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INVITED REVIEW

# Edible spineless cactus (*Opuntia ficus-indica*): A promising alternative forage source for livestock

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**Abstract:** Spineless cactus (*Opuntia fcus-indica*) is a fast growing xerophytic plant well adapted to arid conditions. It remains green even during summer and can serve as a feed resource during scarcity. Its productivity is high in fertile soils and with irrigation facilities but it also grows in poor soils and with little water. It doesn't need well-drained soils and tolerates salinity to a higher extent. Cactus can produce a biomass of 20-200 tonnes DM/ha/year. Cactus pear has the advantage of being a source of water for animals particularly during the dry season. The adaptations enable cactus to convert water 4-5 times more efficiently to DM than the most efficient grasses. Cactus cladodes are high in water, sugars, ash, vitamins A and C but they are low in crude protein and fibre. They exhibit a high Ca: P ratio and are highly palatable. Spineless cactus can be used as a source of alternative green fodder for the livestock particularly in small ruminants and has the capability to combat extreme draught conditions with round the year availability. Though many varieties/clones of spineless cactus have been developed but their potential as animal feed need to be investigated further.

**Keywords:** Alternate feedstuff, Nutrient utilization, Production performance, Ruminants, Spineless cactus

## Introduction

In India, there is deficit of 44% of concentrate feed ingredients, 35.6% of green fodders and 10.95% of dry roughages (IGFRI, Vision 2050). The government labels 30% of India's landmass as wasteland. Against the backdrop of ongoing climate change, frequent and long droughts, land degradation and green fodder scarcity, spineless cactus (*Opuntia fcus-indica*) can be a promising alternative fodder for livestock as it is palatable (Russell and Felker, 1987).

Spineless cactus is a fast growing xerophytic plant well adapted to arid conditions. It remains green even during summer and can be used as a feed during scarcity. Cactus belongs to the family Cactaceae assimilating about 130 genera. It is highly resilient and has high water use efficiency and capability to grow in poor and degraded soils where other plants fail to grow. Cactus is vegetatively propagated and cladodes are used for this purpose. Cactus pear has the advantage of being a source of water for animals particularly during the dry season. It is tolerant to poor soil conditions and produces high biomass yield (Russell and Felker, 1987). Cactus is able to convert water 4-5 times more efficiently to dry matter (DM) than the most efficient grasses (Russell and Felker, 1987). These and other attributes such as ability to remain succulent during drought and produce forage, fruit and other useful products as well as its capacity in preventing long-term degradation of ecologically weak environments have increased the importance of cactus in arid and semi-arid regions. Ruminants adapted to these areas can make efficient use of non-conventional feed resources like *Opuntia ûcus-indica* (Khanum et al. 2007). Different parts of edible cactus have been shown to have antioxidant, anti-inflammatory, anti-diabetic and anti-cholestrogenic activities (Kauthale et al. 2017). Therefore, an attempt has been made to cover aspects like its production and potential as ruminant feed encompassing its effect of inclusion in the ration on feed intake, nutrient utilization and production performance.

### Distribution and production of cactus

Mexico is considered as the centre of cactus origin. Cactus is available in a range of environments from sea level in the Californian deserts to an altitude of 4700 m (above mean sea level) in the Peruvian Andes and from tropical areas of Mexico with temperatures greater than 5°C to parts of Canada where temperature reaches as low as -40°C (Makkar, 2017). It is cultivated in America, Asia, Africa, Europe and Oceania. Argentina has about

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1650 ha under cactus cultivation. Brazil has 500000 ha area under cactus production, Chile covers 935 ha while Mexico has a cultivated area of 54000 ha and a large area (3 million ha) under cactus in the wild as natural plantation. Other countries in America that have cactus plantation are Peru and Bolivia. Brazil and Bolivia use cactus mainly as a fodder crop while Chile, Argentina and Mexico use it for fruit production. Cactus in Peru is used mainly (60%) for production of red dye and the remaining 40% for fruit production. Mexico has started using more intensive production practices , i.e. adoption of drip irrigation and cultivation in more benign areas with better quality soil and good rainfall conditions and use of mechanical fruit cleaning. In Africa, cactus is cultivated in Algeria (30000 ha), Ethiopia (360000 ha, 66% being spiny cactus) and Morocco (120000 ha). South Africa has approximately 4500 ha for fruit (33%) and fodder (67%) production. Cactus area covered in Tunisia is approximately 600000 ha used mainly for fruit production.

In West Asia, Jordan (300 ha), Lebanon, Syria, Gulf countries (Oman, Qatar, Saudi Arabia, the United Arab Emirates and Yemen) and Israel (350 ha) cultivate cactus. In Europe, it is cultivated in Italy (15000 ha), Portugal (200 ha) and Spain. In some countries, cactus cladodes obtained on pruning the cactus orchards established for fruit production are also used for livestock feeding. This has enabled integration of livestock into the cactus production which brings back nutrients and organic matter to cactus cultivation through manure and also complements farmers' income. Due to lack of information on the area under cactus cultivation, it is difficult to assess precisely the importance of cactus in different livestock production systems (Makkar, 2017).

Its productivity is high in fertile soils and with irrigation facilities. It also grows in poor soils and with little water. It doesn't need well-drained soils and tolerates salinity to a higher extent. Cactus can produce a biomass of 20-200 tonnes DM/ha/year (FAO, 2017). With such high biomass yield (60-fold increase over rangeland productivity), it is possible to produce sufficient forage to sustain 4-5 cows per year. In India, commercial cultivation of cactus is yet to start. During the last two decades the research was conducted by many institutes in India especially those in arid and semi-arid regions but the outcome of this work is yet reach to the farmers (Kumar et al. 2018). Cacti can produce 4-5 times DM (Degu et al. 2009) per mm of rainfall than any other type of plant due to their crassulacean acid metabolism (Guevara et al. 2011).

Different clones/varieties of edible cactus (Fig. 1, 2) such as 1269, 1270, 1271, 1280, 1281, 1308, CAZRI Botanical Garden, Mount Abu, 1458, AHCP-2 etc. have been developed (Soni et al. 2015). The study conducted at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India showed that amongst the clones of edible cactus, the highest survival was found in clone 1271 (67%) followed by 1270 (65%), 1280 (64%) and the least in clone 1287 (48%). Clone 1270 took the least time of 69-72 days to sprout followed by 1271 (85 to 96 days) and 1280 (93 to 98 days) while the maximum time taken to sprout was found in the case of clone 1287 (97 to 110 days). Clone 1271 had significantly larger plant height followed by 1280 under different planting methods but clone 1270 recorded more plant height followed by 1271 with variable irrigation treatments. Over all, clone 1287 was shortest among the four clones. The clone (1280) performed well at all the salinity levels but plant height and number of cladodes formation were reduced with increase in salinity from normal (0 mM) to 32 mM and then to 52 mM (Singh et al. 2014).

**Edible spineless cactus as animal feed**

Long dry seasons, characterized by shortage of animal feed, both in quality and quantity are the major factors that affect animal production. The low nutritive value of the forage during the dry season is the main obstacle to increasing animal productivity (Abidi et al. 2009). Use of non-conventional feed resources that are available and adapted in dry areas and use water more efficiently can be the best option to ensure viability of livestock in these drought prone areas. Ruminants adapted to these areas can make efficient use of non-conventional feed resources like *Opuntia fcus-indica* (Khanum et al. 2007).

There are different types of *Opuntia spp*. namely native thorny, less thorny, thorn-less and regular type. All the four varieties are palatable with variable nutrient composition. They exhibit different acidic pH of 5.53, 5.27, 5.09 and 5.55, respectively. The native thorny and less-thorny varieties are adapted well to semi-arid regions like Rajasthan, Haryana, Maharashtra and other states. ICAR- Central Arid Zone Research Institute, Jodhpur has successfully established a thornless variety of cactus called 'CAZRI Botanical Garden' with very good yield and palatability to livestock (Kumar et al. 2017). However, cactus cladodes have high water content which results in high ruminal degradability and laxative effects when fed alone (Ben Salem et al. 2004, Souza et al. 2009, Santos et al. 2010) but this laxative action has no detrimental effect on the animal's health. It is the result of a fast passage through the digestive system (De Kock, 2001). Also, *O. ûcus-indica* cladodes are not nutritionally balanced as it is low in crude protein, ûbre, phosphorus and sodium (Souza et al. 2009). The low CP content may affect its use as a complete feed as it may cause protein deficiency in livestock. Ensiling it with dried forage legumes could increase DM content making it suitable for ruminant feeding.

**Chemical composition of edible spineless cactus**

The cactus cladodes are high in water, sugars, ash, vitamins A and C but they are low in crude protein and fibre (Ben Salem et al. 1996, Batista et al. 2003). They exhibit a high Ca: P ratio and are highly palatable (Nefzaoui and Ben Salem, 2001). Cladodes (1 to 3 year) are high in water during winter and spring (85-90%), less

**Fig.** 1 Edible spineless cactus

in summer (75-85%). Younger cladodes have higher water content (Le Houérou, 1996). Cladodes as forage can solve the problem of livestock watering but attention should be paid to their low DM content with regard to diet composition. To compensate for low DM content, the ruminant consumes large quantities of cladodes which may lead to diarrhoea. It is, therefore, recommended to include a fibrous feedstuff and appropriate supplements particularly N richer ones. De Kock (2001) reported that penned sheep could be kept alive for 500 days without drinking water provided they had free access to fresh cactus. Compared with conventional feedstuffs, *Opuntia* cladodes have high ash content. Depending on the species and cultivar, the ash content ranges from 10-25% on DM basis. Calcium followed by K is the most abundant mineral in the cladodes but the availability of Ca to rumen microflora and the host animal is compromised by the high content of oxalates and the extremely high Ca: P ratio (Sawyer et al. 2001).

**Table 1** Chemical composition (% DM basis) of different accessions of spineless cactus

| Parameter | Cactus accession | | | |
|---|---|---|---|---|
| | 1270 | 1271 | 1280 | 1308 |
| Dry matter | 7.51 | 7.95 | 11.44 | 11.15 |
| Crude protein | 6.09 | 5.48 | 5.45 | 5.33 |
| Crude fibre | 11.57 | 13.73 | 17.22 | 20.66 |
| Ether extract | 2.54 | 1.98 | 2.60 | 1.60 |
| Ash | 13.11 | 18.05 | 12.10 | 12.95 |
| Silica | 1.23 | 1.01 | 1.65 | 0.96 |
| NDF | 26.34 | 26.52 | 26.43 | 25.26 |
| ADF | 17.66 | 18.24 | 17.42 | 17.16 |
| Ca | 0.53 | 0.47 | 0.50 | 0.53 |
| P | 0.36 | 0.41 | 0.38 | 0.37 |
| K | 0.27 | 0.26 | 0.29 | 0.30 |
| Mg | 0.11 | 0.10 | 0.09 | 0.10 |
| Na | 0.20 | 0.19 | 0.22 | 0.21 |

Cactus cladodes are high in carbohydrates (60%) and β-carotene (6.5 mg/kg DM) as per reports (Ayadi et al. 2009, FAO, 2017). Mucilage level is high in the cladodes of spineless (6-13 g/kg fresh material) and spiny (6-14 g/kg fresh material) cactus (Abidi et al. 2009). Mucilage concentration increases at least twofold in summer compared to winter. It reduces salivation in ruminants thus avoiding a rapid decrease in rumen pH. In general, neutral detergent fibre (NDF) ranges from 18-30% on DM basis although the cladodes of a spiny cactus (*Opuntia imbricate*) contain 40% NDF. 12-20% acid detergent fibre (ADF) and 1.5-4.0% lignin. Carotenes, titratable acidity and carbohydrate contents increase during development while protein and fibre levels decrease. Cladodes are high in malic acid and its content fluctuates due to a CAM-based diurnal rhythm (FAO, 2017). Salem et al. (2004) reported that cactus cladodes contained 17.7% DM. The concentration of OM, TDN, CP, NDF, ADF, ADL, Ca, P, Na, K and Mg were 76.2, 65.0, 4.6, 33.8, 16.8, 5.2, 5.21, 0.1, 0.06, 2.6 and 1.09%, respectively. The levels of Cu, Fe, Mn and Zn were 6.5, 170.8, 248.9 and 31.0 ppm, respectively. The nutritional evaluation of four cactus accessions namely 1270, 1271, 1280 and 1308 was undertaken at BAIF laboratory, Urulikanchan, Maharashtra and is given in the Table 1 (Kauthale et al. 2017).

**Effect of inclusion of edible spineless cactus in diet on DM intake**

Sirohi et al. (1996) indicated that cactus was preferred by the animal over the conventional roughages like cenchrus hay and baru grass (*Sorghum halepense*). Total DM intake was the least in animals offered the highest level of cactus DM inclusion compared to the other treatments (Gebremariam et al. 2006).

Sheep supplemented with cotton seed cake and peanut cake had higher intake of cactus and tef straw DM than those supplemented with noug (*Guizotia abyssinica*) seed cake. Moreover, cotton seed cake and peanut cake promoted higher DM and OM intake compared to noug seed cake and the control treatment (Degu et al. 2009). Thus, supplementation increased total CP intake. Mixing cactus and browse in silage making improved both DM and N content in the product (Gusha et al. 2015). Cactus can serve as a link between legume forage and hays by supplying a degradable source of OM. Also, cactus-browse silages improved microbial protein flow to the lower gut for digestion thus increasing amino acid supply for maintenance, growth and production. Poor quality roughage utilization was improved with the addition of cactus-browse silages as supplements. These silages could be used in livestock feeding to improve livelihood in drier and resource constrained farming sections.

Spineless cactus can be an alternative feed in semi-arid regions replacing up to 80% of wheat bran in sheep diet without affecting DM intake (Lins et al. 2016). Spineless cactus plus urea was found to be a useful alternative feed option in semi-arid regions during the shortage of feed and water in prolonged drought. Spineless cactus could replace up to 80% of wheat bran in sugarcane-based diets for sheep promoting a higher intake of DM and TDN and consequently could reduce dependence on feed concentrates and the feeding costs (Lins et al. 2017). Makkar (2017) reported that 70% cactus and 30% concentrate could maintain a cow with daily milk yield of 20 L. The effect of replacement of 1/3rd dietary DM through *Opuntia* on DM intake and digestibility in sheep (Kumar et al. 2017) has been shown in Table 2.

**Effect of edible spineless cactus on nutrient utilization and rumen fermentation**

Salem et al. (2005) reported that barley diet and cactus diet showed similar OM digestibility, however, CP digestibility was lower in cactus diet. Dry matter intake and total tract apparent digestibility of DM, OM, CP and GE were lower in sheep fed *Opuntia* + *Cenchrus* hay diets than those on *Cenchrus* hay + Concentrate or *Opuntia* + *Cenchrus* hay + GN cake diets which were similar (Mishra et al. 2006). The NDF and ADF digestibility were similar in different groups. However, *Opuntia*+*Cenchrus* hay + GN cake diets had lower cellulose digestibility than *Opuntia* + *Cenchrus* hay diets than those on *Cenchrus* hay + Concentrate diets which showed similar digestibility values. The inclusion of cactus upto 24-36% in diets had pronounced effects on feed and water intake and urine excretion in Dorper wethers (Menezes et al. 2010). Due to its relatively high soluble carbohydrate and low fibre contents, inclusion of sun-dried *Opuntia* cladodes in diets increased the digestibility (particularly DM) and tended to stimulate voluntary intake. Despite an increase in the watery fresh faeces, less faecal DM was excreted. De-Kock (1980) reported that this laxative action due to high mucilage content is not a disease symptom and has no detrimental effect on the animal's health but it is due to faster passage through the digestive system.

The digestibility of *Opuntia* cladodes is comparable with high quality hay (Shoop et al. 1977). The replacement of wheat bran by spineless cactus provided a higher nutrient intake explained by the concentration of easily fermentable carbohydrates and lower indigestible NDF content of diets promoting better dry matter digestibility (Lins et al. 2016). The plant is extremely variable in its nutritive value which depends mainly on species, variety, age of plant, season and plant part (Hanselka and Paschal, 1990). Sirohi et al. (1997) showed that *Opuntia* from semi-arid regions in India contained 9.2% CP which is higher than the commonly used dry roughages (straw, strovers and grasses) in ruminant feeding. Although *Opuntia* feeding with conserved fodder maintained adult sheep, however, high N loss in urine led to negative N balance (Sirohi et al. 1997).

The faecal N loss was higher in lambs than kids and higher with barley than cactus diets (Abidi et al. 2009). The amount of N voided in urine was not affected by supplement type and animal species. Replacement of wheat bran with spineless cactus did not alter N loss from faeces. However, there was a quadratic effect on N loss from urine representing approximately 19.7% of the ingested N in the diets with spineless cactus plus urea. The maximum N loss from urine was estimated with a 70.3% replacement of wheat bran (Lins et al. 2017).

The total N content of ruminal ûuid was the highest in *Opuntia* + *Cenchrus* hay + GN cake diet compared to *Opuntia* + *Cenchrus* hay and *Cenchrus* hay + concentrate diet. *Opuntia* feeding increased ruminal pH but decreased total volatile fatty acid (TVFA) and fractional VFA concentration (Mishra et al. 2006). Supplemented groundnut meal improved ruminal N and $NH_3$-N whereas impaired microbial N supply needs further research to optimize P and other nutrient supplements for better animal performance. There was a reduction in microbial protein synthesis at above 46% replacement of wheat bran with spineless cactus (Lins et al. 2017) which could be due to greater amount of urea added to the diets. The lower microbial protein production with replacement of 100% of wheat bran could be explained by the presence of low ruminal digestibility of fibre of sugarcane resulting in lower energy available for rumen bacteria.

**Effect of edible spineless cactus on blood parameters**

Blood Ca concentration was affected by the supplementation of cactus and barley at 1 week prior to and at 2 weeks after lambing. The ewes supplemented with cactus had a higher concentration

**Table 2** Effect of replacement of dietary DM through *Opuntia*, on DM intake and digestibility in sheep

| Experimental Group(n=8) | DMIntake (% of body weight) | DM Intake (g/kg $W^{0.75}$ of body weight) | DM digestibility (%) |
|---|---|---|---|
| Opuntia* + Green Napier | 3.39 | 79.32 | 61.49 |
| Opuntia* + Berseem hay | 4.15 | 100.11 | 62.00 |
| Opuntia* + Lathyrus straw | 4.35 | 101.71 | 60.09 |
| Opuntia* + gram straw | 3.45 | 82.24 | 55.95 |

*1/3rd dietary DM was replaced through *Opuntia*

of Ca than ewes supplemented with barley (Rekik et al. 2010). They also reported that plasma glucose concentrations at 2 weeks prior to lambing were higher in ewes that were supplemented with cactus and the situation was reversed 2 weeks after lambing, the ewes supplemented with barley having a higher plasma glucose concentration. However, insulin level was lower in ewes of both feeding regimes and not different at any sampling time with no clear trend before or after lambing. Nitrogen retention increased by 0.10 g/d while plasma urea nitrogen (PUN) increased by 0.20 mg/d for every 1% level of replacement of wheat bran with spineless cactus in sugarcane based diet (Lins et al. 2017). Despite the gradual increase in PUN, toxicity was not recorded in the animals that received up to 39.4 g urea/kg ration. However, above 31.6 g/kg of supplied urea, there was a reduction in DM intake with 80% replacement.

**Effect of inclusion of edible spineless cactus in rations on production performance**

Inclusion of cactus in the diet or other comparable diets up to 50% on DM basis for sheep fed tef straw promoted weight gain without causing digestive disturbances common in diets with high cactus inclusion (Gebremariam et al. 2006). Cactus supplementation with cottonseed cake and peanut cake resulted in higher daily BW gain than the non-supplemented sheep. Sheep supplemented with cactus alongwith cottonseed cake had higher slaughter weight and dressing percentage on empty BW basis than the non-supplemented ones. They opined that the basal diet consisting of cactus and tef straw promoted body weight gain in sheep indicating their usefulness under conditions of feed scarcity (Degu et al. 2009).

Forage cactus meal showed the capability of replacing ground corn in the diet of ovines without affecting biological yield and weighted value of prime and choice cuts or the yield of viscera and organs (Santos et al. 2011). The results on use of cactus-legume diets were comparable to those of commercial diet (Gusha et al. 2015). Provision of cheaper quality protein from browse hay and readily fermentable sugar from cactus to animal feeding on poor quality roughage had improved roughage intake. Slaughter weight could be reached earlier if these supplements were used leading to higher and quick turn over in goats production. Gusha et al. (2015) reported that throughout 1$^{st}$ month of life, lambs born to barley and cactus fed ewes had a similar growth pattern. At 10 days of age, cactus fed lambs weighed 6.8 kg compared with 6.2 kg for barley lambs. At 30 days of age, average BW of lambs of both treatments was 9.5 kg. Cactus feeding in Osmanabadi kids also enhanced the performance of kids in terms of total body weight gain and average daily gain in body weight without any adverse effect (Kauthale et al. 2017). Spineless cactus could be included up to the level of 30% on DM basis in the finishing diets of lambs to increase the fat content of meat without compromising its sensorial properties (Lima et al. 2019).

Cactus fed ewes tended to accumulate more colostrum at birth and yielded higher colostrum at 24 h than barley fed ewes (Rekik et al. 2010). The milk yield at 10 days from lambing in ewes receiving the barley and cactus based diets averaged 1441 and 1580 g/d, respectively. For both feeding regimes, milk yield decreased at 30 days to 1030 and 1041 g/d for barley and cactus based diet fed ewes, respectively. Lipid extracted from animals on the cactus diet contained more C18:2 and conjugated linoleic acid (CLA). The composition was similar for the other fatty acids. Furthermore, animals fed cactus based diet showed a higher proportion of poly-unsaturated fatty acid (PUFA) and PUFA: SFA ratio than those in the control group (Atti et al. 2006). The lipid content of the goat milk underwent a linear reduction (3.84 to 2.97%) with the substitution of corn meal by cactus pear (Costa et al. 2010). The milk total solids content decreased linearly with the substitution of the energetic concentrate by cactus ranging from 12.08 to 10.76%. The percentages of medium-chain SFA in goat milk increased linearly with the substitution of corn meal by cactus pear. The total fatty acid concentration in the goat milk also increased linearly with the substitution of corn meal for cactus pear ranging from 62.80 to 71.93%. While mono-unsaturated fatty acid (MUFA) presented a linear reduction with values ranging from 26.73 to 16.11%, while the total PUFA concentration in goat milk did not change that averaged 2.30%.

Cactus feeding reduced proportion of stearic and oleic acids but did not affect linoleic (C18: 2) and linolenic (C18: 3) acids. Abidi et al. (2009) found that cactus diet increased the accumulation of trans-11C18:1 as compared to the meat from the animals fed the barley-based diet. Feeding cactus resulted in a lower percentage of n-3 fatty acids in sheep compared to goats. Despite the higher level of total n-6 fatty acids in kid meat, the n-6/n-3 ratio had a very low value which is favourable for human health.

## Conclusions

Edible spineless cactus can be grown easily in the lands with low water content due to its higher water conversion efficiency. Moreover, it has more tolerance to higher soil salinity. Therefore, growing cactus as a forage source for livestock can lead to a proper utilization of waste lands. Nutritional value of spineless cactus cladode is almost similar to some of the other conventional cereal fodders. Use of its cladodes as ruminant forage source reduces the water requirement as its cladodes are significantly high in moisture content which is of significance to the livestock farmers particularly in draught prone areas. Therefore, edible spineless cactus could be an alternate source of green fodder for livestock particularly small ruminants with due supplementation of nutrients especially protein, however, nutritional worth of different clones/varieties in different ruminant species need to be evaluated for formulation of balanced rations.

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RESEARCH ARTICLE

# Characterization of *Mohanthal* – A traditional sweet from Gujarat, India

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**Abstract:** *Mohanthal* is a popular sweet consumed in Gujarat state of India. In order to characterize the typical attributes of this traditional sweet, samples were collected from Ahmedabad, Anand, Vadodara and Palanpur cities of Gujarat and evaluated for sensory, physico-chemical, textural and microbiological characteristics. Wide variations in the composition of the market samples were found to exist. Moisture, fat, protein, lactose, sucrose, ash, other carbohydrates and free fatty acid contents of the market samples were in the range of 8.64-13.86, 18.21-28.12, 8.27-13.00, 0.00-12.10, 19.00-29.56, 0.81-1.81, 17.61-32.95% and 0.85-1.56 % (oleic acid). Microbial analysis showed moderate standard plate counts (SPC) 3.0 – 4.0 log(cfu/g) and yeast and mould counts 1.0 – 2.3 log(cfu/g) in market samples of Mohanthal. Sensory attributes of highly preferred *Mohanthal* sample were: light brown to medium brown colour with rectangular shape, soft, cohesive and firm body with little crumbliness, small grains preferred over smooth texture, pleasant, nutty, roasted and caramelized flavor with rich ghee flavour. There is a need for further study to optimize a process for the preparation of *Mohanthal* so that dairy industry can produce this besan and milk solids based sweet on a large scale. This overall characterization of *Mohanthal* will also help in any further works to be taken up on Mohanthal for example cottage level manufacture, mechanization, setting of standards etc.

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**Keywords:** *Mohanthal*, Characterisation, Gujarat, Market survey, Traditional sweet, Composition, Textural characteristics

## Introduction

India is a country known for its rich cultural heritage of various social and historical aspects including food and cuisines. It is a home to hundreds of varieties of sweetmeats on whose taste generations of people grew up. Many of traditional products find mention in oldest relics, which are popular even today (Pagote and Rao, 2012). Naturally, such sweet items have embedded in the psyche of the people here and continue to do so even in Indian diaspora abroad. But many of them could not withstand the onslaught of western products' publicity and became overshadowed so much so, that recent and present generations are blissfully unaware that so many varieties of sweets indeed existed in earlier years! It is only recently that the government realized the need to survey and document Indian traditional products, otherwise these products might be lost forever. Further, documentation of such products creates awareness among people in other parts of the country and may spur their commercial production as well. As such, Government of India lays emphasis on promoting indigenous technologies and encourages patent protection. It is in this direction that Indian Council of Agricultural Research has mandated Institutes to survey and characterize traditional Indian dairy products and National Dairy Research Institute is a part of such nationwide endeavour. This is possible by techniques like survey works, which were used by many workers earlier to characterize products like basundi, khoa, sweets etc. Such studies help to define the typical characteristics of products and will be of immense help in patenting processes. These results will serve as a base platform for the future works on the product. Also, the industry may think of manufacturing and marketing the traditional products because of their consumer appeal.

During our survey, we observed that traditional sweetmeats are basically prepared using three or four base materials such as milk, cereal flours, wheat/legumes particularly Bengal gram *(Cicer arietinum)* and fat. Using the above ingredients, the sweetmeat manufacturers are able to provide large variety of sweetmeats in

the market place with their inherited art/skill and effective subtle changes in processing conditions. For example, the products like Laddu, Sohn papri, Mysore pak, *Mohanthal* and *Besan* burfi prepared using Bengal gram flour (*besan*) have similar ingredients, but the textures of these products are entirely different. Several of these region-specific traditional dairy products have been surveyed, characterized and documented. Mysore pak was launched by Karnataka Milk Federation and today it is one of the major revenue earners for the organization. In spite of such efforts, many products still remain unknown to other parts of the country. There is a need to document and preserve such traditional products. Several products have their origin in specific areas like Mysore pak (Mysore), Dharwad peda (Dharwad), Mathura peda (Mathura), kunda (Belgaum), basundi, khoa jalebi and shrikhand (Maharashtra), payasam (Karnataka and Kerala) etc. *Mohanthal* is one such region specific milk sweet.

*Mohanthal* is a traditional sweet product popular in Gujarat and some parts of Rajasthan. Mohan refers to '*Lord Krishna'* and Thal refers to '*thali or plate'. Mohanthal* is offered to Lord Krishna at various festivals. It is also a favourite at weddings. *Mohanthal* may be categorized under 'Cereal and pulses based dairy product'. Other Cereal and pulses based dairy products are: Mysore pak, doda burfi, pinni, kheer, payasam, halwasan etc. The history of *Mohanthal* origin is obscure and only a few scattered literatures are available related to *Mohanthal*. Traditionally *Mohanthal* is prepared at house hold levels and also by *halwai's*. It is observed on the basis of preliminary observations that the product *Mohanthal* is famous for its rich pleasant roasted flavor along with the rich ghee aroma. It is marketed in the form of small rectangular shaped pieces. The product showed wide variation from shop to shop. *Mohanthal* produced and marketed by local *halwai's* has a limited shelf life of 1-2 weeks.

## Materials and Methods

### Procurement of *Mohanthal* samples

*Mohanthal* is prepared in many households in Gujarat. It is also being sold in various sweet shop outlets in towns and cities. Based on survey work, and through various sources and media, well known manufacturers of *Mohanthal* were identified for the study. In all sixteen samples of *Mohanthal* were procured from selected cities namely Ahmedabad (C-1), Anand (C-2), Vadodara (C-3) and Palanpur (C-4). Four samples of *Moahnthal* from reputed manufacturers from each city were collected. As good number of product manufacturing units was present in these cities, they were able to meet local market demand as well as supply the product to nearby regions too. A few selected samples from well-known manufacturers were brought to Southern Regional Station of ICAR-NDRI in their packaging containers and evaluated for sensory, textural, microbial and physico-chemical parameters.

### Sensory evaluation

Sensory evaluation of the procured *Mohanthal* samples was carried out by semi-trained faculty of Dairy Technology Section of the institute using 9-point hedonic scale and descriptive sensory evaluation technique, in hygienic and well ventilated laboratory. The judges were asked to evaluate and describe various product attributes viz. colour and appearance, flavour, body and texture, sweetness and overall acceptability.

### Analytical

#### *Texture profile*

Texture profile analysis of the samples of *Mohanthal* was carried out using Texture Analyzer, TA-XT plus Stable Micro System, England (Bourne, 1975). The instrumental test protocols maintained were: Option: return to start; Test mode : compression; Pre-test speed: 1 mm/s; Test speed : 5 mm/s; Post-test speed: 5 mm/s; Target mode : Distance; Distance: 10 mm; Time : 5 sec; Trigger type: Auto (Force); Trigger force : 5 g; Break Mode : Off; Advanced option: ON; Probe: P/75 plunger probe

*Mohanthal* sample, gently patted into cubes of 20 x 20 x 20 mm and tempered at 30°C for about an hour, was kept positioned centrally over the platform of Texture Analyser and the computer was allowed to execute the program to run the test, then the sample was compressed (50% compression) by the plunger twice (resembling two bites) and the force exerted back by the sample onto the plunger was sensed by the machine generating a two peak force – time curve. Different textural parameters like hardness (Newtons), cohesiveness (no units), spinginess (no units), gumminess (Newtons), and chewiness (Newtons) were computed from the force-time graph as described by Patel and Rao (2013).

#### *Proximate composition*

The market samples of *Mohanthal* were thoroughly mixed using pestle and mortar and the homogeneous mass was used for all the analyses. The nuts embedded on the surface as well as silver coating, if any were carefully removed before grinding. The following physico-chemical estimations were made by the methods mentioned: moisture content-gravimetric method (BIS, 1981), fat content-Mojonnier method (AOAC, 2005), ash content - gravimetric method (AOAC, 2005), total protein - Kjeldahl method (AOAC, 2005), lactose and sucrose - volumetric method (BIS, 1981), and other carbohydrates- by difference, FFA – Thomas et al. (1954).

#### *Colour*

The properly mixed sample of *Mohanthal* was stuffed into scratchless petriplate (Diameter: 3 cm) and the bottom surface exposed to scanner (Hewlett-Packard Scan jet 5370c). The scanned image was opened in Adobe Photoshop and colour parameters

measured as described by and Vyawahare and Rao (2011). Browning Index (BI), Yellowness Index (YI) and Whiteness Index (WI) were computed as described by Yam and Papadakis (2004).

### *Microbiological*

All the market samples of *Mohanthal* were analyzed for standard plate count (SPC) and yeast and mould count by BIS (1981) method (pour plate method).

### Statistical

The data of various attributes of sixteen *Mohanthal* samples from different markets were statistically analyzed employing one way ANOVA along with Tukey test using SPSS software, to know the variations in samples between markets.

## Results and Discussion

The market samples collected from different cities are shown in Fig.1 and their sensory descriptions are given in Table 1. It may be seen that many samples are mixed and garnished with nuts. Natural colour of *Mohanthal* was observed to be light to medium brown, however, added colour and silver foil coatings are adopted by some manufacturers for consumer appeal. As a traditional practice as well as for typical body and textural attribute, *Mohanthal* is always sold in rectangular or cubical shaped.

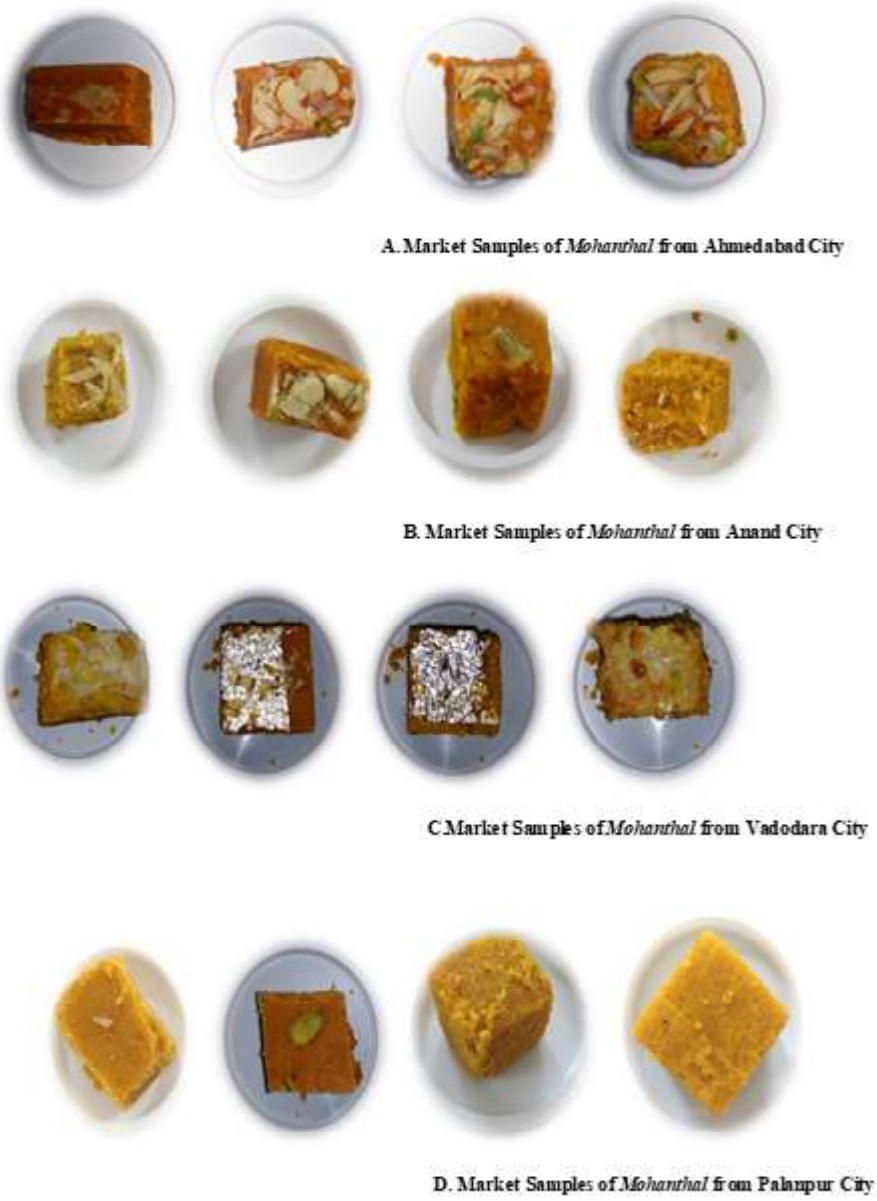
### Chemical composition of *Mohanthal*

The fat content of *Mohanthal* collected from different markets varied from 18.21 – 28.12% (Table 2), with an average for all markets of 23.74%, and statistical analysis indicated a significant variation between the markets. All other constituents also varied significantly ($P<0.05$), the protein content ranging from 8.27 – 13.00, lactose 0.00 – 12.10, sucrose 19.00 – 29.56, moisture 8.64 – 13.86 and ash 0.81 – 1.81, % respectively. These variations in the samples from market to market could be attributed to the following reasons: 1) *halwais* use *besan* of different ages; as per *halwais*, fresh *besan* gives different texture and taste than stored one 2) use of milk solids other than skim milk powder and khoa 3) extent of roasting is arbitrary and manually controlled depending on the time availability and personal preferences; 4) sugar syrup concentration varies, because sugar and water are not weighed properly and used in arbitrary quantities. It is also boiled for

**Table** 1 Descriptive sensory characteristics of market samples of *Mohanthal* collected from different markets

| Sensory attributes | markets | | | |
|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 |
| Colour and appearance | Light orange; brown to dark brown in colour, garnished with almonds & pista etc. | Light yellow to reddish brown colour, smooth to granular appearance, coated with few pieces of nuts | Golden yellowish to dark brown in colour, garnished with nuts & silver foils | Light yellow to brown in colour, smooth appearance, no garnishing |
| Body and texture | Soft & slightly chewy body, cohesive, smooth to slightly grainy texture | Slightly soft to hard body, moist to dry, slightly granular & crumbly | Slightly soft to hard, brittle & crumbly body | Smooth to slightly granular texture<br>dry, hard, brittle & crumbly body |
| Flavour | Pleasant, cooked to caramelized, ghee flavor, less raw *besan* flavor Compare to other | Good, nutty & caramelized, ghee flavor | Raw *besan* flavor to ghee flavor, lacks in typical caramel flavor | Raw *besan* flavor to cooked roasted ghee flavor |
| Sweetness | Slightly less to optimum sweet | Optimum to slightly more sweet | Slightly less to optimum sweet | Optimum to slightly more sweet |
| Overall acceptability | Most Preferred (1st Rank) | (2nd Rank) | (3rd Rank) | Least Preferred (4th Rank) |

**Fig. 1** Market Samples of *Mohanthal* from different cities of Gujarat



A. Market Samples of *Mohanthal* from Ahmedabad City

B. Market Samples of *Mohanthal* from Anand City

C Market Samples of *Mohanthal* from Vadodara City

D. Market Samples of *Mohanthal* from Palanpur City

such times as per convenience and 5) Use of other optional ingredients like nuts.

The variation in the moisture content of *Mohanthal* among manufacturers within a particular city may be due to the difference in their manufacturing techniques, sugar syrup concentration and the marketing requirements. The lower or higher moisture content may also be responsible for its higher or lower storage life. The fat content obtained in *Mohanthal* was comparable to the fat content found in the various other heat desiccated dairy products such as peda (Patel and Gandhi 1980; Vijayakhader and Patel 1983; Miyani 1988; Patel 1996; Ray et al. 2002), burfi (Rastogi et al. 1966; Miyani et al.1982; Garg and Mandokhot 1987), kalakand (Moulick et al. 1996), kunda (Kulkarni et al. 2001) and milk cake (Madhava Rao et al. 2003; Kumar et al. 2016) the values ranging from 3.3-28.54 %. During analysis of market samples of *Mohanthal* for lactose, it was found that some of the samples did not contain any lactose or had in it in very minor level which was not easily detectable. This may be due to the reason that milk or khoa was not used in those samples because milk solids are the only source of lactose in nature (Nickerson, 1974). But in a few samples, lactose was found very high, around 12.10%, which indicates that skim milk powder may have been used. The 'other carbohydrates' content of *Mohanthal* mainly comes from bengal gram flour (*besan*), which is one of the major ingredients of *Mohanthal.* Free fatty acids (FFA) are the measure of the fatty acids liberated from the triglycerides due to fat degradation occurring in the product prior to or after manufacturing. It is responsible for the

**Table 2** Chemical composition* of *Mohanthal* collected from different markets

| Constituent | Markets | | | | Range for all markets, n=16 | Average of all markets |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | | |
| Moisture (%) | 12.22±0.98$^{d}$ | 9.98±0.80$^{b}$ | 10.99±0.54$^{c}$ | 9.25±0.51$^{a}$ | 8.64-13.86 | 10.61±1.33 |
| Fat (%) | 25.70±2.04$^{c}$ | 24.66±3.15$^{bc}$ | 22.77±2.47$^{ab}$ | 21.80±3.35$^{a}$ | 18.21-28.12 | 23.74±3.17 |
| Protein (%) | 9.53±1.72$^{ab}$ | 10.47±1.15$^{bc}$ | 9.21±0.82$^{a}$ | 11.02±1.33$^{c}$ | 8.27-13.00 | 10.05±1.47 |
| Sugar (%) | 22.13±2.27$^{a}$ | 26.35±2.27$^{c}$ | 24.41±2.09$^{b}$ | 27.62±1.33$^{c}$ | 19.00-29.56 | 25.13±2.89 |
| Lactose (%) | 1.45±2.63$^{ab}$ | 0.00±0.00$^{a}$ | 3.95±4.37$^{b}$ | 4.50±5.14$^{c}$ | 0.00-12.1 | 2.48±4.05 |
| Ash (%) | 1.11±0.18$^{a}$ | 1.44±0.28$^{b}$ | 1.25±0.36$^{ab}$ | 1.40±0.20$^{b}$ | 0.81-1.81 | 1.29±0.29 |
| Other Carbohydrates (%) | 27.85±2.80$^{a}$ | 27.11±3.76$^{a}$ | 27.44±4.69$^{a}$ | 24.41±6.78$^{a}$ | 17.61-32.95 | 26.69±4.85 |
| FFA (% oleic acid) | 1.14±0.22$^{ab}$ | 1.09±0.07$^{a}$ | 1.27±0.19$^{b}$ | 1.13±0.20$^{ab}$ | 0.85-1.56 | 1.16±0.18 |
| pH | 6.47±0.04$^{b}$ | 6.42±0.04$^{ab}$ | 6.38±0.10$^{a}$ | 6.39±0.10$^{a}$ | 6.25-6.53 | 6.41±0.08 |

Values in a row with different superscripts are significantly different between markets (cities) at p≤0.05, *mean values ±SE, n=4

**Table 3** Textural characteristics* of *Mohanthal* collected from different markets

| Textural Characteristics | Markets | | | | Range for all markets, n=16 | Average of all markets |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | | |
| Hardness (N) | 4.64±0.56$^{a}$ | 10.40±1.84$^{b}$ | 9.17±1.25$^{b}$ | 14.63±3.49$^{c}$ | 3.44-20.24 | 9.71±4.18 |
| Cohesiveness | 0.132±0.020$^{b}$ | 0.071±0.014$^{a}$ | 0.140±0.022$^{b}$ | 0.077±0.008$^{a}$ | 0.051-0.178 | 0.105±0.035 |
| Springiness | 0.203±0.021$^{a}$ | 0.225±0.015$^{b}$ | 0.200±0.017$^{a}$ | 0.197±0.031$^{a}$ | 0.158-0.265 | 0.206±0.020 |
| Gumminess (N) | 0.610±0.095$^{a}$ | 0.720±0.105$^{a}$ | 1.274±0.184$^{b}$ | 1.127±0.318$^{b}$ | 0.464-1.562 | 0.932±0.328 |
| Chewiness (N) | 0.123±0.021$^{a}$ | 0.161±0.023$^{b}$ | 0.256±0.046$^{d}$ | 0.215±0.042$^{c}$ | 0.088-0.325 | 0.188±0.057 |

Values in a row with different superscripts are significantly different between markets (cities) at p≤0.05
*mean values ±SE, n=4

development of rancidity during storage. The pH of all the market samples ranged from 6.25 to 6.53. The highest pH was found in C-1 market samples, which was significantly (p≤0.05) higher than C-3 and C-4 market samples. There was significant (p≤0.05) difference found among samples from same market (Table 2).

**Textural characteristics of *Mohanthal***

The instrumental textural attributes varied widely depending on the market as well as the manufacturer. Hardness of *Mohanthal* varied from 3.44 – 20.24 N and cohesiveness from 0.051 – 0.178 (Table 3). The variations in the textural attributes are indicated by standard error values. The least hardness was observed in C-1 (Ahmedabad samples) (4.64 N) and the highest in C-4 (Palanpur samples) (14.63 N). It was noticed that cohesiveness, springiness, gumminess and chewiness values were much less than those of other similar dairy products (Chawla et al. 2011). This can be attributed to the presence of roasted *besan* particles. Raw *besan* particles have a very good water binding ability and strength, but on roasting, they lose the water binding, hence impart typical brittleness desirable to *Mohanthal* coupled with presence of sugar (Wani and Kumar, 2014). The variation in hardness may be ascribed to the variation in moisture, fat and total sugar content of the samples. Samples of C-1 city contained maximum amount of moisture and maximum amount of fat amongst others and could probably be correlated with minimum hardness. During TPA testing procedure, after first bite of TPA, *Mohanthal* lost its original shape and texture and became little bit floury, which shows brittle nature of the product and lower cohesiveness value in C-2 and C-4 samples.

**Sensory and physico-chemical characteristics of *Mohanthal***

*Mohanthal* is generally rectangular or cubical in shape with a thickness of about 2.0-3.0 cm. It resembles 'coloured' burfi sold in the market, but with a higher thickness. The colour is light brown to medium brown. This of course depends on the extent and uniformity of roasting during manufacture. When cut, it could be found that the interior of the pieces was uniformly brown. The colour was measured instrumentally in terms of Browning Index (BI), Yellowness Index (YI) and Whiteness Index (WI) all of which are related inversely with each other. Higher BI values indicated more brown colour caused by roasting of *besan* during manufacture and resulted in lower WI values. However, YI values have more to do with added colour. Further, added colour might decrease WI values. But, interestingly consumers still like added colours because of more visual appeal. The body of *Mohanthal* is firm, but slightly brittle, crumbling when pressed hard. However, because of roasted *besan*, the body is grainy thus imparting chewiness in mouth. The taste of *Mohanthal* is pleasantly sweet, slightly caramelized and has typical roasted *besan* flavour. There

was not much variation in the flavor of *Mohanthal* collected from different markets, but those brought from C-1 (Ahmedabad) had better quality.

Thus, the sensory attributes of *Mohanthal* were found to be typical of the product and had profound effect on the consumers'preference. These attributes are the result of compositional constituents and the processing methodology employed during manufacturing. All the market samples were more or less well accepted by judges, but some samples were more acceptable than others because of typical *Mohanthal* characteristics. The average colour and appearance, flavour, body and texture, sweetness and overall acceptance scores were: 8.01±0.31, 7.81±0.44, 7.75±0.31, 8.13±0.14 and 7.84±0.31, respectively. All the colour and appearance values for the samples collected from different cities showed significant ($p \leq 0.05$) difference between cities (Table 2).

