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Supplementation of tomato pomace in *lassi* (a traditional Indian dairy product) and its effects on physico-chemical, functional attributes and shelf-life of *lassi*

Tikesh Kumar, Sunil Meena, Dinesh Chandra Rai and Raj Kumar Duary

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Abstract: Utilization of agriculture waste as food additives increased significantly due to their potential health and nutritional attributes. Tomato crop is widely grown across the globe and generates large amount of waste in the form of tomato pomace during the processing of tomato. Dried tomato pomace has a higher shelf-life and easier for food fortification. *Lassi* is a traditional Indian dairy product consumed as a refreshing beverage during summer. Considering the various health and nutritional benefits of tomato pomace present study was undertaken to supplement tomato pomace in *lassi*. Tomato pomace powder (TPP) was prepared by tray drying at 55-60 °C for 16-18 hrs. Pomace had 16.70% protein, 57.53% crude fibre, and 49.80% 2, 2 diphenyl-1-picryl hydroxyl (DPPH) inhibition activity, 311.7 mg gallic acid equivalents (GAE)/100g total phenolic content (TPC). Supplementation of tomato pomace was done at different rates of 0.5, 1.0, 1.5, and 2.0% (w/v) of milk during the heating of milk. 1% tomato pomace added *lassi* had a better sensory score and physico-chemical properties with an overall acceptability score of 7.5. In, addition 1 % tomato pomace *lassi* had 2.53% fat, 2.67% protein, 18.81% total solids, 0.66% ash, 21.81% DPPH inhibition antioxidant activity and 12.37 mg GAE/100 g. Tomato pomace powder added *lassi* was found stable up to 12 days of storage study while the control sample (without tomato pomace) was unacceptable after 9 days of storage. *Lassi* supplemented with tomato pomace rich in fibre and antioxidant activity. Utilization of tomato pomace in *lassi* fortification can be better alternative for tomato processor as *lassi* consumed very large segment of population.

Keywords: Antioxidant activity, Fibre, Fermentation, *Lassi*, Milk, Pomace, Tomato

Introduction

India is the largest producer and consumer of milk and milk products and has a nearly 23% contribution to global milk production. A major share (approximately 60%) of milk is utilized in the manufacturing of traditional dairy products due to their huge demand across the country. Several technological and functional advances attempted to popularize or commercialize these traditional dairy products. Various functional ingredients are incorporated into traditional dairy products for their value addition (Arora et al. 2022, Aneja, 2002). Numerous traditional fermented dairy products *i.e.* *Dahi*, *Mishti doi*, *Shrikhand*, *Buttermilk*, *Lassi*, etc. popular due to their organoleptic, nutritional and functional attributes. Fermentation of milk improved nutritional value and bioavailability of nutrients. Lactic acid bacteria used in fermentation helps in eliminating toxic and anti-nutritional compounds available in different food formulations (García-Burgos et al. 2020).

Lassi is a popular refreshing beverage that is commonly consumed in the Northern part of India. It is prepared by fermentation of milk by specific strain of starter culture (lactic acid bacteria), followed by mixing with sugar, water and flavouring ingredients (optional). *Lassi* possess various positive effects on human health by improving the immune and gastrointestinal health (Dahir et al. 2022). Consumer perception changes towards foods and food habits, they believe that food played important role in health (Mollet and Rowland, 2002). Food also prevents different nutritional-related diseases and improves the physical and mental health (Betoret et al. 2011).

Tomato (*Lycopersicon esculentum* L.) is the second-largest grown horticultural crop, worldwide. Annual tomato production is nearly 186.821 million metric tonnes across the globe (Branthôme, 2022) and India contributes around 11% of total tomato production (Kushwaha et al. 2018). As per World Processing Tomato Council (WPTC) in the year 2020, nearly 130 MT of tomatoes were processed and around 8 MT of tomato pomace was generated as waste. Presently, tomato pomace is used in many applications such as wheat flour mixes, dairy products, meat products and cattle feeds (Choo et al. 2021). Tomato pomace is rich in protein, fibre, antioxidants, carotenoids,

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and polyphenolic compounds (Isik and Topkaya, 2016). Tomato skin had a high amount of lycopene and polyphenolic compound compared to the pulp of tomato (George et al. 2004). Demand for fibre-incorporated products increased significantly due to their health benefits. Dietary fibre had several health-promoting properties against different diseases such as cardiovascular disease, diabetes - ii, obesity, improves immune systems and laxation effects (Barber et al. 2020). Tomato pomace had a good amount of fibre and antioxidant activity, which makes it a functional ingredient for food incorporation (Mudgil and Barak, 2016).

Alqahtani et al. (2020) prepared fibre and antioxidant enriched yoghurt by fortification of tomato pomace powder (0, 0.5, 1.0, 1.5 and 2.0% of milk). Study reported that TPP incorporated yoghurt had higher total phenolic content and radical scavenging activity, 6.47 ± 0.38 mg GAE/100 g (control); 12.56 ± 0.33 mg GAE/100 g (2% TPP) and 15.82 ± 0.35 μ mol TE/100 g (control), 17.56 ± 0.31 μ mol TE/100 g (2% TPP), respectively. In, addition other parameter such as acidity, colour value a^* and b^* significantly increased on TPP fortification. Yoghurt enriched with 1% TPP showed highest sensory scores and acceptable up to 15 days of storage (4°C). Several authors attempted to incorporated tomato pomace in various food preparation for functionality improvement such as addition of tomato pomace solids in low fat cake as fat substitute (Namir et al. 2015); Gluten-free ready-to-cook snack's enriched with tomato pomace (Rehal et al. 2022), Tomato pomace incorporated cookies (Bhat and Ahsan, 2015).

Considering the several nutritional and health-promoting properties of tomato pomace, the presents study was undertaken to prepare tomato pomace powder (TPP) and analysis of nutritional and functional properties of the powder was also carried out. Optimization of the level of tomato pomace powder in *lassi* was done based on physico-chemical, antioxidant activity and sensory properties of *lassi*. Shelf-life study of developed product evaluated and compared with control product.

Material and Methods

Material

Fresh and matured tomato (*Solanum lycopersicum L.*) of *Kashi aman* variety procured from local market of Varanasi, Uttar Pradesh in morning. Fresh cow milk collected from Dairy Farm, BHU and standardized at 3.0% Fat and 8.5% SNF and *lassi* were prepared by using a mixed starter culture of *Lactococcus lactis*, *Lactococcus diacetylactis* and *Lactococcus cremoris* (NCDC-217) procured from National Collection of Dairy Cultures (NCDC), Karnal. Cane sugar of commercial grade was brought from the local market of Varanasi, India. All the chemicals used during study were of Analytical Grade (AR) and were procured from reputed companies.

Preparation of Tomato Pomace Powder (TPP)

Fresh and matured tomato of *kashi aman* variety were sorted and properly washed using water and subjected to tomato pulper (Bajaj Processpack Pvt. Ltd., Noida, India) where the pulp is collected in a container and on completion of pomace remains in pulper strainer were segregated and dried at 55-60°C in air circulated tray drier (Make: Balaji Processpack Pvt. Ltd., India) for 16-18 hr and further ground at room temperature by circulating tap water around jacket of low-temperature grinder, (Make: Balaji Processpack Pvt. Ltd., India) into fine particle (passed through 40 mesh size sieve).

Optimization of the level of TPP in Functional *Lassi*

The standardized milk is heated to 85°C for 10 min and TPP added (at different levels 0%(T₀), 0.5%(T₁), 1.0%(T₂), 1.5%(T₃), 2.0%(T₄) (w/v)) and mixed properly by continuous mixing and cooled to 30-37°C. Milk is inoculated @ 2% with mixed starter culture (NCDC-217) and mixed properly. Further, incubated at 37± 1°C for 12 h and stored at 5°C for further use. *Lassi* was prepared by blending Dahi (75%), Water (25%) and Sugar (12%) by using a Hand Blender (Khera Instrument Pvt. Ltd. Delhi, India) and packed in 100 ml Polypropylene plastic cups. Optimization of TPP level was done on the basis of sensory (n=10), physico-chemical and antioxidant properties (n=3) of *lassi*.

Physico-chemical analysis of TPP and Functional *Lassi*

Chemical analysis

Fat, protein, total solids, crude fibre, ash and pH of TPP and developed *lassi* was determined by AOAC (2000) methods. Estimation of acidity and whey syneresis of *lassi* was done by protocol given by IS 1166: 1986 and Parnell-clunies et al. (1986), respectively. All the analysis done in triplicate (n=3).

Anti-oxidant activity

Antioxidant activity of tomato pomace powder (TPP) and TPP added *lassi* was determined by 2, 2 diphenyl-1-picryl hydroxyl (DPPH) free radical scavenging potentiality following method of Brand-Williams's (1995) with slight modification. In brief, 80 mg/ml (w/v) solution of the sample was prepared with absolute methanol and placed in a shaker for 2 hr, further centrifuge at 6000 rpm/10 min at 27°C. Supernatant (2.5 ml) was mixed with 5 ml of 2mM DPPH in methanol solution and vortexed. The mixture was incubated for 30 min in dark conditions and absorbance of sample and blank (80% Methanol solution) was measured at 517 nm. Antioxidant activity was calculated by the following equation and results are expressed as % free radical scavenging activity.

$$\text{Free radical scavenging activity (per cent)} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100$$

-equation 1

Total phenolic compound (TPC)

Total phenolic content of TPP and TPP added *lassi* was determined by method given by Zheng and Wang, (2001) using

Folin-Ciocalteus reagent. 100 mg/ml (w/v) solution of sample was prepared with 70% Acetone and placed in a shaker for 2 h, further centrifuge at 6000 rpm/15 min at 27°C. 20 µl supernatant was

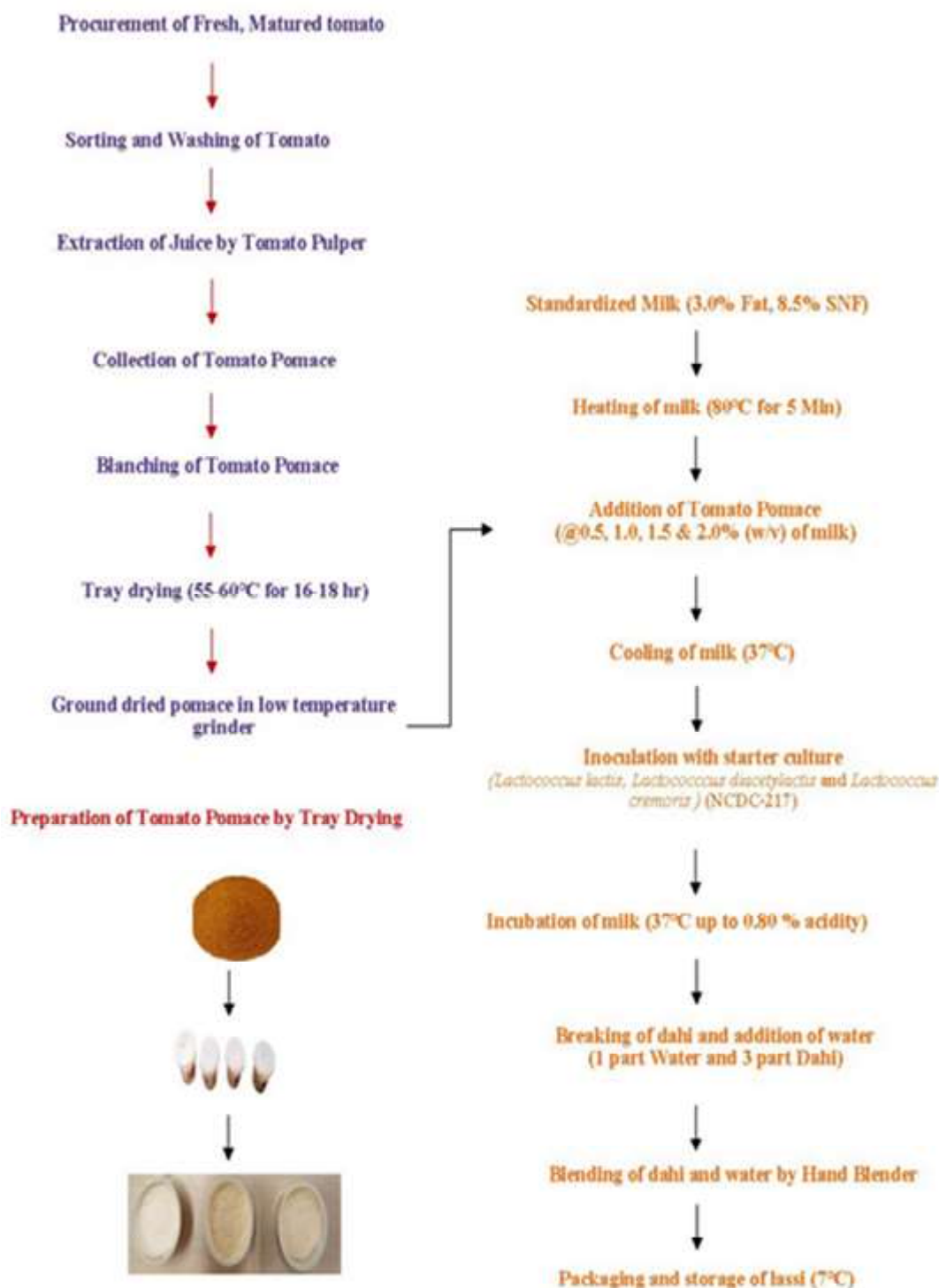


Fig. 1: Process flow diagram of Tomato Pomace incorporated *lassi*

mixed with 1.58 ml distilled water followed by addition of 100 μ l Folin–Ciocalteus reagent. The mixture was vortexed vigorously and left to stand at room temperature for 15 min and 300 μ l of sodium carbonate solution (20%) was added. Further, the mixture was stored at room temperature for 2 h and absorbance was measured at 765 nm.

