left(MR_{exp,i} - MR_{pre,i}\right)^2 \right]^{\frac{1}{2}} \quad (10)$$

Where,

$MR_{exp,i}$ = Experimental moisture ratio

$MR_{pre,i}$ = Predicted moisture ratio

N = No. of observations

z = No. of constants in model.

Results and Discussion

Weight of *chhana*

Higher yield of *chhana* is always desirable due to higher economic returns. It also implies either higher moisture content, more whey retention or lower solid losses in whey. The real time measurement of *chhana* mass was recorded during simultaneous whey removal due to gravimetric separation. In the present study, the progressive decrease in the *chhana* weight could be quantified. The weight of chhana decreased with whey draining time during the first 15 min and thereafter remained nearly constant during the entire trial period. It can be attributed to the fact that the majority of whey draining occurred during the first 15 min. The measured *chhana* weight also included the loss of moisture due to evaporation during 135 minutes hanging of *chhana* mass in the open air. The results of yield of *chhana* are in agreement with the findings in which *chhana* yield was in the range of 15.8 - 17.75 % (Kumar et al. 2015). In a similar study, Choudhury et al. (1998) used whey protein denaturation as an indicator for heat treatment. And increase in whey protein denaturation from 0.485 to 6.642 resulted in recovery of milk solids from 0.512 to 0.649 kg/kg and 0.535 to 0.649 kg/kg for cow's and buffalo's milk, respectively. The method of delayed straining greatly helps to improve the soft and smooth texture of *chhana*. It results in higher retention of moisture and better recovery of milk solids.

Moisture content

Whey removal phenomenon was studied in terms of whey removal rate, *chhana* weight and moisture content during *chhana* production process. Of these, moisture content is considered to be the important one to maintain its soft texture and suitability for manufacturing of diversified products. Moisture content decreased with time for draining. The average moisture content decrease of *chhana* for trials conducted for 5 to 20 kg milk was from an initial moisture content value of 58.8 % to 57.96 % wb after 135 min (Fig. 1 a). Pandey et al. (2004) prepared *chhana* from cow milk using mixture of citric acid, lactic acid, papaya and ginger extract. The average yield and moisture content of *chhana* was in the range of 18.98-21.12% and 52.47-59.63%, respectively. Other literatures showed that the average moisture content of *chhana* is in the range of 54-57%. Moisture content is significantly affected (p<0.05) by the dilution medium, type of coagulant and coagulant concentration as well as by their interactive effects (Bandyopadhyay et al. 2005).

Whey draining rate

A steep increase in whey drainage rate was observed during the first 15 min of whey draining and followed by a gradual decrease in whey draining rate with increasing time. It showed that majority of whey draining occurs during the first 15 min (Fig. 1 b). Whey separation is essentially a filtration process which drains the liquid and retains the curd. A constant rate of whey drainage was observed with increase in time when the curd was not pre-strained. Whey contains about 50% of the nutrients present in milk. Whey is rich in lactose, proteins, minerals and vitamins (De Witt, 2001).

The maximum whey draining rate observed was 0.321 kg/min with an average moisture content of 58.3 % wb during the first 15 minutes of whey draining. An exponential decrease in the whey draining rate was observed after the initial peak of first 15 minutes. During heating, the conformational alteration of whey proteins allows the protein structure to unfold. Heat induced denaturation of whey proteins immobilizes more water inside the structure. Due to the better water holding capacity of denatured whey proteins, retention of moisture in the product is higher. It is therefore necessary to quantify whey draining rate. Such

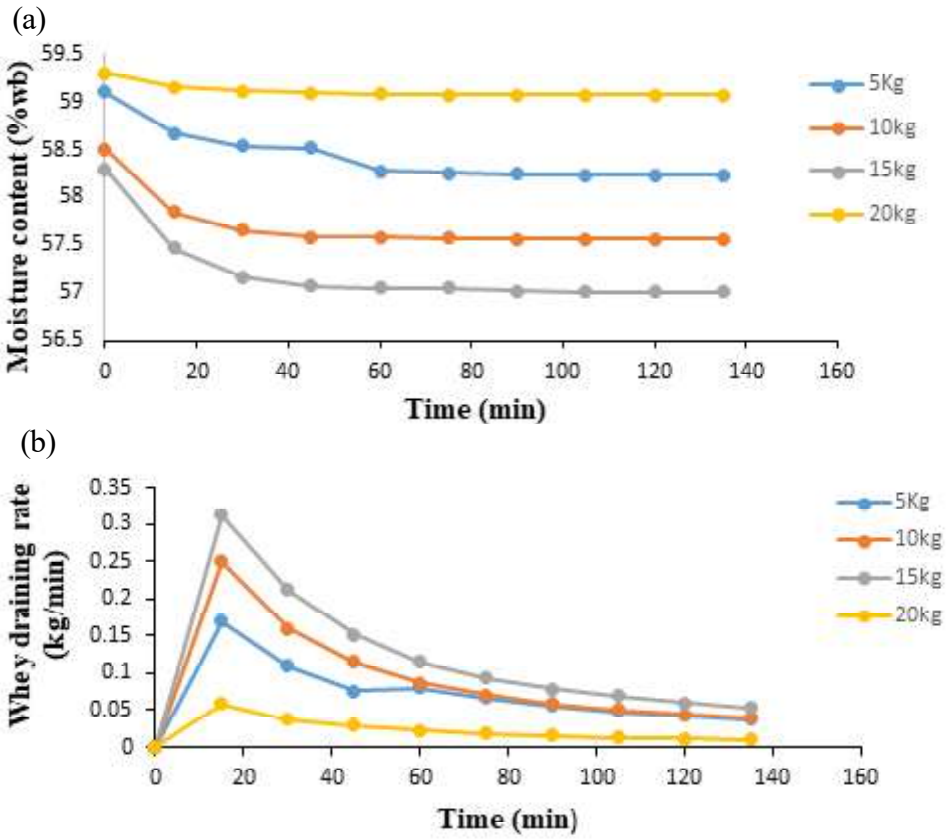

Fig. 1 Whey syneresis parameter (a) Variation of moisture content (% wb) of *chhana* with time(b) Whey draining rate (kg/min) with time

Table 1 Statistical parameters for mathematical models to predict moisture ratio of *chhana*

Models	Quantity of milk (kg)	R^2	Chi square	SSE	RMSE
Page	5	0.9059	0.0269	0.0215	0.1466
	10	0.6678	0.0316	0.0252	0.1589
	15	0.8014	0.0651	0.0521	0.2282
	20	0.8824	0.0033	0.0026	0.0511
Logarithmic	5	0.8985	0.0000*	0.0000*	0.0019
	10	0.6893	0.0000*	0.0000*	0.0018
	15	0.8353	0.0000*	0.0000*	0.0021
	20	0.9064	0.0000*	0.0000*	0.0003
Newton	5	0.7604	0.0004	0.0003	0.0184
	10	0.5229	0.0010	0.0009	0.0294
	15	0.5859	0.0016	0.0014	0.0378
	20	0.6743	0.0000*	0.0000*	0.0067

* value < 0.0001

information will help in design of equipment for mechanized production of *chhana* and whey downstream processing.

Mathematical modeling

Mathematical modeling of whey draining process of *chhana* aims at selecting an efficient as well as best model that can design the process of whey removal so as to predict the relevant draining characteristics such as moisture ratio, moisture content, drying rate etc. Whey removal phenomena during *chhana* production was studied using mathematical modeling and conventional whey draining characteristics of *chhana* were determined. The observations of whey draining characteristics during *chhana* production revealed that whey draining rate decreased with draining time as well as decrease in moisture content. It was also noted that moisture content in *chhana* decreased exponentially with draining time. Mathematical modelling helped in describing the time dependent dynamics of whey removal for designing an efficient whey draining system. It helped in predicting moisture content of *chhana* and thereby helping to design process for rapid removal of whey from *chhana*. The experimental data for whey draining characteristics of *chhana* had showed that logarithmic model had satisfied the requirements of high R square and low chi square and SSE and hence logarithmic model can be

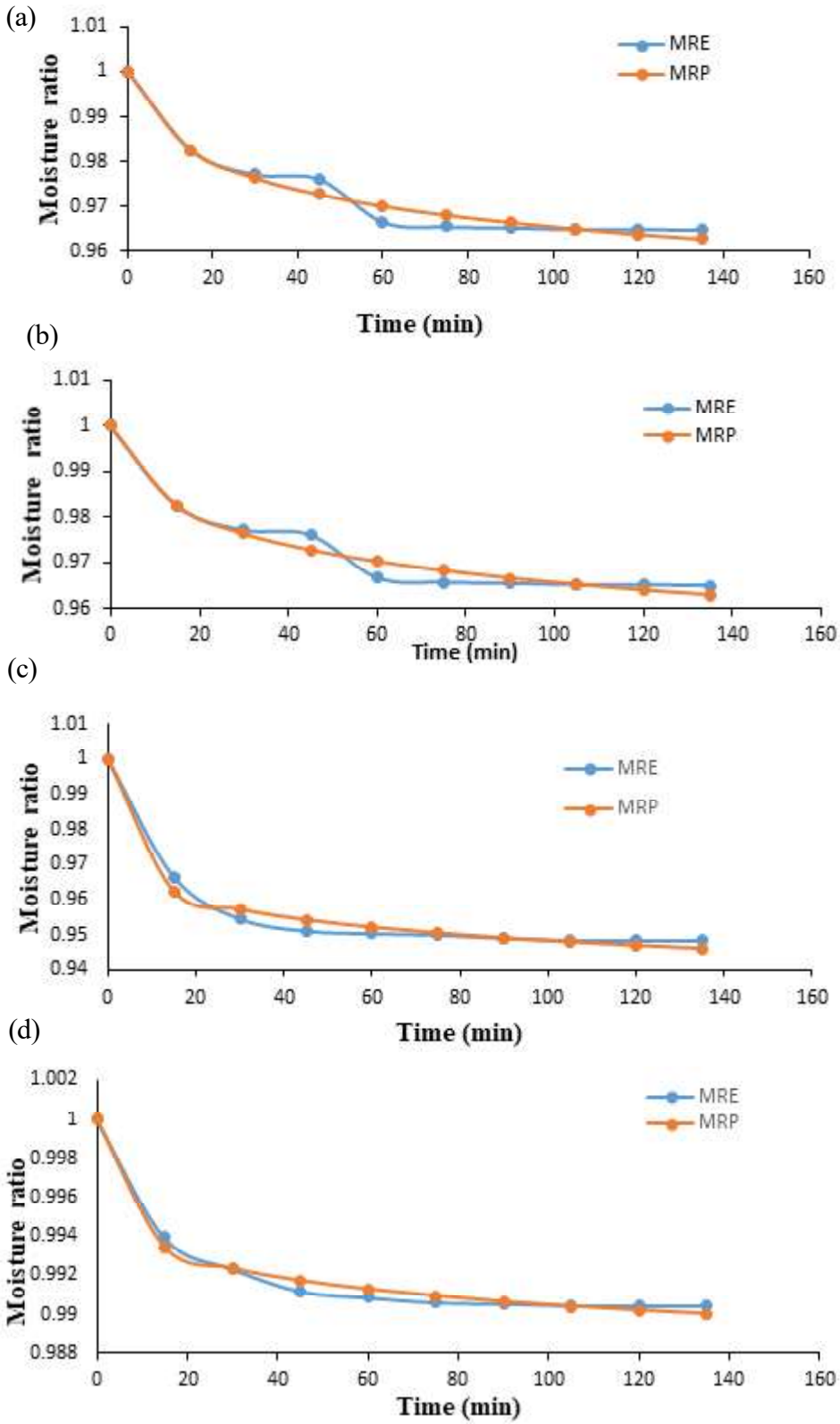

Fig. 2 Moisture ratio v/s time for experimented (MRE) and predicted values (MRP) in logarithmic model a) Milk quantity 5 kg (b) Milk quantity 10 kg (c)Milk quantity 15 kg (d) Milk quantity 20 kg

considered as the best model in describing draining characteristics of *chhana* (Table 1). The logarithmic model serves as the suitable model in predicting the moisture content of *chhana* at any time during draining of whey. The comparison between expected and predicted values of moisture ratio using logarithmic model clearly depicts that the values obtained experimentally were in reasonable agreement with the values obtained by prediction (Fig. 2). Drainage is a dynamic process that results in whey

removal and gel hardening. It is a critical stage in the production process with a major influence on the quality of chhana.

Conclusions

Whey removal phenomena during *chhana* production was studied as a function of time. Analysis of the current study data revealed that the whey draining rate decreased exponentially with draining time. Mathematical modeling helped in predicting draining characteristics such as moisture ratio, which gives better understanding of the moisture removal from *chhana*. Compared to Page and Newton model, Logarithmic model was found best in estimating moisture ratio of *chhana.* The results from the study will be useful in designing equipment for rapid draining of whey as traditional process of *chhana* production is time consuming.

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Conflict of interest

All authors have no conflict of interest to report.

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RESEARCH ARTICLE

Characterization of Ladakhi *churpe* enriched with apricot and spinach

Anwar Hussain[1], Sheikh Rafeh Ahmad[2] and Maheshwar Singh Kanwar[3]

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Abstract: The aim of this study was to produce novel *churpe*-products with apricot and spinach supplementation. These products being rich in antioxidants along with good source of minerals, vitamins and other essential nutrients could have enormous potentials to guard against many human diseases. To the tribal people of Ladakh, these could overcome the deficiency of micronutrients caused due the shortage of fruits and vegetables during the winter season. The nomads of Changthang region of Ladakh could carry these products easily as nutritional supplements during their year round migration. Dried dairy products *viz. churpe*-balls and *churpe*-strips were developed with the incorporation of apricot powder into cottage cheese at different levels (05, 10, 15 and 20%) and spinach powder at levels (03, 06, 09 and 12%), respectively. Physico-chemical, antioxidant, colour, sensory and microbial attributes of the products were studied. The addition of apricot powder in balls resulted in significant ($P < 0.05$) increase in ash, hydrosoluble vitamins (thiamine, riboflavin and ascorbic acid), titratable acidity and antioxidant activity and decrease in protein, fat, minerals (calcium, magnesium and sodium) and pH. Incorporation of spinach powder in strips resulted in increase ($P < 0.05$) in ash, calcium, magnesium, hydrosoluble vitamins (thiamine, riboflavin and ascorbic acid), titratable acidity and antioxidant activity and decrease in protein, fat, sodium and pH. A non significant increase in moisture and lactose contents of both the value added products was also observed. In color analysis, L* and a* increased in both the products with the increase in level of supplementation (05 to 20% in case of balls and 03 to 12% in case of strips), however, b* decreased in balls and increased in strips. Rise in the supplementation levels resulted in decline in microbial load in the products whereas, with the advancement of storage period up to 120 days, there was rise in microbial population but was found within safe limits. The overall acceptability of the *churpe*-balls was highest at 15% level and that of *churpe*-strips was highest at 09% level of blending.

Keywords: *Churpe*, Apricot, *Churpe*-balls, *Churpe*-strips, Ladakh, Spinach

Introduction

Ethnic foods are expression of culture, history and lifestyle of a particular region. *Churpe*, a traditional dried cottage cheese, is one of the popular traditional fermented dairy products consumed by the people of Ladakh. Ladakh region, a trans-Himalayan tribal part of India, is a cold desert with extreme climatic conditions like fluctuating temperature (-30°C in winter and reaches 35°C in summer), very low moisture content in the atmosphere, intensive sunlight, etc. *Churpe* is also very popular among different ethnic groups in the Himalayan region of India *viz*. Darjeeling, Lahaul-Spiti, Sikkim and Arunachal Pradesh and also in Nepal, Bhutan and Tibet (China). In Ladakh, *churpe* is generally prepared during summer when the milk production is surplus, for consumption during harsh winter season when the region got cut off from outside world due to heavy snowfall. The purpose of drying cheese i.e *churpe* is mainly to increase the storability which is not possible in the fresh products. Local people consume it by cooking with *thukpa*, a thick soup and also moistened it in mouth and masticate to get its health benefits as well as to enjoy its characteristic flavor. Its flour is mixed with *kholaq* and *chasrul* and is also an important ingredient of *thut*, a sweet. Therefore, *churpe* is not only a food product but also an integral part of Ladakhis. Like other dairy products, *churpe* is also considered as a nutrient capsule containing quality proteins, vitamins and minerals. The probiotic properties of indigenous microorganisms isolated from the *churpe* have been reported (Tamang et al. 2000). Probiotic milk products can have health-promoting benefits such as modulation of the immune system, maintenance of gut flora,

[1]Krishi Vigyan Kendra, Nyoma (Leh-II)-SKUAST-K, Nyoma-194404, Ladakh, India

[2]Division of Livestock Products Technology, FVSc & AH, SKUAST-K, 190006, J & K, India

[3]High Mountain Arid Agriculture Research Institute, SKUAST-K, Leh-194101, Ladakh, India

Anwar Hussain (✉)
Krishi Vigyan Kendra, Nyoma (Leh-II)-SKUAST-K, Nyoma-194404, Ladakh, India
E-mail: anwarfoodtech101@gmail.com

regulation of bowel habits, alleviation of constipation, and curing of gastrointestinal infections (Tamang, 2010). Yeast, mold, LAB, and Bifidobacterium sp. not play an important role in *churpe* preparation but their synergistic actions convert the milk sugar into beneficial compounds, such as vitamins, lactic acid, etc. (Panda et al. 2016). However, it lacks polyphenols like fruits and vegetables which play important role as antioxidants in the human body.

Fruits and vegetables are good sources of nutrients as well as health promoting agents. Apricot is a carbohydrate-rich stone fruit and is a good source of fibers, minerals and vitamins (Hussain et al. 2012; Hussain et al. 2013). The major minerals are potassium, calcium, phosphorus, magnesium, iron and selenium (Ali et al. 2011) and vitamins are Vitamin A, C, K, E, B_1, B_2, B_3, B_6, and B_9 (Fatima et al. 2018). Soluble fiber lowers blood cholesterol, maintains blood sugar level, prevents constipation and helps in reducing body weight. It is also rich in bioactive phyto-chemicals that have certain roles in the biological system and effective in preventing oxidative stresses (Leccese et al. 2007). The polyphenols and carotenoids because of antioxidant properties have possible ability to alleviate chronic diseases (Gardner et al. 2000). Spinach is an extremely nutritious vegetable, rich both in core nutrients and phyto-chemicals. The major micronutrients in spinach are Vitamin A (from β-carotene), C, K and folate (Guha and Das, 2008) and the minerals, calcium, iron and potassium (Anonymous, 2004). It also provides fiber and is low in calories. Apart from having nutritional value, it has been also credited with various biological activities. Spinach is known for antimicrobial, anti-carcinogenic and antioxidant activity (Vazquez et al. 2013). Anti-aging properties associated with spinach leaves with considerable amount of β-carotene (2-6 mg/100g), folic acid (120mg/100g) and riboflavin (0.25 mg/100g) make this leaf a unique food material (Anon, 2011). Apart from the benefits, spinach powder may be used as replacer of artificial food colour as demand for natural pigments is increasing in present era.

It has been reported that fortification of food products using natural resources like fruits, vegetables, herbal extracts, cereals, nuts, seeds, etc. is necessary to improve nutrient intake (Granato et al. 2017). To the best of our knowledge, addition of apricot and spinach powders to dried cheese, *churpe* (Fig. 1a) has not been studied yet. Therefore, the aim of this study was to produce novel *churpe* products with apricot and spinach supplementation. The effect of additives on the physico-chemical properties, sensory attributes, colour properties, bioactivities of products, such as antioxidant activity and microbial load were investigated. Cheese snacks as cheese balls and chips are very popular worldwide (Rakcejeva et al. 2009). The value added products developed were apricot added *churpe*-balls (Fig. 1b) and spinach added *churpe*-strips (Fig. 1c). These products can be used to solve the problem of nutrient deficiency among this tribal population of Ladakh.

Materials and Methods

Materials

The raw buttermilk, a byproduct obtained during the production of butter from cow milk was procured from the herders of Nyoma and Nidder villages of Changthang region, Leh Ladakh. Dried apricots without stone were procured from the local market of Leh. Fresh spinach leaves (moisture content 94.8%) were obtained from Vegetable Farm, Krishi Vigyan Kendra, Nyoma (Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir).

Drying of spinach

The destalked spinach leaves were washed with tap water. Before drying, the leaves were blanched for 15 seconds at 98°C with a spinach-water ratio of 1:4 with slight modification of blanching time as recommended by Sharma et al. (2011). The blanched leaves were then shade dried.

Preparation of products

The method given by Hussain et al. (2022) was followed for the preparation of the value added dairy products. Dried spinach and dried apricot were converted into powder in a food processor (HR-7629, Philips, China). A sieve with 750 µm pore size was used to sieve the powders. The buttermilk was boiled for 10 min at 70 °C and the coagulum so obtained was subjected to cooling. The holding time in whey was about 5 minutes. The solid mass (cottage cheese) was separated from the whey by straining through a cheese cloth. It is then incorporated with the additives, apricot powder for balls and spinach powder for strips. Blends were prepared by replacing the mass with apricot powder at 5%, 10%, 15% and 20% with 10% ground sugar for balls. For strips, the mass was replaced with spinach powder at 3%, 6%, 9% and 12%. The mixture was then kneaded. Balls were made by rolling the mass between the palms. Strips were made by pressing the mass between the palm and fingers. The products so obtained were dried in a solar *churpe* dryer. After drying, the products were stored in cotton bags at an average temperature of 18 °C and relative humidity of 30 %. The *churpe*-strips without any addition were taken as a control in this study. The flow diagram for preparation of *churpe* products is given in Fig. 2

Proximate composition

Analysis of moisture, protein, fat, and ash contents of the samples were performed according to the method described by the Association of Official Analytical Chemists (1990).

Lactose

Titrimetric method as described by Adolf Lutz Institute (2005) using Fehling licor (solution containing cupric ions in alkaline

Fig. 1 (a) *churpe* (b) *churpe*-balls and (c) *churpe*-strips

medium) was applied to measure lactose in the products. A solution of each product was made using 50 g dissolved in 2 ml acetic acid (2% v/v) and distilled water. The mixture was heated for 5 minutes at 80 °C. After this, the samples were transferred to volumetric flask of 200 ml and volume was completed with distilled water. After filtration, the solutions were used to react with 20 ml of standard Fehling licor.

Minerals

The determination of calcium, sodium and magnesium was carried out by flame photometry (direct method) as given by Kravic et al. (2012) using flame photometer (Systronics, India) in air-butane flame. After the homogenization by mixing, 2.5 g of the sample was transferred to a calibrated flask, 2 cm^3 of 10% solution of lanthanum was added and diluted to a final volume of 50 cm^3 with distilled water.

pH

The pH of the products was estimated according to the method of Panda et al. (2016). The pH of the product (10 g) was determined by homogenizing the sample with sterile distilled water (100 mL) in a ratio of 1:10, followed by shaking for 5 minutes. The pH of the fermented substrate was then measured by a glass probe digital pH meter (Eutech, Singapore).

Hydrosoluble vitamins

The protocol given by Ghosh et al. (2015) was followed for the quantification of hydrosoluble vitamins. These vitamins were analyzed by reverse phase-HPLC using an Agilent HPLC system (Agilent Technology) equipped with a Zorbax SB-C18 column and the mobile phase was 0.05M KH_2PO_4 (pH 2.5) and acetonitrile (A). The solvent gradient was as follows: at 0 minutes 0.6% A, at 0.5 minutes 0.6% A, at 4 minutes 6% A, at 12 minutes 0.6% A, at 17 minutes 0.6% A, and the stop time was 20 minutes. The temperature was kept at 15 °C and a constant flow rate of 1 mL/min was maintained. The effluent from the column was monitored by variable wavelength UV detector (204 nm).

Antioxidant activity

The antioxidant activity was determined by DPPH (1,1, diphenyl-2-picryihydrazyl) scavenging activity using DPPH as a free radical as per the method given by Brand-Williams et al.(1995). 100 µl of sample extract solution was added to 1ml of 0.01 percent methanolic solution in a cuvette. The sample was then incubated for 30 minutes at room temperature. The reaction solution was examined at 515 nm using a spectrophotometer. The inhibition percentage of DPPH solution was calculated according to the below equation:

$$\text{Inhibition (\%)} = \frac{(Abs_{t0min} - Abs_{t30min})}{Abs_{t0\ min}} \times 100$$

Where, $Abs_{t0\ min}$ = absorbance of DPPH at initial stage and $Abs_{t30\ min}$ = absorbance of DPPH after 30 minutes of incubation

Titratable acidity

Acidity in dried cheese products was estimated by Titration Method No. 920.124 of AOAC (1990). 1 g of each cheese sample was mixed with warm water and volume was made up to 10 ml in 100 ml conical flask; Sample containing flask was shaken vigorously and filtered. The filtrate was titrated with 0.1 N NaOH using phenolphthalein as indicator. The percent of titratable acidity was calculated as percent (%) of lactic acid (as lactic acid is a major producing acid in cheese) according to the following expression:

$$\text{Titratable acidity (\%)} = \frac{0.0090 \times \text{volume of NaOH used} \times 100}{\text{Weight of sample}}$$

Colour analysis

Colour was measured according to the protocol of Noh et al. (2013), using a Hunter lab (Hunter Lab, Mini Scan XE Plus, and Reston, Virginia, USA). The colour was reported as L* (dark to light), a* (red to green) and b* (yellow to blue) values. Total colour differences (ΔE) were calculated using the equation shown below, where L_0, a_0 and b_0 are the control values for the cheese. In these equations, ΔL is the difference of lightness (L*- L_0); Δa is the difference of redness (a*- a_0); and Δb is the difference of yellowness (b*- b_0).

$$TCD\ (\Delta E) = \text{“}\ (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2$$

Microbial load

For total plate count, spread plate technique, described by Palczar and Chan (1991) was followed. 1 g of sample was aseptically transferred into test tube containing 9 ml of sterile water and was mixed vigorously. After mixing, 1 ml of this mixture was again transferred to a test tube containing 9 ml sterile water for further dilution. The process was continued until 6th diluents (10^{-6}). Potato dextrose agar (PDA) was inoculated with 0.1 ml of the diluted sample (10^{-6}), by spread plating technique and incubated at 37 ÚC for 24 hours. Colonies were counted and multiplied by dilution factor.

$$\text{Microbial load (cfu/g)} = N \times \frac{1}{V} \times D$$

Where:

N = No. of colonies counted
V = Volume of inoculums
D = Dilution factor

Sensory evaluation

The organoleptic attributes of the value added dairy samples were assessed by semi-trained panelists comprising of professionals, non-professionals and consumers using the evaluation criteria described by Demirci et al. (2017). The panel was asked to evaluate the samples using a graduated scale from 1 to 9 (1, Very bad; 2, Bad; 3, Imperfect; 4, Sufficient; 5, Mediocre; 6, Satisfactory; 7, Good; 8, Very good; and 9, Excellent) for appearance, flavor, texture, and overall acceptability. The balls were analyzed as such but the strips were soaked in water prior to analysis.

Statistical analysis

Results of determinations reported in this study constitute a mean from three replications. For the purpose of objectivity of inference, the recorded results were subjected to statistical analysis. For the determination of significance of differences between means, analysis of variance (ANOVA) was conducted using the OP-Stat software (Version 1.0). Dependencies were considered statistically significant at the level of significance $P < 0.05$.

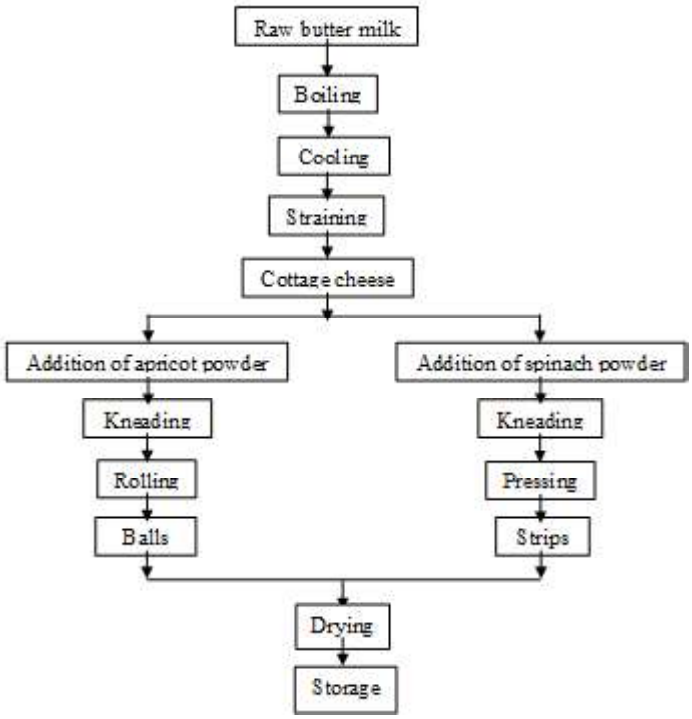

Fig.2 Preparation of *churpe*-products

Results and Discussion

Proximate composition

The proximate composition as given in Table 1, shows that the supplementation of *churpe* with apricot and spinach did not affect the moisture and lactose contents, significantly. However a significant difference ($P < 0.05$) was observed in protein, fat and ash content of the products. The average protein content of control was 36.16 ± 0.14%, whereas that of supplemented products (balls) varied from 32.97 ± 0.10% to 28.73 ± 0.12% at 05 to 20% levels of apricot powder and 36.04 ± 0.06% to 34.09 ± 0.07% (strips) at 03 to 12% levels of spinach powder. The average fat content of control *churpe* was 07.65 ± 0.16% which got decreased from 07.33 ± 0.15% to 04.75 ± 0.19% in balls at 05 to 20% levels of apricot powder and from 07.51 ± 0.39% to 06.33 ± 0.16% in strips at 03 to 12% levels of spinach powder. The average ash content of control was 07.02 ± 0.21%. The addition of apricot powder from 05 to 20 % resulted into increase in ash content of *churpe*-balls from 07.02 ± 0.06% to 09.68 ± 0.04%, whereas the addition level of spinach powder from 03 to 12% resulted into increase in ash of *churpe*-strips from 07.12 ± 0.13% to 09.11 ± 0.09%. These results indicated that, the addition of apricot and spinach powders adversely affected the protein, fat and ash contents of the

supplemented products. The decrease in protein and fat contents of supplemented *churpe* products might be the result of the appreciably lower protein and fat contents of the supplements. The same reason is applicable to the increase in the ash content of the products. Roy et al. (2015) also reported significant ($P < 0.05$) decrease in protein and fat contents of value added yogurts prepared from blends of fresh yogurt and fruit pulp (banana, papaya and water melon) and the same workers also reported significant ($P < 0.05$) increase in ash contents of yogurt-banana pulp. Addition of spinach powder to UF-soft cheese resulted in increase in its ash and decrease in fat contents (El-Sayed, 2020). Mohamed and Shalaby (2016) in cheese analogue made with apricot pulp and Awad et al. (2003) in cheese made with guava, mango and banana pulps, observed decrease in protein content.

Titratable acidity

Titratable acidity in control as well as supplemented *churpe* products are presented in Table 1. Control *churpe* had titratable acidity of 06.27 ± 0.11% and demonstrated an excellent improvement in it with the rise in the levels of supplements. The maximum increase of 10% over the control was found in *churpe*-strips containing 12% spinach powder (06.90 ± 0.07%) while minimum increase of 8% was found in *churpe*-balls containing 20% apricot powder (06.83 ± 0.12%) over the control. The increased titratable acidity of the supplemented *churpe* products seems to be the direct effect of the higher amount titratable acids present in apricot and spinach powders. The addition of apricot to fruit-cheese bar, spinach powder to UF-soft cheese and *Moringa oleifera* leaves powder to soft cheese enhanced acid development (Jabeen et al. 2020; El-Sayed, 2020; Hassan et al. 2017).

pH

It was observed that pH of *churpe*-balls decreased with increase in the level of fortification of apricot powder in fresh cheese (Table 1). Control *churpe* had pH of 04.17 ± 0.23 which got reduced significantly ($P < 0.05$) from 04.33 ± 0.38 to 04.02 ± 0.13 at 05 to 20% levels of apricot powder. This effect may be attributed to higher acid contents in apricot. The acetic acid, citric acid, malic acid and tartaric acid are the main organic acids that are present in apricots (Ghnimi et al. 2018) thus conferring high acid content to the product. Similar results were reported for probiotic yogurt supplemented with passion fruit or pineapple peel powders (Espirito et al. 2012; Sah et al. 2016). The addition of apricot to fruit-cheese energy bar significantly reduced the pH (Jabeen et al. 2020). The pH increased significantly ($P < 0.05$) on increasing the fortification of spinach powder in *churpe*-strips. It was found that, at 12% level of supplementation, there was increase of 18% in pH. This could be contributed to higher pH in spinach. Lucera et al. (2018) also observed increase in pH of fortified spreadable cheese with the enrichment of broccoli and artichoke.

Minerals

Incorporation of apricot and spinach powder resulted into the significant effects ($P < 0.05$) on mineral contents of value added *churpe* products (Table 2). Calcium content decreased from 614.31 ± 0.09 mg/100g to 601.75 ± 0.21 mg/100g; magnesium content decreased from 503.74 ± 0.07 mg/100g to 413.69 ± 0.39 mg/100g; sodium content decreased from 776.64 ± 0.16 mg/100g to 612.22 ± 0.36 mg/100g, when supplementation level of apricot powder increased from 05% to 20% in balls. However, the decreasing rate is less in calcium content as compared to the magnesium and sodium contents of balls. Decrease in sodium was also observed by Mohamed and Shalaby (2016) in apricot-cheese analogue with the increase in the ratio of apricot pulp. Calcium and magnesium contents of strips increased from 623.67 ± 0.16 mg/100g to 854.04 ± 0.30 mg/100g and from 549.53 ± 0.21 mg/100g to 632.55 ± 0.15 mg/100g, respectively, when supplementation level of spinach powder increased from 03 to 12%. However, sodium content in the same product decreased from 872.12 ± 0.24 mg/100g to 816.08 ± 0.11 mg/100g. From the results it is clear that, due to low sodium contents, the products could be healthy for those who are suffering from frequent hypertension. El-Sayed (2020) reported increase in calcium and magnesium contents in UF-soft cheese supplemented with spinach powder and El-Taweel et al. (2017) observed similar results in dried parsley incorporated Kareish cheese.

Hydrosoluble vitamins

Apricot possesses good quantities of thiamine, riboflavin and ascorbic acid. Increasing addition of apricot powder (05 to 20%) has shown enhancement of hydrosoluble vitamins in *churpe*-balls as thiamin increased from 37.02 ± 0.26 µg/g to 37.68 ± 0.04 µg/g, riboflavin from 105.97 ± 0.10 µg/g to 106.73 ± 0.12 µg/g and ascorbic acid from 10.42 ± 0.03 µg/g to 11.73 ± 0.12 µg/g (Table 2). The same trend is also true with the *churpe*-strips. The thiamine content of spinach incorporated strips ranged from 37.00 ± 0.13 µg/g to 37.91 ± 0.09 µg/g, riboflavin ranged from 106.04 ± 0.06 µg/g to 106.95 ± 0.07 µg/g and ascorbic acid ranged from 10.48 ± 0.06 µg/g to 11.71 ± 0.09 µg/g at 03 to 12% levels of spinach powder. The hydrosoluble vitamins of both the value-added products are higher than the control which might be due to higher contents of these vitamins in the additives. Thiamine, riboflavin and ascorbic acid contents of control were 37.02 ± 0.21 µg/g, 106.16 ± 0.14 µg/g and 10.43 ± 0.10 µg/g, respectively. Ascorbic acid increased with the addition of green pea puree in vegetable enriched yogurt (Yildiz and Ozcan, 2018).

Antioxidant activity

Table 2 depicted the effect of supplementation of apricot and spinach powder on antioxidant activity (DPPH radical scavenging activity) of *churpe* products was significant ($P < 0.05$). As mentioned by Sochor et al. (2010) and Fatima et al. (2018) most of

Table 1: Proximate composition, titratable acidity and pH of value-added *churpe* products (balls and strips)

Supplementation level (%)	Parameters						
	Moisture (%)	Protein (%)	Lactose (%)	Fat (%)	Ash (%)	Titratable acidity (%)	pH
Control (Cottage cheese)	07.43 ± 0.10	36.16 ± 0.14	42.29 ± 0.29	07.65 ± 0.16	07.02 ± 0.21	06.27 ± 0.11	04.17 ± 0.23
CC:AP:GS							
90:00:10	07.31 ± 0.16	34.02 ± 0.19	42.06 ± 0.16	07.47 ± 0.11	07.02 ± 0.10	05.05 ± 0.17	04.26 ± 0.26
85:05:10	07.42 ± 0.03	32.97 ± 0.10	40.64 ± 0.11	07.33 ± 0.15	07.02 ± 0.06	05.82 ± 0.15	04.33 ± 0.38
80:10:10	07.43 ± 0.12	31.73 ± 0.15	39.76 ± 0.08	06.42 ± 0.06	07.57 ± 0.08	06.41 ± 0.08	04.18 ± 0.05
75:15:10	07.48 ± 0.26	29.56 ± 0.06	38.04 ± 0.14	05.12 ± 0.21	08.12 ± 0.17	06.67 ± 0.26	04.12 ± 0.07
70:20:10	07.46 ± 0.17	28.73 ± 0.12	36.45 ± 0.25	04.75 ± 0.19	09.68 ± 0.04	06.83 ± 0.12	04.02 ± 0.13
Mean	07.42 ± 0.14	31.40 ± 0.12	39.43 ± 0.14	06.21 ± 0.14	07.88 ± 0.09	06.15 ± 0.15	04.18 ± 0.17
CD (P < 0.05)	N.S.	0.23	N.S.	0.27	0.22	0.28	0.39
CC:SP							
97:03	07.48 ± 0.06	36.04 ± 0.06	42.13 ± 0.21	07.51 ± 0.39	07.12 ± 0.13	06.06 ± 0.09	04.18 ± 0.28
94:06	07.50 ± 0.25	35.53 ± 0.08	41.33 ± 0.06	07.17 ± 0.03	07.58 ± 0.27	06.30 ± 0.05	04.29 ± 0.19
91:09	07.50 ± 0.15	35.71 ± 0.18	40.82 ± 0.14	06.89 ± 0.18	08.69 ± 0.18	06.68 ± 0.19	04.68 ± 0.18
88:12	07.51 ± 0.09	34.09 ± 0.07	40.29 ± 0.29	06.33 ± 0.16	09.11 ± 0.09	06.90 ± 0.07	04.93 ± 0.04
Mean	07.49 ± 0.13	35.34 ± 0.09	41.14 ± 0.17	06.97 ± 0.19	08.12 ± 0.16	06.48 ± 0.10	04.52 ± 0.17
CD (P < 0.05)	N.S.	0.21	0.40	0.40	0.34	0.20	0.37

Table 2: Minerals, hydrosoluble vitamins and antioxidant activity of value-added *churpe* products (balls and strips)

Supplementation level (%)	Parameters						
	Calcium (mg/100g)	Magnesium (mg/100g)	Sodium (mg/100g)	Thiamine (µg/g)	Riboflavin (µg/g)	Ascorbic acid (µg/g)	Antioxidant activity (%)
Control (Cottage cheese)	616.14 ± 0.21	530.66 ± 0.16	883.47 ± 0.18	37.02 ± 0.21	106.16 ± 0.14	10.43 ± 0.10	37.16 ± 0.13
CC:AP:GS							
90:00:10	615.42 ± 0.07	524.18 ± 0.14	880.21 ± 0.23	36.02 ± 0.10	105.02 ± 0.19	10.31 ± 0.16	35.08 ± 0.43
85:05:10	614.31 ± 0.09	503.74 ± 0.07	776.64 ± 0.16	37.02 ± 0.26	105.97 ± 0.10	10.42 ± 0.03	37.25 ± 0.09
80:10:10	610.39 ± 0.03	488.44 ± 0.25	712.06 ± 0.05	37.12 ± 0.17	106.13 ± 0.15	10.46 ± 0.17	42.17 ± 0.11
75:15:10	608.43 ± 0.15	460.63 ± 0.10	689.13 ± 0.03	37.57 ± 0.28	106.56 ± 0.06	10.48 ± 0.26	47.72 ± 0.21
70:20:10	601.75 ± 0.21	413.69 ± 0.39	612.22 ± 0.36	37.68 ± 0.04	106.73 ± 0.12	11.73 ± 0.12	50.09 ± 0.06
Mean	611.08 ± 0.12	486.89 ± 0.18	758.95 ± 0.16	37.08 ± 0.09	106.08 ± 0.12	10.68 ± 0.14	41.57 ± 0.17
CD (P < 0.05)	0.22	0.38	0.36	0.35	0.23	0.28	0.38
CC:SP							
97:03	623.67 ± 0.16	549.53 ± 0.21	872.12 ± 0.24	37.00 ± 0.13	106.04 ± 0.06	10.48 ± 0.06	37.55 ± 0.17
94:06	668.35 ± 0.14	580.26 ± 0.08	851.38 ± 0.06	37.38 ± 0.27	106.53 ± 0.08	10.50 ± 0.25	39.46 ± 0.07
91:09	780.52 ± 0.09	601.74 ± 0.17	832.57 ± 0.08	37.69 ± 0.18	106.71 ± 0.18	11.50 ± 0.15	41.28 ± 0.16
88:12	854.04 ± 0.30	632.55 ± 0.15	816.08 ± 0.11	37.91 ± 0.09	106.95 ± 0.07	11.71 ± 0.09	44.86 ± 0.13
Mean	708.54 ± 0.16	578.94 ± 0.17	851.12 ± 0.15	37.49 ± 0.16	106.55 ± 0.09	11.04 ± 0.13	40.06 ± 0.16
CD (P < 0.05)	0.32	0.35	0.33	0.34	0.21	0.26	0.34

CC = Cottage cheese, AP = Apricot Powder, GS = Ground sugar, SP = Spinach powder
Values are means ± SD of three independent determinations

phenolic compounds occurring in fruits and vegetables display antioxidant activity, which is defined as the ability of a compound or a mixture thereof to inhibit oxidative degradation of various substances via scavenging of reactive species, including free radicals. Increase in the incorporation levels of apricot and spinach powder resulted into the enhancement of DPPH radical scavenging activity of strips (37.25 ± 0.09% to 50.09 ± 0.06%) and strips (37.55 ± 0.17% to 44.86 ± 0.13%), respectively. Dried apricots are a valuable source of bioactive compounds other than phenolic and flavonoids including anthocyanins (3.08 ± 0.40 mg CGE/100 g) and β-carotene (0.56 ± 0.03 mg/100 g) that could also be responsible for the increase of antioxidant activity (Ali et al. 2011). (Jabeen et al. 2020) and Sharma et al. (2011), reported similar results in fruit-cheese based energy bar and broccoli-cheese powder blends, respectively. Earlier studies on antioxidant activity of spinach supplemented biscuits and cheddar cheese fortified with *Inula britannica* extract also exhibited increase in DPPH scavenging activity (Narsing et al. 2017; Lee et al. 2016).