The wide range in values of colour and appearance score of samples collected from each city clearly revealed manufacturer to manufacturer variation. This may be due to lack of proper care during preparation, wide variations in ingredients, use of different type of garnishing and colouring materials and also poor packaging materials. In the present study, some of the observations were also described by sensory judges which included light yellow orange to dark brown colour, smooth to granular appearance and no garnishing to well garnished with nuts and silver foil coat (Table 1). Similar variations were reported by Patel (1996) for market peda and Londhe (2006) for brown *peda* and Anon. (2006) for kunda. The variation in colour and appearance score of samples collected from each city was also attributed to level of ghee, sugar addition and moisture content of the product.

The wide range in values of body and texture score of samples collected from each city could be mainly ascribed to variation in moisture and fat contents. In the present study, judges described *Mohanthal* as having moist and soft to hard and dry, cohesive to brittle and crumbly body with small to medium grains and granular texture. All the flavor scores obtained for the samples collected from different cities showed significant ($p \leq 0.05$) difference (Table 5). The highest and lowest mean scores 8.35 and 7.46 were obtained from C-1 and C-4 cities, which were significantly ($p \leq 0.05$) different from other three cities, while mean score of C-2 and C-3 didn't show any difference. The wide range in values of flavour score of samples collected from each city clearly revealed shop to shop variation. This may be due to variation in preparation method and composition of the ingredients, particularly, *besan*, ghee and sugar levels in the final product. In the present study, some of the observations were also described by sensory judges, which included pleasant roasted ghee with caramelized and nutty flavour with added

**Table 4** Colour parameters* of *Mohanthal* collected from different markets

| Colour Parameters | Markets | | | | Range for all markets, n=16 | Average of all markets |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | | |
| Browning Index | 186.82±6.60$^{ab}$ | 185.54±30.51$^{ab}$ | 189.81±9.47$^{b}$ | 169.66±26.85$^{a}$ | 140.00-222.00 | 182.96±22.40 |
| Yellowness Index | 126.95±5.05$^{b}$ | 126.41±12.38$^{b}$ | 125.43±3.48$^{ab}$ | 118.74±10.43$^{a}$ | 107.00-139.60 | 124.38±9.21 |
| Whiteness Index | 30.58±1.12$^{ab}$ | 31.47±3.72$^{ab}$ | 29.66±1.83$^{a}$ | 32.89±4.09$^{b}$ | 27.00-37.33 | 31.15±3.17 |

Values in a row with different superscripts are significantly different between markets (cities) at $p \leq 0.05$; Colour parameters measured by computer vision *mean values ±SE, n=4

**Table 5** Sensory acceptance scores* of *Mohanthal* collected from different markets

| Sensory attributes | Markets | | | | Range for all markets, n=16 | Average of all markets |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | | |
| Colour and appearance | 8.34±0.30$^{c}$ | 8.06±0.42$^{b}$ | 7.95±0.42$^{b}$ | 7.69±0.47$^{a}$ | 6.75-8.75 | 8.01±0.31 |
| Body and texture | 8.08±0.23$^{b}$ | 7.69±0.42$^{a}$ | 7.74±0.25$^{a}$ | 7.60±0.36$^{a}$ | 6.75-8.50 | 7.75±0.31 |
| Flavour | 8.35±0.30$^{c}$ | 7.72±0.39$^{b}$ | 7.73±0.29$^{b}$ | 7.46±0.42$^{a}$ | 6.75-9.00 | 7.81±0.44 |
| Sweetness | 8.17±0.20$^{b}$ | 7.99±0.27$^{a}$ | 8.23±0.21$^{b}$ | 8.13±0.29$^{ab}$ | 7.75-8.50 | 8.13±0.14 |
| Overall acceptability | 8.19±0.18$^{c}$ | 7.80±0.25$^{b}$ | 7.83±0.33$^{b}$ | 7.54±0.32$^{a}$ | 6.75-8.50 | 7.84±0.31 |

Values in a row with different superscripts are significantly different between markets (cities) at $p \leq 0.05$
*mean values ±SE; maximum, 9.0, n=4

**Fig.2** Flow chart of manufacture method of *Mohanthal*

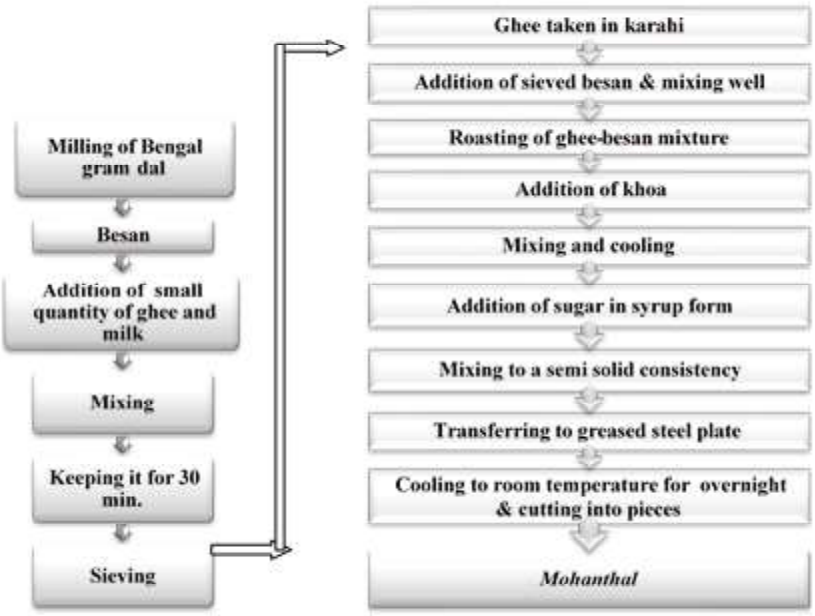



**Table 6** Microbiological counts* of market samples of *Mohanthal* collected from different cities of Gujarat

| Microbial Count log(cfu/g) | Markets | | | | Range for all markets, n=16 | Average of all markets |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | | |
| Standard Plate Count | 3.75±0.18[a] | 3.72±0.10[a] | 3.72±0.33[a] | 3.96±0.04[b] | 3.00-4.00 | 3.79±0.21 |
| Yeast and Mould Count | 1.83±0.11[a] | 1.96±0.21[a] | 1.86±0.39[a] | 1.96±0.22[a] | 1.00-2.30 | 1.90±0.18 |

Values in a row with different superscripts are significantly different between markets (cities) at p≤0.05, *mean values ±SE, n=4

cardamom. In some samples, raw *besan* flavor was also observed which was liked least. From overall acceptability scores, it was clearly revealed that city C-1 samples were better in comparison to other 3 cities in sensory attributes.

**Ideal characteristics of *Mohanthal***

Sensory attributes of highly preferred *Mohanthal* sample include light brown to medium brown colour, soft bodied but enough firmer and cohesive to cut in rectangular pieces, holding its shape without becoming floury, and small *besan* grains preferred over smooth texture. It must have pleasant, nutty, roasted and caramelized flavor with ghee flavour but raw *besan* flavour is not preferable. Garnishing with nuts such as almonds and pistachio or addition of saffron, cardamom or nutmegs enhances visual appearance and flavour of product, respectively.

**Microbiological quality of *Mohanthal***

All the food products sold in market contain microbes, because microorganisms enter the product at various stages during the processing and manufacture, and through packages from environment, if not packed properly. However, the number of microorganisms depends on the moisture content of the product, because at low water contents, they multiply slowly. In case of *Mohanthal*, moisture is low and sugar content is high, so we expect lower counts than other dairy products. The average values for SPC of *Mohanthal* samples collected from C-1, C-2, C-3 and C-4 markets were 3.75, 3.72, 3.72 and 3.96 log(cfu/g), respectively. The highest SPC was found in C-4 (Palanpur). In general, there was significant (p≤0.05) difference found among samples from the same market (Table 6). Wide variations in SPC counts of market samples of plain peda were reported by earlier workers. Ghodekar et al. (1974), Kamat and Sulebele (1974), Dwarkanath and Srikanta (1977) and Garg and Mandokhot (1987) reported $2x10^3$-$3x10^5$, $6x10^2$-$1x10^7$, $7.6x10^2$-$5x10^3$ and $1.1x10^3$-$5.6x10^3$ per gm, respectively. The average values for yeast and mould count of *Mohanthal* samples collected from C-1, C-2, C-3 and C-4 markets were 1.83 ± 0.11, 1.96 ± 0.21, 1.86 ± 0.39 and 1.96 ± 0.22 log(cfu/g), respectively and the range was from 1.60 - 2.00, 1.60 - 2.30, 1.00 - 2.30 and 1.48 - 2.26 log(cfu/g), respectively. There was no significant (p>0.05) difference in the samples found among the cities (Table 6). However, there was variation found in yeast and mould counts in samples collected from each market. Similar findings have been reported in khoa by Singh et al. (1975) in Mysore pak and kalakand by Dwarkanath and Srikanta (1977) and in burfi by Garg and Mandokhot (1984). Lower SPC and yeast and mould counts were found in all the samples of *Mohanthal* when compared to other traditional dairy products, which may be due to high roasting temperature employed during

*Mohanthal* preparation as well as low moisture of the product. The SPC and yeast and mould counts found in samples may be due to post processing contamination; unhygienic practices followed by manufacturers, using inappropriate packaging materials and long duration kept between manufacturing and packaging of product.

**Ingredients and manufacturing aspects**

Based on the survey work, the most common ingredients used for manufacture of *Mohanthal* were found to be *besan*, sugar, ghee and khoa. The critical factor agreed up on by all the *halwais* is the extent of roasting of *besan* during manufacture. *Besan* contains major constituents (17–22%) protein and (50%) carbohydrate (Saleh and Tarek, 2006). Roasting of *besan* releases compounds which provide relishing flavour, however more roasting results in charring of proteins and carbohydrates which may cause slight bitterness (Hodge et al. 1972). However, their proportions varied depending on the manufacturer. The method of preparation is shown in Fig. 2. The ingredients are mixed well till the mixture attains a bread crumb consistency. After proper mixing, the mixture is kept undisturbed for half an hour. This mixture is named *dhrabo*. Ghee is taken in a separate vessel and heated till it melts completely. Then *dhrabo* is added to the molten ghee and heated till the mixture develops a light brown colour and pleasant roasted flavor. The ghee *besan* mixture is then allowed to cool for some time. Sugar syrup (one thread consistency) is added to the prepared mixture and continuous mixing is done till it attains a semi solid form. Finally, the product is spread on a greased dish and garnished with nuts and cardamom. The product is then allowed to cool by keeping it overnight. Next day, the product is cut into pieces and served.

## Conclusions

*Mohanthal* is a versatile traditional product of Gujarat state with sensory characteristics of its own. Use of pulse flour (*besan*) imparts its typical colour and flavor. The study of market samples of *Mohanthal* showed variations in its quality characteristics and quality of best *Mohanthal* has been described. Presence of SPC, yeast and mold counts in market samples indicated the lack of proper hygienic practices adopted for preparation and handling of the product. Results obtained during the present investigation are of great importance in determination of product quality. To add a new item in the food basket of the global consumers with uniform quality and improved safety, large scale production and marketing of this product can be taken up by organized sector. Consequently, optimization of process parameters will act as a pre-requisite to showcase this product as a typical Indian traditional product in various forums. Based on the results, industry can manufacture *Mohanthal* for commercial activity and popularize the product throughout the country for the benefit of consumers.

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RESEARCH ARTICLE

# Physicochemical, antioxidant and in-vitro release behaviour of *burfi* added with curcumin as a source of functional ingredient

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**Abstract:** The present study was conducted to explore the benefits of curcumin and to enhance the antioxidant potential of *burfi*. Three different forms of curcumin *viz*. native curcumin, curcumin emulsion, and dried curcumin encapsulate powder were added to *burfi*. Addition of curcumin in *burfi* resulted in significant (P<0.05) increase in the antioxidant potential. A highest antioxidant potential with minimal decrease in the sensorial and colour attributes was observed in dried encapsulate added at patting stage. The functional properties were enhanced with the extent of dried curcumin encapsulate in *burfi*. The in-vitro study revealed that encapsulated curcumin was the most suitable form for addition in *burfi* as 63% of curcumin retention was observed after intestinal digestion. Curcumin encapsulate added *burfi* was found effective against fungal spoilage-causing microorganisms due to the reduction of 0.35 Log CFU/ml *Aspergillus flavus* and 0.47 Log CFU/ml *Aspergillus niger*. The available curcumin restricts the growth of spoilage-causing microorganisms and thus extends the shelf life of curcumin encapsulate added *burfi* by 3 days when stored at 30±1°C. Thus, curcumin in the form of dried encapsulate was found to be effective for enhancement of antioxidant potential and shelf life of *burfi*.

**Keywords**: Antioxidant, *Burfi*, Curcumin, Shelf life, Sensory attributes

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## Introduction

Turmeric (*Curcuma longa* L.) is a herbal plant that belongs to the ginger family, originated and grown in the Indian subcontinent. Curcumin is the bioactive component present in turmeric, comprise of about 3-5% of total rhizome and is responsible for the yellow colour (Jayaprakasha et al. 2006). It has a wide array of functional attributes *viz*., antioxidant, antimicrobial, antifungal, neuroprotective and anticarcinogenic activity and over the past few years, there has been an increasing interest in curcumin due to its medicinal properties (Maheshwari et al. 2006). Curcumin possess Generally Recognized As Safe (GRAS) status by the FDA for food applications. Despite of many benefits, the lipophilic nature of curcumin limits its application in many of the food products, thus restricts the exploration of all benefits of this bioactive compound. In addition to its solubility, the lower bioavailability, another critical issue which hinders its application in food products (Kakkar et al. 2011).

To explore the functional benefits of curcumin, extensive research has been carried out to enhance its water solubility and stability to make it available for food application. Many novel technologies like complexation, emulsion, and encapsulation were used to protect curcumin from harsh processing conditions, makes it more stable, mask undesirable taste and enable it for food application (Madene et al. 2006). Encapsulation is the promising technology to protect bioactive components and it is extensively used in dairy as well as in the food industry. The various phytochemicals have been successfully encapsulated using nanoemulsions (Garti, 2003). Spray-drying is considered as an effective technique for encapsulating curcumin in milk system as being a fat soluble substance, curcumin gets entrapped with fat globules inside a solid mix of lactose and protein and thus protected against harsh processing condition during food application (Neves et al. 2019). Despite of many successful preparations of curcumin emulsion and encapsulates, only a few researchers have studied the actual behaviour of prepared curcumin emulsion in dairy products. Lodh et al. (2018) reported that *ghee* with enhanced antioxidant property and overall sensorial acceptability could be prepared by incorporation of curcumin.

*Burfi* is one of the most popular traditional *khoa* based sweet prepared by heat desiccation of milk followed by the addition of sugar. However, it is highly susceptible to the chemical as well as microbial spoilage (Prasad et al. 2017b). With increased demand, the lower shelf life imparts an additional burden on the dairy industry. Thus, addition of naturally occurring bioactive compounds such as curcumin to improve functionality and shelf life. The incorporation of curcumin in *burfi* not only enhances its shelf life but also contributes to better human health of consumer due to the beneficial health attributes of curcumin. Thus, the present work has been undertaken with the objective of application of encapsulated curcumin as a source of functional ingredient in *burfi* to enhance its antioxidant potential and shelf life and its comparison assessment with native curcumin added *burfi*.

## Materials and Methods

### Materials

Fresh milk was procured from the Experimental Dairy of the ICAR-National Dairy Research Institute, Karnal, India and was standardised to 6% fat and 9% SNF using Pearson's square method. Curcumin powder with 93.03% total curcuminoids was procured from M/s Plant Lipids Pvt. Ltd. Kolenchery, Cochin, Kerala. Whey protein-80 (WPC- 80) was procured from M/s Globex Enterprises, Delhi, India. Maltodextrin (maximum 20.00% reducing sugar) and Gum acacia were procured from M/s HiMedia Laboratories Pvt. Ltd., Mumbai. Butteroil was prepared from cream containing 80% fat and 2.3% SNF obtained from Experimental Dairy, National Dairy Research Institute, Karnal using direct evaporation method (De, 1989). Partially concentrated skimmed milk with 13.85% total solids was taken from Experimental Dairy, National Dairy Research Institute, Karnal.

### Preparation of oil-in-water (O/W) curcumin emulsion

The curcumin emulsion (O/W) was prepared in three stages using the method described by Meena (2018). In the first stage, the core phase was prepared by solubilisation of curcumin in butteroil with continuous stirring at 60°C for 10 min with the help of magnetic stirrer. In second stage, the wall phase was prepared by mixing 1:2 parts of protein (WPC-80) and carbohydrate (maltodextrin and gum acacia in 90:10 proportions) in partially concentrated skimmed milk. In third stage, the core material (oil phase) and the wall material were properly mixed using a blender and the emulsion was prepared by high-speed homogenisation using Ultra-Turrax mixer at 10,000 rpm at 60°C for 10 min. In the final stage to dried curcumin encapsulate was prepared by spray drying the emulsion at 180° and 85°C inlet and outlet air temperature, respectively.

### Effect of different processing conditions on emulsion stability

The stability of curcumin emulsion was tested against diverse processing conditions like pH, ionic concentration, and heat treatment using the method described by Sari et al. (2015) with certain modifications. For effective heating, 10 ml emulsion was taken in test tubes and subjected to different heat treatments *viz*., pasteurization (63°C for 30 min) and boiling (95°C for 10 min). The effect of pH was assessed by adjusting the pH (3, 6 and 9) of emulsion using either 0.1 N HCl or 0.1 N NaOH solution. A different concentration of salt (0.35M, 0.70M, and 1M NaCl) was added to prepare the emulsion with different ionic concentration. After exposing to the above mentioned treatment, the stability of curcumin emulsion was evaluated on the basis of particle size distribution, viscosity and zeta potential using Master sizer 3000 version 3.30 (Malvern Instruments Ltd, Malvern, Worcs WR14)).

### Preparation of *burfi*

The control *burfi* was prepared using the standard batch method as proposed by Bhatele (1983). During the preparation of curcumin *burfi*, different forms of curcumin *viz*., native curcumin (free form), curcumin emulsion, and dried curcumin encapsulate powder in quantity equivalent to 100 ppm free curcumin (100 ppm native curcumin, 25% liquid emulsion, and 10% dried curcumin encapsulate on *khoa* basis) were added separately at different stages of *burfi* preparation (initially in standardized milk, after the first boil, initially in milk along with sugar, and at patting stage).

### Antioxidant potential and total phenolic content

The antioxidant potential of control and curcumin added *burfi* was evaluated by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method given by Brand-Williams et al. (1995). 0.1 mL of prepared methanolic extracted sample solution was mixed with 2.9 mL of freshly prepared DPPH working solution in amber coloured test tube and was properly vortexed and incubated in dark at 37°C for 30 min. After incubation, absorbance of the solution was measured spectrophotometrically at 517 nm. For blank, 0.1 mL methanol was taken instead of the sample.

ABTS [2, 2'-azinobis (3 ethyl benzothiazoline) -6-sulfonic acid] free radical scavenging activity of control as well as curcumin added *burfi* was evaluated by the method given by Re et al. (1999). 0.1 mL of prepared sample solution was mixed with 3.9 mL of ABTS working solution and incubated in dark at 37°C for 6 min. After incubation, absorbance of the solution was measured spectrophotometrically at 734 nm. For blank, 0.1 mL methanol was taken instead of the sample.

$$\text{Inhibition (per cent)} = \frac{(\text{Blank absorbance} - \text{Sample absorbance})}{\text{Blank absorbance}} \times 100$$

Total phenolic content of *burfi* samples (control and curcumin *burfi*) were analysed by Folin-Ciocalteu's method of Maizura et al. (2011) with slight modification. 0.4 mL of methanolic extracted sample solution was mixed with 2 mL of 0.2N Folin Ciocalteu's phenol reagent. After 3 minutes, 1.6 mL of sodium carbonate solution (7.5%) was added into it and incubated at 37°C for 2 hr. The absorbance was measured spectrophotometrically at 730 nm.

**Estimation of curcumin retention in *burfi***

The curcumin retention in *burfi* was estimated according to the method of ASTA (1985) with slight modification. Exactly, 1 g of *burfi* sample was mixed with 10 mL of 95% ethyl alcohol and mixed thoroughly using pestle and mortar to facilitate colour extraction. The solution was subjected to centrifugation at 4000 rpm for 30 minutes followed by filtration through 0.22µ syringe filter. The absorbance of filtrate was measured at 425 nm.

**Colour analysis**

Colour measurement of *burfi* samples was estimated using Hunter-Lab model Colour Flex® (Mini Scan XE plus, Hunter Associates Laboratory Inc. Reston, Virginia, U.S.A.) and the results were expressed in terms of CIE-LAB (L*, a* and b*) system.

**Sensory evaluation**

Control and curcumin added *burfi* samples were organoleptically evaluated on a 9-point hedonic scale by a panel of semi-trained judges selected from the Faculty of Dairy Technology Division.

**Texture analysis**

The texture profile analysis of *burfi* sample (1 cm height and 1 cm in diameter) was evaluated using TA-XT2i Texture Analyzer (M/s Stable Micro Systems, UK) using P75 Probe, test speed of 1 mm/s, Compression of 80 %, 25 ± 1ºC testing temperature, Load cell 25 kg.

**In vitro release kinetics of encapsulated curcumin added *burfi***

In-vitro release kinetics of encapsulated curcumin *burfi* was evaluated under simulated gastrointestinal conditions as per protocol given by Herrero-Barbudo et al. (2009).

**Microbial analysis**

For microbial analysis, Total plate count, coliform count, yeast and mould count were determined by the standard procedure given by Indian Standards IS: SP: 18(Part XI)-1981.

**Physicochemical analysis**

The free fatty acids (FFA) content of *burfi* was estimated using the method of Deeth and Fitz-Gerald (1975). Total Hydroxy Methyl Furfural (HMF) content in *burfi* was determined by the method recommended by Keeney and Bassette (1959).

**Antifungal activity**

Fungal cultures NCDC-224 (*Aspergillus niger*) and NCDC- 226 (*Aspergillus flavus*) were subjected for proper activation by three subculturing. Curcumin *burfi* (11 g) was properly mixed in 99 mL saline solution added with 100µL fully activated fungal culture and allowed to react with curcumin present inside *burfi* for 4 h. The subsequent plating was performed by standard protocol given in Indian Standards IS: SP: 18 (Part XI)-1981.

**Statistical analysis**

Data obtained from various experiments were recorded as mean ± standard error (SE) and were subjected to analysis of variance (ANOVA) using Tukey's post hoc comparison test to establish the significance of differences among the mean values at 5 per cent level of significance by employing SPSS statistical package.

## Results and Discussion

**Characterization and process stability of curcumin nanoemulsion**

The effect of process conditions on emulsion stability was evaluated in terms of particle size distribution, zeta potential, and viscosity and the results are presented in Table 1.

**Effect of pH**

The particle size and zeta potential were significantly affected with change in pH of emulsion, while the viscosity showed a non-significant difference between all the pH adjusted emulsions. The emulsion adjusted to pH 3 was completely destabilized with increased particle size to 1603.67 nm (Figure 1) and reduction in zeta potential to -0.313 mV. This could be due to coagulation and aggregation of milk proteins with the decrease in pH caused due to the shifting of electrical charge on particles that result in the reduction of electrostatic repulsive forces between protein molecules. A similar trend was observed by Sari et al. (2015) in curcumin nanoemulsion adjusted to pH 3 where the zeta potential was significantly reduced from - 6.9 to + 2.03 mV. Rao and Mcclements (2011) studied the effect of processing conditions on nano/microemulsions and found destabilization and aggregation of emulsion at lower pH values, which was attributed to the reduction of electrostatic repulsive forces.

**Effect of heat treatment**

Heat treatment (63°C/30 min) caused a slight change in the particle size and zeta potential, but the destabilization effect was significantly higher in extremely heated emulsions (95°C/10 min) with increased particle size to 1099.83 (Figure 2) while the zeta

potential decreased to -16.84 mV. A tremendous increase in viscosity from 16.57 to 1623.33 mPa.s was observed in boiled emulsion (95°C/10 min). The destabilizing effect was associated with the denaturation and aggregation of whey proteins due to intense heating that destroyed the native structure of the emulsion. Sari et al. (2015) reported a slight increase in the particle size of curcumin nanoemulsion after pasteurization (63°C/30 min) that increased with increased heat treatment (95°C/10 min).

**Effect of ionic concentration**

A non-significant difference in the viscosity of curcumin emulsion was observed in comparison to control emulsion due to variation in ionic concentration, but the zeta potential and particle size distribution were significantly affected. The average particle size was increased to 394.43 nm at 1M NaCl concentration (Figure 3). A non-significant difference in zeta potential was observed in control curcumin emulsion (-20.97 mV) and emulsion added with 0.35 M NaCl (-20.20 mV), but with increased ionic concentration to 1 M NaCl a significant decrease in the zeta potential to -17.73 mV was observed. This could be due to the alteration in the repulsive forces between the proteins at higher salt concentration. Sari et al. (2015) reported a significant alteration in the zeta potential of curcumin nanoemulsion with changes in ionic concentration.

**Effect of a different form of curcumin and stages of addition on free radical scavenging activity (ABTS and DPPH) and total phenolic content of *burfi***

The results of the antioxidant activity and total phenolic content of *burfi* added with different forms of curcumin at various stages of preparation are presented in Table 2. The ABTS and DPPH free radical scavenging activity of *burfi* was significantly increased with the addition of curcumin. Antioxidant activity of both emulsion and dried encapsulate added *burfi* was significantly higher than native curcumin added *burfi*. The increase in antioxidant potential of encapsulation was observed as compared with native curcumin added *burfi*, with an approximate 25 to 30 µg Trolox equivalent (depends on the stage of addition). The different stages of curcumin addition had a significant effect on antioxidant potential with the highest activity observed in *burfi* added with curcumin encapsulate at patting stage. The total phenolic content in curcumin added *burfi* was significantly affected by different forms of curcumin. The highest phenolic content was found in *burfi* added with liquid curcumin emulsion at patting stage. The lowest antioxidant potential and phenolic content of native curcumin added *burfi* could be due to the interactive effect of curcumin and milk constituents that decreased the curcumin concentration in *burfi*. The hydrophilic and hydrophobic interactions were the binding force behind the complexation of polyphenolic compounds and caseins (Pan et al. 2014). Encapsulation protects curcumin and restricts its interaction with casein, thus enhances the potential functional benefits of curcumin. Addition of curcumin encapsulate at patting stage caused less destabilization of curcumin encapsulate (due to heating and stirring) thus results in minimal interaction with milk solids. Pan et al. (2014) observed the interaction of curcumin with casein micelles that limits the actual functional benefits of curcumin when added in milk system. Bourassa et al. (2013) also reported the interaction of casein (α-casein and β-casein) with curcumin and other polyphenolic compounds when added in the milk system.

**Effect of a different form of curcumin and stages of addition on curcumin stability in *burfi***

The effect of different form of curcumin and stages of addition on curcumin stability in *burfi* were compared and listed in Table 2. Emulsion and dried curcumin encapsulate added *burfi* retained significantly higher amount of curcumin (approximately 90%) than native curcumin added *burfi*. Among all the stages, the highest curcumin retention was observed at patting stage. The results indicate the resistance of encapsulation against the interaction of curcumin and milk components due to the shielding effect of the coating material. A same shielding effect of the coating material against the interaction of curcumin and milk components was observed by Maurya (2012), who prepared curcumin-

**Table 1** Effect of processing conditions on emulsion stability

| Treatment | Average particle size distribution (nm) | Zeta potential (mV) | Viscosity (centipoises) |
|---|---|---|---|
| Untreated emulsion (pH- 6.5) | 155.70±10.51$^{a}$ | -20.97±0.44$^{b}$ | 16.57±0.78$^{a}$ |
| pH -3 | 1603.67±16.90$^{e}$ | -0.31±0.04$^{e}$ | 13.03±0.29$^{a}$ |
| pH -6 | 307.00±31.97$^{ab}$ | -17.90±0.46$^{cd}$ | 11.63±0.90$^{a}$ |
| pH -9 | 505.80±47.35$^{c}$ | -24.50±0.26$^{a}$ | 12.57±0.09$^{a}$ |
| Ionic concentration 0.35 M NaCl | 382.03±34.69$^{bc}$ | -20.20±0.57$^{b}$ | 12.37±0.32$^{a}$ |
| Ionic concentration 0.70 M NaCl | 334.06±37.20$^{bc}$ | -19.33±0.30$^{bc}$ | 9.34±0.16$^{a}$ |
| Ionic concentration 1 M NaCl | 394.43±34.98$^{bc}$ | -17.73±0.52$^{cd}$ | 10.15±0.74$^{a}$ |
| Heat treatment (63°C/30 min) | 286.77±21.47$^{ab}$ | -19.60±0.40$^{bc}$ | 20.97±0.75$^{a}$ |
| Heat treatment (95°C/10 min) | 1099.83±54.54$^{d}$ | -16.84±0.29$^{d}$ | 1623.33±8.82$^{b}$ |

Values are Mean ± standard error (n=3)

a,b,c,d,e: Mean with different superscripts are significantly different within the column ($p<0.05$)

**Table 2** Effect of a different form of curcumin and stages of addition on antioxidant activity, total phenolic content, curcumin stability and hunter colour profile of *burfi*

| Form of curcumin | Stages of addition | Antioxidant free radical scavenging activity (µg Trolox equivalent/g sample) | | Total phenolic content (µg Gallic acid equivalent/g sample) | Curcumin stability and retention in *burfi* (ppm) | Hunter colour profile | | |
|---|---|---|---|---|---|---|---|---|
| | | ABTS | DPPH | | | L* | a* | b* |
| Native curcumin (free form) | Initially in standardized milk | 127.435±3.40$^{Bb}$ | 88.717±3.66$^{Bbc}$ | 35.883±0.32$^{Ab}$ | 76.224±2.51$^{Aab}$ | 67.57±0.09$^{Bc}$ | -1.45±0.01$^{Ca}$ | 55.51±0.05$^{Bc}$ |
| | After the first boil | 127.072±4.42$^{Bb}$ | 89.009±4.44$^{Bb}$ | 36.517±0.47$^{Ab}$ | 77.628±2.11$^{Aa}$ | 64.39±0.21$^{Bb}$ | -1.64±0.01$^{Ca}$ | 53.41±0.03$^{Bb}$ |
| | Initially along with sugar | 131.633±1.22$^{Bb}$ | 92.848±5.72$^{Bb}$ | 35.667±0.22$^{Ab}$ | 81.630±0.32$^{Abc}$ | 65.73±0.05$^{Bb}$ | -2.53±0.04$^{Ca}$ | 52.93±0.04$^{Bb}$ |
| | Patting stage | 136.087±1.39$^{Bc}$ | 95.431±2.14$^{Bc}$ | 36.033±0.20$^{Ab}$ | 82.497±0.30$^{Ac}$ | 58.31±0.04$^{Ba}$ | 3.40±0.04$^{Cb}$ | 49.43±0.21$^{Bb}$ |
| Curcumin emulsion | Initially in standardized milk | 140.194±1.08$^{Cb}$ | 112.684±2.16$^{Cbc}$ | 44.500±0.62$^{Cb}$ | 89.635±0.60$^{Cab}$ | 69.22±1.17$^{Bc}$ | -2.66±0.05$^{Ba}$ | 65.70±1.08$^{Dc}$ |
| | After the first boil | 146.754±2.70$^{Cb}$ | 107.548±1.37$^{Cb}$ | 44.617±1.11$^{Cb}$ | 89.795±0.79$^{Ca}$ | 65.16±0.87$^{Bb}$ | -4.04±0.12$^{Ba}$ | 56.51±0.72$^{Db}$ |
| | Initially along with sugar | 144.759±3.63$^{Cb}$ | 106.094±2.65$^{Cb}$ | 41.567±0.53$^{Cb}$ | 91.895±0.59$^{Cbc}$ | 63.84±0.89$^{Bb}$ | -3.28±0.09$^{Ba}$ | 58.59±0.89$^{Db}$ |
| | Patting stage | 155.234±3.65$^{Cc}$ | 120.760±2.70$^{Cc}$ | 43.700±1.69$^{Cb}$ | 90.685±0.46$^{Cc}$ | 58.19±0.62$^{Ba}$ | 3.55±0.06$^{Bb}$ | 59.33±0.64$^{Db}$ |
| Dried curcumin encapsulate powder | Initially in standardized milk | 145.930±1.51$^{Cb}$ | 110.143±1.53$^{Cbc}$ | 40.800±0.23$^{Bb}$ | 86.689±0.61$^{Bab}$ | 67.87±0.84$^{Bc}$ | -4.25±0.06$^{Aa}$ | 60.93±1.11$^{Cc}$ |
| | After the first boil | 144.488±2.64$^{Cb}$ | 109.745±1.02$^{Cb}$ | 40.400±0.29$^{Bb}$ | 84.178±0.46$^{Ba}$ | 65.65±0.93$^{Bb}$ | -3.16±0.08$^{Aa}$ | 59.16±0.31$^{Cb}$ |
| | Initially along with sugar | 148.509±2.29$^{Cb}$ | 108.720±1.39$^{Cb}$ | 40.217±0.22$^{Bb}$ | 85.639±0.42$^{Bbc}$ | 65.51±0.54$^{Bb}$ | -3.57±0.06$^{Aa}$ | 56.93±0.57$^{Cb}$ |
| | Patting stage | 158.994±1.80$^{Cc}$ | 118.295±2.15$^{Cc}$ | 41.717±0.15$^{Bb}$ | 87.397±0.64$^{Bc}$ | 63.83±0.85$^{Ba}$ | 1.34±0.06$^{Ab}$ | 55.46±1.21$^{Cb}$ |
| Control | | 112.707±1.70$^{Aa}$ | 71.780±0.88$^{Aa}$ | 35.180±0.60$^{Aa}$ | - | 60.79±1.53$^{Aa}$ | 6.27±0.75$^{Dc}$ | 27.23±0.49$^{Aa}$ |

Values are mean ± standard error (n=3)

a,b,c: Mean with different superscripts are significantly different within stage of addition ($p<0.05$)

A,B,C,D: Mean with different superscripts are significantly different within form of curcumin ($p<0.05$)

**Fig. 1** Particle size distribution of curcumin emulsion of varied pH (3, 6 and 9)

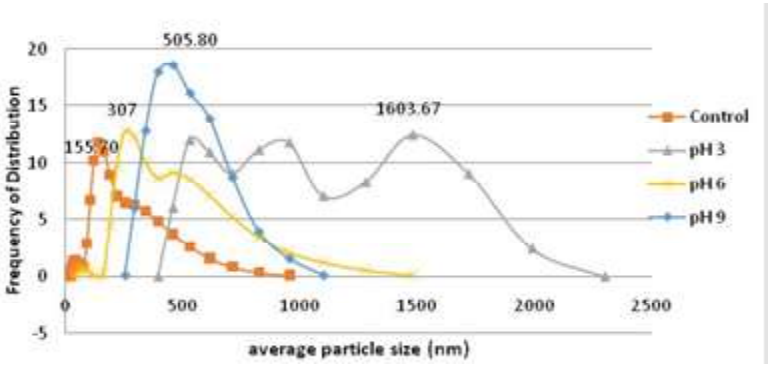



**Fig. 2** Particle size distribution of curcumin emulsion with varied heat treatment (63°C/30 min and 95°C/10 min)

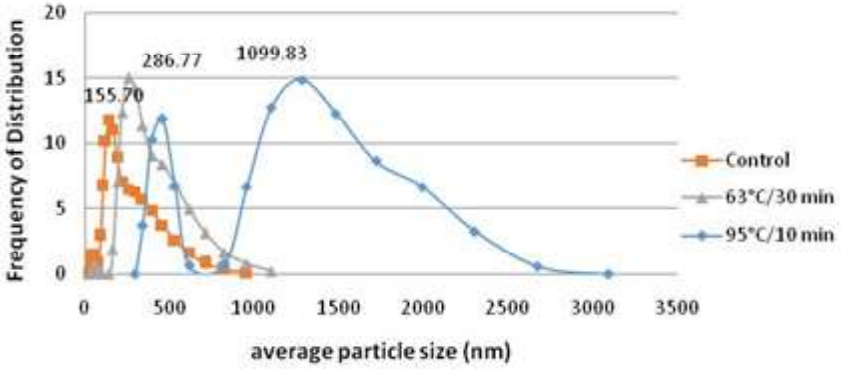



**Table 3** Effect of a different form of curcumin and stages of addition on sensory attributes of *burfi*

| Form of curcumin | Stages of addition | Flavour | Texture | Colour and appearance | Overall acceptability |
|---|---|---|---|---|---|
| Native curcumin (free form) | Initially in standardized milk | 7.20±0.27$^{Aa}$ | 7.30±0.15$^{Aa}$ | 7.23±0.37$^{Aa}$ | 7.31±0.35$^{ABab}$ |
| | After the first boil | 7.20±0.18$^{Aa}$ | 7.12±0.16$^{Aa}$ | 7.24±0.04$^{Aa}$ | 7.22±0.12$^{ABa}$ |
| | Initially along with sugar | 7.23±0.09$^{Aa}$ | 6.99±0.07$^{Aa}$ | 7.48±0.08$^{Aa}$ | 7.21±0.15$^{ABa}$ |
| | Patting stage | 7.28±0.15$^{Aab}$ | 7.13±0.19$^{Aa}$ | 7.37±0.19$^{Aa}$ | 7.36±0.07$^{ABab}$ |
| Curcumin emulsion | Initially in standardized milk | 6.90±0.26$^{Aa}$ | 7.26±0.06$^{ABa}$ | 7.48±0.29$^{Aa}$ | 7.18±0.16$^{Aab}$ |
| | After the first boil | 7.22±0.02$^{Aa}$ | 7.18±0.04$^{ABa}$ | 7.20±0.22$^{Aa}$ | 7.03±0.09$^{Aa}$ |
| | Initially along with sugar | 7.10±0.06$^{Aa}$ | 7.16±0.23$^{ABa}$ | 6.88±0.51$^{Aa}$ | 6.92±0.19$^{Aa}$ |
| | Patting stage | 7.53±0.15$^{Aab}$ | 7.29±0.05$^{ABa}$ | 7.18±0.31$^{Aa}$ | 7.08±0.20$^{Aab}$ |
| Dried curcumin encapsulate powder | Initially in standardized milk | 7.37±0.20$^{Aa}$ | 7.19±0.18$^{ABa}$ | 7.30±0.05$^{Aa}$ | 7.20±0.09$^{Aab}$ |
| | After the first boil | 7.19±0.11$^{Aa}$ | 7.26±0.13$^{ABa}$ | 7.13±0.14$^{Aa}$ | 7.17±0.11$^{Aa}$ |
| | Initially along with sugar | 7.13±0.13$^{Aa}$ | 7.51±0.07$^{ABa}$ | 7.13±0.07$^{Aa}$ | 7.08±0.04$^{Aa}$ |
| | Patting stage | 7.45±0.23$^{Aab}$ | 7.51±0.07$^{ABa}$ | 7.47±0.08$^{Aa}$ | 7.49±0.20$^{Aab}$ |
| | Control | 7.83±0.03$^{Bb}$ | 7.55±0.13$^{Bb}$ | 7.73±0.16$^{Aa}$ | 7.69±0.15$^{Bb}$ |

Values are mean ± standard error (n=3)
a,b: Mean with different superscripts are significantly different within stage of addition ($p<0.05$)
A,B: Mean with different superscripts are significantly different within form of curcumin ($p<0.05$)

cyclodextrin complex and reported 85 to 90% of total curcumin retention in *lassi*.

**Effect of a different form of curcumin and stages of addition on hunter colour values of *burfi***

The lightness (L*), redness-greenness (a*) and yellowness-blueness (b*) index of the control and curcumin added *burfi* were presented in Table 2. The L* value of all the curcumin added *burfi* samples varied from 58.19 to 69.22 which was significantly higher than control *burfi*. The lightness value of *burfi* was associated with the extent of heat treatment which mainly depends upon the stage of addition. The initial fortification in standardized milk showed the highest lightness index with L* value of 69.22 whereas fortification at patting stage showed the least (58.19). The a* value of control sample was 6.27 which was significantly higher than all curcumin added *burfi* and ranged from -4.25 to

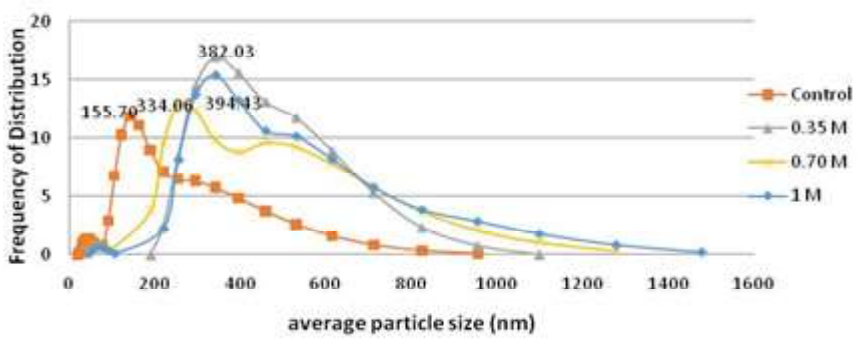



**Fig. 3** Particle size distribution of curcumin emulsion with different ionic concentration (0.35 M NaCl, 0.70 M NaCl, and 1M NaCl)

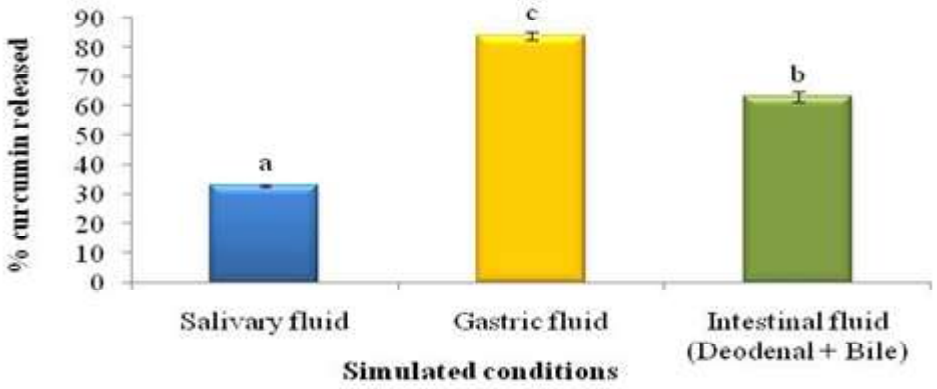



**Fig. 4** *In-vitro* release kinetics of curcumin encapsulated *burfi*

+3.55. Both (different forms of curcumin and the stage of addition) were responsible for decreased a* value of *burfi*. The b* value (yellowness-blueness index) of control sample was 27.23 that significantly increased to 65.70 in curcumin emulsion added *burfi* at the initial stage of addition. The emulsion and encapsulate curcumin added *burfi* showed significantly higher b* value than native curcumin added *burfi*. This could be due to the lower retention of curcumin in *burfi* when added in native form. The change in the colour value of *burfi* was due to the addition of encapsulated curcumin. Meena (2018) has observed the L*, a*, and b* value of dried curcumin encapsulate to be 87.44, -7.49, and 72.02, respectively that further decreased with storage period. The addition of curcumin in milk at the initial stage of *burfi* preparation resulted in higher destabilization of curcumin thus released more curcumin due to the higher exposure of heat and scrapping that affected the colour values of curcumin added *burfi.*

**Effect of a different form of curcumin and stages of addition on sensorial attributes of *burfi***

The average flavour score of control *burfi* was 7.83, which was reduced to 6.90 upon addition of curcumin (Table 3). A significant difference was observed in *burfi* samples fortified with different forms of curcumin added at various stages during preparation. The decreased flavour score of curcumin *burfi* may be attributed to the bitter taste of curcumin that mask the flavour of *burfi*. The body and textural scores of all the curcumin added *burfi* were significantly less than the control sample. A non-significant difference was observed in the colour and appearance score of curcumin added *burfi* and control sample. The intense yellow coloured *burfi* was least accepted by the sensory panellists than light yellow coloured *burfi*. The overall acceptability scores of curcumin added *burfi* ranged from 6.92 to 7.49 which were significantly less than control *burfi* with a mean score of 7.69. The bitter after taste and intense yellow colour decreased the overall acceptability of curcumin added *burfi.* The bitter taste was more pronounced in *burfi* added with native curcumin which was reflected in the flavour score. *Burfi* added with dried curcumin encapsulate at patting stage was highly acceptable with average overall acceptability score of 7.49 than other curcumin added *burfi* but comparatively less than the control sample. Similar, results of bitterness in *burfi* added with turmeric essential oil that decreased the consumer acceptability were reported by Prasad et al. (2017a). Maurya (2002) reported a decreased overall acceptance of curcumin fortified *lassi* due to intense yellow colour. Based on above observation the dried curcumin encapsulate was the most suitable form for incorporation in *burfi* that enhance the antioxidant potential but the enhancement was much higher during the patting stage of preparation.

**Optimization of the level of addition of dried curcumin encapsulate in *burfi***

To optimize the level of addition, the *burfi* was incorporated with dried curcumin encapsulate at levels of 5%, 7.5%, 10%, 12.5%,

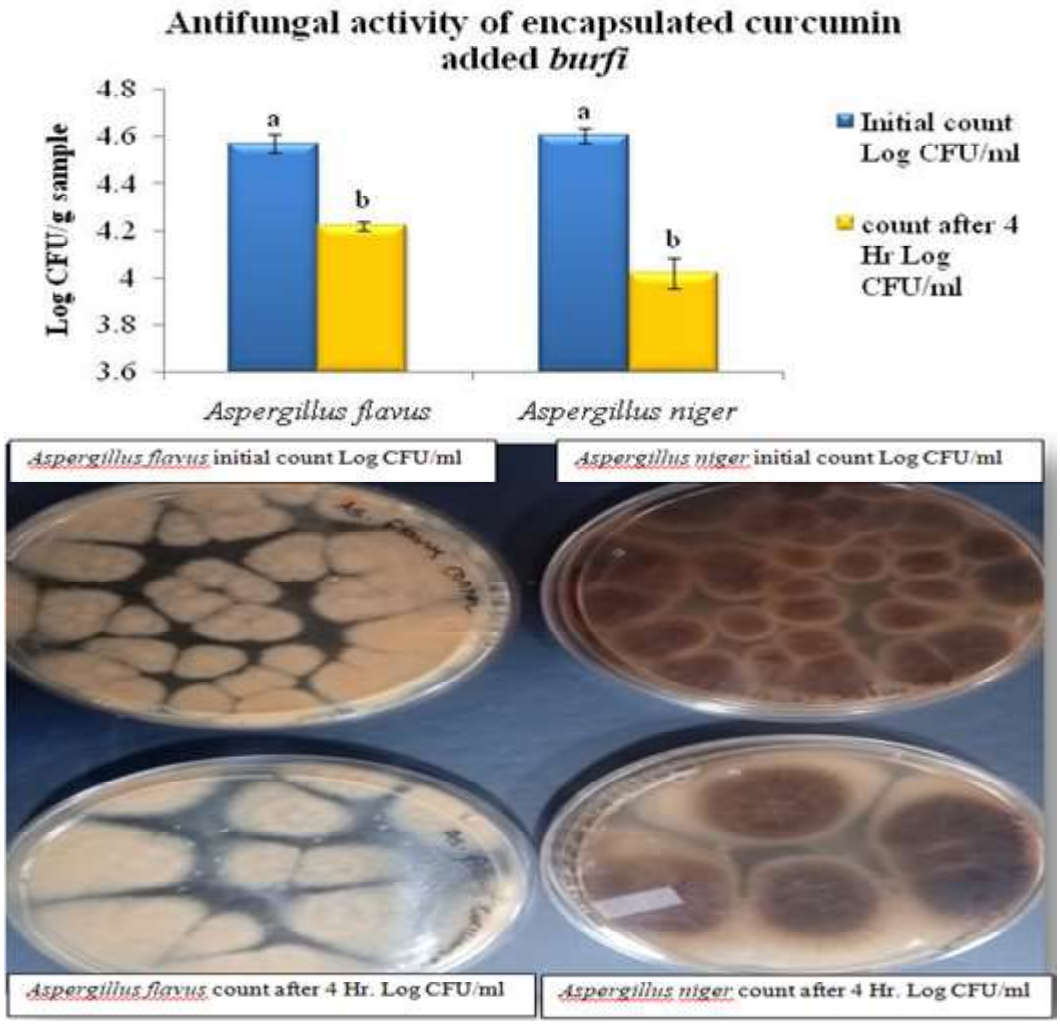



**Fig. 5** Antifungal activity of curcumin encapsulate added *burfi*

and 15% and the responses were measured in terms of antioxidant potential, hunter colour value and sensorial attributes.