Microbiological analysis

The Coliform and Yeast and mold count were estimated using Violet Red Bile (VRBA) agar and Potato Dextrose (PDA) agar (added 1% Tartaric acid) at suitable dilution of sample. Colonies in the plates were counted and the count was expressed as cfu/g (IS: 1479, 1962) after incubation at 30 ± 1 and $25 \pm 1^\circ\text{C}$, respectively. All the analysis done in triplicate (n=3).

Sensory evaluation

TPP incorporated *lassi* were subjected to sensory evaluation by an expert and semi-trained panel of judges (Trained according to ISO: 8586-2: 2008) (n=10) for various sensory attributes, viz., flavour, color and appearance, body and texture, sweetness, and overall acceptability criteria using a 9-point hedonic scale described by Stone and Sidel, (2004). Labelled samples of freshly prepared products were given to the panel of judges.

Statistical analysis

The data relating to chemical, sensory and functional aspects of tomato pomace powder incorporated *lassi* were analysed using one-way ANOVA using SPSS 16.0 software (SPSS INC, Chicago, IL, USA) and all the tests were done in triplicate (n=3).

Results and Discussion

Chemical composition of TPP prepared by tray drying

Different chemical and functional properties analyses of TPP are shown in Table 1. The average composition of TPP was $4.59 \pm 0.09\%$ moisture, $3.19 \pm 0.08\%$ fat, $16.70 \pm 0.05\%$ protein, $57 \pm 0.08\%$ crude fibre, and $4.06 \pm 0.04\%$ ash. The prepared powder had a good amount of antioxidant compound that possess strong

Table 1: Chemical and functional analysis of Tomato Pomace Powder prepared by Tray drying

Parameters	Percentage
Moisture (%)	4.59 ± 0.09
Fat (%)	3.20 ± 0.08
Protein (%)	16.72 ± 0.05
Crude fibre (%)	57.53 ± 0.08
Ash (%)	4.09 ± 0.04
DPPH activity (% of inhibition)	49.80 ± 5.55
Total phenolic content (mg GAE/100 g)	311.7 ± 34.03

Values reported as Mean $\pm$ SE (n=3)

antioxidant activity in terms of DPPH inhibition activity and TPC with $49.80 \pm 5.55\%$ inhibition and 311.7 ± 34.03 mg GAE/100g TPC, respectively. Similar finding was reported by Isik and Topkaya, (2016); Jafari et al. (2006) for dried tomato pomace chemical composition and antioxidant activity.

Optimization of the level of tomato pomace powder in *lassi* on the basis of sensory, physico-chemical and functional attributes

Effect of tomato pomace incorporation on different physico-chemical and functional parameters of *lassi*

Functional *lassi* was prepared by incorporating tomato pomace powder at a different level in preparation of *lassi* and optimized the level of TPP based on physico-chemical analysis, antioxidant activity and sensory evaluation of prepared products. Different physico-chemical parameter used in optimization of tomato pomace level in *lassi* such as Fat (%), Protein (%), Total Solids (%), Ash (%), pH, Acidity (% lactic acid), Whey Syneresis (mL), DPPH antioxidant activity (% Inhibition), TPC (mg GAE/100g). Different physico-chemical and functional analysis values shown in Table 2.

Changes in TPP incorporated *lassi* was shown in Table 2. From the data different physico-chemical properties of *lassi* observed that fat content of TPP fortified *lassi* was non-significantly different ($p < 0.05$) at a different level of addition, it is probably due to lesser amount of fat content in tomato pomace powder, Alqahtani et al. (2020) also reported non-significant ($p < 0.05$) changes in fat content of stirred yoghurt fortified up to 2% level of tomato pomace.

Protein content of control and tomato pomace added *lassi* was significantly different ($p < 0.05$) at different level of tomato pomace incorporation. Protein content of *lassi* was increased with TPP addition; increased level of protein content may be attributed to higher protein content in tomato pomace, Similar finding reported by Alqahtani et al. (2020). Increased protein content improved the functionality of *lassi* as in general *lassi* consider as low protein product, but little increase protein percentage ultimately improves nutrition quality. In, addition protein had water binding properties that improve consistency of *lassi* and increase acceptability of *lassi* (Schkoda et al. 2001). Desai et al. (2013) reported that high protein yoghurt had better sensory and texture attributes in terms of creaminess, viscosity and smoothness. In addition, Total solids content of tomato pomace incorporated *lassi* was significantly different ($p < 0.05$) for control as well as different level of tomato pomace addition. Increased total solids in *lassi* improved consistency of *lassi* and higher thicker *lassi* more like by consumers and high solids sold at higher price in India. TPP incorporated *lassi* ash percentages was non-significantly differ ($p < 0.05$) among the different rate of addition. It may be due to low ash content in tomato pomace and lesser rate of fortification

in *lassi*. Results reported by Alqahtani et al. (2020) in-line with the current study.

Acidity of TPP fortified *lassi* was increased with the tomato pomace addition, it may due to organic acids present in pomace (Tikhonova et al. 2021) that contribute in acidity increase and that results in decrease of pH on addition of TPP. Whey Syneresis was decreased on addition of tomato pomace, it may due to increased protein in *lassi*, and protein and fibre helps in binding more that ultimately decreased that free water in *lassi*. Staffolo et al. (2004) reported that cultural dairy products fortified with dietary fibre had better sensory and textural properties. High concentrations of TPP incorporation visible sedimentation occur and that affect acceptability of *lassi*.

TPP added *lassi* were possess good antioxidant activity, it may attribute to antioxidant and phenolic compounds present in tomato pomace. DPPH inhibition activity were increased significantly at initial level of TPP fortification, but at higher level DPPH inhibition activity is constant, it may be due to insolubility of tomato pomace in *lassi* at higher concentration (1.5 and 2.0% TPP). Because, higher level of TPP sedimentation was occurs. Similar trend was observed for total phenolic compound (TPC). Varnaite et al. (2022); Alqahtani et al. (2020) reported similar trend of TPC and DPPH for cranberry and tomato pomace incorporated yoghurt.

Effects of tomato pomace addition on the sensory parameter of *lassi*

For, sensory analysis products were subjected to judging and grading for sensory attributes viz., (i) flavour, (ii) body and texture, (iii) sweetness, (iv) colour and appearance and (v) overall acceptability by experts and semi-trained panel (Age: 23-40 year) of judges using 9-point Hedonic scale. Scores obtained for sensory attributes of TPP incorporated *lassi* are shown in Table 3.

Overall acceptability of control and different level of tomato pomace addition varied with 8.1 ± 0.19 , 7.3 ± 0.22 , 7.5 ± 0.18 , 6.8 ± 0.26 , and 6.1 ± 0.42 for control, 0.5, 1, 1.5 and 2.0% tomato pomace addition in *lassi*. Overall sensory score of control and different level of tomato pomace added *lassi* was significantly different ($p < 0.05$). The highest acceptability was observed for the control sample, as tomato pomace addition affects flavour, colour and appearance and sweetness parameter of *lassi* extremely, while in tomato pomace added *lassi*, 1% TPP added *lassi* was having better sensory quality compared to other levels of pomace addition.

The flavour score of control and tomato pomace added *lassi* was non-significantly ($p < 0.05$) differ up to 1% of pomace addition

Table 2: Physico-chemical parameters and antioxidant activity of the Tomato Pomace Powder incorporated *lassi*

Parameters	Treatment				
	TP ₀	TP ₁	TP ₂	TP ₃	TP ₄
Fat (%)	2.49 ± 0.02^a	2.51 ± 0.04^a	2.53 ± 0.02^a	2.55 ± 0.01^a	2.56 ± 0.03^a
Protein (%)	2.48 ± 0.04^a	2.58 ± 0.02^b	2.67 ± 0.01^c	2.74 ± 0.04^d	2.83 ± 0.03^e
Total solid (%)	17.85 ± 0.02^a	18.32 ± 0.04^b	18.81 ± 0.01^c	19.55 ± 0.04^d	20.01 ± 0.05^e
Ash (%)	0.63 ± 0.01^a	0.64 ± 0.01^a	0.66 ± 0.03^a	0.71 ± 0.04^a	0.77 ± 0.02^a
Acidity (% lactic acid)	0.65 ± 0.09^a	0.67 ± 0.16^a	0.71 ± 0.21^a	0.75 ± 0.11^a	0.78 ± 0.18^a
pH	4.54 ± 0.18^a	4.49 ± 0.08^a	4.45 ± 0.11^a	4.40 ± 0.17^a	4.35 ± 0.25
Whey Syneresis (mL)	55.37 ± 2.85^a	52.17 ± 2.55^a	49.64 ± 1.55^a	45.97 ± 1.21^b	44.08 ± 1.89^b
DPPH (% Inhibition)	13.11 ± 2.57^a	16.65 ± 1.02^a	21.81 ± 1.89^b	27.02 ± 2.06^c	28.12 ± 3.19^c
TPC (mg GAE/100g)	6.16 ± 1.17^a	9.02 ± 0.94^b	12.37 ± 1.24^c	13.76 ± 1.06^c	15.08 ± 0.87^c

Values are reported as Mean $\pm$ SE (n=3), a-e different superscript differ significantly within the row ($p < 0.05$)

Table 3: Sensory attributes of the Tomato Pomace Powder incorporated *lassi* evaluated using 9-point hedonic scale (1-9)

S.N.	Treatment	Flavour	Colour and Appearance	Body and Texture	Sweetness	Overall Acceptability
1	TP ₀	8.0 ± 0.26^a	7.8 ± 0.18^a	7.1 ± 0.17^a	8.2 ± 0.17^a	8.1 ± 0.19^a
2	TP ₁	7.9 ± 0.33^a	7.6 ± 0.22^a	7.4 ± 0.09^b	7.8 ± 0.29^b	7.3 ± 0.22^b
3	TP ₂	7.8 ± 0.21^a	7.4 ± 0.38^a	7.3 ± 0.25^b	7.5 ± 0.15^b	7.5 ± 0.18^c
4	TP ₃	7.1 ± 0.19^b	6.9 ± 0.14^b	6.9 ± 0.29^c	6.5 ± 0.32^c	6.8 ± 0.26^d
5	TP ₄	6.5 ± 0.12^c	5.7 ± 0.24^b	5.9 ± 0.31^d	5.2 ± 0.49^d	6.1 ± 0.42^e

Values are reported as Mean $\pm$ SE (n=8), a-e different superscript differ significantly ($p < 0.05$)

Table 4: Changes in physico-chemical parameters and microbial count of TPP incorporated and control *lassi* during the storage at 5°C

Parameters	Sample	0 days	3 rd day	6 th day	9 th day	12 th day
pH	Control	4.33±0.08 ^{aa}	4.17±0.03 ^{ba}	3.96±0.02 ^{ca}	3.71±0.09 ^{da}	ND
	Optimized	4.35±0.04 ^{aa}	4.21±0.01 ^{ba}	4.01±0.05 ^{cb}	3.86±0.01 ^{db}	3.51±0.03 ^e
Acidity (%LA)	Control	0.74±0.0 ^{aa}	0.79±0.02 ^{aa}	0.86±0.03 ^{ba}	0.93±0.04 ^{ba}	ND
	Optimized	0.77±0.04 ^{aa}	0.81±0.02 ^{aa}	0.89±0.01 ^{ba}	0.94±0.03 ^{ca}	1.05±0.04 ^d
Coliform (cfu/ml)	Control	Nil	Nil	Nil	Nil	ND
	Optimized	Nil	Nil	Nil	Nil	Nil
Yeast & Mould (cfu/ml)	Control	Nil	Nil	4	10	ND
	Optimized	Nil	Nil	NIL	11	21