Colour properties

Both *churpe*-balls and *churpe*-strips showed significant ($p < 0.05$) differences in color values enriched with different levels of apricot powder and spinach powder, respectively (Table 3). Apricot enriched *churpe* showed a significant decrease in L* and b* and an increase in a* with the increase in addition of apricot powder from 05 to 20%. L* ranged from 82.00 ± 1.27 to 66.30 ± 2.15; a* ranged from 21.90 ± 2.21 to 78.00 ± 0.25; b* ranged from 22.50 ± 0.13 to 11.70 ± 3.25. The significant variations in color profile of *churpe*-balls may be due to the brownish colour of apricot powder. The colour values L* decreased and a* and b* increased with the addition of spinach powder. L* ranged from 86.00 ± 6.20 to 71.00 ± 1.32; a* ranged from -08.70 ± 4.14 to -62.30 ± 3.23; b* ranged from -03.00 ± 1.16 to -25.60 ± 0.37. Similar findings were reported for colour changes in UF-soft cheese supplemented with spinach powder (El-Sayed, 2020) and biscuits supplemented with spinach powder (Narsing et al. 2017). These changes in colour of cheese products may be due to the green colour of spinach powder coincided with the results of Mohamed et al. (2018). The total colour differences (ΔE) of both the balls and strips increased with the increase in the incorporation of apricot and spinach powder, respectively.

Microbial evaluation (Total Plate Count)

The value added dairy products after drying were packed in cotton bags and stored at room temperature (20 °C) and higher temperature (26 °C) for 150 days (Table 4). These were analyzed initially and at one month interval for microbiological parameter. The results showed that, the mean microbial load of apricot supplemented *churpe*-balls decreased from $4.0 \times 10^3 \pm 0.14$ cfu/g to $3.2 \times 10^3 \pm 0.16$ cfu/g at 05 to 20% supplementation levels. However, storage period for 120 days resulted into increase in microbial load of the same product from $3.3 \times 10^3 \pm 0.17$ cfu/g to $4.5 \times 10^3 \pm 0.16$ cfu/g. Both the cases are also true with the *churpe*-strips. The microbial load declined with the increased addition ratios (03 to 12%) of spinach powder and increased with the advancement of storage period (120 days). The microbial load ranged from $5.2 \times 10^3 \pm 0.15$ cfu/g to $4.7 \times 10^3 \pm 0.15$ cfu/g in the former case and ranged from $3.7 \times 10^3 \pm 0.14$ cfu/g to $6.1 \times 10^3 \pm 0.11$ cfu/g in the latter case. Microbial studies indicated that the

Table 3: Colour properties of value-added *churpe* products (balls and strips)

Supplementation level (%)	Parameters			
	L*	a*	b*	ΔE
Control (Cottage cheese)	91.34 ± 5.18	03.01 ± 1.27	25.29 ± 2.18	-
CC:AP:GS				
90:00:10	89.30 ± 3.15	05.20 ± 2.32	24.20 ± 1.28	03.18 ± 2.45
85:05:10	82.00 ± 1.27	21.90 ± 2.21	22.50 ± 0.13	21.25 ± 4.51
80:10:10	75.80 ± 6.22	46.09 ± 4.35	20.66 ± 3.10	45.88 ± 3.37
75:15:10	71.50 ± 5.15	63.00 ± 3.18	18.50 ± 1.26	63.35 ± 3.93
70:20:10	66.30 ± 2.15	78.00 ± 0.25	11.70 ± 3.25	80.21 ± 3.37
Mean	79.37 ± 3.84	36.20 ± 2.26	20.47 ± 1.86	42.77 ± 3.52
CD (P < 0.05)	07.63	04.70	03.89	06.60
CC:SP				
97:03	86.00 ± 6.20	- 08.70 ± 4.14	- 03.00 ± 1.16	23.61 ± 3.63
94:06	79.70 ± 4.31	- 21.96 ± 8.18	- 08.89 ± 2.29	27.63 ± 6.96
91:09	74.50 ± 2.12	- 36.90 ± 1.14	- 16.00 ± 1.31	38.96 ± 3.18
88:12	71.00 ± 1.32	- 62.30 ± 3.23	- 25.60 ± 0.37	62.68 ± 4.69
Mean	77.80 ± 3.48	- 32.46 ± 4.17	- 13.37 ± 1.46	38.22 ± 4.61
CD (P < 0.05)	07.15	08.13	02.99	09.25

CC = Cottage cheese, AP = Apricot Powder, GS = Ground sugar, SP = Spinach powder
Values are means ± SD of three independent determinations

products stored at room temperature up to 5 months had better stability as the microbial count remained within permissible limits of Indian Food Standard, 2006 (http://www.fssai.gov.in/portals/0/pdf/food-act). The decline in the microbial population due to incorporation of dried parsley in Kareish cheese is also reported by El-Taweel et al. (2017) and according to them, the decline in microbial population due to incorporation of fruits and vegetables might be due to the presence of phenol and flavonoid contents which are known for their antimicrobial activity. The increase in microbial population with the passage of time might be due to

Table 4: Microbial load (cfu/g) of value-added *churpe* products (balls and strips)

Supplementation level (%)	Storage period (days)					
	0	30	60	90	120	Mean
Control (Cottage cheese)	$3.7 \times 10^3 \pm 0.23$	$3.9 \times 10^3 \pm 0.20$	$4.1 \times 10^3 \pm 0.05$	$4.6 \times 10^3 \pm 0.13$	$5.8 \times 10^3 \pm 0.06$	$4.4 \times 10^3 \pm 0.13$
CC:AP:GS						
90:00:10	$3.6 \times 10^3 \pm 0.27$	$3.7 \times 10^3 \pm 0.18$	$3.9 \times 10^3 \pm 0.07$	$4.4 \times 10^3 \pm 0.29$	$5.1 \times 10^3 \pm 0.08$	$4.1 \times 10^3 \pm 0.17$
85:05:10	$3.5 \times 10^3 \pm 0.09$	$3.6 \times 10^3 \pm 0.16$	$3.8 \times 10^3 \pm 0.17$	$4.4 \times 10^3 \pm 0.15$	$4.9 \times 10^3 \pm 0.16$	$4.0 \times 10^3 \pm 0.14$
80:10:10	$3.3 \times 10^3 \pm 0.17$	$3.4 \times 10^3 \pm 0.21$	$3.7 \times 10^3 \pm 0.26$	$4.1 \times 10^3 \pm 0.17$	$4.7 \times 10^3 \pm 0.22$	$3.8 \times 10^3 \pm 0.20$
75:15:10	$3.1 \times 10^3 \pm 0.27$	$3.1 \times 10^3 \pm 0.35$	$3.2 \times 10^3 \pm 0.15$	$3.9 \times 10^3 \pm 0.18$	$4.1 \times 10^3 \pm 0.15$	$3.4 \times 10^3 \pm 0.22$
70:20:10	$3.0 \times 10^3 \pm 0.05$	$3.0 \times 10^3 \pm 0.19$	$3.2 \times 10^3 \pm 0.28$	$3.3 \times 10^3 \pm 0.13$	$3.7 \times 10^3 \pm 0.19$	$3.2 \times 10^3 \pm 0.16$
Mean	$3.3 \times 10^3 \pm 0.17$	$3.3 \times 10^3 \pm 0.21$	$3.5 \times 10^3 \pm 0.18$	$4.0 \times 10^3 \pm 0.18$	$4.5 \times 10^3 \pm 0.16$	$3.7 \times 10^3 \pm 0.18$
CD ($P < 0.05$)	Supplementation = 0.13		Storage = 0.12		Supplementation x Storage = 0.31	
CC:SP						
97:03	$3.8 \times 10^3 \pm 0.19$	$4.3 \times 10^3 \pm 0.19$	$5.1 \times 10^3 \pm 0.03$	$6.0 \times 10^3 \pm 0.30$	$6.8 \times 10^3 \pm 0.06$	$5.2 \times 10^3 \pm 0.15$
94:06	$3.7 \times 10^3 \pm 0.16$	$4.3 \times 10^3 \pm 0.17$	$4.8 \times 10^3 \pm 0.08$	$5.6 \times 10^3 \pm 0.31$	$6.2 \times 10^3 \pm 0.15$	$4.9 \times 10^3 \pm 0.17$
91:09	$3.7 \times 10^3 \pm 0.14$	$4.1 \times 10^3 \pm 0.04$	$4.3 \times 10^3 \pm 0.05$	$4.9 \times 10^3 \pm 0.10$	$5.8 \times 10^3 \pm 0.05$	$4.5 \times 10^3 \pm 0.07$
88:12	$3.6 \times 10^3 \pm 0.08$	$4.1 \times 10^3 \pm 0.14$	$4.6 \times 10^3 \pm 0.07$	$5.6 \times 10^3 \pm 0.26$	$5.9 \times 10^3 \pm 0.21$	$4.7 \times 10^3 \pm 0.15$
Mean	$3.7 \times 10^3 \pm 0.14$	$4.2 \times 10^3 \pm 0.13$	$4.7 \times 10^3 \pm 0.05$	$5.5 \times 10^3 \pm 0.24$	$6.1 \times 10^3 \pm 0.11$	$4.8 \times 10^3 \pm 0.13$
CD ($P < 0.05$)	Supplementation = 0.11		Storage = 0.11		Supplementationx Storage = 0.26	

CC = Cottage cheese, AP = Apricot Powder, GS = Ground sugar, SP = Spinach powder
Values are means ± SD of three independent determinations

Table 5: Sensory parameters of value-added *churpe* products (balls and strips)

Supplementation level (%)	Parameters			
	Appearance	Texture	Flavour	Overall acceptability
Control (Cottage cheese)	08.30 ± 0.18	06.70 ± 0.27	05.60 ± 0.18	06.30 ± 0.32
CC:AP:GS				
90:00:10	08.30 ± 0.15	06.20 ± 0.32	07.20 ± 0.28	07.40 ± 0.21
85:05:10	07.50 ± 0.15	05.90 ± 0.21	07.50 ± 0.13	07.60 ± 0.30
80:10:10	08.00 ± 0.27	05.30 ± 0.35	08.00 ± 0.10	07.90 ± 0.14
75:15:10	08.50 ± 0.22	05.00 ± 0.18	08.50 ± 0.26	08.50 ± 0.19
70:20:10	06.30 ± 0.15	05.00 ± 0.25	08.70 ± 0.25	06.20 ± 0.13
Mean	07.70 ± 0.18	05.40 ± 0.26	07.90 ± 0.20	07.50 ± 0.19
CD ($P < 0.05$)	0.34	0.48	0.38	0.40
CC:SP				
97:03	08.00 ± 0.20	08.70 ± 0.14	08.00 ± 0.16	06.10 ± 0.18
94:06	08.50 ± 0.31	07.20 ± 0.18	07.20 ± 0.29	07.00 ± 0.17
91:09	08.50 ± 0.12	06.90 ± 0.14	06.00 ± 0.31	07.70 ± 0.23
88:12	07.00 ± 0.32	06.30 ± 0.23	05.60 ± 0.37	06.90 ± 0.18
Mean	08.00 ± 0.23	07.20 ± 0.17	06.70 ± 0.28	06.90 ± 0.19
CD ($P < 0.05$)	0.44	0.36	0.50	0.41

CC = Cottage cheese, AP = Apricot Powder, GS = Ground sugar, SP = Spinach powder
Values are means ± SD of three independent determinations

increase in moisture content of the products. Results are similar to those of Paul et al. (2020) and Gupta et al. (2011) in freeze dried cow milk cheese and soy cheese and in barley incorporated cookies, respectively.

Sensory properties

The sensory properties of the *churpe* supplemented with apricot and spinach powders are given in Table 5. Supplementation showed significant ($P < 0.05$) effect on the sensory properties of the value-added balls and strips. The control *churpe* showed the lowest flavor score of 05.60 ± 0.18. There was decrease in texture score (05.90 ± 0.21 to 05.00 ± 0.25) and increase in flavor score (07.50 ± 0.13 to 08.70 ± 0.25) of balls with the increase in the supplementation levels of apricot. Sucrose, γ-decalactone, β-Ionone and citrate are the key flavouring compounds while alcohols are identified as the main volatiles compounds present in apricot that contribute to the consumer acceptance of the balls (Xi et al. 2016). An increase in the acidic taste was detected in the products due to apricot supplementation. However, appearance and overall acceptability scores first increased upto 15% supplementation of apricot powder (08.50 ± 0.22 in case of appearance and 08.50 ± 0.19 in case of overall acceptability) followed by decrease in these parameters. Appearance of the balls was pale cream upto 10% level thereafter it was brown in color when 15% and 20% apricot powder was substituted. Feng et al. (2019) showed a potential improvement in sensory properties of goat yogurt supplemented with jujube pulp and concluded that 3% jujube pulp conferred a higher perceived overall acceptability than that obtained with 1% or 9% jujube pulp. A similar trend in overall acceptability was also found by Mohamed and Shalaby, (2016) in apricot-cheese analogue. The texture and flavor scores showed decreasing trend (08.70 ± 0.14 to 06.30 ± 0.23 in case of texture and 08.00 ± 0.16 to 05.60 ± 0.37 in case of flavor) with the increase in supplementation levels (05% to 20%) of spinach powder in *churpe*-strips. The loose texture (low firmness) could be due to higher concentration of spinach powder which has fibrous and graininess attribute and also responsible for savory-sour taste of the product (Lucera et al. 2018). The appearance and overall acceptability scores increased upto 09% level with values 08.50 ± 0.12 and 07.70 ± 0.23, respectively, followed by decreasing trends. Similar observations were informed by other workers for the changes in sensory parameters due to the incorporation of spinach powder. Overall scores were highly acceptable in low concentration of spinach supplemented soft cheese (El-Sayed, 2020) and biscuits (Narsing et al. 2017). The addition of moringa plant extract in yogurt (> 0.05%) and rice bran (1%, 2%, and 3%) improved the bioactivity but exhibited a negative impact on the sensory characteristics (Demicri et al. 2017; Zang et al. 2018). Sharma et al. (2011) observed significant decrease in appearance, flavor and overall acceptability scores of broccoli-cheese powder blends. Based on the above results *churpe*-balls and *churpe*-strips containing 15% apricot powder and 09% spinach powder, respectively, were found to be most acceptable by the panelists. However, overall acceptability scores of the all supplemented products were still in the category of 'like'.

Conclusions

This study highlights the possibility of development of innovative products from the traditional dairy product, *churpe* of Ladakh. It has been successfully established that apricot and spinach powder could be condensed to obtain functional *churpe* products extremely rich in antioxidants and containing a high concentration of minerals, vitamins and other essential nutrients. Thus these products have enormous potentials to guard against many human diseases. To the tribal people of Ladakh, these could overcome the deficiency of micronutrients caused due the shortage of fruits and vegetables during the winter season. The nomads of Changthang region of Ladakh could carry these products easily as nutritional supplements during their year round migration. The balls could be moistened and masticated as a chewing gum while moving with livestock and the strips could be cooked with *thukpa*, a local dish.

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RESEARCH ARTICLE

Effect of incorporation of Generally Regarded as Safe (GRAS) carbohydrate derivatives on quality attributes of skim milk

Dnyaneshwar Shinde[1*], B Surendra Nath[2], Rahila MP[3] and P Heartwin Amladhas[4]

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Abstract: Reducing the fat content of dairy products adversely affects their flavour, texture and mouthfeel which consequently affect the consumer acceptance of skim milk. It was observed in the present study that pasteurized toned milk (3% fat, 8.5% SNF) had better sensory properties and higher viscosities than pasteurized skim milk, showing the importance of fat in consumer acceptance of milk samples. In commercial practice, skim milk is therefore traded only upon UHT treatment as it was much better in sensory properties than pasteurized skim milk. Since carbohydrate derivatives are usually used as fat mimetics, Inulin fruitafit text and fruitafit HD and polydextrose were added at different levels (0-2.5%) to skim milk to improve its quality attributes. It is observed that incorporation of a 1% level of polydextrose in combination with either of inulins (@ 1%) had improved viscosity and sensory properties. The rheological properties of such milk were comparable to that of toned milk and better than that of UHT skim milk. The quality of the skim milk added with carbohydrate derivatives was not affected by the normal processing treatments, such as homogenisation, pasteurization, boiling and sterilization. The gross constituents such as fat, protein, lactose and ash contents did not differ much with the control skim milk sample. The pH, acidity, and color (measured as % reflectance) of the skim milk samples added with the derivative were within the normal range. The samples were heat-stable, had high MBR time and when packed in LDPE pouches and stored at 5^0C kept well for two days. After appropriate approvals from the Food Safety and Standards Authority of India (FSSAI) pasteurised skim milk added with GRAS carbohydrate derivatives can be commercialised with improved consumer acceptance and affordability.

Keywords: Carbohydrate Derivative, Inulin, Polydextrose, Skim Milk, Sensory Properties, Physico-chemical properties

[1]Dairy Chemistry Section, ICAR-NDRI (SRS)
Adugodi, Bengaluru-560 030, India

[2]Dairy Chemistry Section, ICAR-NDRI (SRS), Adugodi, Bengaluru-560 030 India

[3]Dairy Chemistry Department, College of Dairy Science and Technology, Kerala Veterinary and Animal Sciences University Pookode 673576 Kerala India

[4]Dairy Engineering Section ICAR-NDRI (SRS), Adugodi, Bengaluru-560 030 India

Dnyaneshwar Shinde (✉)
Dairy Chemistry Section,
ICAR-NDRI (SRS), Adugodi, Bengaluru-560 030, India
Email: dshinde032@gmail.com

Introduction

Dietary fat contributes to key sensory, nutritional and physiological benefits. It contributes to flavor, or the combined perception of mouthfeel, taste, and aroma/odor. Fat also contributes to creaminess, appearance, palatability, texture, and lubricity of foods and increases the feeling of satiety during meals. However, due to an established relationship among health, diet and maintenance of healthy weight have boosted the market of foods with reduced energy value (Santos, 2009; Boff *et al.* 2013). Therefore, it is important to identify commercially viable strategies that are capable of removing or reducing the fat content of food products (Wu *et al.* 2013) without altering their sensory and nutritional characteristics (Boff *et al.* 2013). Thus, it is pertinent to investigate the use of potential carbohydrate derivatives to improve the textural properties of pasteurized skim milk. This would help to include skim milk in the diet of health-conscious people without sacrificing the mouthfeel and psychological satisfaction during their consumption. Accordingly, the present work was carried out to add Generally Regarded as Safe (GRAS) carbohydrate derivatives to skim milk and analyze its physico-chemical, textural and sensory properties, to evaluate the acceptability of pasteurized skim milk.

Reducing the fat content of milk adversely affects its flavor, texture and mouth feel thereby reduces the consumer acceptance of skim milk that is either pasteurized or boiled. In this regards Ultra High Temperature (UHT) processing improves the body, texture and mouthfeel of skim milk and several commercial brands of skim milk are available in the market for health-conscious consumers. But UHT processing is not affordable for small-scale

operations and many consumers. Therefore, several attempts have been made to use fat mimetic or fat substitutes or fat replacers, which are known to improve the sensory attributes of low-fat foods. Some of the carbohydrate derivatives perform the function of hydrocolloids to provide several fat-like attributes such as structure, viscosity, smoothness and opacity to reduce and/or essentially replace the fat content in foods. Carbohydrate-based fat-substitutes provide some of the functions of fat by binding water and providing texture, mouthfeel, and opacity (Giese, 1996).

Further, some of the carbohydrate derivatives such as inulin, β glucan, polydextrose, oat ingredients offer many health benefits, besides improving the textural attributes. Hond (2000) and Kleessen (1997) reported that consumption of 15–20 g inulin per day contributes to counteracting constipation. Schneeman (1999) suggested that an increase in defecation frequency decreases the risk of colon cancer. Some studies indicate that inulin lowers the level of cholesterol in blood plasma (Roberfroid, 2005) and there are plenty of studies confirming that inulin consumption increases calcium, magnesium and iron absorption (Bosscher *et al.* 2003). Polydextrose is recognised as a valuable source of fibre in Japan and many countries. Japanese regulations allow polydextrose to be used as a source of soluble fibre in many beverages and other products (Hamanaka, 1987). Based on EFSA scientific opinion, polydextrose is suitable for those who want to follow a low glyceimic diet when it is used as a sugar replacer (EFSA, 2011). The US Food and Drug Administration allow a health claim to be made on food labels containing 0.75 g of β-glucans, the dietary fibre present in oats per serving (FDA, 1997). These derivatives exhibit diverse functional, structural and sensory characteristics and hence, it is appropriate to consider their application to improve the sensory properties of skim milk.

Materials and Methods

Milk Samples

Fresh cow milk obtained from the Experimental Dairy of National Dairy Research Institute, Bengaluru was used for experimental samples. For comparative study, UHT skim milk of four different brands along with one brand of toned milk was collected from the Bengaluru market.

Carbohydrate Derivatives

Two types of Inulin- obtained from DKSH India Private Limited, Powai, Mumbai developed to improve texture and mouthfeel in various food applications are used in this study. Inulin I is an inulin type with enhanced technological properties. It is a natural powdered food ingredient based on chicory inulin with high purity. Inulin II is a native inulin/oligofructose. It is a natural powdered food ingredient extracted from chicory roots. Polydextrose powder (Brand name: Litess® 2), was obtained from Danisco (India) Pvt. Ltd. Haryana, India.

Preparation of Skim Milk

Fresh cow milk was separated (using Lakshmi cream separator Noe Tech International (p) Ltd. Sonepat, Haryana) at 40^0C to skim milk and cream. Skim milk was then added with a 2 % level of polydextrose and inulin individually or in combination with each at a 1% level of addition. This level of addition was selected from results obtained during preliminary trials of sensory scores and the viscosity of each sample. Incorporation of both the carbohydrate derivatives was done at 50^0C with a very slow rate of addition and continuous stirring. It was observed that at low temperature and a higher rate of addition, clumps were formed which remain insoluble even after immediate stirring. After the addition of carbohydrate derivatives, the samples were subjected to pasteurization (72^0C/15 S) in a hot water bath and cooled to 4^0C in a cold water bath.

Compositional Analysis

Reference methods were used to determine total solids, fat, lactose by adopting the Lane - Eynon procedure (IS: SP (part 11) - 1981), solids not fat (IS: SP: 18 (Part I,) 1980), protein (semi-automated Kjeldahl digestion and distillation unit), ash **(**as per the AOAC 2005) procedure.

Physico-chemical Analysis

Titratable acidity was determined as per the method described in BIS for milk. The pH was determined with a calibrated digital pH meter by dipping electrode directly in milk samples taken in a 5 ml beaker.

Viscosity was determined using Brookfield viscometer (Brookfield LVDV-**ÀÀ** + Pro, Middleboro, MA, USA) with a jacketed small sample adaptor and S18 spindle. Five milliliters of the sample was added to the sample cup and kept for 60 seconds before the measurement was taken and the temperature of the sample was maintained at 30^0C. Readings were taken over 20 rpm and the result was expressed in centipoise (cP).

For color measurement reading on the reflectance scale of the reflectance meter (Elico Model CL-28 Elico Pvt. Ltd. Hyderabad) was adjusted to zero using a completely opaque plate (Black) and to a 100 value using white plate under color mode. The flat bottomed glass bottle containing the milk sample was placed under the lamp of the reflectance meter and the reflectance as shown by the pointer was recorded. The same flat bottomed glass bottle was used throughout the analysis. The test was repeated for two more readings and the mean was considered for results.

For heat-stability (Alcohol test) five ml of milk sample was added to a test tube using a graduated pipette. An equal amount of 68 % of ethyl alcohol was added to the same tube. Contents in the test tube were mixed well by inverting the test tubes several times. The test tube was observed for the appearance of any flakes on the wall of the test tube. ISI method was used for determining the methylene blue reduction time (MBRT).

Sensory Evaluation of Samples

The organoleptic quality of samples was evaluated by a panel of judges on a 9 points hedonic scale under standard conditions of product evaluation. The panelist rated each sensory attribute on 9 point hedonic scale where 1 corresponded to "dislike extremely" and 9 corresponded to "Like extremely". The beakers (250 ml) containing milk samples were taken out from the incubator at 30^0 C and poured into small size beakers (50 ml). The Beakers (50 ml) were coded properly and served to judges in the sensory evaluation laboratory. The judges provided scores and comments on color and appearance (CA), body and texture (BT), flavor (FL) and overall acceptability (OA) of the milk samples.

Storage Studies

The skim milk samples added with selected levels of carbohydrate derivatives were packed in LDPE pouches of 55 µ thickness and stored in the refrigerator (~ 5^0C). The samples were drawn at 24 hours intervals and analyzed for sensory parameters and the physico-chemical changes.

Statistical Analysis

The results of the physic-chemical and sensory evaluation were statically analyzed using SPSS 16.0 software (SPSS INC, Chicago, IL, USA).

Results and Discussion

Analysis of Commercial Milk Samples

Sensory Parameters and Physico-chemical Properties

Commercial UHT skim milk and pasteurized toned milk, and pasteurized skim milk and toned milk samples prepared in the laboratory were subjected to sensory analysis in two batches of three trials each. The four commercial UHT milk were coded as UHTA, UHTB, UHTC, UHTD, while commercial toned milk sample as TMA; the pasteurized skim milk and toned milk processed in the laboratory were coded as PSM and TMB respectively. The SNF content in PSM and TMB were adjusted to match with that in UHT skim milk. Scores for sensory parameters such as color and appearance (CA), body and texture (BT), flavor (FL) and overall acceptability (OA), of samples obtained on a nine-point hedonic scale, from a panel of judges are shown in Table 1.

It may be seen from the data in the table that the scores obtained for the toned milk samples differed significantly from those obtained for the pasteurized skim milk samples for all the sensory attributes. Van *et al.* (2011) observed that an important sensory attribute of dairy products is the perceived creaminess or fat film formation, which is associated with the amount of fat present. Bayarri and Costell (2009) reported that elimination or reduction of fat in foodstuffs not only modifies composition and structure but also causes interaction among various constituents, giving rise to clear perceptible changes in color, flavor and texture, and possibly to a less acceptable product. The difference in the scores between the toned milk samples and UHT milk samples was less and statistically not significant. Thus, it can be concluded that UHT treatment has considerably improved the important attributes such as flavor and body and texture of skim milk. Similar results were proposed by Datta (2002) that UHT processing of milk enhances flavor and mouthfeel of milk over that of traditionally processed counterparts. Following the initial heat-induced changes, other changes occur slowly in UHT milk during storage and result in the formation of a three-dimensional protein network which causes the milk to thicken and then gel (Datta and Deeth, 2001). It may be seen that the pasteurized skim milk obtained considerably low scores, which were significantly different from those of UHT skim milk and toned milk. Thus, it may be observed that fat is an important constituent for rendering the milk palatable.

It may be seen from the data in Table 1 that the mean fat and SNF contents in TMA were 3.14 and 8.86%, respectively. The TMB had 3.19% fat and 9.41% SNF content. The SNF content in laboratory toned milk was adjusted matching that of commercial UHT skim milk. The fat content of commercial UHT skim milk samples ranged from 0.14 to 0.39% and SNF content ranged from 9.22 to 9.47%. The fat (0.23%) and SNF (9.43) contents of the pasteurized skim milk samples did not differ from those of the UHT skim milk samples. The pH and acidity of toned milk samples were about 6.87 - 6.84 and 0.12 – 0.125 % LA, respectively and were well within the normal range. The pH and acidity of UHT skim milk samples were significantly different from those of toned milk samples. The pH (range: 6.71 – 6.74) was lower and acidity was higher (range: 0.152 – 0.159% LA). This increase in acidity and decrease in pH of UHT skim milk may be correlated to an increase in the concentration of lactic acid and other organic acids which resulted from the degradation of lactose. These results are consistent with the previous findings reported by Augustin (1991) and Erdem (2005). Husain (2003) also reported that acidity increased in UHT processed buffalo skim milk. The pH and acidity of pasteurized skim milk samples were 6.79 and 0.144 % LA.

It may be seen from the data in Table 1 that the viscosity of toned milk samples was higher (1.77 and 1.83cp) than the pasteurized skim milk (1.36cp) and UHT skim milk samples (1.42 - 1.50cp). It is known that higher levels of total solids, caseins and fat increase the viscosity of milk (Mathur, 1999). Since SNF content of toned

Table 1 Study of sensory score and Physico-chemical properties of commercial milk samples

Attributes / Properties	Toned milk TMA	TMB	PSM	UHTA	Skim milk UHTB	UHTC	UHTD
CA	7.68±0.21^{bc}	7.79±0.24^{c}	7.16±0.41^{a}	7.30±0.26^{ab}	7.40±0.12^{abc}	7.65±0.09^{bc}	7.63±0.25^{bc}
BT	7.55±0.2^{bc}	7.79±0.07^{c}	6.59±0.1^{a}	7.49±0.1^{bc}	7.41±0.02^{b}	7.44±0.33^{c}	7.5±0.29^{bc}
FL	7.68±0.07^{cd}	7.73±0.01^{d}	6.38±0.09^{a}	7.29±0.23^{b}	7.44±0.11^{bcd}	7.18±0.35^{b}	7.38±0.08^{bc}
OA	7.66±0.04^{cd}	7.80±0.03^{d}	6.63±0.19^{a}	7.15±0.25^{b}	7.43±0.06^{bc}	7.34±0.1^{bc}	7.34±0.25^{bc}
Fat (%)	3.14±0.21^{c}	3.19±0.13^{c}	0.23±0.04^{a}	0.14±0.04^{a}	0.24±0.05^{a}	0.39±0.13^{ab}	0.18±0.06^{a}
SNF (%)	8.86±0.05^{a}	9.41±0.03^{b}	9.43±0.05^{b}	9.47±0.03^{b}	9.22±0.02^{ab}	9.29±0.04^{b}	9.44±0.06^{b}
TS (%)	12±0.72^{b}	12.29±0.1^{b}	9.66±0.14^{a}	9.61±0.07^{a}	9.46±0.34^{a}	9.78±0.24^{a}	9.62±0.69^{a}
pH	6.87±0.01^{d}	6.84±0.05^{d}	6.79±0.01^{c}	6.74±0.05^{b}	6.73±0.5^{ab}	6.73±0.01^{ab}	6.71±0.01^{a}
Acidity (%LA)	0.12±0.02^{a}	0.125±0.3^{a}	0.144±0.01^{b}	0.152±0.01^{c}	0.153±0.01^{c}	0.152±0.08^{c}	0.159±0.04^{d}
Color (% Whiteness)	78.16±1^{abc}	79±0.5^{abc}	81.33±1.2^{c}	76.16±0.5^{ab}	74.5±0.5^{a}	79.33±0.7^{bc}	77.5±3.7^{ab}
Viscosity (Cp)	1.77±0.02^{c}	1.83±0.03^{c}	1.36±0.11^{a}	1.5±0.13^{b}	1.49±0.05^{b}	1.44±0.04^{ab}	1.42±0.05^{ab}

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) differ significantly ($P<0.05$).

Table 2 Study of sensory score and viscosity of skim milk added with different levels of carbohydrate derivatives

Attributes / Properties	Ingredients	Level of addition (%) 0	0.5	1	1.5	2	2.5	Combination of inulin and polydextrose PSM	SM with PD+Inulin-I (@ 1%each)	SM with PD+ Inulin-II (@ 1%each)
CA	Inulin-II	7.55±0.41^{Aa}	7.63±0.28^{Aa}	7.53±0.28^{Aa}	7.65±0.28^{Aa}	7.68±0.28^{Aa}	7.7±0.28^{Aa}	7.61±0.19^{a}	7.58±0.08^{a}	7.64±0.18^{a}
	Inulin-I	7.5±0.21^{Aa}	7.53±0.25^{Aa}	7.56±0.25^{Aa}	7.66±0.2^{Aa}	7.6±0.2^{Aa}	7.65±0.1^{Aa}			
	PD	7.58±0.31^{Aa}	7.56±0.15^{Aa}	7.66±0.15^{Aa}	7.66±0.15^{Aa}	7.6±0.15^{Aa}	7.64±0.15^{Aa}			
BT	Inulin-II	6.68±0.09^{Aa}	7.17±0.16^{Aa}	7.33±0.05^{Aab}	7.53±0.23^{Ab}	7.73±0.15^{Ac}	7.9±0.36^{Ac}	6.71±0.25^{a}	7.75±0.1^{b}	7.76±0.04^{b}
	Inulin-I	6.55±0.09^{Aa}	7±0.1^{Aa}	7.43±0.05^{Ab}	7.5±0.1^{Ab}	7.76±0.15^{Ac}	7.88±0.15^{Ac}			
	PD	6.64±0.09^{Aa}	7.26±0.2^{Aa}	7.4±0.1^{Ab}	7.43±0.11^{Bb}	8.43±0.05^{Bc}	8.25±0.17^{Bc}			
FL	Inulin-II	6.49±0.32^{Aa}	6.96±0.15^{Aa}	7.36±0.15^{Ab}	7.53±0.05^{Abc}	7.66±0.15^{Bc}	7.15±0.01^{Aa}	6.65±0.14^{a}	7.8±0.1^{bc}	7.7±0.1^{b}
	Inulin-I	6.59±0.32^{Aa}	7±0.1^{Aa}	7.35±0.2^{Ab}	7.46±0.15^{Ab}	7.5±0.15^{Ab}	7.26±0.05^{Aa}			
	PD	6.51±0.32^{Aa}	6.96±0.15^{Aa}	7.42±0.2^{Ab}	7.48±0.2^{Ac}	7.38±0.05^{Bab}	7.3±0.1^{Aab}			
OA	Inulin-II	7.02±0.31^{Aa}	7.43±0.05^{Ba}	7.53±0.05^{Ba}	7.7±0.1^{Ab}	7.76±0.05^{Ac}	7.46±0.05^{Aa}	7.18±0.38^{a}	7.79±0.01^{b}	7.76±0.01^{b}
	Inulin-I	6.82±0.31^{Aa}	7.06±0.05^{Aa}	7.16±0.15^{Aa}	7.63±0.05^{Abc}	7.7±0.1^{Ac}	7.5±0.1^{Ab}			
	PD	7.08±0.31^{Aa}	7.25±0.05^{Ba}	7.35±0.08^{Ba}	7.66±0.05^{Ab}	7.58±0.05^{Bb}	7.45±0.15^{Ab}			
Viscosity	Inulin-II	1.45±0.02^{Aa}	1.57±0.05^{Aa}	1.58±0.02^{Aa}	1.63±0.05^{Ab}	1.74±0.025^{Ac}	1.75±0.05^{Ac}	1.5±0.03^{a}	1.76±0.01^{bc}	1.74±0.01^{b}
	Inulin-I	1.42±0.04^{Aa}	1.55±0.01^{Aa}	1.57±0.01^{Aa}	1.58±0.01^{Aa}	1.75±0.01^{Ab}	1.77±0.05^{Ab}			
	PD	1.38±0.05^{Aa}	1.57±0.05^{Aa}	1.61±0.01^{Ab}	1.69±0.02^{Bc}	1.77±0.01^{Bd}	1.79±0.01^{Bd}			

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) is significantly different ($P<0.05$) from each other. Means in each row with different superscripts (A, B) is significantly different ($p<0.05$) from each other.

and skim milk samples did not vary considerably, it is the fat present in toned milk, which is responsible for its higher viscosity. Creamy or fatty perception is related to the apparent viscosity of the product. The UHT skim milk samples showed significantly ($P<0.05$) higher viscosity (1.42-1.50Cp) than pasteurized skim milk samples (1.36 Cp). This might be because of the heat-induced changes in milk components that cause thickening. When milk is heated, the whey proteins denature and associate with the casein micelles (Smits and van Brouwershaven, 1980). This has two different effects: first, the micelle grows in size (Morr, 1969), and second the interaction between micelles changes. Under certain conditions both the change in size and the change in interactions can be determined from viscosity measurements. The increase in viscosity of skim milk found after UHT is due to the denaturation of whey proteins (Walstra, 1984).

The whiteness measured as reflectance was higher in pasteurized skim milk than in toned milk and UHT skim milk samples. The yellow fat present in toned milk and heat-induced color change in UHT skim milk might have contributed to lower reflection in these samples. Milk has its natural color due to the reflectance of light by dispersed milk fat globules, proteins, and natural milk pigments like riboflavin and carotenoids (Solah, 2007). The level of fat also affects the whiteness of milk. Among the components present in milk, casein is responsible for the light scattering. On reduction of fat as happens in skim milk, the effect of casein increases leading to a higher degree of scattering of light, which results in increased whitishness of skim milk. The decrease in the degree of whiteness of UHT treated skim milk may be related to heat-induced browning in these samples.

It may be observed from the above analysis that while fat played a vital role in imparting flavor and mouthfeel (viscosity) to milk. The UHT treatment had significantly contributed to these characteristics in skim milk samples. The sensory properties and viscosity of pasteurized skim milk samples were considerably lower. Thus, there is a scope and need to improve the sensory attributes, especially the mouthfeel of the pasteurized skim milk, to prepare it by small dairies and make it available and affordable for the consumer.

Screening and Selection of GRAS Carbohydrate Derivatives

The preliminary trials have shown that inulins and polydextrose, when added to skim milk, improved the mouth feel characteristics and did not pose problems with regards to solubility and off-flavor. Hence, these additives were selected for further trials. Silva (1996) suggested that inulin is a rheology modifier and can be used to enhance the organoleptic properties of foods. It can form a creamy, fat-like gel when dissolved in water. Kappas *et al.* (1993) reported that polydextrose, a complex carbohydrate, provided some of the functionality of the oil by providing mouthfeel, viscosity and bulk.