**Effect of level of dried curcumin encapsulate on antioxidant activity and total phenolic content of *burfi***

The antioxidant potential of *burfi* samples incorporated with different levels of dried curcumin encapsulate were analyzed by ABTS and DPPH free radical scavenging method and compared with the positive control sample containing 200 ppm BHA. The ABTS and DPPH free radical scavenging activity of curcumin encapsulate added *burfi* was significantly less than the positive control but increased with the extent of curcumin (Table 4). Moreover, the highest antioxidant potential was obtained at 15% level of dried curcumin encapsulate. In addition, the *burfi* added with 15% level of dried curcumin encapsulate was observed to have highest phenolic content of 48.317 µg gallic acid equivalent/ g sample. Polyphenolic compounds are naturally present in many plants and are potential source of antioxidants (Soong and Barlow, 2004; Balasundram et al. 2006). A similar trend of increased antioxidant potential was observed in *burfi* with the addition of different polyphenol-rich herbs (Prasad et al. 2017a). The increase in the antioxidant potential of bread with incorporation of turmeric was observed by Lim et al. (2011).

**Effect of level of dried curcumin encapsulate on Hunter colour profile of *burfi***

*Burfi* samples with different concentrations of curcumin encapsulate were evaluated for colour profile using Hunter lab colorimeter and the results were presented in Table 4. A non-significant difference in L* value was observed in *burfi* with highest lightness (63.268) value in 10% curcumin encapsulate *burfi*. However, the a* (redness-greenness) value of *burfi* showed a significant decrease with increased level of curcumin

encapsulate. With the increased level of curcumin encapsulate a significant increase in the b* (yellowness-blueness) value was observed with the highest yellowness (58.174) in *burfi* with 15% curcumin encapsulate. The oversaturation of yellowness at 15% curcumin encapsulates exhibits unnatural appearance of *burfi,* thus less accepted. Cheese curcumin showed higher yellowness index while yoghurt curcumin showed a slight greenish-yellow colour (Marcolino et al. 2011). Manoharan et al. (2012) observed increased yellowness of ice cream with addition of curcumin.

**Effect of level of dried curcumin encapsulate on sensorial attributes of *burfi***

The sensory scores of *burfi* in terms of flavour, texture, colour and overall appearance perceived by sensory panellists, were reported in Table 5. The level of curcumin encapsulate showed a non-significant effect on the sensory attributes of *burfi* and the scores decrease beyond 10 % level of curcumin encapsulates due to the over saturation of yellowness. At higher concentration curcumin encapsulate induced intense yellow colour and bitter taste in *burfi* thereby, limiting its overall acceptance. Kumar et al. (2016) observed a non-significant difference in sensory analysis on 9 point hedonic scale of control and curcumin emulsion ice cream. The overall acceptability of bread was observed to be decreased with the increase in the curcumin concentration due to intense yellow crumb colour (Lim et al. 2011).

Based on antioxidant and sensory analysis, 10% level of curcumin encapsulate was found to be effective for incorporation in *burfi* and hence, was considered to be the optimized product with chemical composition of 14.72% moisture, 21.23% fat, 14.82% protein, 18.09% lactose, 2.81% ash and 28.33% sucrose in the final product.

***In-vitro* release kinetics of curcumin encapsulate added *burfi***

Functional benefits of optimized *burfi* primarily depends on the release of curcumin from encapsulate which was evaluated in simulated gastro-intestinal conditions. A 33% of total curcumin from encapsulate present in *burfi* was released in salivary fluid which was further increased to around 83% in gastric fluid. The alkaline conditions in the intestinal fluid caused around 20%

**Table 4** Effect of level of dried curcumin encapsulate on antioxidant activity (ABTS and DPPH), total phenolic content and hunter colour profile of curcumin encapsulate added *burfi*

| Level of addition curcumin encapsulates (%) | Antioxidant free radical scavenging activity (µg Trolox equivalent/g sample) | | Total phenolic content (µg Gallic acid equivalent/g sample) | Hunter colour profile | | |
|---|---|---|---|---|---|---|
| | ABTS | DPPH | | L* | a* | b* |
| Control | 112.707±1.70$^{a}$ | 71.780±0.88$^{a}$ | 35.183±0.60$^{a}$ | 60.787±1.53$^{a}$ | 6.269±0.75$^{b}$ | 27.230±0.49$^{a}$ |
| BHA (200 ppm) | 200.515±3.46$^{f}$ | 195.304±1.20$^{g}$ | 34.633±0.59$^{a}$ | 58.910±1.92$^{a}$ | 4.121±0.24$^{ab}$ | 49.508±1.75$^{b}$ |
| 5 % | 119.182±1.96$^{a}$ | 103.148±0.78$^{b}$ | 41.167±1.11$^{b}$ | 62.210±0.47$^{a}$ | 4.424±0.34$^{ab}$ | 53.698±0.71$^{bc}$ |
| 7.5 % | 134.922±2.38$^{b}$ | 111.909±0.95$^{c}$ | 42.017±0.62$^{b}$ | 63.268±0.48$^{a}$ | 2.727±0.26$^{a}$ | 56.201±0.92$^{c}$ |
| 10 % | 150.634±1.75$^{c}$ | 125.439±1.41$^{d}$ | 43.667±0.57$^{bc}$ | 59.600±1.53$^{a}$ | 3.822±0.64$^{a}$ | 55.971±1.05$^{c}$ |
| 12.5 % | 168.404±2.12$^{d}$ | 140.746±2.27$^{e}$ | 45.300±0.29$^{cd}$ | 58.762±0.79$^{a}$ | 3.766±0.37$^{a}$ | 58.174±0.91$^{c}$ |
| 15 % | 183.439±2.61$^{e}$ | 152.410±1.93$^{f}$ | 48.317±0.39$^{d}$ | | | |

Values are mean ± standard error (n=3)

a,b,c,d,e,f,g: Mean with different superscripts are significantly different within the level of curcumin encapsulates ($p<0.05$)

**Table 5** Effect of varied levels of curcumin encapsulate on sensory attributes of *burfi*

| Level of addition curcumin encapsulates (%) | Flavour | Texture | Colour and Appearance | Overall acceptability |
|---|---|---|---|---|
| Control | 7.81±0.04$^{a}$ | 7.73±0.02$^{a}$ | 7.79±0.09$^{a}$ | 7.72±0.06$^{ab}$ |
| 5 % | 8.01±0.07$^{a}$ | 7.71±0.11$^{a}$ | 7.73±0.04$^{a}$ | 7.70±0.06$^{ab}$ |
| 7.5 % | 7.99±0.20$^{a}$ | 7.79±0.15$^{a}$ | 7.76±0.18$^{a}$ | 7.79±0.10$^{ab}$ |
| 10 % | 7.88±0.25$^{a}$ | 8.12±0.07$^{a}$ | 7.84±0.22$^{a}$ | 7.93±0.12$^{b}$ |
| 12.5 % | 7.69±0.04$^{a}$ | 7.66±0.22$^{a}$ | 7.47±0.06$^{a}$ | 7.45±0.06$^{a}$ |
| 15 % | 7.84±0.05$^{a}$ | 7.89±0.21$^{a}$ | 7.30±0.03$^{a}$ | 7.45±0.04$^{a}$ |

Values are mean ± standard error (n=3)

a,b: Mean with different superscripts are significantly different within the level of curcumin encapsulates ($p<0.05$)

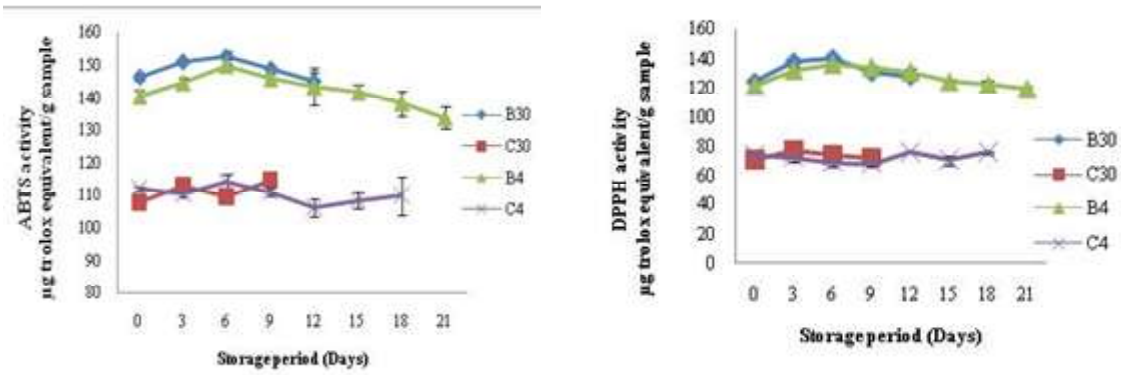



**Fig. 6** Changes in the antioxidant activity of control and encapsulated curcumin *burfi* during storage

degradation of free curcumin released from encapsulate and the remaining 63% free curcumin was found to be stable at the end of intestinal digestion (Figure 4). Sari et al. (2015) determined the *in-vitro* release behaviour of curcumin nanoemulsion and observed that the emulsion had excellent stability in gastric fluid with the release of only 8.48 % of total curcumin, but undergoes destabilization in intestinal digestion with the release of 77.75% curcumin from the emulsion. The release of curcumin from encapsulated added burfi in gastric fluid is based on the behaviour of whey protein during the processing of *burfi*. The heat treatment results in the denaturation of whey protein (WPC-80) known for higher digestibility, readily digested in gastric condition and thus released the curcumin present inside encapsulate.

**Antifungal activity of curcumin encapsulate added *burfi***

The curcumin encapsulate added *burfi* was evaluated for its antifungal activity against two major milk sweet spoilage causing moulds *viz*., *Aspergillus flavus* and *Aspergillus niger* and with 0.35 Log CFU/ml and 0.47 Log CFU/ml reduction in the *Aspergillus flavus* and *Aspergillus niger*, the curcumin encapsulate present inside the *burfi* was found to be effective against fungal spoilage (Figure 5). Wang et al. (2009) studied the inhibitory effect of microcurcumin encapsulate against spoilage microorganisms. The highest inhibition was found in *Aspergillus niger* and thus indicates the efficacy of curcumin against fungal microorganisms.

**Storage stability and shelf life of curcumin encapsulate added *burfi***

***Changes in the antioxidant activity of control and curcumin encapsulate added burfi during storage***

The ABTS and DPPH free radical scavenging activity of curcumin encapsulate added *burfi* was increased up to 6 days followed by continuous decrease throughout the storage period as depicted in Figure 6. A non-significant difference in the antioxidant activity of curcumin encapsulate *burfi* was observed with storage irrespective of storage temperature. The initial increase in the antioxidant potential of curcumin encapsulate *burfi* was due to the release of remaining curcumin from encapsulate with the storage. This released curcumin scavenges free radicals with storage period thus, caused further reduction in the antioxidant activity. A similar finding of control release of curcumin from hydrogel during storage was observed by Nakagawa et al. (2013).

***Changes in the physicochemical parameters of control and curcumin encapsulate added burfi during storage***

Control and the curcumin encapsulate added *burfi* were analysed for the physicochemical changes *viz*., free fatty acid (FFA) content, hydroxylmethylfurfural (HMF), thiobarbituric acid (TBA) value, water activity and acidity during storage. The FFA content was significantly increased with storage period and higher FFA content was observed at 30±1°C than 4±1°C storage temperature (Figure 7). At 30±1°C, the initial FFA content in control and curcumin encapsulate added *burfi* was 3.68 and 4.6 µ equivalent oleic acid/g *burfi* sample respectively which was significantly increased to 6.88 and 8.12 µ equivalent oleic acid/g *burfi* sample within 9 days of storage. The HMF content indicates the extent of Maillard browning reaction during storage. The HMF formation was significantly higher in control and curcumin encapsulate *burfi* stored at 30±1°C than the samples at 4±1°C storage. At 30±1°C, HMF content of curcumin encapsulate *burfi* reached to 28.98 µmoles/100g TS in 12 days of storage. The non-significant difference in the HMF content of control and the curcumin encapsulate added *burfi* indicates a similar extent of browning reaction in both samples. The lipid oxidation (measured in terms of TBA value) was observed to be higher at 30±1°C temperature as compared with the oxidation at 4±1°C storage. The water activity showed a non-significant difference throughout the storage period with initial decrease in the activity of *burfi* samples up to 3 days of storage. A significant increase in acidity of control and curcumin encapsulate *burfi* was observed with increased storage period at both temperatures. The results are in line with the observations of Chawla et al. (2015) who reported a significant increase in the FFA content, HMF and acidity with decreased water activity throughout the storage period in Doda *burfi.*

***Changes in the sensory attributes of control and curcumin encapsulate added burfi during storage***

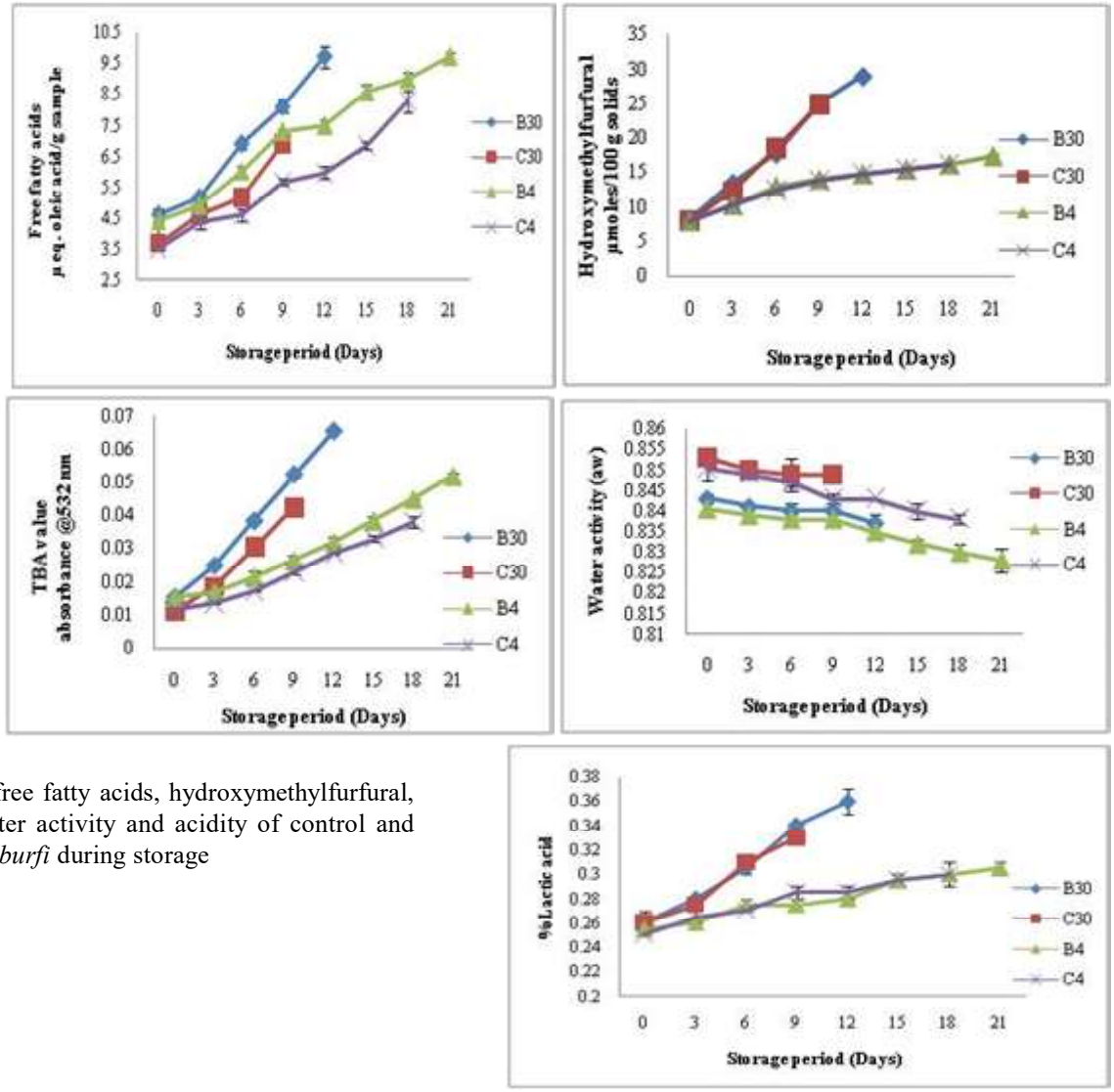



**Fig. 7** Changes in the free fatty acids, hydroxymethylfurfural, thiobarbituric acid, water activity and acidity of control and encapsulated curcumin *burfi* during storage

As depicted in Figure 8, the flavour score of control and the curcumin encapsulate added *burfi* stored at 4±1°C was significantly decreased from 7.55 and 7.60 to 6.85 and 6.65 within 18 and 21 days of storage respectively. A significant variation in the body and texture score of samples was observed during the storage period and was decreased to as low as 6.50 in curcumin encapsulate added *burfi* after 21 days, while the control *burfi* had scored 6.70 at the end of 18 days of storage. Moreover, a similar trend in colour, appearance score and overall acceptability was also observed throughout the storage period. The overall acceptability score declined from 7.60 to 6.80 in control *burfi* after 18 days of storage, whereas it was reduced to 6.65 after 21 days of storage in the *burfi* added with curcumin encapsulate. A non-significant difference was observed in all the sensorial attributes of control and curcumin encapsulate added *burfi* during the entire storage period at 30±1°C. The decrease in the sensorial attributes of *burfi* samples during storage was due to the evaporative loss of moisture and the oxidative and physical-chemical changes. Prasad et al. (2017b) observed a decrease in the sensorial attributes of herbal *burfi* during storage due to the excessive hardness of sample in the refrigerated condition. The results observed are in agreement with the findings of Chawla et al. (2015) in Doda *burfi*.

***Changes in the instrumental textural attributes of control and curcumin encapsulate added burfi during storage***

The results revealed that the hardness of control and curcumin encapsulate added *burfi* stored at 4±1°C was significantly increased from 54.58 N and 46.78 N to 73.87 N and 80.25 N in 18 and 21 days of storage respectively as shown in Figure 9. A similar increased trend was observed in adhesiveness, cohesiveness, gumminess and chewiness while springiness was found to be decreased throughout the storage. The resilience showed a non-significant difference in *burfi* sample stored at different temperatures. These textural changes were less

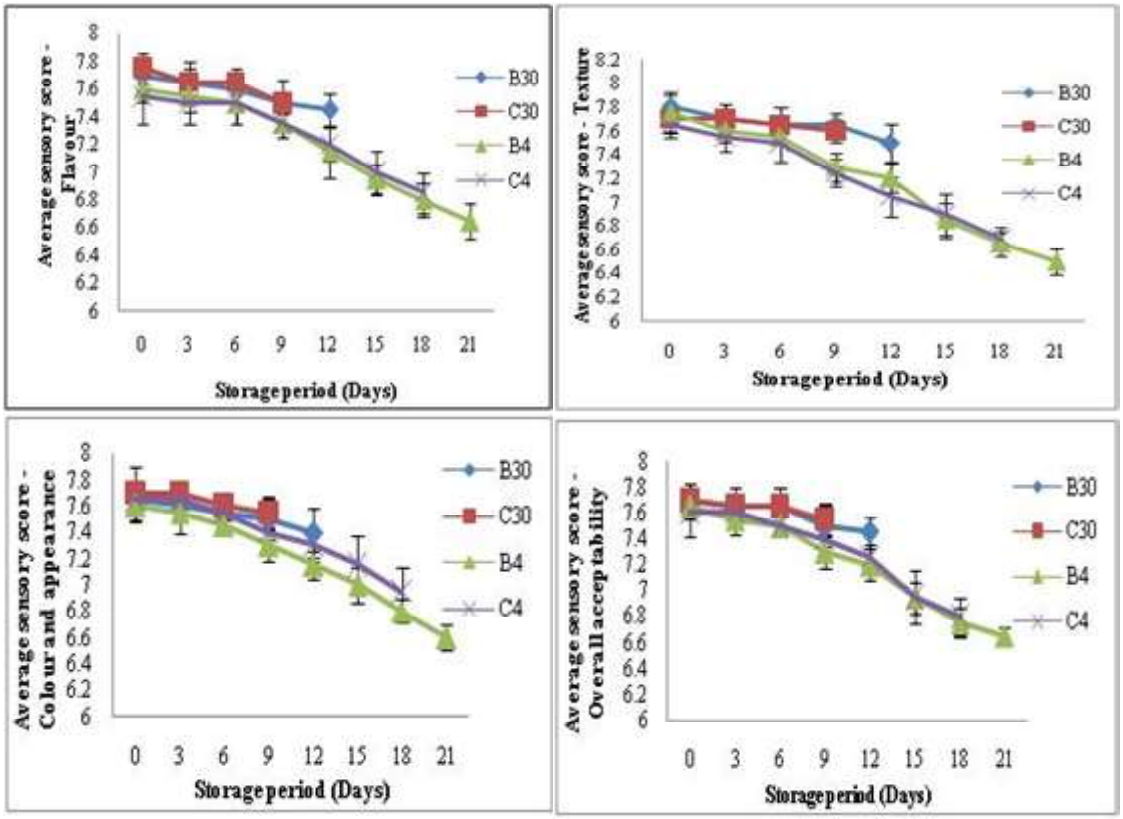



**Fig. 8** Changes in the sensorial attributes of control and encapsulated curcumin *burfi* during storage

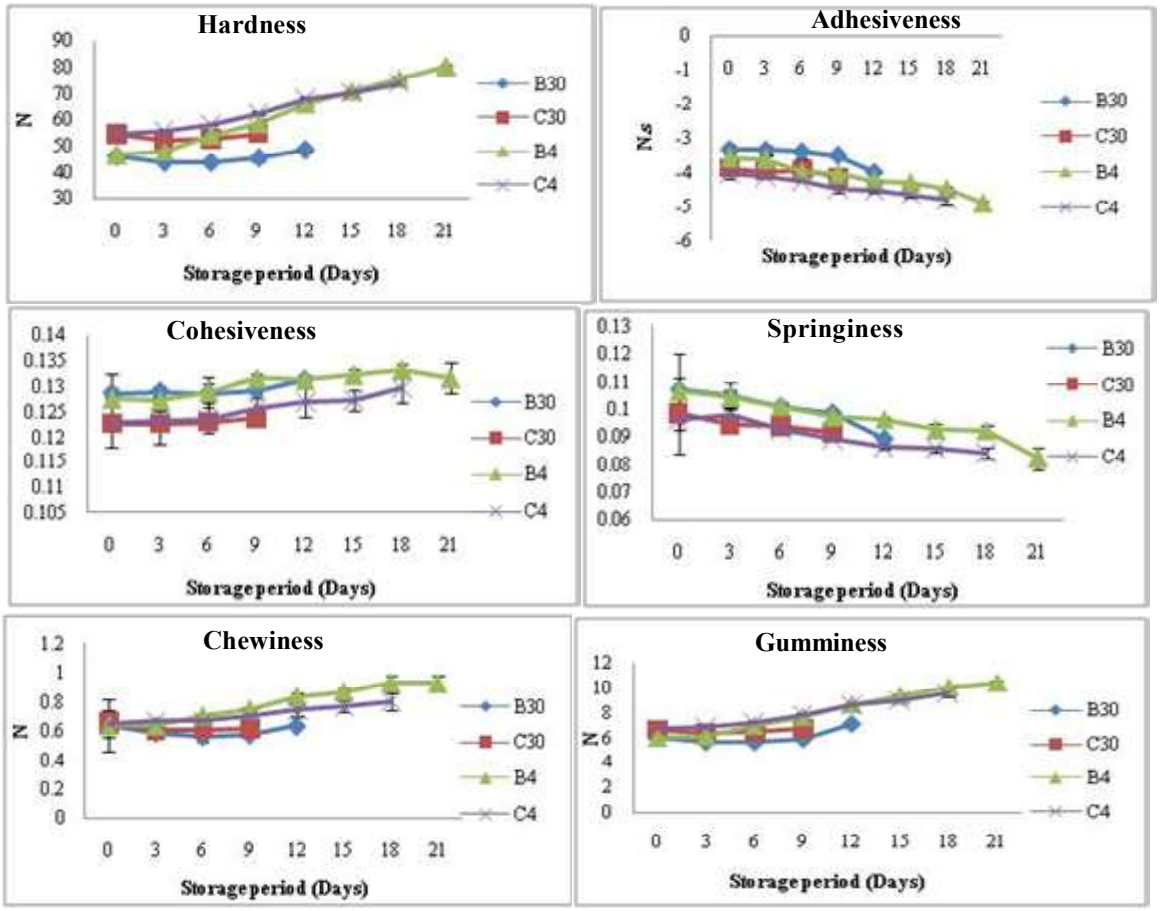
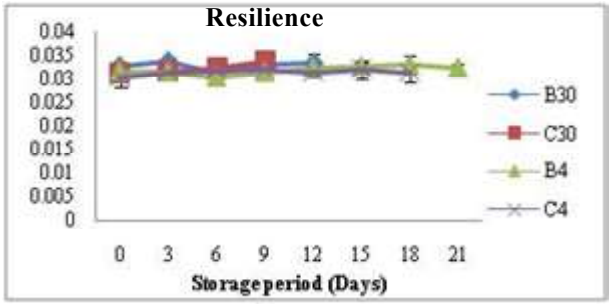



**Fig. 9** Changes in the textural attributes of control and encapsulated curcumin *burfi* during storage




pronounced in *burfi* stored at 30±1°C as compared to sample kept at refrigerated temperature

(4±1°C). The textural change observed in burfi samples was due to the evaporation of moisture from the product. Arora et al. (2007) reported a significant increase in the hardness of low-calorie sweetener added *burfi* from 62.21 N to 89.18 N during one week storage under refrigerated temperature. Chawla et al. (2015) observed a significant variation in hardness, adhesiveness, cohesiveness and gumminess of Doda *burfi* kept at 30±1°C storage temperature.

***Changes in the hunter colour profile of control and curcumin encapsulate added burfi during storage***

The results presented in Figure 10 revealed that the lightness value (L*) of the control and curcumin encapsulate added *burfi* decreased significantly with storage. The extent of decrease in lightness value was more pronounced at 30±1°C as compared to 4±1°C storage temperature. The a* value of curcumin encapsulate

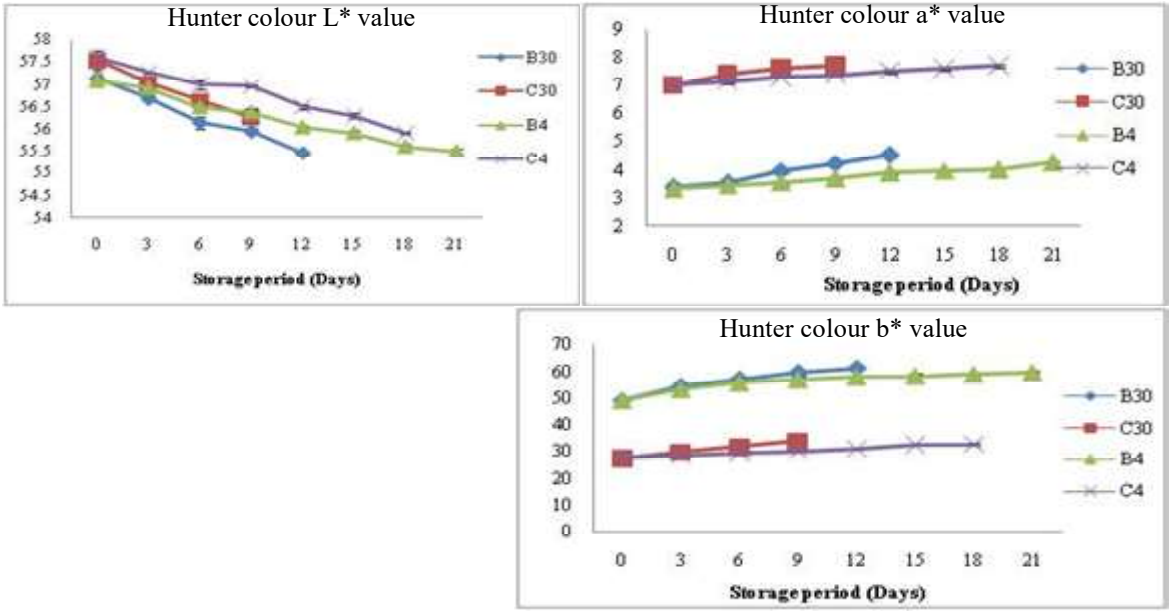



**Fig. 10** Changes in the hunter colour profile of control and encapsulated curcumin *burfi* during storage

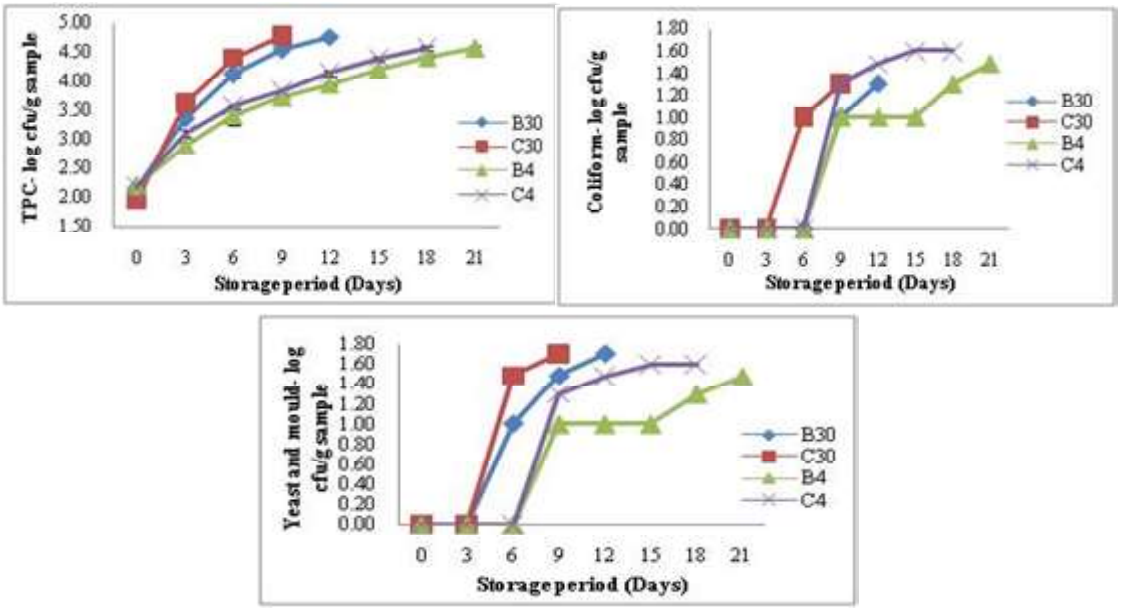



**Fig. 11** Changes in the microbiological count of control and encapsulated curcumin *burfi* during storage

added *burfi* was significantly increased from 3.38 and 3.31 to 4.52 and 4.32 within 12 and 21 days of storage at 30±1°C and 4±1°C temperature respectively. The b* value was found to be increased during storage period. The changes in *burfi* samples may be due to Maillard browning reactions. Arora et al. (2010) observed a decreased lightness value of artificial sweetener added *burfi* with increased storage period. The increase in a* value with increase in storage temperature and time have been reported by Gothwal and Bhavdasan (1991) and Arora et al. (2010) in *khoa* and artificial sweetener added *burfi,* respectively.

***Changes in the microbiological count of control and curcumin encapsulate added burfi during storage***

The changes in the microbiological counts *i.e*., total plate count (TPC), coliform count, yeast and mould count of *burfi* samples were evaluated throughout the storage period as revealed in Figure 11. A rapid microbial growth was observed in the control *burfi* sample as compared to the curcumin encapsulate added *burfi*. A similar total plate count was observed in control (after 9 days) and curcumin encapsulate added *burfi* (after 12 days) at 30±1°C storage. The visible yeast and mould growth was also observed after 9 and 12 days of storage at 30±1°C in control and curcumin encapsulate added *burfi*. The coliform count was observed to be increased to 1.30 Log CFU/g in curcumin encapsulate added *burfi* after 12 days of storage but no count was seen upto 6 days of storage at 30±1°C. Thus, the results revealed that the antimicrobial and antifungal activity of curcumin restricts or delays the growth of spoilage-causing microorganisms and thus extends the shelf life of curcumin encapsulate added *burfi* upto 3 days as depicted in Figure 11. Hosny et al. (2011) observed the curcumin addition in *Karish* cheese could be an effective mean to protect it from spoilage and pathogenic microorganisms.

## Conclusions

In this work, different forms of curcumin were added in burfi. The study concluded that the antioxidant activity and total phenolic content of curcumin added *burfi* were higher than the control. The highest antioxidant activity was observed in *burfi* sample added with curcumin encapsulate at patting stage. It was also observed that the curcumin addition affected the hunter colour profile of *burfi* with the least colour variation observed at patting stage. The dried curcumin encapsulate added *burfi* was highly accepted by the sensory panel. Thus, the dried curcumin encapsulate added at patting stage of *burfi* was selected for incorporation due to higher antioxidant potential. The curcumin encapsulate added at different concentrations showed increased b* value with extent of addition. The overall acceptability of curcumin encapsulate added burfi significantly decreased beyond 10% addition and thus, the addition of curcumin encapsulates at 10% level was selected for incorporation in *burfi*. The encapsulated curcumin *burfi* was found to be effective against fungal spoilage-causing microorganisms. The in-vitro release behaviour revealed that 63% curcumin was found to be stable after the end of intestinal digestion. The free radical scavenging activity of encapsulated curcumin *burfi* was increased upto 6 days of storage. The encapsulated curcumin added *burfi* was observed with a shelf life of 12 days at 30±1°C. The storage life was observed to be 18 and 21 days for control and curcumin encapsulated *burfi* at 4±1°C.

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RESEARCH ARTICLE

# Storage studies on textural aspects of selected Indian dairy products

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**Abstract:** The present study was undertaken to study the effect of storage temperature on the textural properties of market samples of *Paneer, Kaju Katli and Rajmalai*. The investigation was done to know the significance of the effect of ambient (fluctuating) versus steady storage temperature conditions on the textural properties of products. Ambient storage in the glass shelves or jar is the most common and preferred method of storage by sweet vendors in India. So the market samples of products were procured and stored at designated temperature conditions, (5 ± 1 $^0$C, 80± 5% RH) incubator at (18 ± 1 $^0$C, 55± 5% RH) and (30 ±1 $^0$C, 55± 5% RH), and at ambient condition (18 – 30 $^0$C, 70 ± 5% RH) for different time periods. Such products are subjected to fluctuating environmental conditions. The result indicates that, the primary textural characteristics of *Paneer* and *Kaju Katli* were not significantly affected by the fluctuation of ambient storage temperature for the short shelf life at that temperature but the adhesiveness, cohesiveness and springiness of *Rajmalai* were significantly affected by the fluctuation of ambient storage temperature for the long shelf life at that temperature.

**Keywords:** *Kaju Katli*, *Paneer*, *Rajmalai*, Texture-analyser

## Introduction

In India, of the total milk processed, around 65 % to 70 % milk is sold as liquid milk while rest is processed in to various dairy products like cheese, butter, ghee, *Paneer*, ice-cream, curd etc (ICFA, 2019). Variation in the ingredients, their proportion and processing condition affect the quality of product. Lack of knowledge in these aspects is a serious limitation for the processing standardization and quality control. Instrumental analysis of texture in food provides fast and relatively inexpensive indications on product characteristics and consumer acceptance (Anton and Luciano, 2007).

The literature shows very sparse data on engineering properties of indigenous milk products. Texture is one of the major criteria which consumers used to judge the quality and freshness of many foods. *Paneer*, essentially had a characteristics harder texture than Chhana. Fresh or fried *Paneer* had usually been analysed for its textural profile parameters with Instron (Desai, 1988; Zanjad and Mathur, 1990; Rao, 1993). Khan and Pal, (2011) stated that *Paneer* was highly perishable product. Freshness of *Paneer* remains intact only for 3 days at refrigeration temperature. At room temperature, *Paneer* does not stay good for more than a day. *Paneer* packaged in laminated pouches had a shelf of about 30 days at refrigerated storage. Several compositional and environmental factors have influence on the quality attributes of cashew nut sweets. Moisture reduction below 9.5 per-cent (dry weight basis) renders the product hard in body and coarse in texture, thereby affecting its acceptability (Aneja et al. 2002). *Kaju Katli* is one of the most popular Indian sweets because of its delicate texture, good flavor and excellent mouthfeel. Major ingredients are milk solids, cashew nut and sugar and it is typically prepared in the form of thin slices cut into diamond shape with silver foil coating. *Rajmalai* is a khoa based popular sweet in Vidarbha region of Maharashtra state, marketed in the form of chocolate with approximate dimensions 2.5 inch X 1.0 inch X 0.25inch. The base material of *Rajmalai* is the khoa. Present study will discuss the effect of storage conditions on textural properties of *Paneer*, *Kaju Katli* and *Rajmalai.*

### Materials and Methods

*Paneer* and *Kaju Katli* samples were obtained in a lot from the same batch packed and sealed in polythene and cardboard boxes. These boxes were placed in polythene bag to prevent moisture migration during transport. *Rajmalai* samples were purchased at the same time from the same lot and packed in sealed plastic

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bottles. Samples of all these products were analysed for chemical qualities and textural parameters. Moisture, titratable acidity, ash, and protein content were determined for each product in triplicate by BIS (SP: 18 (Part XI)-1981) while; fat content of *Kaju Katli* and *Rajmalai* was determined by BIS (SP: 18 (Part XI)-1981), but fat content of *Paneer* was determined by (IS:10484, 1983). Total carbohydrate content of all these samples was determined by subtracting the sum of moisture, ash, protein and fat from 100. Total solid content of all the products was determined by subtracting moisture content from 100. Chemical composition of *Paneer*, *Kaju Katli* and *Rajmalai* are shown in Table1.

**Texture profile analysis**

Texture properties of the *Paneer*, *Kaju Katli* and *Rajmalai* samples were determined by TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., UK) equipped with 50 kg load cell. Cylindrical samples of each product measuring 10 mm in length and height were subjected to uniaxial compression to 50 per cent of the initial sample height using the Texture Analyzer. At least five replications were performed on each observation.

The deformation curve obtained for a two-bite deformation cycle, using 75 mm diameter compression plate was used to determine the textural characteristics of samples of the product tempered at 25 $^0$C. Various textural characteristics of the product viz., hardness, fracturability resilience, cohesiveness, springiness, gumminess and adhesiveness were measured from force-time curve (Bourne, 2002). The analyzer settings that were employed to determine the textural attributes of products are presented in Table 2.

**Statistical Analysis**

The data obtained from the analysis of all these samples were statistically analyzed to identify the change in textural parameters at different temperature treatments using "One Way ANOVA". Means were compared according to methods described by Snedecor and Cochran (1967).

Treatments given to *Paneer*, *Kaju Katli* and *Rajmalai* samples were as follows:

Control = Ambient temperature storage

T1 = Storage at steady and controlled temperature of 18 ± 1 $^0$C

T2 = Storage at steady and controlled temperature of 30 ± 1 $^0$C

All statistical analyses were performed using Microsoft Office Excel® 2007 software utilizing the add-on Data Analysis tools to calculate the means, variances, correlations and for performing one-way ANOVA. Results are presented in means ± standard error of mean (SEM), and statistical significance was set at $p < 0.05$. Analysis of variance (ANOVA) was used to determine the main effects of treatments (Gacula and Singh, 1984). Further verification of statistical results was done by making use of Daniel's XL toolbox version 5.08, an MS Excel add-on.

## Results and Discussion

**Paneer**

The moisture content of *Paneer* stored at all temperature conditions showed a decrease with increase in storage period. The product, initially had 54.09 ± 3.79% moisture content, lost moisture to the environment as function of storage temperature. The hardness of *Paneer* stored at refrigeration temperature showed slight variation with increase in storage period, but that stored at ambient and other temperatures showed a definite increase with increase in storage period. The product becomes harder and this is attributed to the corresponding decrease in moisture content, indicated in figure 1 and figure 2. Other primary textural characteristics viz. Adhesiveness, Cohesiveness and Springiness and the secondary textural characteristics viz. Gumminess and Chewiness varied in diverse manner with storage period.

The correlations amongst primary textural characteristics and with moisture content were analyzed. For the refrigeration temperature (5 ± 1$^0$C), non-significant correlation between moisture content and hardness, and moderately high inverse correlation between moisture content and adhesiveness (-0.742) were observed. For the controlled storage temperature of 18 ±1 $^0$C, strong inverse correlation between moisture content and hardness (-0.972) and between moisture content and adhesiveness (-0.831) is exhibited. For the controlled temperature condition of 30 ± 1 $^0$C, there is very strong inverse correlation

**Table 1** Chemical composition of *Paneer*, *Kaju Katli* and *Rajmalai*

| Constituent | *Paneer* | *Kaju Katli* | *Rajmalai* |
|---|---|---|---|
| Moisture(%) | 54.09±0.16 | 12.05±0.23 | 14.85±0.25 |
| Fat (%) | 23.60±0.12 | 16.60±0.12 | 8.70±0.31 |
| Protein (%) | 17.60±0.13 | 10.99±0.23 | 6.92±0.41 |
| Acidity (%) | 0.39±0.04 | 0.37±0.02 | 0.43±0.03 |
| Ash (%) | 1.79±0.03 | 1.13±0.07 | 1.72±0.06 |
| Total carbohydrate (%) | 2.92±0.23 | 59.23±1.26 | 67.81±1.07 |
| Total solid (%) | 45.91±0.65 | 88.00±0.75 | 85.15±1.28 |

Mean±S.D., n=5

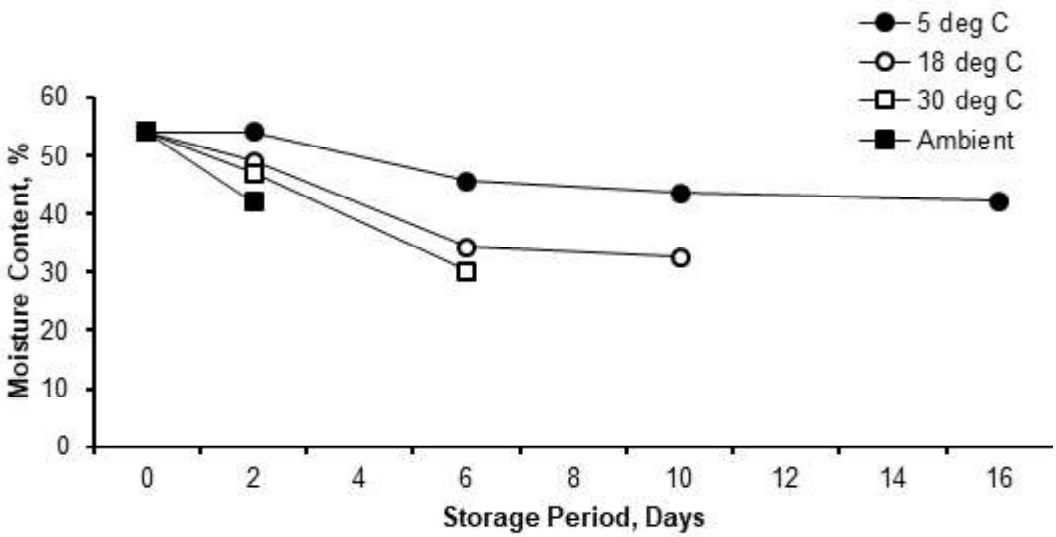


**Fig. 1** Variation in the moisture content of *Paneer* with storage period at different storage temperature conditions

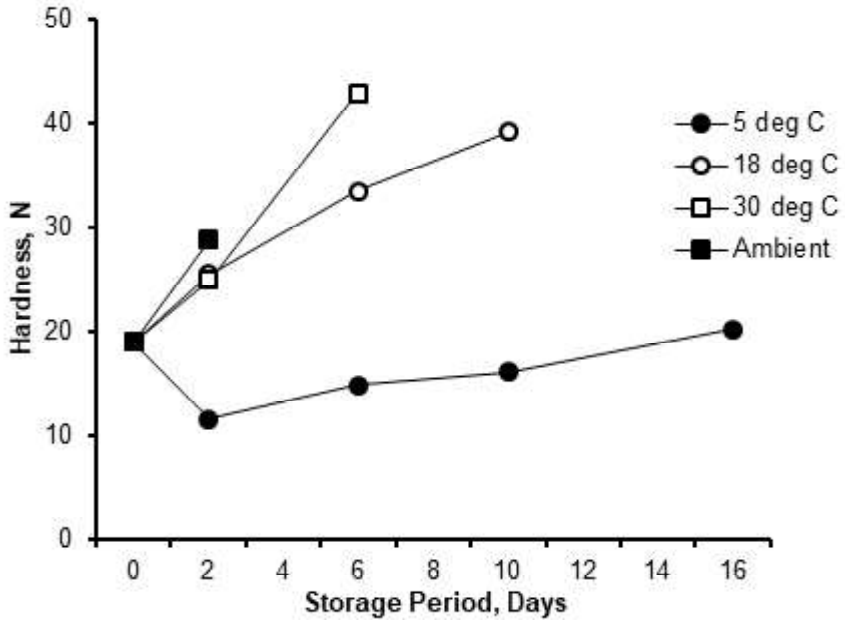


**Fig. 2** Variation in the hardness of *Paneer* with storage period at different storage temperature conditions

**Table 2** Texture analyzer TA-XT2i settings for the present study

| Texture analyzer parameters | Values |
|---|---|
| Pre-test speed | 2 mm/sec |
| Test and Post-test speed | 0.5 mm/sec |
| Trigger type | Auto |
| Threshold | 0.049 N |
| Time | 5 s |
| Distance | 5 mm |
| Data acquisition rate | 200 pps |

between moisture content and hardness (-0.999). Adhesiveness exhibited strong inverse correlation with moisture content (-0.964). Cohesiveness is strongly correlated with moisture content (0.936). For the fluctuating ambient temperature condition (18 ~ 30$^0$C), very strong inverse correlation between moisture content and hardness (-0.996) and between moisture content and adhesiveness (-0.997) is observed. Cohesiveness is highly correlated with moisture content (0.946). Springiness exhibited non-significant relationship with other textural parameters at all temperature conditions. It is concluded that the variation profile of the moisture content over a period of time is one of the important determining factors for the consumer acceptance of *Paneer* based upon its texture.