Values are reported as Mean±SE, a-e and a-b different superscript used to denote differ significantly among the row and column (p<0.05). (*ND: Not done)

but at an increased rate of addition statistically different (p<0.05). Flavour score of TPP *lassi* was decreased with increasing the level of addition, it may be due to typical intense flavour of tomato pomace and the highest flavour score was obtained in the case of control sample. Similarly, Colour and appearance of *lassi* were non-significantly different (p<0.05) at an initial level of fortification, but at the increased level (above 1% TPP addition) of tomato pomace more darken the colour of *lassi* was observed that significantly decreased the score. Similar study of the addition of grape pomace in fermented probiotic goat milk reported that colour is intensified due to grape colour and overall acceptability is affected due to the higher flavour and intense colour of grape pomace extract (dos Santos et al. 2016). Sensory score of body and texture was significantly different (p<0.05) for control and tomato pomace *lassi*, which was improved on the addition of tomato pomace compared to the control sample, up to 1% pomace addition. Higher amount of fibre and protein content in tomato pomace probably improved better body and texture of *lassi*. The study of Varnaite et al. (2022) conclude that dietary fibre-rich cranberry pomace yoghurt had good water binding capacity and better rheological properties. No visible sedimentation was observed but above 1% TPP addition body and texture score reduces as sedimentation of tomato pomace observed in *lassi*, it may be due to insoluble portion of tomato pomace in milk. The sweetness score of TPP incorporated *lassi* decreased significantly with pomace fortification. Sweetness score may reduce due to the astringent compound present in tomato pomace.

The functional *lassi* with 1% of TPP had maximum overall acceptability due to the better scores in flavour, body and texture, sweetness, and, colour and appearance, which were significantly higher than any other levels of fortification. However, the TPP added *lassi* having up to 1% supplementation level with tomato pomace also exhibited average sensorial attributes, but beyond that level, the overall acceptability scores declined. Several researcher Alqahtani et al. (2020); Dabija et al. (2018); Sendra et al. (2010); Marchiani et al. (2016) attempted supplementation of plant fibre and pomace in yoghurt and similar fermented dairy

products and reported that its positive or negative effects on sensory parameter of products depends on type of pomace and fibre and rate of addition.

Changes during storage in physico-chemical parameters and microbial count of TPP incorporated and control *lassi*

Control and optimized (1% of TPP) samples were packed in 100 ml Polypropylene (PP) and stored in refrigerated condition (5±1 °C). Changes in different physico-chemical (pH, Acidity) and microbial (Coliform and Yeast and Mold count) parameters of control and TPP incorporated *lassi* were observed and shows in Table 4.

Control *lassi* (without tomato pomace addition) was found stable up to 9 days in refrigerated condition, while 1% tomato pomace incorporated *lassi* was found stable up to 12 days in sensory analysis. pH of control and optimized sample were decreased significantly with storage period and acidity of both the sample increased during storage, these changes attributed to production of lactic acid by growth of lactic acid bacteria and reduction of lactose in *lassi*. In, addition Du et al. (2021) reported that organic acids, polysaccharides and phenolic compounds presents in pomace served as prebiotics for LAB during yoghurt storage. Krishna et al. (2019) reported similar increased in pH and acidity of probiotic *lassi* up to 12 days storage period. Coliform count was not detected in control as well as optimized samples. Initially, yeast and mold count were absent in control and optimized sample of *lassi*. The presence of coliform count in dairy products indicates that production done under unsanitary condition and low hygiene practices followed, but absence of coliform count in present study indicate that *lassi* production done under hygienic and sanitary condition. Result reported Pawar et al. (2010) completely agreed with present study, in which coliform count absence up to 7 days of storage (at 5°C), it may be attributed to inhibition of lactic acid bacteria (LAB) (Debnath, 2017) and antimicrobial properties of LAB (Adeniyi et al. 2015). In control sample after 6 days of storage, yeast and mold observed and count increased significantly. In optimized sample yeast and mold count was observed after 9 days of storage significantly. Pawar et al. (2010)

reported similar trend of yeast and mold count for *lassi* (control) and nisin added *lassi* at 5°C storage temperature.

Conclusion

Functional ingredient in form of tomato pomace is incorporated in popular dairy beverages, *lassi*. In general, *lassi* has very low total solids by incorporation of tomato pomace several chemical constituents increased that improves consistency and acceptability of *lassi*. In, addition on tomato pomace at higher rate leads to sedimentation in *lassi* that had negative impact on acceptability. Tomato pomace added *lassi* had good amount phenolic compound and shows antioxidant activity properties. Incorporation of tomato pomace in dairy product be better alternative for tomato waste utilization and nutrition enrichment in similar products.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could be construed as a potential conflict of interest.

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RESEARCH ARTICLE

Development and evaluation of ginger-honey *shrikhand* – a fermented sweet delicacy

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Abstract: *Shrikhand* is a semi-soft, pleasant sweetish-sour, whole milk fermented product popular in the western parts of the India and is prepared by mixing *chakka* with sugar, cream and other ingredients. Levels of milk fat content, sugar: honey ratio and ginger juice concentration were optimized using central composite rotatable design of response surface methodology. Optimized formulation of ginger-honey *shrikhand* contained 3.16 % milk fat, 79.61 sugar: honey ratio (i.e. 20.39 % sugar replaced with honey) and 3.79 % ginger juice concentration (on *chakka* basis) and gave an overall acceptability score of 8.07 ± 0.06 on a 9-point hedonic scale. Ginger-honey *shrikhand* samples packed in polystyrene cups remained acceptable on 30th day of storage at 7 ± 1 °C with overall acceptability score of 7.83 ± 0.06 . Responses from 90 consumers indicated wide acceptability of ginger-honey *shrikhand* among consumers with mean overall liking score of 8.25 on 9-point hedonic scale.

Keywords: Fermented product, *shrikhand*, ginger, honey, RSM

Introduction

Shrikhand is a traditional fermented milk product popular in the western parts of the India and is prepared by mixing *chakka* (strained yoghurt) with sugar, cream and other ingredients (Aneja et al. 1977; Upadhyay and Dave 1977). Several varieties of *shrikhand* include plain, mango, dry-fruit, saffron, pineapple etc., the name of which are derived from the added ingredients.

Honey is a sweet and syrupy liquid collected by bees from the nectar of flowers (Haydak et al. 1942; Aparna and Rajalakshmi 1999). It contains small amounts of vitamins and minerals, including niacin, riboflavin, thiamine and pyridoxine, pantothenic acid, ascorbic acid and nicotinic acid (Bogdanov et al. 2008). According to Jonathan and White (1978), honey is the only sweetening material that can be stored and used exactly as produced in natural form. In foods, honey is used as flavouring and colouring agents, natural sweetener, binding agent, curing agent, and humectant. Honey has been reported to exhibit various health beneficial properties such as antioxidant (Kek et al. 2017), anti-hypertensive, anti-diabetic (Erejuwa et al. 2012; Erejuwa 2014), anti-inflammatory (da Silva et al. 2016), immunomodulatory (McLoone et al. 2016) and antimicrobial properties (Mercan et al. 2007).

Ginger (*Zingiber officinale*) is most commonly used herbal supplement reported for alleviating the symptoms of motion sickness, nausea and vomiting (White 2007). Ginger has also been reported to exhibit anti-hypertensive effects, cancer prevention, regulation of blood glucose levels, anti-oxidant and antimicrobial actions (Shukla and Singh 2007; Khan et al. 2019). Ginger is commonly used as a spice in the preparation of Indian cuisine dishes. It is also preferred as an ingredient in tea preparation due to its pleasant aroma in various parts of Asia. Present research work is an attempt to explore the use of honey as sweetening agent and ginger as flavouring agent in *shrikhand* making.

Materials and Methods

Raw Materials

Milk, ginger, cane sugar, honey (Dabur India Limited) and polystyrene cups were procured from the local market of Amreli district. Ginger was washed with plenty of tap water, peeled off to remove the skin and grated using stainless steel grater. Grated ginger was pressed on a strainer to obtain the juice. Ginger juice was indirectly heated to 80 °C for 15 seconds and chilled immediately below 5 °C. Sugar was ground using mixer-grinder and used in the product preparation. *Dahi* culture (NCDC-324) was procured from National Collection of Dairy Cultures of Dairy

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Preparation of ginger-honey *shrikhand*

Milk was filtered through nylon milk strainer, and standardized for fat content as per the experimental design matrix indicated in Table 1. Milk was heated to 60 °C and homogenized using laboratory milk homogenizer. Homogenized milk was heated to 90 °C for 15 min and cooled to 42 ± 1 °C. Milk was then inoculated with *dahi* culture at the rate 2 %, and incubated at 42 ± 1 °C till acidity reaches 0.80 % of lactic acid. Curd was then transferred to sterilized muslin cloth and drainage of whey was allowed for overnight under refrigerated conditions. Calculated amount of ground sugar, honey and ginger juice as per the experimental design matrix was added and mixed thoroughly using planetary mixer to get homogenous consistency (Aneja et al. 1977; Patel and Chakraborty 1985). Calculation of sugar was made at the rate of 60 % on *chakka* weight basis and replacement of sugar with honey was made as per sugar: honey replacement ratio indicated in experimental design matrix (e.g. 60 indicates: 40 % of calculated sugar amount replaced with honey).

Optimization of the formulation

Levels of three variables namely milk fat content, sugar: honey ratio and ginger juice concentration were optimized using the Response Surface Methodology (Myers 1971) provided in Design-Expert® V-10 (Stat-Ease Corporation, USA). Based on preliminary trials, lower and higher levels for milk fat content; sugar: honey replacement ratio; and ginger juice content were decided as 1.5 % and 4.5 %; 60:40 and 100:0; and 2 % and 6 %, respectively (on *chakka* weight basis); and experimental design matrix obtained is presented in Table 1. Experiments were conducted in a random manner and replicated three times at least.

Effect of milk fat content (x_1), sugar: honey ratio (x_2) and (x_3) ginger juice concentration were analysed on the sensory quality of ginger-honey *shrikhand*. Results obtained for each responses were statistically analysed using Design Expert® software. Levels of milk fat, sugar: honey replacement ratio and ginger juice concentration were optimized using numerical optimization tool provided in the software. Constraints for variables i.e. milk fat content, sugar: honey ratio and ginger juice concentration were: “in-range”, while for the critical sensory responses i.e. flavour and overall acceptability were: ‘maximized’. Solution with the highest desirability was selected as optimized formulation.

Physico-chemical analysis

Milk was analysed for fat and solids-not-fat (SNF) content as per methods of IS: SP:18 (Part XI). Acidity of *dahi* was estimated by titration method as suggested in IS:9617 (1980). Acidity of *chakka* and *shrikhand* was estimated by using titration method as per the procedure of described in Appendix D of IS:1166 (1986).

Total solids, ash and fat content of ginger-honey *shrikhand* and market sample was determined by adopting the methods of FSSAI (Manual of methods of analysis of foods, Milk and milk products 2015). Protein content of *shrikhand* was estimated by Kjeldahl method according to the procedure described in IS: SP:18 (Part XI). The total carbohydrate was estimated using phenol-sulphuric method (Dubois et al. 1956; Krishnaveni et al. 1984) as described in book Biochemical Methods (Sadasivam and Manickam 1996).

Colour and firmness analysis

The colour of samples were measured using Colorflex EZ instrument (HunterLab, USA) and measurements were obtained in Hunter L a b scale (Hunter and Harold 1987) using D65 as an illuminant. Firmness test for *shrikhand* samples were carried out using TA.XT plus texture analyzer (Stable Micro Systems Limited, UK). *Shrikhand* samples were filled in plastic beakers (dia. 5 cm) up to 4 cm and tapped 10 times. A 35 mm aluminum cylinder probe with a flat end was used and allowed to travel up to 20 mm at speed of 2 mm/s with trigger force of 5 g at 10 °C. Test data were analysed using the Exponent Lite (Version 6) software for determination of firmness.