Selection of Level of Addition

Study of sensory Parameters and Viscosity

Two types of inulin, I and II and polydextrose were added at a level ranging from 0.5 to 2.5% with an increment of 0.5%. The samples were stirred well and pasteurized. The samples equilibrated to room temperature were subjected to sensory analysis and the scores are presented in Table 2. It may be seen from the data that for all the additives and at all levels of addition, the color and appearance scores were almost similar and the differences were not significant. Body and texture scores of the samples added with inulins and polydextrose increased. The scores increased with the level of addition for all the additives. However, the increase was not significant ($P<0.05$) at 0.5% of addition of inulins and polydextrose from control skim milk. Further, it was observed that the body and texture scores did not differ significantly ($P<0.05$) for skim milk samples added with 2.0 and 2.5% levels.

The flavor score of skim milk samples added with inulins increased with the level of addition up to 2% for inulins and up to 1.5% for polydextrose. The increase in flavor scores was significantly ($P<0.05$) different with control samples at a 1.0% level of addition for inulins and polydextrose. At a 2.5% level, the inulins exhibited bitter flavor in skim milk. However, polydextrose gave a slightly sweet taste at 2.0% addition which markedly increased at a 2.5% level of addition. It may be the reason for a lower flavor score of skim milk containing polydextrose @ 2%, though; body texture score was higher among the other derivatives.

The overall acceptability scores, which were reflections of the perceptions of flavor and body and texture, also showed a similar trend. However, due to a sweet note, the overall acceptability score of polydextrose at the 2% level was lower and significantly different from that of both inulins at the same levels. Among the additives, the difference in the sensory scores was marginal except that of flavor and overall acceptability score of polydextrose at 2 % level.

The skim milk samples added with the carbohydrate derivatives at a level ranging from 0 to 2.5% were subjected to viscosity analysis and the data are presented in Table 2. The viscosity of skim milk samples increased with the addition of inulins and polydextrose. The viscosity also increased with an increase in the level of addition. The observed variation of viscosity, body texture score of milk with inulin could be explained by different factors. Soukoulis *et al.* (2009) attributed it to the capacity of inulin to retain water. Schaller-Povolny and Smoth (2001) reported that the interaction of inulin with milk proteins could lead to an increase in molar mass which increased viscosity. Using a new approach based on tribological measurements, Meyer *et al.* (2011) determined the effect of inulin on the texture of various types of milk. The effect of the addition of long or medium chain length

inulin (1, 3 or 5%) on the friction coefficient and sensory profile of skimmed milk could be decreased to the value of full-fat milk, with long-chain inulin being more effective than native inulin. It was observed in the present study that the viscosity of the samples added with inulins and polydextrose was highest at a 2.5% level of addition. However, the values did not differ significantly (P<0.05) from those obtained at the 2% level of addition. The values obtained at the 2% level were closer to those obtained for toned milk (Table 1).

As may be seen from the sensory scores given in Table 2 the flavor scores of skim milk containing either of the inulins at a 2% were higher than those with a 2.5% level. That might be due to the observations that at 2.5% addition, as mentioned earlier, the inulins exhibited a bitter taste in skim milk. However, at 2% level, polydextrose gave a slightly sweet note. Veena (2014) also reported that polydextrose above 1 % level imparted slight sweetness to milk samples. Hence, combinations of polydextrose with either of the inulins each @ 1% were subjected to sensory analysis and viscosity measurement and the data are presented in Table 2. As may be seen from the data, the sensory scores for body and texture, flavor and overall acceptability and viscosity of the skim milk samples added with polydextrose @ 1% in combination with either of inulins @ 1% increased considerably and were significantly different (P<0.05) with that of the control skim milk (PSM). The viscosity of these samples was also found to be similar to that of the samples with the derivatives, added singly @ 2%. Hence, based on the above-mentioned observations, both the inulins at 2% and a combination of polydextrose @ 1% with either of the inulins @ 1% were tried for further studies.

Effect of Heat Treatments on Skim Milk added with Carbohydrate Derivatives

Since milk samples are subjected to heat treatment as routine practice, the effect of different heat treatments such as pasteurization, boiling and sterilization on the sensory and physico-chemical properties were studied.

As may be seen from the sensory scores given in Table 3 that the samples had acceptable scores with all the heat treatments and thus they were found to be stable even after heat treatment. This

Table 3 Study of effect of heat treatments on sensory score and physicochemical properties of skim milk samples added with different carbohydrate derivatives

Treatment	Attributes / Properties	PSM	SM with Inulin-I (@ 2%)	SM with Inulin-II (@ 2%)	SM with PD+ Inulin-I (@ 1% each)	SM with PD+ Inulin-II (@ 1% each)
Pasteurization	CA	7.61±0.19^{a}	7.59±0.18^{a}	7.72±0.2^{a}	7.58±0.08^{a}	7.64±0.18^{a}
	BT	6.57±0.25^{a}	7.62±0.12^{b}	7.64±0.2^{b}	7.72±0.1^{b}	7.73±0.04^{b}
	FL	6.26±0.14^{a}	7.49±0.12^{b}	7.51±0.14^{b}	7.69±0.1^{bc}	7.72±0.1^{c}
	OA	6.68±0.18^{a}	7.55±0.12^{b}	7.51±0.14^{b}	7.69±0.01^{bc}	7.72±0.01^{c}
Boiling	CA	7.38±0.21^{a}	7.45±0.13^{ab}	7.36±0.23^{a}	7.6±0.02^{ab}	7.61±0.04^{ab}
	BT	6.68±0.04^{a}	7.46±0.05^{b}	7.43±0.12^{b}	7.75±0.03^{c}	7.62±0.01^{bc}
	FL	6.31±0.04^{a}	7.43±0.06^{b}	7.41±0.06^{b}	7.69±0.01^{d}	7.61±0.03^{c}
	OA	6.71±0.06^{a}	7.49±0.06^{bc}	7.45±0.06^{b}	7.79±0.01^{d}	7.58±0.04^{c}
Sterilization	CA	7.24±0.19^{a}	7.23±0.15^{a}	7.23±0.11^{a}	7.21±0.14^{a}	7.28±0.14^{a}
	BT	6.61±0.09^{a}	7.40±0.07^{b}	7.35±0.11^{b}	7.75±0.08^{c}	7.64±0.1^{bc}
	FL	6.52±0.04^{a}	7.21±0.02^{b}	7.17±0.03^{b}	7.51±0.08^{c}	7.46±0.06^{c}
	OA	6.7±0.11^{a}	7.25±0.06^{b}	7.08±0.1^{a}	7.57±0.05^{c}	7.56±0.04^{c}
Pasteurization	pH	6.73±0.03^{a}	6.78±0.02^{a}	6.78±0.07^{a}	6.82±0.05^{a}	6.81±0.06^{a}
	Acidity (% LA)	0.15±0.013^{a}	0.135±0.01^{a}	0.14±0.01^{a}	0.13±0.01^{a}	0.138±0.01^{a}
	Color (% whiteness)	82±0.5^{bc}	81.5±0.5^{bac}	81±1^{b}	81.16±0.7^{a}	81.83±0.2^{bc}
	Viscosity (Cp)	1.48±0.03^{a}	1.65±0.01^{b}	1.66±0.03^{b}	1.69±0.01^{bc}	1.73±0.01^{c}
Boiling	pH	6.71±0.02^{a}	6.77±0.03^{ab}	6.79±0.03^{bc}	6.73±0.02^{a}	6.81±0.02^{bc}
	Acidity (% LA)	0.162±0.09^{a}	0.159±0.01^{a}	0.15±0.09^{a}	0.15±0.05^{a}	0.156±0.01^{a}
	Color (% whiteness)	83±2.64^{a}	82.5±2.17^{a}	82.33±1.5^{a}	81±2^{a}	83.33±2.08^{a}
	Viscosity (Cp)	1.63±0.02^{a}	1.77±0.08^{bc}	1.69±0.03^{b}	1.82±0.2^{c}	1.81±0.08^{c}
Sterilization	pH	6.46±0.005^{b}	6.52±0.01^{ab}	6.52±0.01^{ab}	6.51±0.04^{ab}	6.51±0.01^{a}
	Acidity (% LA)	0.189±0.09^{a}	0.17±0.09^{a}	0.18±0.01^{a}	0.18± 0.01^{a}	0.18±0.09^{a}
	Color (% whiteness)	73.66±1.44^{a}	72±1.32^{a}	72.83±1.5^{a}	73.5±1^{a}	72.66±1.52^{a}
	Viscosity (Cp)	1.65±0.05^{a}	1.78±0.07^{b}	1.78±0.07^{b}	1.85±0.13^{bc}	1.89±0.13^{c}

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) is significantly different (P<0.05) each other.

Tables 4 Effect of homogenization on sensory score and physico-chemical properties of skim milk samples added with carbohydrate derivatives

Attributes / Properties	PSM	Homogenized PSM	SM with PD+Inulin-I (@ 1% each)	Homogenized SM with PD+Inulin-I (@ 1% each)	SM with PD+Inulin-II (@ 1% each)	Homogenized SM with PD+Inulin-II (@ 1% each)
CA	6.65±0.05^{a}	6.68±0.18^{a}	7.64±0.20^{a}	7.67±0.18^{a}	7.608±0.18^{a}	7.612±0.21^{a}
BT	6.56±0.09^{a}	6.702±0.07^{ab}	7.543±0.12^{bc}	7.637±0.16^{c}	7.614±0.27^{c}	7.715±0.22^{c}
FL	6.7±0.10^{a}	6.81±0.05^{a}	7.557±0.08^{b}	7.703±0.14^{b}	7.603±0.27^{b}	7.673±0.06^{b}
OA	6.57±0.02^{a}	6.68±0.04^{a}	7.68±0.03^{c}	7.69±0.08^{c}	7.62±0.23^{c}	7.71±0.065^{c}
pH	6.76±0.03^{a}	6.76±0.05^{a}	6.81±0.01^{b}	6.80±0.01^{ab}	6.79±0.03^{ab}	6.79±0.03^{a}
Acidity (%LA)	0.141±0.05^{ab}	0.144±0.09^{ab}	0.132±0.05^{a}	0.139±0.07^{ab}	0.138±0.05^{ab}	0.141±0.05^{ab}
Viscosity (Cp)	1.549±0.017^{a}	1.584±0.017^{a}	1.717±0.04^{b}	1.7517±0.05^{b}	1.74±0.062^{b}	1.768±0.06^{b}

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) is significantly different (P<0.05) from each other.

trial has again shown that the carbohydrate derivatives have significantly improved the body and texture, flavor and overall acceptability of the samples when compared to control skim milk. Among the heat treatments, pasteurized and boiled samples scored almost the same score. On the other hand, sterilized samples showed a perceptible brown color and cooked flavor because of which they scored slightly lower for color and appearance and flavor (P<0.05). Product browning is more pronounced with increases in process severity and storage temperature (Burton 1988).

The effect of different heat treatments on some of the pertinent physico-chemical properties was also studied and the data are presented in Table 3. It may be seen from the data that the pH and acidity of the samples added with the derivatives were within the normal range. With a particular heat treatment, the acidity value did not differ (P<0.05) among the samples. However, as the intensity of heat treatment increased, the pH of the samples decreased and acidity increased. These changes were more perceptible in the case of sterilized samples. These results are consistent with the previous findings of Augustin (1991) and Erdem, (2005). Among the heat treatments, the viscosity increased with an increase in the intensity of heat treatment.

Effect of Homogenization on Skim Milk added with Carbohydrate Derivatives

Among the different carbohydrate derivatives tried, both inulins (@1%) along with polydextrose (@1%) were found to be the best in terms of sensory score and viscosity data as can be seen in table 3. Ingredients and levels were selected and the trials were conducted to see the effect of homogenization on the quality of skim milk by studying sensory and physico-chemical properties.

It may be seen from the data in Table 4 that homogenization has only marginally improved (P<0.05) the sensory properties of milk. Homogenization is known to improve the color and appearance, flavor and body and texture scores of whole milk. Sanguansri (2006) reported that homogenization enhanced product texture and mouthfeel.

As may be seen in Table 4, pH and acidity did not differ (P<0.05) between homogenized and un-homogenized samples. Viscosity was slightly higher, but without significant difference, in samples that were homogenized. As observed earlier, viscosity was higher in samples containing the carbohydrate derivatives.

Final Selection of Carbohydrate Derivatives

In this trial, the selected combination (polydextrose and either of the inulins at 1% level each) was again compared for sensory score and physico-chemical properties with skim milk, UHT skim milk and toned milk for final selection to carry out shelf-life studies.

Table 5 gives information about the sensory scores of the samples. From the data, it may be seen that the body and texture, flavor and overall acceptability scores of skim milk added with the combination of polydextrose with either of the inulins (@ 1% each) improved considerably (P<0.05) when compared to the control (skim milk) sample and the differences were statistically significant. The properties were even slightly better than UHT skim milk, though the differences were not significant (P<0.05). The sensory properties of skim milk added with the carbohydrate derivatives were also not significant with those of the toned milk, although the toned milk showed higher scores. The color and appearance scores of all the samples were almost the same without any significant difference (P<0.05).

It may be seen from the data in Table 5 that the addition of polydextrose and inulin fruitafit HD or text, has decreased acidity and increased the pH of the samples. It was concluded that the textural quality of skim milk containing a combination of polydextrose and either of the inulins was comparable to that of the toned milk and better than that of UHT skim milk.

Physico-chemical Analysis of Skim Milk added with Carbohydrate Derivatives

It may be observed from the data in Table 6 that the constituents such as fat, protein, lactose and ash of all the skim milk samples were within the range for the normal skim milk. Skim milk samples added with the carbohydrate derivatives had slightly lower acidity and higher pH compared to the control skim milk sample. As reported in the previous sections, the viscosity of skim milk samples added with the carbohydrate derivatives (1.78 and 1.75 Cp) was higher than the control milk sample (1.52 Cp). These differences were statistically significant (P<0.05). The viscosity of skim milk added with the derivatives was almost similar to that of the toned milk (1.8 Cp). It was observed that the addition of carbohydrate derivatives did not promote the microbial load of

Table 5 Sensory score and physico-chemical properties for final selection of derivatives

Attributes / Properties	PSM	SM with PD+Inulin-II (@ 1% each)	SM with PD+Inulin-I (@ 1% each)	UHT	TMB
CA	7.63±0.16^{a}	7.7±0.1^{a}	7.71±0.08^{a}	7.57±0.11^{a}	7.72±0.13^{a}
BT	7.09±0.16^{a}	7.59±0.09^{b}	7.65±0.16^{b}	7.59±0.09^{b}	7.74±0.14^{b}
FL	6.97±0.03^{a}	7.51±0.1^{bc}	7.54±0.1^{bc}	7.3±0.22^{b}	7.66±0.09^{c}
OA	6.98±0.16^{a}	7.6±0.08^{bc}	7.62±0.08^{bc}	7.46±0.15^{b}	7.71±0.03^{c}
pH	6.73±0.01^{a}	6.79±0.01^{bcd}	6.77±0.02^{bc}	6.72±0.04^{a}	6.82±0.02^{d}
Acidity (% LA)	0.168±0.05^{c}	0.139±0.07^{b}	0.138±0.05^{b}	0.169±0.04^{c}	0.125±0.04^{a}
Viscosity (Cp)	1.505±0.02^{a}	1.698±0.01^{bc}	1.741±0.01^{d}	1.68±0.02^{bc}	1.78±0.01^{e}
Color (%whiteness)	81.33±0.7^{b}	80.66±0.57^{b}	81.66±0.57^{b}	74.13±1.1^{a}	75.33±1.15^{a}

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) is significantly different (P<0.05) from each other.

Table 6 Physico-chemical analysis of skim milk samples added with carbohydrate derivatives

Parameter	PSM	SM with Inulin-1+PD (@ 1% each)	SM with Inulin-II+PD (@ 1% each)	TMB
TS (%)	9.23 ± 0.24^{a}	10.92 ± 0.46^{b}	10.85 ± 0.45^{b}	11.75 ± 0.44^{b}
SNF (%)	8.7 ± 0.25^{a}	10.52 ± 0.52^{b}	10.55 ± 0.41^{b}	8.66 ± 0.44^{a}
Fat (%)	0.53 ± 0.02^{a}	0.49 ± 0.05^{a}	0.5 ± 0.04^{a}	3.18 ± 0.04^{b}
Protein (%)	3.258 ± 0.04^{a}	3.26 ± 0.09^{a}	3.24 ± 0.18^{a}	3.256 ± 0.07^{a}
Lactose (%)	4.75 ± 0.24^{a}	4.73 ± 0.13^{a}	4.79 ± 0.07^{a}	5.06 ± 0.3^{a}
Ash (%)	0.74 ± 0.03^{b}	0.72 ± 0.04^{b}	0.71 ± 0.02^{ab}	0.65 ± 0.01^{a}
pH	6.73 ± 0.01^{a}	6.8 ± 0.01^{b}	6.79 ± 0.005^{b}	6.8 ± 0.01^{b}
Acidity (% LA)	0.142 ± 0.06^{a}	0.134 ± 0.02^{ab}	0.122 ± 0.06^{b}	0.116 ± 0.05^{b}
Viscosity (Cp)	1.52 ± 0.02^{a}	1.78 ± 0.005^{c}	1.75 ± 0.001^{b}	1.8 ± 0.01^{c}
Alcohol test	Negative	Negative	Negative	Negative
MBRT (Hours)	6.35±0.35^{a}	6.25±0.48^{a}	6.18±0.4^{a}	6.30±0.54^{a}

Results are expressed as mean±SD of three replications. Mean in each column with different superscripts (a, b, c, d) is significantly different (P<0.05) from each other.

Table 7 Sensory score and physico-chemical properties of skim milk samples added with carbohydrate derivatives during storage at 5°C

Attributes / Properties	Days	Sample PSM	SM with Inulin-I+PD (@ 1% each)	SM with Inulin-II+PD (@ 1% each)	TMB
CA	0th	7.71±0.15^{Aa}	7.8±0.11^{Aa}	7.72±0.06^{Aa}	7.75±0.05^{Aa}
	1st	7.54±0.13^{Aa}	7.55±0.7^{Aa}	7.56±0.2^{Aa}	7.7±0.26^{Aa}
	2nd	7.61±0.18^{Aa}	7.63±0.2^{Aa}	7.66±0.22^{Aa}	7.65±0.22^{Aa}
	3rd	7.5±0.17^{Aa}	7.6±0.1^{Aa}	7.7±0.17^{Aa}	7.56±0.07^{Aa}
BT	0th	7.36±0.17^{Aa}	7.73±0.1^{Ab}	7.77±0.12^{Ab}	7.86±0.06^{Ab}
	1st	7.11±0.48^{Aa}	7.65±0.15^{Ab}	7.68±0.08^{Ab}	7.74±0.07^{Ab}
	2nd	7.15±0.18^{Aa}	7.63±0.07^{Ab}	7.68±0.02^{Ab}	7.85±0.13^{Ab}
	3rd	7±0.17^{Ba}	7.25±0.18^{Ba}	7.26±0.05^{Ba}	7.63±0.15^{Bb}
FL	0th	7.14±0.15^{Aa}	7.6±0.09^{Ab}	7.63±0.18^{Abc}	7.88±0.13^{Ac}
	1st	7.22±0.19^{Aa}	7.47±0.2^{Aab}	7.64±0.05^{Abc}	7.91±0.22^{Ac}
	2nd	7.14±0.22^{Aa}	7.35±0.17^{Ab}	7.48±0.11^{Ab}	7.93±0.11^{Ac}
	3rd	6.1±0.07^{Ba}	6.3±0.3^{Ba}	6.13±0.2^{Ba}	7.26±0.15^{Bb}
OA	0th	7.34±0.09^{Aa}	7.7±0.1^{Ab}	7.73±0.09^{Ab}	7.83±0.04^{Ab}
	1st	7.32±0.17^{Aa}	7.56±0.07^{Ab}	7.62±0.02^{Abc}	7.78±0.12^{Ac}
	2nd	7.26±0.16^{Aa}	7.43±0.04^{Ab}	7.55±0.02^{Ab}	7.81±0.06^{Ac}
	3rd	7.1±0.02^{Ba}	7.21±0.05^{Ba}	7.22±0.11^{Ba}	7.51±0.18^{Bb}
pH	0th	6.73±0.01^{Aa}	6.77±0.02^{Aa}	6.76±0.01^{Aa}	6.79±0.02^{Ab}
	1st	6.73±0.01^{Aa}	6.76±0.03^{Aa}	6.77±0.03^{Aa}	6.79±0.01^{Ab}
	2nd	6.72±0.01^{ABa}	6.75±0.01^{ABa}	6.74±0.05^{ABa}	6.78±0.03^{ABa}
	3rd	6.71±0.08^{Ba}	6.73±0.02^{Ba}	6.72±0.02^{Ba}	6.75±0.03^{Ba}
Acidity (% LA)	0th	0.149±0.06^{Ac}	0.13±0.04^{Ab}	0.13±0.09^{Aab}	0.12±0.04^{Aa}
	1st	0.152±0.01^{Ac}	0.139±0.08^{Ab}	0.133±0.02^{Aab}	0.126±0.09^{Aa}
	2nd	0.152±0.04^{Ac}	0.14±0.09^{Abc}	0.139±0.04^{Aab}	0.127±0.01^{Aa}
	3rd	0.169±0.01^{Ba}	0.162±0.06^{Ba}	0.165±0.04^{Bab}	0.148±0.05^{Ba}
Viscosity(Cp)	0th	1.52±0.02^{Aa}	1.73±0.05^{Ab}	1.74±0.05^{Abc}	1.77±0.02^{Ac}
	1st	1.49 ± 0.05^{Aa}	1.73±0.01^{Ab}	1.74±0.01^{Ab}	1.78±0.05^{Ac}
	2nd	1.50±0.02^{Aa}	1.74±0.02^{Ab}	1.73±0.02^{Ab}	1.75±0.02^{Ab}
	3rd	1.52±0.02^{Aa}	1.73±0.01^{Ab}	1.73±0.01^{Ab}	1.75±0.05^{Ab}

milk, as the MBRT of the pasteurized samples was found to be in the range exhibited by good quality milk and all samples were similar in this respect. The alcohol test was found to be negative for all the samples which showed that samples added with carbohydrate derivatives were suitable for heat processing.

Shelf Life of Skim Milk added with Carbohydrate Derivatives

In order to find out the effect of the added carbohydrate derivatives on the shelf-life of the skim milk, 200 ml of the pasteurized samples were packed in LDPE pouches and kept at 5°C along with control (pasteurized skim milk) and pasteurized toned milk. The samples were analyzed periodically for sensory and pertinent physico-chemical properties.

Table 7 shows the sensory scores of the stored samples. It may be observed that color and appearance scores did not differ ($P<0.05$) among the samples as well during storage. Scores for body texture, flavor and overall acceptability did not change much during the first two days of storage in all the samples; but they were significantly ($P<0.05$) lower on the third day of the storage. It was observed that on the third day, flavor scores of all the samples decreased considerably, though they were acceptable. On the third day, skim milk samples containing added carbohydrate derivatives had fruity flavor and control skim milk and toned milk slightly had acidic flavor. The rate of decrease in sensory scores was more in control skim milk samples followed by skim milk added with carbohydrate derivatives and toned milk.

Table 7 gives information on the physico-chemical changes occurring in the milk samples during storage. It may be observed that acidity significantly increased and pH decreased on the third day of storage in all the samples. It was observed that the rate of rise in acidity was higher in control skim milk samples followed by skim milk added with carbohydrate derivatives and toned milk. The viscosity of all the milk samples did not change

significantly ($P<0.05$) during storage. From the results on the sensory scores and pH and acidity, it could be concluded that the shelf life of pasteurized skim milk samples added with carbohydrate derivatives was two days when packed in LDPE pouches and stored at 5^0C, which was similar to that of the control skim milk and toned milk.

Conclusions

Viscosity and sensory score of skim milk samples added with the carbohydrate derivatives was higher than the control milk sample. These differences were statistically significant. The results obtained of improved viscosity and higher sensory score for skim milk samples added with carbohydrates derivatives were reproducible can be seen throughout the results of research trials conducted during the multiple stages of the study. Reproducible results obtained signifies that the method adopted for incorporation of GRAS carbohydrate derivative is robust and can be readily adopted for improving quality attributes of skim milk. It may be concluded that the mouthfeel (body and texture scores and viscosity) of pasteurized skim milk could be considerably improved by adding polydextrose (@ 1%) along with inulin (@ 1%) without affecting consumer acceptability. This could help to commercialize the pasteurized skim milk for health or fat conscious people. Though the carbohydrate derivatives used in this study have GRAS status, appropriate approval from FSSAI is needed before their commercial use in skim milk.

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RESEARCH ARTICLE

Thermal and electrical energy analysis of scraped surface heat exchanger during *Kheer* making

Sapna Jain[1], S Kartikeyan[2], P Purohit, AG Bhadania[3], AK Agrawal[1] and C Sahu[1]

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Abstract: *Kheer* is a cereal based sweetened and concentrated milk confection, very well-liked in India. The present investigation was undertaken to study the energy consumption of the developed batch type scraped surface heat exchanger (SSHE) for *kheer* making. The *kheer* was prepared by using SSHE at different levels of steam pressure (1.0, 1.5 and 2.0 kg/cm^2), scraper speed (10, 20 and 30 rpm) and batch size (10, 15 and 20 kg). The thermal and electrical energy consumptions were determined. The rate of evaporation increased with increase in operating variables. The average rate of evaporation ranged from 11.84 to 25.65 kg water evaporated/h. The specific steam consumption by the SSHE during *kheer* making ranged between 1.568 and 1.702 kg steam/kg of water evaporated under different operating conditions of the machine. The total heat losses during manufacture of *kheer* in SSHE ranged from 12.9 to 18.9 %. The electrical power consumption of the machine during manufacturing of *kheer* varied from 230.1 to 260.5 W.

Keywords: Electrical, Energy analysis, *Kheer*, Scraped surface heat exchanger, Thermal

Introduction

Kheer is the India's popular dessert. It is a partially heat desiccated cereal-based sweetened indigenous dairy dessert. It is characterized by a sweet, nutty and pleasant flavour that is highly acceptable to the Indian palate. *Kheer* is considered nutritious as it is a rich source of protein, minerals, vitamins and other nutrients (Aneja et al. 2002). Indian sweet market is estimated to be worth Rs. 8,000 crores , out of which the branded segment has grown at 25%, while the marke as a whole 10-12% (Jayaraj Rao, 2020). Many small scale dairy entrepreneurs are interested to adopt mechanization in production of many Traditional Indian Dairy Products (TIDP) in order to get uniform and improved product quality. The proper design, ease of operation and energy efficient process make the scraped surface heat exchangers suitable for manufacturing dairy product at small scale set-up. Energy analysis of equipment is a tool that can be applied to evaluate efficiency of a process/ machine and design improve without compromising the product quality. Thermal energy requirement depends upon the thermal properties of product being handled, operation carried out, thermal losses and thermal efficiency of the system. Electrical power requirement of SSHE is product and process dependent. The mechanical energy required to drive the scraper assembly depends largely upon the rheological properties of the product, speed of rotation, etc.

Abhichandani et al. (1995) developed a continuous ghee making system of capacity 500 kg/h *ghee*. The values of specific steam consumption during *basundi* reported by Patel et al. (2007) under different operating conditions using 180º angle blades in a three-stage SSHE ranging from 1.30 to 1.40 kg, 1.35 to 1.42 kg and 1.40 to 1.45 kg of steam per kg of water evaporated in SSHE-1, SSHE-2 and SSHE-3 respectively. The major components of power consumption in SSHEs are shear stress created by the liquid for the blade action, the scraping action of the blades, friction in the bearings, the rotation of the mass of the fluid within the cylinder and blades (Rao and Hartel, 2006). The power required to rotate the shaft and blades is mainly determined by the design of the blades (Rajasekhar et al. 2001). Abichandani and Sarma (1988) studied power requirement in SSHEs during thin-film operation of milk and cream. The power requirement during evaporation of milk was consumed in three principle operations: accelerating the product to the rotor speed (inertial force), overcoming the viscous force and surface tension force to form the film on the heating surface and agitating the product film. Dhotre et al. (2009) evaluated the electrical power consumption under different

[1] Dairy Engineering Department, College of Dairy Science and Food Technology, Dau Shri VashudevChandrakarKamdhenuViswavidhyala, Durg, Chhattisgarh, India

[2] Dairy Technology Department, College of Dairy Science and Food Technology, Dau Shri Vashudev Chandrakar Kamdhenu Viswavidhyala, Durg, Chhattisgarh, India

[3] Dairy Engineering Department, SMC College of Dairy Science, Anand Agricultural University, Anand-388 110, Gujarat, India

Sapna Jain (✉)
Dairy Engineering Department, College of Dairy Science and Food Technology, Dau Shri VashudevChandrakarKamdhenuViswavidhyala, Durg, Chhattisgarh, India
E-mail:er.sapnajain@gmail.com

operating conditions for *shrikhand* thermization machine and found the value varied from 64.4 to 127.8 W.

Literature pertaining to process optimization of continuous *kheer* making has been reported by Kadam et al. (2013), however, its adaptability at small scale is less viable. In view of this, Chauhan et al. (2018) reported a design and development of a batch type multipurpose SSHE for preparing *kheer* at small scale. Singh et al. (2017) investigated a process modification of conventional method for production of rice *kheer* using single stage SSHE in combination with Conical Process Vat (CPV) and reported 24 % TS concentrated milk in SSHE with 2.0 kg/cm^2 steam pressure in CPV yielded rice *kheer* with highest overall acceptability score. Jain et al. (2021) studied the heat transfer behaviour of SSHE during *kheer* making; however, data on energy evaluation of SSHE during *kheer* making could not be traced. Therefore, the present study was conducted, to evaluate energy consumption required to operate the developed batch type SSHE for preparing *kheer* at small scale.

Materials and Methods

The experimental set up for mechanized production of *kheer* was the batch type multipurpose Scraped Surface Heat Exchanger (SSHE) without insulation. The batch type scraped surface heat exchanger(SSHE) with scraper assembly and inner side of product barrel are depicted in Fig 1(a, b and c). It consisted of semi jacketed product tube of AISI-304 grade stainless steel, four spring loaded teflon edged scraper assembly, vapour hood, variable frequency drive arrangement for the scraper assembly, measuring and control instruments. The SSHE was mounted on stainless steel frame and has a provision for steam supply and removal of condensate from the jacket (Chauhan et al. 2018).The developed SSHE requires electrical energy for operation of three phase induction motor which drives scraper assembly and exhaust fan operated on single phase power supply. The steam generated from Non-IBR boiler is supplied at required pressure by regulating the hand operated steam valve used in the seam pipeline. The heat and mass balance equations were developed to carry out thermal analysis of the SSHE. The experimental trials were carried out at different operating parameters that are scarper speeds (S_1=10 rpm, S_2=20 rpm, S_3=30 rpm), operating steam pressures (P_1=1.0 kg/cm^2, P_2=1.5 kg/cm^2, P_3=2.0 kg/cm^2) and batch sizes (B_1=10 kg, B_2=15 kg, B_3=20 kg). The different combination of process parameters was taken as treatments.

Preparation of *Kheer*

The standardised (4.5% fat and 8.5 % SNF) pasteurized milk of Amul was used for preparation of *kheer*as per the method described (De *et al.* 1976) with slight modification. A local variety of rice *(Oryzasativa*), Gujarat 17, was used; a fine crystalline sugar and other additives like saffron and cardamom were used for *kheer* preparation and were procured from the local market. The *kheer* was prepared in cleaned SSHE using standardized milk through feed hopper and the scraper was started. Steam at required pressure was admitted in the jacket of the SSHE for heating and concentration of milk. When milk attained almost boiling, rice was added (@ 3% of milk quantity) and the operation of heating together with the action of scraper continued till rice was cooked properly. The evaporated water vapour was continuously removed through the vapour hood. The cooking stage was judged by taking the sample of the product from the outlet of the SSHE. The sugar was added (@ 8% of milk quantity) when rice grains are cooked properly and in order to dissolve the sugar, the content was continuously heated for 2-3 minutes. The steam supply was stopped at this point and the content was removed from the SSHE. The product was cooled and stored in the refrigerator before serving for sensory evaluation. The control sample of *kheer* was prepared in the lab using S.S. *karahi* and LPG stove following the same ingredient

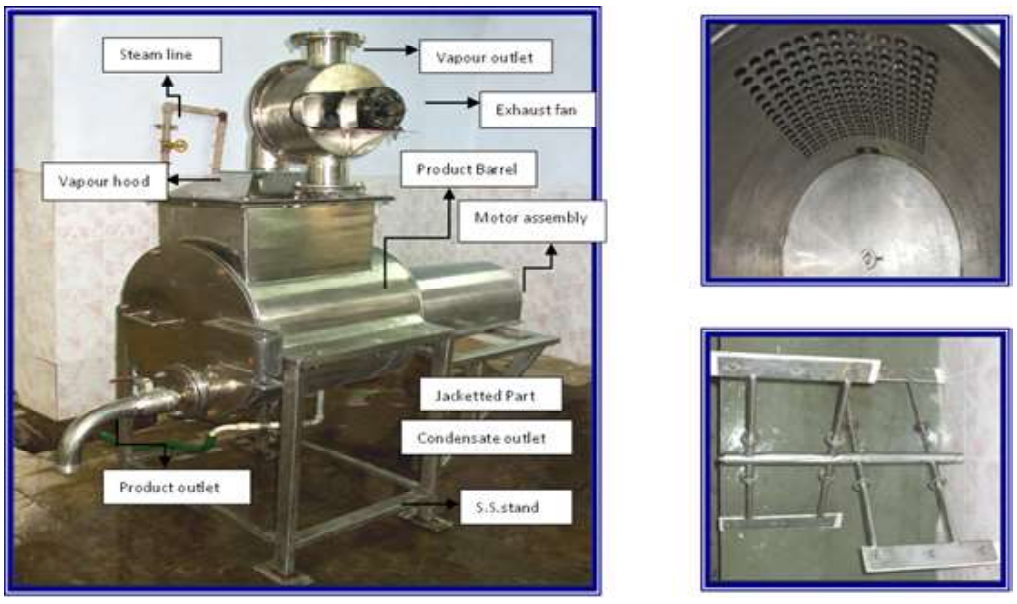

Fig 1: (a)Batch type scraped surface heat exchanger (b) Inner product barrel design, (c) Scarper assembly

ratios as for the experiment. The sensory attribute of product prepared in the machine was evaluated with a 9-point hedonic score by a panel of 8 judges.

Measurement of operating variables

The steam consumption of the SSHE was estimated by measuring the quantity of condensate coming out from the steam trap of the jacket. The dryness fraction of steam was also estimated. The electrical energy consumption (input voltage, current and power) of the SSHE was measured by power analyzer (MICO, 3-ϕ, 4-wire power analyzer, 440 volt) and energy meter installed in the electrical circuit.

Energy analysis

The thermal and electrical energy input were measured during preparation of *kheer* in SSHE. The thermal energy analysis of the SSHE was carried out in order to determine the extent of energy losses. Steam is supplied in the jacket through a steam valve and the condensate leaving the steam trap of the SSHE was measured. The heat balance equation (1) for the multipurpose SSHE is as under.

Heat energy with the feed material+Heat energy of steam = Heat energy of condensate+Heat energy of evaporated water+Energy going with the product +Energy losses

Rate of heat energy input = Rate of heat energy leaving the SSHE + rate of energy losses$(m*C_{pm}*T_i) + S_1 (h_s + x*hL) = (C_1*C_{pc}*Ts_1) + (E*H_v) + (m_p*Cp_p*t_1) + E_l$ (1)

Where,

m = mass of material

h_s = sensible heat of steam, kJ/kg

x = dryness fraction of steam

H_L = latent heat of steam, kJ/kg

C_{pc} = specific heat of condensate, kJ/kg°C

C_{pm} = specific heat of milk, kJ/kg°C

S_1 = quantity of steam used kg/h

C_1 = quantity of condensate, kg/h

T_{s1} = saturation temperature of condensate, °C

T_i = initial temperature of feed, °C

E = evaporation rate of water, kg/h

Table 1: Average values of rate of evaporation during *kheer* making

Steam Pressure(P) (kg/cm^2)	Batch size(B) (kg)	Evaporation at different scraper speeds(S) #					
		Kg water evaporation/batch			Kg water evaporation/hour		
		10 rpm	20 rpm	30 rpm	10 rpm	20 rpm	30 rpm
1. 0	10	5.83	5.84	5.83	11.84	12.72	14.28
	15	8.35	8.34	8.40	12.37	13.15	14.83
	20	11.14	10.95	11.16	12.98	13.70	15.39
1.5	10	6.08	5.79	5.85	16.20	17.39	19.49
	15	8.78	8.37	8.5	16.98	17.94	20.07
	20	11.30	11.04	11.15	17.76	18.40	20.78
2.0	10	6.10	5.79	6.00	20.34	21.74	24.00
	15	8.90	8.73	8.28	20.94	22.76	24.83
	20	11.73	11.18	11.33	21.9	23.53	25.65
	Source		SEM		C.D.		CV%
	P		0.0663		0.19*		1.588%
	B		0.0664		0.20*		
	S		0.1152		NS		
	PxB		0.0665		0.19*		
	PxS		0.1151		0.33*		
	BxS		0.1152		NS		
	PxBxS		0.1994		NS		

#Each value is an average of two replications
* significant effect ($p<0.05$), NS-Non significant

E_l = energy loss, kJ/h

H_v = enthalpy of evaporated water, kJ/kg

m_p = mass of the product leaving the SSHE, kg/h

Cp_p = specific heat of the product, kJ/kg°C

t_l = temperature of the product leaving the SSHE, °C

The sensible heat of the milk and other ingredients were accounted for calculating the sensible energy required to raise the temperature to evaporating temperature.

Statistical Analysis

Data obtained were subjected to statistical analysis to determine the effect steam pressure, batch size and scraper speed on the rate of evaporation during *kheer* making. The collected data were analyzed statistically using Factorial Completely Randomized Block Design described by Snedecor and Cochran (1994).

Results and Discussion

The thermal and electrical energy input were measured during preparation of *kheer* in multipurpose batch type SSHE at different combinations of operating parameters.

Rate of Evaporation

The rate of evaporation is one of the important factors in deciding the quality of the *kheer* made in SSHE. The rate of evaporation should be in controlled manner so that adequate time is made available for cooking of rice grains. It depends on the variety of rice, proportion of ingredients and configuration of SSHE as well as operating condition of machine. The rate of evaporation ranged between 11.84 to 25.65 kg water evaporated/h during manufacturing of *kheer* at different operating conditions. The mean values of evaporation rate (kg water evaporated/batch and kg water evaporated/hour) were calculated using the time taken for completion of one batch at different operating conditions is indicated in Table 1. It was noticed that the rate of evaporation increased with the increase in batch size and scraper speed at all steam pressures studied. The p-values from ANOVA to indicate significance of steam pressure, batch size and scraper speed individually and interactively on rate of evaporation.. There was significant increase ($p<0.05$) in rate of evaporation with the increase in steam pressure, batch size and scraper speed and combination of steam pressure and scarper speed.

During *kheer* making, it was found that at the higher rate of evaporation resulted in product with higher TS content, however with uncooked rice which was not acceptable by the sensory panel. Therefore, the controlled rate of evaporation enabled to proper cooking of rice, in turn lead to acceptable quality of the final product.