The TPA values of *Paneer* primary textural characteristics under the fluctuating ambient temperature conditions (18 ~ 30 $^0$C) and the two comparable controlled temperature conditions during the short shelf-life period were subjected to Two-factor Analysis of Variance at 5 per cent level of significance. It was observed that values of hardness changed significantly over the short storage period (P: 0.02576 < 0.05) as expected for high-moisture food, but there is statistically insignificant variation amongst the

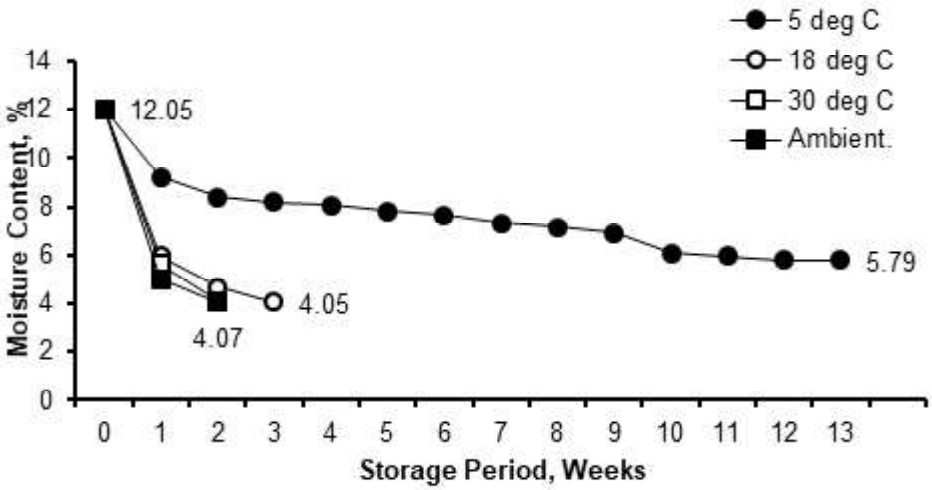


**Fig. 3** Variation in the moisture content of *Kaju Katli* with storage period at various temperature conditions

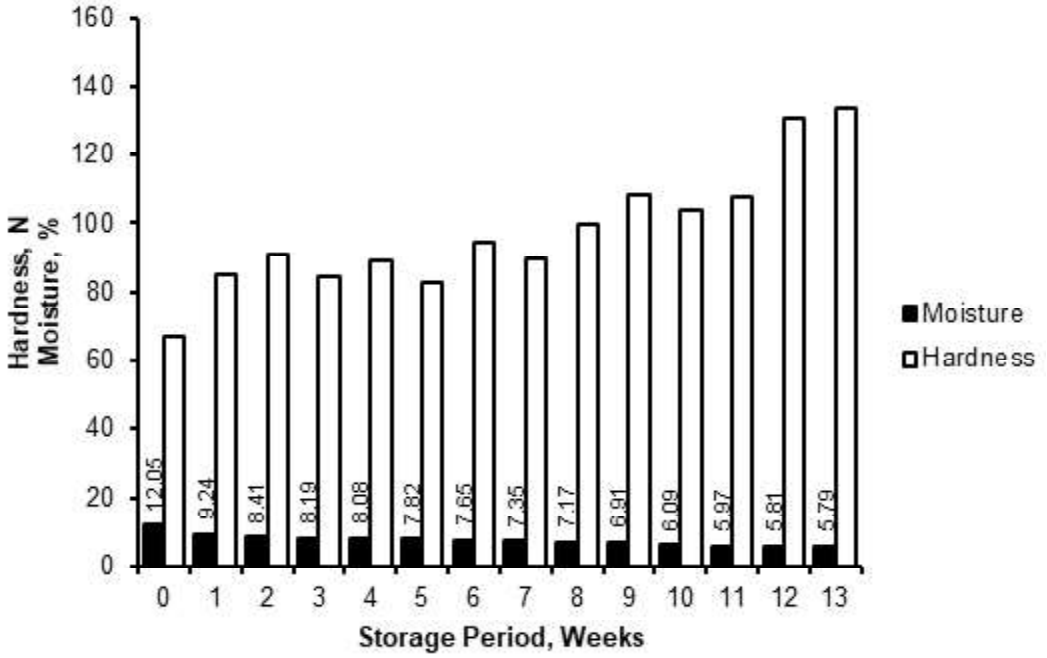


**Fig. 4** Variation in the hardness and moisture content of *Kaju Katli* with storage period at refrigeration temperature condition

storage temperature conditions (P: 0.5 > 0.05). This implies that the hardness of *Paneer* is not significantly affected by the fluctuation of ambient storage temperature, for the short shelf life at that temperature. The other primary textural characteristics of *Paneer* are also not significantly affected by the fluctuation of ambient storage temperature, for the short shelf life of about 2 days at that temperature. Bargale and Jha (1992) concluded that as the storage period increased, hardness, chewiness, and gumminess increased significantly, while springiness and cohesiveness remained almost unchanged.

***Kaju Katli***

The correlations amongst primary textural characteristics and with moisture content were analyzed (indicated in figure 3 and figure 4). For the refrigeration temperature (5 ± 1 $^0$C),), high inverse correlation of moisture content with hardness (-0.849) and moderately high inverse correlation with adhesiveness (-0.763) was observed. Cohesiveness is moderately correlated with moisture content (0.712). For the controlled storage temperature of 18 ± 1 $^0$C, strong correlation of moisture content with cohesiveness (-0.910) and with springiness (0.943) was exhibited. For the controlled temperature condition of 30 ± 1 $^0$C, moderately high inverse correlation of moisture content with hardness (-0.749) and moderate correlation with cohesiveness (0.751) were observed. Springiness exhibited strongly significant relationship with the moisture content (0.978). For the fluctuating ambient temperature condition (18 ~ 30$^0$C), very strong inverse correlation of moisture content with hardness (-0.966), strong correlation with springiness (0.960), and moderately inversely with adhesiveness (-0.736) and cohesiveness (-0.735) was observed. The hardness of *Kaju Katli* was found to be inversely correlated

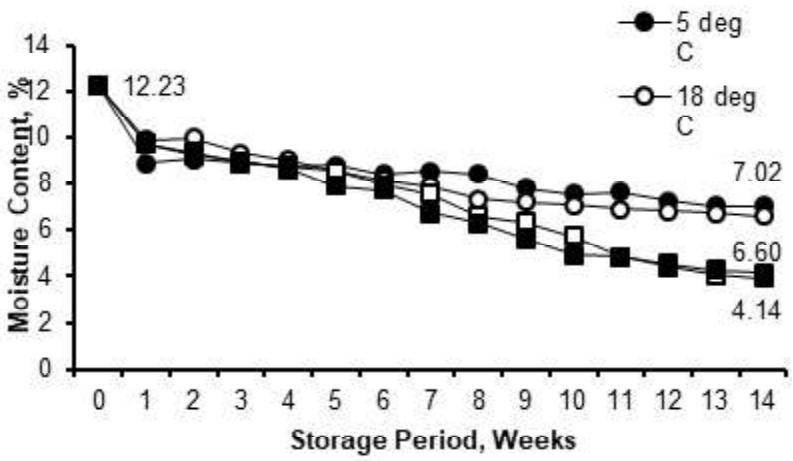


**Fig. 5** Variation in the moisture content of *Rajmalai* with storage period at different storage temperature conditions

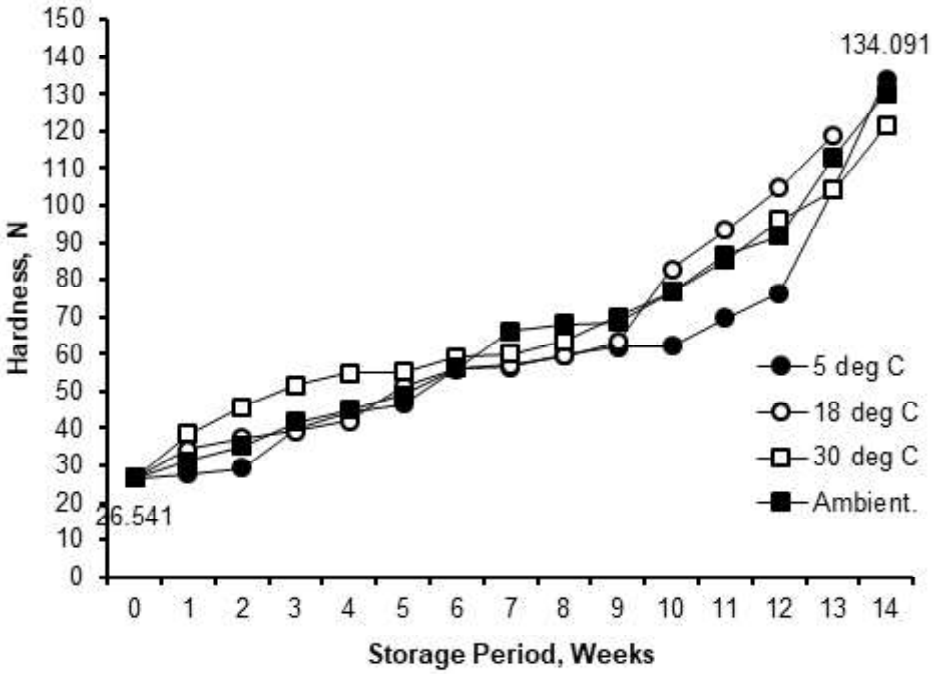


**Fig. 6** Variation in the hardness of *Rajmalai* with storage period at different storage temperature conditions

with the moisture content in moderate to highly strong manner, while other primary textural parameters were also observed to be correlated with the moisture content with varying intensity. Sharma et al. (2017) observed that the hardness of *Kaju Katli* was found to be increased with decrease in free moisture content over a longer storage period. Hence it is concluded that the variation in moisture content over a period of time significantly affects the consumer acceptance of *Kaju Katli* based on its texture.

The TPA values of *Kaju Katli* primary textural characteristics under the fluctuating ambient temperature conditions (18 ~ 30 $^0$C) and the two comparable controlled temperature conditions during the shelf-life period were subjected to Two-factor Analysis of Variance at 5% level of significance. It is observed that the values of hardness changed insignificantly for the short storage period (P: 0.1096 > 0.05) as well as there is statistically insignificant variation amongst the storage temperature conditions (P: 0.1506 > 0.05), implying that the hardness of *Kaju Katli* is not significantly affected by the fluctuation of ambient storage temperature, for the short shelf life at that temperature. Also, there are insignificant changes in primary textural characteristics on account of temperature fluctuations under ambient conditions. The common market practice of storage of *Kaju Katli* under ambient temperature conditions does not adversely affect its textural quality for the indicated shelf life.

***Rajmalai***

The correlations amongst primary textural characteristics and with moisture content were analyzed (indicated in figure 5 and figure 6). For the refrigeration temperature (5 ± 1$^0$C), it is seen that there is moderate inverse correlation of moisture content

with hardness (-0.732) and moderately high correlation with adhesiveness (0.849). For the controlled storage temperature of 18 ± 1$^0$C, high inverse correlation of moisture content with hardness (-0.835) and moderate inverse correlation with springiness (-0.759) and adhesiveness (-0.771) is exhibited. For the controlled temperature condition of 30 ± 1$^0$C, there is very strong inverse correlation of moisture content with hardness (-0.955) and strong correlation with adhesiveness (-0.888). For the fluctuating ambient temperature condition (18 ~ 30$^0$C), very strong inverse correlation of moisture content with hardness (-0.911) and with adhesiveness (-0.909) is exhibited. The hardness of *Rajmalai* was found to be inversely correlated with the moisture content in a strong manner, while adhesiveness was also observed to be highly inversely correlated with the moisture content. However, cohesiveness and springiness did not exhibit any significant relationship with the moisture content. It is concluded that the variation profile of the moisture content over a period of time is important for the consumer acceptance of *Rajmalai based* upon texture.

The TPA values of *Rajmalai* primary textural characteristics under the fluctuating ambient temperature conditions (18 ~ 30$^0$C) and the two comparable controlled temperature conditions during the shelf-life period were subjected to Two-factor Analysis of Variance at 5% level of significance. It is observed that the values of hardness changed significantly for the long storage period (P:2.71x10-17 < 0.05) but there is statistically insignificant variation amongst the storage temperature conditions (P: 0.47696 > 0.05), implying that the hardness of *Rajmalai* is not significantly affected by the fluctuation of ambient storage temperature, for the studied shelf life at that temperature; but there are statistically significant changes at 5% level of significance in other primary textural characteristics viz. adhesiveness (P: 0.000108 < 0.05), cohesiveness (P: 0.018688 < 0.05) and springiness (P: 0.018562 < 0.05) on account of temperature fluctuations under ambient conditions were. Nightingale et al. (2011) reported the textural changes in dark chocolate stored at ambient (storage room) and temperature fluctuations. The percent change in hardness, cohesiveness and springiness after 8 weeks of storage was less than 10 per cent for temperature fluctuations environment. Chand et al. (2011) prepared and packed jaggery chocolate and then subjected them to different storage conditions, including ambient (25-35$^0$C) and reported increased in hardness with increased in storage period.

The common market practice of storage of *Rajmalai* under ambient temperature conditions does adversely affects its textural quality for the indicated long shelf life, and textural variations over the storage period can be minimized by storing the product in steady temperature environment.

## Conclusions

Moisture content of a product has direct bearing on its textural characteristics. The variation profile of the moisture content over the storage period is an important factor for determining consumer acceptance of the product based upon texture. The common market practice of storage of Indian dairy products under ambient temperature conditions affects the textural quality for long shelf life products but for short shelf life products, the textural variations over the storage period are insignificant.

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RESEARCH ARTICLE

# Effect of incorporation of Finger millet (*Eleusine coracana*) on the antimicrobial, ACE inhibitory, antioxidant and antidiabetic potential of a milk-millet composite probiotic fermented product

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**Abstract:** The aim of this research was to evaluate the effect of incorporation of finger millet on anti-microbial, ACE inhibitory, antioxidant and antidiabetic potential of milk-millet composite product. Malted, heated finger millet flour was incorporated in the milk @20%. The mix was fermented using culture comprising of *Streptococcus thermophilus* MTCC 5460 and probiotic strain *Lactobacillus helveticus* MTCC 5463 @ 2%. Product without finger millet was the control. Products were evaluated for antimicrobial, ACE inhibitory, antioxidant and antidiabetic potential using standard procedures. Incorporation of finger millet significantly ($P<0.05$) improved the antimicrobial, antioxidant and antidiabetic activity of the composite product. The product showed a significantly ($P<0.05$) higher antimicrobial activity against all test pathogens in comparison to control. The antioxidant activity of composite food and control were 95.59 % and 52.37 % respectively. Antidiabetic potential measured in terms of α-amylase inhibition and α-glucosidase inhibition revealed that incorporation of finger millet significantly improved the α-glucosidase inhibition (69.89 %) in comparison to control (51.89%), whereas the α-amylase inhibition was not significant. The ACE inhibitory activity of composite product and control were found to be 51.54 and 51.11% respectively, which did not differ significantly. Incorporation of finger millet adds to the functional value of resulted composite food. The results of this study open promising prospects for use of such composite product as functional food.

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**Keywords:** Antioxidant, Antidiabetic, Finger millet, Functional food, Milk-millet composite product

## Introduction

The worldwide market for functional foods continues to increase in response to the demands of an increasingly health conscious public. Fermented milk products containing probiotics have grown into one of the leading categories of functional foods (Begum et al. 2017). Both milk and probiotics are reported to play significant roles in the prevention and management of non-communicable diseases. Even though fermented milk is considered superior in its nutritional and health benefits, it is deficient in micronutrients such as iron and vitamin C and dietary fiber components known for its prebiotic effect. Such deficiency can be overcome to a great extent through fortification of milk with cereals such as finger millet (*Eleusine coracana*). Finger millet is a low cost millet with practically no reports of its adverse effect. Further, it is reported to have higher dietary fiber content, several micronutrients and phytonutrients (Tripathi and Platel, 2010; Shobana et al. 2013). Hence, the cereal can be a good ingredient for use along with milk for developing a milk cereal-based functional food.

Finger millet (*Eleusine coracana*) is a very common millet found in different parts of world. This millet has recently been assigned the status of nutri-cereals (The Gazette of India, 2018). Finger millet is very rich in calcium (some genotypes of finger millet have been reported to contain calcium as high as 450 mg/100 g of grains), phosphorus (283 mg %), potassium (408 mg %) and contains iron (3.9 mg %), dietary fiber, vitamins, as well as useful amounts of copper and comparatively higher chromium, magnesium, molybdenum, zinc and selenium. (Gopalan et al. 2009; Tripathi and Platel, 2010; Gupta et al. 2011; Gupta et al. 2017). Finger millet is comparable to rice with regard to protein (6-8%) and fat (1-2%) and is superior to rice and wheat with respect to mineral and micronutrient contents and essential amino acids such as methionine and tryptophan (Verma and Patel, 2013; Gupta et al. 2017). The seed coat of finger millet is reported to be rich in phytochemicals like polyphenols (Devi et al. 2014) which contributes to antioxidant, anticancer and antidiabetic activities. High fiber in the millet is reported to promote slow digestion and

prevent constipation, high cholesterol formation, diabetes and intestinal cancer (Devi et al. 2014). Being a rich source of calcium and iron, and the fact that the bioavailability can be improved by simple processing such as germination and fermentation, finger millet is considered as a good supplement for improving bone health and haemoglobin. It is recognized for its health benefitting properties such as antimicrobial (Chethan and Malleshi, 2007; Varsha et al. 2009), antioxidant (Chandrasekara and Shahidi, 2011; Veenashri and Muralikrishna, 2011), cholesterol lowering (Pore and Magar, 1976), blood glucose lowering effect (Shobana et al. 2009), nephroprotective and anti-cataractogenic (Shobana et al. 2013). Earlier it was believed that polyphenols, phytates, tannins and dietary fiber contents of finger millet act as anti-nutrients because of their metal chelating and enzyme inhibition activities but now it has been confirmed that these constituents can contribute to antioxidant activity, which is an important factor in resisting aging and metabolic diseases (Chandra et al. 2016). Regular consumption of finger millet product is reported to decrease fasting glucose by 32% and eliminate insulin resistance by 43% (Chandra et al. 2016).

Looking to the nutritional and functional aspects of finger millet, its incorporation in milk and the probiotic fermentation of this milk-millet mixture can result in a composite fermented food having improved nutritional and functional value. Many *in vitro* and *in vivo* study reports are available on the health benefits of fermented milks and finger millet. But very few research works have been carried out in combining the nutritional aspects of milk, finger millet, probiotics and lactic acid bacterial fermentation as well as *in vitro* functional evaluation of millet enriched probiotic fermented milk. Hence in the current research, the effect of incorporation of finger millet on the sensory attributes, physico-chemical parameters, microbial count, status of phytic acid and tannin and functional properties such as antimicrobial, ACE inhibitory, antioxidant and antidiabetic potential of the milk-millet composite probiotic fermented product was studied.

## Materials and Methods

### Bacterial strains

Starter culture (*Streptococcus thermophilus* MTCC 5460 and probiotic strain *Lactobacillus helveticus* MTCC 5463) used in the study was obtained from the culture collection of Dairy Microbiology department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. Individual strains were propagated in sterilized reconstituted skim milk (11% total solids) medium by incubation at 37 ± 1ºC for 6 h and stored at 5 ± 2ºC. Three successive transfers of cultures were given in the same medium prior to their use to ensure activity of cultures during the course of study.

### Materials

Toned milk (Fat-3 %, SNF-8.5 %) was obtained from Vidya shoppe, Anand, Gujarat. Finger millet variety PRM 9802 (dark brown colored) of AGMARK (Grade I) was procured from local supermarket of Anand, Gujarat, India.

### Preparation of malted finger millet flour

Malting of finger millet was carried out according to the procedure suggested by Shaikh et al. (2017). Finger millet grains were cleaned to remove extraneous matter. The grains were washed and steeped in water for 12 h at 30 ± 2 ºC (Grains to water proportion 1:3). Water was changed after every 4 h. Steeped grains were then drained and spread on perforated trays lined with muslin cloth and kept in the BOD incubator at 25 ± 2 ºC/24h for germination. The germinated grains were then vacuum tray dried (42 ± 3 °C at 640 to 660 mm Hg of vacuum), ground to form a fine powder and sieved (100 µ mesh size) to obtain malted finger millet flour.

### Preparation of milk-millet composite probiotic fermented product

Toned milk (3.0% Fat, 8.5% SNF) was heated to 90°C/10 min and cooled to 40°C. This milk was added with malted, heated (70°C/2 min) finger millet flour @ 20% on milk basis. It was then mixed to ensure uniform mass. The mixture was then heated to 70ºC for no hold and further cooled to 40ºC and inoculated with starter culture @2%. It was incubated at (37 ± 2°C) till titratable acidity reached to about 0.7% lactic acid (LA). The product was then cooled (5 ± 2°C) and the curd was broken to obtain a uniform viscous product. It was filled in HDPE bottles and stored at refrigeration temperature (7±1ºC).

### Titratable acidity and pH

Titratable acidity of the product was determined after mixing 10 g of samples with 10 ml of distilled water and titrated against 0.1 N NaOH using 1 per cent (w/v) phenolphthalein as an indicator. The results were expressed as per cent lactic acid (IS: 1479-1, 1960). pH of the product was measured using a pH meter (Oakton pH 700 Benchtop Meter, Mumbai, India). Fermentation time was measured as time required to reach pH 4.7 ± 0.1 or 0.7 per cent lactic acid expressed as hours.

### Viscosity

Viscosity of the fermented milk products (200g each) at 25°C was measured using Brookfield viscometer (LVDV-E Viscometer, Brookfield, Borivali East, Mumbai, India) with a constant shear rate using spindle No. 61s at 100 RPM. Viscosity of the samples was expressed as centipoise.

**Microbiological analysis**

Eleven grams of product was aseptically weighed and transferred to 99ml sterile phosphate buffer to obtain 1:10 dilution. Subsequently, 1 ml of above dilution was used for making further dilutions in 9 ml phosphate buffer tubes. Suitable dilutions were prepared and poured in a set of sterile Petri dishes in duplicates. For the enumeration of Probiotic count, 1.0 ml from selected dilutions were poured in duplicate plates and mixed with sterile cooled deMan, Rogosa and Sharpe (MRS) agar. After setting of the agar, another layer of the same medium (5-7ml) was poured. The plates were then incubated at 37 ± 2°C for 72 h. After incubation, the typical lactobacilli colonies in the plates were counted and the count was expressed as log cfu/g (IS: 1479, 1962). For the enumeration of Streptococcal count, in place of MRS agar, M17 agar was used.

**Sensory evaluation**

Fermented products were subjected to sensory evaluation by expert panel of judges (n=8) for various sensory attributes, *viz*., flavor, body and texture, acidity, color and appearance, and overall acceptability criteria using 9-point hedonic scale described by Stone and Sidel (2004). Coded samples of freshly prepared products were given to the panel of judges. The judges were asked to rank the products from 1 to 9 according to their liking preference using 9-point hedonic scale rating.

**Tannin estimation**

Tannin content of malted finger millet flour, unmalted finger millet flour and probiotic fermented milk enriched with finger millet were estimated by the Folin-Denis method (Swain and Hillis, 1959). Standard solution of tannic acid (0.1 mg/ml) was taken in aliquots of 0, 1.5, 2.0, 2.5 and 3ml in five volumetric flask of 100 ml each containing 75ml distilled water. 5 ml of Folin-Denis reagent was added and the contents were shaken well. 10 ml of saturated $Na_2CO_3$ solution was added to each flask, and the volume made up to 100 ml with distilled water. The contents were mixed thoroughly and the absorbance was measured at 760 nm using spectrophotometer (Systronics 2206, Ahmedabad, India). For the preparation of sample, 2.5 g of probiotic fermented milk enriched with finger millet, 0.5 g malted and 0.5 g unmalted finger millet flours were weighed separately in 250 ml conical flasks and 75 ml of distilled water was added to each conical flasks. Contents were mixed properly and the samples were boiled for 30 min. Then the contents were filtrated using Whatman no.2 filter paper and the volume made up to 100 ml with distilled water. 0.5 ml of this solution was used for estimation of tannin content in a 10 ml volumetric flask. 0.5 ml Folin-Denis reagent and 1 ml of saturated solution of $Na_2CO_3$ was added and the volume made up to 10 ml. The contents were mixed thoroughly and kept undisturbed for 30 min. The absorbance was measured at 760 nm using a spectrophotometer. The percent tannin content was calculated using the formula given below.

$$\text{Percent tannin} = \frac{\text{Gross factor x Absorbance reading of sample x Total volume of solution made x } 10^{-4}}{\text{volume taken for estimation x weight of sample}}$$

**Phytic acid estimation**

Phytic acid content of the product and finger millet flours was estimated by titrimetric method (Lolas and Markakis, 1975). 2.5 g of probiotic fermented milk enriched with finger millet, 0.5 g each of malted and unmalted finger millet flours was taken in separate 100 ml flasks and 10 ml of 2% hydrochloric acid was added to each flasks. The contents were mixed well and kept for 3 h. It was filtered properly using Whatman no.2 filter paper and 5ml filtrate was taken into 100 ml flask and 1 ml distilled water was added, mixed well and 10ml 0.3% ammonium thiocynate indicator was added. This was titrated against iron dichloride solution containing 0.00195 g iron per ml. The end point noted was yellow colour. Phytic acid content was calculated using formula given below.

Per cent Phytic acid = y×1.19×100

Where,y = Titre value × 0.00195 g

**Antimicrobial activity**

Antimicrobial activity of the fermented product was tested by agar well diffusion method (Delgado et al. 2001) with some modifications. The antimicrobial activity of the products were tested against *Bacillus cereus, Escherichia coli*, *Salmonella typhi, Listeria monocytogenes,* and *Staphylococcus aureus* (All the strains were obtained from Department of Dairy Microbiology, S.M.C. College of Dairy Science, Anand, Gujarat, India). 100 µl of each indicator strains were poured into the petri dish followed by adding 15 to 20 ml of Nutrient agar and allowed to solidify or the plates were refrigerated at 5 °C for 10-15 min before wells were punched out of the agar with sterile borer (Himedia, diameter-15 mm). 100 µl fermented milk samples were then filled into the wells to check their inhibition activities on all five strains. The plates were once again refrigerated at 5 °C for 10 to 15 min to facilitate the diffusion of fermented milk samples and were incubated at 37°C for 24 h. The inhibition activities of fermented milk samples on the indicator strains were indicated by the presence of a clear zone surrounding the agar wells. The zone of inhibition around the wells was measured in mm.

**Angiotensin Converting Enzyme (ACE) inhibitory activity**

ACE-inhibitory activity (ACEi %) of the fermented products was determined according to the method described by Hati et al. (2015) with some modifications. An aliquot of 200 µl of product supernatant collected after centrifuging at 14000 rpm for 20 min

was added to 20 µl of ACE (4mU in 250 µl) and then incubated for 10 min at 37°C in eppendorf, before 50 µl 5mM N-Hippuryl-His-Leu (HHL) was added. The mixture was gently stirred and then incubated at 37 °C for 60 min. The reaction was ended by adding 500 µl of 1 N chilled HCl. The 635 µl of mixture was transferred into another eppendorf, added with 850 µl of ethyl acetate followed by centrifugation at 14000 RPM for 20 min. The aqueous layer was transferred into another eppendorf and then dried on water bath at 85 °C. The residual hippuric acid was dissolved in 2 mL of deionized water and it was filtered through 0.45 µm membrane filter (Millex® - HV, MERK Ireland). The absorbance of the solution was measured spectrophotometrically (Systronics 2206, Ahmedabad, Gujarat, India) at 228 nm. The activity of each sample was tested in triplicate. The percent ACE inhibition was calculated using the following formula.

$$ACEi\% = \frac{(\text{Absorbance of HA control} - \text{Absorbance of HA sample})}{\text{Absorbance of HA control}} \times 100$$

Where, HA control was the absorbance of hippuric acid produced by the ACE in buffer without lactic cultures. HA sample was the absorbance of hippuric acid produced by the ACE in the presence of lactic cultures.

**Antioxidant activity**

Antioxidant activity was measured by using 2, 2'-Azino-bis 3-ethylbenzothaizoline-6-sulfonic acid (ABTS) Assay with some modifications. Total radical scavenging capacity was based on the ability of a compound to scavenge the stable ABTS radical in 10 min (Re et al. 1999). The ABTS working solution was prepared by mixing 88 µl of 140mM potassium persulphate with 5 ml of 7mM ABTS stock solution and incubating overnight in dark bottles for generation of radicals. Then it was diluted with phosphate buffer saline (PBS) to adjust the absorbance at 734 nm to 0.7 ± 0.02. An aliquot of 25 µl of product (for control 230 µl) supernatant collected after centrifuging at 14000 rpm for 30 min was mixed with 2270 µl ABTS and made up to 2500 µl with PBS solution. The decrease in absorbance at 734 nm was recorded over period of 6 min at 30 sec interval using spectrophotometer. Percent inhibition of absorbance at 734 nm was calculated using the following formula.

$$\text{ABTS}^{\cdot+} \text{ scavenging activity (\%)} = \frac{\text{Control}_{(A734nm)} - \text{Sample}_{(A734nm)}}{\text{Control}_{(A734nm)}} \times 100$$

**Antidiabetic activity**

Antidiabetic activity was measured in terms of α-Amylase and α-Glucosidase inhibition.

**α-Amylase inhibition assay**

Amylase activity was assayed by the spectrophotometric method using 3, 5 dinitrosalicylic acid (Pinto et al. 2010). Sample extracts (300 µl) were taken in test tubes, to that, 70 µL of 50% methanol, 50 µL of enzyme solution and 1mL of starch solution was added and incubated at 37 °C for 5 minutes. 3, 5 Dinitrosalycylic Acid (DNSA) reagent (2ml) was added and the tubes were heated in boiling water bath for 5 minutes followed by cooling to room temperature. The absorbance of the colour developed was read at 540 nm. Blank and control tubes were also set up simultaneously without enzyme and sample, respectively.

$$\% \text{ inhibition of } \alpha\text{-amylase} = \frac{(\text{O.D. Control} - \text{O.D. Sample})}{\text{O.D. Control}} \times 100$$

**α-Glucosidase inhibition assay**

The assay was performed according to the method given by McCue et al. (2005). The assay mixture consisting of different concentrations of sample extracts (500 µg/ml) and 1000 µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/ml) was incubated at 25 °C for 10 min. After pre-incubation, 500 µl of 5mM para nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added and incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm using UV spectrophotometer and compared to a control containing 500 µl buffer solutions instead of the extract. The α-glucosidase inhibitory activity (%) was calculated as following:

$$\% \text{ inhibition of } \alpha\text{-glucosidase} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

**Statistical analysis**

The data related to chemical, sensory and functional aspects of probiotic fermented products were analyzed using statistical design CRD and Factorial completely randomized design (FCRD) as per Steel and Torrie (1980). The values for microbial counts were log transformed before analysis.

## Results and Discussion

The results of analyses of finger millet enriched product and control for sensory attributes, physico-chemical parameters, microbial count, phytic acid and tannin are shown in Tables 1, 2 and 3. The products were subjected to judging and grading for sensory attributes *viz*., (i) flavour, (ii) body and texture, (iii) acidity, (iv) colour and appearance and (v) overall acceptability by expert panel of judges using 9-pont Hedonic scale. Scores obtained for sensory attributes of fermented products are shown in Table 1.

Both products were liked by the judges and the difference in their flavor scores were found to be not significant. But a significant (P<0.05) difference was seen in the scores of all other attributes, which were higher in case of finger millet enriched product (T1). This may be because, the finger millet enriched product was found more viscous and uniform in body and texture in comparison to control (T2) sample. Acidity scores of the products as perceived by the judges differed significantly (P < 0.05). The mean values for T1 and T2 were 8.45 and 7.45 respectively. Colour and appearance is one of the important attributes of sensory quality. A significant (P < 0.05) difference was seen in the colour and appearance score of both products. Because of its viscous and uniform nature, finger millet enriched product scored higher for color and appearance. Overall acceptability of T1 (8.43 ± 0.160) was significantly (P < 0.05) higher than T2 (7.64 ± 0.238) indicating the high sensory acceptability of the product.

Statistically there was no significant (P>0.05) difference found in the pH and titratable acidity of products (Table 2). pH of the freshly prepared fermented products ranged from 4.77 to 4.78 while the titratable acidity ranged from 0.71 to 0.73 per cent lactic acid. The *Lactobacillus* (probiotic) and streptococcal counts were significantly (P<0.05) higher in finger millet enriched product compared to control. This may be due to the better nutrient availability to the cultures in the milk- millet composite food. Lactic acid bacteria are fastidious in their nutritional requirements, and cereals in general are reported to have higher content of some of the essential vitamins, minerals and dietary fibre than in milk. Further, malted millet is reported to be a good source of amylases and during germination, the amylases partially hydrolyze the starch to lower molecular weight carbohydrates such as oligo- and disaccharides. When this malted flour is mixed with milk and heated, the amylases hydrolyze the starch to simple sugars which the lactic acid bacterial strains can utilize for their growth (Shobana et al. 2013; Shaikh et al. 2017). There was no scientific published data on similar kind of work available to compare the effect of finger millet incorporation on streptococcal and probiotic counts of fermented milk product. The apparent viscosity (cp) of T1 and T2 were 33.29 cp and 29.64 cp respectively.

Status of phytic acid and tannin in the unmalted finger millet flour, malted finger millet flour and finger millet enriched probiotic fermented product is shown in Table 3. In comparison to the unmalted finger millet flour, malted finger millet flour has shown a reduction of 40.21 % and 18.21 % in the phytic acid and tannin content respectively. In the finger millet enriched probiotic fermented product, the reduction in the phytic acid and tannin content was 45.97 % and 81.35 % percent respectively in comparison to malted finger millet flour and the reduction was 67.69 % and 84.76 % respectively when compared to unmalted finger millet flour. In our product preparation, we have used malted finger millet flour. The processes such as soaking, germination and fermentation are reported to reduce the anti-nutritional factors like phytic acid and tannin in finger millet (Devi et al. 2014; Chaudhary and Vyas, 2014). It has been reported that tannin and phytic acid in finger millet might reduce the bio-availability of nutrients (iron, calcium, zinc, magnesium) and significantly influence the functional and nutritional properties of millet containing foods (McDonough et al. 2000). Tannin is considered undesirable because it precipitate protein, inhibit digestive enzymes and affect the utilization of vitamins and minerals. The dosage of tannin is critical to these effects (Chung et al. 1998). Phytic acid binds to minerals and makes them unavailable due to its chelating property. It has been reported that phytic acid inhibits absorption of iron, zinc, calcium, magnesium and manganese (Phillippy, 2006). Hence removal of phytic acid increases bioavailability of many cations and thus nutritional value of food. Mbithi-Mwikya et al. (2000) reported that sprouting of finger millet results in lowering of the

**Table 1** Sensory attributes of the fermented products evaluated using 9-point hedonic scale (1-9)

| Test Products | Flavor | Body & Texture | Acidity | Color & Appearance | Overall Acceptability |
|---|---|---|---|---|---|
| T1 | 8.19±0.12[a] | 8.42±0.24[a] | 8.45±0.25[a] | 8.52±0.16[a] | 8.43±0.16[a] |
| T2 | 8.10±0.29[a] | 7.78±0.24[b] | 7.45±0.42[b] | 7.97±0.09[b] | 7.64±0.24[b] |

The results are indicated as mean ± standard deviation (n = 7). T1= Product with finger millet, T2= product without finger millet. Different letters in the same column indicate significant differences (p < 0.05).

**Table 2** Physico-chemical parameters and microbial count of the fermented products

| Test products | Titratable acidity (% LA) | pH | Probiotic count (log cfu/g) | Streptococcal count(log cfu/g) | Viscosity (cp at 25ºC) |
|---|---|---|---|---|---|
| T1 | 0.73 ± 0.01[a] | 4.77± 0.09[a] | 10.07 ± 0.09[a] | 9.88 ± 0.07[a] | 33.29 ± 0.37[a] |
| T2 | 0.71 ± 0.01[a] | 4.78 ± 0.08[a] | 9.34 ± 0.07[b] | 9.64 ± 0.04[b] | 29.64 ± 0.21[b] |

The results are indicated as mean ± standard deviation (n = 7). T1= Product with finger millet, T2= product without finger millet. Different letters in the same column indicate significant differences (p < 0.05).

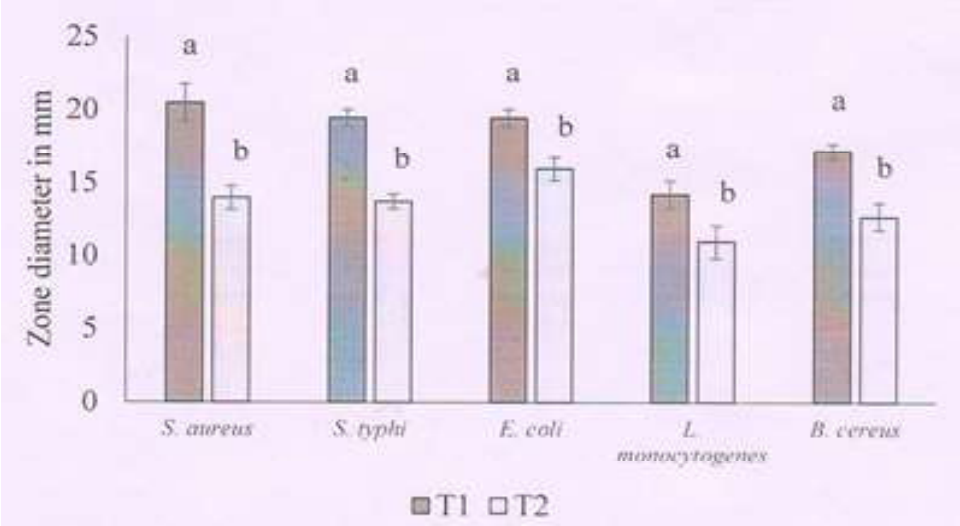


**Fig 1.** Antimicrobial activity of product with finger millet (T1) and without finger millet (T2) against test pathogens. Values are given as mean ± SD (n=4). Letters (a, b) indicate significant differences ($p < 0.05$).

**Table 3** Phytic acid and tannin content of finger millet flour and finger millet enriched fermented milk

| Samples | Anti-Nutritional Factors | |
|---|---|---|
| | Phytic acid (%) | Tannin (%) |
| Unmalted finger millet flour | 0.291±0.005[a] | 0.433±0.01[a] |
| Malted finger millet flour | 0.174±0.005[b] | 0.354±0.02[b] |
| Finger millet enriched probiotic fermented milk | 0.094±0.002[c] | 0.066±0.00[c] |

The results are indicated as mean ± standard deviation (n = 7). Different letters in the same column indicate significant differences ($p < 0.05$).

**Table 4** ACE Inhibitory, antioxidant and antidiabetic potential of fermented products

| Functional property | Finger millet enriched product (T1) | Product without finger millet (T2) |
|---|---|---|
| ACE Inhibitory Activity (ACEi %) | 51.54 ± 0.03[a] | 51.11 ± 0.04[a] |
| Antioxidant activity [ABTS·+ Scavenging Activity (%)] | 95.59 ± 0.50[a] | 52.37 ± 6.29[b] |
| α- Amylase inhibition (%) | 57.43 ± 5.02[a] | 55.46 ± 2.67[a] |
| α- Glucosidase inhibition (%) | 69.89 ± 3.12[a] | 51.89 ± 8.06[b] |

Values show mean and standard deviation (n=7). Different letters in the same row indicate significant difference ($p < 0.05$).

antinutritional factors like tannins and phytate. Geetha et al. (1997) found that malting reduced tannins and phytic phosphorus up to 54% and 58 % respectively in brown finger millet. Antony and Chandra (1998) reported that phenolics decrease by 26-29%, while tannins showed a more marked decrease of 44-52% by 48 h of fermentation. They attributed the reduction to the release of fiber bound tannins and polyphenol oxidase activity by fermenting microbes.

**Effect of incorporation of finger millet on antimicrobial activity**

The antimicrobial activity of finger millet enriched probiotic product and control is shown in Figure 1. The products showed promising antimicrobial activity against test pathogens. But the antimicrobial activity of finger millet enriched product (T1) was significantly ($p<0.05$) higher than that of control (T2) towards all the test pathogens. The zone of inhibition by T1 and T2 against *S. aureus* was 20.50 mm and 14 mm respectively; and it was 19.50 and 13.75 mm respectively against *S. typhi*. The inhibition zone against *E. coli* by T1 and T2 were 19.50mm and 16 mm respectively; and it was 14.25 mm and 11 mm against *L. monocytogenes* respectively. The inhibition zone against *B. cereus* by T1 and T2 were 17.25mm and 12.75 mm respectively. Our study results showed that incorporation of finger millet significantly improved the antimicrobial activity of the resultant product compared to control which may be due to the action of antimicrobial substances from both milk and millet during fermentation of milk-millet mixture. The antimicrobial activity of fermented milk, in general, is said to be due to the lactic acid bacterial metabolites such as organic acids like lactic acid, bacteriocins, diacetyl, carbondioxide, etc (Mudgal, 2015). The probiotic strain used in this study is reported to possess significant antimicrobial activity against *Bacillus*

*cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Typhi, and *Escherichia coli (*Prajapati et al. 2011). With respect to finger millet, its phenolic compounds, such as quercetin, gallic, caffeic, protocatechuic, para-hydroxy benzoic acid are reported to be responsible for its antimicrobial activity. (Chethan and Malleshi , 2007). Antimicrobial activity of germinated and ungerminated millet phenol extract against *Bacillus cereus, Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Listeria monocytogenes, Streptococcus pyogenes, Pseudomonas aeruginosa, and Serratia marcescens* and *Klebsiella pneumonia* was reported by Chethan and Malleshi (2007). Quercitin is found to inhibit the growth of all the pathogenic bacteria, whereas gallic, caffeic, protocatechuic, para-hydroxy benzoic acid showed their antimicrobial activity towards few bacterial strains (Chethan and Malleshi , 2007). Seed coat phenolic extract from finger millet have been reported to inhibit pathogens including *Bacillus cereus and Aspergillus niger* (Viswanath et al. 2009). Antony et al. (1998) reported that fermented finger millet extract suppressed the growth of *Salmonella* sp. and *Escherichia coli*.

**Effect of incorporation of finger millet on ACE Inhibitory, antioxidant and antidiabetic activity**

***Effect on ACE Inhibitory activity***

ACE Inhibitory activity, antioxidant activity and antidiabetic activity of the finger millet enriched product (T1) and control (T2) is shown in Table 4. Average values for ACE inhibitory activity of T1 and T2 were found to be 51.54 and 51.11 per cent respectively. The difference in the ACE inhibitory activity of the samples were statistically not significant. Our results were in accordance with the reported literature. Research reports say that bioactive amino acid sequence displaying antihypertensive activity is mainly isolated from bovine and human milk caseins. Sun et al. (2009) reported that milks fermented by several strains of *L. helveticus* showed antihypertensive activity. ACE inhibitory peptides such as Val-Pro-Pro and Ile-Pro-Pro were identified from such fermented milks made using *L. helveticus.* Hati et al. (2015) evaluated ACE-inhibitory activities of eight different cultures. They suggested that *Lb. helveticus* MTCC 5463 and *Lb. delbruckii* (009) had ACE inhibitory activities and produced highest proteolytic zone in skim milk agar plate under optimized growth conditions. Sathya et al. (2017) studied the ACE inhibitory activity of fermented milk samples prepared by using seven different lactic acid bacterial cultures such as *Lactobacillus helveticus*, *L. rhamnosus*, *L. delbruckii* subsp. *bulgaricus*, *L. plantarum*, *L. acidophilus*, *L. casei* and *L. paracasei* subsp. *paracasei*. They found that ACE inhibitory activity ranged from 65.12 to 90.70 per cent. In their review on the current knowledge on Lactic Acid Bacteria (LAB) as cell factories for the production of bioactive peptides from a variety of food protein sources, Brown et al. (2017) mentioned that, LAB containing cell envelope-associated proteinases are used as biocatalysts for the first step of casein breakdown releasing bioactive peptides during milk fermentation. Our study results showed that incorporation of finger millet do not have much contribution to the ACE inhibitory activity of the composite food. At the same time, the presence of finger millet did not interfere with the release of such peptides.

***Effect on antioxidant activity***

The products differed significantly ($p<0.05$) in their antioxidant activity. Average values for antioxidant activity of millet enriched product (T1) and control (T2) were found to be 95.59 and 52.37%, respectively. Higher antioxidant activity of T1 may be due to the polyphenols present in the finger millet which is said to possess free radical scavenging activity. Phenolic acids and their derivatives, flavonoids and tannins present in the finger millet seed coat are of multifunctional and can act as reducing agents, metal chelators and singlet oxygen quenchers (Sripriya et al. 1996; Devi et al. 2014). Finger millet arabinoxylans have been shown to exhibit high antioxidant activities as a result of their bound phenolic acids such as ferulic acids (Chandrasekara and Shahidi 2011; Lafiandra et al. 2014). Ferulic acid is reported to exhibits very strong antioxidant, free radical scavenging and anti-inflammatory activity (Devi et al. 2014).

***Effect on antidiabetic activity***

Incorporation of finger millet resulted in a significantly ($p<0.05$) higher α-glucosidase inhibition activity, whereas the α-amylase inhibition was not significant. The average values for α-amylase inhibition of T1 and T2 were found to be 57.43 and 55.46%, respectively. α-glucosidase inhibition of T1 and T2 were found to be 69.89 and 51.89%, respectively. Regular consumption of finger millet is known to reduce the risk of diabetes mellitus (Okoyomoh et al. 2013) and such property is attributed to its high polyphenols and dietary fiber contents (Shobana et al. 2013). Kavitha and Prema (1995) reported that the carbohydrates present in finger millet are slowly digested and assimilated than those present in other cereals. The beneficial effect of finger millet phenolics is due to partial inhibition of amylase and α-glucosidase during enzymatic hydrolysis of complex carbohydrates and delayed absorption of glucose, which ultimately controls the postprandial blood glucose levels (Shobana et al. 2009). Beneficial effect of dietary fiber is usually attributed either to slower gastric emptying or formation of unabsorbable complexes with available carbohydrates in the gut lumen and these two properties might result in delayed absorption of carbohydrates and in the reduction of absolute quantity absorbed (Kawai et al. 1987; Rasmussen et al. 1991). Dietary fibers are categorized as water soluble and water insoluble. Chethan and Malleshi (2007) reported 15.7% insoluble dietary fiber, 1.4% soluble dietary fiber in finger millet grain, while Shobana and Malleshi (2007) reported 22.0% total dietary fiber, 19.7% insoluble dietary fiber and 2.5% soluble dietary fiber in finger millet.