Sensory evaluation and consumer survey

The samples were presented to the panel of seven trained panelists from the faculty members of Postgraduate Institute of Dairy Education and Research, Amreli. The sample was analysed for different sensory parameters like colour and appearance, body and texture, sweetness, flavour and overall acceptability using a 9-point hedonic scale (Amerine et al. 1965); where, score of 1 indicates ‘dislike extremely’ and 9 indicates ‘like extremely’.

For assessment of consumer responses, optimized formulation of ginger-honey *shrikhand* was distributed to 90 probable consumers (Alvensleben and Schrader 1998) belonging to mixed age group, gender and location of residence. A questionnaire along with the sample was presented to the consumers for collecting data regarding personal information (age, gender and location) and acceptability of the optimized product. Responses were obtained on a 9-point hedonic scale and results were presented in average value.

Storage study

Samples of ginger-honey *shrikhand* drawn on 0, 10, 20 and 30th day of storage and analysed for changes in sensory (colour and appearance, body and texture, sweetness, flavour and overall acceptability), physico-chemical (total solids, acidity, free fatty acids and proteolysis) and microbial quality (standard plate count, coliform count and yeast and mould count) at 7 ± 1 °C. Total free fatty Acids (FFA) content was estimated using extraction titration method of Deeth and Fitzgerald (1976). The extent of proteolysis in the fresh as well as stored samples of ginger-honey *shrikhand* was assessed by adopting the procedure of Church et al. (1983)

using o-phthaldialdehyde (OPA) method. The extent of proteolysis was assessed by plotting standard curve with L-leucine and expressed as mg/g sample. Standard plate count, yeast and mold count and coliform count were enumerated as per methods described in IS SP: 18 (Part: XI) 1981.

Statistical analysis

Results are expressed as mean $\pm$ standard error (SE). The data obtained during storage analysis were subjected to analysis of variance test in a completely randomized design followed by Duncan's Multiple Range Test ($p < 0.05$) for multiple sample comparison.

Results and Discussion

Sensory quality of ginger-honey *shrikhand* as affected by varying the levels of milk fat, sugar: honey ratio and ginger juice concentration is presented in Table 1. Variation in milk fat content was not more than ± 0.05 % of the values indicated in the experimental design matrix, whereas SNF content varied from 8.63 to 9.11. Acidity of *dahi* for the preparation of *chakka* varied from 0.80 to 0.85 % of lactic acid. Product were prepared in random manner as per experimental design matrix (Table 1).

Effect of fat, sugar: honey ratio and ginger juice concentration on the sensory quality of ginger-honey *shrikhand*

The colour and appearance score of ginger-honey *shrikhand* ranged from 7.45 to 8.09 (Table 1). Sugar: honey ratio and ginger juice concentration indicated significant influence ($p < 0.05$) on the colour and appearance of ginger-honey *shrikhand* on quadratic level. Colour and appearance scores of ginger-honey *shrikhand* at intermediate levels of ginger juice and honey were higher (Figure 1a). Waghmare et al. (2021) who studied the effect of ginger powder incorporation on the sensory properties of *shrikhand* also observed increase in the colour and appearance score up to certain level with increasing levels of ginger powder, further increasing the levels of ginger powder led to decrease in the scores. The decrease in colour and appearance scores at higher levels of ginger and honey could be attributed to the perceivable changes in the colour and appearance of *shrikhand* from milky white to off white.

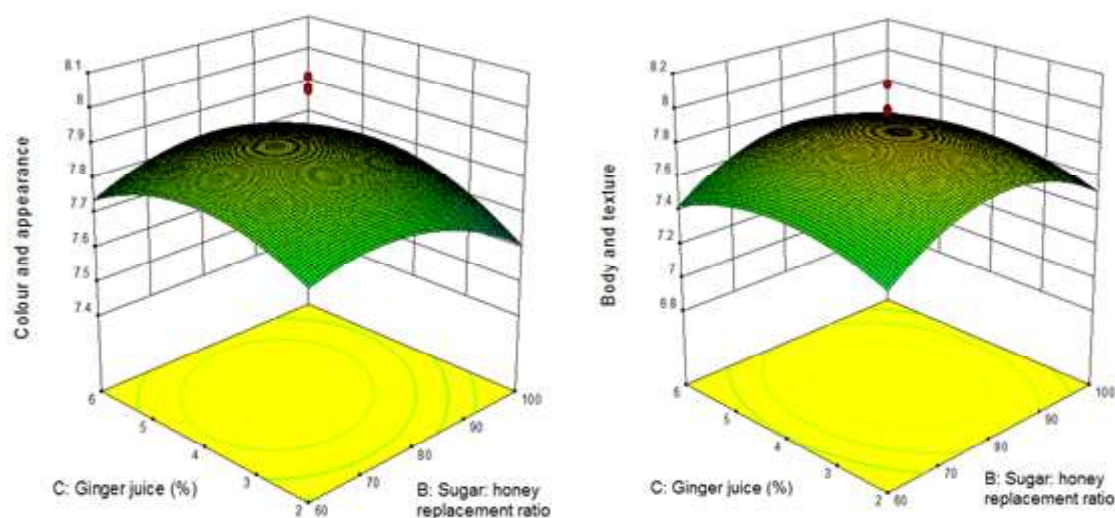
Levels of sugar: honey ratio and ginger juice significantly affected the body and texture score ginger-honey *shrikhand* on quadratic level at $p < 0.01$ and $p < 0.05$ level of significance, respectively (Table 2). At intermediate levels of sugar: honey replacement ratio and ginger juice concentration, body and texture score were higher, but a gradual decrease was observed with either increasing or decreasing the levels (Figure 1b). Chorage et al. (2018) reported decrease in the total solids content of *shrikhand* with increasing concentration of ginger juice. The loose body of ginger-honey *shrikhand* at higher levels of honey and ginger juice was not

Table 1 Experimental design matrix for the effect of milk fat, sugar: honey ratio and ginger juice concentration on sensory responses

Standard order	Actual factors			Sensory scores				
	Milk fat (%)	Sugar: honey*	Ginger juice(%)	C & A	B & T	Sweetness	Flavour	OA
1	1.5	60	2	7.63	7.48	7.70	7.26	7.52
2	4.5	60	2	7.77	7.55	7.59	7.27	7.50
3	1.5	100	2	7.45	7.41	7.61	7.80	7.45
4	4.5	100	2	7.70	7.58	7.65	8.02	7.58
5	1.5	60	6	7.71	7.35	7.52	7.30	7.46
6	4.5	60	6	7.74	7.74	7.87	8.03	7.76
7	1.5	100	6	7.68	7.50	7.52	7.54	7.48
8	4.5	100	6	7.80	7.57	7.70	7.45	7.70
9	0.48	80	4	7.70	8.00	7.83	7.35	7.91
10	5.52	80	4	7.80	7.65	7.65	7.32	7.70
11	3	46.36	4	7.68	6.95	7.52	7.19	7.30
12	3	113.64	4	7.56	7.27	7.40	7.41	7.21
13	3	80	0.64	7.58	7.47	7.47	7.88	7.45
14	3	80	7.36	7.63	7.63	7.25	7.80	7.50
15	3	80	4	7.83	7.78	7.89	8.00	7.78
16	3	80	4	8.09	8.00	8.00	7.66	8.04
17	3	80	4	8.06	7.98	7.90	7.58	8.02
18	3	80	4	7.73	7.71	7.79	7.33	7.73
19	3	80	4	8.05	8.14	8.14	7.71	8.05
20	3	80	4	7.60	7.64	7.70	7.34	7.68

x_1 : milk fat content; x_2 : sugar: honey ratio, x_3 : ginger juice concentration; C & A: colour and appearance; B & T: body and texture; OA: overall acceptability; *ratio (e.g. 60 indicates: 40 % of calculated sugar amount replaced with honey)

Fig. 1 Response surface plot of sensory attributes: (a) colour and appearance (b) body and texture as influenced by ginger juice concentration and sugar: honey ratio



preferred by the panelists, thus led to decrease in body and texture scores.

Formulation containing maximum level of ginger juice concentration yielded minimum sweetness score (Table 1), which could be attributed to the pungent aroma of ginger masking the sweetness of ginger-honey *shrikhand*. Sweetness score of ginger-honey *shrikhand* significantly varied with the sugar: honey ratio ($p < 0.05$) and ginger juice concentration ($p < 0.01$) at quadratic level (Table 2).

Flavour score of ginger-honey *shrikhand* ranged from 7.19 to 8.03. Differences in the flavour score of ginger-honey *shrikhand* due to sugar: honey ratio and ginger juice concentration were significant ($p < 0.01$) at quadratic level, however, none of the variables indicated significant ($p < 0.05$) influence on flavour score at linear level. Milk fat content indicated positive influence on the flavour score of ginger honey *shrikhand*, and is also evident from the positive coefficient of estimate for milk fat content at linear level (Table 2). Higher fat content improved the richness and mouthfeel of ginger-honey *shrikhand*. Figure 2a indicated higher scores for flavour at intermediate levels of ginger-juice concentration and sugar: honey ratio. However, Waghmare et al. (2021) reported decrease in flavour scores from 7.7 to 6.9, when increased the levels of ginger powder in *shrikhand* formulation from 98:02 to 94:06 for *chakka*: ginger powder ratio. The persistent decrease in the reported result due to ginger powder was attributed to the strong pungent aroma of ginger powder. In our study, flavour scores at lower concentration of ginger juice were low indicating ginger flavour were not perceived at lower levels of juice, whereas higher levels has contributed to the pungency thus led to decrease in flavour scores. The form of addition of ginger i.e. juice or powder influenced the intensity of flavour compounds thus differences in observations from previous reports were noted.

None of the formulation variables indicated significant influence on the overall acceptability of ginger-honey *shrikhand* at linear level, however, effect of sugar: honey ratio and ginger juice concentration was significant ($p < 0.01$) at quadratic level (Table 2). Figure 2b indicates that overall acceptability of ginger-honey *shrikhand* increased with the increase in milk fat content, whereas intermediate levels of ginger juice concentration resulted higher acceptability scores. Findings of Waghmare et al. (2021) who worked on sensory properties of *shrikhand* using ginger powder supported our observations. Similar trend for overall acceptability of herbal ice cream prepared using incorporation of ginger juice (Jadhav et al. 2017) was reported with increasing ginger juice levels in ice cream formulation. Pungent aroma at higher ginger juice levels; loose body at higher levels of honey and ginger juice; and changes in colour and appearance due to addition of honey and ginger juice were the reasons affecting overall acceptability of ginger-honey *shrikhand*.

The R^2 values for the models obtained for sensory parameters varied in the range of 0.59 to 0.85, being the lowest for colour and appearance and highest for flavour scores. High R^2 values generally indicates the how better variability in the responses can be explained by the model. Lack of fit test was non-significant for all the sensory parameters that confirmed the fitness of model.

Optimized formulation

Optimum formulation obtained as a result of numerical optimization using Design Expert® V-10 contained 3.16 % milk fat, 79.61 sugar: honey ratio (i.e. 20.39 parts of sugar replaced with honey in calculated amount of sugar) and 3.79 % ginger juice concentration. The desirability of selected optimum formulation was 0.82. Sensory evaluation of the optimized product indicated an overall acceptability score of 8.07 ± 0.06 . Comparatively higher scores in market *shrikhand* were reported for colour, texture, flavour and overall acceptability which were

Fig. 2 Response surface plot of sensory attributes: (a) flavour score as influenced by ginger juice concentration and sugar:honey ratio (b) overall acceptability score as influenced by ginger juice concentration and milk fat concentration.