Thermal energy requirement

The thermal energy analysis of the SSHE was carried out in order to determine the extent of energy losses. The estimation of steam consumption is one of the important factor influences the manufacturing cost of the product. The steam consumption was determined and it was co-related with the rate of evaporation in the SSHE under various operating conditions. The steam and electrical power requirement during *kheer* making are shown in Table 2.The values of steam consumption under different operating conditions were found in the range of 19.20 to 42.10 kg/h. It can be clearly interpreted from the tables, that the steam consumption increased with batch size, scraper speed and steam pressure. The specific steam consumption (kg steam/kg water evaporated) under different operating conditions during manufacturing of *kheer* ranged from 1.568 to 1.702 kg steam/kg water evaporated. The increased batch size, not only led to higher steam consumption but non-insulated batch type SSHE was also

Table 2: Steam and electrical power consumption of the SSHE during *kheer* making

Steam Pressure(P)(kg/cm^2)	Batch size(B) (kg)	Steam consumption (kg/h)			Specific steam consumption (kg steam/ kg water evaporated)			Electrical power consumption (W)		
		10 rpm	20 rpm	30 rpm	10 rpm	20 rpm	30 rpm	10 rpm	20 rpm	30 rpm
1.0	10	19.20	20.4	22.40	1.622	1.604	1.568	243.2	244	245.6
	15	20.00	21.1	23.30	1.617	1.605	1.571	244.2	245.1	247.2
	20	21.00	21.9	24.20	1.602	1.598	1.572	245.1	246.5	249.5
1.5	10	27.10	28.8	31.40	1.663	1.656	1.611	242.8	244.2	255.2
	15	28.00	29.7	32.50	1.667	1.655	1.619	244.1	245.5	255.9
	20	29.30	30.3	33.40	1.663	1.646	1.622	245.4	247.1	259.4
2.0	10	34.60	36.7	39.50	1.701	1.688	1.646	242.7	245.2	253.5
	15	35.60	38.1	41.30	1.700	1.673	1.663	243.8	247.1	256.2
	20	36.70	39.2	42.10	1.702	1.666	1.641	246.3	247.9	260.2

Each value is an average of two replications

Table 3: Heat energy input, output and heat losses during manufacture of *kheer*

Steam Pressure (P)(kg/cm²)	Batch size(B) (kg)			Heat energy (x 10^3)kJ/h																	
	Feed			Steam			Condensate			Evaporated water			Product			Energy losses (%)					
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3			
P_1	B_1	2.15	2.35	2.65	47.76	50.75	55.72	7.55	8.10	8.90	31.68	34.04	38.21	3.22	3.45	3.96	15.6	14.7	13.1		
	B_2	2.36	2.51	2.80	49.75	52.49	57.96	7.94	8.38	9.25	33.10	35.19	39.69	3.69	3.94	4.32	14.8	14.2	12.9		
	B_3	2.39	2.49	2.86	52.24	54.48	60.72	8.34	8.70	9.61	35.09	36.66	41.18	3.89	4.11	4.64	14.0	13.7	13.4		
P_2	B_1	2.86	3.18	3.50	67.73	71.98	78.48	11.10	11.68	12.73	43.62	46.54	52.16	4.08	4.78	5.21	17.4	16.9	15.1		
	B_2	3.09	3.36	3.74	69.98	74.23	81.23	11.47	12.04	13.18	44.93	48.01	53.71	4.59	5.25	5.74	17.2	16.5	15.2		
	B_3	3.19	3.41	3.78	73.23	75.73	83.48	12.00	12.29	13.54	47.12	49.24	55.10	5.06	5.60	6.09	16.7	15.8	15.0		
P_3	B_1	3.51	3.98	4.24	86.82	92.08	99.11	14.47	15.03	16.18	54.43	58.18	64.22	4.96	5.98	6.13	18.9	18.3	16.9		
	B_2	3.74	4.14	4.77	89.32	95.60	103.63	14.88	15.61	16.92	56.04	60.91	66.45	5.47	6.19	7.55	18.6	17.8	16.8		
	B_3	3.84	4.29	4.50	92.08	98.36	105.63	15.34	16.06	17.25	57.70	62.97	68.64	5.92	6.96	7.19	18.4	16.9	16.1		

associated to it. Moreover due to single stage the vapour generated during process of *kheer* making couldn't be reutilized. These could be possible reasons for higher steam consumption. Since published literature is not traceable with respect to present study, the data couldn't be compared.

However, Abhichandani et al. (1995) reported steam consumption of 35 kg/h against that of 68 kg/h required in jacketed ghee kettle. Bhadania et al. (2005) observed that the steam consumption of the three stage *khoa* making machine was 65.97 kg/hand specific steam consumption ranged from 1.446 to1.618, 1.275 to 1.360 and 1.278 to 1.380 kg steam/kg water evaporated in$SSHE_1$, $SSHE_2$ and $SSHE_3$ respectively. The values of specific steam consumption during *basundi* reported by Patel et al. (2007) under different operating conditions ranged from 1.30 to 1.40 kg of steam/kg of water evaporated for SSHE-1, 1.35 to 1.42 kg of steam/kg of water evaporated for SSHE-2 and 1.40 to 1.45 kg of steam/kg of water evaporated for SSHE- 3. Dodeja and Deep (2012) observed an average steam consumption of 1.017 kg steam/kg of milk processed in three stage SSHE for *danedar khoa* making..

The energy components of heat input, heat output and heat losses are presented in Table 3. Heat losses were determined based on heat and mass balance equation in order to provide data or actual level of heat losses during *kheer* making operation in the SSHE. These components of energies showed increasing values with increase in batch sizes, steam pressure and scraper speed. The total energy of steam, condensate and water evaporated depends on the steam pressure which caused evaporation of water. The total heat losses during manufacture of *kheer* in uninsulated multipurpose SSHE ranged from 12.9 to 18.9 % of the heat input by the steam, at different operating conditions studied. Patel et al. (2007) reported radiant heat loss from the uninsulated jacket ranged between 1.0 to 1.3 % of total heat input, while it was negligible in case of insulated jacket for *basundi* making machine.

Electrical energy requirement

The estimation of electrical power consumption is important for sizing the motor required for the SSHE and to determine the operating electrical cost of SSHE. The power consumption is not only required for rotating the rotor scraper assembly but also to overcome all frictional forces existing in the SSHE. In the SSHE, three phase induction motor was used to drive the scraper assembly. The electrical power consumption of the SSHE during manufacturing of *kheer* is given in Table 3. The electrical power consumption of the machine during *kheer* making varied from 243.2 to 260.5 W at P_1, P_2 and P_3 conditions; 242.4 to 241.5 W at B_1, B_2 and B_3 conditions and were 230.1, 239.4 and 254.6 W at S_1, S_2 and S_3 conditions respectively. The average power consumption to drive the scraper assembly of the SSHE during *kheer* making was 0.25 kW. The data indicated rise in power consumption with increase in scraper speed and batch size as

the product became viscous with advancement in the processing time that directly exerts resistance on scraper assembly, thus draws more current to overcome the resistance. Though the power consumption is very less (< 0.01%) in comparison with steam energy. Similar results was reported by Bhadania et al. (2005) reported the electrical power consumption to drive the scraper assemblies ranged from 292.68 to 324.48 W which is about one per cent of the steam energy required in evaporation during manufacture of *khoa* in continuous SSHE. The electrical power consumption of batch type *khoa* making machine developed by More (1987) was 0.093 kWh per kg of milk handled. Cuevas and Cheryan (1982) indicated that the contribution of electric power required for driving the rotor in a vertical liquid full scraped surface heat exchanger was very small (< 0.5 %) as compared to steam required for evaporation of water.

Conclusion

The study revealed that controlled rate of evaporation was required for proper cooking of rice, so as to obtain acceptable quality of *kheer*. The maximum value of specific steam consumption (kg steam/kg of water evaporated) and electrical power consumption of the SSHE during *kheer* making were 1.702 kg steam/kg of water evaporated and 260.5 W respectively. The power consumption was low as compared to thermal energy requirement of the machine. The total heat losses during manufacture of *kheer* in batch type multipurpose SSHE (uninsulated) was 18.9 %. The losses can be reduced by providing proper insulation to the equipment. The vapour emerged during *kheer* making through hood can be reutilized for preheating purpose in the dairy operations.

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RESEARCH ARTICLE

Biopreservation of *paneer* using chitosan

RS Waghchaure[1], AS Khojare [2] and AB Jadhav[3]

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Abstract: The effect of chitosan on microbial, sensory, chemical, and textural characteristics of paneer was investigated for biopreservation and packaging alternatives. Five different concentrations of chitosan solution viz. 0.2%, 0.4%, 0.6%, 0.8%, 1% and three different temperatures *viz*. 3°C, 20°C and 37°C were selected for the studies along with control. Paneer samples coated with 0.8% concentration of chitosan solution observed to bear the least total viable count when compared with other concentrations. Yeast, mold, and coliform count were found nil in all the samples under study. 0.8% chitosan coated paneer samples also achieved higher sensory score as compared with control samples. The paneer samples coated with 0.8% chitosan solution subjected to proximate compositional analysis viz. moisture, fat, protein, ash, and acidity of the coated paneer samples are reported. The textural analysis also carried out for the attributes viz. hardness (1.1N), springiness (2.81mm), cohesiveness (0.43), gumminess (0.48N), and chewiness (1.40mJ) and found comparable with market samples of paneer.

Keywords- Biopreservation, Chitosan, Paneer

[1]Department of Agricultural Engineering, Maharashtra Institute of Technology, 431001, Aurangabad, Maharashtra, India

[2]Department of Dairy Science and Technology, Vivekanand College, Samarthnagar 431001, Aurangabad, Maharashtra, India

[3]Asily Agro & Food Processing Private Limited, Hansapore, Navsari, Gujrat, India

AS Khojare (✉)
Department of Dairy Science and Technology, Vivekanand College, Aurangabad, Maharashtra, 431 001, India
Email: khojare@yahoo.com

Introduction

Paneer is a very popular Indian heat and acid coagulated milk product. The market of paneer is mostly dominated by the unorganized players due to its limited shelf life and packaging issues. Due to the ever-growing demand of paneer, researchers were encouraged to develop new techniques for the manufacture and preservation of paneer. The characteristics of good quality paneer are white color, mildly acidic, nutty flavor, spongy body and a closely-knit texture (Kumar et al. 2011). Paneer is a rich source of high-quality proteins, fat, minerals, and vitamins (Shrivastava and Goyal, 2007).

The high moisture content (about 55%) of paneer is responsible for its deterioration. The shelf life of paneer is 6 days at refrigeration (8-10°C) and 24 hours at room temperature (30°C) (Sachdeva et al. 1991). The bacterial proliferation on the surface of paneer leads to spoilage (Sachdeva and Singh, 1990).

Many antimicrobial agents are used as a preservative compound which is incorporated into food products to increase its shelf life by inhibiting microbial growth. The use of packaging film based on chitosan could prove to be more efficient by maintaining a high concentration on food surface with the low migration of active substances (Coma et al. 2003). Chitin is the second most natural polysaccharide on the earth after cellulose. Chitosan is the principle derivative of chitin having properties such as non-toxicity, biodegradability, and biocompatibility. Chitosan is derived by the deacetylation of chitin in 40% sodium hydroxide at 120°C for 1-3 hours (Datta et al. 2011).

Chitosan has film-forming properties, antimicrobial action and lack of toxicity. Biochemical properties and biodegradability proved that it is one of the best edible and biologically safe preservative coatings for various types of foods (Shiekh et al. 2013). A wide range of unique applications offered by chitosan is the formation of a biodegradable film, bioconversion for the production of value-added food products, recovery of waste material from food processing discards and preservation of foods from microbial deterioration (Shahidi, 1999). The use of chitosan in dairy products was also reported by Coma et al. (2003). Tripathi et al. (2008) prepared chitosan/poly(vinyl alcohol)/ pectin ternary

film and confirmed that the antimicrobial effect of chitosan occurred without migration of active agents and organisms in direct contact with the active sites of chitosan are inhibited by microbiological screening.

Honarkar and Barikani (2009) also reported the antimicrobial activity of chitosan solution for different food components (starch, whey protein, and oil) and recommended that gram-negative bacteria are very sensitive to the applied chitosan while the sensitivity of gram-positive bacteria is highly variable. Inmaculada (2009) reported the potential of chitosan as a preservative coating in reducing or preventing moisture loss, lipid oxidation, and microbial growth.

The application of different concentrations of chitosan coating on paneer samples was carried out and its effect on microbial, physicochemical and textural attributes was investigated.

Methods and Materials

The paneer was prepared according to the standard method as reported by Sachdeva et al. (1991). Fresh and good quality cow milk standardized to Fat/SNF ratio of 1:1.65 was transferred to vessel and heated to 82°C for 5 min and cooled to 70°C. The coagulant (1% citric acid solution at 70°C) was added with gentle stirring till clear greenish whey appeared. Whey was drained off using a pre-cleaned muslin cloth. The coagulum obtained was pressed in a paneer press for 30 minutes. The paneer block obtained was immersed in the chilled water (4°C) for 15 min. The excess moisture was wiped out from the surface of paneer and the paneer block was cut into 2 x 1 cm pieces of equal sizes. This was done in the sterile environment for dipping the paneer pieces into the chitosan solution of different concentrations prepared as below:

The chitosan solutions were prepared according to the method reported by Islam et al.(2011). The edible chitosan purchased from M/s. Himedia Laboratories with a degree of deacetylation greater than 75% was used. The chitosan flakes were weighed accurately in five different beakers for the preparation of five different concentrations of chitosan solutions i.e. 0.2%, 0.4%, 0.6%, 0.8%, and 1%. 40 ml of distilled water was added in each beaker and stirred by a magnetic stirrer to form a solution. The citric acid of food-grade quality purchased from the local market was used for the preparation of 0.1% citric acid solution. 10ml of 0.1% citric acid solution was added in each beaker containing different concentrations of chitosan solution and stirred using magnetic stirrer till a clear solution obtained. The solution obtained was stored at 3°C for further use.

In a laminar airflow sterile environment, the paneer cubes previously cut into standard sizes were dipped for a second into the beakers containing different concentrations of chitosan solutions and taken out using sterile stainless steel forceps. The excess solution was allowed to drain back in a beaker. The coated samples were kept in the petri dishes for further analysis.

The microbial analysis in terms of Total Viable Count (TVC) was carried out to assess the antimicrobial effect of various concentrations of chitosan on paneer samples. The analysis was carried out in a sterile environment by spread plate technique using serial dilutions of 10^{-2},10^{-4} and 10^{-6}. The samples were stored for a week at different temperatures *viz*., 3°C, 20°C, and 37°C and subjected to the enumeration of TVC every day.

Sensory evaluation of paneer samples coated with different concentration of chitosan solution was carried out using the sensory score card method as suggested by Bureau of Indian Standards (IS15346:2003). The trained sensory panel consisted of the faculties of Dairy Science & Technology, Vivekanand College Aurangabad and Department of Agricultural Engineering of MIT College, Aurangabad. The sensory panel judged each sample of paneer independently based on product attributes listed in scorecard viz, flavor, body and texture, color and appearance. The product qualifying score has also been obtained on a nine-point hedonic scale.

The proximate compositional analysis of the coated paneer, as well as control samples, were carried out for moisture, fat, protein, ash, and acidity as per the methods suggested in BIS: SP: 18 (Part XI)-1981.

The texture analysis of the paneer sample coated with 0.8% concentration was done using a texture analyzer (Make: M/s. Brookfield, CT3) with 20 kg load cell and textural responses viz., hardness, springiness, adhesiveness, cohesiveness, gumminess, and chewiness were compared with the control sample.

Statistical Analysis

All experiments were carried out in triplicate and the experimental data were statistically analyzed using Microsoft Office Excel Worksheet 2007. The total viable count, as well as sensory score obtained for different attributes of paneer samples coated with different concentrations of chitosan solutions, were analyzed in terms of its ANOVA using p-value as 0.05 as the cut off for significance.

Results and Discussion

Microbial Analysis

The microbial analysis in terms of the total viable count (TVC) was carried out to assess the antimicrobial effect of various concentrations of chitosan solution on paneer samples. The samples were stored for a week at different temperatures viz., 3°C, 20°C, and 37°C and subjected to the microbial enumeration every day. The effect of different concentrations of chitosan solution on the total viable count of paneer at three temperatures

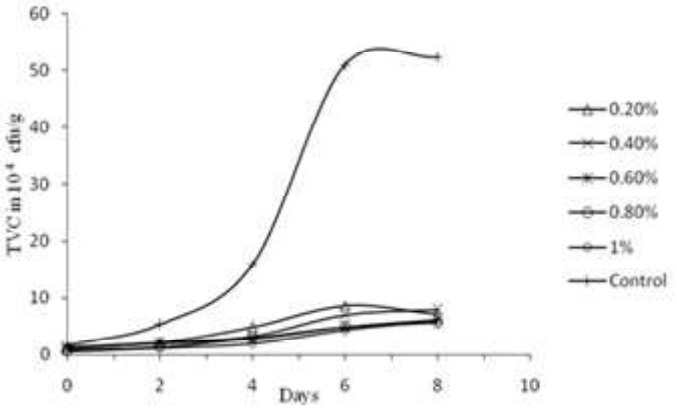

Fig. 1 Effect of chitosan on TVC in paneer at 3°C

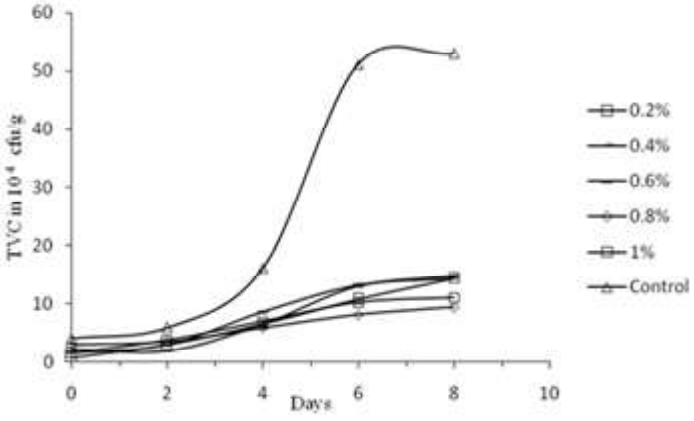

Fig. 2 Effect of chitosan on TVC in paneer at 20 °C

is presented in Fig. 1 to 3. It is observed from Fig 1 that at 3°C the coated paneer samples bear low initial total viable count in 10^4 cfu/g as 1.35, 1.35, 1.08, 0.81, and 0.54 on zero-hour of storage when subjected to different concentrations of chitosan solution. The TVC increased to 7.06, 7.92, 6.17, 5.64, and 5.98 in 10^4 cfu/g on 8th days of storage. However the control sample without any chitosan treatment stored at 3°C temperature showed the initial TVC as 1.62×10^4 cfu/g on zero-hour of storage and increased enormously to 52.26×10^4 cfu/g on eighth days of storage.

Fig 2 shows the effect of different concentrations of chitosan solution on the total viable count of paneer at 20°C. It was observed that at zero hour of storage the total viable count in 10^4 cfu/g in paneer at different concentrations of chitosan solution were 0.81, 2.16, 1.62, 2.97, and 1.62 respectively. Altieri et al. (2005) treated mozzarella cheese with 0.075% chitosan concentration and reported the microbial count as 3.75×10^4 cfu/g on the first day of storage. In the present study, the total viable count in paneer after evaluation on eighth days of storage was observed as 14.32, 14.86, 14.32, 9.45 and 11.08×10^4 cfu/g at 0.2%, 0.4%, 0.6%, 0.8%, and 1% concentration of chitosan solution respectively. The TVC in the control sample of paneer stored at 20°C rose from 4.05 to 52.97×10^4 cfu/g on zero to eight days of storage. It is also observed from Fig 2 that on the eighth day of storage the paneer sample coated with 0.8% chitosan solution showed the least microbial count as 9.45×10^4 cfu/g as compared to other concentrations of chitosan solution and the control sample.

The chitosan treated paneer samples stored at the temperature of 37°C observed visible fungal growth on third days of storage hence considered spoiled. It was reported by Sachdeva and Singh (1990) that mostly aerobic microorganisms are responsible for the spoilage of paneer. Therefore, Fig 3 shows the effect of different concentrations of chitosan on total viable count of paneer at 37°C for consecutive two days of storage. It is observed from Fig 3 that the paneer samples stored at 37°C and coated with different concentrations of chitosan solution showed the initial total viable count in 10^4 cfu/g as 2.16, 1.89, 1.62, 0.81 and 0.81 respectively. On the very second day of storage the total viable count was observed as of 3.24, 3.24, 2.70, 1.89, and 2.16 in 10^4cfu/g. The TVC in the control sample stored at 37°C rose from 2.7 to 7.56×10^4cfu/g from zero to the second day of storage. At 37°C also the paneer sample coated with 0.8% concentration of chitosan solution showed the least total viable count as 1.89×10^4 cfu/g on the second day of storage.

As per the Bureau of Indian Standards (BIS 1983) the standard plate count (SPC) of marketable paneer should be less than 5×10^5 cfu/g. The total viable count of the paneer sample under study was significantly lower than the standards available.

It was also been observed that the yeast and mold count were absent in both treated and untreated paneer samples of paneer at all the temperatures. Ghodekar (1989) reported that the market paneer was contaminated with yeast and mold led to deterioration in the sensory quality of paneer during storage.

It was also observed in the study that the coliform count was nil in all the samples of paneer at all temperatures. Kumar and Sinha (1989) reported that more than 60% of the paneer samples from organized dairies and markets in India were contaminated with coliforms. The chitosan solution also has an inhibitory effect on coliform. Tsai and Su (1999) reported that the E.coli cells in the exponential phase are most sensitive to chitosan. Tripathi et al. (2008) reported chitosan-starch solution shows a stronger inhibitory effect against E. coli and B. subtilis than S. aureus.

From Fig. 1 to 3 it was observed that untreated paneer samples stored at 3, 20 and 37°C temperatures were showing significant increase in TVC which is likely associated with fast spoilage of paneer and less shelf life at all the temperature conditions as compared with chitosan coated paneer samples. Paneer cubes coated with various concentrations of chitosan solution are showing significant slower growth of microbes and low TVC at

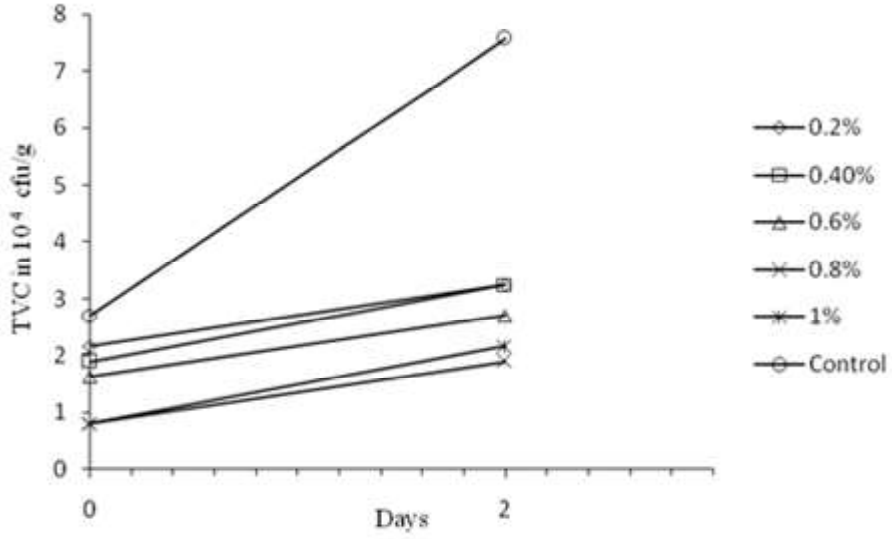

Fig. 3 Effect of chitosan on TVC in paneer at 37°C

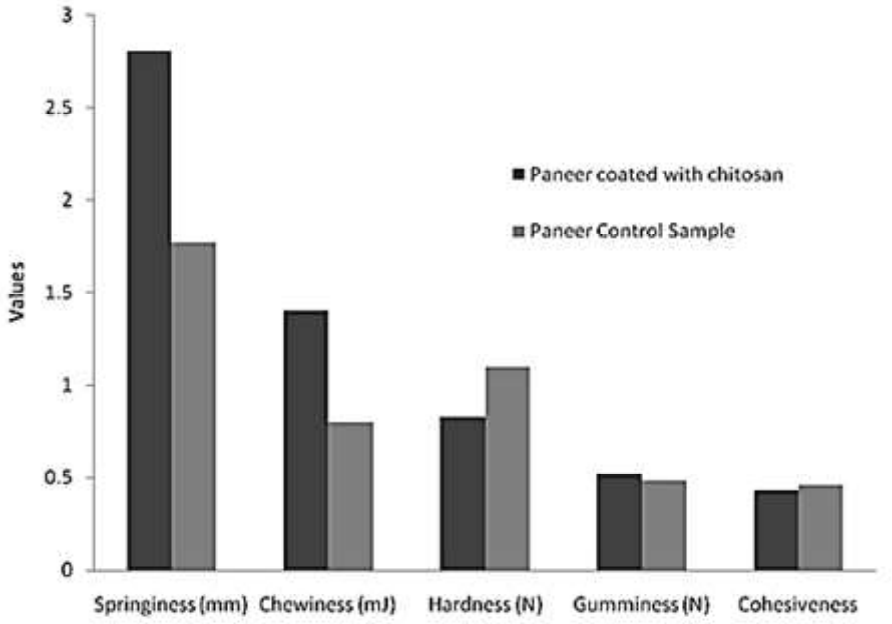

Fig. 4 Textural profile analysis of paneer coated with chitosan

all the three storage temperatures which definitely increases the shelf life of paneer. Hence, the difference in slowing down of microbial growth and slow increase in TVC in treated and untreated samples is likely due to use of chitosan as bio preservative agent in this study. Study results also stand similar with earlier research findings discussed above which have already proven the antimicrobial properties of chitosan and its derivatives against pathogenic and food spoiling microorganisms.

Furthermore, it was observed that at all the three temperatures under study 0.8% concentration of chitosan solution showed the least total viable count. Therefore, it can be inferred that 0. 8% concentration of chitosan has a better effect on the shelf life of paneer.

Sensory Evaluation

The paneer samples coated with various concentrations of chitosan were subjected to sensory evaluation through the expert panel of judges. The results analyzed are presented in table 1. It was observed that there was significant increase in overall sensory score for paneer samples coated with 0.8% chitosan solution. For rest of the other concentrations the sensory score remain insignificant when compared with the control samples. The hedonic scale also indicated the same trends for the overall acceptability of all the samples. It was observed that the chitosan solution of all the concentration helps to retain the overall acceptability of paneer.

Table 1 Sensory score for different attributes obtained by scorecard for paneer samples

Sample coated with chitosan solution	Flavour (Max score = 50)	Body & Texture (Max score = 30)	Colour & Appearance (Max score = 15)	Overall Score (Max score =95)
0.2%	38.8 ± 6.4[a]	24.6 ± 2.78[a]	11.9 ± 1.83[a]	75.3 ± 8.21[a]
0.4%	39.3 ± 4.94[a]	24.3 ± 2.59[a]	12 ± 1.93[a]	76.4±6.36[a]
0.6%	40.8 ± 4.06[a]	24 ± 3.49[a]	12.62 ± 0.86[a]	77.8 ± 6.72[a]
0.8%	40.5 ± 3.87[a]	27 ± 3.04[b]	13.12 ± 1.11[b]	79.7 ±7.01[b]
1%	38.37 ± 4.41[a]	24.5 ± 2.97[a]	13.5 ± 2.96[b]	75.8 ± 6.61[a]
Control Sample	39.75 ± 6.53[a]	24.87 ± 3.04[a]	11.62 ± 2.06[a]	76.5 ± 9.45[a]

Values indicates mean ± SD, different superscript a, b indicates significantly different ($p < 0.05$) from each other, SD = Standard Deviation, n=3, Average of three trials

Table 2 Chemical analysis of paneer samples

Parameters (% wet basis)	Sample coated with 0.8% chitosan solution	Control Sample
Moisture	53.80 ± 0.00[a]	54.54 ± 0.90[a]
Fat	21.53 ± 0.02[a]	22.34 ± 1.5[a]
Protein	16.73 ± 0.01[a]	17.3 ± 0.5[a]
Acidity (% L A)	0.15 ± 0.00[b]	0.12 ± 0.01[a]
Ash content	1.32 ± 0.00[b]	1.64 ± 0.01[a]

Values indicates mean ± SD, different superscript a, b indicates significantly different ($p < 0.05$) from each other, SD = Standard Deviation, n=3, Average of three trials

Chemical Analysis

The compositional analysis of paneer coated with 0.8% chitosan solution was carried out and presented in table 2. The moisture content in chitosan-coated paneer and the control sample were observed as 53.80% and 54.54% respectively. There was no significant difference in the moisture content of the coated paneer samples when compared with control one. These observations are also in line with Shashikumar and Puranik (2011) who reported the moisture content of paneer treated with lactoferrin as 54.50%.

The fat content in coated paneer was obtained as 21.53% and it was 22.34% in the control sample. Sachdeva et al. (1991) reported the fat content in paneer added with calcium chloride as 22.0% which are comparable with the fat content obtained in the present study. Inmaculada (2009) reported that the chelation of metal ions is one of the reasons why chitosan may be considered as a potential natural antioxidant for stabilizing lipid-containing foods to prolong shelf life and therefore it had been used in multiple nutritional supplement products due to its ability to bind fat.

The protein content of the chitosan-coated paneer was obtained as 16.73% whereas it was 17.3% in control sample. However, both the samples were comparable with the protein content reported by Sachdeva et al. (1991) which was 16.9% to 18.4%. There was significant increase in acidity content of the coated (0.15 %LA) paneer when compared with the control one (0.12%LA). The increase in acidity of paneer may be ascribed to the use of citric acid during the preparation of the chitosan solution. Reeta and Kumar (2015) reported the acidity value for paneer as 0.22%. The ash content in the coated paneer was significantly differed (1.32%) than the control sample (1.64%). The nearest value for ash content of paneer (1.40%) prepared from cow milk was reported by Sachdeva et al. (1991). Shahnawaz et al. (2014) also reported the ash content in paneer from 1.8 to 2% using different coagulants.

Texture analysis

The textural profile analysis (TPA) entails the parameters viz., cohesiveness, springiness, gumminess, chewiness, and hardness. The values obtained are presented in Figure 4. From the figure it is observed that the values of cohesiveness for the paneer treated with chitosan solution obtained as 0.43 and that for the control sample it was 0.46. Narayan (2014) reported the cohesiveness values for designer paneer as 0.48 which was comparable with the present study. Kanawjia and Singh (1996) reported the cohesiveness values for paneer as 0.68 measured using the Instron machine with 1000 N load cell.

The chitosan-coated paneer sample in the present study showed the springiness value as 2.81 mm and the control sample gave the springiness value as 1.77. The gumminess of the treated paneer was obtained as 0.48N and for a control sample of paneer it was 0.52N. The chewiness values for chitosan treated paneer and the control sample of paneer were 1.40mJ and 0.80mJ respectively. The chitosan treated paneer showed the hardness values as 1.1N and the control sample had the hardness value as

0.825N. The hardness value is the function of moisture content in paneer (Desai et al. 1991). Since the control sample has higher moisture content as compared to the coated paneer, the control sample was softer as compared to the chitosan-coated paneer. It was concluded from the present study that the selected concentration of chitosan could not affect the textural parameters of paneer. Therefore, chitosan may be considered as the promising material for biopreservation of paneer.

Conclusions

Chitosan is emerging as an important bio-preservative for dairy foods. Microbial and sensory analysis of the paneer sample coated with different concentrations of chitosan solutions were carried out. The paneer samples coated with 0.8 % of the chitosan solution observed to bear lower values of total viable count. From the sensory evaluation it was observed that the chitosan solution of all selected concentrations retained the overall acceptability of the product. However, the overall sensory score of 0.8% chitosan coated paneer achieved significantly increased score. The paneer samples coated with 0.8% chitosan solution subjected to compositional and textural analysis. The acidity and ash content of paneer samples differed significantly when compared with the control one. The textural analysis of 0.8% chitosan coated paneer also been reported. The outcome of the study was an important step towards the development of technologies for enhancing the shelf life of paneer. However, more detailed research study needs to be undertaken to find out precise mechanism involved in inhibition of microbial growth due to chitosan coating of paneer.

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RESEARCH ARTICLE

Quality assessment of milk in supply chain of Parbhani sub-division

Manisha Jawale[1], Anita Chappalwar[2] and Ashok Devangare[3]

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Abstract: Aim of the present study was to detect the common adulterants and physico-chemical properties of milk samples collected from different levels viz. farmer/vendors, collection centre (CC) and milk processing plants (Govt./Co-operative dairy) in Parbhani sub-division (Parbhani, Gangakhed, Jintur, Selu, Purna and Palam taluka). During the study, average minimum and maximum values for water, total solids (TS), fat, solid not fat (SNF), protein and lactose content 79.05%, 10.90% .3.43%, 5.36%, 2.57%, 3.47% 88.88%,12.15%, 11.18%, 9.17%, 4.01%, 5.36% were observed in the milk samples of Parbhani sub division. Statistical analysis revealed significant difference ($P<0.05$) in water, fat, protein content, % acidity and non significant difference in TS, SNF, lactose content, pH value of milk samples collected from farmer/vendors, collection centre and milk processing plants (Govt./Co-operative dairy) of Parbhani subdivision. However, density of milk samples did not differ significantly among both different levels as well as taluka. Most of the compositional parameters were found below the FSSAI standards for buffalo milk. More or less amount of extraneous water was added in all the milk samples of Parbhani subdivision. Adulterants viz, detergents, skim milk powder (SMP), sucrose, nitrate and urea were detected in vendor's milk samples. None of the adulterant was detected in dairy of Parbhani subdivision; however, CC milk samples of Jintur and Purna taluka were only positive for presence of detergent. From the study, it is concluded that adulteration of milk is a generally practiced by vendors which results in the deterioration of nutritive and microbiological quality of milk.

[1] Department of Livestock Products Technology,
College of Veterinary and Animal Sciences Parbhani, Maharashtra Animal and Fishery Sciences University, Nagpur (M.S.) 440006

[2] Department of Livestock Products Technology,
College of Veterinary and Animal Sciences Parbhani, Maharashtra Animal and Fishery Sciences University, Nagpur (M.S.) 440006

[3] Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Udgir, Maharashtra Animal and Fishery Sciences University, Nagpur (M.S.) 440006

Anita Chappalwar (✉)
Dept of LPT, COVAS Parbhani, M.S.-431402
Email: anitachappalwar@gmail.com

Keywords: Adulteration, Dairy, Milk, Milk CC, Parbhani subdivision Vendor's Milk,

Introduction

In India, agriculture is the main stay of living for people, however animal Husbandry and dairying is the subsidiary to agriculture constituting both organized and unorganized dairy sector (75%). As per economic Survey 2020-21, India is a leading milk producer in the world, contributing 198.4 million tonnes of milk, with a growth rate of 5.68 per cent and per capita availability of 407 grams milk per day in 2019-20.

Milk is a product of biological evolution obtained from different species of animal which contributes about 24% of milk from indigenous cows, 55% from buffalo and 21% from cross bred cows (Sunjay 2012). Since time immemorial, milk has been accepted worldwide as food of choice, because of its high biological value. Wholesome milk and milk products are palatable, nutritious, economic and convenient food for human being, growing children and nursing mothers as it contains almost all food ingredients in right proportion and easily digestible form. However unorganized and non-regulated marketing system, hardly maintains the quality of milk upto the consumer level due to adulteration practices.

Water adulteration is the most commonly practiced in India either by addition of ice for lowering the temperature of milk or water with or without soluble substances to increase the SNF/skimming, due to which milk composition does not meet to the FSSAI standards and may pose a great risk to consumers health. However adulteration of milk with contaminated water gradually deteriorates the quality of milk (Izhar and Masud 1991).

Urea also named as carbamide, resin, isourea, carbonyldiamide, and carbonyldiamine (Jenkins et al. 2002) addition is also a common malpractice in the dairy industry to extend the shelf life and whitening, thickening of milk, so that it gives resemblance to rich milk (Walker et al.2004). However urea adulterated milk is low in fat, SNF content and poisonous (toxic) to the human health (Renny et al. 2005). According to ICMR, consumption of urea adulterated milk leads to gradual impairment of the body and cancerous effect on the human system (Fox 1992).

Nitrates are rarely used as a preservative; however presence of nitrate in milk may serve as a confirmatory test for added water. Traces amount of nitrate in milk seems to be due to secretary and post-secretary contamination of milk sample. The secretary contamination occurs via dietary and water intake which is usually of minor significance, while the post-secretary contamination may occur due to addition of natural water containing nitrate. Detergents adulteration enhances the cosmetic nature of milk but creates gastro – intestinal complications (Gawali 2021). Addition of starch is done to increase solid-not-fat (SNF) content of milk, however consumption of milk containing higher amount of starch induces diarrhoea due to accumulation of undigested starch in colon. Its accumulation in the body may be very fatal for diabetic patients (Singuluri et al. 2014). However the hazards caused by milk adulteration are monumental and incalculable which produces a cumulative burden to public exchequer because of treatment and hospitalization. Detection and prevention of adulterant is a demand of the day, therefore the target of the present study was urged to detect milk adulteration at different levels (production to processing) in Parbhani sub-division.

Material and Methods

Collection of milk samples

In a present study, total of 468 Milk samples were collected in the chain of milk production to processing i.e. from milk producers (vendors) before dispatching milk to collection centres, different collection centres and chilling/ processing centers (CC) and govt/ cooperative dairy of Parbhani subdivision (Parbhani, Gangakhed, Jintur, Selu, Purna and Palam). The collected milk samples were analyzed for compositional parameters, physicochemical properties and adulterants. Twenty six milk samples were collected from a source i.e. a taluka [Vendors (24), CC (1) and Dairy (1)] of Parbhani sub-division and replicated thrice for all talukas of Parbhani sub-division. Each sample was collected in 250ml capacity sterilized glass bottle, labelled and brought immediately at 4°C by using icebox to the laboratory of COVAS, Parbhani for further analysis.

Analytical methods

Compositional parameters

Water, total solids and protein content of milk was calculated according to the methods of AOAC 2000 and percent acidity according to AOAC 1990. The lactose content in the milk sample was determined according to the method of BIS 1981. Fat content of milk was determined using Gerber's method, however solids not fat (SNF) content was determined by difference as reported by (Harding 1995).

SNF content (%) = TS (%) – Fat (%)

pH

The pH of milk was determined by using a digital pH meter (model -920 LMPH-12). Density of milk samples was calculated by using milk analyzer Lactoscan SL (Manufactured by milkotronic Ltd., Bulgaria).

Added Water

Water adulteration in milk was detected by using milk analyzer Lactoscan SL (Manufactured by milkotronic Ltd., Bulgaria). Adulterants-formalin, nitrates, urea, starch, sucrose, detergent, skim milk powder, caustic soda in milk samples were detected as per the manual of methods of Analysis of Foods, Milk and Milk Products (FSSAI 2012). Prevalence study for different adulterants was calculated as percentage of positive milk samples collected from the chain of production to processing in Parbhani sub division.

Statistical analysis

Statistical analysis of data obtained during the study was done by analysis of variance using Completely Randomized Design (CRD) and Random Bulk Design (RBD) as suggested by Snedecor and Cochran (1989).

Results and Discussion

Physicochemical properties of milk samples

The data regarding physico-chemical properties of milk samples collected from different levels in Parbhani sub division presented in table 1.

Table 1 revealed, similar water content in vendors and dairy milk samples which was differed significantly ($P<0.05$) with CC milk samples. Similar non-significant difference for water content observed between Jintur, Gangakhed, Palam, taluka, while water content of milk samples collected from Parbhani, Selu, Purna taluka differed significantly ($P<0.05$) from all taluka of Parbhani sub-division. There was gradual decline in the water content of Jintur taluka milk from vendors to dairy. However Selu, Purna and Parbhani taluka CC milk contains less water followed by vendors and then dairy milk. Contrarily water content of Parbhani taluka milk declined from vendors to dairy and then to CC. The study showed minimum (79.05%) and maximum (88.88%) water content in CC milk samples of Purna and Gangakhed respectively. This might be due to wide water adulteration practices carried out by different vendors in wide range to increase volume and profit, which was supplied at CC and dairy. However lower results were recorded by Chappalwar et al. (2014) for vendors milk samples collected from four different areas of Parbhani city.

There was non-significant difference in the total solid content of milk samples collected from different levels as well as between Jintur, Palam and Gangakhed taluka of Parbhani subdivision. In contrast TS content of Parbhani, Selu and Purna taluka milk

samples differed significantly (P<0.05) with the values of each taluka. Dairy milk sample of Parbhani taluka showed higher TS content followed by Jintur, Gangakhed and Palam taluka, the values for dairy were higher than the vendors and CC milk sample of respective talukas. Higher TS content was observed for CC milk samples of Selu, Purna taluka and lower for Palam and Gangakhed taluka than other levels. However there was increasing trend in TS content of Jintur and Parbhani taluka milk samples from vendors to dairy. The present findings are in agreement with the Shaikh et al.(2013) noted more total solids (13.8±0.4%) in the milk samples collected from surrounding areas than sale points of Hyderabad city (13.56±0.1%). Lower TS content in water adulterated milk is very oblivious, however higher TS content might be due to addition of soluble substances to increase the SNF/skimming.