According to the current study results, incorporation of finger millet significantly increased the α-glucosidase inhibitory activity. Reports have shown that the natural α-amylase and α-glucosidase inhibitors from plants to have lower inhibitory effect against α-amylase activity and a stronger inhibitory activity against α-glucosidase. Phytate is known to have α-amylase inhibitory properties (Knuckles and Betschart, 1987) and a regulatory role in insulin secretion from pancreatic β-cells. Shobana et al. (2009) reported that finger millet phenolics are non-competitive inhibitors of intestinal α-glucosidase and pancreatic amylase. As these inhibitors are proven modulators of postprandial glycaemia, they play a significant role in the management of diabetic complications. To our knowledge, there are no reports available on the *in vitro* anti-diabetic activity of milk-millet composite probiotic fermented product.

## Conclusions

Enrichment of milk with finger millet and fermentation with starter culture enhanced the functional properties of resultant milk-millet composite probiotic fermented product. Incorporation of finger millet significantly improved the anti-microbial, antioxidant and antidiabetic potential of the composite product. However, incorporation of finger millet did not contribute to ACE inhibitory activity of the product. The process of malting and fermentation has significantly reduced the antinutritional factors phytic acid and tannin content in the product. The product was organoleptically acceptable with adequate probiotic count in that. The milk-millet composite probiotic fermented product has shown potential as a functional food.

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RESEARCH ARTICLE

# A comparative study of automated TEMPO® rapid method with IS/ISO method for enumeration of microorganisms in different dairy products

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**Abstract:** Recently numerous automated microbiological methods have been developed which aims to provide accurate, precise, and rapid test results, but their performance needs to be established before being used for sample analysis. The objective of the study is to evaluate the comparison between an automated rapid technique "TEMPO" and a conventional method for enumeration of microorganisms in the Skimmed Milk Powder, Milk, Infant Milk Food and Paneer. The samples were artificially contaminated with reference microbial cultures for the study. The study was done for Total Aerobic Count as per IS 5402, enumeration of Coliform as per IS 5401 (Part-1), Yeast and Mould count as per IS 5403, and enumeration of *Escherichia coli* as per IS 5887 (Part-3). All samples were analyzed by both TEMPO and IS methods. The Acceptability Limit (AL) is set at ± 0.5 log10 units as per ISO 16140-2: 2016. Acceptability Limit is expressed as a difference between the reference and the alternative method. Logarithmic differences between the results obtained from two methods are d" 0.5 log for Aerobic count, Yeast and Mould count, Total Coliform count and *Escherichia coli* count. Statistical analysis of the results have shown good rate of agreement for all artificially contaminated samples for the two methods, when counted for Aerobic count at 30°C for 40-48 hours, Coliform count at 30°C for 24-27 hours, Yeast and Mould count at 25°C for 72-76 hours and *Escherichia coli* count at 37°C for 22-27 hours.

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**Keywords:** Artificial contamination, Conventional methods, Rapid method and IS method, TEMPO®,

## Introduction

Foodborne diseases have become a major public health problem worldwide due to the significantly increased incidence of foodborne illness over the last 20 years (Oliver et al. 2005). Although it is difficult to estimate the global incidence of foodborne illness as some of the cases are under-reported especially in developing countries, the increased incidence of foodborne diseases was reported in many parts of the world (Van de Venter, 2000).

Consumption of contaminated food by microorganisms has not only caused numerous infections but also resulted in foodborne outbreaks. Prevention of these foodborne diseases is a major concern in the food industry.

The presence of microorganisms in milk and dairy products is a major concern for safety, quality, regulations, and public health. Recent outbreaks of foodborne illness, implicating milk and dairy products contaminated with the microorganism like Coliform, *Escherichia coli,* Yeast and Mould, *Listeria spp*., and Salmonella have underscored the need for rapid methods for microbiological analysis of dairy products. Microbiological analysis is a critical assessment for safety and quality conformation with standards or specifications, and regulatory compliances as well.

To control the microbial contamination of food and consequently to reduce foodborne illnesses, rapid and accurate microbiological detection methods are required for effective monitoring of microbial contamination in food supplies in a short time. Conventional methods for the enumeration of quality indicators such as Aerobic count, Yeast and Mould count, Coliform and *Escherichia coli* in foods are laborious and require more material, manpower and time. Besides, quality assurance in the food industry requires rapid test methods that allow a fast reaction to mitigate risks (Kawasaki et al. 2003).

For these reasons, several alternative rapid methods have been developed recently for the enumeration of quality indicators in

foods. These methods are generally based on the utilization of chromogenic or fluorogenic substrates for the detection of specific enzyme activities (Manafi et al. 1991). Rapid methods are more time- efficient, labor-saving and are able to reduce human errors (Mandal et al. 2011).

Traditionally, these organisms have been enumerated using a pour plate or surface spread technique on selective agar and confirmed result is usually obtained in 8-10 days. To meet the need for rapid microbial analysis, different methods have been developed to reduce the time required for the detection of microorganisms. This study undertakes a comparison of the rapid technique versus conventional methods for various food matrices for four different parameters to ensure the effectiveness of rapid methods against conventional.

TEMPO was used as a rapid technique for microbial analysis of Total Aerobic Count, *Escherichia coli*, Yeast & Mould and Coliform parameters and its performance was checked by comparing its results with the results obtained from conventional methods based on IS/ISO methods. This study was conducted using 4 different food products, which was artificially contaminated for better and controlled evaluation of TEMPO. In this paper, we propose a fully automatic rapid technique TEMPO and compared its performance with conventional methods.

## Materials and Methods

### Samples

In this study, 4 samples i.e., Milk, Infant Milk Food, Paneer & Skimmed Milk Powder (SMP) were used. All samples were collected from the local market of Anand, Gujarat, India. These samples were analyzed by both

TEMPO and IS methods respectively.

### Bacterial strains and growth conditions

*Escherichia coli* (MTCC-1687) was used to contaminate the samples artificially for the verification test of Total Aerobic Count, *Escherichia coli* and Total Coliform count and *Candida albicans* (NCPF 3179) was used to artificially contaminate the samples for verification test of Yeast and Mould count.

*Escherichia coli* working culture was prepared in Nutrient broth, incubated at 37°C for 24 hours and *Candida albicans* working culture was prepared in Sabouraud Dextrose Broth, incubated at 25°C for 72 hours. Serial tenfold dilutions of each test organism were prepared; 1ml from the $10^{-4}$ dilution from each culture tube was transferred to 90 ml diluent containing 10 gm sample.

### Sample preparation

10 gm or ml of sample was homogenized with 90 ml of sterile peptone salt solution in a sterile bag with a lateral filter using stomacher and further analyzed by TEMPO and IS methods respectively.

### Conventional Method (IS/ISO method)

#### *Total aerobic count*

Total aerobic count was performed as per IS 5402: 2012. Plate Count Agar (PCA) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plate in duplicates and PCA was poured. After solidification, plates were incubated at 30 ± 1°C for 72 hours. After completion of the incubation period, colonies were counted.

#### *Coliform count*

Coliform count was performed as per IS 5401 (Part-1):2012. Violet Red Bile Agar (VRBA) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plate in duplicates and VRBA was poured. After solidification of media, overlay was done with VRBA. Plates were incubated at 37 ± 1°C for 24 hours. After completion of the incubation period, colonies were counted.

#### *Yeast and mould count*

Yeast and Mould count was performed as per IS 5403: 1999. CYGA (Chloramphenicol Yeast Glucose Agar) was previously cooled at 44°C - 46°C. 1.0 ml aliquots of the dilutions were transferred to petri plates in duplicate and CYGA was poured. Plates incubated at 25 ± 1°C for 5 days. After completion of the incubation period, colonies were counted.

#### *Escherichia coli count*

*Escherichia coli* was performed as per IS 5887 (Part-3): 1976. 1.0 ml of aliquot was spread over the three Tergitol-7 agar plates. The plates were incubated at 37°C for 24 hours. After completion of the incubation period, yellow colonies were counted.

### TEMPO method

The TEMPO test kit consists of a vial of a specific culture medium and a card, which are specific to a particular test. The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred by the TEMPO Filler instrument into the card containing 48 wells of three different volumes. The card contains 3 sets of 16 wells (small, medium and large wells) with a one log difference in volume for each set of wells. The card is designed to simulate the Most Probable Number (MPN) method (Cochran et al. 1950). The card is then hermetically sealed to avoid any risk of contamination during subsequent handling. The specific organism present in the card reduces the substrate in the culture medium during incubation and causes a fluorescent

signal to appear, which is detected by the TEMPO Reader instrument. Depending on the number and type of the positive wells, the TEMPO system calculates the number of microorganisms present in the original sample according to a calculation based on the MPN method.

***Primary diluents use for sample preparation***

Peptone Saline Diluent (90 ml) and di-potassium hydrogen phosphate (for dried milk products).

***The secondary diluent used for Aerobic Count (AC), Total Coliform (TC) and Yeast and Mould (YM)*** Sterile distilled water.

The secondary diluent used for *Escherichia coli* is sterile distilled water. The Mandatory diluent used for milk powder is MOPS buffer (0.4M). MOPS buffer was prepared by dissolving 13.60 gm MOPS acid and 31.21 gm MOPS sodium salt in 500 ml distilled water. Sterilized by autoclaving.

***Total aerobic count***

TEMPO AC medium vial reconstituted by dispensing 3.9 ml of secondary diluent per vial using the sterile pipette. 0.1 ml of prepared sample in primary diluent added into the reconstituted 3.9 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at 30 ± 1°C for 40-48 hours.It is recommended to incubate card at 30 ± 1°C upto 48 hours for pasteurized milk. (TEMPO Aerobic Count package insert 9301732B-en-2013/01).

***Total coliform count***

TEMPO TC medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using a sterile pipette. 0.1 ml of prepared sample in primary diluent added into the reconstituted 3.0 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at 30 ± 1°C for 24-27 hours. (TEMPO Total Coliform package insert 12599H-en-2014/03)

***Yeast and mould count***

TEMPO YM culture medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using a sterile pipette. 1.0 ml of prepared sample in primary diluent added into the reconstituted 3.0 ml vial. Then Card was filled by the TEMPO filler system automatically. Filled card incubated at 25 ± 1°C for 72-76 hours. (TEMPO Yeast and Mould package insert 12594E-en-2010/12)

***Escherichia coli count***

TEMPO EC culture medium vial reconstituted by dispensing 3.0 ml of secondary diluent per vial using the sterile pipette. 1.0 ml of prepared sample in primary diluent added into the reconstituted 3.0 ml vial. Then card was filled by the TEMPO filler system automatically. Filled card incubated at 35 ± 1°C for 22-27 hours. (TEMPO *E. coli* package insert 12587F-en-2007/06). After completion of incubation time, the instrument read cards, calculates and gives the result in CFU/gm.

**Statistical analysis**

All samples were analyzed in three replicates using both the methods for all parameters. For the statistical analysis, decimal logarithms of the results were used. The statistical equivalence of the two method groups converted in log10 values and evaluated by their difference in log values.

## Results and Discussion

The variation in data obtained by using the TEMPO technique and IS methods for all 4 parameters (Total Aerobic Count, Total Coliform Count, Yeast and Mould Count and *Escherichia coli* Count) in triplicates are shown in tables.

The log difference calculated in table 1 for Total Aerobic Count is - 0.20 in SMP, 0.11 in Milk, 0.03 in Infant Milk Food and - 0.26 in Paneer. The log difference calculated in table 2 for the enumeration of *Escherichia coli* is - 0.05 in SMP, - 0.02 in Milk, and 0.00 in Infant Milk Food and - 0.07 in Paneer. The log difference calculated in table 3 for Total Coliform Count is - 0.14 in SMP, - 0.05 in Milk, - 0.10 in Infant Milk Food and -

0.17 in Paneer. The log difference calculated in table 4 for Yeast and Mould Count is 0.13 in SMP, - 0.11 in Milk, 0.09 in Infant Milk Food and 0.02 in Paneer. The log difference of results between the IS method and the TEMPO method are not exceeded 0.5 logarithmic units in any case. Hence, the above data indicate that the results correlated with the two levels of artificial

**Table 1** Represents the enumeration results of Total Plate Count obtained by IS 5402:2012 and TEMPO

| Product | Mean Counts of IS/ISO Method, log cfu/g | Mean Counts of TEMPO Method, log cfu/g | log difference |
|---|---|---|---|
| SMP | 4.37 | 4.57 | -0.20 |
| Milk | 4.22 | 4.11 | 0.11 |
| Infant Food | 4.34 | 4.30 | 0.03 |
| Paneer | 4.34 | 4.59 | -0.26 |

**Table 2** Represents the enumeration results of *Escherichia coli* obtained by IS 5887 (Part-3): 1976 and TEMPO

| Product | Mean Counts of IS Method, log cfu/g | Mean Counts of TEMPO Method, log cfu/g | log difference |
|---|---|---|---|
| SMP | 4.37 | 4.42 | -0.05 |
| Milk | 3.89 | 3.91 | -0.02 |
| Infant Food | 2.43 | 2.43 | 0.00 |
| Paneer | 4.57 | 4.64 | -0.07 |

**Table 3** Represents the enumeration results of Total Coliform obtained by IS 5401 (Part-1):2012 and TEMPO

| Product | Mean Counts of IS/ISO method, log cfu/g | Mean Counts of TEMPO Method, log cfu/g | log difference |
|---|---|---|---|
| SMP | 4.25 | 4.39 | -0.14 |
| Milk | 3.93 | 3.97 | -0.05 |
| Infant Food | 4.01 | 4.10 | -0.10 |
| Paneer | 4.14 | 4.30 | -0.17 |

**Table 4** Represents the enumeration results of Yeast & Mould count obtained by IS 5402:1999 and TEMPO

| Product | Mean Counts of IS method, log cfu/g | Mean Counts of TEMPO Method, log cfu/g | log difference |
|---|---|---|---|
| SMP | 3.01 | 2.88 | 0.13 |
| Milk | 2.87 | 2.97 | -0.11 |
| Infant Food | 3.16 | 3.07 | 0.09 |
| Paneer | 4.43 | 4.41 | 0.02 |

contamination, with no significant difference between the counts estimated by the two methods.

To ensure the quality check of food, we require rapid techniques that can help to achieve quick and reliable results. Recently, different rapid methods for microbiological analysis with high sensitivity and specificity have been developed to overcome the limitations of conventional methods. We have studied 4 different microbiological parameters of various food products using the TEMPO method. To evaluate the efficiency of TEMPO results, we analyzed all the 4 parameters of 4 different food products in IS methods (conventional methods) as well; 4 readings in triplicates are taken for each food product for individual parameters. The results obtained from both the methods of TEMPO and IS are compared statically and are highly correlated with less significant variation. Log difference for the results of TEMPO and IS methods were not exceeding 0.5 logarithmic units for all the tested parameters, which confirms the excellent performance of TEMPO for artificially contaminated samples. The results are comparable and within acceptable criteria, as can be seen from the above-mentioned data. Thus, from the study, we can conclude that the TEMPO method can help to achieve the results more rapidly and precisely, but it is not advisable to use rapid techniques without its verification. Sometimes it is found that the results may vary in the TEMPO method due to food matrixes effect, sample homogeneity, sample weighing error, pipetting error, etc. It is better to verify the rapid technique first for various parameters in different food products and assure confidence for the rapid techniques, than it may be used for daily analysis and can be replaced by conventional methods. Thus, the TEMPO system eliminates the disadvantages of the conventional methodology while at the same time allowing precise results. The number of miniaturized tubes in the TEMPO card increases the enumeration range and the precision of results compared to a traditional methodology. The TEMPO system can give results over quite a wide enumeration range. The advantages of the MPN methodology and the chromogenic substrate are combined in the TEMPO method.

## Conclusions

The comparison of the TEMPO technique for Total Aerobic Count, Total Coliform Count, Yeast & Mould Count, and *Escherichia coli* Count was equivalent to the corresponding IS/ISO methods with a very good rate of agreement, manufacturer instructions and GLP are strictly followed. This system offers improved standardization as well as economic savings in terms of manpower and time by eliminating serial dilutions, media preparation, tedious plate reading, calculations, confirmation tests, etc. Many samples can be analyzed quickly and precisely, involving minimum manpower and man-day's; thereby reducing the turnaround time (TAT). Hence, from the study, it can be concluded that the TEMPO method is a suitable and reliable alternative method for microbiological testing of various food products provided its performance is verified for respective food matrixes.

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RESEARCH ARTICLE

# Isolation and characterization of oleaginous yeasts from dairy waste

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**Abstract:** Microbial lipids (biodiesel) can be produced by oleaginous yeasts via converting carbohydrates into lipids under certain conditions. Sudan black 'B' staining technique was used to screen cellular lipid accumulation. Extraction of the bacterial lipids was carried out by Bligh and Dyer method and fatty acid methyl esters were analysed by GC. Primary screening of sample resulted in 22 isolates, out of which 6 isolates were found to generate lipid. After detection of good oleaginous lipid productivity, one best yielding isolate was preferred for upward spring. Secondary selection of potential isolate was based on the production in addition to parameters like pH and nutritional requirements. Lipid from selected isolate CNK 1 was subjected to determination of saponification value and PUFA screening. The isolate CNK1 was identified as *Zygrosaccharomyces rouxii* by FAME analysis. The above results were promising hence it has importance for supplementary development of the yeast isolate *Zygrosaccharomyces rouxii* as another source of lipid for biodiesel production.

**Keywords:** FAME-GC, PUFA, Oleaginous Yeast; Sudan Black B, *Zygrosaccharomyces rouxii,*

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## Introduction

Lipid is the brief and storage form of energy require for metabolism. However, it is not only the energy contributor for an organism but also the imperative building block. Phospholipids are one of the most important compounds of biological membrane. Under certain situation, some microorganisms convert carbohydrate, hydrocarbon and normal lipid into lipids within the cells. Prior studies suggested that oleaginous microorganisms are mainly bacteria, yeast, filamentous fungi and microalgae. It was also reported that the lipid content in microalgae, yeasts and filamentous fungi was higher (70%-90%) than that in bacteria (20%-50%) (Yi Cao et al. 2012). Lipid accumulation in oleaginous yeasts and molds has been demonstrated to occur when a nutrient in the medium (i.e. the nitrogen or the phosphorus source) becomes limited and the carbon source is present in excess. Nitrogen limitation is the most efficient condition for inducing lipogenesis. In oleaginous species, the excess carbon preferentially channelled toward lipid synthesis, leading to the accumulation of TAG within intracellular lipid bodies. The ability to accumulate high amounts of lipid depends mostly on the regulation of the biosynthetic pathway and the supply of the precursors (i.e. acetyl-CoA, malonyl-CoA, and glycerol-3-phosphate) and the cofactor NADPH (Ratledge and Wynn 2002). The most deeply investigated oleaginous yeasts belong to the genera *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporodium*, *Cryptococcus*, and *Lypomyces*. Exploitation of oleaginous filamentous fungi for biodiesel production has a more recent history with few exceptions, derives from studies focused to poly-unsaturated fatty acid production (PUFA), such as arachidonic acid and linolenic acid (Rossi et al. 2011). Among heterotrophic microorganisms, oleaginous fungi, including both molds and yeasts, are increasingly been reported as good triglycerides (TAG) producers (Certik et al. 1999). The impact of drastic increase in energy consumption, concerns about green house gas (GHG) emissions, the rising price of fossil fuels and the projected decrease and insecurity of their existence in the fossil fuel reserves, have led to high attention microbial lipid production for bio-diesel production, so present work is undertaken.

## Materials and Methods

### Soil sample collection

Soil sample were collected from district milk collection centre, Nanded (industry waste area).

### Enrichment of microorganism

1gm soil sample was added in to 250 ml flask containing 100 ml sterile enrichment medium (composition (g/L): Glucose-100, Yeast extract-1, $NH_4Cl$-1, $KH_2PO_4$-2, $MgSO_4$-0.75, $CaCl_2$-0.05, $ZnSO_4$-0.01, $FeCl_3$-0.01 and $Na_2HPO_4$-1). This mixture was cultured at 28 C, 120 rpm for 48 hrs. Serially diluted soil samples were spread on sterile enrichment medium plate. Then plates were kept in incubator at 28 C for 1-4 days. After incubation each separate colonies were picked and stored on same medium agar slant for further study. Screening was done by lipid staining (Burdan K 1946).

### PUFA producers screening / $H_2O_2$ plate assay method

PUFA producers screening was done as per the method mentioned by (Ashwini et al. 2012). YPD medium containing (1mM) $NaN_3$ were prepared and sterilized and isolated strains were spread on plates. After spreading sterile filter paper disc (5mm) were placed on medium. 10 µl of $H_2O_2$ solution having different concentration (0.5% and 1%) were added on separate filter paper disc on the plate. Plates were incubated at 28º C for 24- 48 hrs.

### FAME analysis

The fatty acids were extracted by a procedure which consists of saponification in sodium hydroxide/methanol solution followed by derivatization with hydrochloric acid/methanol solution to give the respective fatty acid methyl esters (FAMEs). The FAMEs are then extracted from the aqueous phase by the use of an organic solvent and the resulting extract was analysed by GC. FAMEs are more volatile than their respective fatty acids and therefore more suitable to GC analysis. The Sherlock software automates all analytical operations and uses a sophisticated pattern recognition algorithm to match the unknown FAME profile to the stored library entries for identification (Anju et al. 2011).

### Production and extraction of lipid

100 ml fermentation medium were prepared and sterilized at 121 C for 15 mins and cultures of positive screened organism were inoculated into fermentation medium. Flasks were incubated at 28 C, 120 rpm for 6 days. These set were used to determine biomass, dry weight and lipid content. Extraction of lipid was done by (Bligh and dyer 1959) method. 100 ml cultured medium were centrifuged at 5000g for 10 mins. Supernatant was discarded, pellet was washed with sterile distilled water, dried and weight was taken. 15 ml of 4M HCl was added and kept at room temperature for 30 mins. This mixture was kept in freezer (-20 C) for 20 minutes and immediately transferred to boiling water bath for 10 min. 30 ml chloroform : methanol mixture was added and centrifuged at 5000 g for 10 min. Lower layer chloroform contained lipid were collected and used for further study.

### Estimation of lipid

Estimation of total lipid was carried out by (Barnes and blackstock 1973) method.

### Production media optimization study

#### *Effect of pH on biomass lipid production*

100ml fermentation medium of different pH (4.5, 5.5, 6.5, 7.5 & 8.5) were prepared. Isolated strains were inoculated into each different pH medium and incubated at 28 C, 120 rpm for 6 days for lipid production. After incubation the biomass and lipid content were checked in each pH medium. The lipid content was determined by lipid estimation. The optimum pH medium suitable for isolated strain was determined.

#### *Effect of carbon source on biomass and lipid production*

100ml of fermentation medium of different carbon source (glucose, sucrose, lactose, maltose, and fructose) were prepared. Isolated strains were inoculated into each different carbon source medium and incubated at 28 C, 120 rpm for 6 days for lipid production. Biomass and lipid content were checked in each carbon source medium. The high lipid content was determined by lipid estimation. The optimum carbon source medium suitable for isolated strain was determined.

#### *Effect of nitrogen source on biomass and lipid production*

100ml fermentation medium of different nitrogen source (organic nitrogen source: yeast extract, peptone and inorganic nitrogen source: $NH_4Cl$, $KNO_3$, $(NH_4)_2SO_4$, $NH_4NO_3$, $NaNO_3$). Isolated strains were inoculated into each different nitrogen source medium and incubated at 28 C, 120 rpm for 6 days for lipid production. After incubation the biomass, lipid content was checked in each nitrogen source medium. The high lipid content was determined by lipid estimation and the optimum nitrogen source medium suitable for isolated strain was determined.

### Saponification value

Saponification value was determined by the methods of Association of Official Analytical Chemists (1990). 4-5 gm lipid sample was added in to flask and 50 ml alcoholic KOH was added. Blank was prepared by taking only 50 ml alcoholic KOH. Air condenser was connected to the flask and reaction mixture was boiled for 60 min. 1ml phenolphthalein indicator was added and

reaction mixture was titrated against 0.1 N HCl up to pink colour disappears.

**Statistical analysis**

All the incubations were performed at triplicates and the data was analysed using MS-Excel and the result were expressed as the mean ± SD.

## Results and Discussion

Soil sample were collected from district milk collection centre, Nanded (industry waste area) and successfully used for isolation of lipid producers. Total 43 isolates were obtained on nitrogen limited medium. Out of these isolates, 22 isolates were recovered and used for screening of lipid producers. Twenty two isolates preliminary screened trough Sudan black B staining method and six isolates showed lipid producers (Figure 1). Extraction of lipid was done by using advanced Bligh and dyer method. Amount of lipid produced in test sample was calculated by interpolating optical density of test sample on standard lipid curve and the estimated concentration of lipid was found to be 0.098gm/100ml. The Microbial Identification System (MIDI) for fatty acid methyl ester (FAME) analysis is a standard method for identification of microorganisms (Schutter et al. 2000). Whole cell fatty acids are converted to methyl esters and analysed by gas chromatography. The fatty acid composition of the unknown is compared to a library of known organisms in order to find the closest match. The list of the fatty acids composition like straight Chain fatty acids 21.84%, branched chain fatty acid 25.50%, Mono Unsaturated Fatty Acid 12.68% and oleic acid (C18:1w9c) 38.36 % was given clearly according to the GC report. The FAME GC analysis data obtained (Table 1) is more descriptive and elaborative. Our experimental data matches and establish the similar result mentioned in the report of MIDI Sherlock software databases and the similarity was matched with organism *Zygosaccharomyces rouxii* with selected ion monitoring (SIM) index 0.49 (Figure 2). Based on morphological characteristics and FAME-GC analysis it was identified as *Zygosaccharomyces rouxii*. Ravikumar et al. (2012) worked on biodiesel production from oleaginous fungi which involves the mixture of fatty acyl methyl/ethyl esters, produced from transesterification of neutral lipids. In similar way, Mrinal et al. (2011) has studied the comparative lipid profiling of endophytic fungi by FAME analysis techniques. Gao et al. (2010) also studied the screening, fermentation and optimisation of microbial lipid producing molds.

**Production media optimization study**

***Effect pH on biomass and lipid production***

Effect of pH on biomass and lipid production were studied, the strains was incubated at varying pH (4.5 to 8.5). Outcomes of the stated reports (Figure 3) shows increase in the biomass as well as lipid yield with respect to the increase in $p^H$ value but the relevant increase was maximum at 7.5 and shows minimum at 8.5 $p^H$ respectively.

***Effect of carbon source on biomass and lipid production***

Effect of carbon source on biomass and lipid production was studied; different carbon sources were used in the production medium (Figure 4). The result showed lactose, maltose, glucose, sucrose and fructose were suitable carbon source for the growth and lipid accumulation of the isolated strain among which glucose is the most suitable. The lipid production was 0.092 g/100ml.

***Effect of Nitrogen source on biomass and lipid production-***

The effect of nitrogen source on biomass and lipid production was shown in (Figure 5). Organic nitrogen source (yeast extract and peptone) whereas inorganic ($KNO_3$,( $NH_4)_2SO_2$ , $NH_4NO_3$, $NH_4Cl$ and $NaNO_3$) were used. In the organic nitrogen source yeast extract is the suitable and lipid production was 0.098 g/100 ml. Similarly, $NH_4Cl$ is the most suitable inorganic nitrogen source; lipid production was found to be 0.098 g/100ml.

**PUFA screening**

As shown in (Figure 6), yeast cells were grown in presence of $H_2O_2$ to check membrane shielding effect of PUFA. PUFAs are the most vulnerable to oxygen and ROS. To confirm this, growth of yeasts in presence of $H_2O_2$ was in reality mainly due to presence of PUFA; $NaN_3$ was added into the media which is a very powerful inhibitor of catalase. If microorganism is producing catalase enzyme, $NaN_3$ inhibits catalase enzyme. Out of selected 6 strains, strain of *Zygosaccharomyces rouxii* has given false positive results at all $H_2O_2$ concentrations used during plate assay method.

**Table 1** FAME GC analysis

| RT | Response | Ar/Ht | RFact | ECL | Peak Name | Percent (%) |
|---|---|---|---|---|---|---|
| 1.000 | 24951 | 0.100 | 1.000 | 1.000 | Straight | 21.84 |
| 2.000 | 3865 | 0.100 | 1.000 | 2.000 | Branched | 25.50 |
| 3.000 | 5564 | 0.100 | 1.000 | 3.000 | Hydroxy | 1.62 |
| 5.000 | 2485 | 0.100 | 1.000 | 5.000 | MUFA | 12.68 |
| 9.000 | 168 | 0.100 | 1.000 | 9.000 | 18:1w9c | 38.36 |
| 12.000 | 353 | 0.100 | ———- | 12.000 | Other | ———- |

**Fig. 1** Sudan black B staining

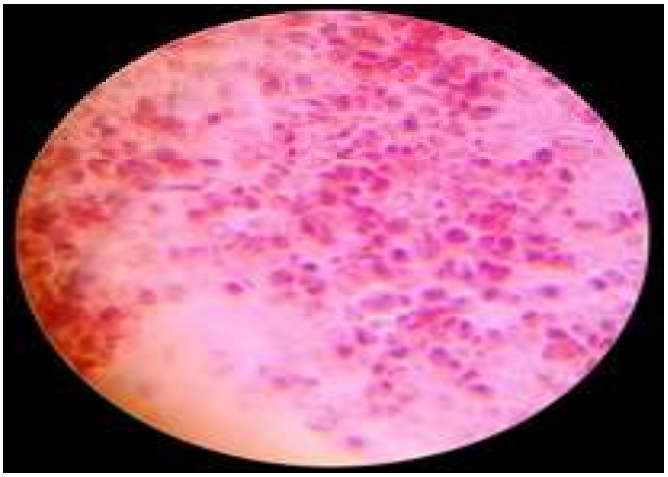
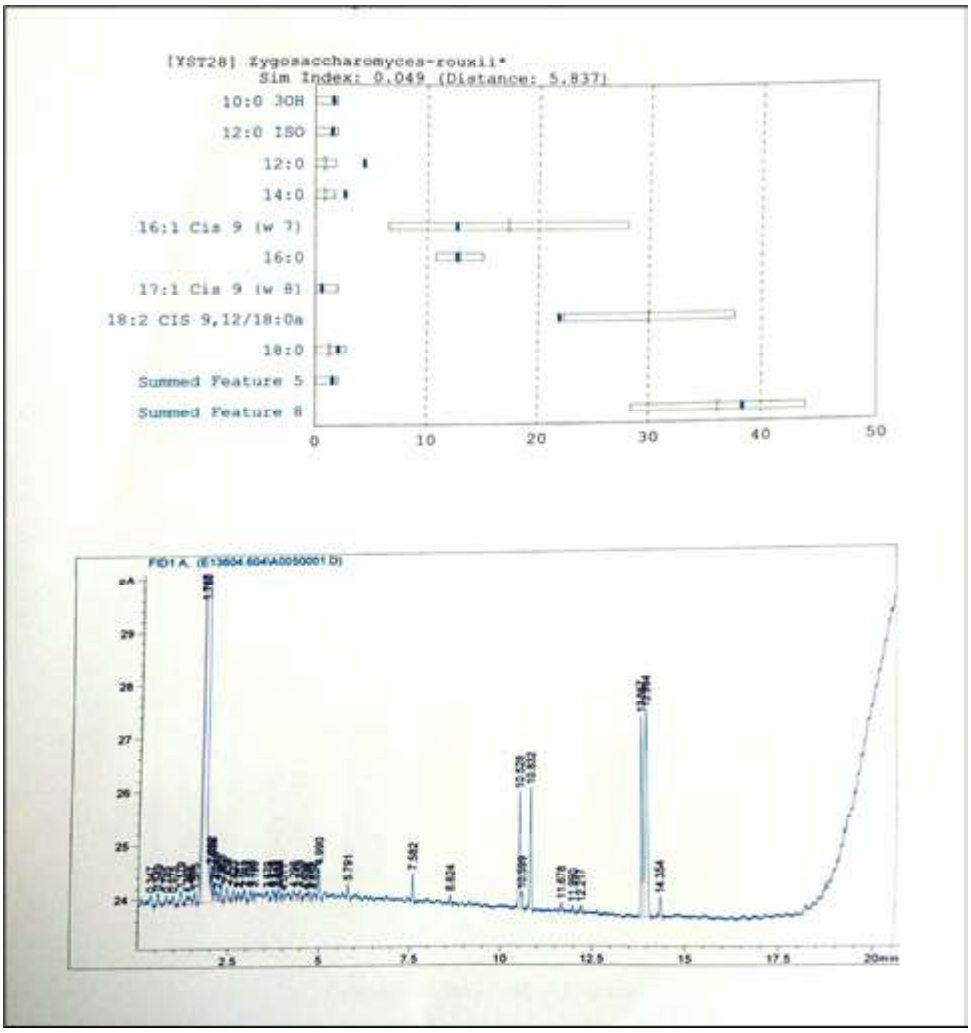


**Fig. 2** FAME-GC Analysis




**Saponification value**

Saponification value for *Zygrosaccharomyces rouxii* has calculated 1122. Saponification value is one of the chemical properties of biodiesel which contribute to fatty acid profile. Saponification value indicates amount of triacylglycerol present in total lipid. Since, the proportion of neutral lipid is the major component in total lipid content of *Zygrosaccharomyces rouxii* capable of yielding high amount of lipid biomass. The basic mechanism of lipid accumulation in microorganisms has been well studied. It was observed that when the culture medium lacks the nitrogen source, the isocitric dehydrogenase (ICDH) get

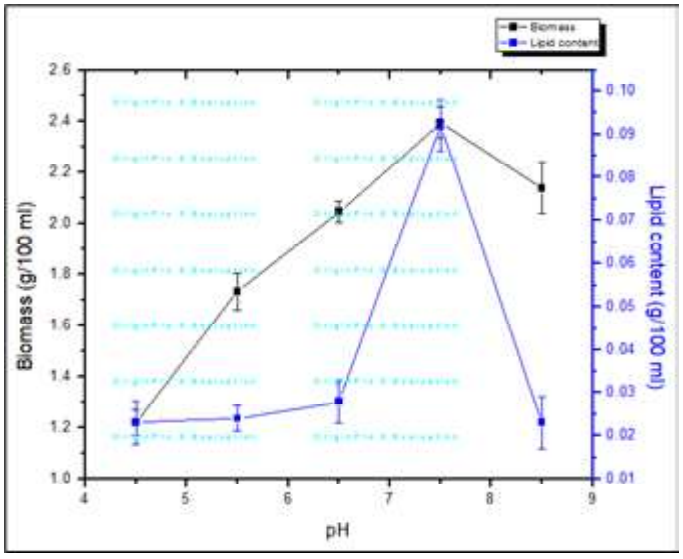



**Fig. 3** Effect of pH on biomass and lipid production

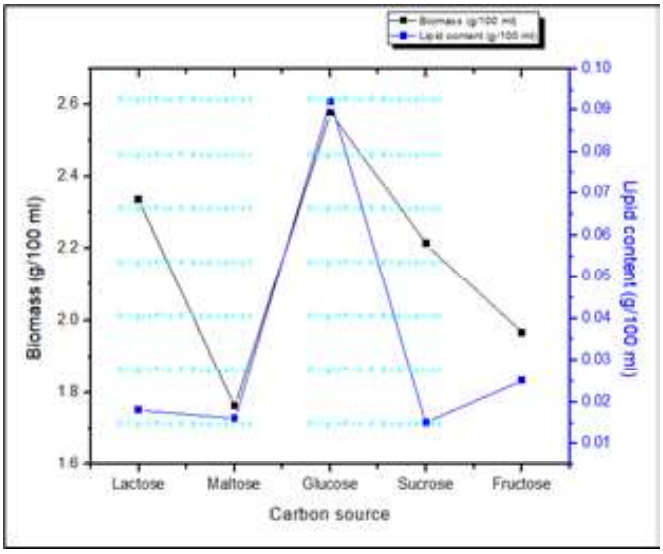



**Fig. 4** Effect of carbon source on biomass and lipid production

**Fig. 5** Effect of Nitrogen source on biomass and lipid production

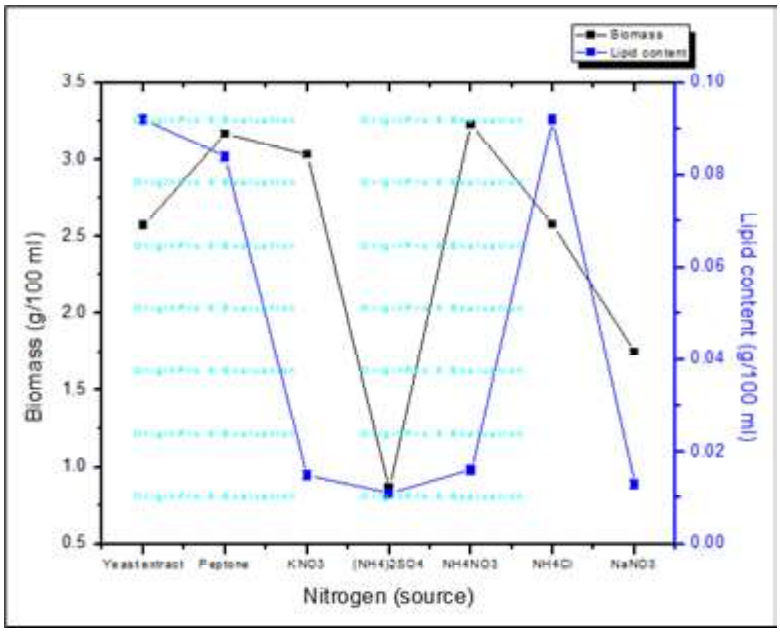



**Fig. 6** $H_2O_2$ Plate assay method

suppressed, therefore the tricarboxylic acid (TCA) was blocked. Extra carbon source was transformed to triglyceride (TAG) by a series of enzymes like the citric acid lytic enzyme, the malic acid enzyme, the fatty acid synzyme, thus completed the fat accumulation. When the culture medium contains sugar but low nitrogen, lipid accumulates in order to isolate oleaginous

microorganism, Sudan Black B staining is usually used to determine lipid content. However, this method roughly indicates the presence of microbial lipids.

## Conclusions

A methodical study to search the soil sources for eukaryotes, producing lipid was performed. The study involved isolation and screening of PUFA (poly unsaturated fatty acid) producing oleaginous yeast, identification of the yeast with fatty acid profile by FAME-GC, physico-chemical parameters, optimization and saponification value from dairy waste soil sample. Primary screening of sample resulted in 22 isolates. Among them 6 isolates were found to generate lipid. Based on lipid productivity, the potent isolate CNK1 was selected and identified as *Zygrosaccharomyces rouxii* by FAME analysis. The lipid yield of *Zygrosaccharomyces rouxii* was found 0.098gm/100ml in optimized media and at optimized condition. *Zygrosaccharomyces rouxii* has shown saponification value 1122. In addition to that *Zygrosaccharomyces rouxii* is good PUFA producing and with high percent oleic acid side chain which are the best characteristics of oleaginous yeast used for biodiesel production. The above results are promising but still there is a need for protein sequencing, gene identification and other bioinformatics parameters study for further establishment of value of the yeast isolate *Zygrosaccharomyces rouxii*.

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RESEARCH ARTICLE

# Oxidative stress molecules as indicators of uterine health in Murrah buffaloes during peripartum period

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**Abstract:** The present study was carried out to know the changes in concentration of oxidative stress molecules in peripheral blood during peripartum period in relation to uterine health in Murrah buffaloes(n=24). Blood samples were collected from day -7 to day +35 of calving and serum was used for analysis. Based on assessment of uterine fluid scoring, trans-rectal USG, clinical signs uterine health of buffaloes were determined and they were classified into healthy and uterine infected groups. Results indicated that nitric oxide (NO) concentration was significantly (P<0.05) higher in serum during peripartum period i.e. on day -7 to +35, whereas malondialdehyde (MDA) concentration in serum was elevated throughout peripartum period in uterine infected Murrah buffaloes. Total antioxidant capacity(TAC) was significantly higher (P<0.05) on day -7, 0, +7, +14, +21, +35 postpartum in healthy buffaloes as compared to uterine infected buffaloes. From this study it can be concluded that in Murrah buffaloes NO and TAC in serum may be used as promising indicators for predicting uterine health before onset of clinical infections.

**Keywords:** Nitric oxide (NO), Malondialdehyde (MDA), Total antioxidant capacity (TAC)

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## Introduction

India is considered as the dairy hub of the world as it holds the largest stocks of cattle and buffaloes. But the poor production performance is associated with compromised reproductive efficiency such as peripartum uterine infection, infertility, subfertility, anestrus, delayed onset of puberty, mastitis, metritis, endometritis, [1]; whereas the incidence of metritis, endometritis and subclinical endometritis has been reported to be about 20, 20 and 30%, respectively (Markusfeld, 1987; LeBlanc et al. 2002; Kasimanickam et al. 2014; Goshen and Shpigel, 2006;Hammon et al. 2006). Presence of high progesterone (Tizard, 1991) during gestation period and high cortisol around parturition (Magnusson and Fossum, 1992) results in immune suppression and animals become susceptible to various infectious diseases during this stage (Castillo et al. 2005). Further it has been reported that during peripartum period there is enhanced production of reactive oxygen and nitrogen species (ROS and RNS)(Rizzo et al. 2012)which cause damage of macromolecules such as proteins, lipids and DNA (Trevisan et al. 2001) and it is controlled by cellular antioxidant defence systems. Oxidative stress results when ROS are produced faster than their neutralization by antioxidant mechanisms (Trevisan et al. 2001). There are reports that during the peripartum period oxidative stress is a major threat and the incidence of health problems is clearly a huge complicating factor for subsequent reproductive performance . As a part of immune response phagocytes produce Nitric oxide (NO) during inflammatory diseases. Productions of NO occurs as a result of inflammation and is produced by a wide range of cells including macrophages, neutrophils, epithelial and endothelial cells and have an important role in immunity and inflammation (Frean et al. 1997; Korhonen et al. 2005). A well-established mechanism of oxidative damage caused by reactive oxygen species is Lipid per-oxidation and estimation of the melondialdehyde (MDA) provides a convenient index of lipid peroxidation(Nielsen et al. 1997). When MDA reacts with thiobarbituric acid formation of a red pigment occurs in the form of thiobarbituric acid reactive substance (TBARS) which is measured by spectrophotometry (Janero, 1990). By measuring the total antioxidant capacity (TAC) of body the plasma antioxidant status can be measured. It is the outcome of the interaction of many different compounds and systemic metabolic

interactions (Ghiselli et al. 2001). Early prediction of diseases before occurrence of clinical signs will be helpful in improving management strategies and productivity of Murrah buffaloes. Keeping this in view the present study was undertaken to examine the changes in concentration of oxidative stress molecules NO, MDA and TAC in peripheral blood of Murrah buffaloes during peripartum period.

## Materials and Methods

### Location and climatic conditions

The present research was conducted at Livestock Research Centre (LRC), National Dairy Research Institute (NDRI), Karnal, Haryana, India. The experimental duration was mid September to end of May, 2018. Climatic condition of the place touches both the extremes i.e. cold (approximately 3°C) and hot (approximately 48°C). Relative humidity ranges between 15 and 80 %. Average annual rainfall is about 90 to 120cm.

### Experimental animals and collection of blood samples

Twenty four Murrah buffaloes of 2$^{rd}$ parity having body weight around 400kg were selected at peripartum period (10 days before expected date of calving to 35 days after calving). Optimum conditions were maintained for all experimental animals and were kept in general herd in open housing system.Blood samples were collected on day -7, 0, 7, 21 and 35 of calving from jugular vein of all the experimental animals both in anticoagulant containing vacuum tubes for serum separation and non vacuum coagulation tubes for differential count of blood . After that coagulation tubes are kept in slanting manner for 1hr and serum was separated by centrifugation at 3000rpm for 10 minutes at 4$^{o}$C. Serum samples were stored at -20$^{o}$C for further analysis of different parameters. Uterine fluid was collected using blue sheath (IMV technology, France) fitted in Universal AI gun by inserting it into vagina till it reaches the uterine horn guided by per rectal palpation on day +7, +14,+21,+35 of parturition and it was scored as the method described by Sheldon et al. 2009. Buffaloes which had undergone normal puerperium without any postpartum complications were classified as healthy animals.

### Estimation of NO concentration by modified Greiss method

Using modified Griess reaction by Shoker et al. (1997) the NO concentration in serum was estimated. Precipitation of serum protein was done using acetonitrile (1:1 volume) and vortexed thoroughly, followed by holding at room temp for 1hr. Then centrifugation was done at 7000rpm for 5min and the supernatant was transferred to a 2ml eppendorf tube followed by evaporation at 37°. Adding equal amount of milli Q water (same as serum sample taken initially for deproteinization) the contents were kept in room temperature for 1hr to ensure complete solubilisation. Standard was prepared using 1.56 to 100µM concentration of Sodium nitrate (NaNOƒ ). For estimation of NO 100µl of samples and standards were taken in the wells of 96 well flat bottomed plate and 100µl vanadium chloride was added in each well following addition of 100µl Greiss reagents(Greiss 1 reagent; 2% sulphanilamide in 5% HCl and Greiss 2 reagent; 0.1% N-Naphthylethylendiaminedihydrochloride). Then the plate was kept in incubator for 30 min. at 37°C and absorbance was taken at 540nm wavelength in spectrophotometer (TECAN, Seestrasse 103, Switzerland). Using linear regression equation the final concentration of nitric oxide (µM) in samples was estimated.

### Estimation of serum malondialdehyde (MDA) using TBARS method

Malondialdehyde (MDA) in serum was estimated by the method of Kaushal and Kansal (2012). MDA concentration was measured by this method due to its reactivity with thiobarbituric acid (TBA) in acidic conditions to generate a pink colour chromophore which was read at 535nm. 0.2ml of serum was added to 2.8ml of TCA (Trichloro acetic acid):TBA:HCl reagent solution and the mixed solution was heated for 15 min. in boiling water. Then the contents were mixed vigorously after cooling in room temperature. After centrifuging at 1000rpm for 10 min at room temperature supernatant was taken in cuvet and absorbance was taken at 535nm against the blank. A series of standard solution (8-40nmol) were also treated in similar manner. The MDA content was calculated from the calculation curve and expressed as nmoles/ mg protein (1,1,3,3- tetraethoxypropane was used as a standard).