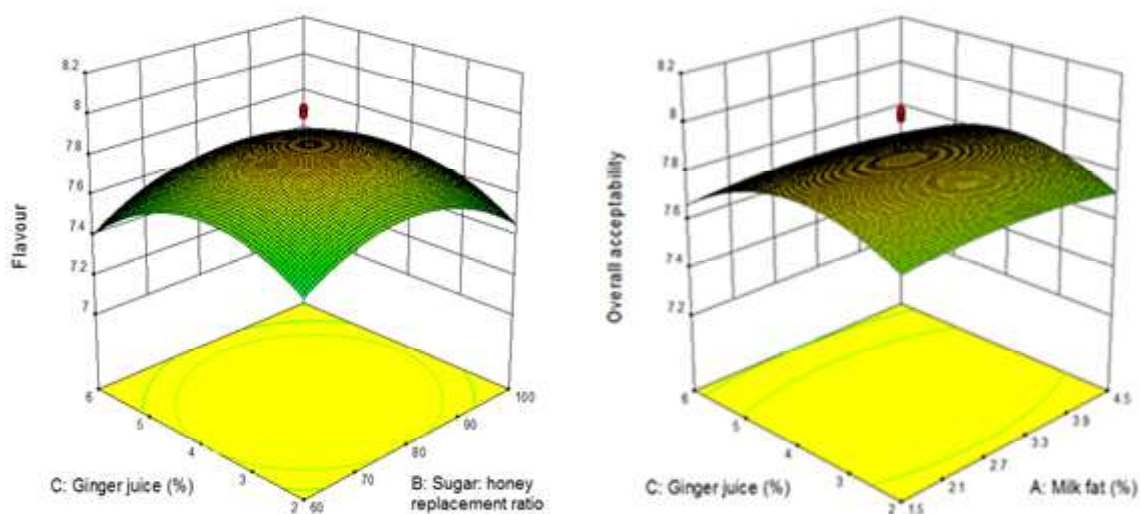


Table 2 Regression coefficients of the quadratic model to predict sensory quality in ginger-honey *shrikhand*

Model term	Coefficient estimate				
	C & A	B & T	Sweetness	Flavour	OA
Intercept	7.892012	7.871655	7.898546	7.885594	7.884758
x_1	0.051855	0.008155	0.011516	0.027093	-0.00829
x_2	-0.03089	0.035013	-0.02942	-0.01651	0.003561
x_3	0.033982	-0.00699	-0.0227	-0.04337	-0.03338
x_1x_2	0.025	-0.0275	-0.0025	0.02875	-0.025
x_1x_3	-0.03	0.0275	0.075	0.00375	0.0025
x_2x_3	0.035	0.0025	-0.0175	0.02375	0.0225
x_1^2	-0.04204	0.004181	-0.02646	-0.04424	-0.03701
x_2^2	-0.088*	-0.24861**	-0.12546*	-0.20687**	-0.23146**
x_3^2	-0.09331*	-0.14608*	-0.16081**	-0.22278**	-0.15368**
R^2	0.59	0.74	0.70	0.85	0.81

**significant at $p < 0.01$, *significant at $p < 0.05$, x_1 : milk fat content; x_2 : sugar: honey replacement ratio, x_3 : ginger juice concentration; C & A: colour and appearance; B & T: body and texture; OA: overall acceptability

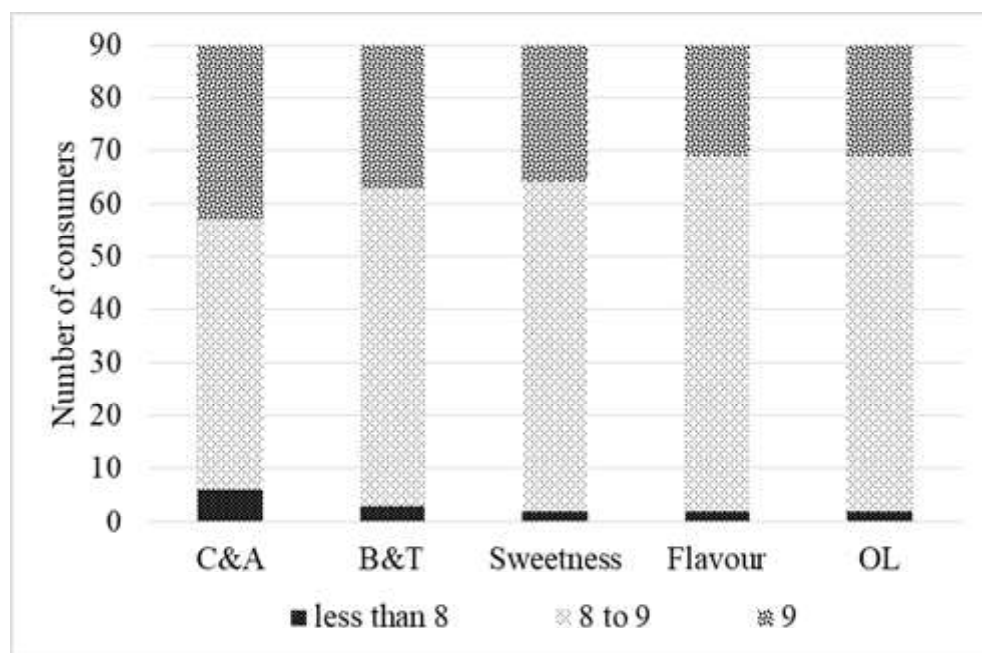
8.70, 8.67, 8.40 and 8.50, respectively (Gupta et al. 2015). Quality analysis of *shrikhand* sold in Kolhapur city indicated colour and appearance, body and texture, flavour, sweetness, and overall acceptability score in the range of 6.46 to 7.50, 6.05 to 7.84, 6.32 to 7.77, 6.01 to 7.29 and 6.50 to 7.35, respectively (Bhagavan et al. 2020). A large variation among the acceptability market samples was noted from previous reports. Such variations are normal to be seen, as large proportion of *shrikhand* is produced by traditional methods at cottage scale, thus leading to lack of uniformity in the production techniques.

Physicochemical, microbial and sensory quality of optimized ginger-honey *shrikhand*

Results for physico-chemical, microbial and sensory quality of ginger-honey *shrikhand* prepared using optimized formulation are presented in Table 3. In our study, optimized product contained 52.75 % total solids which is lower than the current prescribed legal standards for *shrikhand*, which could be

attributed to the partial replacement of sugar with honey and addition of ginger juice containing higher moisture content. Mehta (2013) reported total solids content in few market samples *shrikhand* varied in range of 47.55 to 55.77 %. Analysis of the market samples of *shrikhand* sold in Kolhapur city of Maharashtra state indicated total solids in the range of 53.28 to 57.27 % (Jaybhay et al. 2019). Total solids content in symbiotic *shrikhand* samples blended with different levels of papaya pulp and dextrine varied in the range from 51.56 to 54.22 %, whereas control samples were reported to contain 57.35 % total solids (Suryawanshi et al. 2022) when prepared using traditional method. Process interventions such as efficient whey separation by mechanical methods using basket centrifuge or quarg separator, will be helpful in increasing final total solids of the *shrikhand*.

Firmness of optimized ginger-honey *shrikhand* and market sample were 0.52 and 0.48 kg, respectively. In spite of containing higher total solids firmness value in the market samples was lower than

Fig. 3 Acceptability of ginger-honey *shrikhand* among consumers**Table 3** Physico-chemical, microbial and sensory quality of ginger-honey *shrikhand*

Characteristics	Ginger-honey <i>shrikhand</i>	Market sample*
<i>Physico-chemical quality</i>		
Fat (%)	5.64±0.07	5.36±0.05
Protein (%)	6.12±0.08	7.70±0.05
Total carbohydrate (%)	39.57±0.45	47.38±0.26
Total solids (%)	52.75±0.25	61.09±0.25
Ash (%)	0.82±0.01	0.76±0.01
Acidity (% LA)	1.11±0.01	1.22±0.06
Firmness (kg)	0.52±0.03	0.48±0.01
L	83.90±0.51	78.27±0.15
a	-0.68±0.03	-0.55±0.02
b	13.03±0.09	13.70±0.05
<i>Microbial quality</i>		
Standard plate count (log cfu/g)	7.84±0.27	3.49±0.23
Yeast and mould count (log cfu/g)	2.43±0.12	2.18±0.30
Coliform count (log cfu/g)	nd	nd
<i>Sensory quality</i>		
Colour and appearance	8.21±0.10	7.78±0.05
Body and texture	8.17±0.08	7.70±0.06
Sweetness	8.11±0.07	7.78±0.06
Flavour	8.04±0.06	7.62±0.06
Overall acceptability score	8.07±0.06	7.78±0.05

*cardamom flavoured *shrikhand* ; nd: not detected

that of ginger-honey *shrikhand*, which could be attributed to the differences in unit operations involved in manufacturing process. Some manufacturers adopt *chakka* production from skim milk curd, whey separation using quarg separator, mixing and thermization of cream and other ingredients using scraped surface heat exchanger (Aneja 2002) causing shear sensitivity on protein network of *shrikhand*. Firmness value of dietetic amrakhand

prepared by incorporating different levels of stevia leaf extracts and mango pulp reported in the range of 1.21 to 1.28 N when estimated using 25 mm cylindrical probe at 23 °C (Tondare and Hembade 2021). Firmness of market sample *shrikhand* was reported as 29.03 g, when analysed using P/0.5 Cylindrical Telrin probe (Gupta et al. 2015). The reported values of firmness were lower than our findings, the reason could be attributed to the

Table 4 Changes in physico-chemical, microbial and sensory quality of ginger-honey *shrikhand* during storage

Storage interval	0 day	10 day	20 day	30 day
<i>Chemical Quality</i>				
Acidity (% lactic acid)	1.10 ± 0.01 ^a	1.13 ± 0.01 ^b	1.15 ± 0.01 ^c	1.18 ± 0.01 ^d
Free fatty acid (µeq/g)	1.43 ± 0.01 ^a	1.46 ± 0.01 ^b	1.50 ± 0.01 ^c	1.53 ± 0.01 ^d
Total solids (%)	52.33 ± 0.02 ^a	52.38 ± 0.01 ^b	52.50 ± 0.01 ^c	52.55 ± 0.01 ^d
Proteolysis	0.49 ± 0.005 ^a	0.53 ± 0.005 ^b	0.57 ± 0.005 ^c	0.65 ± 0.005 ^d
<i>Microbial quality</i>				
Standard plate count (log cfu/g)	7.38 ± 0.02 ^a	7.89 ± 0.02 ^b	8.19 ± 0.04 ^c	8.44 ± 0.06 ^d
Yeast and mould count (log cfu/g)	2.04 ± 0.02 ^a	2.38 ± 0.04 ^b	2.56 ± 0.06 ^c	2.70 ± 0.03 ^d
Coliform count	nd	nd	nd	nd
<i>Sensory quality</i>				
Colour and appearance	8.21 ± 0.10 ^a	8.16 ± 0.09 ^a	7.88 ± 0.06 ^b	7.76 ± 0.07 ^b
Body and texture	8.17 ± 0.08 ^a	8.09 ± 0.06 ^{ab}	7.90 ± 0.06 ^{bc}	7.76 ± 0.07 ^c
Sweetness	8.11 ± 0.07 ^a	8.07 ± 0.05 ^{ab}	7.92 ± 0.05 ^{bc}	7.80 ± 0.06 ^c
Flavour	8.04 ± 0.06 ^a	8.02 ± 0.05 ^a	7.95 ± 0.07 ^{ab}	7.78 ± 0.07 ^b
Overall acceptability	8.07 ± 0.06 ^a	8.04 ± 0.06 ^a	7.97 ± 0.06 ^{ab}	7.83 ± 0.07 ^b

Means with different superscripts in the same raw differ significantly ($p < 0.05$); Values in each cell represent mean $\pm$ SE, $n=3$ for physico-chemical and microbial quality, $n=7$ for sensory quality; nd: not detected

variation in selection of probe used during textural analysis, sample temperature, compositional differences, ingredients used in *shrikhand* making and action of shear forces during mixing of ingredients.

Colour values on Hunter L, a and b scale for optimized ginger-honey *shrikhand* and market *shrikhand* is presented in Table 3. Mean colour values L*, a* and b* for goat milk *shrikhand* were reported as 77.34, 4.47 and 7.19, respectively (Sahu et al. 2021), where CIE L*, a* and b* indicate lightness, red/green coordinate and yellow/blue coordinate, respectively. Gupta et al. (2015) observed differences in the colour values of market *shrikhand* and experimental samples. L*, a* and b* values of market *shrikhand* were 82.70, -2.73 and 13.39, respectively. Colour of *shrikhand* sample can enhance the acceptability of *shrikhand*. However, the colour preference may vary according to the variety of *shrikhand*. Conclusion from previous reports indicate variation in the colour values, which is influenced by the type of milk, ingredients used in the manufacture and colour scale adopted to represent colour values.

Suvera et al. (2017) during development of fiber fortified probiotic *shrikhand* reported standard plate count (SPC) in the range of 5.23 to 5.27 log cfu/g, whereas coliform count was reported nil. In the present study, market sample of *shrikhand* showed lower SPC, which could be due to thermization of *shrikhand* in industrial method of production, which also enhances the shelf life of the product (Aneja et al. 2002; Dhotre and Bhadania 2016).