There was no significant difference in the milk SNF content between vendors, CC and dairy milk samples of Parbhani sub-division, however the milk SNF content of Gangakhed and Selu taluka differed significantly (P<0.05) from SNF content all talukas milk. There was no significant difference in the SNF content of Palam and Jintur taluka as well as Parbhani and Purna taluka milk. Solid not fat content was found lower in Jintur, Gangakhed, Selu and Palam CC milk, while highest in dairy milk of Parbhani and Purna taluka. In Selu taluka, vendor's milk sample had higher SNF content than CC and dairy. Contrarily Parbhani taluka vendor's milk sample had lowest SNF content than other sources. Least (5.37%) and highest (9.76%) SNF content recorded in Jintur and Purna taluka dairy milk respectively. Irrespective of milk collection source, SNF content of Jintur, Palam, Gangakhed and Selu taluka milk samples was far away from the standards. Similarly SNF content sample of Selu taluka CC milk was also lower than standard. The findings are in close agreement with Chappalwar et al. (2014) who noted lower SNF content in the milk samples collected from different areas of Parbhani city.

The data obtained during the study revealed that the fat% in milk samples collected from different levels i.e. Vendor, CC and Dairy of Parbhani sub-division differ significantly (P<0.05). Fat percentage of Jintur and Selu taluka milk sample did not differs significantly, while fat % in milk sample of Palam, Gangakhed, Purna and Parbhani taluka differs significantly (P<0.05) with all taluka of Parbhani sub-division. It is also noticed from the table that irrespective of source, milk fat percentage of Palam and Gangakhed taluka milk samples was below the standards. Whereas in Jintur taluka, vendors and CCs milk samples collected had fat percentage lower than the FSSAI standard. Highest (11.18%) and lowest (3.42%) fat percentage noted respectively in Purna and Selu taluka dairy milk samples. Variation in fat percent of milk sample might be due to addition of water/skimming/ mixing of cow milk with buffalo milk. Prasad et al. (2018) observed 6.42, 4.06 and 3.59 % fat respectively in buffalo, cow and goat milk samples collected from Allahabad City of India. However Chappalwar et al. (2014) observed fat percentage in the range of 6.83-7.5% for the milk samples collected from different areas of Parbhani city.

The protein content of dairy milk samples of Parbhani sub-division were differed significantly (P<0.05) from vendors and CC, but the mean values of vendors and CC did not differ significantly amongst each other. Protein content of Jintur and Gangakhed taluka milk sample were significantly (P<0.05) higher than other talukas. However, protein content of Selu taluka milk samples were differed significantly (P<0.05) from milk samples of other talukas. Milk samples of Selu, Palam, Purna taluka had Slightly lower milk protein content however the protein content of Jintur and Gangakhed taluka had noticeable lower protein content than the standard. Highest protein content i.e. 4.01 noted in dairy milk sample of Palam taluka, while lowest content i.e. 2.58 in CC milk sample of Selu taluka. Results are in close agreement with Shoaib et al. (2010) and Chappalwar et al. (2014) who found slightly lower protein content in the milk samples collected from different areas of Parbhani city. Lower protein content in milk samples of Parbhani subdivision might be due to addition of water/ mixing of cow milk with buffalo milk.

Lactose content in different levels i.e. vendor, CC and dairy milk sample of Parbhani sub-division differs non-significantly. Lactose content values of Selu taluka milk sample were differed significantly from the lactose content values of all taluka. Amongst all taluka lactose content was highest in CC milk samples of Parbhani taluka i.e. 5.43, whereas least values in Jintur dairy samples i.e. 3.47 which is slightly lower than standard value. Present findings are similar with the result noted by Shaikh et al. (2013) and Chappalwar et al. (2014) who found 4.9% and 3.91-4.52 % lactose content in market milk samples respectively.

Acidity and pH is the indicator of milk quality and hygiene. Result showed significant difference (P<0.05) in the percent acidity of milk sample collected from vendors (0.15%), CC (0.16%) and dairy (0.17%), of Parbhani subdivision. It is confirmed from the taluka wise data that the acidity (%) of Palam taluka milk sample differed significantly (P<0.05) from other talukas of Parbhani sub-division. Least acidity recorded in vendor's milk of Jintur (0.15%) and Palam (0.14%) taluka which is one of the characteristic of fresh milk. Acidity of all milk samples showed increasing trend from vendors to dairy. Increase in milk acidity (%) from vendor to dairy is obliviously due to development of lactic acid in milk samples, which might results due to increase in storage time, temperature and mishandling of milk from the point of production to dairy. The results are also in close agreement with Chappalwar et al. (2014) who noted milk acidity in the range of 0.15 to 0.17%.

It is clear from the table that the pH values declines from vendor's milk sample to dairy milk sample which goes towards acidic condition which was correlated with acidity value. The milk pH value did not differ significantly between Gangakhed, Selu and Palam talukas as well as between Purna and Parbhani taluka,

while the milk pH value of Jintur taluka differed significantly ($P<0.05$) from pH values of all talukas. Walstra et al. (2006) noted that the milk pH should not be <6.6 or >6.8 when milk temperature is 20°c. Similarly, pH readings were reported as between 6.44 - 6.99 by Miloga et al. (2010). However, there was no significant difference in milk density values of Parbhani sub-division. Jintur and Purna dairy milk samples had highest density value than vendor and CC. While CC milk sample of Parbhani taluka were

Table 1 Physico chemical properties of milk samples collected from Parbhani sub-division

Milk Collection Centers	% Milk samples						Treatment Mean
	Parbhani	Jintur	Gangakhed	Selu	Palam	Purna	
			Water				
Vendors	82.39	88.78	88.66	86.00	88.48	82.15	86.07^{a}
CC	80.08	88.60	88.88	84.21	89.09	79.05	84.98^{b}
Dairy	81.55	87.03	87.62	87.03	87.97	87.84	86.50^{a}
Taluka Mean	81.34^{c}	88.13^{a}	88.38^{a}	85.75^{b}	88.51^{a}	83.01^{d}	
			Total solids				
Vendors	13.55	11.37	11.33	13.99	11.52	17.84	13.27^{a}
CC	13.59	11.39	11.12	15.78	10.90	20.94	13.95^{a}
Dairy	18.24	12.97	12.17	11.65	11.65	12.15	13.13^{a}
Taluka Mean	15.12^{b}	11.88^{d}	11.54^{d}	13.31^{c}	11.36^{d}	16.98^{a}	
			Solid not fat				
Vendors	8.81	6.32	7.48	8.97	6.97	8.89	8.07^{a}
CC	8.88	6.47	7.35	7.76	6.89	8.67	7.83^{a}
Dairy	9.17	5.36	8.05	8.22	7.34	9.76	7.98^{a}
Taluka Mean	8.95^{a}	6.72^{d}	7.63^{c}	8.32^{b}	7.07^{d}	9.11^{a}	
			Fat (%)				
Vendors	8.67	3.89	3.85	5.02	4.54	8.95	5.82^{c}
CC	8.44	3.91	3.76	8.02	4.01	8.51	6.11^{b}
Dairy	9.27	7.61	4.12	3.43	4.68	11.18	6.71^{a}
Taluka Mean	8.79^{b}	5.13^{c}	3.91^{e}	5.49^{c}	4.41^{d}	9.54^{a}	
			Protein				
Vendors	3.65	2.68	2.76	3.59	3.09	3.31	3.18^{b}
CC	3.73	2.73	2.70	2.58	3.37	3.71	3.14^{b}
Dairy	3.81	2.57	2.88	3.31	4.01	3.81	3.40^{a}
Taluka Mean	3.73^{ba}	2.66^{d}	2.78^{d}	3.16^{c}	3.49^{a}	3.61^{a}	
			Lactose (%)				
Vendors	5.18	3.99	4.17	4.71	4.16	5.27	4.58^{a}
CC	5.43	4.16	4.03	4.23	4.08	5.21	4.52^{a}
Dairy	5.36	3.47	4.28	4.51	3.75	5.33	4.45^{a}
Taluka Mean	5.32^{a}	3.87^{d}	4.16^{c}	4.49^{b}	4.00^{cd}	5.27^{a}	
			Acidity				
Vendors	0.160	0.150	0.163	0.160	0.143	0.163	0.157^{c}
CC	0.170	0.163	0.173	0.167	0.150	0.173	0.166^{b}
Dairy	0.190	0.173	0.183	0.180	0.170	0.170	0.178^{a}
Taluka Mean	0.173^{a}	0.162^{ab}	0.173^{a}	0.169^{a}	0.154^{b}	0.169^{a}	
			pH				
Vendors	6.93	6.03	6.23	6.13	6.30	6.90	6.42^{a}
CC	6.80	6.03	6.23	6.33	6.20	6.66	6.37^{ab}
Dairy	6.72	5.96	6.17	6.26	6.16	6.48	6.29^{b}
Taluka Mean	6.81^{a}	6.01^{c}	6.21^{b}	6.24^{b}	6.22^{b}	6.68^{a}	
			Density				
Vendors	1018	1018	1028	1029	1025	1018	1023^{a}
CC	1028	1025	1026	1014	1024	1028	1024^{a}
Dairy	1027	1026	1025	1027	1022	1029	1026^{a}
Taluka Mean	1024^{a}	1023^{a}	1026^{a}	1023^{a}	1024^{a}	1025^{a}	

Overall means bearing different superscripts between rows (A, B, C, D.) differ significantly ($P<0.05$)

found with higher density values than vendor and dairy. Lowest milk density value 1018g mL-1 recorded in Parbhani, Jintur and Purna taluka vendor's milk sample however highest values for dairy of Purna taluka i.e.1029. Chappalwar et al. (2014) and Mihaela et al. (2011) also found respectively 1022 to 1026g mL-1 and 1.029 g/cm^3 density values Sibiu dairy farm and Parbhani city market milk. Slight variation milk density and values water adulteration observed which might be due to the effect of skimming or addition of different adulterants.

Extent of common adulterants in milk samples of Parbhani sub-division

Table 2 Added water (%) in milk samples collected from Parbhani sub-division

Milk Collection	Added water (%)						Treatment
Centers	Parbhani	Jintur	Gangakhed	Selu	Palam	Purna	mean
Vendors	2.46	11.20	6.13	0.90	14.16	1.01	5.96^b
CC	0.20	15.90	8.55	0.77	8.22	0.00	5.60^b
Dairy	2.33	2.87	0.75	0.25	5.21	2.36	2.29^a
TalukaMean	1.66^c	9.99^a	5.14^b	0.64^c	9.19^a	1.12^c	

Overall means bearing different superscripts between rows (a, b, c, d.....) differ significantly (P<0.05)

Table 3. Milk adulteration status in Parbhani subdivision

Milk Collection	% Milk samples					
Centers	Parbhani	Jintur	Gangakhed	Selu	Palam	Purna
			Formalin			
Vendors	-	-	-	-	-	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Nitrates			
Vendors	-	16.66	4.16	-	12.5	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Urea			
Vendors	-	13.88	1.38	4.16	-	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Starch			
Vendors	-	-	-	-	-	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Sucrose			
Vendors	-	-	5.55	8.33	5.55	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Detergent			
Vendors	18.50	31.94	4.16	16.6	22.22	44.44
CC	-	100	100	-	-	-
Dairy	-	-	-	-	-	-
			Skim milk powder			
Vendors	-	-	4.16	-	-	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-
			Caustic soda			
Vendors	-	-	-	-	-	-
CC	-	-	-	-	-	-
Dairy	-	-	-	-	-	-

Adulteration of milk with Water

All the milk samples of Parbhani- subdivision were analyzed to detect extent of water adulteration and obtained results presented in table 2. Result showed that water adulteration in dairy milk samples differs significantly ($P<0.05$) with other sources, but not among vendors and CC milk samples of Parbhani sub-division. There was non-significant difference in water adulteration of milk sample between Parbhani, Selu and Purna as well as between Jintur and Palam talukas. However, Jintur and Palam taluka milk samples had significantly ($P<0.05$) higher water addition than the Parbhani, Selu and Purna taluka. Water adulteration significantly ($P<0.05$) differed in Gangakhed and all other talukas milk samples of Parbhani sub-division. There was declining trend in water adulteration from vendors to dairy milk sample of Palam and Selu taluka, indicating that vendors adulterate milk with higher amount of water for their personal benefit. While the CC milk samples of Jintur and Gangakhed taluka have slightly higher water adulteration than vendors and dairy milk samples. Contrarily slightly lower water adulteration was found in the CC milk samples of Parbhani taluka than vendors and dairy milk samples. Purna taluka showed 1.01% and 2.36% water adulteration in vendors and dairy milk sample respectively, however none of the milk sample from CC adulterated with water. Highest water adulteration i.e.15.90% observed in CC milk sample of Jintur taluka in Parbhani subdivision. Chappalwar et al. (2014) also detected water as common adulterant in the market milk samples of Parbhani city. However, Sudarshan et al. (2011) noted 3.33%, 13.33% and 90% water adulteration in cooperative, organized private dairy and vendor's milk sample collected in and around greater Hyderabad municipal corporation respectively. Ali et al. (2012) also detected 5.6 to 47.9%. 4.2 to 47.9% and 5.1 to 32.6% added water in dairy farms, street vendors and dairy shops milk samples of Assiut governorate respectively. While Swathi and Naazia (2015) reported 100% water adulteration in milk samples collected from traditional vendors in Telangana State. In India, water adulteration of milk is commonly practiced to increase profit by increasing volume, the practice not only decreases nutritive value of milk but also increases the risk to consumer's health, if added water is contaminated.

Adulteration of milk with common adulterants

Present study showed the presence of detergent in 31.94%, 4.16%, 18.05%, 16.6%, 22.22% and 44.44% vendors milk samples of Jintur, Gangakhed, Parbhani, Selu, Palam and Purna taluka respectively, while all milk samples of CC in Jintur and Gangakhed taluka found positive for detergents adulteration. Dairy milk samples collected from Parbhani sub-division were free from detergent. Similarly Hemanth and Sukumaran (2014) found detergent adulteration in 44% of milk samples. Ghulam et al. (2014) detected detergent as an adulterant in 32% milk samples and found the risk of adulteration at dairy shops significantly higher than milk collector and milk producer. Prevalence rate of the detergent varies with different level and taluka of Parbhani subdivision which might be either due to mixing of milk sample collected from different sources at CC and dairy or lack of hygiene and sanitation. Whereas Kandpal et al. (2012) noted poor quality milk at Jolly grant, Dehradun due to presence of detergent as common adulterant in all milk samples.

It is noticed from the result outlined in Table 3 that urea is used as an adulterant in 13.88% vendors milk samples of Jintur, in 1.38% sample of Gangakhed and in 4.16 % sample of Selu taluka. Similarly Ghulam et al. (2014) detected urea adulteration in 10% milk samples and found the risk of adulteration at DS significantly higher than MC and M). Whereas Shaikh et al. (2013) found urea adulteration in 25% and 10% milk samples of Hyderabad city and surrounding areas respectively. Milk samples collected from vendors of Palam, Purna, Parbhani taluka, as well as collection centre and dairy of Parbhani subdivision found negative for presence of urea. Results are in agreement with Eman et al. (2012) who also found absence of urea in all tested milk samples. Urea detection in milk sample may be due use of urea as an adulterant to increase shelf life and solid not fat value /its total nitrogen content.

The result indicated very less percentage of milk samples with sucrose adulteration i.e. 5.55 %, 8.33% and 5.55% in vendor's milk sample of Gangakhed, Selu and Palam taluka respectively. It is interesting to note that rest of the samples were tested negative for the presence of sucrose. Similarly Ghulam (2014) recorded cane sugar adulteration in 14% milk samples respectively. However Nirwal et al. (2013) detected glucose adulteration in 80% milk samples.

Skim milk powder was absent in all different levels milk sample (vendors, CC and dairy) of Parbhani subdivision except in Gangakhed vendors milk sample (4.16 %). Results are in agreement with Gahlawat et al. (2012) who noted absence of SMP in market mlk. Contararily Thakre (2014) detected SMP in skim milk of Akola city.

The study indicated nitrate presence in 16.66%, 4.16%, 12.5% vendor's milk samples of Jintur, Gangakhed and Palam taluka of Parbhani sub-division respectively. Presence of nitrate in milk samples might be due addition of natural water in milk. The test was negative for presence of an adulterant nitrate in rest of the milk sample collected from different levels of Parbhani subdivision. Similarly Ali et al. (2012) also observed nitrate adulteration in 12.5% buffalo's milk samples of dairy shop and 17.5% of street vendors.

It is interesting to note that cent percent screened milk samples were found to be negative for formalin, starch and caustic soda adulterants. Results with respect to formalin are in agreement with Ali et al. (2012) who also noted that all dairy farms milk

samples were free from caustic soda. While Ghulam et al. (2014) detected starch adulteration in 12% milk samples of Mirpurkhas city in Pakistan.

Conclusions

Intentionally adulteration of milk practiced during production to processing to increase the benefit. Milk handling is a barrier in milk processing, mishandling and storage of milk changes the pH value and acidity of milk. Common adulterants viz., water, detergent, urea, nitrates and skim milk powder are added by milk producers which alter the composition, density of milk and does not accomplish FSSAI standards. Adulteration practices generally practiced in milk industry for personal benefit which reduces the nutritional value of milk and causes human health hazards.

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RESEARCH ARTICLE

Molecular characterization of α-lactalbumin (LALBA) protein in Indian buffalo (*Bubalus bubalis)*

Vinay Kumar Mehra, Dhruba Malakar and Satish Kumar

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Abstract: The full open reading frame (ORF) of the buffalo α-lactoglobulin (Bu_LALBA) gene was characterized. Results showed that Bu_LALBA ORF consisted of 429 bp long (142 aa residues) with three nucleotide variations at 111bp, 147 bp, and 291 bp but no change in amino acid sequence. The MSA showed that the Bu_LALBA was different from the pig and human LALBA sequences. The phylogenetic tree showed that the cow, yak, and buffalo formed one cluster, while the buffalo was closer (94%) to domestic yak (*Bos grunniens*). Results of ExPASy analysis showed that the Bu_LALBA protein was acidic (pI, 4.81), thermo-tolerant, and hydrophilic. However, the presence of random coil (33.80%) and α-helix (41.5%) in Bu_LALBA protein suggest that the protein was flexible and thermostable. Thirty liner motifs were identified, indicating that the Bu_LALBA act as regulatory protein. The tertiary structure of Bu_LALBA predicted by I-TASSER showed a more stabilized nature of LALBA protein. Further, the Ramachandran plot validated the 3-D structure of Bu_LALBA, which was of decent quality. The presence of four ligand-binding sites in Bu_LALBA (calcium ion, glycine, N-acetyl-L-glutamate, and N-acetylglucosamine) proposed that the LALBA binds to several fatty acids and ions. The presence of four serine, four threonine, two tyrosine residues, and six methylated lysine and five acetyl-lysine sites in Bu_LALBA indicated that the protein was involved in post-translational modification processes. IEDB analysis showed the presence of five and one epitope sites in Bu_LALBA protein for B-cell and T-cell, respectively, which suggest that this protein has certain immunological roles.

Keywords: Buffalo, Milk, α-lactalbumin, Protein structure, Molecular

Introduction

Buffalo milk is a rich source of vitamins, minerals, lipids, carbohydrates, and amino acids, which constitutes a complete food for the neonates and children. Majority of farm ruminant's milk contains approximately 80% as casein proteins (αS1, αS2, β, and κ) and 20% as whey proteins (β-lactoglobulin, α-lactalbumin, serum albumins) (Hoffman J R and Falvo M J 2004). These milk constituents vary across various cattle and buffalo breeds and among the individuals within the breed. β-lactoglobulin (βLG or LGB) and α-lactalbumin (LALBA) accounts for about 9% and 3% of the total milk protein in cattle, respectively (Ding et al. 2011). whey milk of cattle constitutes a mixture of βLG (~65%), LALBA (~25%), bovine serum albumin (~8%), several immunoglobulins and other components (~2%) (Haug et al. 2007). On the other hand, women milk contains approximately 60% of whey proteins and 40% of casein proteins (Luhovyy et al. 2007). The women milk comprises of caseins, α-lactalbumin, lactoferrin, immunoglobulin IgA, vitamins, lysozyme, and serum albumins (Lönnerdal B 2004), but absent the βLG protein in human, lagomorph, and rodent milk (Sawyer L and Kontopidis G 2000; Picariello et al. 2019).

The α-lactalbumin (LALBA) constitutes about 22% and 3.5% of the total milk protein in human and bovine milk, respectively (Layman et al. 2018). The LALBA protein is involved in lactose synthesis, hence it facilitates the production and secretion of milk in the mammary gland (Layman et al. 2018). The LALBA protein binds to divalent cations (Ca^{+2} and Zn^{+2}), where it promotes the absorption of essential minerals and provides a well-balanced supply of essential amino acids to the growing infants. In cattle and buffalo, the LALBA gene is located on chromosome 5, which consisted of about 3061bp long sequence

Animal Biotechnology Centre, ICAR-National Dairy Research Institute, Karnal -132 001, India

Vinay Kumar Mehra (✉)
Animal Biotechnology Centre, ICAR-National Dairy Research Institute, Karnal -132 001, India
Email: vinay28mehra@yahoo.com

with 4 exons (Ensembl database). It has been reported that the cattle have two genetic variants of the LALBA gene i.e. A and B. Variant A differs from the variant B due to a change of arginine into glutamine at 10th position of the protein (i.e. glutamine in variant B) (Mitra et al, 1998). Further, several genetic polymorphisms in the LALBA gene have been reported in goat, pig, and buffalo (Yang et al, 2019). Thus, the single nucleotide polymorphisms (SNPs) may change the expression of the LALBA transcript and alter the yield and quality of the buffalo milk (Ramesha et al. 2008; Manzoor et al. 2020).

The safety and quality standards of milk used for human consumption need to be assessed before the commercialization of milk and milk products. The safety aspects of milk are necessary for improving the health of milk consumers. Therefore, the molecular characterization of milk α-lactalbumin protein is necessarily required for improving the quality of milk and milk products. Although various milk protein constituents have been analyzed in buffalo milk, the information on structural characterization of LALBA protein in Indian buffalo is lacking. Therefore, the present study was undertaken to characterize the nucleotide and protein sequences and analyze certain physiological attributes such as α-helices, β-turn, different motifs, tertiary structure, post-translational modification, and antigenic behavior of the LALBA protein in Indian buffalo.

Materials and Methods

RNA isolation and cDNA synthesis

Buffalo mammary gland tissue was obtained from the slaughterhouse and the mammary epithelial cells (BuMEC) were isolated as per the protocol described by Anand et al. 2012 with minor modifications. Total RNA was prepared from buffalo mammary tissue and BuMECs using the TRIzol method (Invitrogen, USA). The purity of RNA was checked through a nanodrop spectrophotometer by using optical density (OD) at 260 nm and 280 nm. The cDNA was prepared using a RevertAid first-strand cDNA synthesis kit (Thermo Scientific, USA). Briefly, 10 ng RNA was reverse transcribed using 1µL M-MuLV reverse transcriptase (200 U/µL), 1µL RiboLock (20 U/µL), 2µL 10 mM dNTPs mix, 1µL oligo dT primer, and 4µL 5X reaction buffer in 20 µL reaction volume. The quality of cDNA was ascertained by agarose gel electrophoresis using GAPDH primers.

Primer designing and PCR amplification

The primers for buffalo LALBA were designed using Primer3 software based on the conserved sequences of cattle, sheep, and goat through multiple sequence alignment (MSA). These primer pairs (LALBA forward: 5'-GGGGTAACCAAAATGATGTCC-3', LALBA reverse: 5'-GCACCCCTGGAGATTAGTCC-3') were designed to flank the full coding region (ORF) of the LALBA gene. The buffalo LALBA gene was amplified using gene-specific primers in Gene Pro Thermal Cycler (BIOER, China). The PCR reaction components were as follows; 1x PCR buffer, 1.66 U Taq DNA polymerase (5U/µl), 1.5mM $MgCl_2$, 250µM dNTPs mix (10mM), 4µl cDNA, and 0.5µM of each forward and reverse primer in 25µl reaction volume.

Gene cloning and sequence analysis

The PCR products of the LALBA gene were purified by using the QIAquick Gel Extraction Kit (QIAGEN) and cloned in pJET1.2 blunt cloning vector (Thermo Scientific, USA). The PCR products with 32 -dA overhangs were blunted with a blunting enzyme and then ligated to a linearized pJET1.2 blunt cloning vector. The recombinant plasmid DNA was transformed into Top10 *E. coli* competent cells. The single transformed *E. coli* colony was streaked on LB agar plate and subjected to colony PCR using a gene-specific primer. The amplified PCR products were resolved on 1.5% agarose gel and the representative bacterial clone showing the positive bands were transferred into LB broth. The plasmid DNA was purified by using QIAprep Spin Miniprep Kit (QIAGEN) and sequenced through Sanger sequencing. The acquired Bu_LALBA gene sequence was submitted to NCBI and the Gene Acc. No viz. MT130465 was received. Further, the Bu_LALBA sequence was analyzed through the Bio-Edit tool and translated into a protein sequence by 'Sequence Manipulation Suite' (www.bioinformatics.org). Phylogenetic analysis of the Bu_LALBA gene was performed by using MEGAX software (version 10.1.5) to determine the evolutionary relationship between closely related species.

Primary and secondary structure of Bu_LALBA protein

The physicochemical properties of the protein sequence of Bu_LALBA were analyzed by using ExPASy-ProtParam tool (http://web.expasy.org/protparam/) (Gasteiger et al. 2003), which computes the number of amino acids and its composition, theoretical isoelectric point (pI), molecular weight, grand average of hydropathicity (GRAVY), instability index, and aliphatic index. The secondary structure of Bu_LALBA protein was examined through SOPMA (Self-Optimized Prediction Method with Alignment) server (Geourjon C and Deleage G 1995) which computes the percentage of α-helices, β turn, and β-sheet in the protein.

Prediction of the eukaryotic linear motif (ELM)

The eukaryotic linear motif prediction tool (Gouw et al. 2018) was used to examine the Bu_LALBA protein sequence to match the regular expressions defined in the ELM tool. This tool provides a wide range of functionality to the proteins which play a crucial role in cell regulation if any.

Homology modeling and validation of Bu_LALBA protein

The 3-D structure of Bu_LALBA protein was predicted through the I-TASSER (Iterative Threading ASSEmbly Refinement) server. I-TASSER predicts a structure based on the confidence score (C-score) and template modeling score (TM-score). I-TASSER acknowledges the normalized B-factor for structure prediction. The more negative value of the B-factor reveals more stability of the protein structure (Zhang Y 2008). To validate the I-TASSER model, the Bu_LALBA protein model was predicted through SWISS-MODEL software (Waterhouse et al. 2018), which produces the 3-D structures based on the homology template from the PDB. Further, the predicted structure of Bu_LALBA protein was validated through the Ramachandran plot by PROCHECK server (Laskowski et al. 1993) to visualize the energetically allowed regions for backbone dihedral angles ψ against φ of the amino acid residues of the protein structures.

Prediction of phosphorylation, glycosylation, methylation, and acetylation, and immunological sites

The different phosphorylation sites of Bu_LALBA protein was predicted by NetPhos 3.1 Server (Blom et al. 1999). The glycosylation sites of Bu_LALBA protein were determined through NetNGlyc 1.0 Server (Blom et al. 2004) and the potential methylation and acetylation sites of Bu_LALBA protein were also predicted by *in-silico* PLMLA (Prediction of lysine methylation and lysine acetylation) tool (Shi et al. 2012). Immunological sites play an important role in determining the antigenic nature of the protein. Hence, the B-cell and T-cell linear epitopes for Bu_LALBA protein were predicted through the IEDB tool (Larsen et al. 2006; Paul et al. 2014).

Results and Discussion

Sequence analysis of Bu_LALBA gene and construction of the phylogenetic tree

The full ORF of the Bu_LALBA gene was amplified and produced the 604 bp PCR product. Results showed that the Bu_LALBA ORF consisted of 429 bp long sequence with three base pair changes at 111bp (TAC to TAT), 147 bp (ACG to ACA), and 291 bp (GAC to GAT) as compared to published buffalo sequence in Figure 1(A), but no change was observed in amino acid sequence in Figure 1(B).

The MSA of Bu_LALBA protein was carried out with domestic yak, exotic cow, sheep, goat, pig, and human, which is shown in Figure 1B. Although, the amino acid sequence of Bu_LALBA showed higher similarity with the published sequences of buffalo as shown in GenBank. The Bu_LALBA protein sequence (mature portion: 123aa residues) was similar to the cattle LALBA sequence, but it was more different from the human and pig sequence as shown in Figure. 1B. It was indicated that the buffalo LALBA amino acid sequence was more variable from the pig and human sequences. The acquired nucleotide sequence of Bu_LALBA was analyzed for homology through NCBI BLASTN tools. The nucleotide sequence of the Bu_LALBA gene showed 99.77% identity with published buffalo sequence. Further, the Bu_LALBA mRNA sequence similarity with Exotic cow (*Bos taurus),* Domestic yak (*Bos grunniens*), Sheep (*Ovis areas*), Goat (*Capra hircus*), Pig (*Sus scrofa*), and Human (*Homo sapiens*) was about 98.83%, 98.60%, 97.67%, 97.20%, 85.15%, 84.92% respectively. It is concluded that the Bu_LALBA has more identity with an exotic cow in the study.

The phylogenetic tree was constructed based on buffalo LALBA gene sequence against the LALBA gene of Cattle (*Bos taurus),* Sheep (*Ovis aries*), Goat (*Capra hircus*), Yak (*Bos grunniens*), Pig (*Sus Scrofa*) and Human (*Homo sapiens*) using MEGAX program. Phylogenetic tree in Figure 2(A) showed that the cow, yak, and buffalo formed a cluster with 100% similarity, while buffalo was closer to domestic yak (94%). The sheep and goat formed another cluster (98%) showing a close relationship with each other. However, humans and pigs were placed as an out-group in a tree. The results of the present study indicated that the LALBA gene has more recumbences with exotic cow and domestic yak; therefore the buffalo LALBA protein may have more similarity with cow and yak protein rather than the sheep, goat, pig and human protein.

Evaluation of primary structure

The primary structure of Bu_LALBA protein was evaluated for the number of amino acids, molecular weight, iso-electric point

Table 1 Physicochemical characteristics of Bu_LALBA protein

Physicochemical		Parameters
Bu_LALBA		
Number of amino acids		142
Theoretical pI		4.81
Molecular weight		16304.64
Instabiliy index		28.77
Aliphatic index		91.27
Grand average of hydropathicity (GRAVY)	-	0.191
Total number of negatively charged residues (Asp + Glu)		21
Total number of positively charged residues (Arg + Lys)		13

(pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY) as shown in Table 1. The full coding region of Bu_LALBA protein contains the 142 aa residues, while the mature portion of Bu_LALBA protein contains 123 aa residues. Nineteen amino acid residues were found as a signal sequence in the translated Bu_LALBA protein. Earlier workers (Nitta K and Sugai S 1989) suggested that the human, bovine, goat, camel, equine, guinea pig and rabbit LALBA protein also contains 123 amino acid residues, which was similar to buffalo LALBA protein as shown in our study. The molecular weight of LALBA protein has been given in Table 1.

The ExPASy-ProtParam analysis showed that the Bu_LALBA protein was acidic (pI, 4.81), which was similar to bovine LALBA protein (pI, 4 to 5) (Permyakov E A and Berliner L J 2000). Our data revealed that the Bu_LALBA protein was more stable since the instability index was about 28.77 (Table 1). It is sound that the LALBA protein provides a binding site for several metal ions such as Ca^{2+}, Mg^{2+}, Mn^{2+}, $Na^{+,}$ and K^{+}. Hence, the binding of Mg^{2+}, Mn^{2+}, $Na^{+,}$ and K^{+} in the presence of Ca^{2+} ions in milk may increase the stability of the LALBA protein in bovine milk (Permyakov E A and Berliner L J 2000). In addition to metal binding, the thermal stability of LALBA protein might be due to temperature regulation and function in the mammary gland (Permyakov E A and Kreimer D I 1986). It is indicated that Bu_LALBA protein was more stable, which might be due to an interaction of Ca^{2+} ions with other metal ions in buffalo milk. In the present study, the aliphatic index of Bu_LALBA protein was analyzed, hence LALBA contains a higher percentage (91.27) of aliphatic amino acids showing the thermostable nature of the protein (Table 1). However, negative GRAVY value (-0.191) was found for Bu_LALBA which showed the hydrophilic nature of the protein (Table 1). It is concluded that the Bu_LALBA was acidic, hydrophilic, and thermo-tolerant nature of the protein.

Evaluation of secondary structure of Bu_BLG

The secondary structure of Bu_LALBA protein was predicted by the SOPMA server, which is shown in Figure 2(B). The secondary structure of the protein is stabilized by hydrophobic, ionic, and hydrogen bond interactions between the peptide chains. Protein flexibility is defined by the presence of more random

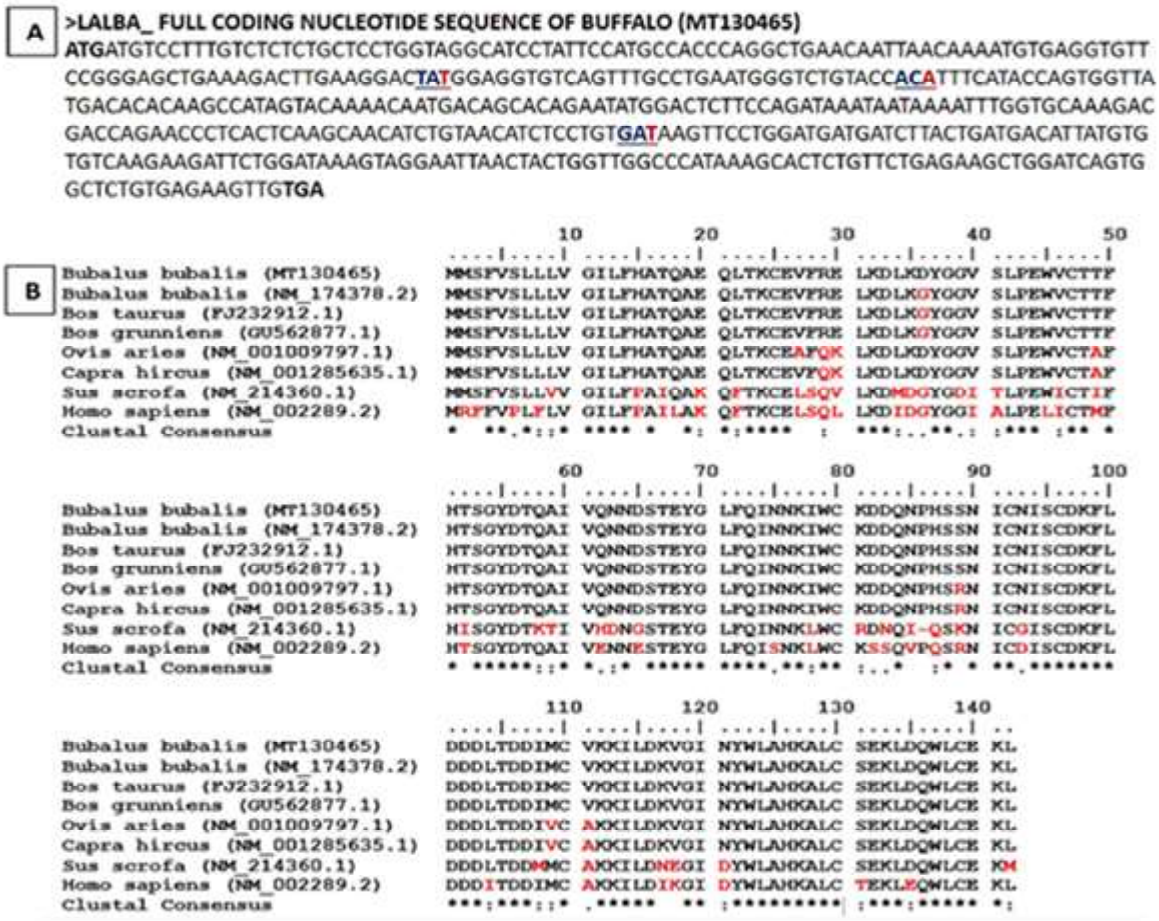

Fig. 1 (A) The full coding nucleotide sequence of buffalo LALBA gene; (B)Multiple sequence alignment of amino acid sequence of Bu_LALBA protein (142aa) with the LALBA sequences of domestic yak, exotic cow, sheep, goat, pig, and human through Bio-Edit tools.

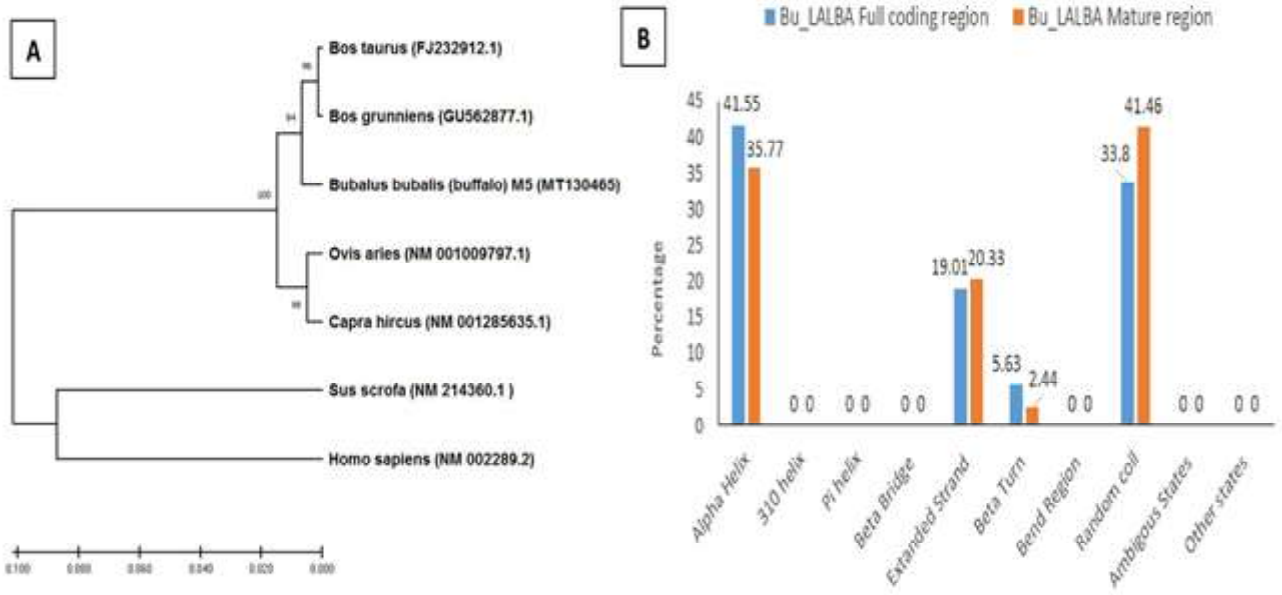

Fig. 2 (A) Phylogenetic tree showing the evolutionary relationship of Buffalo with different species based on the nucleotide sequences of α-lactalbumin gene; (B) Graphical representation of the analysis of the secondary structure of full coding region and mature region of Bu_LALBA protein using SOPMA server.

coils (Berjanskii MV and Wishart D S (2008). In the present study, the low percentage (33.80%) of random coils was observed in Bu_LALBA protein which confirmed that this protein is slightly rigid and less flexible (Figure 2B). Similarly, the high percentage (41.55%) of α-helix in Bu_LALBA suggests that the protein is thermostable since the thermophilic nature of protein has more abundance of alpha-helices as suggested by previous workers (Kumar et al. 2000).