### Estimation of Total antioxidant capacity (TAC) using ELISA kit:

Standards were prepared, optical densities of samples were taken and TAC was calculated as per the protocol given in the ELISA kit(CAYMAN total antioxidant assay kit).

### Statistical analysis

Descriptive statistics were calculated for different biochemical and blood parameters for both healthy and uterine infected group and the results were expressed as mean± SE. Within group comparisons were performed using independent sample T test. One way ANOVA was used to compare between groups. Group wise multiple comparisons were performed using Tukey's post hoc test. The difference of means was considered significant when the probability (P value) was <0.05. All the analysis was performed using IBM SPSS Statistics 22, Prism.

## Results and Discussion

Based on the uterine discharge scoring and per rectal examination the Murrah buffaloes were classified into healthy and uterine infected groups. The healthy group consisted of animals that had undergone normal puerperium without development of uterine infection. Buffaloes with mucopurulent or purulent or fetid uterine discharge during postpartum period i.e. up to day 35

**Table 1**. Nitric oxide (NO) conc.(µmolar/L), Malondialdehyde(MDA) conc.(nmol/ml) and TAC conc. (µmol)in blood serum of both healthy and uterine infected Murrah buffaloes during peripartum period

| Group | Day -7 | Day 0 | Day +7 | Day +14 | Day +21 | Day +35 |
|---|---|---|---|---|---|---|
| | | Serum nitric oxide(NO) conc.(µmolar/L) | | | | |
| Healthy buffaloes | 22.24$^{b}$±5.90 | 13.82$^{abA}$±1.75 | 8.77$^{aA}$±0.87 | 6.6$^{aA}$±0.61 | 6.29$^{aA}$±.94 | 4.32$^{aA}$±0.72 |
| Infected buffaloes | 28.88$^{a}$±3.28 | 25$^{aB}$±2.29 | 25.92$^{aB}$±2.62 | 28.47$^{aB}$±2.29 | 26.47$^{aB}$±2.53 | 34.45$^{aB}$±6.98 |
| | | Serum malondialdehyde(MDA) conc.(nmol/ml) | | | | |
| Healthy buffaloes | 2.09$^{aA}$±0.29 | 2.42$^{aA}$±0.56 | 2.00$^{aA}$±0.56 | 1.94$^{aA}$±0.34 | 1.26$^{aA}$±0.15 | 1.16$^{aA}$±0.25 |
| Infected buffaloes | 7.45$^{aB}$±0.84 | 9.10$^{aB}$±1.32 | 9.81$^{aB}$±1.54 | 10.80$^{aB}$±2.08 | 11.96$^{aB}$±1.66 | 11.02$^{aB}$±1.36 |
| | | TAC conc. (µmol) | | | | |
| Healthy buffaloes | 127.86$^{a}$±12.36 | 113.01$^{A}$±4.92 | 148.20 $^{aA}$±14.51 | 121.72±9.01 | 138.09$^{A}$±13.88 | 172.78±31.02 |
| Infected buffaloes | 102.28$^{a}$±5.56 | 68b$^{B}$±8.04 | 93.68$^{abB}$±7.35 | 96.23±5.24 | 102.94$^{aB}$±5.79 | 113.20$^{a}$±7.15 |

Means bearing different superscripts (A,B,C,D) in column and superscripts (a,b,c,d) in row differs significantly(P<0.05)

postpartum with or without systemic signs were classified as uterine infected animals. In Murrah buffaloes (n=24), a total number of 11 were healthy and 13 animals developed uterine infection.

**Nitric oxide (NO)**

The mean serum NO concentration was significantly (p<0.05) higher on day -7 and 0 as compared to other days of sampling period i.e. on day +7, +14, +21 and +35 in healthy group of buffaloes. When comparison was made between two groups, serum NO concentration was significantly higher (P<0.05) on day 0, +7, +14, +21 and +35 in uterine infected buffaloes as compared to healthy buffaloes. The pattern of changes in NO concentration as observed in the present study was similar to the observations of previous studies in Sahiwal cows (Baithalu et al. 2016). Enhanced NO level has been observed in uterine infected buffaloes than in healthy buffaloes (Mili and Pandita, 2014). In the present study, the elevated levels of NO from the day of parturition to day 35 in serum might have shown the damaging effect on uterine health resulting into uterine infection.

**Malondialdehyde (MDA)**

The mean serum MDA concentration was significantly (p<0.05) higher on day -7, 0, +7, +14, +21, +35 in uterine infected buffaloes in comparison to healthy group of buffaloes. Serum MDA concentration remains elevated throughout peripartum period and didn't vary significantly among different days in both healthy and uterine infected groups. The pattern of changes observed in MDA concentration in healthy animals in this study was similar to the findings of Baithalu et al. (2016) in Sahiwal cows. Measuring MDA levels enables to better comprehend the status of oxidative damage caused by free radicals. In the present study, the increased level of MDA was positively correlated with the uterine infection in Murrah buffaloes indicating a higher level of production of ROS in leukocytes during inûammation. Similar findings also observed by Kaya et al. 2017 and Heidarpour et al. 2012.

**Total antioxidant capacity (TAC)**

The mean serum TAC concentration was significantly (P<0.05) higher on days 0, +7, and +21 in healthy buffaloes when compared with uterine infected buffaloes. When comparison was made across the days in healthy group of buffaloes, serum TAC concentration although decreased (P>0.05) on day 0 from that of day -7 but remain elevated throughout postpartum period. However, in uterine infected buffaloes TAC concentration was significantly decreased (P<0.05) on day 0 from that of day -7 and concentration elevated afterwards and maximum concentration was obtained on day +35. Ghiselli et al. (2001) reported the decreased levels of TAC to provide information regarding the dynamic equilibrium between pro-oxidants and antioxidants in the plasma. Reduction in the level of antioxidants renders system incapable to protect the cellular components resulting in reduced immunity and onset of various infections (Miller et al. 1993; Sordillo and Atiken, 2009). The pattern of changes in TAC concentration observed in the present study was similar to the observations made by earlier workers (Castillo et al. 2005; Baithalu et al. 2016).

## Conclusions

From the above study it can be concluded that higher concentration of TAC and lower production of MDA and NO in healthy buffaloes indicate better anti-oxidant status to combat

against oxidative stress. On the other hand, the higher concentration of serum NO and MDA in buffaloes that developed uterine infection indicate damaging effects on uterine health. Further it may be concluded that serum NO and TAC can be used as indicators for monitoring of uterine health in Murrah buffaloes.

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RESEARCH ARTICLE

# Effect of different levels of sodium sesquicarbonate on *in vitro* rumen fermentation parameters

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**Abstract:** This experiment was conducted to study the effects of sodium sesquicarbonate on in vitro rumen fermentation parameters. Sodium sesquicarbonate (NaSc) was added at different levels (0.5, 1, 1.5, 2, 2.5 and 3%) to the substrates having concentrate: roughage ratio (concentrate: sugargraze fodder) of 50:50 and 60:40. There was no significant effect of addition of sodium sesquicarbonate (upto 3%) on in vitro DM digestibility and organic matter digestibility in different treatments. Average value of $NH_3$-N, IVFA, MBP, PF, molar proportion of acetate, propionate and butyrate also remained unaffected due to NaSc supplementation.

**Keywords:** Buffer, In vitro, Rumen fermentation, Sodium sesquicarbonate

## Introduction

There is decline in milk yield in many dairy herds which appear to be temporarily increased by overfeeding of grains (Kmicikewyez and Heinrichs, 2014; Abdela, 2016) and such diets may potentially exacerbate subacute acidosis which represents one of the most important metabolic disorders that affects rumen fermentation resulting in decrease in DMI, milk yield, and milk fat content, animal welfare and farm profitability (Morgante et al. 2007; Mao et al. 2017). Such rations also tend to support less rumination which reduces the production of salivary bicarbonate. Subacute ruminal acidosis is characterized by low ruminal pH (5.8-5.0) and an alteration in ruminal biohydrogenation of dietary polyunsaturated fatty acids (Bauman and Griinari, 2003; Plaizier et al. 2008).

Different approaches have been made for improving the ruminant production by searching the alternatives for stabilizing the rumen pH to minimize the occurrence of rumen acidosis and related disorders (Owens and Basalan, 2016). Rumen buffer could be one such alternative considering facts that addition of dietary buffers to control rumen pH can be justified if bunk management and nutritional factors cause low pH (Kang and Wanapat, 2013). Buffers can neutralize the excessive acidity due to increased production of volatile fatty acids but the effects depend upon type of buffer, dose and type of animal in which it is supplemented (Sen et al. 2006). Bicarbonates are commonly used as exogenous buffer as their dissociation constant (pka =6.25) is close to normal rumen pH thus possessing high acid consuming capacity (Marden et al. 2008) and thus prevent further depression in pH (Humer et al. 2018). Sodium bicarbonate increases rumen pH, produces a more desirable rumen fermentation and increases rumen fluid outflow. Dietary supplementation of sodium sesquicarbonate could be one of the alternative as it is a mixture of sodium bicarbonate and sodium carbonate and is naturally occuring an alkalizing agent. The pH of a one percent sodium sesquicarbonate solution is 9.9 as compared to sodium bicarbonate which is 8.4 thus expected to have better potential for buffering action besides being cost effective. Therefore, the present experiment was conducted to study the effects of sodium sesquicarbonate on in vitro rumen fermentation parameters.

## Materials and Methods

### Substrate composition, treatments and parameters estimated

Sugargraze (moderately draught tolerant sweet sorghum hybrid) fodder and concentrate mixture were dried in hot air oven at 60°C for 48 h and ground using a hammer mill to pass thtrough 1 mm sieve. The substrate was prepared by mixing concentrate mixture and sugargraze fodder in the ratio of 50:50 and 60:40. Sodium sesquicarbonate was added in treatment groups at different levels viz. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% of substrate whereas the control group comprised of only substrate. Proximate principles and fibre

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fractions of the concentrate mixture and the substrate were determined according to AOAC (2005) and Van Soest et al. (1991). Rumen fluid and particulate matter were collected from the goats (n=4) with the help of stomach tube in the morning before feeding (sugargraze and concentrate in 70:30 ratio) and watering of animals, in a pre-warmed thermo flask. The incubations were carried out as described by Menke and Steingass (1988) to study *in vitro* gas production. Three sets of in vitro trials in triplicates were conducted to estimate the effect of addition of different levels of sodium sesquicarbonate in diet/substrate on the various parametrs such as IVGPT, partition factor, pH and microbial protein synthesis along with in vitro DM/OM degradability to obtain a complete picture for rumen fermentation pattern.

**Analytical procedure**

The substrate (sugargraze and concentrate; 200 mg) was weighed and placed into the bottom of the 100 mL graduated glass syringes without sticking it to the sides of syringe. The medium mixture solution and rumen liquor were mixed in the ratio 2:1 and immediately after thorough mixing, 30 mL of this incubation medium was injected to glass syringes (Haberle, Germany) using an auto dispenser. The level of piston was recorded (initial reading) and the syringes were placed in the incubator preadjusted at 39±0.5$^0$C for 24 h. After the completion of incubation period, total gas production was calculate after correcting corresponding blank values and pH of syringe contents was estimated with the help of digital pH meter (Model: pH Spear, Eutech Instruments, Malaysia). The contents of the syringe were then emptied into centrifuge tubes and centrifuged at 3000 rpm for 15 min till clear supernatant was obtained which was then preserved at -20ºC for the estinamtion of individual volatile fatty acids (Erwin et al. 1961) using gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with chromosorb 101 mesh 80 – 100 (length 1.5 m; o.d 3.175 mm; i.d. 2 mm). An aliquot of supernatant was acidified with equal volume of 0.5 M HCl and kept at -20ºC for estimation of ammonia nitrogen by Kjeldahl method. True DM and OM digestibility of samples were estimated as per method described by Van Soest et al. (1991). The pellet left after centrifugation was refluxed in beakers with neutral detergent solution for 1h and thereafter, contents were filtered through the sintered glass crucible (G-1) washed with hot water and kept in hot air oven at 100$^o$C for drying. *In vitro* true dry matter digestibility IVTDMD was calculated as the difference between the weights of the DM incubated and the DM residue left. The residue in each crucible was ashed in muffle furnace at 550$^o$C for 2 h to determine the in vitro true organic matter digestibility (IVTOMD). The partitioning factor (PF) and microbial biomass production (MBP) were calaculated with the help of equations based on parameters like IVTDMD, IVTOMD, total gas and net gas volume (Blummel et al. 1997; Blummel and Lebzien, 2001).

**Equations**

$$PF = \frac{\textit{In vitro} \text{ true DM digested (mg)}}{\text{Total gas produced (mL)}}$$

$$MBP\ (mg) = TDOM\ (mg) - (\text{Net gas volume} \times 2.20)$$

**Statistical analysis**

The statistical analysis of the data was done using one-way analysis of variance (ANOVA) by SPSS, 2010 version 16.

## Results and Discussion

Detailed chemical composition of the feed ingredients and the substrate prepared from them has been given in Table 1. The same substrate was used in both the control and treatment groups. The effect of different levels of sodium sesquicarbonate in 50C: 50R and 60C:40R ration in vitro digestibility, pH, net gas production, PF and MBP is presented in Table 2 and IVFA and $NH_3$ in Table 3. In the present study, the mean value of pH in 60C:40R was found to be significantly ($P<0.001$) higher than 50C: 50R and there was no effect of buffer addition on pH value. Similarly, net gas production, IVDMD and IVOMD were also higher in the diet with 60C:40R composition. Diet with 50C:50R had higher values of MBP, PF and higher proportion of acetate ; and lower in propionate. However, there was no difference in the levels of $NH_3$ and butyrate concentrations between the two diets. Hence, supplementation of sodium sesquicarbonate (0.5 upto 3%) and the interaction between diet and treatment had no effect on rumen fermentation parameters. The findings of the present study are consistent with those of Xu et al. (1994) who reported that supplementation of rumen buffers in lactating Holstein cows (1.5% and 2.2% of DMI) and observed no change in rumen fluid

**Table 1** Chemical composition (% DM basis) of the substrate

| Attribute | Sugargraze fodder | Concentrate mixture |
|---|---|---|
| DM | 28.00 | 89.56 |
| CP | 10.20 | 19.53 |
| EE | 1.29 | 3.74 |
| Ash | 8.64 | 11.22 |
| ADF | 37.29 | 12.38 |
| NDF | 58.65 | 26.37 |
| NDICP | 5.30 | 1.94 |
| ADICP | 1.14 | 0.69 |
| Hemicellulose | 21.40 | 13.99 |
| Cellulose | 21.00 | 6.40 |
| ADL | 7.75 | 2.32 |
| CHO | 79.87 | 65.51 |
| td NFC | 26.00 | 40.26 |
| td CP | 9.70 | 19.25 |
| td FA | 0.30 | 2.74 |
| td NDF | 24.8 | 13.13 |
| TDN | 54.10 | 71.81 |

**Table 2** Effect of different levels of sodium sesquicarbonate on in vitro digestibility, pH, net gas production, IVFA, PF and MBP

| Parameter | Substrate | Level of supplementation (%) | | | | | | | Mean | SEM | *P value* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | | | S | T | S*T |
| pH | 50 R:50 C | 6.64 | 6.58 | 6.54 | 6.60 | 6.56 | 6.56 | 6.59 | 6.58[a] | 0.02 | <0.001 | 0.548 | 0.629 |
| | 40 R:60 C | 6.84 | 6.83 | 6.87 | 6.82 | 6.79 | 6.83 | 7.08 | 6.86[b] | 0.04 | | | |
| | Mean | 6.74 | 6.71 | 6.71 | 6.72 | 6.67 | 6.69 | 6.83 | | | | | |
| Net gas | 50 R:50 C | 35.00 | 34.00 | 35.33 | 34.66 | 35.33 | 35.00 | 35.33 | 34.95[a] | 0.21 | <0.001 | 0.985 | 0.773 |
| (mL/24h) | 40 R:60 C | 39.33 | 40.00 | 39.33 | 39.67 | 39.67 | 39.00 | 39.50 | 39.50[b] | 0.26 | | | |
| | Mean | 37.17 | 37.00 | 37.33 | 37.17 | 37.50 | 37.00 | 37.42 | | | | | |
| IVDMD | 50 R:50 C | 61.48 | 62.19 | 61.90 | 61.21 | 61.29 | 61.19 | 61.23 | 61.50[a] | 0.29 | <0.001 | 0.804 | 0.966 |
| (%) | 40 R:60 C | 65.07 | 65.81 | 66.80 | 66.10 | 65.49 | 65.18 | 65.84 | 65.76[b] | 0.27 | | | |
| | Mean | 63.27 | 64.00 | 64.35 | 63.65 | 63.39 | 63.19 | 63.54 | | | | | |
| IVOMD | 50 R:50 C | 62.02 | 62.96 | 62.57 | 62.36 | 61.82 | 61.91 | 62.05 | 62.24[a] | 0.29 | <0.001 | 0.888 | 0.991 |
| (%) | 40 R:60 C | 65.93 | 66.71 | 67.38 | 66.89 | 66.81 | 66.40 | 66.45 | 66.65[b] | 0.28 | | | |
| | Mean | 63.97 | 64.84 | 64.98 | 64.62 | 64.32 | 64.15 | 64.25 | | | | | |
| PF | 50 R:50 C | 3.36 | 3.49 | 3.37 | 3.41 | 3.31 | 3.34 | 3.33 | 3.37[b] | 0.02 | <0.001 | 0.728 | 0.528 |
| | 40 R:60 C | 3.16 | 3.12 | 3.20 | 3.21 | 3.15 | 3.21 | 3.13 | 3.17[a] | 0.02 | | | |
| | Mean | 3.27 | 3.31 | 3.28 | 3.31 | 3.23 | 3.28 | 3.23 | | | | | |
| MBP (mg) | 50 R:50 C | 38.98 | 42.42 | 39.48 | 40.11 | 37.56 | 38.28 | 38.29 | 39.31[b] | 0.63 | 0.005 | 0.766 | 0.696 |
| | 40 R:60 C | 35.91 | 34.98 | 37.46 | 37.70 | 35.61 | 37.32 | 34.78 | 36.25[a] | 0.68 | | | |
| | Mean | 37.45 | 38.70 | 38.47 | 38.91 | 36.58 | 37.80 | 36.54 | | | | | |

C: Concentrate mixture, R: Roughage, S: Substrate, T: Treatmnet

**Table 3** Effect of different levels of sodium sesquicarbonate on in vitro ammonia and volatile fatty acid concentration

| Parameter | Substrate | Level of supplementation (%) | | | | | | | Mean | SEM | *P value* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | | | S | T | S*T |
| $NH_3$-N | 50 R:50 C | 9.71 | 9.89 | 9.52 | 9.24 | 9.52 | 9.52 | 9.33 | 9.53 | 0.12 | 0.404 | 0.833 | 0.658 |
| (mg/dL) | 40 R:60 C | 9.15 | 9.80 | 9.05 | 10.08 | 9.98 | 10.55 | 9.80 | 9.77 | 0.23 | | | |
| | Mean | 9.43 | 9.85 | 9.29 | 9.66 | 9.75 | 10.03 | 9.57 | | | | | |
| Acetate | 50 R:50 C | 38.53 | 39.47 | 39.24 | 37.53 | 38.32 | 37.68 | 38.17 | 38.42[b] | 0.29 | <0.001 | 0.704 | 0.467 |
| (mM) | 40 R:60 C | 34.22 | 33.33 | 35.88 | 34.86 | 36.47 | 35.81 | 33.36 | 34.85[a] | 0.52 | | | |
| | Mean | 36.38 | 36.40 | 37.56 | 36.19 | 37.39 | 36.74 | 35.76 | | | | | |
| Propionate | 50 R:50 C | 10.84 | 11.36 | 10.80 | 10.13 | 11.72 | 10.80 | 10.13 | 10.83[a] | 0.21 | <0.001 | 0.883 | 0.696 |
| (mM) | 40 R:60 C | 16.02 | 15.70 | 16.58 | 17.13 | 16.78 | 17.44 | 16.35 | 16.57[b] | 0.35 | | | |
| | Mean | 13.43 | 13.53 | 13.69 | 13.63 | 14.25 | 14.12 | 13.24 | | | | | |
| Butyrate | 50 R:50 C | 7.51 | 7.04 | 6.97 | 7.64 | 8.09 | 6.97 | 7.29 | 7.36 | 0.16 | 0.091 | 0.753 | 0.857 |
| (mM) | 40 R:60 C | 7.01 | 6.68 | 7.12 | 6.41 | 7.20 | 6.82 | 7.00 | 6.89 | 0.18 | | | |
| | Mean | 7.26 | 6.86 | 7.04 | 7.02 | 7.65 | 6.89 | 7.15 | | | | | |

C: Concentrate mixture, R: Roughage, S: Substrate, T: Treatmnet

pH and VFA molar percentage. The results of this study are in accordance with those of Umucalilar and Seker (2000) who carried out an *in vitro* experiment with different ratios of $NaHCO_3$ (0, 0.5, 1.0, 1.5%) and MgO (0, 0.25, 0.5 and 1). They found that $NaHCO_3$ supplementation had no effect on pH, buffering capacity, TVFA and gas production but increased the levels of $NH_3$ whereas MgO supplementation increased the values of pH, buffering capacity, TVFA and gas production. However, results are in disagreement with Patra and Yu (2013) who reported an decrease in molar percentage of acetate and acetate-to-propionate ratio, whereas the molar percentage of propionate increased quadratically with increasing bicarbonate concentration. This difference could possibly be due to change in pH in the above study which varied from 6.0 to 6.38 in the above study which was not found in our study because of difference in both dose and souce of bicarbonate added. Furthermore, bicarbonate is regularly now a days being supplemented to dairy cattle rations to reduce incidences of acidosis and to counteract milk fat depression by increasing molar percentage of acetate and decreasing molar percentage of propionate (Cruywagen et al. 2015). Therefore, there has been a

wide discrepancy in results of addition of buffers on comparison of in vitro and in vivo studies indicating that factors other than bicarbonate concentrations in media might also affect these results. In this study there was significant effect of incubation time on fermentation parameters. Our findings were also similar with those of Pereira and Armenanto (2000), Dschaak et al. (2010) and Bougouin et al. (2018) who observed no effect on digestibility supplementing $NaHCO_3$ in the diet. Grant and Mertens (1992) studied the effect of buffer pH (5.2, 6.2 and 6.8) on in vitro digestion kinetics and observed that low pH decreased fiber digestion. Mao et al. (2017) found that sodium bicarbonate supplementation (7% of substrate) under in vitro condition increased the final pH levels and the concentration of total volatile fatty acids and the proportions of acetate, propionate and total branched chain VFA were also affected ($p<0.001$) by incubation time ($p<0.001$) and interaction between incubation time and bicarbonate supplementation. They also found that total gas production was higher in the bicarbonate group but the concentration of $NH_3$–N was almost similar among the control and bicarbonate supplemented group.

## Conclusion

Addition of sodium sesquicarbonate upto 3 %) of substrate did not show any significant effect on *in vitro* rumen fermentation parameters.

## Aclnowledgements

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RESEARCH ARTICLE

# Effect of composition and size of the reference population in genotype imputation efficiency of INDUSCHIP in HF Crossbred cattle

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**Abstract:** The objective of this study was to investigate the effect of composition and size of the reference population in imputation efficiency of INDUSCHIP v2 in Indian HF crossbred cattle. Data set consisted of a total of 869 cattle from 14 Indicine breeds, 2 crossbreds (HF and Jersey crossbreds) and 2 exotic breeds (HF, Jersey) genotyped with Illumina BovineHD (Illumina, San Diego, CA) panel. Post QC, 846 animals and 449955 SNPs remained for imputation study. 3 test groups each with randomly selected 25 HFCB animals with subset genotype of INDUSCHIP v2 were created, whereas with HD genotyping data of remaining animals, 3 different categories of reference groups were created namely reference 1 (HF, Jersey, all 14 Indicine breeds, HF and Jersey crossbreds), reference 2 (HF, HF crossbred, Sahiwal, Gir and Kankrej ) and reference 3 (pure HF, Sahiwal, Gir and Kankrej). Imputation efficiency of INDUSCHIP v2 was expressed in terms of concordance rate and Dosage R2 (DR2). Reference groups 1 and 2 were found to be better than Reference group 3. Further, the size of the reference population had an impact on imputation efficiency. The concordance rate and DR2 decreased with decline about population size. However, a reference population with 280 animals was found to be sufficient to obtain a concordance rate of around 95% or more and DR2 around 0.93. More number of HF, HF crossbred, Sahiwal, Gir and Kankrej animals need to be HD genotyped and incorporated in the reference population to improve the imputation efficiency of INDUSCHIP v2.

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**Keywords:** Crossbred cattle, Genotype Imputation, HD chip, LD chip, Single Nucleotide Polymorphism, Reference population

## Introduction

Under Genomic selection, evenly spaced DNA markers (Single Nucleotide Polymorphism-SNPs) spread across the genome are used to estimate breeding values (GEBV) for the target individuals (Meuwissen et al. 2016). Genomic information from dense SNPs chips provides the opportunity to increase the rate of genetic progress in the breeding programs if a sufficient number of markers and animals with phenotypes are genotyped (Carvalheiro et al. 2014). More number of markers means greater linkage disequilibrium between SNPs and more chances of capturing genomic variation. Since genotyping with HD SNP panels are expensive, it limits the number of animals to be genotyped. Hence, in practice, a cost-effective alternative called genotype imputation is preferred. Genotype imputation makes it possible to extrapolate genotypes from lower to higher density arrays based on a representative sample of individuals genotyped at higher density (Pausch, H, 2013). This not only makes it possible to increase the genomic information and predict missing genotypes (Marchini and Howie,2010) but to reduce genotyping costs and intensify genomic selection (Ventura et al. 2014) by genotyping more number of animals and combine data from different breeds (Larmer et al. 2014). The imputation efficiency of any chip depends upon several factors namely imputation method, software used for imputation, the MAF of the SNP to be imputed, linkage disequilibrium between SNPs, the chromosomal position of the SNP, the quality of SNP maps, size and composition of reference population, etc. (Schrooten et al. 2014).

To implement genomic selection in India for indicus breeds and their taurine crosses a medium-density customized chip i.e INDUSCHIP v1 consisting of 45700 SNPs sampled from HD genotype of the mostly four indicus breeds (Gir, Sahiwal, Kankrej, Redsindhi) and their taurine crosses (HF cross & Jersey cross) have been developed. (Mrode et al. 2019). The genotyping chip contained around 41000 SNPs from HD data having high MAF (0.25), uniformly distributed across the genome for all the breeds under study with an average distance between two consecutive SNPs around 65 kbps. In addition to the above, 2000 ancestry

informative SNPs for above mentioned six breeds, ISAG recommended parentage SNPs and some known open-source genetic markers were also included (Nayee et al. 2017). Subsequently, INDUSCHIP v1 was upgraded to INDUSCHIP v2 (52363 SNPs) incorporating additional 6663 highly polymorphic SNPs.

Keeping this in mind the current investigation was undertaken to study the effect of the composition of the reference population and its size on the genotype imputation efficiency of INDUSCHIP v2, a custom made medium density genotyping chip designed on Illumina platform to genotype crossbred and indigenous cattle of India.

## Materials and Methods

### Source of data

A total of 869 number of cattle belong to 14 different Indicene breeds (Amritmahal, Deoni, Gir, Hariana, Hallikar, Kankrej, Khillar, Kangayam, Kankrej, Ongole, RedSindhi, Rathi, Sahiwal) and 2 crossbred (HFCB and Jersey crossbred) breeds were genotyped with 777K BovineHD BeadChip (Illumina, Inc., San Diego, CA). The genotype data for 2 Taurine breeds i.e. Holstein Friesian (HF) and Jersey, were obtained from Aarhus University, Denmark. The genotype candidates were selected mainly from frozen semen stations in India and certain state-run livestock farms maintaining purebred animals of those breeds.

### Data editing

Quality control checks were applied to raw HD genotype data. Only SNPs located on autosomes, with call rate >95% and genotyping rate >90% were kept. Further, SNPs with a minor allele frequency (MAF) less than 0.01 and Hardy Weinberg equilibrium less than $10^{-4}$ were excluded.

After quality control, out of a total of 869 animals belong to fourteen different breeds (multi-breed) and 777962 SNPs, only 846 animals and 449955 SNPs remained for the imputation study.

### Creation of Test, Reference and Validation data sets

From this data, randomly 25 HFCB animals were selected at a time to form test groups of animals. Only subset genotypes of INDUSCHIP v2 were considered for test animals and the rest of the animals were taken as reference group with HD genotype data. Three such test groups were created. For each test group, 3 different categories of reference groups were created namely Reference 1 (821 animals comprising of HF, Jersey, all 14 Indigenous breeds, HF and Jersey crossbred animals), Reference 2 (404 animals comprising of only HF, HF crossbred, Sahiwal, Gir and Kankrej breed) and Reference 3 (266 animals with only pure HF, Sahiwal, Gir and Kankrej cattle). Imputation accuracy was measured as the concordance of actual HD genotype with imputed genotypes and squared correlation (Dosage R2) between estimated allele dose and true allele doses for test animals. A schematic diagram of the experimental design of this imputation study is presented in Figure.1.

### Imputation using INDUSCHIP v2 SNP panels

Thereafter, 50K SNP panel data (52363 SNP) was retrieved from customized INDUSCHIP v2 manifest file (NDDB_ Induschip2_ 15061153X355693_B1.bpm). Around 2949 SNPs, which were present in INDUSCHIP v2 manifest file but not found to be matching with HD SNPs, were excluded from this study. After quality control, finally, 49399 SNPs remained, whose HD genotyping data was extracted as a subset to study the imputation efficiency of INDUSCHIP.

Imputation was carried out for all the 3 test groups of animals using genotyping information of INDUSCHIP v2 SNP panel considering all the 29 autosomes against 3 different reference populations.

Subsequently, to investigate the impact of the size of the reference population on imputation efficiency, around 70% (121), 50% (202), 40% (242) and 30% (283) animals were retained randomly in the reference population Group 2. Thereafter imputation was carried out for all the 3 test groups of animals.

PLINK (Purcell et al. 2007) software was used for quality control of the data, creation of test, reference and validation data sets as well as for preparing input files for Beagle. Imputation was carried out using Beagle 5.0 software (Browning et al. 2018), which is a population-based imputation program (does not rely on pedigree information) that adopts a stochastic procedure based on a Hidden Markov Monte-Carlo process to infer the probabilities of each haplotype/genotype (Carvalheiro et al.2014).

Imputation accuracy was assessed in terms of concordance rate i.e. the proportion of alleles or genotypes that are correctly imputed (Weigel et al. 2010) and squared correlation between the estimated allele dose and the true allele dose expressed as Dosage R2 (DR2) in Beagle 5.0 (Browning et al. 2018). The animal wise concordance rate between imputed and actual genotype was estimated using R statistical software and squared correlation values between markers were obtained after phasing and imputation using Beagle software.

## Results and Discussion

### Efficiency of INDUSCHIP v2 in imputing missing SNPs in HF crossbred cattle using a different reference population

Average concordance rates and DR2 obtained for 3 test groups of animals using 3 different reference populations are presented in Figure 2. Results obtained from this study revealed (Table 1) that the highest average concordance rate was obtained for

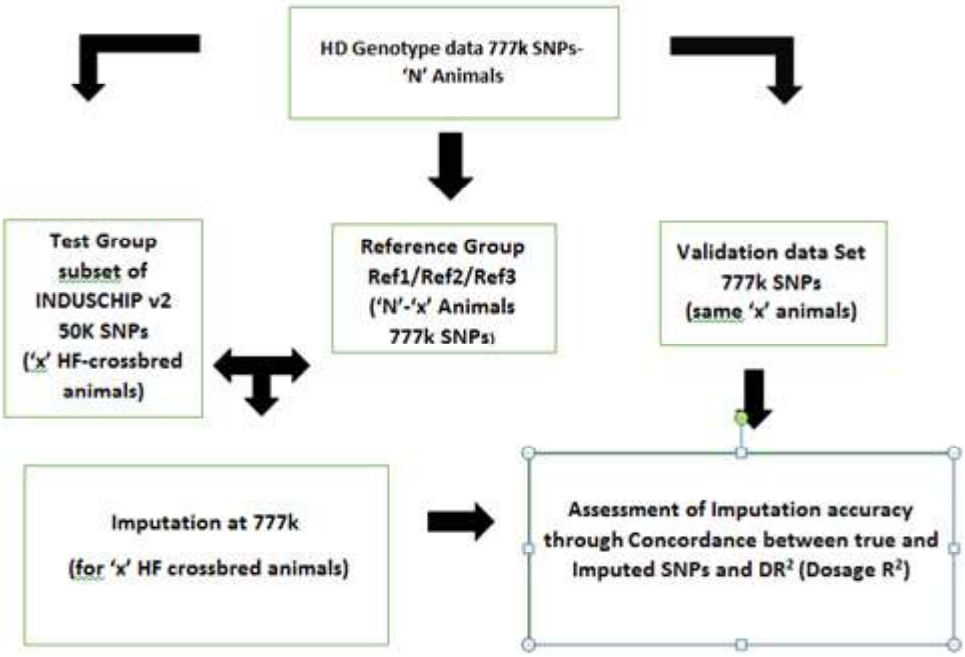


**Fig. 1** Schematic Diagram of the experimental design for imputation study

**Table 1** Average Concordance Rate using three different reference populations

| Test Group | Reference group | | |
|---|---|---|---|
| | Reference-1(821) | Reference-2(404) | Reference-3 (266) |
| Test -1 | 0.978 | 0.973 | 0.898 |
| Test -2 | 0.970 | 0.963 | 0.887 |
| Test -3 | 0.972 | 0.967 | 0.901 |
| Average | 0.974 | 0.968 | 0.895 |

**Table 2** DR2 using three different reference populations

| Test Group | Reference group | | |
|---|---|---|---|
| | Reference-1(821) | Reference-2(404) | Reference-3 (266) |
| Test -1 | 0.952 | 0.946 | 0.861 |
| Test -2 | 0.948 | 0.940 | 0.856 |
| Test -3 | 0.946 | 0.941 | 0.865 |

reference group-1 (0.974) followed by reference group-2 (0.968), while the lowest concordance rate was found for reference group-3 (0.895). DR2 estimates as presented in Table 2 found to vary between 0.946-0.952 for reference group-1, 0.940-0.946 for reference group-2 and 0.856-0.865 reference group-3.

**Efficiency of INDUSCHIP v2 in imputing missing SNPs in HF crossbred cattle for various size of the reference population**

To assess the impact of varying size of reference population on imputation efficiency, average concordance rates and DR2 were estimated using reference group 2 for all three test groups of animals keeping only 70%, 50%, 40% and 30% of the animals, respectively in the said reference groups. The estimates are presented in Table 3 and Table 4, respectively. The results indicate an increasing trend in the average concordance rate as well as DR2 as the size of the reference population increases (Figure 3). The average concordance rates observed were 0.959, 0.946, 0.939 and 0.922 respectively for 70%, 50%, 40% and 30% of animals retained randomly in the reference groups. The DR2 values ranged between 0.931 to 0.934, 0.913 to 0.922, 0.905 to 0.911 and 0.884 to 0.887, respectively, for 70%, 50%, 40% and 30% of the animals retained in reference group.

The present study revealed that the composition of the reference population plays an important role in determining imputation efficiency at the HD level. For HF crossbred cattle, the reference population-1 comprising all the 14 Indigenous breeds, HF, Jersey, HF crossbred and Jersey crossbred resulted in higher concordance rate and DR2, while reference group 2, consisting of HF, HF crossbred and 3 indigenous breeds like Sahiwal, Gir and Kankrej cattle, despite of having nearly half of the HD genotyped animals than reference group-1 (404 against 821) resulted into very minor loss of imputation accuracy with

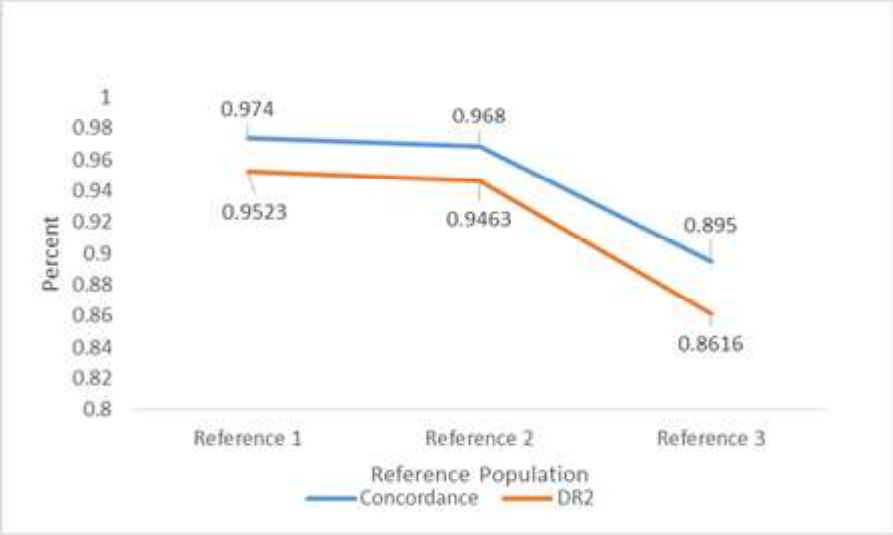


**Fig. 2** Average Concordance rate (%) and DR2 (%) of HF crossbred test group of animals for different composition of the reference population

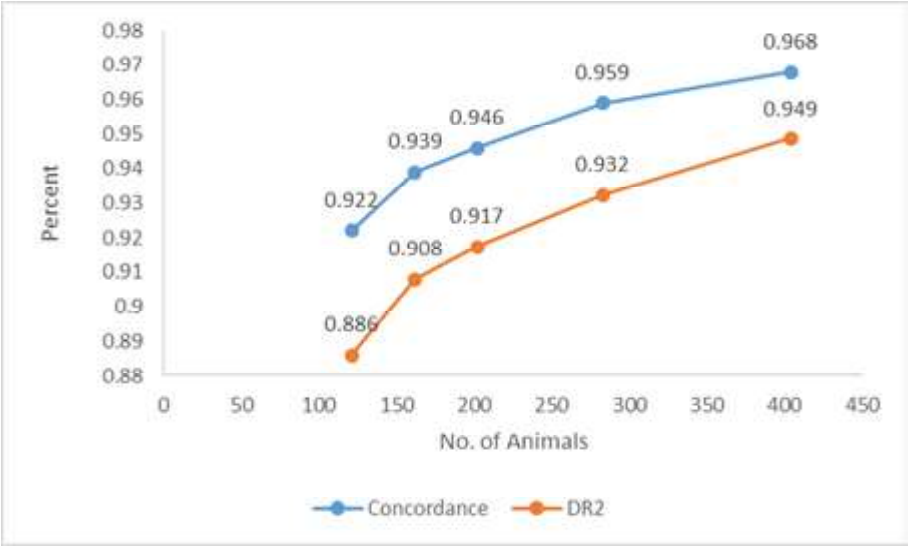


**Fig.** 3 Average concordance rate (%) and DR2(%) for different size of reference populations in HF crossbred cattle

**Table 3** Average Concordance Rate for different size of the reference population

| Test Group | Reference group size | | | | |
|---|---|---|---|---|---|
| | With all available animals | % of animals retained | | | |
| | (404) | 70%(283) | 50%(202) | 40%(162) | 30%(121) |
| Test -1 | 0.973 | 0.963 | 0.953 | 0.944 | 0.925 |
| Test -2 | 0.963 | 0.956 | 0.943 | 0.936 | 0.920 |
| Test -3 | 0.967 | 0.959 | 0.943 | 0.938 | 0.929 |
| Average | 0.968 | 0.959 | 0.946 | 0.939 | 0.922 |

# Figures in the parenthesis indicates no. of animals

concordance rate around 95% between imputed and actual genotype and average DR2 around 0.94. This may be due to the fact that as the HF crossbred cattle were mainly produced by interbreeding of exotic HF with indigenous Sahiwal, Gir and Kankrej cattle, the existing HF crossbreds are expected to be more closely related to HF, Sahiwal, Gir and Kankrej breed and their crosses than other indigenous draft breeds, Jersey and their crosses. As a result, almost similar imputation efficiency was observed despite having almost half the size of the population in reference Group 2. Berry and Kearney (2011), Ma et al. (2013), Moghaddar et al. (2015), Bolormaa et al. (2015) and Ventura et al.

**Table 4** DR2 for different size of the reference population

| Test Group | Reference group size | | | | |
|---|---|---|---|---|---|
| | With all available animals | % of animals retained | | | |
| | (404) | 70%(283) | 50%(202) | 40%(162) | 30%(121) |
| Test -1 | 0.952 | 0.934 | 0.922 | 0.911 | 0.887 |
| Test -2 | 0.948 | 0.932 | 0.917 | 0.908 | 0.887 |
| Test -3 | 0.946 | 0.931 | 0.913 | 0.905 | 0.884 |

(2016) in their studies also reported that genetically closer animals in the reference and imputation population produce higher imputation accuracies.

On the other hand, the present study also revealed that retaining only purebred animals i.e. HF, Sahiwal, Gir and Kankrej breed (excluding of HF crossbred) in reference group-3 resulted in relatively poor imputation efficiency. This indicates the possibility of the presence of crossbred specific haplotypes. The results are in agreement with the findings of Oliviera Junior et al. (2017), where the inclusion of crossbred Girolando in the reference population had a greater effect on the imputation accuracy than the purebred Gyr haplotypes.

The findings indicated that to improve the imputation efficiency of INDUSCHIP v2 by strengthening reference population, more number of HF, HF crossbred, Sahiwal, Gir and Kankrej cattle etc. from varied sources need to be HD genotyped and included in the reference population.

Subsequently, when imputation was carried out for all the three test groups of animals using the same reference (reference group 2) of varying sizes, the average concordance rates and DR2 were found to decrease as the size of the reference population decreases, which agrees with the findings reported by Schrooten, D.T. and De Roos, A.P.W(2010) and Pausch, H. et al. (2013) However, the present study indicated a reference population size of around 280 was sufficient to obtain concordance rate around 95% or more and DR2 around 0.93. Ghoreishifar S.M et al. (2018) in Italian Mediterranean buffaloes also found that increasing the reference population size from small to intermediate (i.e., from 42 to 202) resulted in a greater improvement in imputation accuracy compared to increasing the Reference Population size from intermediate to large (i.e., from 202 to 736).

## Conclusions

Imputation in HF crossbred population in India using custom made microarray INDUSCHIP v2 was found to be affected by both the composition of the reference population and its size. For further improvement in the efficiency of imputation by INDUSCHIP v2, more number of HF, HF crossbred, Sahiwal, Gir and Kankrej animals may need to be HD genotyped and incorporated in the reference population.

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RESEARCH ARTICLE

# Genetic analysis of test days, 305 days and lifetime lactation records in Sahiwal cattle

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**Abstract:** The present research was aimed to perform genetic analysis of first lactation monthly test day milk yields, first lactation 305 days and lifetime milk yield of Sahiwal cattle. Data were collected on 867 Sahiwal cows sired by 76 bulls over a period of 31 years. Complete genetic analysis of all the traits was done using Harvey (model 2). The heritability of MTDMY ranged from 0.12 ± 0.06 (TD10) to 0.48 ± 0.09 (TD4) by LSML. The heritability estimates for FL305DMY and LTMY were found to be 0.40 ± 0.09 and 0.34 ± 0.07 respectively. The estimates of phenotypic and genetic correlation among all the monthly test day milk yields ranged from 0.14 to 0.79 and 0.15 to 0.99 respectively. The genetic correlation of MTDMY and FL305DMY ranged from 0.66 (TD1) to 0.99 (TD7, TD10).The phenotypic correlation of MTDMY with FL305DMY ranged from 0.29±0.04 (TD1) to 0.74±0.03 (TD6). The knowledge of the heritabilities and correlations among the traits help in developing the prediction models for performance traits which assists in developing better, accurate and faster selection strategies for breeding programs.

**Keywords:** First lactation 305day milk yield, Genetic correlation, Heritability, Lifetime milk yield, Phenotypic correlation, Sahiwal cattle, Test day milk yield

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## Introduction

India has a diverse and unique livestock population. As per 20th livestock census, India has 192.49 million cattle out of which 142.11 million belong to indigenous/nondescript category. The recognized importance of indigenous cattle in traits like higher feed conversion efficiency, better adaptability to tropical conditions, resistance to heat and tropical diseases etc. has led to an increase in their population by 10% in 2019 as compared to previous census.

Sahiwal is one of the best indigenous dairy breed with highest milk production compared to other zebu breeds. Pure Sahiwal herds are available in Punjab, Rajasthan and some pockets of U.P. and Chhattisgarh. A fair understanding of genetic and phenotypic parameters of the population is needed for developing a suitable breeding strategy. The genetic makeup of a population is reflected in the parameters like heritability, genetic and phenotypic correlations between the performance traits. Test day (TD) model is a statistical procedure which considers genetic and environmental effect directly on the test day basis (Swalve, 1995). The usefulness of test day milk yield depends on genetic correlation between test day milk yield records and 305 days milk yield and also upon the accuracy with which sire evaluation can be obtained based on test day milk yield records. In the present investigation the heritabilities and correlation among the test day milk yields, first lactation 305 days milk yield and lifetime milk production are analyzed using Harvey.

## Materials and Methods

### Data collection

Data from history cum pedigree sheets and daily milk recording registers of Sahiwal cattle maintained at ICAR – National Dairy Research Institute (NDRI), Karnal, Haryana, were used for this study. The first, second, third, fourth, fifth and sixth lactation production records of Sahiwal over a period of 31years were recorded. Test day milk yield were recorded at monthly interval (6th, 35th, 65th, 95th, 125th, 155th, 185th, 215th, 245th, 275th, 305th day of first lactation).