Sensory quality of ginger-honey *shrikhand* and market sample is presented in Table 3, which indicated ginger-honey *shrikhand* was superior in terms of sensory acceptability. Meena et al. (2016) during study on the sensory preference modelling compared normal and probiotic market *shrikhand* with lab made samples

and observed better sensory acceptability of lab made samples. During development of fiber fortified *shrikhand* total sensory scores for control sample was 89.74 out of 100 (Suvera et al. 2017). Findings of Gupta et al. (2015) indicated better acceptability of market samples compared with experimental samples prepared microencapsulated rice bran oil as fat alternative in *shrikhand* premix. From the published literatures it can be concluded that the acceptability of *shrikhand* varies with the choice of ingredients and practices followed in its manufacture.

Effect of storage on the physico-chemical, sensory and microbial quality of ginger-honey *shrikhand*

The data pertaining to changes in the physico-chemical, sensory and microbial quality of ginger-honey *shrikhand* is given in Table 4. The initial titratable acidity (% lactic acid) of ginger-honey *shrikhand* was 1.10 which increased to 1.18 (% lactic acid) after 30 days of storage (Table 4). Significant ($p < 0.01$) increase in the acidity was observed during refrigerated storage of the samples. Increase in the titratable acidity from 1.19 to 1.38 (% lactic acid) was also reported in the control samples of *shrikhand* when stored at 5 ± 1 °C (Jadhav et al. 2019). Consistent increase in the titratable acidity of *shrikhand* samples is due to action of microbes during storage.

The free fatty acids (FFA) content of fresh ginger-honey *shrikhand* samples was 1.43 ± 0.01 (µeq/g), which increased significantly ($p < 0.01$) to 1.53 ± 0.01 (µeq/g) after 30 days of storage at 7 ± 1 °C (Table 4). In previous reports, increase in average FFA content from 3.49 to 8.56 (% oleic acid) in *shrikhand* samples was reported after 35 days storage under refrigeration at 5 ± 1 °C (Raghuwanshi et al. 2014). Jadhav et al. (2019) also observed increase in the FFA content of *shrikhand* samples from 0.27 to 0.38 (% oleic acid) on the 21st day of storage at 5 ± 1 °C.

Analysis of ginger-honey *shrikhand* samples for proteolysis indicated increase in L-leucine values from 0.49 mg/g to 0.65 mg/g on 30th day of storage. Increase in the soluble nitrogen content of *shrikhand* samples were reported from 0.103 to 0.241 % during 13 days of storage under refrigeration (Sonawane et al. 2007). Soluble nitrogen content in the control samples of *shrikhand* was also reported to increase from 0.50 to 1.19 % after 28 days of storage at 5 ± 1 °C (Jadhav et al. 2019). Increase in the soluble nitrogen content in *shrikhand* samples could be attributed to proteolytic activity of lactic acid bacteria (Wolfschoon 1979; Savijoki 2006).

The initial total solids (TS) content of ginger-honey *shrikhand* increased from 52.33 % to 52.55 % at the end of 30 days of storage under refrigeration (Table 4). Decrease in the moisture content of *shrikhand* from 49.27 % to 47.55 % was reported after 13 days storage under refrigeration (Sonawane et al. 2007). Mehrotra et al. (2014) also observed slight decrease in the moisture content of *shrikhand* from 32.04 to 31.86 % when stored under refrigeration for 21 days. Increase in the total solids content during storage largely depends on the moisture barrier properties of package, temperature and relative humidity of storage conditions.

Changes in the sensory quality of ginger-honey *shrikhand* samples were significant when packed samples were stored at 7 ± 1 °C for 30 days (Table 4). Findings of Mehrotra et al. (2014) supported our observations who reported decrease in colour and appearance, taste and flavour, body and texture, and overall acceptability score of *shrikhand* from 7.86 to 5.81, 7.83 to 5.63, 7.74 to 5.87, and 7.92 to 6.00, respectively during storage up to 21 days at 7 °C. Similar trends for decrease in the sensory scores of *shrikhand* prepared by incorporating cardamom and saffron were also reported when stored at 7 ± 1 °C for 21 days (Dandile et al. 2014). However, extent of changes in sensory quality reported by previous authors were higher. In our study, samples were remained acceptable on 30th day of storage with an overall acceptability score of 7.83, which could be attributed to the antimicrobial properties of honey and ginger which slowed down the growth of lactic acid bacteria (Mercan et al. 2007; Khan et al. 2019). Dhotre and Bhadania (2016) during study on the thermization of *shrikhand* using scraped surface heat exchanger reported gradual decrease in the sensory scores in control and thermized *shrikhand* samples when stored at 8 ± 2 °C. However, thermization of *shrikhand* was reported to extend the shelf life up to 45 days.

Standard plate count (SPC) and yeast and mould count (Y&M) of ginger-honey *shrikhand* samples revealed significant increase from initial count of 7.38 ± 0.02 and 2.04 ± 0.02 log cfu/g to 8.44 ± 0.06 and 2.07 ± 0.03 log cfu/g (Table 4), respectively when stored under refrigeration for 30 days. Coliform count was not detected when the first dilution of samples drawn on 0th, 10th, 20th and 30th day were plated on violet red bile agar (VRBA). Jadhav et al. (2019) reported nil coliform count in *shrikhand* samples during

21 days storage period under refrigeration, which increased to $1.84 \log_{10}$ cfu/g on 28th day of storage.

Consumer's acceptability

A total of 90 consumers provided their responses to the optimized ginger-honey *shrikhand* samples. Responses as a result of the sensory perception obtained from the consumers using a 9-point hedonic scale and summarized in Figure 3. Among 90, 88 consumers rated the optimized product more than or equals to 8, which was nearer to 'liked extremely' region of hedonic scale. Average scores of 90 respondents for colour and appearance (C&A), body and texture (B&T), sweetness, flavour and overall liking (OL) score were 8.31, 8.27, 8.27, 8.38 and 8.25, respectively.

Conclusion

Optimized level of milk fat content, sugar:honey ratio and ginger juice concentrate were 3.16 %, 79.61 (i.e. 20.39 % of sugar replaced with honey) and 3.79 %, respectively. Consumer survey studies indicated wide acceptability of ginger-honey *shrikhand* which combines the health beneficial attributes of lactic acid bacteria, honey and ginger.

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Improvement in quality of cow's raw milk using novel on-farm milk cooling system

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Abstract: The present study was conducted with an aim to check the improvement in raw milk quality at farm level by novel milk cooling system. Bulk milk cooler was taken as control. Optimization of different precooling temperatures viz., 30°C, 20°C, 10°C and 4°C in novel milk cooling system was carried out based on microbial and physicochemical tests. The raw milk samples were stored at 4°C and analyzed at an interval of 24 h for 72 h. A significant ($p < 0.05$) reduction in the aerobic plate count and psychrotrophic count was observed when precooling temperature was decreased from 30°C to 10°C whereas non-significant difference was observed when precooled at 10°C and 4°C. The aerobic plate count and psychrotrophic counts of the raw milk samples ranged from 5.02 ± 0.16 log CFU/ml to 6.87 ± 0.17 log CFU/ml and 1.71 ± 0.13 log CFU/ml to 3.54 ± 0.17 log CFU/ml, respectively throughout the storage period. Whereas, pH and titratable acidity of cow's milk samples ranged from 6.71 ± 0.04 to 6.56 ± 0.08 and $0.14 \pm 0.01\%$ LA to $0.16 \pm 0.02\%$ LA, respectively throughout the storage period. pH and titratable acidity of all milk samples during storage at 4°C for 72 h remained non-significant. Among the treatments, raw milk precooled at 10°C and 4°C showed lower aerobic plate count and psychrotrophic count with non-significant difference, hence, precooling of raw milk was optimized to 10°C. The better quality of raw milk can be obtained at reduced operating expenses using such novel milk cooling system. The farmers can earn higher incentives from superior quality of raw milk.

Keywords: Raw milk, Milk cooling, Storage temperature, Aerobic plate count, Psychrotrophic count, Milk cooling system, Bulk milk cooler

Introduction

In the present scenario of Indian milk collection centre where bulk milk coolers (BMCs) are used, the temperature of blend (milk from morning and evening milk collection in BMC) raw bulk milk reaches about 18 to 20°C when second milking is added to chilled milk (first milking) at 4°C. It takes another one and half hour for a bulk milk (first and second milking) to be chilled to 4°C. Besides the storage temperature, the cooling time to reach storage temperature (4 °C) is also of significance. Keeping milk at elevated temperature for longer time stimulates the microbial proliferation and hence increased microbial load in raw milk. Precooling of milk before it enters the bulk tank by using novel milk cooling system, could restrict the rate of bacterial growth in raw milk thereby gives superior quality of raw milk.

Sameera et al. (2020) evaluated quality of raw milk from two different locations in Hyderabad region, Telangana state, India for a period of six months from January to June. The bacterial count ranged from 7.09 to 8.18 log CFU/ml. Further, the trend of microbial quality of a greater number of milk samples were shifting towards fair, poor and very poor from February to June due to seasonal variation in raw milk quality as affected by variations in milk production practices and ambient temperature with the season. The study concluded that the microbiological quality of most of the milk samples collected from different areas of Hyderabad city were not up to the standards, as evidenced by their high number of microorganisms and also the presence of coliform bacteria. Kakati et al. (2021) assessed the quality of raw milk based on the microbial load, sold in and around Guwahati city of India. All of the raw milk samples had a significantly higher standard plate count and coliform count than the permissible standard. While Dinki and Balcha (2013) evaluated raw milk samples of cattle collected from six different consumers collection centres of Guwahati city, India. It was reported that the mean standard plate count and the mean coliform count of raw milk were 6.38 ± 0.02 and 2.85 ± 0.03 log CFU/ml, respectively. It can be concluded from the above two studies that the raw milk sold in

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Guwahati city do not confer to the legal microbiological standard and may pose a high risk of milk-borne illness among consumers.

Psychrotrophic microbes, particularly *Pseudomonas* spp., are found in the microbiota of chilled milk because they can grow at temperatures below their optimal growth temperature. Psychrotrophic counts ranging from 6.00 to 9.00 log CFU/ml in refrigerated raw milk affect cheese quality, since the synthesized thermoresistant enzymes affect the nutritional value, sensory properties and texture. In addition to significantly affecting cheese yields, the enzymes produced by psychrotrophic microbes cause taste alterations, unfavourable clotting times, increased concentrations of free fatty acids and free amino acids, and a shorter shelf-life. Surprisingly, psychrotrophic bacterial growth may represent a serious defect both for fresh or ripened cheeses (Caputo et al. 2015). Poor quality of raw milk is also known to produce inferior quality dairy products with reduced shelf life.

The pH of milk should be between 6.65 and 6.8 to ensure trouble-free processing and high quality of the final product. A lower pH will risk product stability and cause fouling. A higher pH may indicate mastitis-infected milk. As a result, milk that does not fulfil these requirements is not appropriate for UHT processing (Tetrapak, 2014). The natural acidity of milk is due to casein, mineral substances, and phosphates. The developed acidity is due to the lactic acid produced by lactose degradation because of microorganisms. The titratable acidity test is used to determine whether milk has a high acidity level that affects its keeping quality and heat stability. The acidity of milk is not a true measure of lactic acid present but in practice, gives a good indication of the quality of milk. The superior quality raw milk has a relatively steady Titratable Acidity (TA) value ranging between 0.12 to 0.17% lactic acid (Schmidt et al. 1996).

Even though India stood first in milk production, the quality of raw milk produced is poor. This leaves larger impacts on the finished dairy product prepared out of it. Hence there is a necessity to improve the microbial quality of raw milk at the farm level itself by rapid cooling of milk immediately after milking. This can restrict the growth of microorganisms at the initial level. So, the present study was conducted with an objective to see the effect of rapid cooling on microbiological quality of raw milk compared to BMC cooled raw milk at farm level.

Materials and Methods

The novel milk cooling system was installed at Livestock Research Station (LRS) farm of Anand Agricultural University, Anand. The existing milk cooling system *i. e.*, BMC, available at dairy farm was used as a control.

Novel milk cooling system

The different components of milk cooling system were cooling cum storage tank (350 L), plate cooler for pre-cooling of raw milk

at different temperature, pump for transferring raw milk from plate cooler to cooling cum storage tank, thermal storage system (500 L) for making required quantity of ice in cooling of raw milk to final storage temperature. Temperature measuring sensors were installed at raw milk inlet to plate cooler, cooling cum storage tank, chilled water supply and return. The piping & instrumentation diagram of novel milk cooling system is given in Figure 1.