In the present study, the percentage of α-helix was calculated based on the full coding region of buffalo LALBA protein, which was higher than the α-helix of cattle LALBA protein. Further, the percentage of α-helix in the mature portion of Bu_LALBA protein was higher (35.77%) than that of the mature portion of the LALBA protein in cattle. Similarly, the random coil in full coding and mature portion of Bu_LALBA protein was observed as 33.80% and 41.46%, respectively (Figure 2B). It is indicated that the more random coil was found in a mature portion than the full coding portion of the Bu-LALBA protein. The X-ray crystallography data showed that LALBA protein was very similar to lysozyme and exhibit a little cell lytic activity (McKenzie HA and White F H 1987; Acharya et al. 1991; Acharya et al. 1994). The previous study (Acharya et al. 1991) also proposed that the secondary structure of the buffalo LALBA protein contains two domains: a large α-helical and a small β-sheet domain, which is linked by a calcium-binding loop . This α-helical domain is formed by three major α-helices and two short 3_{10} helices. While a small β-sheet domain contains a series of a loop, a small three-stranded antiparallel β-pleated sheet, and a short 3_{10} helix (Permyakov E A and Berliner L J 2000; Acharya et al. 1991). However, the four disulfide bridges stabilized the overall secondary structure of the LALBA protein (Hendrix et al. 1996). It is concluded from the above discussion that the buffalo LALBA protein has more percentage of α-helix than the cattle LALBA protein. Hence, it is proposed that the Bu_LALBA protein is less flexible than the cattle LALBA protein.

Identification of eukaryotic linear motif (ELM)

The linear motifs are the compact regulatory proteins that display a key role in cell signaling, protein trafficking, and post-translational modification processes (Gouw et al. 2018). Prediction of motifs in various regions of Bu_LALBA protein may be helpful for the identification of distinct sites, which are involved in various cellular processes (Batra et al. 2019). About 30 different linear motifs were identified in Bu_LALBA protein in Figure 3(A), which indicated that the LALBA protein has different kinase and phosphorylation sites and is involved in cell signaling pathways.

It is known that the LALBA is a regulatory protein of lactose synthase, which regulates the affinity of catalytic component, 4-galactosyltransferase I, UDP-galactose β-*N*-acetyl glucosaminide β1 by reversible protein-protein interaction (Chrysina et al. 2000). Further, LALBA has Ca^{2+} ion binding site, which is required for proper folding and native disulfide bond formation as suggested by earlier workers (Chrysina et al. 2000). A previous study (Gao et al. 2018) indicated that the bovine LALBA hydrolysates suppressed the insulin receptor substrate 1 (IRS-1) and serine phosphorylation (Ser307, Ser612), but enhanced the protein kinase B phosphorylation in mouse. In other words, bovine LALBA hydrolysates inhibited the activation of an inhibitor of kappaB

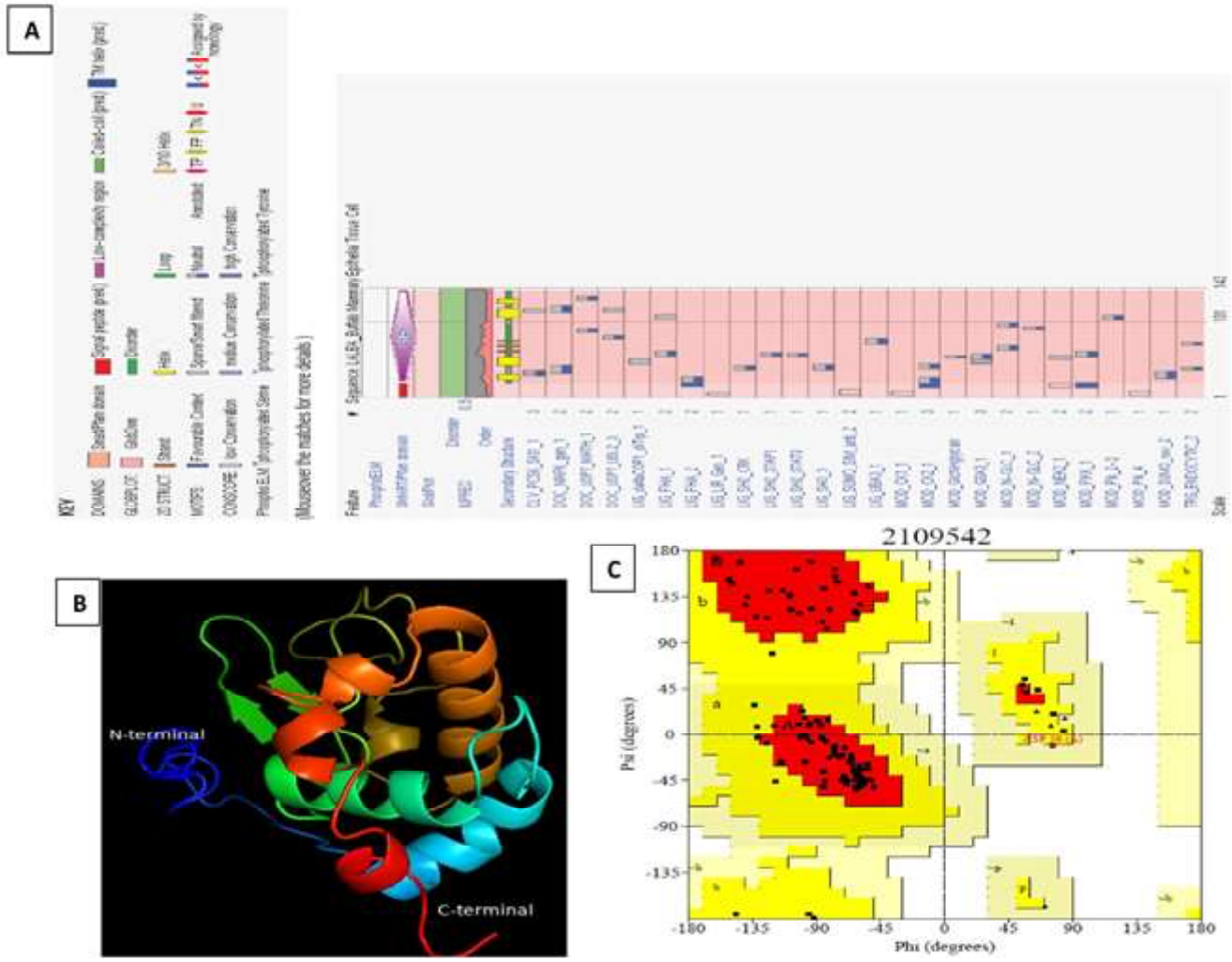

Fig. 3 (A)Graph showing different linear motifs in Bu_LALBA protein sequence; (B) Predicted 3D structure of Bu_LALBA protein by I-TASSER Server; (C) Ramachandran plot of Bu_LALBA protein (White-Region 0: Disallowed; Cream-Region 1: Generous; Yellow-Region 2: Allowed; Red-Region 3: Most favorable core region; Black Dot-Colour of markers in favorable regions; Red DOT-Colour of markers in unfavorable regions).

kinase (IKK) and mitogen-activated protein kinase (MAPK) signaling pathways in mice adipose tissue (Gao et al. 2018). These results indicated that LALBA may serve as a regulatory protein and may involve in several signaling pathways in buffalo.

Prediction of tertiary structure and its validation

The 3D structure of BU_LALBA protein was predicted through I-TASSER. I-TASSER uses the SPICKER program to cluster all the decoys based on the pair-wise structure similarity and generate the models based on high C-score and TM-score (Zhang Y 2008). The 3-D Model-1 of Bu_LALBA was showing in Figure 3(B), which has the C-score and TM-score as 0.04 and 0.72, respectively. The Bu_LALBA protein model contains 48 coils, 27 strands, and 59 helixes and has a negative value of normalized B-factor, indicating the stabilized nature of predicted Bu_LALBA structure as shown by earlier workers (Sun et al. 2018). Previous workers (Hendrix et al. 1996) have generated the 3-D structure of LALBA, which has α- and β-domain. The α-domain has a helical structure, while the β-domain is largely unfolded. The α-domain has two Cys^{6} - Cys^{120} and Cys^{28} - Cys^{111} disulfide bonds and the β-domain have two Cys^{61} - Cys^{77} and Cys^{73} - Cys^{91} disulfide bonds and one calcium-binding site. Twelve aspartic acid and eight glutamic acid residues have been found in LALBA protein in addition to the C-terminal carboxyl group (Rosner H I and Redfield C 2009).

Further, the validation of the predicted 3-D structure of Bu_LALBA protein was made by the Ramachandran plot. The Ramachandran plot of Bu_LALBA protein showed the 88.6% residues in the most favored region and 10.5% in the allowed regions in Figure 3(C), which suggest that the predicted LALBA model was of good quality as these parameters were suitable

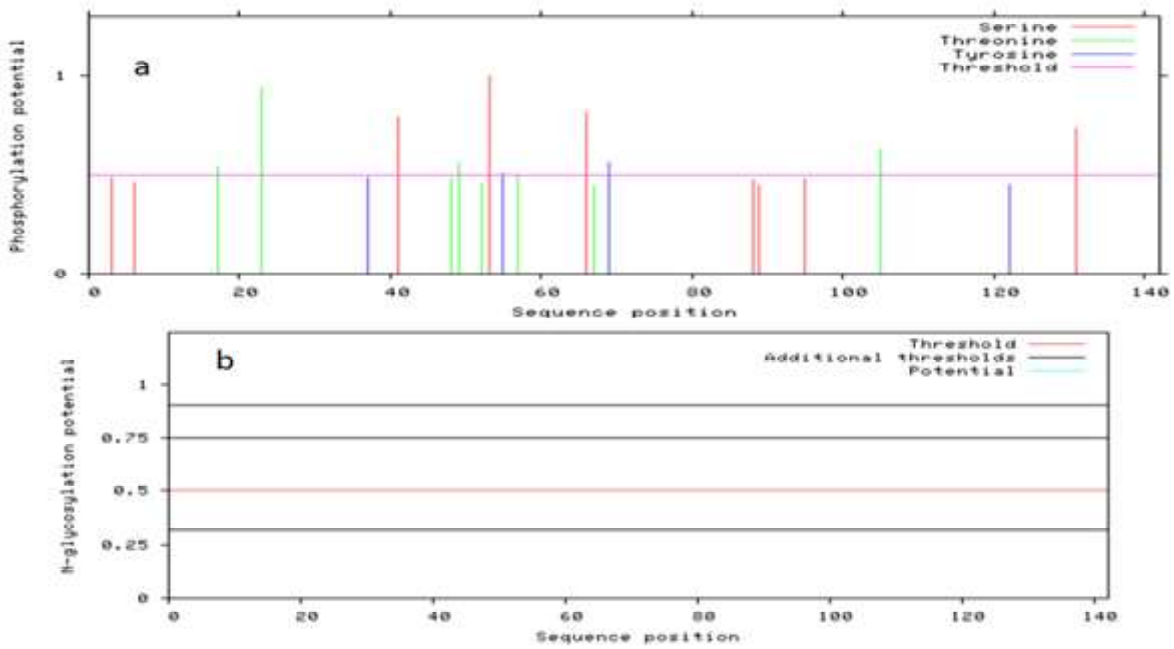

Fig. 4 Graphical representation of phosphorylation sites (A) and glycosylation sites (B) in Bu_LALBA protein predicted by NetPhos 3.1 and NetNGlyc 1.0 Server

indicators for good quality of 3-D models of the protein as suggested by previous workers (Laskowski et al. 1993).

The native LALBA has large α-helical and small β-sheet domains. These two domains are linked by a disulfide bridge between residues 73 and 91, forming the calcium ion (Ca^{2+}) binding loop (Barbana et al. 2011). It has been demonstrated that LALBA protein binds to one calcium ion (Ca^{2+}) per protein (Hiraoka et al. 1980), but when calcium ion is not available it can bind to fatty acid such as oleic acid as suggested by previous workers (Le et al. 2014). In our predicted Bu_LALBA model 1, four potential ligand-binding sites were found namely Calcium ion (Ca^{2+}) (binding site residues: 98,99,101,103,104,106,107), Glycine (binding site residues: 52,68,73,74,75,79,114,122,123), N-acetyl-L-glutamate (NAG) (binding site residues: 52,63,68,73,75,122,123,124), and N-acetyl glucosamine (binding site residues : 78,79,116,118). It is very difficult to discuss and compare the structural features of the Bu_LALBA protein with the other species. The different species may have a variable number of amino acid and most of the structural parameters would be different among the species. It is summarized that the LALBA protein binds to several fatty acids, ions and ligand molecules and acts as a carrier and transport protein for various cell signaling processes in buffalo, however, an alteration in the protein structure may be due to a change of amino acid composition and power of the bioinformatics tools used in the analysis.

Evaluation of phosphorylation, glycosylation, methylation and acetylation sites

Phosphorylation of protein switches the activity of cellular protein quickly from one state to another. Thus, protein phosphorylation is a key step in various cell signaling processes (Blom et al. 2004). The insertion or deletion of the phosphate group resulted in the alteration of the protein function (Batra et al. 2019). None of the information is available on the phosphorylation of LALBA protein in Indian buffalo. The result of NetPhos 3.1 Server showed the four serine, four threonine, and two tyrosine residues in Bu_LALBA protein in Figure 4(A), which evidences that this protein is moderately phosphorylated and is involved in signal transduction processes. The previous study has shown that the LALBA protein had two potential serine phosphorylation sites at positions 47 and 76 in tripeptide Ser-X-Glu and tripeptide Ser-X-Asp, respectively (Bingham et al. 1988).

On the other hand, Eigel et al. 1984 suggested that the LALBA was not phosphorylated in cow milk. It is concluded from the above arguments that the protein is noticeably involved in the phosphorylation process. Glycosylation plays an important role in the folding and stability of the proteins. There are mainly two types of glycosylation of proteins i.e. *N*-linked glycosylation and *O*-linked glycosylation. *N*-glycosylation is defined as the addition of sugar to the amino group (NH_2) of asparagine, while in *O*-linked glycosylation, a sugar molecule is attached to the hydroxyl group (OH) of a serine or threonine (Blom et al. 2004). The NetNGlyc 1.0 Server showed none of the N-glycosylation

Table 2 Different methylation and acetylation sites in Bu_LALBA protein

Position	Flanking residues	Predicted result	SVM probability
24	QAEQLT-K-CEVFRE	methylated lysine	0.607653
24	QAEQLT-K-CEVFRE	acetyl lysine	0.560368
32	EVFREL-K-DLKDYG	acetyl lysine	0.514825
35	RELKDL-K-DYGGVS	methylated lysine	0.667746
35	RELKDL-K-DYGGVS	acetyl lysine	0.526332
77	LFQINN-K-IWCKDD	methylated lysine	0.557465
81	NNKIWC-K-DDQNPH	acetyl lysine	0.516065
113	DIMCVK-K-ILDKVG	methylated lysine	0.571454
117	VKKILD-K-VGINYW	methylated lysine	0.564107
117	VKKILD-K-VGINYW	acetyl lysine	0.518003
133	KALCSE-K-LDQWLC	methylated lysine	0.573940

Table 3 Different B-cell epitopic sites in Bu_LALBA protein

Site No.	Start	End	Peptide	Length
1	19	21	AEQ	3
2	35	41	KDYGGVS	7
3	52	68	TSGYDTQAIVQNNDSTE	17
4	80	89	CKDDQNPHSS	10
5	103	104	DL	2

Table 4 Different T-cell epitopic sites in Bu_LALBA protein

Site No.	Start	End	Peptide	ImmunogenicityScore
1	66	80	STEYGLFQINNKIWC	70.1867

sites in Bu_LALBA protein in Figure 4(B). The results suggested that the LALBA protein is mildly thermo-stable and has poor folding conditions as suggested by previous workers (Shental Bechor D and Levy Y 2008).

Methylation and acetylation sites in Bu_LALBA protein were predicted through PLMLA software (Prediction of lysine methylation and lysine acetylation). Results showed the presence of six methylated lysine and five acetyl-lysine sites in Bu_LALBA protein in Table 2.

It has been suggested that the covalent modification of specific lysine residues in protein may have a role in different cellular processes as suggested by previous workers (Blom et al. 2004). So, the prediction of methylation and acetylation sites in Bu_LALBA protein may be helpful in the identification of the structural and functional properties of the proteins (Batra et al. 2019). It is concluded that methylation and acetylation may alter the structural properties of buffalo LALBA protein for assessing their roles in various cellular processes.

Prediction of immunological sites

In the present study, IEDB analysis showed five and one epitope sites in Bu_LALBA for B-cell and T-cell, respectively in Table 3 and 4. The previous study (Adams et al. 1991; Maynard et al. 1997) has shown that the LALBA protein has larger IgE binding epitopes namely peptides 5-18, 17-58, and smaller epitope peptides 6-10, 115-123, 109-123. Researchers (Jarvinen et al. 2001) identified three IgG binding sites in the LALBA protein of bovine. Further, four peptides were found as T-cell epitopes in bovine LALBA protein (Meulenbroek et al. 2014). The result of the analysis indicated that the Bu_LALBA has immunological epitope sites, which can be used for the generation of the antibodies *in vivo* against these epitopes to reduce the immunoreactivity of LALBA protein if any for the safety aspect of the milk and milk products (Batra et al. 2019).

Conclusions

In the present study, the nucleotide and protein sequence of the buffalo LALBA was characterized. Th study showed the three-nucleotide variations with no change in amino acids of Bu_LALBA protein. The phylogenetic tree showed that the cattle, yak, and buffalo formed one cluster with a close relationship

between exotic cow, domestic yak, and buffalo followed by goat and sheep cluster. Computational analysis showed that the buffalo LALBA protein was slightly acidic, thermos-stable, and hydrophilic. It was found that the LALBA protein has a less flexible structure, but the presence of motifs in the protein may play a key role in various cell signaling processes. The Bu_LALBA protein showed phosphorylation processes, however, the presence of methylation and acetylation specific lysine residues in LALBA protein may suggest that this protein is involved in several physiological processes. The presence of immunological epitopes on Bu_LALBA proved that this protein may act as an antigen and elicit the antibody response against such antigen.

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RESEARCH ARTICLE

A study on cow welfare vis-à-vis sustainability of gaushalas (cow orphanages)

Ajmer Singh[1], ML Kamboj[2] and BS Chandel[1]

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Abstract: As soon as cows become unprofitable, they are abandoned. Since, gaushalas are considered to be the best alternative to manage the ever increasing unprofitable and stray cattle population, it is important to study their sustainability levels vis-à-vis status of cow welfare level. The study finds that size and type of gaushala makes a difference wrt. sustainability indicators as well as welfare levels. The sustainability of Gaushalas could be increased by increasing their sources of income and catering to social needs, besides organizing professional trainings. The cow welfare level in these gaushalas was found to be traversing in harmony with their sustainability levels.

Keywords: Animal welfare, Gaushala, Indigenous cow, Sustainability index.

Introduction

As soon as the productivity of local cow declines, they become unprofitable to the owners, they are abandoned. These abandoned animals become stray and end up foraging in garbage dumps in cities or sold to slaughter-houses (GoI, 2020). The religious sentiments attached to the animal and ban on cow slaughtering take the issue beyond its economic considerations. There is strong need to find an alternative to manage these stray animals and also conserve the decreasing pure indigenous cattle population of the country. One such alternative can be Gaushala; provided they are sustainable and gratify to welfare of animals. At present, there are over three thousands gaushalas in the country registered with different agencies. Largely, these gaushalas are supported by the charity which seems to be difficult to sustain in future (Singh and Kamboj, 2019).

The interaction of direct and indirect factors and actors in socio-economic-cultural environment of Gaushalas influence their sustainability. To discern and determine the factors responsible for making Gaushalas self-sustainable, quantification of their economic viability, extent of animal welfare and comoposite sustainability level of these institutions are required. Although animal welfare is the prime objective for setting up Gaushalas (BIS, 1987) but no scale is available for measuring it. In this backdrop, it becomes indispensable to examine their functioning, and whether they have a potential to propel into self-sustaining institutions. Gaushalas being animal welfare entities, it is therefore, imperative to investigate the current state of cattle welfare therein.

Methodology

Sampling frame of the study

The state of Haryana is selected purposively since it has been pioneer in establishing and maintaining the Gaushalas. For this purpose, a sample of 21 Gaushalas has been selected from different districts of the state based on their track record and data availability. Efforts were made to include prominent and welfare oriented Gaushalas. Information was gathered on such variables from officials of Gau Sewa Aayog and Deptt. of Animal Husbandry & Dairying, the Haryana State Gaushala Sangh and Arya Pratinidhi Sabha. Then the popular literature and folk tales material was also consulted to know about the reputation, working and social base of different Gaushalas. The selected Gaushalas have been categorized into three categories with the help of Cumulative Square Root Frequency method (Ghangurde and Rao, 1969). The average size of small Gaushalas is 1306 animals, that of medium

[1] DES&M Division, ICAR-National Dairy Research Institute, Karnal - 132001

[2]LPM Section, ICAR-National Dairy Research Institute, Karnal -132001

Ajmer Singh (✉)
DES&M Division, ICAR-National Dairy Research Institute, Karnal - 132001
Email:ajmerskundu@gmail.com

Gaushalas is 3003 and large Gaushalas are having 5208 animals. Over all, it was 2535 animals (gauvansh).

Further on the basis of financial backing and social base; Gaushalas have been delineated into two categories; Type A (Khap or Community Gaushalas) i.e. those which have financial backstroking by village communities primarily for animal welfare with no commercial interests and Type B. *Khap* is synonymously used for local self governance among the rudimentary units of the republic. The type B Gaushalas normally run on multiple motives and mainly dependent upon money granted by the government organizations and the public donations.

Construction of Sustainability indices

The sustainability concept is considered to be composed of three dimensions viz. Economic, Social and Environmental sustainability (Sabarathnam, 2002; Waltric, 2003; Chand et al. 2011; Rehman and Khan, 2012). In the study, Composite Sustainability Index (CSI) has been constructed by combining three different indices viz. Economic, Social and Environmental sustainability indices. Since all the variables, used to develop index have different units of measurement; so, the data were normalized before constructing the indices. After normalization, three different indices were calculated by aggregating the weighted normalized variables.

$$ESI = \sum_{i=0}^{n} L_i/n \qquad SSI = \sum_{i=0}^{n} W_i L_i/n \qquad EnSI$$

Where, ESI = Economic sustainability index of each Gaushala

SSI = Social sustainability index

EnSI = Environmental sustainability Index

w_i = Weights assigned to the ith indicator

L_i = Normalized value of i^{th} indicator of respective index

n = Number of indicators

The three indices (Economic, Social and Environmental) are given weights and aggregated to arrive at Composite Sustainability Index (CSI) for each Gaushala.

$$CSI_k = (W_1 * ESI_k + W_2 * SSI_k + W_3 * EnSI_k) / \text{“}W_i$$

where, CSI= Composite index of k^{th} Gaushala

W_i = Weights assigned to individual indicators by expert opinion, K – the gaushala in question.

An experts' brainstorming involving the subjects like Dairy economics, Dairy Extension, Livestock Production & Management, Animal Physiology, Animal Nutrition, etc facilitated the weighting of individual variables and indices. Then, Gaushalas were categorized based on the sustainability levels.

Animal welfare index in Gaushala conditions

After generation of sustainability index, animal welfare was evaluated at Gaushala level. It has been assessed using DCWA (Dairy Cattle Welfare Assessment) scale (Annex-1) which is composed of 20 welfare indicators developed by NDRI Karnal (Anonymous, 2017; Chandra, 2018). The scale encompasses an integrated assessment of two resource based welfare components (housing & other facilities; availability of feed & fodder) and one animal based welfare component (animal health, performance and behaviour) with a weightage of 30, 30 and 40 respectively and aggregated into an overall welfare index with a total score of 100.

Results and Discussion

Before we present the results on sustainability and animal welfare, it is worth to have a glimpse of economic performance of gaushalas. About 81 per cent of total receipts of the Gaushalas were coming from grants and donations and only 19 per cent were generated from sales of outputs and miscellaneous activities. The analysis depicts that the total receipts of Type A Gaushala were Rs 9810 per animal (SAU) as against total expenses of ¹ 9240 with net income of 570 per year per SAU. In Type B Gaushala, net income was ¹ 279 only per SAU per year which was nearly half of Type A. The main reason for the same was uncontrolled expenses especially on labour and veterinary services as discussed by Singh et al, 2019 and also corroborated by Bijla and Singh, 2019. Among size categories, the net income varies from Rs 180 (small) to Rs 618 (large) per SAU /yr. The large category Gaushalas were able to corner large share of grants and donations which was about 85 per cent of the total receipts, higher than the average.

Sustainability of Gaushalas

Using three dimensions viz. Economic, Social and Environmental, the Composite Sustainability Index (CSI) of each Gaushala was estimated (Singh, 2013). The indicators have been described in the following sections.

Economic indicators

To assess the economic dimension of sustainability, the following indicators were used along with their weights as depicted in parentheses. The net income of a Gaushala (calculated as net of gross receipts and total expenditures) on SAU basis per annum, was considered to be the major indicator of economic sustainability by assigning the maximum weight of 30 per cent.

The next important economic indicators was Autonomy (20) expressed as percentage and calculated as a ratio of revenue generation in the gaushala and total income multiplied by 100. Then it was Returns over variable cost (15) which is net of total returns or revenue generation over and above variable cost which was found negative over all the size groups and type categories expressed in INR per SAU per year. It was followed by Productive animals (10) which is equal to proportion of milch females and males engaged in servicing in percentage terms. Then it was Employment generation (10) ie number of man-days created in the gaushala followed by Dependency (10) taken as reverse of autonomy and calculated as 100 minus Autonomy. The Operating ratio (5) arrived at by taking vaiable cost as a fraction of total income and expressed in percentage terms was the least weighted economic indicator though very important.

Surprisingly, the net inco me was significantly greater in Type A Gaushalas. It was also high for large Gaushalas which were generally situated in rural areas and backed by the large village communities. Type A Gaushalas were carrying heavier load of unproductive animals as the proportion of productive animals was lesser in type A and larger gaushalas. Employment generation was in proportion to their size of operation and job volume. Autonomy which was an important variable for sustainability with 20 per cent weightage, was better among Type B and smaller size Gaushalas.

Social indicators

The indicators of social dimension are described below along with their weights and the way how these are measured and expressed.

Cattle protection with highest weightage of 35 per cent, consists of number of cattle left by farmers at Gaushala and the number of cattle saved from butchers and other illegal confinements was the most important social variable impacting the sustainability. Other important indicators were: Tainings imparted (15) means number of training programmes conducted in a year Charity support (15) means number of persons giving donations or rendering charity services Social events (10) describe number of celebrations/ fairs organized in a year Human health (10) variable consists of number of persons getting treatment from Gaushala HRD (10) signifies number of persons getting training in Gaushala Other programmes (5) include number of programmes other than trainings conducted in a year Type A Gaushalas was having the higher proportion of cow protection, training programmes conducted, number of persons getting training and number of donors as compared to Type B Gaushalas. In case of herd size, large sized Gaushalas had higher values of all the indicators followed by medium and small sized Gaushalas. The community services in terms of training programmes conducted and providing various services and social participation were the major variables which were significant in varying degrees while sustaining the indigenous cattle production system as corroborated in earlier studies of Van (2005) and Singh et al. (2007).

Environmental indicators

The major environment related variables used to delineate the sustainability: Sewage used in irrigation and Disposal of dead animals was assigned 20 per cent weight each to calculate environmental sustainability index (ESI). The other indicators used were Dung proportion used in biofuel, Housing space available, Bio-pesticides production, Vermi-compost production and Electricity production.

Analysis of environmental variables shows that, the Type B gaushalas use maximum amount (36.37%) of dung as fuel against 33.8% used in Type A. Because of higher land availability in the rural areas, the other indicators like housing area per Standard Animal Unit (15.23 m^2), the number of dead animals buried (92%) and vermin-compost production (10.0 q/month) were higher in the Type A gaushalas. The environmental indicators displayed a mixed dispensation. Some variables were better among large

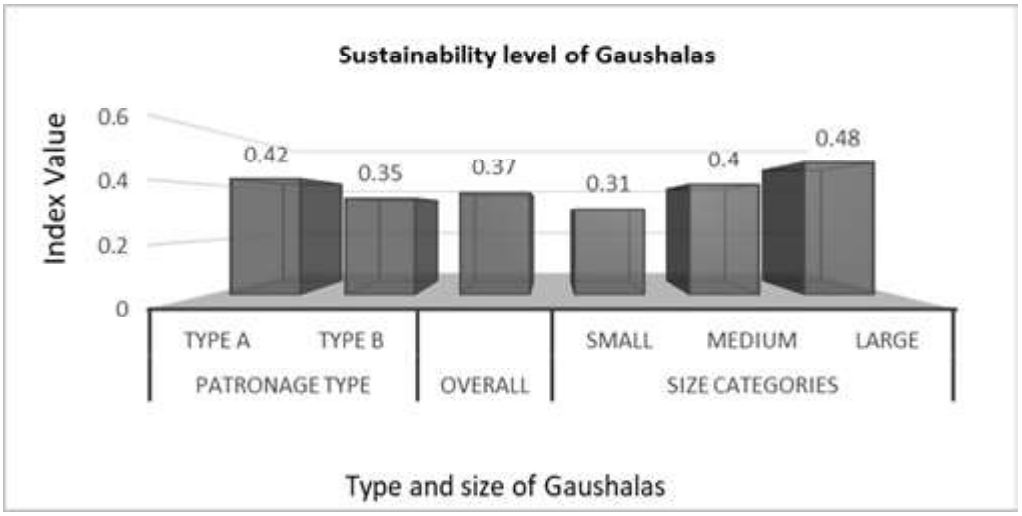

Fig. 1 Composite sustainability index in the Gaushalas

gaushalas like bio-pesticides and vermin-compost production, and the better utilization of dung. In case of other variables no consistent trend was observed.

The Composite sustainability index

Using the three indices namely economic, social and environmental, the Composite Sustainability Index (CSI) of gaushala was developed. The overall CSI value was 0.37 which was less than half of the range of index and revealing thereby low level of overall sustainability. The overall value of ESI was 0.41 followed by SSI (0.38) and EnSI (0.26).The CSI was higher at 0.42 for Type-A Gaushalas and even more for large Gaushalas (0.48) among different categories (Fig.1).

The large Gaushalas were found economically more sustainable with ESI value of 0.48 followed by medium sized Gaushalas (0.41) and small sized Gaushalas (0.39). In terms of social sustainability, similar pattern was observed. As per EnSI values, the larger Gaushalas outperformed the other gaushalas due to availability of material to conduct different operations. Based on the results of CSI, it can be concluded that the large and Type A gaushalas backed by the village communities and social support from the rural folks were better managed and were more sustainable. The same is reflected SSI value of the gaushalas.

Factors affecting the sustainability of Gaushala

Analysing the factors affecting sustainability of gaushalas, it was observed that the large Gaushalas are more sustainable as compared to small sized Gaushalas (dummy coefficient being 0.0957*). It further infers that autonomy has positive and significant effect on sustainability of Gaushalas. The similar positive and significant effect was seen with number of trainings and other programs conducted by the Gaushalas. As values of operating ratio and proportion of protected animals increase, they pose negative effect on sustainability of Gaushalas. Hence there is need to control working expenses of Gaushalas to maintain their sustainability levels.

Type of gaushala alone has not much relevance with the sustainability index but the individual factors and specifically the economic ones have more effect on sustainability level of Gaushalas.

Welfare levels in the Gaushalas

The welfare parameters quantified under the welfare index are presented in the section further but the Animal Housing which is even more important than index parameters, is dealt separately as described below.

Housing practices

The space availability per animal both covered as well as open, is an important indicator of animal welfare which was found to be the higher among type A and large Gaushalas than the standards fixed by Bureau of Indian standards (BIS) i.e. 9.75 m^2 or 105 sq. ft. per SAU. The animals in Gaushala were housed under loose system of housing comprising a covered area and an adjacent loafing/resting area with common feeding and watering arrangements. But if we consider only the covered area, the space per SAU was less than half of the BIS recommendation i.e. 9.75 m^2 or 105 sq. ft. per SAU. Most of the large Gaushala and about a half of small and medium Gaushalas had a provision for ceiling fans inside the sheds for protection against heat stress during summers. Animal housing facilities at Type A and at large Gaushalas were in general better in terms of provisions for separate housing of sick and injured animals and treatment yard; higher floor, feeding and watering space availability and better summer protection

Welfare scores in different Gaushalas

The average welfare scores of animal welfare across different types and sizes of Gaushala are presented in Table 1. The mean welfare score obtained out of a total score of 30 with respect to housing and other facilities (component A) was highest in large sized Gaushalas (24.75) followed by medium (21.5) and small sized Gaushalas (18.64). Similar trend is also evident in case of mean scores of feeds and feeding practices (component B) as well as in case of animal health, performance and behaviour (component C). The overall mean welfare scores obtained across the three components out of a total score of 100 are thus the highest with respect to large Gaushalas (77.0) followed by medium (70.0) and small Gaushalas (60.09).

Furthermore, Type A Gaushalas performed better on animal welfare front with an average score of 72.40, whereas Type B

Table 1 Average animal welfare scores in different sizes and categories of Gaushalas

Animal welfare component	Animal welfare score	Types of Gaushala		Size of Gaushala		
		Type A	Type B	Small	Medium	Large
Housing and other facilities	30	23^a	20.06^b	18.64^b	21.50^a	24.75^a
Feeds and feeding practices	30	23.60^a	20.45^b	18.73^b	23.17^a	24.25^a
Animal health, production and behaviour	40	25.80	24.81	23.73^b	25.33^b	28.00^a
Total	100	72.40	65.32	61.09	70.00	77.00

Source: Estimated by authors

The values with different superscripts indicate the significant difference at P<0.05

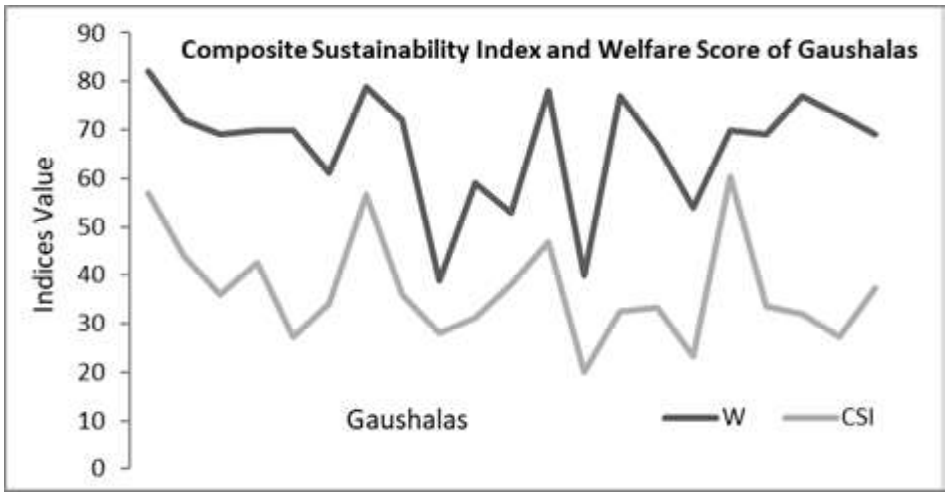

Fig. 2 Relation between CSI and welfare score of Gaushalas

Table 2 Categorization of Gaushalas based on sustainability levels vis-à-vis animal welfare status

Sustainability levels		Gaushala ranking	Animal welfare status	
Percentage of Gaushalas	Composite sustainability index (CSI)		Animal welfare index	Percentage of Gaushalas
14	> 0.52	High/ V. good	>80	4.8
24	0.31 – 0.52	Medium/ good	60 - 79	71.4
62	<0.31	Low/ Average	<59	23.8

Gaushalas scored 65.32 as they faired marginally poor on resource based indicators. If a welfare score of 60 is considered acceptable then two resource based indicators viz., microclimate protection measures and feeding & watering space availability and four animal based indicators viz., cow productivity, body condition scores, breeding practices and reproductive efficiency are the most compromised indicators which needed improvement. The average animal welfare scores of Type A Gaushalas in all three welfare components are higher than those obtained in case of Type B Gaushalas with the overall welfare score of 72.4 and 65.3 out of 100 in Type A and Type B Gaushalas respectively (Table 1). The larger Gaushalas being older and well supported by the village community, have better basic infrastructure in terms of animal housing facilities, better availability of feeds and fodder, healthcare facilities and thus were in a position to ensure better animal welfare conditions.

Ranking of Gaushalas based on sustainability and level of animal welfare

Applying cumulative square root frequency method on the composite sustainability index of the Gaushalas, gaushalas were categorized into low (< 0.31 CSI), medium (0.31 – 0.52 CSI) and highly (> 0.52 CSI) sustainable. The Gaushalas falling under low sustainable category were 62 per cent, in medium sustainable were 24 per cent and the highly sustainable gaushalas were only 14 per cent of the total sample (table-2).

The cattle welfare at an overwhelming majority of selected Gaushalas (71.4%) was good and about one fourth of gaushalas (23.8%) were found lying in average category, out of this, only a small percentage were found in poor category (4.7%) and an equal proportion (4.8%) are in 'very good" category (table-2). The larger Gaushalas performed better with an average welfare score of 77, followed by medium (70) and small sized (61). Based upon the DCWA scale, gaushalas scoring more than 80 points on welfare scale are categorized as very good; those scoring between 60 and 79 as good; between 40 to 59 an average; and those with less than 40 score are categorized as poor but generally, it is not counted seperately.

Relationship between welfare level and sustainability of Gaushalas

Figure-2 shows relationship between sustainability index and welfare score of Gaushalas which indicates more or less fluctuating relationship at low and medium sustainability levels but at high sustainability level the welfare score is seen rising continuously which depicts positive relationship among them. The correlation between sustainability index and is of high degree with correlation coefficient value of 0.58. The welfare index seems to be going in tandem with higher attitudes of CSI but if losses it link at its troughs. It has also implications for the type of Gaushalas as the peaks of W score are reflected mostly by the type A gaushalas which are backed by the village communities.

Conclusions

The study inferred that the Gaushalas backed by village communities and run on the cultural lines (Type A) were

Annexure 1 Animal welfare assessment scale for gaushalas

S No	Welfare indicators	Weightage
	COMPONENT A: Housing & other facilities (Total weightage 30)	
1	Segregation/grouping of different categories of cattle	5
2	System of housing and availability of floor space	5
3	Type and height of roof	3
4	Type of floors	2
5	Microclimate protection measures inside animal houses & other practices for protection against heat and cold stress	5
6	Feeding & watering space availability, feeding & watering systems with frequency	5
7	Facilities for rescue, transport and treatment of abundant/sick animals	5
	COMPONENT B: Feeds & fodders (Total weightage 30)	
1	Availability of quality feeds and fodder	10
2	Availability of feeds and fodder storage/preservation space	5
3	Feeding practices for different categories of animals	10
4	Colostrums and milk feeding to male & female calves	5
	COMPONENT C: Animal health, physiology and behaviour (Total weightage 40)	
1	Average Productivity (AP)(for lactating cows)	8
2	Body Condition Score (BCS) (for lactating cows)	4
3	Cow Comfort Index (CCI)	5
4	Cow Cleanliness Score (CCS)	4
5	Hock Injury Score (HIS)	3
6	Human Animal Relationship (HAR)	3
7	Lameness Score (LS)	4
8	Breeding practices	4
9	Reproductive efficiency (RE) (for milch cows)	3
10	Abnormal behaviours (AB)	2

Source: The DCWA (Dairy Cattle Welfare Assessment) scale as modified by the Project (NDRI/ IRC/G60) team for gaushala condition

performing better on economic and social dimensions of sustainability. On the other hand, size of Gaushalas makes a difference by harnessing economics of scale as well as catering to the social and environment needs. Nevertheless, overall sustainability of Gaushalas was very low as 62 per cent of the Gaushalas were having low CSI value (CSI score less than 0.31). Only 14 percent gaushalas were showing high CSI value (more than 0.52). Thus, for increasing the sustainability, the Gaushalas should be made autonomous by increasing their sources of income and catering to social needs, etc. The significant factors affecting sustainability found in the study were operating ratio, autonomy, cow protection and training components of their social activities. Type and size of Gaushala were having a role in deciding their sustainability. Strong correlation between welfare index of the Gaushala and their sustainability levels show that cow welfare can be increased in symmetry with their sustainability levels.