**Statistical analysis**

The heritability of test day milk yields, were estimated using the genetic (co)variances, permanent environmental (co)variances and homogeneous residual variances of test day milk yield as per the procedures given by Jamrozik and Schaeffer (1997). For the estimation of heritability of test day milk yields one need to estimate-

**Estimation of heritability of test day milk yields**

The heritability of test day milk yield records at different DIM in first lactation was estimated as follows

$$h_{(i)}^2 = \frac{\sigma_{a(i)}^2}{\sigma_{a(i)}^2 + \sigma_{pe(i)}^2 + \sigma_{e(i)}^2}$$

$h_{(i)}^2$ = Heritability of test day milk yield on $i^{th}$ DIM

$\sigma_{a(i)}^2$ = Genetic variance of test day milk yield on $i^{th}$ DIM

$\sigma_{pe(i)}^2$ = Permanent environmental variance of test day milk yield on $i^{th}$ DIM and

$\sigma_{e(i)}^2$ = Homogeneous residual variance of test day milk yield on $i^{th}$ DIM

**Estimation of genetic and phenotypic correlations**

The genetic and phenotypic correlations among different monthly test day milk yield, between test day milk yield and 305 days milk yield, between test day milk yield and life time milk yield were estimated from the analysis of variance /covariance using half sib data.

| Sources of variation | d.f. | SS | MS | EMS |
|---|---|---|---|---|
| Between sires | (S – 1) | $\Sigma_{i=1}=X_i y_i/n_i - X..Y../N$ | MSs | $\sigma_e^2{}_{(XY)}+K\,\sigma_s^2{}_{(XY)}$ |
| Within sires | (N – S) | $\Sigma_i\Sigma_j X_{ij}Y_{ij} - \Sigma_{i=1}X_iY_i/n_i$ | MSe | $\sigma_e^2{}_{(XY)}$ |
| Total | (N – 1) | | | |

**Genetic correlation ($r_g$)**

The genetic correlation was calculated by using the following formula

$$r_g(XY) = \frac{Cov\,S_{XY}}{\sqrt{(\sigma_x^2)(\sigma_y^2)}}$$

where,

$Cov\,S_{XY}$ = Sire component of covariance between traits X and Y

$\sigma_x^2$ = Sire component of variance for trait X

$\sigma_y^2$ = Sire component of variance for trait Y

The standard error of genetic correlation ($r_g$) was estimated by using the following formula as given by Robertson (1959):

$$S.E.(r_g) = \frac{1-r_g^2}{\sqrt{2}}\sqrt{S.E.(h_x^2)\,S.E.(h_y^2)/(h_x^2)(h_y^2)}$$

($h_x^2$) and ($h_y^2$) are the heritability estimates of the two traits X and Y, respectively

**Phenotypic correlation ($r_p$)**

The phenotypic correlations were estimated as

$$r_{p(XY)} = Cov_{s\,(XY)} + Cov_{e(XY)}/\sqrt{[\sigma_s^2(X)+\sigma_e^2(X)]\,[\sigma_s^2(Y)+\sigma_e^2(Y)]}$$

Where,

$Cov_{s(XY)}$ = Sire component of covariance between traits X and Y

$Cov_{e(XY)}$ = Error component of covariance between traits X and Y

$\sigma_S^2(X)$ = Sire component of variance for trait X

$\sigma_S^2(Y)$ = Sire component of variance for trait Y

$\sigma_e^2(X)$ = Error component of variance for trait X

$\sigma_e^2(Y)$ = Error component of variance for trait Y

The standard error of the phenotypic correlations was calculated as (Panse and Sukhatme, 1967)

$$S.E.(r_p)\ \sqrt{1 - r_{p\,(XY)}^2}/N-2$$

Where,

$r_{p\,(XY)}$ = Phenotypic correlation between the traits X and Y

N -2 =Degrees of freedom

The statistical significance of correlations was tested using 't' test as given by Snedecor and Cochran (1967) at (N - 2) degrees of freedom.

## Results and Discussion

**Heritability estimates of monthly test day milk yields, first lactation 305 days milk yield and lifetime milk yield**

The heritability of first lactation monthly test day milk yields ranged from 0.12 ± 0.06 (TD10) to 0.48 ± 0.09 (TD4) (Table 1). As the heritability of any trait is influenced by both genetic effects and the environmental effect so having variations in heritability of monthly test day milk records is quite obvious. The heritability is lower towards the later test days because of more environmental effect as compared to genetic effect. This information also points out that certain test day milk yields whose heritability is

**Table** 1 Heritability estimates for monthly test-day milk yields, first lactation 305 days milk yield and life time milk yield in Sahiwal cattle

| Milk yield traits | Test day | $h^2 \pm SE$ |
|---|---|---|
| TD1 | 6th | 0.16 ± 0.06 |
| TD2 | 35th | 0.30 ± 0.07 |
| TD3 | 65th | 0.27 ± 0.07 |
| TD4 | 95th | 0.48 ± 0.09 |
| TD5 | 125th | 0.24 ± 0.06 |
| TD6 | 155th | 0.29 ± 0.07 |
| TD7 | 185th | 0.17 ± 0.06 |
| TD8 | 215th | 0.15 ± 0.06 |
| TD9 | 245th | 0.30 ± 0.07 |
| TD10 | 275th | 0.12 ± 0.06 |
| TD11 | 305th | 0.13 ± 0.06 |
| FL305DMY | | 0.40 ± 0.09 |
| LTMY | | 0.34 ± 0.07 |

determined more by the genetic effects are much more useful and dependable for predicting the genetic superiority of a particular animal. In a study by Ratwan et al. (2020), heritability estimates for test day milk yields varied from 0.06 (TDMY8) to 0.40 (TDMY2). Gupta (2013) reported the heritability estimates of monthly test day yield ranging from 0.244 ± 0.127 to 0.463 ± 0.172. The heritability estimate for first lactation 305 days milk yield was 0.40 ± 0.09 and that of life time milk yield was 0.34 ± 0.07.Similar estimates for first lactation 305 days milk yield were reported by Gopal and Bhatnagar (1972), Singh (1981), Gandhi and Gurnani (1995), Mohanty (2001), Debbarma et al.(2010). Higher estimates were reported by Tomar et al. (1996), Dongre (2013) and Gupta (2013) The heritability estimates were reported to be low for first (0.072 to 0.079) lactation 305-day milk yield by Ved Prakash et al. (2017).

**Genetic and phenotypic correlations among monthly test day milk yields and 305 days milk yield, lifetime milk yield**

The estimates of genetic and phenotypic correlations among first lactation monthly test day milk yields with first lactation 305 days and lifetime milk yield are presented in Table 2. The genetic correlations among first lactation monthly test day milk yields and first lactation 305 days milk yield were found to be positive and highly significant ($p<0.01$). TD2 to TD5 and TD7 to TD10 showed the highest genetic correlation with first lactation 305 days milk yield which suggests that these first lactation test days can predict the FL305DMY to a better extent and thus can be used for its prediction. Higher the correlation among the independent and dependent variable, higher is the accuracy of the prediction model.

The phenotypic correlation of monthly test day milk yields with FL305DMY ranged from 0.29±0.04 (TD1) to 0.74±0.03 (TD6). Gupta (2013) reported the genetic and phenotypic correlation between the test day milk yields and first lactation 305 days milk

**Table 2** Genetic and phenotypic correlations among monthly test day milk yields with first lactation 305 days milk yield and life-time milk yield ( above diagonal = phenotypic correlation, below diagonal = genetic correlation

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LTMY | 0.05±0.03 | 0.07 ±0.04 | -0.46±0.05 | 0.05 ±0.01 | 0.04 ±0.01 | 0.13 ±0.02 | 0.40 ±0.05 | 0.37 ±0.05 | 0.39 ±0.04 | 0.31 ±0.04 | 0.21 ±0.03 | 0.50 ±0.03 | 1 |
| 305DMY | 0.29±0.04 | 0.60±0.04 | 0.68±0.05 | 0.72±0.05 | 0.72±0.05 | 0.74±0.03 | 0.72±0.03 | 0.61±0.05 | 0.56±0.05 | 0.42±0.04 | 0.31±0.04 | 1 | 0.92 ±0.21 |
| TD11 | 0.14±0.03 | 0.30±0.04 | 0.32±0.04 | 0.33±0.03 | 0.28±0.03 | 0.30±0.05 | 0.36±0.03 | 0.44±0.05 | 0.51±0.04 | 0.61±0.05 | 1 | 0.77± 0.39 | 0.93 ±0.49 |
| TD10 | 0.17±0.04 | 0.38±0.04 | 0.35±0.05 | 0.36±0.05 | 0.37±0.05 | 0.45±0.05 | 0.50±0.03 | 0.58±0.04 | 0.71±0.04 | 1 | 0.64±0.55 | 0.99±0.39 | 0.55 ±0.36 |
| TD9 | 0.18±0.05 | 0.45±0.04 | 0.47±0.04 | 0.49±0.04 | 0.50±0.03 | 0.56±0.03 | 0.65±0.03 | 0.76±0.03 | 1 | 0.98±0.23 | 0.63±0.45 | 0.91±0.15 | 0.69 ±0.26 |
| TD8 | 0.19±0.05 | 0.48±0.04 | 0.50±0.04 | 0.54±0.03 | 0.57±0.03 | 0.63±0.03 | 0.75±0.05 | 1 | 0.99±0.19 | 0.76±0.38 | 0.99±0.61 | 0.94±0.22 | 0.99 ±0.32 |
| TD7 | 0.20±0.04 | 0.51±0.04 | 0.55±0.05 | 0.64±0.04 | 0.70±0.04 | 0.78±0.05 | 1 | 0.99±0.18 | 0.91±0.20 | 0.99±0.40 | 0.66±0.63 | 0.99±0.14 | 0.99 ±0.44 |
| TD6 | 0.21±0.03 | 0.55±0.03 | 0.60±0.05 | 0.71±0.05 | 0.78±0.05 | 1 | 0.90±0.14 | 0.69±0.27 | 0.60±0.25 | 0.88±0.38 | 0.74±0.58 | 0.77±0.15 | 0.70 ±0.29 |
| TD5 | 0.20±0.03 | 0.59±0.03 | 0.67±0.05 | 0.79±0.05 | 1 | 0.99±0.09 | 0.99±0.19 | 0.99±0.26 | 0.75±0.24 | 0.85±0.44 | 0.99±0.68 | 0.93±0.11 | 0.48 ±0.30 |
| TD4 | 0.19±0.04 | 0.69±0.04 | 0.78±0.04 | 1 | 0.99±0.09 | 0.95±0.09 | 0.99±0.21 | 0.99±0.27 | 0.91±0.17 | 0.89±0.40 | 0.54±0.49 | 0.92±0.08 | 0.86 ±0.34 |
| TD3 | 0.26±0.03 | 0.79±0.05 | 1 | 0.99±0.07 | 0.95±0.15 | 0.87±0.17 | 0.92±0.25 | 0.61±0.34 | 0.62±0.27 | 0.74±0.44 | 0.26±0.60 | 0.84±0.14 | -0.53±0.35 |
| TD2 | 0.31±0.04 | 1 | 0.93±0.09 | 0.83±0.13 | 0.80±0.20 | 0.77±0.20 | 0.83±0.27 | 0.69±0.32 | 0.63±0.26 | 0.58±0.43 | 0.06±0.63 | 0.92±0.14 | 0.50±0.33 |
| TD1 | 1 | 0.53±0.40 | 0.23±0.46 | 0.27±0.39 | 0.15±0.52 | 0.17±0.48 | 0.49±0.54 | 0.61±0.55 | 0.69±0.43 | 0.99±0.43 | 0.99±0.42 | 0.66±0.32 | 0.49±0.53 |
| | TD1 | TD2 | TD3 | TD4 | TD5 | TD6 | TD7 | TD8 | TD9 | TD10 | TD11 | 305DMY | LTMY |

yield as -0.12 to 0.99 and 0.21 to 0.84 and Ved Prakash (2017) reported the range as -0.17 to 0.99 and 0.22 to 0.83 respectively in Sahiwal.

## Conclusions

The genetic and phenotypic parameters of monthly test day milk yield, first lactation 305 days and life time milk yield were estimated using LSML software. The heritability of above mentioned traits were estimated using paternal half-sib correlation method. From the above study it can be concluded that the heritability of first lactation monthly test day milk yields ranged from low to medium with highest heritability at fourth test day i.e. on 95$^{th}$ day. First lactation 305 days milk yield was found to be highly heritable and life time milk yield showed medium heritability. The estimates of phenotypic and genetic correlation among all the first lactation monthly test day milk yields ranged from 0.14 to 0.79 and 0.15 to 0.99 respectively. First test day milk yield had the lowest and sixth test day milk yield had the highest phenotypic correlation with FL305DMY. The genetic correlation of seventh and tenth first lactation monthly test day milk yields with first lactation 305 days milk yield was found to be the highest in the present study. TD2 to TD5 and TD7 to TD10 milk records showed higher genetic correlation with first lactation 305 days milk yield. This suggests that better statistical models with higher accuracy can be developed for the prediction of first lactation 305 days and lifetime milk yield of Sahiwal cattle using test day milk records having higher genetic correlation with them. This will also reduce the labor and expenditure of maintaining records by the farmers and help in developing better strategies of selection in cattle breeding programs.

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RESEARCH ARTICLE

# Choice modelling for participation in milk marketing channels: Evidence from Punjab, India

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**Abstract:** Over the last few decades India has witnessed tremendous growth in the dairy sector giving varied market opportunities to the milk producers. There have been growing concerns regarding exclusion of the lower castes and small holder producers from organised milk marketing channels. In this context, the paper studies the choice of milk marketing channel in relation to the demographic variables using a set of household level data from the districts of Ludhiana and Moga districts in Malwa region of Punjab. Choice modelling has been done using Bivariate Probit Model and the econometric model has been operationalized using software R. To overcome selection bias, a two-step analysis is undertaken to explain participation in liquid milk markets and conditioned on that is the choice of milk marketing channel. The study found that Buffalo is the preferred milch animal in this area. The region is dominated by organised milk marketing channel with 69% households selling milk to the organised channel. The study concludes that social barriers which are manifested by caste of the household hamper the capability of the SC households to participate in the organised milk marketing channel.

**Keywords**: Bivariate probit model, Informal marketing channel, Organised marketing channel

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## Introduction

From the shortage of milk in the country at the time of independence (1947), India has since 1998 emerged as a top ranking milk producing country in the world. At present India produces almost 20% of the total liquid milk produced in the world. According to GoI (2017), the country produced 165.4 million tonnes in 2016-17. Milk group is the biggest source of income generation within the agricultural sector with an annual output value of Rs. 416611 crore, which is nearer to the combined value of cereals and pulses in the same period (CSO 2017). Milk and milk products are also an important source of calorie and protein intake for the significant vegetarian Indian population. Between 2000-01 and 2011–12, the share of milk and milk products in the monthly per capita food expenditure increased from 15.4% to 18.7% in rural areas and from 18.9% to 20.3 % in urban areas (GoI 2001; GoI 2012). India is thus witnessing tremendous growth in the dairy sector due to rise in market demand, expanding urban population, changing lifestyles, and increasing health consciousness (Kumar et al. 2014). Due to formalization of Indian dairy sector, food safety regulations are becoming increasingly important in the dairy sector in India. These changes make market access more difficult for the informal sector and encourage a higher involvement of the private sector in organized milk marketing. All these factors have contributed to creating a market interest for the newer players in the dairy sector and are catalytic in expansion of business operations of traditional cooperatives and companies already in dairy business.

Since past three decades, dairy sector in the country transitioned from protectionism to liberalization which led to the growth of the private milk processors (Birthal et al. 2017). The state of Punjab, the domain of our study and one of the most dairy-developed states in India too witnessed this trend. (Appendix I). By 2016-17, the milk processing capacity in the state increased to more than 80 lakh litres per day by 2016-17 from 42 lakh litres per day at the time of operation flood. A major part of the total milk processing capacity, around 75% belongs to the private sector.

Consequently, the newly emerged dairy ecosystem is providing more avenues to the farmers for selling milk at the village level. With multiple players in the field, dairy sector is now witnessing

enormous complexity in the dairy supply chain in rural India. In this context, it is imperative to understand how the demographic factors influence the households' choice for milk marketing channel at the village level.

More specifically, the research questions is: "What are the demographic determinants of milk producing households' participation in organized/ informal marketing channels in the Malwa region of Punjab?"

In India, specifically Punjab, there are few studies which investigated the factors which impact the choice of milk marketing channels at the household level. Some studies provide anecdotal evidence of smallholder participation in organized marketing channels in Punjab (Vandeplas et al. 2013; Sharma 2015; Birthal et al. 2017; Brar et al. 2018). These studies are spread over a wider geographical area and hence miss out on the local nuances. The current literature also captures the impact of both demographic variables and village-specific variables such as distance from the urban centres, road density, etc. on the choice of milk marketing channel. Most of the current literature uses a single step multinomial and binomial probit (Vandeplas et al. 2013) and multinomial logit (Sharma 2015; Birthal et al. 2017) to understand the choice of milk marketing channel. None of these studies incorporate the selection bias in the choice of milk marketing channel. The selection bias becomes important because not all households who have a milch animal, participate in liquid milk markets. Staal et al. (2006) however has carried out a two-step analysis to explain milk market participation and conditioned on that, milk outlet choice in Gujarat.

The present study restricts itself to two important districts in central plains (Malwa region) of Punjab – namely Ludhiana and Moga. Further, the five villages in these two districts were selected such that they have similar agricultural and livestock economy, distance from the urban centre, presence of organized and informal milk marketing channel, number of milk collection centres. This study uses a bivariate probit model – with a selection equation to determine the participation of dairy farmers in liquid milk markets.

## Materials and Methods

The major objective of the research was to understand the determinants of marketing channel choice for milk producing households in the five selected villages in Ludhiana and Moga district of Punjab which have a well-developed milk economy. A two-stage sampling – purposive sampling followed by stratified random sampling was done to arrive at the sampled households.

In order to control the effect of the various village level characteristics and study the impact of only of demographic characteristics on the choice of milk marketing channel purposive sampling was done at the first stage. Five villages – three from Ludhiana district and two from Moga district were selected during this first stage after discussions with various stakeholders. The villages selected in the study were such that they do not have a vast regional and infrastructural difference. All the five villages have similar basic physical infrastructure including anganwadis, primary and secondary schools, primary health clinics, government water tanks and reverse osmosis water purification systems at the village level. The main crops produced are rice, wheat, and fodder in all the five villages. All the villages are at a distance less than 20 Km from the district sub-centre. Market for dairying was well developed in all the selected villages. All the villages were similar in terms of presence of organized/formal and informal milk marketing channels.. The different organized players for milk collection present in these villages are indicated in Appendix II.

At the second stage, given the central importance of caste systems in Indian agriculture, caste-based strata were formed, and then systematic random sampling was used in each strata to select the households at the village level. The data for the study was collected in May, June, and July of 2016 – this is the supposedly lean season of dairy animals.

The dairy farmers have a choice to sell the milk through organized marketing channel which includes cooperatives (Milkfed), MNCs (Nestle, Danone etc.) and domestic private processors; or informal marketing channel (mainly milkman) which includes local traders and vendors; or consumer – households. In the sample, most of the members selling milk to the organized sector did not sell milk to the informal sector and vice versa. However, very few members who sold milk to both the channels and exclusively to consumer households were dropped from the data. Finally we arrived at a sample of 214 households from 5 villages, 107 belonged to Schedule Caste (SC), and 107 belonged to general caste.

Explanatory variable includes various demographic characteristics of the household and head of the household. Dairying is an economic activity which requires two basic assets – land for fodder/grazing and labour for maintenance/upkeep of the dairy animals. Accordingly, the first two variables are agricultural land owned by the household and the number of family members, which is a proxy for labour. Caste of the household signifies social and economic hierarchical position of the household in the rural areas and is included as the third variable in the study. Since the census-2011 does not include OBC/BC as one of the castes, so the study categorizes caste as General and SC only. Since the study focuses on number of milch animals and participation of households in the organized milk marketing channel, the number of cattle and buffaloes are also included as variables. The characteristics specific to the head of the household which are included in the study are – gender, age and education of the head of the household. Dairying is a labour-intensive occupation with a large participation of females in it. Thus, it is important to understand the impact of gender of the household

head on number of milch animals and participation in organized milk marketing channels. With more youth being educated and with more than 65% of Indian population below the age of 35 years, it will also be interesting to understand the impact of age and education on participation of households in organized milk marketing channels. The variables used in the study are detailed in table 1.

**Econometric model and estimation procedure**

The choice of milk marketing channel is a selection process which is either buyer-driven or a result of self-selection of the seller, i.e. milk producing household or a mix of both (Vandeplas et al. 2013). A buyer chooses a particular milk producing household to work with - the households with a larger asset base can make complementary investments, households with a more number of milch animals can lead to reduced transaction costs for the buyer or a household with smaller number of milch animals may reduce the procurement risk faced by the buyer, etc. Certain observable and non-observable characteristics may lead a milk producing household to sell milk to a particular milk marketing channel. In the paper, we investigate how the probability of supplying milk to a particular marketing channel varies across milk producing household as a function of relevant characteristics of the household. The model has a mix of continuous and discrete dependent variables which are indicative of the socio-economic and demographic characteristics of the households.

To overcome selection bias, a two-step analysis is undertaken to explain participation in liquid milk markets and conditioned on that is the choice of milk marketing channel. The model used in the study is bivariate probit with sample selection, which is equivalent to Heckman's selection model except now we have probit model in selection equation and a probit model in the outcome equation (Greene 2015). The model consists of two components: a probit equation for participation in liquid milk markets and a probit equation for the outcome, i.e. choice of a milk marketing channel which is observed only for the sub-sample.

The household is assumed to select the alternative- whether to participate in liquid milk markets or not, that gives the household maximum utility. The expected utility of selling milk, i.e. participation in liquid milk markets is given by:

$$U_i = \alpha X_i + u_i \qquad (1)$$

Where $\alpha$ is a vector of unknown parameters; $X_i$ is the vector of exogenous values of observation, i.e. household and household head specific characteristics namely - namely Members, Land, Caste, Cattle, Buffaloes,GenderHH, AgeHH, and EducationHH and $u_i$ is the unobserved random component which is assumed to be jointly normally distributed. We cannot observe the utility, but we know if a household participates in liquid milk markets or not, $Y_{i1}$.

$$Y_{i1} = \begin{cases} 1 \; if U_i > 0 \\ 0 \; otherwise \end{cases}$$

The probit model for participation in liquid milk markets is given by:

$$Y_{i1} = X_{i1}\beta_1 + \varepsilon_{i1} \qquad (2)$$

On similar lines, the probit equation for the choice of milk marketing channel is:

$$Y_{i2} = X_{i2}\beta_2 + \varepsilon_{i2} \qquad (3)$$

Where $Y_{i2}$ is a vector of two marketing channel choices namely organised sector and informal sector ( = 0 for organized sector and = 1 for informal sector) of the $i^{th}$dairy farmer. is a vector of household and household head characteristics that together reflect the capacity, risks and incentives to supply milk to a particular marketing channel.

Where, and are the unobserved components. It is likely that these components are correlated across individuals. is observed only when =1 and is defined only on sub population for which =1. These issues lead to the problem of selection bias in the study.

There are three types of observation in the sample:

$$Y_{i1} = 0$$
$$Y_{i1} = 1, Y_{i2} = 0$$
$$Y_{i1} = 1, Y_{i2} = 1$$

The probability of each of the observation is given by:

$$\Pr(Y_{i1} = 0) = \Phi(-\beta_1 x_{i1})$$
$$\Pr(y_{i1} = 1, y_{i2} = 0) = \Phi(\beta_1 x_{i1}) - \Phi_2(\beta_1 x_{i1}, \beta_2 x_{i2}, \rho)$$
$$\Pr(y_{i1} = 1, y_{i2} = 1) = \Phi_2(\beta_1 x_{i1}, \beta_2 x_{i2}, \rho)$$

Where Ö is the cumulative distribution function (CDF) of the standard normal distribution,is the distribution to estimate bivariate probit models, parameters and are typically estimated by maximum likelihood and is the correlation coefficient.

For the two-step analysis, in the first step, we use a probit model to understand the milk-producing households' decision to participate in milk markets, which is either a 'Yes' or a 'No'. Inverse mills ratio is calculated in this step and is introduced as an additional explanatory variable in the next step which is a probit model with the dependent variable as the choice of milk marketing channel which can either be organized channel or the informal channel. The marginal effect of each dependent variable is also calculated using the sample mean of all variables.

The procedure is implemented in R (version 3.5.1) using package "sampleSelection" and marginal effects having been calculated using package "margins".

## Results and Discussion

### Descriptive analysis

A summary of household level data captured in the study is presented in table 2 and 3. A total of 214 households – 107 belonging to SC caste and 107 from general caste from 5 villages were surveyed. In the sample, 104 households –44 belonging to SC and 60 from general caste own a milch animal – of which only 69 (66%) households reported selling milk through the available marketing channels. The rest 35 (34%) reared milch animals only for household consumption.

The average household in the study is headed by a male with an average age of 55 years and an average education level of 5.13

**Appendix I** Growth of Milk Processing Sector in Punjab (1989-2017)

| Year | Milk Plants | | | Chilling Centres | | | Capacity ('000 liters per day) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cooperative / Public | Private | Joint sector | Cooperative / Public | Private | Joint sector | Cooperative / Public | Private | Joint sector |
| 1989-90 | 11 | 5 | - | 39 | - | - | 1210 | - | 760 |
| 1996-97 | 11 | 17 | - | 32 | - | - | 1585 | 2645 | - |
| 2016-17 | 9 | 65a | 3 | 49 | - | - | 1975 | 6038 | 500 |

a: data for 2013-14

Source: GoP (1990, 1997, 2017) - Statistical Abstract of Punjab

**Appendix II** Presence of milk collection centres of various organized players in the selected villages

| Name of the Village | Danone | Nestle | Verka | Others |
|---|---|---|---|---|
| Bhundri | Yes | Yes | - | Yes |
| KilliChahal | Yes | Yes | - | - |
| KokriKalan | Yes | Yes | Yes | Yes |
| KokriPhula Singh | Yes | Yes | Yes | - |
| SherpurKalan | Yes | Closed recently | Yes | Yes |

Source: Field Study

**Table 1** Explanatory variables used in the study for primary data collection

| Symbol | Definition | Unit of measurement |
|---|---|---|
| Members | Number of family members in a household. | Number |
| Land | Agricultural land owned by the household in acres. | Acres |
| Caste | Social caste of the household and has been categorized as Schedule Caste (SC) or General | Categorical |
| Cattle | Total number of adult female cattle (includes both indigenous and crossbred/exotic cattle) in the household | Number |
| Buffalo | Total number of adult female buffaloes in the household | Number |
| Milch animals | Total number of adult female bovine animals in the household. It is a sum of cattle and buffalo explained above | Number |
| GenderHH | Gender of the head of household and is categorized as male (M) and female (F) | Categorical |
| EducationHH | Number of years of schooling of the head of the household | Number |
| AgeHH | Age in years of the head of the household | Number |

years. The average herd size is 4.2 animals for General caste households while it is 1.2 for SC households.

From table 2, we observe that there is a positive relationship between asset ownership, i.e. average land holding size and average number of milch animals. A larger land holding size gives easy access to fodder, thus helps in production of surplus milk for sale. As per table 3, average land ownership is higher for the households selling milk to the organized channel, which suggests that organized channel reduces its transaction costs and ensures stability in their operations by working with households with a larger asset base. Average herd size of both categories of farmers – those supplying milk to organized channel as well as informal channel is almost comparable at 7 dairy animals per household.

Contrary to previous studies (Vandeplas et al. 2013; Sharma 2015) which conclude that smallholders are more likely to participate in milk markets, this study suggests that dairying is equally practiced by smallholder and large holder milk producing households in the districts of Ludhiana and Moga, Punjab. As per table 2, 52% of the milk-producing households who sell milk have a herd size of less than 5 dairy animals and own 29% of total dairy animals. Remaining 48% of the households have a herd size greater than 6 animals, and they own 71% of total dairy animals.

The number of milch animals are positively correlated with the number of household members. Households which sell milk have more number of members in the family in comparison to those who do not sell milk, wherein additional members act as surplus labor for dairying activities.

As per table 3, 69% of the households sell milk to the organized channel while the remaining 31% sell milk to the informal channel. Average number of milch animals and an average number of members are almost comparable for the households selling milk to the two different channels.

**Table 2** Demographic data of surveyed households segregated by number of milch animals

| | Households which sell milk (69 households) | | | | | Households which do not sell milk(145 households) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Range of milch animals | 1-2 | 3-5 | 6-10 | >10 | Total | 0 | 1-2 | 3-5 | Total | Overall (214 HHs) |
| Average age of head of the household (years) | 58.46 | 61.78 | 55.00 | 58.70 | 58.45 | 54.05 | 55.42 | 56.78 | 54.46 | 55.75 |
| Average land ownership (acres) | 5.31 | 7.37 | 7.87 | 17.35 | 8.59 | 2.51 | 0.65 | 4.22 | 2.29 | 4.32 |
| Average Education of head of the household (years) | 6.00 | 4.65 | 5.39 | 4.20 | 5.09 | 5.55 | 4.46 | 2.22 | 5.14 | 5.13 |
| Average number of Members | 6.38 | 5.87 | 5.87 | 8.60 | 6.36 | 5.24 | 5.38 | 7.11 | 5.38 | 5.70 |
| Number of SC households | 6 | 4 | 5 | 1 | 16 | 63 | 21 | 7 | 91 | 107 |
| Number of General households | 7 | 19 | 18 | 9 | 53 | 47 | 5 | 2 | 54 | 107 |
| Number of Cattle | 9 | 45 | 76 | 145 | 275 | 0 | 17 | 11 | 28 | 303 |
| Number of Buffalo | 11 | 49 | 101 | 76 | 237 | 0 | 22 | 23 | 45 | 282 |

**Table 3** Demographic data of households selling milk to different marketing channels

| | Organized | | | Informal | | |
|---|---|---|---|---|---|---|
| | General | SC | Overall | General | SC | Overall |
| Average age of head of the household (years) | 60.40 | 66.50 | 61.17 | 51.45 | 53.10 | 52.24 |
| Average land ownership (acres) | 10.40 | 0.50 | 9.17 | 11.45 | 2.70 | 7.29 |
| Average Education of head of the household (years) | 4.76 | 2.50 | 4.48 | 6.91 | 6.00 | 6.48 |
| Average number of Members | 6.17 | 8.67 | 6.48 | 5.64 | 6.60 | 6.10 |
| Average number of milch animals | 7.74 | 4.33 | 7.35 | 10.45 | 4.60 | 7.67 |
| Number of households | 42 | 6 | 48 | 11 | 10 | 21 |
| Average number of Cattle | 4.98 | 0.83 | 4.46 | 3.91 | 1.80 | 2.90 |
| Average number of Buffalo | 2.76 | 3.50 | 2.85 | 6.55 | 2.80 | 4.76 |

The plight of the people who are considered at the bottom of the social caste pyramid i.e. scheduled caste (SC) is clearly visible in the descriptive data. The marginalized section face hurdles not just at one level but at multiple levels – starting from asset ownership in terms of land and number of milch animals to opportunity of participation in organized milk marketing channel. Scheduled caste households have a stronger representation in categories with no or few dairy animals. Scheduled caste households are also less likely to sell milk. Only 41% of scheduled caste households are milk producers out of which only 36% sell milk. In the sample, 44 SC households own a milch animal, with 17 of them having more than 3 milch animals. However, only 6 of these households are able to engage with the organized milk marketing channel. Thus, most of the SC households are not able to engage with an alternative channel for milk sale. On the other hand, amongst general households, 56% of the households own an animal out of which 88% sell milk. Further, most of the SC households sell milk to the informal channel while organized channel is dominated by general caste households. 79% of milk selling general households sell milk to organized channel while only 37% of milk selling SC households sell milk to organized channel. As per the field discussions, many SC households prefer informal channel because of various facilities offered by them such as doorstep collection, the facility of advance payment, better prices especially during the lean season, etc.

Another interesting observation from the data is that milk producing households which sell milk prefer buffaloes over cattle in comparison to those who do not sell milk. The ratio of cattle is to buffaloes is 1.16:1 among households which sell milk while it is 1:1.6 among the households which do not sell milk. Also SC households prefer to keep more buffaloes than cattle. Further, households selling milk to the organized sector have 60% cattle in the total herd; households selling milk to the informal sector

**Table 4** Results of the selection equation (probit model for households' participation in milk markets i.e. those households which own a milch animal)

| Col (1) Independent variables | Col (2) Estimate | Col (3) Std. Error | Col (4) z value | Col (5) Pr(>\|z\|) | Col (6) Marginal effects |
|---|---|---|---|---|---|
| (Intercept) | -1.14932 | 1.10009 | -1.045 | 0.29613 | |
| Members | -0.03088 | 0.10059 | -0.307 | 0.75882 | -0.006876 |
| Land | -0.03794 | 0.02226 | -1.704 | 0.08830 | -0.008447 |
| CasteSC | -1.21389 | 0.41467 | -2.927 | 0.00342 ** | -0.3202 |
| GenderHHM | 0.3627 | 0.49083 | 0.739 | 0.45993 | 0.08023 |
| AgeHH | 0.0153 | 0.01502 | 1.018 | 0.30846 | 0.003406 |
| EduHH | 0.03475 | 0.03792 | 0.916 | 0.35957 | 0.007735 |
| Buffaloes | 0.26652 | 0.09222 | 2.89 | 0.00385 ** | 0.05933 |
| Cattle | 0.4287 | 0.15548 | 2.757 | 0.00583 ** | 0.09544 |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.0(Dispersion parameter for binomial family taken to be 1)Null deviance: 135.012 on 103 degrees of freedomResidual deviance: 83.461 on 96 degrees of freedomAIC: 101.46Number of Fisher Scoring iterations: 9 N=104Pr(>Chi)= 2.055e-08 ***Log likelihood = -41.73066 (df=9)

**Table 5** Determinants for informal milk marketing channel

| Col (1) Independent variables | Col (2) Estimate | Col (3) Std. Error | Col (4) z value | Col (5) Pr(>\|z\|) | Col (6) Marginal effects |
|---|---|---|---|---|---|
| (Intercept) | -0.57575 | 1.351764 | -0.426 | 0.6702 | |
| Members | -0.07806 | 0.113292 | -0.689 | 0.4908 | -0.01907 |
| Land | -0.00537 | 0.021883 | -0.245 | 0.8062 | -0.001312 |
| CasteSC | 1.59005 | 0.702701 | 2.263 | 0.0236 * | 0.4576 |
| GenderHHM | 0.53912 | 0.728976 | 0.74 | 0.4596 | 0.1222 |
| AgeHH | -0.01921 | 0.018348 | -1.047 | 0.2952 | -0.004693 |
| EduHH | 0.062109 | 0.050366 | 1.233 | 0.2175 | 0.01518 |
| Cattle | -0.00573 | 0.038507 | -0.149 | 0.8817 | -0.0014 |
| Buffaloes | 0.134627 | 0.065277 | 2.062 | 0.0392 * | 0.03289 |
| IMR1 | -0.11862 | 0.711554 | -0.167 | 0.8676 | -0.02898 |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05(Dispersion parameter for binomial family taken to be 1) Null deviance: 84.801 on 68 degrees of freedomResidual deviance: 59.061 on 59 degrees of freedomAIC: 79.061Number of Fisher Scoring iterations: 6 N=69Pr(>Chi)= 0.002253 **Log likelihood= -29.53073 (df=10)

have 62% buffaloes in the total herd. Cattle traditionally has been used for producing dung which is used as a fuel and fertilizer, for on-farm operations and finally for milk production. Cattle is also a revered animal in India. However, in current times with advancement in technology and science, the utility of cattle has reduced to being only milk producers. On a commercial scale, rearing of buffaloes is more beneficial to the dairy farmer. Buffaloes offer three main advantages over cattle. Firstly, buffaloes are an efficient converter of low-quality feed and coarse fodders. So they need not be fed with special feeds. Secondly, buffalo milk is higher in fat content compared to cow milk which helps in better price realizations for the farmer, especially if the milk is sold in the informal channel. Pricing of milk is only based on fat content in the informal channel while organized channel includes both fat and SNF percentage in pricing. Finally, there is no social taboo against buffalo slaughter. Households not only find it easy to dispose of male buffalo calves and those buffaloes which have outlived their productive life, but the increasing market of meat export also provides the households with an earning opportunity.

**Results of econometric model**

The estimates of the first step, the selection equation for the bivariate probit model (equation (3)) are presented in table 4. The estimate and average marginal effect of the variable are presented in column (5) and (6) respectively. The results of the probit model bring out that caste, cattle, and buffaloes owned by the household are significant in determining if a household participates in milk markets. Interesting to note here is that caste plays the most significant role. The probability of milk market participation falls by as much as 32% (on an average) by a mere switch from general to SC. SC households are majorly smallholders and the participation of SC may thus be low because of many impeding factors such as lack of market access, lack of market information, lack of infrastructure and compliance to food safety and quality standards (Bardhana et al. 2012).

Increase in a number of any of the milch animals – cattle or buffalo has a positive effect on participation in milk markets. However, the probability of households' participation in milk markets increases more with an increase in the number of cattle in comparison to the number of buffaloes. Probability of participation in milk markets increases by 5.9% with a unit increase in the number of buffaloes while it increases by 9.5% with a unit increase in the number of cattle – be it crossbreed or a local breed. As per the discussion with households, MNCs prefer cow milk over buffalo milk.

Land has a negative influence on milk market participation. If land size increases by one unit, then on average the probability of milk market participation goes down by 0.85%. This implies that households with a larger land holding have an increased focus on primary income-generating activity, i.e. agriculture and their dependence on dairying as a supplementary source of income decreases. Such households retain most the milk for household consumption.

In sync with the findings of Staal et al. (2006), none of the characteristics specific to that of the head of the household are significant. Nonetheless, males with higher education level and higher age are more likely to participate in the milk markets. Contrary to Bardhana et al. (2012) younger male farmers and better-educated farmers are not inclined to milk market participation in the Ludhiana and Moga district of Punjab.

The results of the probit model for marketing channel choice (equation (4)) are presented in table 5. Inverse mills ratio is insignificant, indicating that there is no evidence that selection bias is quantitatively important. Caste and number of buffaloes owned are the two significant factors explaining milk marketing channel choice. Marginal effects indicate that caste of the household followed by the gender of the head of the household has the highest average marginal effects on the choice of milk marketing channel.

The likelihood of supplying milk to the organized sector is considerably lower among the socially disadvantaged households. The probability of selling to an informal player increases by as much as 46% for an SC household in comparison to general households, which indicates that the informal channel is preferred by the SC households. It implies that either social barriers or economic barriers which the SC category is proxying exist for participation in organized marketing channels, which is consistent with the findings of previous researchers namely, Vandeplas et al. (2013) and Kumar et al. (2011). The possible explanation for this is based on our observation during data collection. From our observation and qualitative discussion with different households, we learned that the SC households and general households are grouped in two different clusters in the villages. Almost all the organized players have their collection centres in the cluster where the general households are located. This makes these collection centres "inaccessible" for the SC households – or at least difficult to access. However, the unorganized/informal players go to each household to collect milk – making it easier for the SC households to sell milk. Also, informal players pay marginally higher price of milk during the lean season in comparison to the organized channel which follows transparent and stable pricing policy.

The number of cattle has no significant influence on the choice of milk marketing channel. The negative coefficient of the number of cattle indicates that those households with more number of cattle tend not to sell milk to the informal sector. However, the marginal effect of cattle is very small. A decrease in a unit number of cattle decreases the probability of supplying milk to the informal sector by only 0.14% (Table 5).

Also, the number of buffaloes owned by a household has a significant and positive association with participation in informal marketing channels. An increase of 1 unit in the number of buffalo increases the probability of supplying milk to the informal sector by 3.2% on average. As per qualitative discussions, players in organized channel prefer low-fat cow milk for its conversion into value-added products, while informal channel prefers high fat buffalo milk because most of them sell liquid milk in the urban centres and convert only small quantities into value-added products.

Though insignificant, an increase in the productive assets of the household, i.e., land and members, increases the probability of supplying milk to the organized channel, which is consistent with the findings of Vandeplas et al. (2013). When household heads are male, have a higher education level and lower age group then they tend to supply milk to the informal channel. A change in the gender of the head of the household from female to male increases the probability of supplying milk to the informal sector by 12%.

**Policy implications**

Organized milk marketing channel has emerged as a dominant channel for milk procurement in Malwa region of Punjab. However, it has largely excluded the socially disadvantaged strata of population and procures milk largely from the upper caste households in the village. In order to fulfill the aim of inclusive society, it is imperative to include the people at the bottom of the social hierarchy in the procurement by organized milk marketing channels. Organized milk marketing channel prefers to procure cattle milk – can be indigenous or crossbred. Thus, inclusion of cattle in the herds owned by the households will be seen as an opportunity by organized milk marketing channels for milk procurement from such households.

## Conclusions

With the structural transformations taking place in the dairying landscape in the Malwa region in Punjab, the organized channel has become a dominant sector for milk procurement. Almost 70% of the households sell milk to the organized channel. Though being a dominant sector, it has largely excluded the less endowed and socially disadvantaged SC households. Caste barriers are not only limited to participation in the organized channel, but SC households also have a lesser number of milch animals and are also less likely to sell milk.

Contrary to the trend observed in Punjab, there is an increased preference for buffaloes followed by indigenous cattle in the Ludhiana and Moga districts. Econometric analysis suggests that that participation in milk market rises if the number of cattle and buffalo rises, and there is increased informal market participation if buffalo rises. As buffaloes do well with the coarse feed and also provide high-fat content milk, it is preferred by the informal channels for sale in urban centres. Contrary to it, cow milk is preferred by an organized channel for conversion into products such as packed milk and curd.

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RESEARCH ARTICLE

# Forecasting cattle and buffalo population in India – A time series analysis

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**Abstract:** Cattle and buffalo are known to be the symbol of wealth in rural India from time immemorial. To ensure the attainable socio-economic benefits from cattle and buffalo reaching the rural poor through effective strategies, a more complete extrapolation of cattle and buffalo population in the country for the future using forecasting tools was attempted in this study. Data on cattle and buffalo population from 1950-51 to 2016-17 were collected from various reports of BAHS and FAO. Various time series forecasting models were employed to identify the growth patterns and to predict the future trends in bovine population. The forecasting models used were compared to identify the best fit model. From the results of the study, it could be discerned that the Damped Trend Exponential Smoothing was found to be the best fit model for cattle population and forecasted value indicated that the cattle population in the country would be almost stagnant in the next three decades. The cattle population forecast showed a slightly decreasing trend from 2010-11 with 194.184 million to 187.661, 188.177, 188.191, and 188.192 millions in 2020-21, 2030-31, 2040-41 and 2050-51, respectively. However, in case of buffaloes, the Brown Exponential Smoothing model was found to be the best fit model and the buffalo population was predicted to increase from 2000-01 and the predicted populations were 116.663, 127.787, 138.910 and 148.921 million in 2020-21, 2030-31, 2040-41 and 2050-51 respectively.

**Keywords**: Cattle and buffalo population, Forecasting, Time series analysis

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## Introduction

Cattle and buffalo, providing food products like milk, meat and hide, have been generating productive employment and valuable income (Govt. of India, 2015-16 and Govt. of India, 2019), to the vast majority of rural households, majority of whom are small and marginal farmers, landless labourers and women. Total milk production in the country was 17 million tonnes in the year 1950-51 and since 1970s, milk production continued to rise, taking the country as the largest producer of milk in the world now by producing 13.1 per cent of world's milk, with milk production reaching 176.35 million tonnes in 2017-18 and achieving self-sufficiency in milk production (Govt. of India, 2018-19). This impressive growth could be attributed to the concerted efforts of large number of small dairy farmers, milk cooperatives, and planners who made possible crossbreeding of local low producing bovines with exotic germplasm and high producing buffaloes.

To be able to effectively plan strategies for optimizing milk production and for augmenting rural livelihood in the country, prediction of future bovine population using forecasting tools is required. Hence, this study attempted to untangle the future of Indian bovine population, considering the past and present trends.

## Materials and Methods

Data on bovine population from 1950-51 to 2016-17 were collected from the reports of FAO Statistics (www.fao.org) and the reports of Basic Animal Husbandry Statistics (of different years), Dept. of Animal Husbandry and Dairying, Ministry of Agriculture and Farmers' Welfare, Govt. of India, both of various years. Different forecasting models were employed to predict the future bovine population. The results of forecasting models fitted were also compared to identify the best fit model. Among various forecasting models, Auto Regressive Integrated Moving Average [ARIMA] - p, d, q and Exponential Smoothing [ES] models were fitted.

Various combinations of ARIMA models *viz*., ARIMA (1,1,1), (1,1,0), (0,1,1), (0,1,0), (0,1,2), (1,1,2), (2,1,0), (2,1,1), (2,1,2), (1,2,1),

(0,2,1), (1,2,0), (0,2,0), (0,2,2), (1,2,2), (2,2,0), (2,2,1), and (2,2,2) and ES models *viz*., Simple ES, Holt ES, Brown ES and Damped trend ES were tried. Choudhury and James (2014) used simple exponential smoothing, double exponential smoothing, damped-trend linear exponential smoothing and ARMA models for predicting crop yields.

Then, using the best model, two kinds of forecasts were performed: sample period forecasts and post-sample period forecasts. The former was used to develop confidence in the model and the latter to generate genuine forecasts for use in planning and other purposes. Forecasting accuracy of different models was identified by using the measures of indices like Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE). Ahmad and Ahmad (2013) also compared the ARIMA model and Exponential Smoothing Method in making a prediction, by examining the Mean Squared Error (MSE) and Mean Absolute Percentage Error (MAPE).

**ARIMA model**

The data used in the study were non-stationary and non-seasonal. ARIMA model is a combination of an Auto Regressive (AR) process and a Moving Average (MA) process applied to a non-stationary data series. A combined model that contains p (AR) term and q (MA) term is called ARMA (p,q). If the object series is differenced 'd' times to achieve stationary, the model is classified as ARIMA (p, d, q) model as discussed by Box et al. (2007). The basic criteria for choosing the best fit of ARIMA (p, d, q) model are given in Table 1.

The general form of ARIMA model of order (p, d, q) is

$$Y_t = \emptyset_1 Y_{t-1} + \emptyset_2 Y_{t-2} + \ldots + \emptyset_p Y_{t-p} + \mu - \Theta_1 \varepsilon_{t-1} - \Theta_2 \varepsilon_{t-2} - \ldots - \Theta_q \varepsilon_{t-q} + \varepsilon_t$$

Where,

$Y$ = Value at $t^{th}$ year;
$\varepsilon_t$'s = Error terms which are independently and normally distributed with mean zero and constant variance $\sigma^2$ for t =1, 2..., n;
$\mu$ = Constant and
$\phi_s$ and $\theta_s$ = Coefficients to be estimated.

**Simple Exponential Smoothing**

The Simple Exponential Smoothing (SES) model is a time series forecasting technique that can be defined using an additive model used to analyze data which have no trend and seasonal pattern. This is a method of estimation of forecasts of single weight or parameter. Greater weights are assigned to recent observation and smaller weights to distant observation (Sharpe et al. 2010). The model as given below:

$$F_{t+1} = F_t + \alpha(y_t - F_t)$$

New forecast value at time t+1 = Old forecast at time t + $\alpha$ (Error in the last forecast)

The smoothing constant () value is selected based on error minimization approach (Talwar and Goyal, 2019).