Control milk cooling system (BMC)

The different components of existing milk cooling system were buffer tank (Make: Delaval, volume; 20 L), balance tank (150 L), milk transfer pumps, horizontal closed type bulk milk cooler (Make: Delaval, Capacity: 1000 L) and condensing unit of 3 Hp. The piping & instrumentation diagram of control milk cooling system is given in Figure 2.

Precooling of raw milk using plate cooler in novel milk cooling system

The average daily milk production at cow farm was 1250 L. Raw milk collected through machine and manual milking was transferred into novel and control milk cooling system (BMC). In the evening, raw milk was cooled to 4°C in control milk cooling system whereas in novel milk cooling system, raw milk was first, pre-cooled to different temperatures viz., 30°C, 20°C, 10°C and 4°C through plate cooler and then it was transferred to cooling cum storage tank where raw milk was cooled to final storage temperature (4°C) using chilled water. These milks were stored overnight at 4°C in respective milk cooling systems. In the morning, same procedure was followed for raw milk cooling. Hence, raw milk at 34°C received from morning milking was added to previous day evening's raw milk maintained at 4°C.

Both the systems were operated at 50 per cent storage capacity in the evening and remaining 50 per cent in the morning such that system is capable to cool milk as per the standard ISO-5708. For the novel milk cooling system, the required quantity of ice was formed in thermal storage system well before each milk cooling cycle. Trials were conducted for the optimization of precooling temperatures of raw milk based on the storage stability for 72 h at refrigerator temperature (4°C±0.5°C).

Chemicals and glassware

During the entire study, Borosil brand (Borosil Glass Works Ltd., Mumbai, India) of glass wares and analytical grade chemicals were used. The bacteriological media, chemicals and reagents were purchased from Hi-media (Bangalore) and SD chem (India).

Collection of raw milk sample

The milk sample for the analysis was collected in the morning once the blend raw milk temperature reached to 4°C. It was

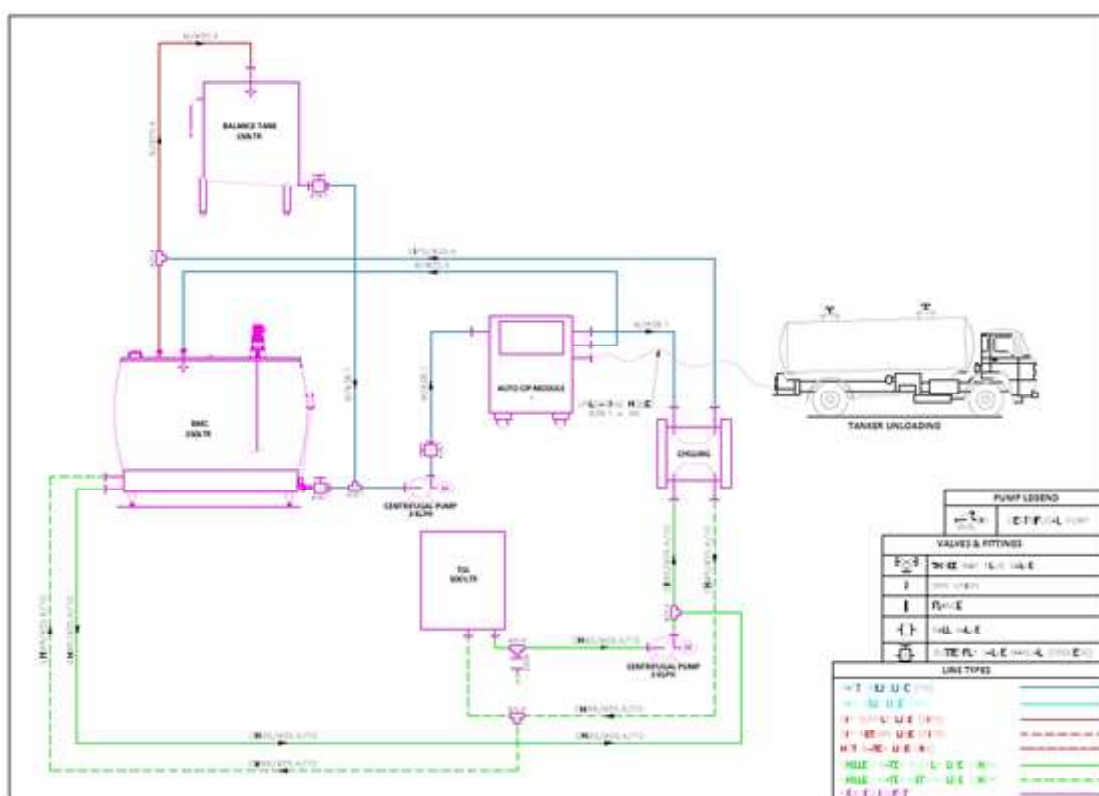
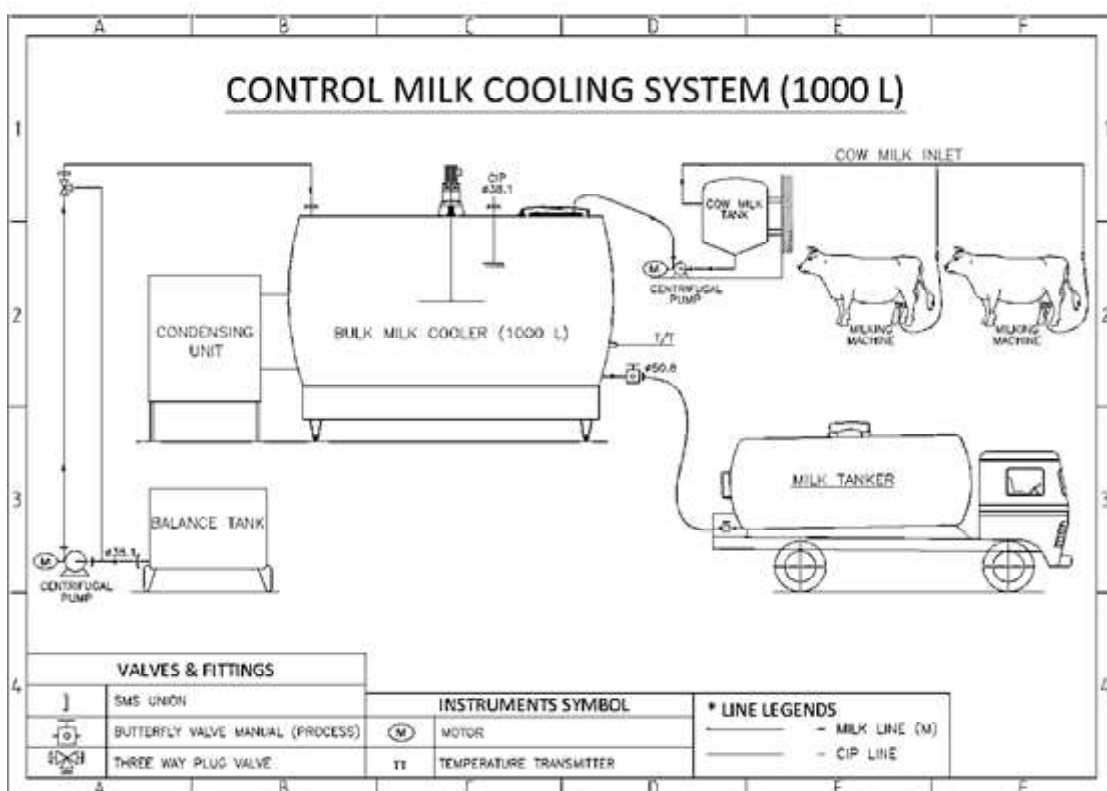


Fig. 2 Bulk milk cooling system



milk was thoroughly mixed and the outlet of the BMC/cooling cum storage tank was disinfected with the wet alcohol swab. Then initial small volume of milk was allowed to drain and the

representative sample of raw milk was drawn into sterilized container. The samples were then stored in refrigerator maintained at $4\pm 0.5^{\circ}\text{C}$ to mimic the storage-conditions used in cold chain. The sample was evaluated for microbiological and physico-chemical tests at an interval of 24 h from 0 h to 72 h.

Determination of fat content of raw milk

The fat content was determined by Gerber method as described in BIS (1981).

Determination of solids-not-fat of raw milk

The SNF of raw milk was determined as follow:

$$\text{SNF of raw milk} = \text{Total solids} - \text{Fat}$$

The total solid content of raw milk was estimated by gravimetric method described in lab manual of FSSAI (2015).

Determination of methylene blue reduction time of raw milk

The MBRT was carried out by following the method of ISO 4833-2:2013. The samples showed > 5 h MBR time were considered as excellent quality raw milk.

Determination of aerobic plate count of raw milk

The aerobic plate count was carried out by following the method of IS 5402-2002 & ISO 4833:1991 with slight modifications. The plates containing colonies between 30-300 were considered for the calculations. The counts were expressed as log CFU/ml.

Determination of psychrotrophic count of raw milk

Psychrotrophic count of raw milk sample was performed by following the method suggested by Júnior et al. (2018) with slight modifications. The counts were expressed as log CFU/ml.

Determination of pH of raw milk

The pH of raw milk was measured using digital pH meter (make: chemi line, India) at 25°C temperature.

Determination of titratable acidity of raw milk

The titratable acidity of raw milk was estimated by the procedure described in IS:1479, Part I (1960). Precooling temperature for raw milk in novel milk cooling system was optimized based on the results obtained from the above tests.

Statistical analysis

All the data were subjected to statistical analysis using Completely Randomized Design (CRD) as per the methods described in Steel and Torrie (1980). The significance was tested at 5 per cent level

of significance using mean value, co-efficient of variance (C.V.) and critical difference (C.D.).

Results and Discussion

Novel milk cooling system and control milk cooling system

The piping and instrumentation diagram (P&ID) of the novel and control milk cooling system is given in figure 1 and 2, respectively. In novel milk cooling system, piping connections for milk flow was provided from balance tank, milk supply pump, plate cooler and cooling cum storage tank. Plate cooler and cooling cum storage tank were provided with chilled water supply and return lines connected to inlet and outlet of thermal storage system. Piping connections for thermal storage was provided such that chilled water circulates either through plate cooler or cooling cum storage tank. Milk supply pump, chilled water supply pump and condensing unit of vapour compression refrigeration system (VCRS) were connected with three phase power supply. Control panel and temperature display screen were mounted on wall. The system was installed on the roof with good ventilation for proper functioning of the condensing unit. Milk outlet at the bottom of cooling cum storage tank was provided for tanker dispatch of raw milk in the morning.

In control milk cooling system (BMC), milk was transferred from milking machine to buffer tank through piped connection. Milk from buffer tank to bulk milk cooler was transferred through milk transfer pump-1. Whereas milk collected in balance tank from manual milking was transferred to bulk milk cooler through milk supply pump-2. Condensing unit for R-22 was connected with bulk milk cooler. Milk outlet at the bottom of bulk milk cooler was provided for tanker dispatch of raw milk in the morning.

In the novel milk cooling system, the size of the different components was selected according to the volume of milk and cooling demand required in milk collection and cooling during evening and morning at the farm. The thermal storage system was designed for cooling 50 per cent volume of milk (150 L) at a given time. The capacity of the novel system was 300 L. While control system was having capacity of 1000 L. Since BMC was already installed at dairy farm with 1000 L capacity. The remaining surplus milk available at dairy farm was handled by novel milk cooling system. Therefore, two milk cooling systems with different capacity was used in study.

Optimization of precooling temperature of raw milk for the novel milk cooling system

The optimization for precooling temperature of raw milk was carried out based on physico-chemical and microbial analysis. The details of raw milk used for the experiments are given in the Table 1. The milk samples were evaluated for MBR time, aerobic plate count, psychrotrophic counts, pH and titratable acidity (%LA).

The MBR time of all the raw milk sample was > 5 h indicating excellent quality of raw milk.

Of the total volume of milk produced at farm, 80% was collected by machine milking and 20% by manual milking.

Determination of aerobic plate count of raw milk

Aerobic plate count enumerates the total number of microorganisms present in the given sample. Aerobic plate count of the raw milk sample was carried out by pour plate technique. Changes in aerobic plate count of raw milk during storage at $4^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 72 h is shown in Table 2. It was found that the aerobic plate count of all the raw milk samples increased significantly ($p < 0.05$) with the storage period. Also, significant ($p < 0.05$) increase in the aerobic plate count was observed in milk samples when precooling temperature increased from 10°C to 30°C . However, non-significant difference in aerobic plate count was observed in milk samples pre-cooled at 10°C and 4°C . The aerobic plate count was the highest in control (5.89 ± 0.15 log CFU/ml) followed by 30°C (5.84 ± 0.22 log CFU/ml), 20°C (5.74 ± 0.23 log CFU/ml), 10°C (5.13 ± 0.16 log CFU/ml) and 4°C (5.02 ± 0.16 log CFU/ml) at 0 h.