These cow welfare institutions ought to be run by giving professional orientation to them through community development programmes. The grants should be decided on per animal basis and their distribution should be regular so that these cow shelters prove to be successful in protecting the indigenous resource base of the country as well as meeting out the objective of cow welfare.

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Conflicts of interest

Authors declare that there are no known financial or any other conflicts of interest associated with the data presented in this publication.

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RESEARCH ARTICLE

Production, consumption and marketing of milk in Eastern Region of India: A farm level analysis

Binita Kumari[1], BS Chandel[2], Tulika Kumari[3] and Priyanka Lal[4]

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Abstract: The present study was undertaken in the eastern region of India comprising the states of Bihar, West Bengal and Jharkhand with the objective to determine milk production, consumption and marketed surplus at farm level. The study found that, inspite of poor dairy development in the region, milk production and productivity was considerably high among the sample households. An average dairy household was producing about 36 litre of milk per day having average productivity of 10 litre per day per animal which was significantly higher than national averages. The milk was drawn more from crossbred cows on Small households and from local cow and buffalo on large households. The milk productivity was highest on large farms which serve as a motivation for removing the resource limitation on small and medium herd size categories farms and promote better feeding and management practices too. The consumption of liquid milk among producer households was 207 gm per day per capita which was higher than per capita availability in the region. Of the 10 per cent of the milk production retained at household level, 40 per cent was consumed as liquid milk and 60 per cent was converted into milk products. The latter share was higher among small herd size category catering to the rural non-milk producing households. The halwais/ dudhiyas/private dairies were the major marketing channels accounting for 61 per cent of the total marketed surplus and only 21 per cent of the milk was sold through cooperatives. The share of milk sold through latter channel by small farmers was still lower (10%) inspite of the fact that the marketed surplus per household was substantially higher (24.02 L/day). An analysis of the factors affecting marketed surplus found that being members of dairy cooperatives had the highest influence on marketed surplus of small farms.

Keywords: Consumption, Marketed surplus, Milk production, Dairy cooperatives

Introduction

India leads the world with the highest milk production of 187.7 million tonnes during 2018-19 (GOI, 2019). The impressive growth is not limited to milk production only, but the processing of milk is also increasing at a compound growth rate of 12 per cent per annum in value terms (AMUL, 2019). The capacity of processing which stands at 53.5 million tonnes (28%) has been designated to double (108 million tonnes) by 2025 (GOI, 2020). The dairy co-operative network which has been mainstay of dairy development in the country covers around 35 per cent of the villages at present. It is evident that the dairy development in the country is regional skewed. The eastern region of the country which is comprised of states of West Bengal, Odisha, Chhattisgarh, Bihar, Assam, Eastern Uttar Pradesh and Jharkhand, accounts for about 12 per cent of the total milk production from a geographical area of around 19 per cent. On the other side, eight states alone with a geographical area of around 54 per cent produce 72 per cent of the milk in the country. On the whole, dairy development in the eastern region is of paramount importance on account of two main reasons. One, the milk production and productivity is very low and have the potential to increase. The milk production potential of the region depends not only on the livestock wealth but also on environment. Secondly, the proportion of poverty (more than 30%) and labour force is comparatively higher in the region and nutritional intake is lower.

It has been intensively debated that the eastern region of the country holds promise for a second Green Revolution, which can be accomplished through holistic management of land, water, crops, biomass, horticultural, livestock, fishery and human

[1]Rashtriya Kisaan (PG) College, Shamli-247776

[2] Department of Dairy Economics Statistics and Management, ICAR-National Dairy Research Institute, Karnal-132001

[3]Dr. Rajendra Prasad Central Agricultural University, Pusa -848125

[4]Department of Dairy Economics Statistics and Management, ICAR-National Dairy Research Institute, Karnal-132001

Binita Kumari (✉)
Rashtriya Kisan (PG) College,
Shamli-247776, India
E-mail: b.binitakumari@gmail.com

resources (Bhatt et al. 2016). The proportion of livestock animals in the region is about 31 per cent mainly comprised of dairy animals. Increasing production and productivity of the dairy animals holds promise to improve livelihood of the farmers and nutritional intake of the people in the area. Milk being a highly perishable commodity, the production should be followed by immediate marketing and processing to insure remunerative prices to the farmers. The information on production, consumption pattern and marketed surplus of milk in the region provides a handful insight to the policy makers to undertake dairy development activities in the region. Keeping the above points in view the present study was undertaken in the eastern region of the country with the following objectives to determine (1) farm level production, productivity and marketed surplus of milk, and (2) factors affecting marketed surplus of milk in the area. Many similar studies have been carried out in this regard by Agarwal et al. 2009; Bairwa et al. 2016; Bhawar et al. 2019; Ghule et al. 2014; Jaiswal et al. 2016; Kumari et al. 2017; Lal et al. 2019; Sivach and Dhaka 1993; Wani and Wani 2010 and many others. These studies were confined to a district or utmost a state and there isn't any study covering the eastern region. Statistics on production, consumption and marketing for eastern region may prove helpful in framing policies and hence the present study was carried out.

Material and Methods

The eastern region of India comprises of the states of West Bengal, Odisha, Chhattisgarh, Bihar, Assam, Eastern Uttar Pradesh and Jharkhand. The per capita availability of milk in the region is very low as it is 195 gm/day in Bihar, Jharkhand (146 gm/day), West Bengal (145 gm/day), Chhattisgarh (130 gm/day), Odisha (122 gm/day) and Assam (69 gm/day). Table 1 shows comparative statistics of milk production, productivity and per capita availability in the major regions of India. As depicted through the table, the regional averages lack deep insights; the study was based on field level survey of 300 dairy farmers selected from six villages according to probability proportional to size. These villages were selected randomly two each from districts of Madhepura (Bihar), Deoghar (Jharkhand) and- Hooghly (West Bengal). A multistage sampling procedure was adopted for selection of states, districts, and villages. After complete enumeration of these villages, the dairy farmers were divided into three strata based on number of milch animals using cumulative square root frequency method. Three herd size categories were formed namely, small (1-3 SAU), medium (4 and 5 SAU) and large (>5 SAU). Every herd comprised of animals of different age groups, types (crossbred, buffaloes, and local cows) and sex (male, female). Joints costs such as fixed costs and labour utilisation is different across these categories of animals, hence, apportionment of joint costs becomes necessary. For this purpose, the different categories of animals were converted into homogenous animal units known as Standard Animal Units (SAUs). SAUs as suggested by Sirohi et al. 2015 (Table 2) were used for this purpose. The difference from traditional practice is that is it region specific. Also, apart from labour utilization, the body weight of the animal was taken into consideration for estimation of SAUs. Based on the expert opinion 60 per cent weight was given to labour utilization and 40 per cent to body weights of animals for the final estimation.

Table 3 shows the sample distribution in the study area. From the total dairy farmers in the study area, nearly 75 per cent of the dairy households were chosen in the sample in each herd size category. The sample comprised 60.66 per cent of small farmers, 29.33 per cent of medium farmers and 10 per cent of large farmers. The average standard animal unit (SAU) of milch animals were 2.54, 4.41 and 7.07 for small, medium and large herd size categories, respectively with an overall average of 3.54. The dairy herds in the study area were dominated by crossbred cows (67.23%), followed by local cows (17.79%) and buffaloes (14.97%).

The marketed surplus is the amount of milk actually sold out by a household. The milk consumed by the households was calculated by subtracting marketed surplus from the total milk production per household. The milk disposal by the households through different marketing channels was also studied to understand the disposal pattern in the sample households.

To determine factors affecting marketed surplus, double log function with following variables was found to be the best fit.

Specification of marketed surplus function

$Y = f(X_1, X_2, X_3, X_4, D, X_5)$

Where,

Y = Marketed surplus of milk (litre/day)

X_1 = Educational level of the head of dairy household (Number)

X_2 = Herd size (SAUs)

X_3 = Family size (man equivalent units)

X_4 = Price received by the farmer (Rs/litre)

D = Membership in dairy cooperatives dummy (1, if member; 0, otherwise (non-member)

X_5 = Distance of household from collection centre (Km.)

Results and Discussion

Milk production and productivity

Table 4 depicts the average daily milk production and productivity in the region. A perusal of table reveals that the average daily milk production per household of the sample households is 36.06

litres. Out of the total average milk production, 87.99 per cent of the total milk produced in the household is from crossbred while only 9.07 per cent is from buffalo and remaining from local cow. The share of milk production from crossbred was the highest (91.48%) for small holding households while for buffalo and local cow, it was the highest for large herd size categories. It was due to highest composition of crossbred cows on the former category of herd size. The study revealed that small farmers were opting more for crossbred cows in the region. On the other hand, medium and large herd size categories were rearing more of the local cows and buffalo mainly to cater to the increasing demand for their milk in urban areas. The latter dairy animals have lower productivity, high cost of maintenance but their milk fetch higher price in the market due to more fat content.

As expected, productivity of crossbreds was the highest (13.15 litre/day/milch animal), followed by buffalo (6.21 litre/day/milch animal) and is the least for local cow (1.65 litre/day/milch animal). If the average productivity of the herd is worked out it is found to be highest (10.62 litre/day/milch animal) for small category while it was 9.72 litre/day/milch animal for medium category and 10.07 litre/day/milch animal for large category. The large herd size category was able to realize highest milk productivity from all categories of dairy animals due to better feeding and management practices. If the same management practices had been followed by lower herd size categories, they would have realized still higher benefits from the milk production.

Milk Consumption and Utilization Pattern:

The quantity of milk retained at the household is equal to total milk production minus marketed surplus (Table 5). This is the quantity of milk used for all consumption purposes, shared with relatives and donations or devotion purposes. The consumption of milk was either in the form of liquid milk or converted into milk products. As evident from the table, on an average 9.59 per cent of the milk was retained at the household and 90.41 per cent was sold in the market. The absolute amount of milk retained per household was the lowest on small herd size category but it was the highest share of its milk production (10.91%) due to more

Table1 Comparison of major regions in milk production, productivity and per capita availability (2018-19)

State/ Region	Milk production (m.t)	In-milk bovine (million)	Milk productivity (kg/ day/ animal)	Per capita availability (kg/ day)
India	181.65	97.61	5.09	397
Eight major states[1]	129.65(71.37)	63.63(65.19)	5.58	670
Eastern Region[2]	21.79(11.99)	17.73(18.16)	3.36	160

Note: Figures in parentheses are percentage of the total at country level (India).
1. Eight major states are Uttar Pradesh, Rajasthan, Madhya Pradesh, Andhra Pradesh, Gujarat, Punjab, Maharashtra and Haryana
2. Eastern Region comprised of Bihar, Jharkhand, West Bengal, Chhattisgarh, Odisha, and Assam.

Table 2 Standard Animal Units for eastern region in India

Region		Adult male	Adult female	Young stock male (<1 yr)	Young stock female (<1 yr)	Young stock male (>1 yr)	Young stock female (>1 yr)	Heifer
East	Crossbred	1.07	1.20	0.25	0.24	0.51	0.38	0.71
	Local cow	0.92	1.00	0.27	0.24	0.41	0.37	0.64
	Buffalo	1.02	0.86	0.25	0.23	0.42	0.38	0.63

Table 3 Population of dairy households and their sample sizes in the study area

Herd Size Category (milch animals)	Total dairy farmers (Number)	Sample Size (Number)	Share of Herd category (%)	Average Herd Size (SAU/ household)	Composition of dairy animals (% of SAUs)		
					Crossbred cow	Local cow	Buffalo
Small (1-3)	241	182(75.52)	60.66	2.54	76.77	11.81	11.41
Medium (4-5)	118	88(74.57)	29.33	4.41	60.77	21.54	17.68
Large (> 5)	38	30(78.95)	10.00	7.07	67.23	17.79	14.97
Total	397	300(75.57)	100.00	3.54	67.23	17.79	14.97

Note: The figures in the parentheses indicate the percentage of household included in the sample to total household in each herd size category; SAU: Standard Animal Unit

practice of converting milk into milk products, the part of which was also sold to the consumers in the village itself.

Table 6 shows the pattern of consumption of milk at home. The total milk retained at home for consumption was used either directly in liquid form or converted into dairy products for consumption. It was observed from the study that on an average about 60 per cent of total milk retained for home consumption was converted into milk products while the remaining 40 per cent was consumed in liquid form. As depicted in the table out of 3.46 litres of milk retained, 1.38 litres was consumed as liquid and remaining 2.08 litres was converted into milk products like curd, ghee or paneer. Using the average family size of 6.65 in the region, per capita liquid milk consumption was worked out to be 207.52 gm/day/person. This consumption was lower than the Indian Council of Medical Research (ICMR) recommendation of an average daily intake of 280 gm per day to deliver the requisite macro and micro nutrients. In case of large herd size category, the consumption was higher (334.72 gm/day) than ICMR recommendation while it was lower in the other categories.

As mentioned above in Table 1, contrary to the lesser per capita average availability of milk (less than 200 gm/ capita) in all states of the region, the per capita average consumption of milk among the producer households in rural areas of the region was higher which implied that consumption of milk among the non-producers in the rural areas and urban consumers may be considerably less.

Market Surplus and Factors affecting it:

Marketed surplus is the total supply of milk in the market. The disposal of milk is done through various agents such as *halwais*, *dudhiyas*, cooperatives and directly to consumer. Table 7 shows of disposal of milk through different agencies for different herd size categories. A perusal of the table reveals that about 54 per cent of households (161 households) disposed off their milk through halwais or dudhiyas, about 20 per cent (59 households) through cooperatives and about 27 per cent (80 households) disposed directly to consumers. It is note worthy that none of the large herd size category was selling milk directly to the

Table 4 Average milk production and productivity in the sample households

Particulars	Small	Medium	Large	Overall
Average milk production (L/day/household)	26.98(100.00)	42.85(100.00)	71.22(100.00)	36.06
Crossbred cow	24.68(91.48)	36.64(85.51)	60.17(85.51)	31.73(87.99)
Local cow	0.48(1.78)	1.57(3.66)	3.07(4.31)	1.06(2.94)
Buffalo	1.82(6.74)	4.64(10.83)	7.98(11.21)	3.27(9.07)
Average productivity (L/day/milch animal)	10.62	9.72	10.07	10.06
Crossbred cow	12.66	13.67	14.57	13.15
Local cow	1.61	1.66	1.80	1.65
Buffalo	6.28	5.96	6.49	6.21

Note: The figures in the parentheses show the percentage of average household daily milk production from each animal type

Table 5 Average milk consumption per household in eastern region of India (l/day/household)

Particulars	Small	Medium	Large	Overall
Average milk production (L/day/household)	26.98	42.85	71.22	36.06
Average marketed surplus (litre/day/household)	24.02(89.03)	39.18(91.43)	65.49(91.95)	32.60(90.41)
Average quantity of milk retained at home (litre/day/household)	2.96(10.97)	3.67(8.57)	5.73(8.05)	3.46(9.59)

Note: The figures in the parentheses shows the percentage of average marketed surplus and average quantity of milk retained at home to average household daily milk production

Table 6 Utilization pattern of milk retained at home

Particulars	Small	Medium	Large	Overall
Average quantity of milk retained at home (litre/day/household)	2.96(10.97)	3.67(8.57)	5.73(8.05)	3.46(9.59)
Average quantity of milk consumed as liquid (litre/day/household)	1.02(34.46)	1.75(47.68)	2.42(42.24)	1.38(39.88)
Average quantity of milk converted into milk products (litre/day/household)	1.94(65.54)	1.92(52.32)	3.31(57.76)	2.08(60.12)
Average family size (number adult equivalent)	6.45	6.85	7.23	6.65
Per capita liquid milk consumption (gm/ day/person)	158.14	255.47	334.72	207.52

Figures in parentheses show the percentage to total quantity retained at home

consumers while it was about 35 per cent of the small households catering directly the consumers. The plausible reason could be that they preferred bulk sale of milk to vendor rather than selling it in small chunks to direct consumer.

Table 7 also shows the quantity of milk disposed through different agencies for different herd size categories. Of the total marketed surplus, about 61 per cent of milk was disposed off through halwais/ dudhiyas/private dairies and nearly 21 per cent to cooperatives and 18 per cent to consumer. The medium and large household sell higher proportion of milk through *halwais/ dudhiyas* and cooperative network. The small farmers were disposing off less than 10 per cent of the milk production per household through cooperatives. Thus, it is evident that a large chunk of marketed surplus was disposed through middlemen, thereby, demanding cooperative network to the extended further for the betterment of small producers, in particular and overall regional dairy development in total. The factors also need to be investigated as what hinders small milk producers from using formal channel inspite of the fact that the marketed surplus per household was substantially higher (24.02 l/day).

In this context, a multiple regression analysis was done to find out the factors affecting the marketed surplus of milk. After using different functional forms, log-log form was selected on the basis of highest R square, maximum number of significant coefficients and least root mean square error. A perusal of Table 8 shows that overall 79.76 per cent of variation in marketed surplus was due to the explanatory variables included in the model.

Among the different explanatory variables, herd size, price of milk, membership in dairy cooperatives and distance of household from collection centre had a positive and significant impact on overall marketed surplus of milk. The coefficients of these

Table 7 Category wise disposal pattern of marketed surplus

Herd size categories	Particulars	Halwais/ dudhiyas/private dairies	Cooperatives	Consumer	Total
Small	Number of households (Number)	97(53.29)	22(12.08)	63(34.63)	182
	Quantity of marketed surplus (litre/day/household)	14.54(60.53)	2.26(9.41)	7.22(30.06)	24.02
Medium	Number of households (Number)	42(47.72)	29(32.95)	17(19.31)	88
	Quantity of marketed surplus (litre/day/household)	20.42(52.12)	13.47(34.38)	5.29(13.50)	39.18
Large	Number of households (Number)	22(73.34)	8(26.66)	-	30
	Quantity of marketed surplus (litre/day/household)	50.19(76.63)	15.30(23.37)	-	65.49
Overall	Number of households (Number)	161(53.67)	59(19.66)	80(26.67)	300
	Quantity of marketed surplus (litre/day/household)	19.82(60.79)	6.85(21.02)	5.93(18.19)	32.60

Figures in parentheses show the percentage to their respective row totals.

Table 8 Determinants of marketed surplus for different herd size categories

Particulars	Coefficients of parameters			
	Small	Medium	Large	Overall
Intercept	4.6183**(0.8167)	7.1587**(1.1658)	5.8319*(0.0986)	5.7269**(0.6531)
Educational level of head of the dairy household	-0.0236(0.0442)	0.0417(0.0767)	0.0476(0.1895)	0.0246(0.5198)
Herd size	0.4411**(0.0454)	0.5935**(0.2404)	0.7780**(0.3199)	0.5391**(0.0264)
Family size	-0.0285(0.0554)	-0.0282(0.0812)	-0.1007(0.1603)	-0.0488(0.0446)
Price of milk	0.4900*(0.2512)	1.3331**(0.3300)	0.8957(1.0216)	0.8548**(0.2005)
Membership in dairy cooperatives	0.9015**(0.0389)	0.1397**(0.0590)	0.1173(0.2236)	0.2466*(0.0327)
Distance of household from collection centre	0.0123(0.0239)	0.0672**(0.0017)	-0.1710(0.1180)	0.4187**(0.0210)
R- square	0.6807	0.7185	0.7745	0.7976
Number of households	182	88	30	300

Figures in parentheses are the standard errors. * 5 per cent level of significance, ** 1 per cent level of significance

variables explain that one per cent increase in herd size, price of milk, membership in dairy cooperatives and distance of household from collection centre increases the marketed surplus by 0.5391, 0.8548, 0.2466 and 0.4187 per cent, respectively. Price of milk had the highest influence on marketed surplus and educational status of head of the dairy household had the least influence. The family size had a negative and non significant influence on marketed surplus. Among the different herd size categories, the influence of price of milk was highest in case of medium farms and least for small farms. Membership of dairy cooperatives had the highest influence on small farms while the influence was least and non significant in case of large farms. This implies that the marketed surplus was high among small households who were member of cooperatives.

Conclusions

The results of the study indicated that, inspite of low dairy development in the region, milk production and productivity was considerably high among the sample households which depict immense scope of dairy in the region. An average dairy household produced about 36 litre of milk per day which was mainly from crossbred cows. On small households more milk was drawn from crossbred cows while on large households, local cows and buffaloes contributed more. Nevertheless, the highest productivity of all dairy animals is on large farms in comparison to other herd categories. This sets an example among the small and medium herd size categories and motivates them to focus on promoting better feeding and management practices as well as setting aside the resource limitation which can be overcome by making efficient utilisation of available resources. The consumption of liquid milk among producer households was 207 gm per day per capita which was higher than per capita availability in the region. Among the herd categories, the per capita consumption of milk was highest among the large herd size category of milk producers. Out of the 10 per cent of the milk retained at home, 40 per cent was consumed as liquid milk while 60 per cent of it was converted into milk products. The latter share was higher among small herd size category catering to the rural non-milk producing households. Of the total marketed surplus, about 61 per cent of milk was disposed off through halwais/ dudhiyas/private dairies and nearly 21 per cent through cooperatives while 18 per cent was sold of directly to the consumer. The medium and large household sell higher proportion of milk through *halwais*/ *dudhiyas* and cooperative network. Despite the fact that the marketed surplus per household was substantially higher (24.02 l/day) among small farmers, they disposed less than 10 per cent of the milk production per household through cooperatives. An analysis into the factor affecting marketed surplus found that herd size, price of milk, membership in dairy cooperatives and distance of household from collection centre had a positive and significant impact on overall marketed surplus of milk. Price of milk had the highest influence on marketed surplus such that farmers tend to sell off more milk if higher prices are offered. Thus better prices of milk to producer farmers ensure better availability of milk in the market. Among the different herd size categories, the influence of price of milk was highest in case of medium farms and least for small farms. The plausible reason could be that the production of milk on small farms are not large enough that marketed surplus, which is indeed the actual supply of milk to the market, could be influenced. Membership of dairy cooperatives had the highest influence on marketed surplus of small farms emphasizing that the strengthening of cooperative network in the region is going to benefit the small producers, in particular and accelerate dairy development, in general.

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RESEARCH ARTICLE

Adoption of livestock insurance in Punjab: Extent and constraints

Simranjeet Singh, Arjinder Kaur and Kashish Arora

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Abstract: The present study has been designed to investigate the extent of insurance coverage of dairy animals in Punjab and highlighting the constraints faced by the dairy households in its adoption. The primary data were collected from 100 dairy households in Patiala and Moga districts of Punjab based on their rank in milk production and secondary data were extracted from central and state government publications. The results of the study showed that Punjab ranked at 8th position among the states in terms of number of beneficiaries availing the dairy loan as well as the insurance with a total of 1877 beneficiaries in the state in the year 2018-19 and contributing to 4.8 per cent of the total number of beneficiaries in India. Out of the total number of animals in the sampled households which have adopted the insurance policy only 24.4 per cent of the animals were insured. The major constraint for non-adoption of insurance coverage was the small herd size of the livestock, while the main reason for discontinuing the policy and problem faced during the claim settlement was the low indemnity level provided by the insurance company. The major problem faced by the adopters of dairy insurance was the gap between the original market price and the sum insured. It was concluded that relevant measures like considering more diseases under insurance coverage, generation of database of dairy animals and sum insured on the basis of market price etc were required for the widespread penetration of the dairy insurance policy in Punjab and more extension services were needed to create awareness about it.

Department of Economics and Sociology,
Punjab Agricultural University, Ludhiana-141 004

Simranjeet Singh (✉)
Department of Economics and Sociology
Punjab Agricultural University, Ludhiana-141 004
Email: simargogia1@gmail.com

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Introduction

Livestock has emerged as an essential constituent of rural livelihood in India. Livestock sector being the most productive enterprise in agriculture is associated with greater risks which include production risk associated with low productivity of cattle, market risk associated with inadequate market infrastructure and price volatility because of highly perishable nature of milk and milk products along with other risks like morbidity losses, mortality losses and losses due to the natural calamities etc. Climatic extremities and disease epidemics increase the risk in the livestock farming. To cope up with different types of risks, there is an obligation of risk reduction strategies such as farm financing, diversification, spot and futures marketing contracts etc., which are somewhat lacking in the state while the other method is to transfer the risk or risk sharing which is only possible by providing the insurance cover for the livestock to the farmer. Hence, livestock being the most fecund asset in rural areas of the country acting as a constructive mechanism for the farmers to subsist the household related problems requires being insured (Ahuja et al. 2000). Livestock insurance in India started about five decades ago with the commencement of Operation Flood in the year 1970. Government of India administered a subsidized Livestock Insurance Scheme in the year 2005-06 which was on the pilot basis and was introduced in 100 districts of the country to demonstrate the benefits of insurance cover to the farmers (Chand et al. 2016). Although many serious measures have been taken by the government for the better implementation of the policy but the progress of Livestock Insurance Scheme has not been as encouraging as in case of Pradhan Mantri Fasal Bima Yojana (PMFBY) and only 6 per cent of the total livestock population has been covered with insurance in the country (Anonymous 2017). Only 1.7 per cent of the milch animals belonging to agricultural households have been insured till 2016 (Anonymous 2017a). Punjab is the dominant agrarian state and dairy sector is an important segment of the agricultural sector of Punjab contributing 10.38 per cent to the Gross State Value Added (GSVA) of the state at current prices (Anonymous 2019; Kashish et al. 2015; Kashish and Kataria 2020). So, a study was undertaken to

analyze the extent of insurance coverage and to assess the various constraints in the adoption of dairy insurance and to find out the reasons for non-adoption and discontinuity of it as well as the perceptions of the farmers about the insurance policy in Punjab.

Materials and Methods

The present study was conducted in two districts i.e. Patiala and Moga selected on the basis of highest milk production in Punjab in the year 2019-20. Multi-stage sampling technique was followed to select a representative sample for the study. In the first stage, in each of the selected districts, two blocks were selected at random. In the second stage, cluster of 5-6 villages from each block were selected. At third stage, a sample of 25 dairy households was selected from each cluster. The farmers who have opted for dairy insurance on their own could not be traced in the clusters, so these were selected with the help of veterinary officers of the selected village and bank officials who have sanctioned the dairy loans as insurance is mandatory for availing bank credit for dairy animals. In this way, the selection of dairy farmers, which was supposed to be random, turned out to be purposive. Thus, 42 farmers were found having the insurance coverage from 2014-20 in the selected villages, while 58 farmers were non-adopters of dairy insurance in the sampled dairy households. Thus, a total sample of 100 farmers covering 2 districts, 4 blocks and 24 villages were selected for the final analysis.

The study was based mainly on primary data while secondary data were used as a supporting base to know the extent of dairy insurance in Punjab state. The secondary data were collected from NABARD and various other published sources like Statistical Abstract of Punjab, official Reports of Department of Animal Husbandry, Dairying and Fisheries, Dairy Development Board, Livestock Census, 2019 about the number of beneficiaries and subsidy component in Punjab as well as in other states. The pertinent variables like insurance coverage, market price of animal, sum insured, detailed characteristics of animals, procedural difficulties pertaining to insurance, other constraints regarding adoption were collected from the sample households. Both Adopters and Non-Adopters were categorized as small, medium and large farmers based on the Adult Cattle Units (ACUs) or the Standard Animal Units (SAUs) using cumulative cube root frequency method. Data regarding the dairy inventory were collected and converted into Standard Animal Units and further divided into three categories based on number of units as small, medium and large using cumulative cube root frequency method. Thus, for this study, the dairy households having less than 15 ACUs were categorized as small farmers, 15-40 as medium farmers, and ≥ 40 ACUs as large farmers.

Garrett's Ranking Technique

Garrett's Ranking Technique has been used to rank the reasons given by the sampled dairy households regarding discontinuity of the policy, non-adoption of the policy and the constraints faced by the farmers who adopted the livestock insurance, constraints faced in claim settlement and for the perceptions of the farmers in availing the livestock insurance as a risk redressal measure.

$$\text{Per cent position} = 100 \times \left(\frac{R_{ij} - 0.5}{N_{ij}}\right)$$

Where,

R_{ij} = Rank given for i^{th} problem by the j^{th} respondent.

N_j = Number of problems ranked by j^{th} respondent.

Cobb-Douglas Production Function

To know the determinants of value of sum insured Cobb-Douglas Function was used.

$$\log Y = b_0 + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + \log D_1 + \log D_2 + \log D_3$$

Where

Y= Sum Insured

X_1= Number of lactations
X_2= Milk Yield
X_3= Adult Cattle Units (ACUs)
X_4= Age of the animal unit
D_1= Insurance Company (1= Public Sector Company, 0= Private Sector Company)
D_2= Species of Dairy Animal (1= Crossbred Cattle and Buffaloes, 0= Indigenous Cattle)
D_3= Caste (1= General, 0= Schedule Caste)

Results and Discussion

Livestock Insurance scheme has been existing in Punjab since 2004, but the data base for its coverage of the state was found to be lacking in terms of dairy cattle. The secondary data for dairy cattle insurance showed that the total number of beneficiaries availing the livestock insurance was recorded as 38865 in India during the year 2018-19 (Table 1).

The highest number of beneficiaries was found in Uttar Pradesh with a share of 18 per cent followed by Rajasthan and Maharashtra with 11.1 per cent and 9.8 per cent, respectively during the year 2018-19. Punjab ranked 8th amongst the states in terms of number of beneficiaries availing the dairy loan as well as the insurance

with a total of 1877 beneficiaries in the state in the year 2018-19 with a share of 4.8 per cent of the total number of beneficiaries (NABARD, 2020)

The socio-economic profile of the sampled dairy households helps to understand the background of the individuals risk bearing ability. It was observed that the average age of the sampled dairy households was observed to be 37.2 years (Table 2). The operational holding was found to be maximum in case of large farmers (19.6 acres) followed by small (14 acres) and medium farmers (13.7 acres). The overall herd size of the sampled dairy households was observed to be 1691 in number out of which crossbred cattle contributes maximum i.e. 54.8 per cent followed by buffaloes (32.22 %). The main reason behind insuring more number of crossbred cattle was observed as the higher milk yield from the crossbred cattle and more lactation lengths and lesser dry periods.

Out of the total herd size in the sampled households which adopted the insurance policy only 24.4 per cent of the animals were found to be insured. Most of the small and large farmers preferred to insure their buffaloes over cattle because of the better returns from the buffalo milk over the crossbred and indigenous cattle due to higher fat content.

The analysis brought out that the scheme of dairy insurance was not as popular in the state as none of sampled household has availed the insurance coverage on voluntary basis. All the 42 households, gone for dairy insurance have availed credit from institutional sources. So, to buy the insurance coverage was mandatory for them.

Reasons for Non Adoption of the Livestock Insurance

Among all the reasons for non-adoption of insurance, the first and foremost problem faced by the sampled dairy farmers was the smaller herd size of the dairy animals with the mean score of 63.53 (Table 3) depicting that livestock not being pursued as a major commercial activity for them, followed by lack of knowledge about the policy norms (54.90), perception of low risk about the

Table 1 Extent of Insurance Coverage in India along with Punjab, 2018-19

State	Total no of beneficiary	Percentage contribution	Amount of subsidy (Rs Crore)	Percentage contribution	Rank
Uttar Pradesh	6984	18.0	43.10	18.8	1
Rajasthan	4325	11.1	27.26	11.9	2
Maharashtra	3827	9.8	18.05	7.9	3
Tamil Nadu	3564	9.2	10.43	4.5	4
Karnataka	2760	7.1	12.20	5.3	5
Punjab	1877	4.8	11.49	5.0	8
Total	38865	-	229.70	-	-

Source: NABARD (National Bank for Agriculture and Rural Development)

Table 2 Socio-Economic Parameters and Extent of Livestock Insurance coverage of selected dairy households in Punjab (2014-19)

Particulars	Small(n_1=10)	Medium(n_2=22)	Large(n_3=10)	Overall(N=42)
Socio-Economic Profile				
Average Age (Years)	35.6	37.5	38.2	37.2
Operational Holding (Acres)	14	13.7	19.6	15.2
Cropping Intensity (%)	202.3	206.3	205.6	205.2
Total Herd Size (No.)	401	702	588	1691
Crossbred cattle	68 (16.95)	364 (51.85)	495 (84.18)	927 (54.82)
Indigenous cattle	38 (9.47)	155 (22.07)	26 (4.42)	219 (12.95)
Buffalo	295 (73.56)	183 (26.06)	67 (11.39)	545 (32.22)
Extent of Insurance Coverage				
Animals Insured (No.)	28* [6.98]	247 [35.18]	138 [23.46]	413 [24.42]
Crossbred cattle	5 [7.35]	148 [40.65]	116 [23.43]	269 [29.01]
Indigenous cattle	-	37 [23.87]	-	37 [16.89]
Buffalo	23 [7.79]	62 [33.87]	22 [32.83]	107 [19.63]

*Heifers are included in the herd size

Figures in the parentheses indicates the percentage to respective total

enterprise (47.41) and no compulsion to buy the policy (46.41). In case of medium farm households, the major constraint was observed to be lack of knowledge regarding the policy norms with a mean score of 64.67. The study conducted by Naidu R, 1989 and Kumar J, 2016 reported that the major reason for the non-adoption of livestock insurance was the lack of proper information and guidance followed by difficulty in getting death certificate from the veterinary officials. While the tedious claim settlement process was found to be the major hurdle in the adoption of the livestock insurance in Haryana (Chand et al. 2016). The lack of knowledge about the insurance policy and lack of interest of insurance companies to work in the remote areas was recorded as the major constraint in the adoption of the policy (Bhandari and Koirala 2018). The other constraints reported by these households were high premium rate, less number of cattle, low income and lack of willingness to adopt insurance cover. On the other hand, large farm households were not willing to adopt the insurance policy because they had better risk management strategies other than insurance which includes vaccination, deworming, improved breeding, adequate i.e. input and effective management of the farm; so they rejected the policy. Lack of knowledge, perception of low risk involved, high premium rate were other reasons cited by the large farmers for not going in to buy the dairy insurance.

Overall for the total non adopters of insurance coverage, small herd size, lack of knowledge about the policy, perception of low risk and non compulsive nature of livestock insurance for those who have not availed credit for the enterprise from institutional sources emerged as the major factors.

Reasons for discontinuity of livestock insurance of dairy animals in Punjab

Policy retention is not an easy phenomenon, it requires payment of premium along with the updation of knowledge at regular intervals. For some households, it proves to be beneficial while for others it gets critical remarks and is considered as an extra financial burden. Some reasons as specified by the dairy households has given in Table 4, who preferred to discontinue the policy rather than trying to renew it. One third of the households i.e. 14 out of 42 households discontinued the policy between the year 2014-20. The first and the most cited reason was the dis-satisfaction of dairy households from the indemnity level provided by the insurance company with the mean rank score of 70.36. While the study conducted in Haryana and Rajasthan cited the major reason for discontinuing the policy were long delays in claim processing followed by high rates of insurance premium (Kumar et al. 2016). In case of small and large farmers, the main reason for discontinuity of the policy was the complex procedure of claim settlement with the mean score of 71.00 and 69.67 respectively.

Table 3 Reasons for non-adoption of livestock insurance of dairy animals in Punjab, 2019-20

Particulars	Small(n_1=54)		Medium(n_2=3)		Large(n_3=1)		Overall(N=58)	
	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank
High Premium Rate	39.07	6	55.67	2	45.00	4	40.03	6
Lack of Knowledge	54.20	2	64.67	1	63.00	2	54.90	2
Low income	46.31	5	46.67	4	36.00	5	46.16	5
Small herd size	64.89	1	52.67	3	23.00	6	63.53	1
Perception of low risk	47.67	3	40.67	5	54.00	3	47.41	3
Not compulsion, so rejected	46.33	4	37.67	6	77.00	1	46.41	4

Table 4 Reasons for discontinuity of livestock insurance of dairy animals in Punjab, 2014-20

Particulars	Small(n_1=3)		Medium(n_2=5)		Large(n_3=6)		Overall(N=14)	
	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank
Complex Procedure	71.00	1	65.80	2	69.67	1	68.57	2
Low Mortality rate	51.67	4	32.20	10	47.67	5	43.00	7
High Premium rates	64.00	3	57.20	4	63.50	3	61.36	3
Less number of Cattle	39.33	10	36.20	9	45.50	6	40.86	9
Low Income	46.33	6	38.60	8	44.50	9	42.79	8
Not satisfied with policy norms	50.67	5	44.40	7	28.67	11	39.00	10
Poor Veterinary services	44.00	7	49.00	6	45.33	7	46.36	6
Insurance is not based on actual market Price	42.00	8	62.40	3	48.50	4	52.07	4
Unsatisfied with the indemnity level	66.67	2	75.20	1	68.17	2	70.36	1
Delay in the Claim Settlement	41.67	9	52.40	5	44.83	8	46.86	5
Repaid Dairy Loan	26.33	11	28.20	11	39.33	10	32.57	11

High premium rate was ranked at 3rd position with the mean score of 61.36, in case of both small (64.00) and large (61.36) farmers. Medium farmers discontinued the policy as the amount of sum insured was always lesser than the market price of the animal because the sum insured of dairy animal is fixed as per the policy norms but most of the times market price of animal is higher. Another reason for discontinuing the policy was observed as the timely repayment of loan and end of the complementary insurance scheme with the end of loan as it was not found useful for future security.

Constraints faced in claim settlement of livestock insurance of dairy animals

Satisfaction from the claim settlement and the indemnity level are the most important aspects for the dairy households, which highly affects the adoption or non-adoption of the insurance policy as well as its continuity. So, in the Table 5, various problems faced at the time of claim settlement by the sampled households have been highlighted. The first and the most important issue was the dis-satisfaction from the level of indemnity paid at the time of claim settlement with a mean score of 59.60. It was reported as always lesser than the amount of sum insured due to faulty investigation by the veterinary doctors. In case of death of higher number of cattle, compensation was not given for all and in some cases diseases causing fatality were not covered in insurance policy.

Most of the dairy households faced the problem of the poor veterinary services (48.95), which were not working efficiently as reported by the sampled dairy households. As per the policy norms, veterinary inspection is required to be undertaken within 24 hours but this is generally neglected and hence the reason for death gets unspecified leading to the lesser claim payments than the sum insured value of the animal.

Constraints pertaining to Adopters of livestock insurance of dairy animals in Punjab

Analysis of the constraints faced by the policy holders after the proper implementation of the policy, is important to enforce regulatory measures for improving in the working of the policy. The problems faced by the adopter category of livestock insurance have been given in Table 6. It was observed that the major problem faced by the adopters was the gap between the original market price and the sum insured with overall mean score of 66.62. Both small and large farmers reported this difference between the market price and sum insured as the major constraint with a mean score of 68.50 and 70.50 respectively, while in case of medium farmers the major constraint faced was poor veterinary facilities in the study area with a mean score of 64.27. High premium rates were not affordable by most of the farmers which didn't allow everyone to buy the insurance cover (Mahboob et al. 2019).

The second major problem faced by the adopters was the poor veterinary services in the study area (59.71) in terms of delays in the inspection of the dead animal to specify the cause of death, so that dairy household can apply for the indemnity. Even the value of sum insured was to be decided by the veterinary officer. Malpractices in the policy implementation which include bribe to the veterinary official for better records of the animals and to the insurance officials for getting higher claim payments were the other constraint cited by the adopters of livestock insurance policy in the state. Limit to the number of cattle insured was found as the third constraint faced by the large farmers with a

Table 5 Constraints faced in claim settlement of livestock insurance of dairy animals in Punjab 2014-20

Particulars	Small(n_1=10)		Medium(n_2=22)		Large(n_3=10)		Overall(N=42)	
	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank
Low indemnity level	49.80	2	60.45	1	67.50	1	59.60	1
Delayed Claim Settlement	46.00	4	47.18	3	47.90	3	47.07	3
Poor access to services	49.50	3	49.05	2	48.20	2	48.95	2
Tedious Claim Procedure	51.10	1	41.32	4	34.40	4	42.00	4

Table 6 Constraints pertaining to Adopters of livestock insurance of dairy animals in Punjab, 2014-20

Particulars	Small(n_1=10)		Medium(n_2=22)		Large(n_3=10)		Overall(N=42)	
	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank	Mean Score	Rank
Insurance only for death	39.20	5	45.32	3	44.50	4	43.67	3
Limits to number of animal insured	42.00	4	38.18	5	48.70	3	41.60	4
Poor Veterinary Services	52.70	2	64.27	1	56.70	2	59.71	2
Malpractices	45.60	3	38.55	4	27.60	5	37.62	5
Insurance not based on the actual market Price	68.50	1	64.00	2	70.50	1	66.62	1

Table 7 Estimates of parameters of Cobb-Douglas Function for the value of sum insured of dairy animals in Punjab (2014-20)

Particulars	Co-efficient	p-value	Standard Error
Intercept	5.61	0.001	0.55
No. Of lactation	-0.26	0.07*	0.14
Milk Yield	0.41	0.01**	0.15
Adult Cattle Units	0.00	0.91	0.04
Age of an animal	-0.05	0.01**	0.03
Insurance Company	0.02	0.56	0.03
Species of dairy animal	0.31	0.006***	0.05
Caste	0.26	0.15	0.09
R^2	0.62		
Adjusted R^2	0.54		

mean score of 48.70. Sahu (2017) studied insurance coverage in weaker sections of Ganjam district of Odisha and found that differential premium subsidy existed there but still the insurance was not taken against livestock neglecting their health condition, milk yield as well as under/over age which acted as a major constraint faced by the adopters of the policy. Mohapatra 2008 conducted a study in Punjab and revealed that some of the respondents faced problems in maintaining the ear tag of the cattle whereas a small per cent of the respondents faced problem in maintaining the documents for the claim aganist loss and considered it as cumbersome.