**Brown's Linear (Double) Exponential Smoothing model**

The double exponential smoothing model is used to model time series data which have trend, but not seasonality (Brown, 1963). Here, F' denotes a simple smoothed value and F" denotes a double smoothed value:

$$F_t' = \alpha Y_t + (1-\alpha) F_{t-1}'$$

$$F_t'' = \alpha Y_t' + (1-\alpha) F_{t-1}''$$

$$\alpha_t = F_t' + \left(F_{t+}' - F_t''\right) = 2F_t'' - F_t'$$

$\alpha_t$ = estimated smoothed level at time t

$$b_t = \frac{\alpha}{1-\alpha}\left(F_t' - F_t''\right)$$

$b_t$ shows the estimated trends at the end of time period t, for m period ahead forecast is

$$F_{t+m} = \alpha_t + m b_t$$

**Holt's Linear (Double) Exponential Smoothing model**

Holt's method can be implemented for the time series data demonstrating a trend (Hanke and Wichern, 2008). This method is appropriate for non stationary data and to make short term forecast. In this technique, level and trend components are smoothed separately using different parameters α and β. Holt's double exponential smoothing method uses three equations one each for level, trend and forecast.

$$L_t = \alpha Y_t + (1-\alpha)(L_{t-1} + b_{t-1})$$

$$b_t = \beta(L_t - L_{t-1}) + (1-\beta) b_{t-1}$$

$$F_{t+m} = L_t + b_t m$$

where,

$L_t$ = Level of time series at period t

$b_t$ = trend (slope) estimate of time series at time period t

$F_{t+m}$ = forecast at m period ahead of time t

α and β are smoothing constants for level and trend with their values lying between 0 and 1.

**Damped Trend Exponential Smoothing method**

The forecasts generated by Holt's linear method display a constant trend (increasing or decreasing) indefinitely into the future. Since empirical evidence indicated that this method tended to over-forecast, especially for longer forecast horizons, Gardner and McKenzie (1985) introduced a parameter that dampens the trend to a flat line sometime into the future.

The smoothing equations are,

$L_t = \alpha Y_{t+} (1-\alpha) (L_{t-1} + \varphi T_{t-1})$

$T_t = \gamma (L_t - L_{t-1}) + (1-\gamma) \varphi T_{t-1}$

The m-step-ahead prediction equation is

$$\hat{Y}_{t+m} = L_t + \sum_{i=1}^{m} \varphi^i T_i$$

This is the forecast y, m-steps ahead by taking the last available estimated level state and multiplying the last available trend (slope)$T_i$, with$\theta i$ =damping factor.

## Results and Discussion

Among various models fitted, the model with the lowest normalized Bayesian Information Criterion (BIC) value and better model fit statistics like higher $R^2$ and the lowest Root Mean Square Error (RMSE), Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE) was selected as the best fit model.

**Forecasting cattle population**

The criteria adopted for model selection for forecasting of cattle population are given in Table 2, like the values of $R^2$, RMSE, MAPE, MAE and Normalized BIC. The BIC value directly compares the information loss of models. Hence, a lower BIC value suggests a better model or best fit model. It can be discerned that the Damped Trend Exponential Smoothing was found to be the best fit model, since its BIC value (28.209) was the lowest among all the models, along with reasonably lower values of RMSE (1247937.145), MAE (539261.451) and MAPE (0.443) and the highest $R^2$ value (0.984).

After this model selection, the model parameters were estimated and the results of the estimates are given in Table 3. Based on the best fit Damped Trend Exponential Smoothing model, forecasting of cattle population was carried out at two stages *viz.,* sample period forecasts for the period from 2000-01 to 2016-17 (to develop confidence in model) and post sample period forecasts for 2020-21, 2030-31, 2040-41 and 2050-51 and the results are displayed in Table 4.

As could be seen, the Indian cattle population forecast shows that the cattle population had been slightly decreasing from 194.184 million in 2010-11 to 187.661, 188.177, 188.191, and 188.192 million numbers in 2020-21, 2030-31, 2040-41 and 2050-51, respectively. The results clearly indicate that the cattle population in the country would be almost stagnant in the next three decades. This underlines the fact that concerted programmes are required to be framed and implemented to ensure higher productivity to continue to satisfy the increasing demand for milk.

**Forecasting buffalo population**

The criteria adopted for model selection for forecasting of buffalo population are given in Table 5, like the values of $R^2$, RMSE, MAPE, MAE and Normalized BIC. From the table, it can be found out that the Brown Exponential Smoothing model was found to be the best fit model, since the BIC value was found to be the lowest (26.606), with the high $R^2$ value of 0.996. RMSE (577912.272), MAPE (0.445) and MAE (336752.723) were also lower in this model. The model parameter was estimated and the results of the estimate are given in Table 6.

Based on the best fit Brown Exponential Smoothing model, forecasting of buffalo population was carried out at two stages

**Table 1** Criteria for choosing the appropriate forecasting model

| Selection Criterion | Notation |
|---|---|
| Bayesian Information Criterion = n log(MSE) +K log n | BIC |
| $\text{Coefficient of Determination} = 1 - \frac{Error\ sum\ of\ square}{Total\ sum\ of\ squares}$ | $R^2$ |
| Root Mean Square Error = $\sqrt{\frac{1}{n-k}\sum \hat{\varepsilon}_t^2}$ | RMSE |
| Mean Absolute Error = $\frac{1}{n}\sum_{t=1}^{n} \lvert\hat{\varepsilon}_t\rvert$ | MAE |
| Mean Absolute Percent Error = $\frac{1}{n}\sum_{t=1}^{n} \left\lvert\frac{\hat{\varepsilon}_t}{y_t}\right\rvert \times 100$ | MAPE |

Where k = Number of parameters in the statistical model; n = Sample size; $y_t$ = Observed value; and $\hat{\varepsilon}_t$ = Difference between the observed and estimated values.

*viz.,* sample period forecasts for the period from 2000-01 to 2016-17 (to develop confidence in model) and post sample period forecasts for 2020-21, 2030-31, 2040-41 and 2050-51. Results are displayed in Table 7. From the forecasted values, it is evident that buffalo population is increasing linearly. The actual value of buffalo population during 2000-01 was found to be 93.831 million against the predicted value of 93.762 million.

The buffalo population was predicted to be increasing from 2000-01 and the predicted populations were 116.663, 127.787, 138.910 and 148.921 million in 2020-21, 2030-31, 2040-41 and 2050-51 respectively. The increasing trend of buffalo population in the past, present and future could be attributed to the higher fat content of its milk which fetches higher price. This inherent characteristic of buffalo must have adequately persuaded the farmers to rear more and more buffaloes over time.

While Prasad et al. (2004) found the average annual growth rates of cattle and buffaloes in India as 0.67 and 1.63 per cent, respectively during the period 1951-92, Prabu et al. (2012) found a positive growth of cattle during 1997 and 2003 census periods also in the country. Borah and Halim (2014) too found a positive average annual growth rate of cattle (1.83 per cent) in India.

**Table 2** Criteria for model selection for cattle population

| Model | $R^2$ | RMSE | MAPE | MAE | BIC |
|---|---|---|---|---|---|
| ARIMA (1,1,1) | 0.982 | 1279634.152 | 0.449 | 848577.423 | 28.343 |
| ARIMA (1,1,0) | 0.982 | 1356200.215 | 0.529 | 1003762.456 | 28.459 |
| ARIMA (0,1,1) | 0.982 | 1356200.231 | 0.529 | 1003762.478 | 28.459 |
| ARIMA (0,1,0) | 0.968 | 1689753.124 | 0.721 | 1372901.548 | 28.826 |
| ARIMA (0,1,2) | 0.981 | 1333415.456 | 0.508 | 964140.245 | 28.498 |
| ARIMA (1,1,2) | 0.982 | 1297745.153 | 0.445 | 842705.654 | 28.517 |
| ARIMA (2,1,0) | 0.982 | 1286037.456 | 0.452 | 855883.968 | 28.426 |
| ARIMA (2,1,1) | 0.983 | 1258719.012 | 0.464 | 876411.743 | 28.456 |
| ARIMA (2,1,2) | 0.984 | 1271794.014 | 0.457 | 862154.756 | 28.549 |
| ARIMA (1,2,1) | 0.982 | 1301902.245 | 0.420 | 795006.123 | 28.454 |
| ARIMA (0,2,1) | 0.979 | 1367974.365 | 0.347 | 654400.431 | 28.479 |
| ARIMA (1,2,0) | 0.979 | 1368205.348 | 0.346 | 652181.654 | 28.480 |
| ARIMA (0,2,0) | 0.979 | 1356819.489 | 0.339 | 639172.698 | 28.389 |
| ARIMA (0,2,2) | 0.979 | 1376650.564 | 0.372 | 703860.124 | 28.566 |
| ARIMA (1,2,2) | 0.980 | 1382529.154 | 0.374 | 707267.423 | 28.648 |
| ARIMA (2,2,0) | 0.979 | 1379807.469 | 0.352 | 665399.000 | 28.570 |
| ARIMA (2,2,1) | 0.980 | 1380997.324 | 0.379 | 715601.123 | 28.646 |
| ARIMA (2,2,2) | 0.982 | 1325554.781 | 0.427 | 808053.465 | 28.638 |
| Simple ES | 0.966 | 1751810.123 | 0.756 | 1444439.146 | 28.824 |
| Holt ES | 0.981 | 1331488.154 | 0.340 | 641808.135 | 28.347 |
| Brown ES | 0.981 | 1318859.215 | 0.335 | 631730.131 | 28.256 |
| Damped trend ES | 0.984 | 1247937.145 | 0.443 | 539261.451 | 28.209 |

**Table 3.** Estimates of the best fit Damped Trend Exponential Smoothing model for cattle population

| Model | Parameters | Estimate | SE | t | Sig. |
|---|---|---|---|---|---|
| Damped trend ES | Alpha (Level) | 1.000 | 0.292 | 3.420 | 0.001 |
| | Gamma (Trend) | 1.000 | 0.946 | 1.050 | 0.295 |
| | Phi (Trend damping factor) | 0.701 | 0.199 | 3.510 | 0.001 |

**Table 4** Forecasts of cattle population (in million)

| Year | Actual | Predicted | LCL(95%) | UCL(95%) | Residual |
|---|---|---|---|---|---|
| 2000-01 | 191.924 | 192.591 | 190.088 | 195.095 | -0.667 |
| 2010-11 | 194.184 | 194.673 | 192.170 | 197.176 | -0.488 |
| 2020-21 | - | 187.661 | 177.929 | 197.394 | - |
| 2030-31 | - | 188.177 | 161.331 | 215.023 | - |
| 2040-41 | - | 188.192 | 150.548 | 225.836 | - |
| 2050-51 | - | 188.192 | 142.963 | 233.422 | - |

**Table 5** Criteria for model selection for buffalo population

| Model | $R^2$ | RMSE | MAPE | MAE | BIC |
|---|---|---|---|---|---|
| ARIMA (1,1,1) | 0.999 | 567161.153 | 0.501 | 381150.716 | 26.788 |
| ARIMA (1,1,0) | 0.999 | 569149.589 | 0.520 | 393204.556 | 26.722 |
| ARIMA (0,1,1) | 0.999 | 577715.345 | 0.539 | 410116.112 | 26.752 |
| ARIMA (0,1,0) | 0.999 | 563598.589 | 0.596 | 401268.973 | 26.789 |
| ARIMA (0,1,2) | 0.999 | 565862.894 | 0.512 | 425689.178 | 26.987 |
| ARIMA (1,1,2) | 0.999 | 602589.241 | 0.536 | 394568.156 | 26.897 |
| ARIMA (2,1,0) | 0.999 | 586947.235 | 0.548 | 375984.679 | 26.698 |
| ARIMA (2,1,1) | 0.999 | 614859.245 | 0.591 | 389125.279 | 26.746 |
| ARIMA (2,1,2) | 0.999 | 598674.259 | 0.459 | 412689.265 | 26.823 |
| ARIMA (1,2,1) | 0.999 | 547785.295 | 0.437 | 338227.316 | 26.723 |
| ARIMA (1,2,0) | 0.999 | 625475.653 | 0.436 | 323676.971 | 26.914 |
| ARIMA (0,2,1) | 0.999 | 545105.745 | 0.439 | 342153.624 | 26.639 |
| ARIMA (0,2,0) | 0.999 | 598647.258 | 0.612 | 396587..415 | 26.789 |
| ARIMA (0,2,2) | 0.999 | 602146.359 | 0.658 | 412689.345 | 26.989 |
| ARIMA (1,2,2) | 0.999 | 576298.345 | 0.589 | 402356.246 | 26.874 |
| ARIMA (2,2,0) | 0.999 | 563897.156 | 0.754 | 396589.125 | 27.215 |
| ARIMA (2,2,1) | 0.999 | 587569.217 | 0.753 | 435689.456 | 27.198 |
| ARIMA (2,2,2) | 0.999 | 589246.256 | 0.659 | 482678.159 | 28.234 |
| Simple ES | 0.996 | 1265498.454 | 1.459 | 1140561.574 | 28.174 |
| Holt ES | 0.999 | 571353.153 | 0.466 | 357039.178 | 26.655 |
| Brown ES | 0.996 | 577912.272 | 0.445 | 336752.723 | 26.606 |
| Damped Trend ES | 0.999 | 576353.100 | 0.492 | 376768.763 | 26.745 |

**Table 6** Estimate of the best fit Brown Exponential Smoothing model for buffalo population

| Model | Parameter | Estimate | SE | t | Sig. |
|---|---|---|---|---|---|
| Brown ES | Alpha (Level and Trend) | 0.671 | 0.065 | 10.31 | 0.000 |

**Table 7** Forecasts of buffalo population (in million)

| Year | Actual | Predicted | LCL (95%) | UCL(95%) | Residual |
|---|---|---|---|---|---|
| 2000-01 | 93.831 | 93.762 | 92.605 | 94.921 | 0.068 |
| 2010-11 | 107.375 | 107.757 | 106.600 | 108.916 | -0.382 |
| 2020-21 | - | 116.663 | 112.815 | 120.513 | - |
| 2030-31 | - | 127.787 | 109.455 | 146.120 | - |
| 2040-41 | - | 138.910 | 100.185 | 177.636 | - |
| 2050-51 | - | 148.921 | 87.957 | 209.886 | - |

Thirunavukkarasu and Rajarathinam (2014), while forecasting milled rice production in India using data from 1960-61 to 2013-14, found that the most appropriate models were the ARIMA, Brown's model and Damped model. Chaudhari and Tingre (2015), while forecasting egg production in India, based on data from 1979-80 to 2010-11, found that the model which had minimum normalized BIC value (i.e., ARIMA (0,1,0)) was found to be the best model for predicting Indian egg production. Celik (2016) used Holt, Brown and Damped Trend exponential smoothing methods for forecasting production of cereals in Turkey based on the data from 1965 to 2015. Celik and Sengul (2016), while predicting the number of poultry in Turkey from 2016 to 2025 found that Damped exponential smoothing method was the best model for predicting the number of turkeys.

## Conclusions

Cattle population would be almost stagnant in the next three decades. This underlines the fact that concerted programmes are required to be framed and implemented to ensure higher productivity to continue to satisfy the increasing demand for milk. However, the buffalo population would increase linearly in the future, due to the much preferred higher fat content of its milk which fetches higher price, adequately persuading the farmers to rear more and more buffaloes. Provision of adequate quality inputs like feed, fodder and health cover needs to be continuously provided to further augment the productivity among cows and to satisfy the increasing demand from the increasing number of buffaloes, if milch bovines are to be continuously exploited to ensure food security and improved rural livelihood in the country.

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RESEARCH ARTICLE

# Constraints faced by the dairy farmers in production and marketing of milk in northern dry zone of Karnataka

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**Abstract:** The present study investigated production and marketing related constraints of dairy farming practice in Northern Dry Zone of Karnataka. The study covered 240 sample households selected randomly from four districts of Northern Dry Zone of Karnataka. About 66 per cent of dairy farmers belongs to marginal (32.50%) and small (33.75%) category. The study also revealed that the 93.75 per cent, 91.66 per cent and 87.91 per cent dairy farmers were facing high cost of feed and fodder, non-availability of quality feed round the year and susceptibility of crossbred animals to disease were the major production related problem, respectively. It was also noted that, the distant location of milk collection center (96%), low price of milk (92.50%), inadequate availability of regular market (91.25%), spoilage of milk due to poor hygiene and storage problem while carrying milk to procurement centre (83.33%) were the major constraints in marketing of milk. Thus efforts should be focused on making availability of quality feed round the year along with the improvement of marketing infrasture is win-win situation for development of dairy sector in the Northern Dry Zone of Karnataka.

**Keywords**: Co-operatives, Dairy, Income, Milk production, Marketed Surplus, Productivity

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## Introduction

The rain-fed farming continues to be critical for meeting the livelihood needs of a vast majority of resource-poor farmers in the chronically drought- prone areas of the country. The recent years have been experiencing recurring droughts and ill-distributed precipitation adversely affecting agricultural production, income and employment. At present, 68 per cent of the total geographical area of the country is prone to drought in varying degrees and about 33 per cent of it is chronically drought-prone (rainfall is less than 650 mm). Hardly, 29 per cent of the total cropped area in drought-prone districts is irrigated as against the all India average of 41 per cent (Kekene, 2014).

The livestock sector holds a great promise in providing income and employment particularly in drought prone areas (Nagarale, et al. 2015). It is interesting to note that the growth in livestock income has always been higher than the growth in crop income, even during the heydays of Green Revolution when the policy emphasis was largely on crop production. Livestock provides livelihood to two-third of rural community. It also provides employment to about 9.20 per cent of the population in India (Livestock Census Report, 2017). Among all the possible livestock enterprises dairy farming is most popular and successful venture. It assumes greater relevance in providing 'drought proofing' and ensuring income and employment for sustainable rural livelihood (Patel, 1993). According to Shukla and Brahmankar (1999) milk production contributes on an average 27 per cent of household income and their contribution varies from about 19 % in case of large scale farmers to about 53 per cent in the landless Category. Thus, dairy production has become an important component of rural development programmes in the rainfed areas of India, and is considered as an instrument for socioeconomic change to improve as income and quality of life (Nagarale, et al. 2015). During 208-19, milk production in the country recorded 187.70 million tonnes. The per capita availability of milk increased from 112 gm per day in 1968-69 to 394 gm in 2018-19 (NDDB, 2019-20). The country has to step up efforts for increasing milk production and other dairy products owing to growing demand for them (Rangnekar, 2006).

The productivity of dairy animals in dry farming conditions is low despite their large contribution to the total milk pool. Prospects of dairying under dry zone conditions would greatly benefit the resource-poor farmers and minimize their migration to cities in search of livelihood. Further, this sector can make significant contribution in promoting redistributive effect on income in favour of weaker sections in general. The examining constraints in dairy farming is not only important for producers but also serves as important bases for planning and policy purposes with a view to generate economic information's useful for projecting development activities in the dairy sector.

Constraints imply the problems faced by dairy farmers while adopting day-today animal husbandry practices in their dairy enterprises. If these constraints are identified, they are helpful to bridge the gap between dairy technology and its adoption by dairy farmers (Rathod et al. 2014). Keeping above facts in view, the present study has been undertaken with the objective to identify the major constraints faced by dairy farmers so that the findings could be used in upliftment of dairy enterprise in Northern Dry Zone of Karnataka.

## Materials and Methods

The proposed study was undertaken in Vijayapura, Bagalakot, Ballary and Koppal districts of Northern Dry Zone of Karnataka which would adequately represent the region. The district were purposively selected owing to the fact that the dairy development pace in selected districts was not as progressive as in the districts of southern region. Yet, dairy farming plays an important role in the rural economy of selected districts; where in about 27 per cent of the total household income is derived from livestock farming. In second stage from each district, four villages were selected randomly. For uniform representation dairy farming practices in each districts villages were selected in a way that, two villages from high bovine density area and two villages from low bovine density area. In third stage random sampling technique were employed in selection of fifteen dairy farming households from each village, and the total sample size was 240 dairy farming households. The required information was recorded with the aid of pre tested & well-structured interview schedules. The total milk produced by all milch animals in households was reckoned as per day milk production for household.

### Herd size

The herd size and the number of milch animals in the study area comprised of crossbred cows, local cows and buffaloes. The cattle population comprised of milch animals, heifers, and young stock (*i.e.* calves <1 year and calves >1 year age). In order to have an appropriate comparison, the herd size maintained by different categories of households were converted into number of Standard Animal Unit (SAU) using the conversion co-efficient factor suggested by Sirohi et al. 2015. (Table 1).

### Garrett's ranking technique

To find out the constraints faced by the farmers in production and marketing of milk in the study area, the Garrett's ranking technique (Woodworth, 1969) was used. Various constraints were framed for the study keeping in view the reports from the available literature. Accordingly, constraints were identified and sub divided into production and marketing related constraints for arriving at the response from the farmers.

The constraints were prioritized by using Garrett's ranking technique in the following manner**:**

$$\text{Percentage position} = \frac{100\,(R_{ij}-0.5)}{N_j}$$

Where,

$R_{ij}$ = Rank given for the $i^{th}$ item by the $j^{th}$, respondent and

$N_j$ = Number of items ranked by the $j^{th}$, respondent

The percentage position of each rank was converted into scores using Garrett table. For each constraint, scores of individual respondents were added together and divided by total number of respondents for whom scores were added. Then, mean score for each constraint was ranked by arranging them in the descending order.

## Results and Discussion

### Socio-economics profile of sample households

Among 240 dairy farming households, about 66 per cent of dairy farmers belongs to marginal and small category. The average size of the family in the study area was 6.80 which varied from 5.11 in marginal farmers to 9.17 in large farmers. About 80 per cent of the sample households had male as head of the family and further it was observed that 55 per cent of the head of the households' age was in the range of 40 to 60 year. Similar findings were reported by Raval and Chandawat (2012). The illiteracy rate was higher (38.46 %) in the case of landless dairy farmers and lowest (31.25 %) in the case large category of dairy farmers. Only 5.83 per cent of dairy farmers studied up to degree and above. Similar findings were observed by Ranganath (2008) found that only7 per cent dairy farmers had attained graduation degree or above. The average operational land holding of the sample households in the study area was 2.91 ha. Agriculture was the main occupation for 46.25 per cent of households. With respect to subsidiary occupation, about 50 per cent of sample households practiced

dairy as subsidiary occupation. Similar findings was reported by Shinde (2014) found 51 per cent of households in both irrigated and non-irrigated region occupied dairying as subsidiary occupation.

**Average herd size across the different categories of farmers**

The average herd size varied from 1.87 Standard Animal Units (SAU) in the case of landless farmers to 6.40 SAU in the case of large farmers for buffaloes. Buffaloes formed larger percentage in the total herd size (Table 2). On an average, the number of buffaloes was 4.02 SAU for all categories of farmers in northern dry zone of Karnataka. Crossbred cattle was the next in dominance after the buffaloes with average herd size of 2.92 SAU which varied from 1.99 SAU in the case of landless farmers to 3.56 SAU in the case of large farmers. The average milch animals (crossbred) in all categories of farmers were 1.64 units whereas in the case of buffaloes it was 2.70 SAU. It was further observed that, in the case of buffaloes, percentage of milch animals to the total animal was around 67.16 per cent and it was 56 per cent and 31 per cent for crossbred and local cow, respectively.

The local cows formed the only 13 per cent of total herd size in the study area (Figure 1). The important reason for paltry share of local cows in the total herd size could be due to greater emphasis of mechanization of agriculture in the study area resulting in lower effective demand of quality draft animals. Another attributable factor is the replacement of less profitable local cows with buffaloes and crossbred cattle for meeting the increasing demand of milk by the organized and unorganized dairy sector.

**Average daily milk yield of different species of milk animal**

The average daily milk yield of local cow varied from 2.58 litres in marginal farmers to 3.01 litres in large farmers. The overall average milk yield for all categories of farmers put together was 2.86 litres per animal per day in the case of local cattle. The average daily milk yield of crossbred cows varied from 7.55 litre in the case of landless farmers to 8.83 litre in the case of medium farmers (Table 3). The overall average milk productions for all categories of households were found to be 8.38 litre per animal per day. In the case of buffaloes, milk yield was observed to be marginally higher at 4.58 litres in medium farmers followed by large farmers, small farmers, marginal farmers and landless farmers with a milk yield of 4.56 litre, 4.49 litre, 4.41 litre and 4.02 litre, respectively. Average milk production per day was observed to be highest in crossbred cows (8.38 litre) followed by buffaloes (4.51 litre) and local cows (2.86 litre) (Table 3).

**Constraints in dairy farming practices**

Constraints imply the problems or difficulties faced by dairy farmers while adopting animal husbandry practices in their dairy enterprise. The constraints in milk production and disposal in the present study refer to all factors which may be social; economic organizational that individually or collectively hinder farmers from going for scientific dairy farming practices. Constraints are studied under two categories *i.e.,* constraints in the milk production and constraints in marketing of milk.

**Constraints in the milk production as perceived by the households**

From the Table 4, it was observed that majority of respondents *i.e.,* 93.75 were facing high cost of feed and fodder. This may be due to the fact that the demand for dry fodder and green fodder

**Table 1.** Standard animal units for southern India

| Animals | Localcow | Crossbredcow | Buffalo |
|---|---|---|---|
| Adult male (≥3 years) | 0.97 | 1.12 | 1.04 |
| Adult female (≥3 years) | 1.00 | 1.62 | 1.24 |
| Young stock male (<1 year) | 0.22 | 0.24 | 0.24 |
| Young stock female (<1 year) | 0.27 | 0.3 | 0.28 |
| Young stock male (>1 year) | 0.54 | 0.63 | 0.6 |
| Young stock female (>1 year) | 0.47 | 0.52 | 0.51 |
| Heifer | 0.82 | 0.86 | 0.77 |

**Table 2.** Average herd size on sample households

| Categories | Crossbred cattle | | | | | Local cattle | | | | | Buffaloes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry | Milch | Young stock | Other | Total | Dry | Milch | Young stock | Other | Total | Dry | Milch | Young stock | Other | Total | Grand total |
| Landless | 0.36 | 1.3 | | 0.01 | 1.99 | 0.32 | 0.12 | | 0.06 | 0.72 | 0.25 | 1.32 | | 0.08 | 1.87 | |
| Marginal | 0.32 | 1.38 | 0.39 | 0.8 | 2.89 | 0.36 | 0.14 | 0.22 | 0.11 | 0.83 | 0.32 | 1.74 | 0.24 | 0.03 | 2.33 | 6.05 |
| Small | 0.69 | 1.52 | 0.58 | 0.16 | 2.95 | 0.38 | 0.38 | 0.16 | 0.06 | 0.98 | 0.82 | 2.82 | 0.32 | 0.16 | 4.12 | 8.05 |
| Medium | 0.68 | 1.92 | 0.52 | 0.12 | 3.24 | 0.36 | 0.48 | 0.32 | 0.14 | 1.3 | 1.12 | 3.66 | 0.38 | 0.24 | 5.40 | 9.94 |
| Large | 0.72 | 2.12 | 0.58 | 0.14 | 3.56 | 0.48 | 0.52 | 0.25 | 0.11 | 1.36 | 1.38 | 3.98 | 0.56 | 0.48 | 6.40 | 11.32 |
| overall | 0.55 | 1.64 | 0.47 | 0.24 | 2.92 | 0.38 | 0.32 | 0.23 | 0.09 | 1.03 | 0.77 | 2.70 | 0.34 | 0.19 | 4.02 | 7.97 |

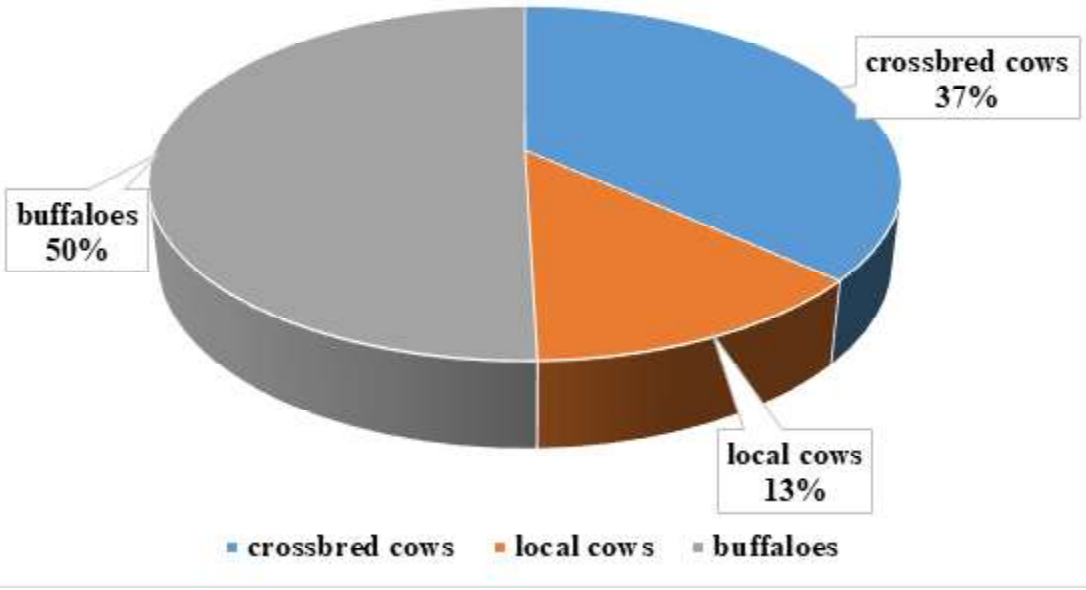


**Fig. 1** Proportion of different bovine species in total herd size

**Table 3.** Average daily milk yield of different milch animal species (litre/animal/day)

| Type of Animal | Categories | | | | | |
|---|---|---|---|---|---|---|
| | landless | Marginal | Small | Medium | Large | Overall |
| Local Cows | 2.58 | 2.85 | 2.98 | 2.88 | 3.01 | 2.86 |
| Crossbred Cows | 7.55 | 8.26 | 8.46 | 8.83 | 8.81 | 8.38 |
| Buffaloes | 4.02 | 4.41 | 4.49 | 4.58 | 4.56 | 4.41 |

**Table 4.** Constraints in milk production as perceived by the households

| Sr. No. | Particulars | Meanscores | Rank |
|---|---|---|---|
| 1 | High cost of feed and fodder | 93.75 | I |
| 2 | Non- availability of quality feed and fodder round the year | 91.66 | II |
| 3 | High susceptibility of crossbred animal to disease | 87.91 | III |
| 4 | Lack of A.I. and veterinary facilities near to village | 83.75 | IV |
| 5 | High cost of obtaining veterinary aid at doorstep | 79.58 | V |
| 6 | Poor knowledge of improved management practices (feeding, breeding *etc*) | 77.91 | VI |
| 7 | Low productivity in local cows and buffaloes | 74.16 | VII |
| 8 | High purchase value of crossbred cow | 68.33 | VIII |
| 9 | Inadequate of availability of extension | 67.08 | IX |
| 10 | Non-availability of grazing land | 65.41 | X |

rise in summer season would cause price rise of fodder and farmers would use more concentrate at high rate. Whereas 91.66 per cent respondents stated that non-availability of quality feed and fodder round the year. This might be because of the fact that northern dry zone of Karnataka more prone to drought and successive drought for the past two years brought down the production of green fodder which lead to scarcity of green fodder. About 87.91 per cent of farmers conveyed that susceptibility of crossbred animals to disease followed by lack of artificial insemination and veterinary facilities near to village (83.75%), high cost of obtaining of veterinary services at door step (79.58%), poor knowledge of improved management practices (77.91%). Low productivity in local cows and buffaloes (74.16%), high purchase value of crossbred cows (68.33%). Whereas 67.08 per cent of respondents were facing inadequate availability of extension services and only 65.41 per cent of respondents stated their constraint as non-availability of grazing land (Table 4). Similar findings were also reported by Michael et al. (2012) high cost of feed and fodder and non-availability of green fodder were major constraint of dairy farming. Rathod et al. (2009) reported that, non-availability of fodder around the year and lack of timely AI facility as major institutional constraint. Sonpasare et al. (2011) found lack of availability of green fodder as major constraint faced by the dairy farmers. Mohapatra et al. (2012) found lack of veterinary facilities was important constraint.

**Table 5.** Constraints in milk marketing as perceived by the households

| Sr. No. | Particulars | Meanscores | Rank |
|---|---|---|---|
| 1 | Distant location of milk collection center | 96.25 | I |
| 2 | Low price of liquid milk | 92.5 | II |
| 3 | Inadequate availableof regular market | 91.25 | III |
| 4 | Spoilage of milk due to poor hygiene and storage problem | 83.33 | IV |
| 5 | Delay in payment from co-operative society | 79.58 | V |
| 6 | High transport cost to delivery of milk to procurement center | 74.58 | VI |
| 7 | High penalty from co-operative society | 71.25 | VI |
| 8 | Nepotism in the society | 69.58 | VII |
| 9 | Price of milk is always based on the fat content from co-operative society | 63.75 | IX |
| 10 | Lack of transparency in fat measuring and pricing of milk | 61.25 | X |

**Constraints in the milk marketing as perceived by the households**

The study also revealed that that distant location of milk collection center (96.25%) was the main marketing constraint (Table 5). Other constraints are low price of milk (92.50%) followed by inadequate availability of regular market (91.25%), spoilage of milk due to poor hygiene and storage problem while carrying milk to procurement centre (83.33%) were the major marketing related constraints. Whereas, 79.58 per cent of respondents stated their constraint as delay in payment from co-operative societies. This may be due to the fact that the society credits the amount once in week or fortnight. About 74.58 per cent conveyed their constraint about high transportation cost or lack of transport facility for selling of milk to collection center. About 71.25 per cent of respondents stated their constraint as high penalty from co-operative society for spoilage or poor hygiene of milk. This may be because of majority of respondents had poor knowledge of scientific and clean milking method. Whereas 69.58 per cent of respondents expressed their constraints as nepotism in the societies followed by price of milk is always based on the fat content of milk from co-operative society (63.75%) and lack of transparency in fat measuring and pricing of milk (61.25%). The findings of constraints in marketing are in conformity with the results of Rathod et al. (2009), Jaya et al. (2012), Michael et al. (2012), Shisode et al. (2009) found lack of transport facilities as important constraint, Subhadra et al. (2009) reported low price of milk as major marketing constraint, Mohapatra et al. (2012) reported low milk price and poor marketing facilities as major constraint

## Conclusions

The study concluded that, the important constraints faced by the dairy farmers in Northern Dry Zone of Karnataka were, high feed cost (93.75%), non-availability of quality feed round the year (91.66%). Therefore government must initiate adequate steps to increase area under fodder crops and to resolve constraints faced by dairy farmers through development of veterinary facilities as majority farmers expressed that susceptibility of crossbred animals to disease, lack of artificial insemination and veterinary facilities near to village. Whereas in case of marketing of the milk, distant location of milk collection center, low price of milk, inadequate availability of regular market were the major constraints. Taking these constraints in consideration, effort should made to increasing the number of procurement centers, providing the remunerative price by ensuring the regular market is an need of the hour to improve the dairy farming in Northern Dry Zone of Karnataka.

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SHORT COMMUNICATION

# Faecal score and dry matter content after feeding synbiotics to neonatal Jersey crossbred calves

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**Abstract:** In order to observe the effect of synbiotic feeding to neonatal calves (treatment group) for 42 days, the fecal score ranged from 1 to 3 with weekly variation ($p<0.01$) among the calves. No difference was found in the fecal score and dry matter percent in both the groups control (synbiotic not fed) and treatment (synbiotic fed) calves. However, the faecal score was negatively correlated ($r=-0.564$, $P<0.01$) to the dry matter percent in faeces. Bacterial (*Lactobacillus sp.)* load count recovered from faeces was negatively correlated ($r=-0.072$) to the FS (faecal score improved) and positively correlated ($r=0.012$) to the DM percent in faeces. Therefore, faecal score and dry matter in faeces was better in calves fed synbiotics and *Lactobacillus sp.* persist ($P<0.01$) to be recovered from feces even after the post feeding period of synbiotics (43- 90 days).

**Keywords**: Faecal score, Fecal dry matter, *Lactobacillus rhamnosus* NCDC 298, Synbiotics,

Calves are susceptible to diarrhoea predominantly during the second week of life (Moran 2002). On feeding synbiotics, it is known to increase feed intake, body weight gain, milk digestibility, improve faecal consistency (Pranckute et al. 2016; Marcondes et al. 2016) with decreased coliform count and increase in IgG level in neonatal calves (Roodposhti and Dabiri, 2012). Accordingly, *L. rhamnosus* NCDC 298 in combination with FOS was found to be effective against toxin produced by *E. coli* (Anand et al. 2017) and was effective in preventing secretory diarrhoea in vitro (Mandal and Anand 2016). Therefore, subjective observation has been an important tool to assess severity of diarrhoea in young calves. Faecal scoring on 1 to 4 point scale increases numerically with the fluid content widely been used in a variety of studies (Moore et al. 2003 and Le Jambre et al. 2007). Faecal consistency scores of 1, 2, 3 and 4 had a dry matter percent of 20.9, 16.3, 9.6 and 5.8 respectively (Bellosa et al. 2011). The combination of the probiotic (*Streptococcus faecium)* and prebiotic (MOS) increased the feed intake and faecal consistency in calves (Morrison et al. 2010). The objective of the present study is to determine faecal consistency by means of scores and analyzing dry matter percent in faeces after feeding synbiotics to the young calves.

The study was carried out during the month of September to November 2017 at National Dairy Research Institute (NDRI), Eastern Regional Station, Kalyani, West Bengal, India. The latitude and longitude position of Kalyani is 22° 58'30"N and 88° 26'4"E, respectively with hot and humid climatic conditions. The average annual maximum and minimum temperatures is 39°C and 12°C, respectively with average annual rainfall of 1250 mm and relative humidity of 90%.

Twelve Jersey crossbred calves born in September to November 2017 were selected randomly and divided into two groups: treatment and control, consisting of 6 calves each. All the calves selected were separated immediately after birth from their mother. Animal in the treatment (T) group was offered synbiotics @ 100 ml/calf/day, dissolved in whole milk for 42 days while the control (C) group received only whole milk without synbiotics. The calves were fed colostrum and whole milk @ $1/10^{th}$ of the body weight through feeding bottles. Besides milk the calves were offered *ad lib* supply of concentrate, green fodder and water. Synbiotic was formulated using *Lactobacillus rhamnosus* NCDC 298 ($3.4x10^9$ CFU/ml) and Fructo oligosaccharides (FOS) (10%), in skimmed milk. It is then incubated for 12-16 h to get the final product. Feed intake and growth of the calves were recorded on weekly basis throughout the study period. Faecal quality (faecal score, dry matter and *lactobacillus* count) was analysed during

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the synbiotic feeding period and post feeding period of synbiotics. Faecal scour was recorded daily as 1= normal faeces, 2= slight liquid consistency, 3= denoted moderate diarrhoea and 4 indicated severe diarrhoea (Morrison et al. 2010). For estimating dry matter (%) and bacterial load count in faeces (log, CFU/g), faecal sample was collected from rectum by using sterile rubber gloves Bacterial load *(Lactobacillus sp.)* was recovered in the faeces by total plate count in MRS (HiMedia®) (Hasunuma et al. 2011). The colonies were further confirmed by bacterial morphology and gram staining techniques (Ewaschuk et al. 2004).

All the data were analyzed using SPSS software (Version 20) and Analysis of variance (ANOVA) was carried out accordingly. Pearson's linear correlation method (Steel and Torrie 1980) was used to correlate faecal score to faecal quality (percent faecal dry matter and faecal *lactobacillus* count).

Feeding synbiotic to the calves had no significant effect on dry matter intake (Table 1). The result was in compliance with the study of Simon et al. (2001).They stated that the non-significant finding was may be due to variations in the individual reactions of the animals. However, significantly higher ($P$<0.05) total body weight gain and average daily gain (Table 1) were found in the synbiotic fed group (29.08 ± 0.90kg and 323.14±0.01g, respectively) during 90 days experimental period. The result was in close correspondence to the findings of Dar et al. (2017) and they stated that higher weight gain may be due to better intestinal microbial balance which leads to efficient digestion and absorption of nutrients from the gastrointestinal tract.

The faecal consistency score of 1 to 3 was reported from both the treatment and control groups which had a median percent dry matter of 22.23, 17.66 and 8.72 respectively. The median for *lactobacillus* count (CFU/g) for faecal consistency scores in both the groups were 1, 2 and 3 was $1.1x10^7$, $3.2x10^7$ and $2.7x10^8$, respectively. The faecal sample with higher numeric scores (3) had high water content, indicating diarrhoea in calves. However, Bellosa et al. (2011) has reported faecal dry matter percent for scores of 1, 2, 3 and 4 as 20.9, 16.3, 19.6 and 5.8 respectively when the calves infected with *C. parvam.* Table 2. Represents the faecal scoring, percent dry matter and *Lactobacillus* load count in synbiotic fed calves at weekly intervals. No difference in faecal score and dry matter percent has been observed in treatment and control group (Table 2). On the contrary, Kehoe et al. (2008) reported lower faecal scores and scour incidence when probiotics was offered to the calves. No differences were observed for faecal score and percent dry matter in faeces between the treatment and control groups. But, faecal bacteria load count was higher in the calves in the treatment group while *Lactobacillus* count in faeces was higher (P<0.01) than the control group even after 42 days of feeding (Table 3, 4). The findings are in agreement to Heinrichs et al. (2009) who also reported an increase in *Lactobacillus* count in faeces of calves when the respective synbiotic formula were fed.

**Table** 1 Dry matter intake and body weight gain of the experimental calves up to 90 days period

| S.N | Parameters | Control | Treatment |
|---|---|---|---|
| 1 | DMI/100 kg Body weight | 3.06±0.19 | 3.01±0.16 |
| 2 | Total body weight gain in 90 days (kg) | $23.58 \pm 2.38^x$ | $29.08 \pm 0.90^y$ |
| 3 | Average daily gain in 90 days (g) | $262.03 \pm 0.03^x$ | $323.14 \pm 0.01^y$ |

$^{xy}$Differences in superscript in row indicate significance at $P$<0.05

**Table** 2 Weekly changes in the faecal scoring (1 to 4), faecal dry matter (%) and bacterial load in faeces (CFU/g) of the experimental calves

| Days | Fecal score (1-4) | | Dry matter (%) | | Bacterial load (log, CFU/g) | |
|---|---|---|---|---|---|---|
| | Control | Treatment | Control | Treatment | Control | Treatment |
| 7 | 1.40±0.32 | 1.02±0.16 | 23.37±3.44 | 26.02±2.75 | 5.39±0.20 | 6.56±0.71 |
| 14 | 1.07±0.04 | 1.00±0.22 | 30.67±1.65 | 25.63±3.33 | 5.62±0.69 | 6.82±0.52 |
| 21 | 1.33±0.25 | 1.35±0.16 | 21.99±2.03 | 22.44±3.63 | 4.60±0.61 | 5.98±0.46 |
| 28 | 1.14±0.07 | 1.25±0.17 | 31.20±4.47 | 21.06±1.13 | 4.40±0.54 | 5.63±0.38 |
| 35 | 1.45±0.18 | 1.21±0.02 | 19.40±0.79 | 22.39±1.62 | 4.97±0.70 | 7.02±0.48 |
| 42 | 1.30±0.14 | 1.19±0.01 | 21.64±1.20 | 23.86±1.73 | 5.21±0.67 | 6.25±0.39 |
| 49 | 1.07±0.04 | 1.38±0.32 | 21.641.28 | 20.34±0.89 | 5.44±0.35 | 6.88±0.25 |
| 56 | 1.21±0.15 | 1.33±0.22 | 23.42±2.14 | 20.60±1.25 | 5.04±0.31 | 6.11±0.51 |
| 63 | 1.19±0.16 | 1.19±0.15 | 19.68±0.63 | 22.28±2.25 | 4.71±0.31 | 5.71±0.53 |
| 70 | 1.69±0.20 | 1.16±0.16 | 18.27±0.70 | 24.28±2.16 | 4.61±0.31 | 5.12±0.43 |
| 77 | 1.38±0.19 | 1.50±0.20 | 20.39±0.70 | 19.04±1.02 | 5.22±0.40 | 5.56±0.22 |
| 84 | 1.57±0.18 | 1.16±0.21 | 20.02±1.72 | 20.71±0.85 | 4.58±0.37 | 5.26±0.25 |
| 90 | 1.33±0.21 | 1.16±0.16 | 21.37±1.03 | 20.65±0.69 | 3.17±0.45 | 4.86±0.12 |
| Overall | 1.32±0.05 | 1.24±0.44 | 22.54±0.67 | 22.25±0.05 | 4.84±0.40 | 5.98±0.13 |

**Table** 3 Faecal characteristics of the experimental calves during synbiotic feeding period of (42days) and after 42 up to 90 days period

| Parameters | Faecal score (1-4) | | Dry matter (%) | |
|---|---|---|---|---|
| Days | Feeding period (4-42 days) | Post feeding period (43-90 days) | Feeding period (4-42 days) | Post feeding period (43-90 days) |
| Control | 1.35±0.06 | 1.27±0.06 | 20.68±0.50 | 21.13±0.54 |
| Treatment | 1.28±0.07 | 1.21±0.08 | 24.71±1.25 | 23.57±1.00 |
| Overall | 1.32±0.05 | 1.24±0.45 | 22.54±0.67 | 22.25±0.05 |
| Sig. | | NS | | NS |

**P<0.01 significance

**Table** 4 Faecal *Lactobacillus* load count (log CFU/g) during synbiotic feeding period and post feeding period in experimental calves

| Parameters | Faecal *lactobacillus* load count (log CFU/ml) | |
|---|---|---|
| Days | Feeding period (4-42 days) | Post feeding period (43-90 days) |
| Control | 4.68±0.16 | 5.64±0.16 |
| Treatment | 5.03±0.23 | 6.38±0.20 |
| Overall | 4.84±0.40 | 5.98±0.13 |
| Sig. | ** | |

**P<0.01 significance

**Table** 5 Correlation between Faecal score with percent dry matter and bacterial load

| Parameter | Faecal score | Dry matter | Bacterial load |
|---|---|---|---|
| Faecal score | 1 | | |
| Dry matter | -0.564** | 1 | |
| Bacterial load | -0.072 | 0.012 | 1 |

**P<0.01

Not only between groups, weekly variation ($p<0.01$) in percent dry matter in faeces and faecal *Lactobacillus* load count has been found within the groups.

The faecal score was negatively correlated ($r=-0.564$, $P<0.01$) to percent dry matter content in faeces while bacterial *(Lactobacillus sp.)* load count recovered in faeces was negatively correlated ($r=0.072$) to the faecal score and positively correlated ($r=0.012$) to the dry matter percent in faeces (Table 5). The lower dry matter percent in faeces reflects the incidence of diarrhoea in calves. Similar trend was reported by Bellosa et al. (2011) and Agazzi et al. (2015) in calves fed probiotics and synbiotics, respectively. The faecal score was found to be higher in the first two weeks of life which then decreased at 3rd week and became normal by 4th and 5th week of age. Similar findings were reported by Bayatkouhsar et al. (2013) however, Quezadamendoza et al. (2011) did not find any effect of probiotics on diarrhoea.

## Conclusions

Hence faecal quality (faecal score, faecal dry matter and bacterial load) improved when synbiotics was fed to the neonatal calves. Further investigation with larger sample size over extended period of synbiotic feeding is necessary to give us the true picture of the trial.

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