Difference between the sum insured and market price of the animal

The major constraint faced by the sampled adopters was reported as the difference between the sum insured and the market price of the animal. So, it was found essential to determine the factors on which sum insured was dependent. This has been undertaken by using the Cobb-Douglas Function as presented in Table 7.

A close perusal of the Table 7 revealed that the adjusted co-efficient of multiple determination (Adj R^2) was found as 0.54 for the value of sum insured of the animal by selected dairy households. The co-efficient of multiple determinations was estimated at 0.62 which indicates that about 62 per cent variation in the value of sum insured of an animal was explained by the variables included in this regression model. The co-efficient of milk yield and species of dairy animal were positive and significant indicating that the animal with higher milk yield will be insured at a higher price and if the species of bovine is crossbred cattle or the buffalo, it will be insured at higher price than the indigenous cattle because of higher milk yields of both the significant species. It was estimated that the co-efficient of the variables such as age of an animal and number of lactations were negative and significant indicating that with more number of lactations and increasing age of an animal the value of the sum insured decreases due to decrease in their market value and this may be due to decline in the productivity of cattle with increase in age.

Conclusions

Punjab, despite being a major milk producing state the dairy insurance coverage was found to be low as indicated by the number beneficiaries availing credit for dairy as well as insurance coverage for dairy animals. Voluntary adoption of dairy insurance was found to be lacking as coverage was mandatory for farmers availing credit for dairy animals. The extent of lesser dairy credit due to small homebred herd size was the major constraint for non-adoption of insurance coverage along with low awareness regarding the policy norms. Low indemnity level provided by the insurance company in case of cattle loss, complex procedure and high premium rates, etc. were found to be the major reasons for discontinuing the policy. The gap between the original market price and the sum insured was found as the major hurdle followed by poor access to veterinary services. The factors determining the value of sum insured of the animal like milk yield, species of dairy animals, age of an animal and number of lactations was found to be significant. There is a dire need of making easy accessibility in terms of creating widespread awareness of the policy and to bring technological advancements in its implementation so that the process of availing the livestock insurance cover becomes easier along with the process of claim settlement.

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RESEARCH ARTICLE

Impact of agromet advisory services on farmers' operational decisions related to dairy farming in Thiruvananthapuram

Manjusree RV[1], Sanjit Maiti[1], Sanchita Garai[1], Manjunath KV[1], Mukesh Bhakat[2], AK Dixit[3], SK Jha[4] and KS Kadian[1]

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Abstract: Climate factors has an impact on numerous aspects of productivity, reproduction, health, and adaptation in dairy animals. Therefore, the present study was undertaken to assess the impact of the agromet advisory services on farmers' decision making for different farm operations related to dairy farming. The study was conducted in Thiruvananthapuram District of Kerala state. Two blocks were selected randomly from the district and from each block two experimental and two control villages selected after verifying that villages were not receiving any kind of agromet advisory services from Agromet Field Unit (AMFU) in Thiruvananthapuram. From each experimental village 15 beneficiary farmers were selected and provided with treatment i.e. Agromet Advisory Bulletins were disseminated twice a week (Tuesday and Friday) regularly for 6 months through Whatsapp group and from each control village 15 non-beneficiary farmers were selected. To assess the impact Difference in difference design and regression models were used. The findings revealed that most of the agromet advices were only relevant for crop farmers and the advices relevant for dairy farmers were included less frequently. Hence advisory services pertinent to dairy farmers must be appropriately included in the agromet advisory services disseminated from AMFUs.

[1]Dairy Extension Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana

[2]Livestock Production and Management, ICAR-National Dairy Research Institute, Karnal-132001, Haryana

[3]Dairy Economics, Statistics and Management Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana

[4]ICAR-Indian Institute of Soil and Water Conservation (ICAR-IISWC), Regional Research Centre, Chandigarh

Sanjit Maiti (✉)
Dairy Extension Division
ICAR-National Dairy Research Institute, Karnal-132001, Haryana
Email: Sanjit.Maiti@icar.gov.in/sanjit.ndri@gmail.com
Mobile: 9466967086; Phone: 0184-2259216

Keywords: Agromet Advisory Services, Difference in Difference Research design, Climate Change, Dairy Farming

Introduction

Climate change is one of the major threats for the sustainability of livestock production systems in tropical countries. The major environmental factors affect livestock production system include temperature, relative humidity (RH), solar radiation, precipitation and wind speed (WS) (Hahn et al. 2003). Strategies to ameliorate negative impact of heat stress on production and reproduction in dairy animals include improved housing and management intervention to reduce climatic impacts on livestock. Weather forecasting is essential for dairy farming in a variety of ways, including disease and parasite transmission, feed grain availability, pasture and forage crop productivity, animal health, animal development, and reproduction, according to studies. Frisvold and Murugesan (2013) reported that both crop producers and diversified producers used data for more decisions than did livestock producers. It was also revealed that producers used weather forecasting advisory services most frequently for timing of irrigation, followed by livestock management. Ranchers used weather forecasting data more frequently for decisions about moving livestock. Use of weather forecasting information controlled commodity specialization and household income diversification. In India, only around 5 percent of farmers use meteorological information while rearing cattle, whereas, a quarter of the farmers said they didn't need the information, and a quarter said they didn't know where to get it (Bhan, 2018). Therefore, in animal husbandry, around one-sixth of the farmers rely on their own experience.

Kerala, a state on India's tropical Malabar Coast, having nearly 600 km of Arabian Sea shoreline and between latitude 10.850516, and the longitude 76.271080 is prone to major climate contingencies such as regular drought, flood causing transient water logging/ partial inundation and sea water intrusions, heavy rainfall with high speed winds in a short span, continuous high rainfall in a short span leading to water logging and outbreak of pests and diseases due to unseasonal rains Anonymous (2010). Farmers need to be more aware of weather contingencies and harmful weather occurrences or catastrophes, such as drought,

flood, cyclones, and heat waves, which occur often Anonymous (2011). A significant restriction for successful farm planning operations is the lack of reliable and timely agrometeorological advice. As a result, weather-based agromet advisory services are critical for weather-tuned farm operations. Hence there is a need for making the farmers realize the utility of agromet advisories in management of day-to-day farm activities and thereby reducing the loss and enhancing the production (Rathore and Chattopadhyay, 2016). The National Centre for Medium Range Weather Forecasting (NCMRWF) under the Ministry of Earth Sciences (MoES), Government of India in collaboration with India Meteorological Department (IMD), Indian Council of Agricultural Research and State Agricultural Universities had been providing Agrometeorological Advisory Services (AAS) at the agro-climatic zone level and even to the district level to the farming community based on location specific medium-range weather forecast (MRWF) (Chaubey et al. 2018). Hence, agromet advisory may be a mechanism to make climate sensitive dairy farming into climate resilient dairy farming. Thus, the present study was designed to determine the impact of agromet advising services on farmers' decision-making for various dairy farm operations in Thiruvananthapuram District of Kerala.

Materials and Methods

Sampling

The study was purposively conducted in Thiruvananthapuram district of Kerala. Two blocks namely Nemom and Athiyanoor selected randomly and from each block two villages were selected as experimental villages and two villages were selected as control villages after verifying that the villages were not receiving any kind of agromet advisory services from Agromet Field Unit (AMFU) in Thiruvananthapuram. Respondents were the dairy farmers with smart phone and internet connectivity and 15 such respondents were selected following random selection making a sample of 120. The farmers in the experimental villages were provided with treatment i.e., block level agromet advisory bulletins prepared from the AMFU, Thiruvananthapuram was disseminated regularly for 6 months (1st Jan – 30th June 2021) through Whatsapp. The experimental and control group was undergone pre-test as well as post-test before and after the treatment was administered.

Research design

Difference in difference (DiD) quasi-experimental research design (Fig. 1) was used to compare the outcome of groups exposed to different factors at different times. After-before difference in outcomes in the treatment group was calculated, and from this difference the after-before difference in the control group subtracted. The indicators used for the assessment of impact were shelter management, feeding schedule, water management, reproductive management and vaccination schedule.

If pre intervention outcome of the treatment group is D0, Post intervention outcome is D1 & Pre intervention and Post intervention out comes of control group is C0 and C1 respectively then,

Table 1 Socio-economic profile of the sample respondents

Variable	Categories	% of farmers in experimental group (n = 60) under the category	% of farmers in control group (n = 60) under the category
Age	Young	41.67	40.00
	Middle aged	51.67	50.00
	Older	6.67	10.00
Gender	Male	88.33	86.89
	Female	11.67	13.11
Education	Primary	13.33	11.67
	Secondary	48.33	43.33
	Higher secondary	33.33	30.00
	Graduation and above	18.33	15.00
Farming experience	Low	68.33	66.70
	Medium	23.33	23.33
	High	8.33	10.00
Caste	SC	3.33	10.00
	OBC	75.00	80.00
	UR	21.67	10.00
Occupation	Farming	60.00	51.67
	Others	40.00	48.33
Average operational landholding	Landless	3.33	5.00
	Marginal	88.33	81.67
	Small	8.33	13.33

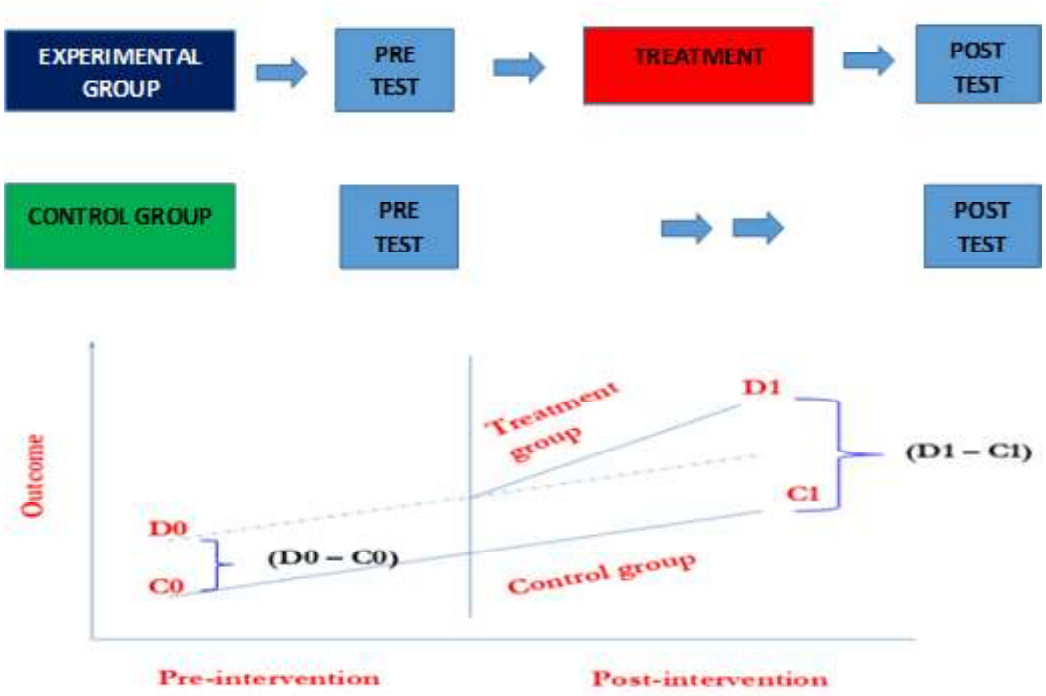

Fig.1 Difference in difference research design

IMPACT = (D1- D0) – (C1- C0)

Difference in difference quasi experimental research design was usually implemented as an interaction term between time and treatment group dummy variables in a regression model which is as follows:

$$Y = \beta0 + \beta1*[Time] + \beta2*[Intervention] + \beta3*[Time*Intervention] + \varepsilon$$

Results and Discussion

Socio-economic profile of the respondents

It is evident from the Table1 that half of the respondents were middle aged farmers with low farming experience and all the respondents were literates having at least primary education. It is also clear that majority of the respondents were marginal landholders and belonging to other backward caste category

Table 2 Possession of the dairy animal

Particulars	Experimental (n=60)		Control (n=60)	
	Mean	Range	Mean	Range
Herd Size (in SAU)	6.99	1.64 – 28.69	4.91	0.82 – 14.68
Species in the herd	Cattle, Buffalo		Cattle, Buffalo	
	Details of the breed and type of animal present in the herd			
Indigenous Cattle	Vechur, Cheruvally, Kasargod dwarf			
Cross-bred	HF cross, Jersey, Jersey cross, Brown Swiss cross, Sunandini, Upgraded Gir cross, Upgraded Red sindhi			
Buffalo	Upgraded Murrah			

Table 3 Herd composition maintained by the respondents

	Experimental(n=60)			Control (n=60)
	Indigenous cattle(n=15)	Cross bred cattle(n=60)	Buffalo(n= 4)	Cross bred cattle(n= 54)
Particulars	F(P)	F(P)	F(P)	F(P)
In milk	7(40.00)	86(143.33)	3(75.00)	14(25.92)
Dry	4(26.67)	48(80.00)	6(150.00)	34(62.96)
Heifer	9(60.00)	41(68.33)	3(75.00)	8(14.81)
Calf	(33.33)	78(130.00)	3(75.00)	11(20.37)

Table 4 Average milk production (in liter)

Particulars	Experimental group(n=60)	Control group(n=60)
Mean	11.6	8.85
SD	2.27	1.83

Table 5 Average treatment impact of the Agromet Advisory Services (AAS) on the operational decision in Dairy farming practices

Particulars	Experimental Group(n=60)		Control Group(n=60)		Treatment Effect
	Pre-Test	Post-Test	Pre-Test	Post-Test	
Watering management	1.00	1.06	1.00	1.00	0.06
Feeding management	1.62	2.04	1.68	1.84	0.27
Shelter management	2.40	3.23	1.05	1.79	0.09
Vaccination schedule	1.38	1.77	1.00	1.26	0.12
Reproductive management	1.15	1.15	2.05	2.05	0.00
Overall effect	1.00	1.65	1.00	1.00	0.65

Table 6 Regression analysis on impact of the Agromet Advisory Services (AAS) on the operational decisions in dairy farming

Particulars	Constant	Δ	T	δT	R^2
Watering management	1.00** (0.03)	0.001(0.04)	0.001 (0.05)	0.06 (0.05)	0.04
Feeding management	1.68** (0.31)	-0.07(0.36)	0.16(0.44)	0.26 (0.51)	0.02
Shelter management	1.05** (0.32)	1.35**(0.37)	0.74(0.45)	0.10(0.53)	0.22
Vaccination schedule	1.00** (0.24)	0.38 (0.28)	0.26(0.34)	0.12(0.39)	0.06
Reproductive management	2.05** (0.18)	-0.99** (0.21)	0.001(0.25)	0.001(0.29)	0.21

Values in parenthesis indicates standard error; ** & * indicates significant at 1 percent and 5 per cent level of significance, respectively. "- Treatment; T- Time; δT- Interaction effect

and depends on farming for their livelihood. It is also revealed that both the experimental and control group farmers were having a similar socio-economic profile.

Dairy production scenario

Table 2 shows that experimental group farmers were having average herd size of 6.99 standard animal units while the average herd size of control group farmers was 4.91 standard animal units. The herd include indigenous breeds of cattle such as Vechur, Cheruvally and Kasargod Kullan and cross bred cattle breeds such as Sunandini, HF cross, Jersey, Jersey cross, Brown Swiss cross, Upgraded Gir and Upgraded Red sindhi, etc. It was also found that few of the farmers were maintaining upgraded Murrah buffaloes. Table 3 depicts the number of in milk and dry animals as well as heifer, calves and dry animals maintained in the herd. It can be seen that the number of dairy animals in the experimental group was more compared to control group

From Table 4, it is clear that the average milk production among experimental group farmers was 11.6 liter while in case of control group farmers, the average milk production was found to be 5.85 liter. It can also be noted that the average milk yield did not significantly differ between experimental and control group farmers.

Impact of agromet advisory services on dairy farmers' operational decisions

Table 5 clearly depicts that almost all the operational decisions in dairy management had a positive treatment effect apart from reproduction management which was found to have zero treatment effect. The results in the Table 6 depicts that there was no significant treatment effect of agromet advisory services on any of the operational decisions related to dairy farming practice. Major portion of the agromet advisory bulletin was relevant for crop production and very little information was related to the dairy farming. Further, bulletins were prepared mainly by the agriculture professionals and they emphasized on crop related advisories. Therefore, agromet advisory services did not have any significant on the farmers' operational decision in any dairy herd management practices.

Conclusions

Dairy animals are vulnerable to climate change and weather related advisory services relevant for dairy farmers may make the dairy farming as climate resilient. But, agromet advisory services did not have significant impact on the operational decision in any of the dairy herd management practices. At the same time, treatment effect was positive in all the operational decisions of dairy herd management. Therefore, inclusion of appropriate advisory

services related to the dairy herd management may transform climate sensitive dairy farming into climate resilient. Regular training and awareness programmes for the farming community may have significant impact of the agmromet advisory services on farmers' livelihood improvement.

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SHORT COMMUNICATION

Detection of A1 and A2 milk in Tiruchirappalli district using TANUVAS A1A2 detect kit

V Jayalalitha[1*], K Shibi Thomas[2] and PN Richard Jagatheesan[3]

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Abstract: Milk contributes a vital role in nutritional security in most of the countries of the world. Recently a debate is going on worldwide that, A1 milk is harmful and A2 milk is beneficial. Though there was no supporting clinical evidence to claim these, milk is selling in the name of A2 milk for high cost without proper identification in the field level. To address this issue to both consumer and producers, scientists are in a position to rule out the A1 and A2 milk. In this study TANUVAS A1A2 detect kit was used in the detection of A1 or A2 type milk with dried blood or milk spot. 31 samples were collected from farms and individual farmers from different places of Tiruchirappalli district. All the samples were analysed with the ready to use primer based PCR test TANUVAS A1A2 detect kit. This study revealed that 20% of the crossbred cows, 78.95% of the indigenous breed cows, 66.67 % of non descriptive cows and 100 percent of the buffaloes had A2A2 genotype. The results of this study is giving the fact that, milk needs to be checked out with proper test like TANUVAS A1A2 detect before claiming for A2 milk.

Keywords: A2 milk, Genotype, TANUVAS A1A2 detect

Milk is the primary food for kids due to its nature of having all type of nutrients. Milk and milk products are playing vital role in balanced diet, recommended by many scientific authorities across the world. WHO, ICMR (India), NHC (China), has recommended a minimum of 300 g of milk and dairy product in daily diet ICMR-NIN has recommended keeping 100 mL milk in breakfast, 100 mL milk or curd in lunch, 50 mL milk (as milk or tea) in evening tea and 50 mL of milk or curd in dinner. One glass of milk satisfying the daily requirement of up to 30% of calcium, 26% of riboflavin, 25% of vitamin D, 25% of phosphorus, 22% of vitamin B-12, 16% of protein, 10% each of potassium, vitamin A and Niacin and hence milk can very well be termed a complete nutrient dense food.

Bovine milk contains around 3.4% protein, of which 30–35% is â-casein. There are 13 different allelic variants of B-casein (Farrell et al. 2004) and most A1 and A2 types are common genetic variant with a chain of 209 amino acids. A1 beta-casein contains histidine molecule in 67th position while A2 milk contains proline in that position.

The prevailing condition about A1 and A2 milk gives less clarity to the consumers because of the association between A1 milk consumption and disease risk. Upon digestion, A1 betacasein released the bioactive peptide BCM-7 (Beta-casomorphin) which is responsible for cardiovascular diseases. (Nguyen et al. 2015). However, there is no evidence of clinical trials linking A1 milk to the risk of disease. Still there is a big demand for A2 milk with higher market price in some places. In addition, there is no specific standard for A1 and A2 milk available with FSSAI. Hence there is a need of clear demonstration of A1 and A2 milk identification at the field level to make awareness about A1 and A2 milk. A1A2 Detect kit is one of the TANUVAS (Tamilnadu Veterinary and Animal Sciences University) technologies to identify A1 or A2 by identifying genotype of cow using milk or blood sample. Venkatesh and Gopal, 2016 reported an alternative non invasive detection of A1A2 using dried milk spot on EDTA (Ethylene diamene tetra acetic acid) treated filter paper. With this background this study is aimed to screen the samples from bovines in farms, and individual farmers for A1 and A2 identification in Tiruchirappalli district.

This study has been conducted in various places of Tiruchirappalli district including farms and individual farmers. Samples were collected in the form of dried blood spot from ear peripheral veins or dried milk spot using ready to use A1A2 detect kit cards. Each sample is properly identified, marked and noted with animal details viz., identification number, breed, physiological status etc. As and when the samples collected, they were packed well and sent to Translational Research Platform for Veterinary Biologicals

Veterinary University Training and Research Centre,
7/2, Kozhipannai Road, Kottapattu Tiruchirappalli 620 023

V Jayalalitha (✉)
Veterinary University Training and Research Centre,
7/2, Kozhipannai Road, Kottapattu
Tiruchirappalli 620 023
Email: jayav99@gmail.com

(TRPVB) laboratory, TANUVAS. Allelic variation was analysed using ready to use PCR kit available with TANUVAS A1A2 detect. Results and the relevant data of the animals were combined and tabulated for further inference.

In total 31 samples were screened for A1 A2 detection. All the results of the samples are depicted in the Table -1 with the animal details. Type of beta-casein (A1 or A2) protein in milk depends on the genes inherited by the cow. There are two alleles of this gene A1 and A2 and they are codominant. It means they are fully expressed resulting in an offspring with a phenotype that is neither dominant nor recessive. Hence, if the typing is A1A1means, it is genotypically homozygous for A1 betacasein production in milk and A2A2 means, genotypically homozygous for A2 betacasein. Whereas genotype is A1A2, it is genotypically heterozygous for A1 and A2 betacasein. Further if the animal is having A2A2 genotype only, it produces A2 milk otherwise it is termed as A1 milk.

With the above results, it is tabulated according to the type of animal in table.2. The percentage of A1A2 and A2A2 genotypic frequency in different breeds could be analysed.

In this study, crossbred cows like Jersey cross, Holstein Fresian cross, Sindhi cross and Gir cross have been used. Out of all, Gir crossbred heifer only showed the A2A2 genotype. Similarly in the indigenous breeds, Gir, Rathi, Sahiwal and Ongole have been included. Out of 19 samples 4 samples showed A1A2 genotype

Table 1:A1A2 typing of sample with their relevant animal details

TANUVAS sample ID	Breed	Physiological status of the animal	A1A2 typing
1350	ND	4th calving	A1A2
1352	Jersey cross	2nd calving	A1A2
1353	ND	2nd calving	A1A2
1355	Sindhi cross	4th calving	A1A2
1441	Rathi	3rd calving	A2A2
1442	Rathi	3rd calving	A2A2
1443	ND	2nd calving	A2A2
1444	ND	2nd calving	A2A2
1445	Rathi	2nd calving	A2A2
1446	Sahiwal	4th calving	A2A2
1447	Rathi	3rd calving	A2A2
1448	Rathi	4th calving	A2A2
1449	Rathi	3rd calving	A2A2
1450	Gir	3rd calving	A2A2
1474	Ongole	3rd calving	A2A2
1475	Ongole	2nd calving	A2A2
1476	Ongole	Heifer	A2A2
1477	Gir	1st calving	A1A2
1478	ND	5th calving	A2A2
1479	Ongole	4th calving	A2A2
1480	Gir	Heifer	A1A2
1481	Ongole	Heifer	A1A2
1482	Gir cross	Heifer	A2A2
1483	ND	Heifer	A2A2
1339	Sahiwal	Heifer	A2A2
1340	Sahiwal	Heifer	A2A2
1341	HFX	3rd calving	A1A2
1342	Gir	3rd calving	A1A2
1343	HFX	4th calving	A1A2
1344	Gir	2nd calving	A2A2
1345	Murrah	5th calving	A2A2

Inference
A1A1: genotypically homozygous for A1 betacasein production
A2A2: genotypically homozygous for A2 betacasein production
A1A2: genotypically heterozygous for A1 and A2 betacasein production

Table. 2 Percentage of animals having A2A2/A1A2 genotype

Type of animals	A2A2	A1A2
Crossbred cows (5)	20.00	80.00
Indigenous breed cows (19)	78.95	21.05
Non descriptive cows (6)	66.67	33.33
Murrah Buffaloe (1)	100.00	-

Values given in the paranthesis are total number of samples analysed

which means around 21.05 percentage of the indigenous breeds produced A1 milk. Non descriptive local cows were also included in this study and 2 out of 6 samples showed A1A2 genotype.

'Similar results were obtained by Sodhi and others (2012), out of 180 bulls tested from different regions only 11% bulls had A1/A1 genes. Among HF bulls, 22% had A1/A1 genes, whereas 45% had A1/A2 genes and 33% had A2/A2 genes. Among Jersey breed, 60% bulls had A1/A2 genes and 37.5% had A2/A2 genes, with only 2.5% having A1/A1 genes. Among crossbred bulls, only 1% had A1/A1 genes, while 50.6% had A2/A2 genes and 39% bulls had A1/A2 genes.

Truswell, 2005 screened 618 cattle from 15 breeds and reported that 98% cattle were of A2 type and in two breeds namely Malnad Gidda and Kherigarh had 20% animals with A1A2 genotypes. All 8 breeds of buffaloes were of A2A2 genotypes. This finding is support of the present report that whatever the breed, genotype will vary to the animal.

Conclusions

Narayan, 2019 reported in his review article, the role of A2 Corporation could be seen in creating scare about A1 type milk. No authorities have agreed adverse impact of A1 milk on human health including FSSAI, Government of New zealand etc. By ignoring EFSA Report, 2009 wider publicity has been created about health hazards in consuming A1 type milk and blaming the crossbred cattle. Since A2 milk is sold at higher price in the market, both consumers and producers are should be aware of A2 milk identification and its concept. Without knowing the identity of the milk, the consumers are paying very high price and nowadays there is a rise of good amount of A2 milk products too. Though FSSAI has not fixed any specific standards for A2 milk, its demand in the market is always as progressive curve. This study concludes that, before claiming of A2 milk, it needs to be identified with validated tests like TANUVAS A1A2 detect

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SHORT COMMUNICATION

Effect of parity on back fat thickness, body condition score and milk yield in Jersey crossbred cows of lower Gangetic region

Abhishek Paul[1], Champak Bhakat[2], Santu Mondal[1] and Ajoy Mandal[2]

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Abstract: The experiment was carried out to know the effect of parities on body score, back fat thickness and milk yield of Jersey crossbred cows. A total of 43 crossbred jersey cattle were examined for 120 days postpartum and divided into four groups, based on their parity number. Correlation and relationship study has revealed significant effect of parity on various parameters. The overall correlation coefficient between BCS and BFT were found to be 54%, 92%, 94% and 90% for P1, P2, P3 and P4 respectively. However, positive to negative significant correlation between BFT and milk yield by parity 1,2,3,4 were 46%, 26%, -10% and -12% respectively. Increased BCS at calving was associated with greater MY in first and second parity cows and reduced MY in cows of third parity or greater. Furthermore, the correlation coefficient between BFT and BW were found to be low in P1 (r=0.58) and high in P4 (r=0.78). The changes in body fat thickness (BFT) and body weight (BW) by parity difference were most distinct in younger cows i.e. -47.92 percent and -12.05 percent respectively. Relationship (R^2) between BFT and BCS in different parity group were 0.29, 0.87, 0.85 and 0.83 respectively ($p<0.001$). Finally, in case of primiparous cow BFT is valid for estimation of subcutaneous fat and second parity onwards BCS can be adopted to predict the body fat reserve of crossbred animals.

Keywords: BCS, Back fat thickness (BFT), Jersey crossbred, Milk yield, and Parity

[1]Abhishek Paul
ICAR-NDRI, Karnal,Haryana, PO-132001
E-mail: abhishek2011v01@gmail.com

[2] Champak Bhakat, ICAR-NDRI (ERS-Kalyani), West Bengal.
Email: bhakat56@gmail.com

[3]Santu Mondal, ICAR-NDRI, Karnal,Haryana, PO-132001
Email: saanvet@gmail.com

[4]Ajoy Mandal, ICAR-NDRI (ERS-Kalyani), West Bengal.
Email: mondalak@gmail.com

Abhishek Paul (✉)
LPM Section, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana
Email: abhishek2011v01@gmail.com

Introduction

The difference in body lipid between the start and end of lactation represents the body energy lost or gained in support of maintaining lactation. Female cows require adequate body fat reserve in order to supply their basal metabolism, growth, lactation and reproductive function (Edmonson et al. 1989). Effect of parity on curves of body condition during lactation of Danish red, Danish HF and Jersey has been studied by Friggers and Badsberg (2007). They reported that first lactation cows had shallower curves and had greater body condition scores at the nadir of the curve. This is also supported by the findings of Dechow et al. (2002) and Ji-yeon lee (2006). Whereas, Jersey and Danish Holstein had lost more body condition than the Danish red. In other study, Meikle et al. (2004) compared the change in body condition score based on parity during postpartum period and stated that primiparous cows had a steeper decline in body condition score than multiparous animals but recuperate faster.

The quality of the manual BCS depends on the observers and scoring protocol (Kristensen et al. 2006). The subjective nature of the condition scoring makes it difficult for inexperienced herd managers to make use of management recommendations. A study by Evans (1978) and Nicoll (1981) determined the factors causing variation in BCS and found that 60 to 70 % was due to animal variation 5% from the evaluator, 10 % happened animal-evaluator variation. An individual or group of observers may not get similar results on one cow and or over time. This is not because the nutritional status of the cow has changed over time, but the different observer may assign a different scores to a cow on same day (Paul et al. 2020).

The measurement of back fat thickness is an effort to objectively (noninvasive) quantify endogenous energy reserves, which result from nutritional status. Ultrasound machines with a linear transducer and 5 MHz frequency can be used in such evaluation. Staufenbiel (1992) and Klawuhn (1997) evaluated different sites of the carcass by ultrasonography and observed that the rump is an appropriate site to evaluate the subcutaneous fat.

It is often said that replacement heifers have greater nutritional requirements than all other females in the herd because they are young and still growing. Younger cows tend to score higher than the older cows (Schwager- Suter et al. 2000).

Since BCS is subjective and sometimes not repeatable, there may be need to develop and establish another practical method for assessing energy status of cows that would also ensure the pattern of changes in BCS across different parity. The aim of this study was to assess the degree of relationship between BCS and BFT in different parities of dairy animal.

The present study was carried out at Eastern Regional Station, National Dairy Research Institute (ERS-NDRI), cattle yard located in Kalyani, West Bengal. A total of 43 crossbred jersey cattle were examined for 120 days postpartum and divided into four groups, based on their parity number. All experimental cattle were reared under loose housing system with common feeding and watering management. The BCS assessment made in the visual method and took 1 to 6 point scales, adopted from (Prasad, 1994) for each cattle whereas BFT was measured by Ultrasonography machine (Mindray, Model- DP6600vet). The data were collected two consecutive days in a week for three months. The USG images were taken in B-mode, using 5.0 MHz frequency with a linear transducer. The images were taken to measure length of subcutaneous fat as BFT which demarked between skin and deep fascia above gluteus muscle. The USG images obtained from both side of rump regions and made the average for statistical analysis. The BCS and USG images were collected on the same day after morning milking whereas body weight were taken in every fortnight after completion of morning milking.

Statistical analyses of the present study were performed using statistical program R, version 3.2.4. The change in BFT and BW from calving to 120 days postpartum were analyzed by, lost divided by starting value. Then, multiply the result by 100.

Data on Body condition score, back fat thickness, body weight and milk yield from calving to 120 days of lactation are presented in Table 1, 2 and 3 respectively. The relationship between BCS and BFT have been presented in Figure1. The overall least squares mean of BCS in P1, P2, P3 and P4 was 3.93, 3.28, 3.40 and 3.93 respectively (Table: 1). The overall least squares mean of BFT in P1, P2, P3 and P4 was 14.24, 16.62, 16.08 and 21.90 respectively(Table:1).

The overall correlation coefficient between BCS and BFT were found to be 54%, 92%, 94% and 90% for P1, P2, P3 and P4 respectively (Table:2). The medium correlation for P1 indicates overestimation of Body scoring than the actual body fat present over the rump region.

Primiparous cows produced less milk than multiparous cows during the experimental period. Correlation coefficient between BFT and MY by parity 1,2,3,4 were 46%, 26%, -10% and -12% respectively (Table: 2). It is interesting to note that the primiparous fat cows produces more milk than older fat cows and vice versa. The impact of BCS on subsequent milk production had been evaluated by many researchers who recorded that higher milk yield was strongly supported by moderate BCS (Mushtaq et al. 2012) while others reported that cows with high BCS had no advantage in milk production (Flamenbaum et al. 1995) or negative correlation between milk yield and body condition (Mikone et al. 2013).

The changes in body fat thickness (BFT) and body weight (BW) by parity difference were most distinct in younger cows i.e. -47.92 percent and -12.05 percent respectively in P1 than that of

Table 1 Descriptive statistics

Parity		N	Mean (+SE)	SD	Median	Min.	Max.	Range
1	BCS	72	3.93±0.09	0.80	4	2	6	4
	BFT	72	14.24±0.59	4.99	14.78	4.52	25.2	20.68
2	BCS	117	3.28±0.09	0.97	3.50	1.50	6	4.5
	BFT	117	16.62±0.52	5.64	16.97	7.04	36.21	29.17
3	BCS	81	3.40±0.15	1.34	3.50	1	6	5
	BFT	81	16.08±0.91	8.18	15.47	3.33	33.1	29.87
4	BCS	117	3.93±0.13	1.36	4	1	6	5
	BFT	117	21.90±0.81	8.72	22.89	5.29	39.57	34.28

Table2 Correlation and relationship among various parameters

Parity	BCS & BFT			BFT & MY			BFT & BW.		
	r	R2	P- Value	r	R2	P- Value	r	R2	P- Value
P1	0.54	0.29	0.01	0.46	0.76	0.01	0.58	0.46	0.01
P2	0.92	0.84	0.01	0.26	0.62	0.01	0.64	0.37	0.01
P3	0.94	0.87	0.01	-0.10	0.40	0.05	0.68	0.40	0.01
P4	0.90	0.84	0.01	-0.12	0.54	0.05	0.78	0.60	0.01

Table3 Changes in BFT and BW

	Initial		After 120 days		Change (%)	
	BFT	BW	BFT	BW	BFT	BW
P1	18.82	352.5	9.8	307.06	-47.92	-12.05
P2	20.16	368.53	14.3	346.4	-28.91	-6.05
P3	18.68	376.22	15.64	360.8	-16.27	-4.04
P4	24.53	402.65	20.66	376.8	-15.77	-6.41

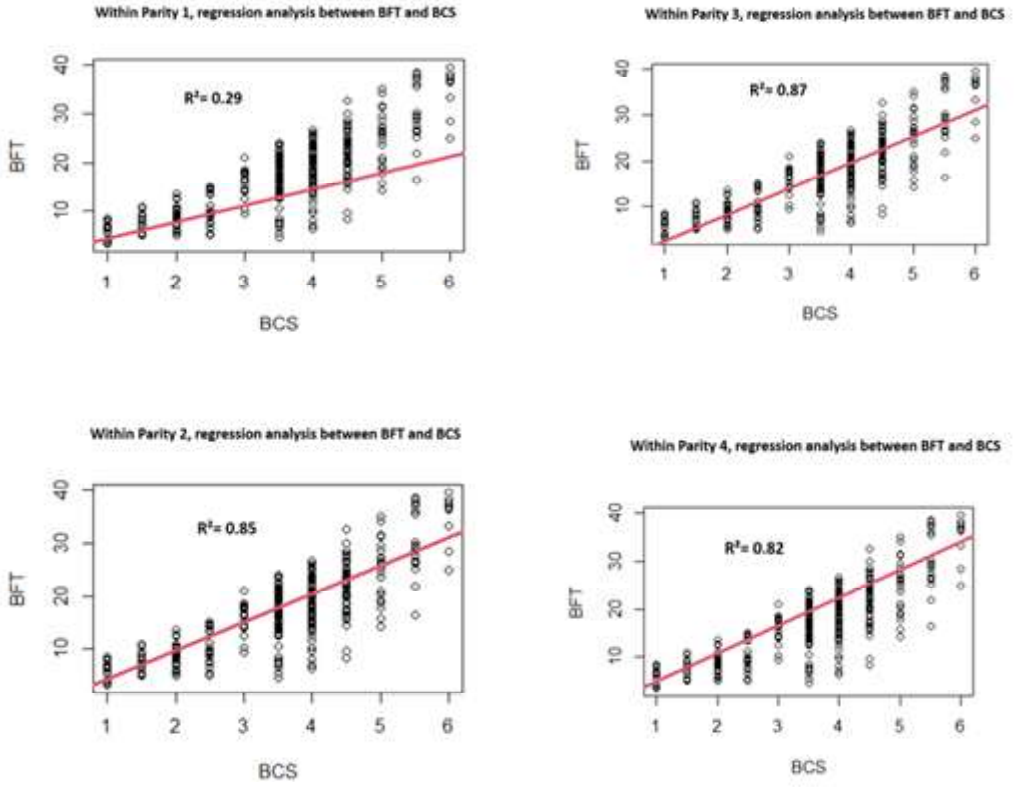

Fig. 1- Relationship between BFT and BCS in different parity group

older cows (Table:3). Meikle et al. (2004) also reported, Primiparous cows had a steeper decline in BCS than multiparous cows but they recuperated faster.

This is probably related to the increased needs for growth in primiparous cows occurring simultaneously with the demands of lactation and their lower feed intake capacity as described previously (Re´mond et al. 1991). Effects of parity and BCS at calving were found in IGF-1 concentrations. Whereas, primiparous cows had a higher concentrations of IGF-1 than multiparous cows (Taylor et al. 2003 and Wathes et al. 2003) are good indicators of the growth of animals. However, by the second lactation, depletion of BFT (-28.91% P2, -16.27% P3 and -15.77% P4) and BW (-6.05% P2, -4.04% P3, and -6.41% P4) reduced as growth is almost complete and milk production potential has increased considerably (Coffie et al. 2006). Furthermore, the correlation coefficient between BFT and BW were found to be low in P1 (r=0.58) and high in P4 (r=0.78) (Table: 2). Some of the earlier observations in cattle have suggested there is no correlations (Wildman et al. 1982; Maltz et al, 1997) or low correlations (Foschi, 2009) as it is influenced by rumen fill, while others suggest moderate correlations (Berry et al. 2006) and or high correlations (Veerkamp, 1998) between BCS and body weight.

Conclusions

There was a tendency for BCS loss from calving to nadir to be related to BFT across different parity of dairy cows. Correlation and relationship among BCS and BFT in primiparous cows, although significant, were weak, implying overestimation of body reserve than actual body fat present over the rump region. Mobilization of body fat was maximum in primiparous than multiparous dairy cows. The consensus is that cows that mobilize more tissue are more susceptible to health and fertility problems. Finally, in case of primiparous cow BFT is valid for estimation of

subcutaneous fat and second parity onwards BCS can be adopted to predict the body fat reserve of crossbred animals.

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