Similar results were reported by Malacarne et al. (2013). They evaluated raw milk samples for aerobic plate count during storage at refrigerator temperature. The aerobic plate count of raw milk,

when stored at $4-6^{\circ}\text{C}$ temperature was 5.02 ± 0.37 log CFU/ml at 0 h and 5.12 ± 0.49 log CFU/ml at 48 h storage. In another similar study conducted by Abd Elrahman et al. (2009), 4.80 ± 0.02 log CFU/ml aerobic plate count of fresh raw cow milk was reported. While Tan et al. (2020) reported 5.18 log CFU/ml aerobic plate count of fresh raw cow milk and 7.84 log CFU/ml aerobic plate count of fresh raw goat milk.

Determination of psychrotrophic count of raw milk

Psychrotrophs can grow in raw milk when kept at refrigerator storage temperature. They are sensitive to pasteurization but capable of producing heat stable protease and lipases enzymes. These enzymes remain active in the finished dairy product leading to reduced shelf life of the dairy products. So, it is necessary to detect the presence of psychrotrophs in the given sample of raw milk. Changes in psychrotrophic count of raw milk during storage at 4°C for 72 h is shown in table 3. It was found that psychrotrophic count of raw milk samples increased significantly ($p < 0.05$) upon storage at refrigerator temperature ($4^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) from 0 h to 72 h. The highest psychrotrophic count was observed in control compared to the treatment samples. A non-significant difference was observed between control and 30°C precooling treatment. Similarly, non-significant difference was observed between 10°C and 4°C precooling treatments. The lowest count was observed when raw milk was pre-cooled at 10°C and 4°C . The psychrotrophic counts of the raw milk samples ranged from

Table 1 Details of raw milk procured for the experiment

Type of milk	Cow
Type of breed	Cross bred of Holstein Friesian, Gir and Kankrej
Type of milking	Machine (80%) and manual (20%)
Frequency of collection	Twice a day (morning and evening)
Fat* (%)	4.51 ± 0.08
SNF* (%)	8.70 ± 0.05
MBR time	> 5 h

*Observation is a mean $\pm$ SD of four replicate experiments (n=4)

Table 2 Changes in aerobic plate count of raw milk (log CFU/ml) during storage at $4^{\circ}\text{C}/72$ h

Storage time (h), P	BMC (Control)	Precooling, T				Period Average (P)
	4°C	30°C	20°C	10°C	4°C	
0	5.89 ± 0.15	5.84 ± 0.22	5.74 ± 0.23	5.13 ± 0.16	5.02 ± 0.16	5.52
24	6.11 ± 0.13	5.98 ± 0.12	5.98 ± 0.13	5.14 ± 0.32	5.12 ± 0.16	5.67
48	6.46 ± 0.23	6.28 ± 0.11	6.03 ± 0.15	5.18 ± 0.08	5.16 ± 0.15	5.82
72	6.87 ± 0.17	6.83 ± 0.22	6.26 ± 0.13	5.26 ± 0.19	5.21 ± 0.11	6.09
Treatment Average (T)	6.33	6.23	6.01	5.18	5.13	
Source	SEm	CD (0.05)		CV (%)		
T	0.06	0.18				
P	0.06	0.16		3.03		
T $\times$ P	0.12	0.37				

Each observation is a mean $\pm$ SD of three replicates (n=3)

1.71±0.13 log CFU/ml to 3.54±0.17 log CFU/ml throughout the storage period.

Similar study was conducted by Malacarne et al. (2013). They evaluated raw milk samples for psychrotrophic count during storage at refrigerator temperature. The psychrotrophic count of raw milk was 3.72±1.64 at 0 h and 4.05±1.84 at 48 h of storage at 4-6°C temperature. The psychrotrophic counts were higher compared to present study. In another similar study conducted by Abd Elrahman et al. (2009), 0.84±0.03 log CFU/ml psychrotrophic counts of fresh raw cow milk was reported. While Tan et al. (2020) found 4.96 log CFU/ml psychrotrophic counts of fresh raw cow milk and 5.90 log CFU/ml psychrotrophic counts of fresh raw goat milk.

The wide variation in psychrotrophic count of raw milk, reported by different researchers can be due to the factors like variation in source of raw milk, place of milking, hygiene maintained during milking, cleanliness of milking shed, season of the year, type of milking (manual and machine), etc.

Determination of pH of raw milk

Changes in pH of raw milk during storage at 4°C for 72 h is shown in table 4. The pH of cow milk samples ranged from 6.71 to 6.64 at 0 h of storage in refrigerator (4°C±0.5°C). The non-significant difference in pH of milk was observed for all the raw milk samples including control during storage at 4°C±0.5°C for 72 h. Similar observation was reported by Malacarne et al. (2013). They evaluated raw milk samples for pH during storage at refrigerator temperature. The pH of raw milk was 6.72±0.03 at 0 h and 6.79±0.05 at 48 h of storage at 4-6°C temperature. In another similar study conducted by Abd Elrahman et al. (2009), reported 6.91±0.003 pH of fresh raw milk of cow. While Tan et al. (2020) found 6.59±0.01 pH of fresh raw milk of cow and 6.34±0.00 pH of fresh raw milk of goat.

In our study, the non-significant increase in milk pH values during cold storage at 4°C±0.5°C was the result of two phenomena with conflicting effects: 1) the production of lactic acid by the growing microflora (mainly psychrotrophic bacteria), which decreased milk pH and 2) the dissociation of calcium from the casein micelle, which increased milk pH. Hence, the pH remained in the normal

Table 3 Changes in psychrotrophic count of raw milk (log CFU/ml) during storage at 4°C for 72

Storage time (h), P	BMC (Control)	Precooling, T				Period Average (P)
	4°C	30°C	20°C	10°C	4°C	
0	2.54±0.10	2.40±0.20	2.13±0.10	1.81±0.13	1.71±0.13	2.12 ^c
24	2.62±0.16	2.50±0.09	2.24±0.13	1.86±0.11	1.76±0.19	2.20 ^c
48	2.99±0.14	2.82±0.19	2.33±0.17	1.97±0.18	1.84±0.11	2.39 ^b
72	3.54±0.17	3.25±0.22	2.52±0.13	2.05±0.16	1.98±0.18	2.67 ^a
Treatment Average (T)	2.92 ^A	2.74 ^B	2.30 ^C	1.92 ^D	1.82 ^D	
Source	SEm	CD (0.05)		CV (%)		
T	0.05	0.16				
P	0.05	0.14		6.56		
T×P	0.11	0.32				

Each observation is a mean ± SD of three replicates (n=3)

Table 4 Changes in pH of raw milk during storage at 4°C for 72 h

Storage time (h), P	BMC (Control)	Precooling, T				P Average
	4°C	30°C	20°C	10°C	4°C	
0	6.64±0.08	6.65±0.06	6.66±0.08	6.70±0.07	6.71±0.04	6.67
24	6.60±0.07	6.62±0.08	6.63±0.04	6.69±0.05	6.70±0.07	6.65
48	6.61±0.06	6.59±0.07	6.59±0.09	6.68±0.04	6.67±0.07	6.63
72	6.56±0.08	6.56±0.08	6.57±0.07	6.65±0.08	6.65±0.06	6.60
T Average	6.60	6.60	6.61	6.68	6.68	
Source	SEm	CD (0.05)		CV (%)		
T	0.02	NS				
P	0.02	NS		1.05		
T×P	0.05	NS				

Each observation is a mean ± SD of three replicates (n=3)

Table 5 Changes in titratable acidity (% LA) of raw milk during storage at 4°C for 72 h

Storage time (h), P	BMC (Control)		Precooling, T			P Average
	4°C	30°C	20°C	10°C	4°C	
0	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14
24	0.16±0.02	0.15±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.15
48	0.16±0.02	0.16±0.01	0.15±0.02	0.15±0.01	0.14±0.01	0.15
72	0.16±0.01	0.16±0.01	0.16±0.01	0.15±0.01	0.15±0.02	0.15
T Average	0.16	0.15	0.15	0.15	0.14	
Source	SEm	CD (0.05)		CV (%)		
T	0.004	NS				
P	0.003	NS		7.35		
T×P	0.008	NS				

Each observation is a mean ± SD of three replicates (n=3) range during initial period of storage and/or until growing psychrotrophs overcomes the buffering capacity of raw milk.

Determination of titratable acidity of raw milk

Titratable acidity indicates the acid produced in the milk in terms of lactic acid. Increased acidity indicates the increased microbial load in the milk. Changes in titratable acidity (% LA) of raw milk during storage at 4°C±0.5°C for 72 h is shown in table 5. In a study, the titratable acidity of all the milk samples remained in the normal range i.e., 0.14 % LA to 0.16 % LA. Increased acidity in milk samples upon storage at 4°C for 72 h was non-significant (p>0.05) for all treatments. According to IS 1479-1(1960) standards, acidity of raw milk varies from 0.10 % LA to 0.17 % LA. Any value in excess of 0.17 % LA can safely be thought as developed lactic acid.

Similar study was conducted by Schmidt et al. (1996). They evaluated raw milk samples for titratable acidity during storage at refrigerator temperature. The titratable acidity of raw milk was 0.15±0.01 %LA at 0 h, 0.16±0.01 %LA at 48 h and 0.17±0.02 %LA at 96 h of storage at 6°C temperature. In another similar study conducted by Abd Elrahman et al. (2009), 0.145±0.000 %LA of fresh raw cow milk was reported. While Tan et al. (2020) found 0.16±0.01 %LA of fresh raw cow milk and 0.22±0.01 %LA of fresh raw goat milk.

MBR time, pH and titratable acidity of all the raw milk samples were in the normal range and non-significant difference observed during storage study. From the microbial analysis of pre-cooled raw milk samples stored at 4°C for 72 h, it was found that the microbial count for 10°C and 4°C were lower and remained non-significant among the pre-cooling treatments. Hence, the pre-cooling temperatures for raw milk were optimized to 10°C and 4°C.

Conclusion

Pre-cooling of raw milk using novel system showed the significant improvement in the microbial quality of milk compared to the traditional milk cooling in BMC. Significant (p<0.05) reduction in

the aerobic plate count and psychrotrophic count was observed from 5.89±0.15 log CFU/ml and 2.54±0.10 log CFU/ml in control sample to 5.13±0.16 log CFU/ml and 1.81±0.13 log CFU/ml in 10°C pre-cooled treatment sample, respectively. Such novel system can help farmers to receive better price of raw milk with improved quality and it could be installed at village cooperative society and/or farm to improve raw milk quality. Further, detailed study can be conducted for the different type of microorganisms present in the raw milk e.g., spore formers, lipolytic, proteolytic, thermotolerant, thermophilic.

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Design of micro-perforated PTFE mould for manufacture of *Paneer*, *Cheese* and *Tofu* using finite element analysis

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Abstract: The idea of utilizing a micro-perforated PTFE (polytetrafluoroethylene) mould to manufacture paneer, cheese, and tofu was conceived and mould was designed, developed and tested. The micro-perforated PTFE mould is intended to work at temperature 65 to 110°C, 200 kPa inside pressure. This new generation pressing mould contains micro perforations of 300µm diameter which are drilled using advanced high precision 4 and 3 axis CNC machines. There will be failure (due to induced hoop stress) in the mould when Von Mises (equivalent) stress generated is more than yield stress of virgin PTFE (30 MPa). Wall thickness of mould was optimized by Finite Element Analysis (FEA) by hyper tetrahedron meshing using ANSYS- 22. The results obtained from stress analysis were expressed as Von Mises stress, deformation and factor of safety. A design software developed by ASME was employed to validate shell thickness. The model prediction was shown to be in good agreement with the analytical calculation. Micro-perforated PTFE mould was fabricated as per FDA, c-GMP standards from 6.00 mm thick virgin PTFE material. The FEA resulted in Von Mises Stress of 14.7 MPa, deformation of 2.1535×10^{-10} m and factor of safety of 2.04. The working drawings were developed and actual fabrication was carried out adopting the prescribed sanitary standards. Satisfactory production of paneer was carried out in the newly designed and developed PTFE paneer mould resulted in excellent quality product.

Keywords: Cheese hoop, FEA Micro-perforated PTFE mould, Paneer mould

Introduction

A notable technological improvement in Dairy and Food Industry have taken place by the utilization of food-grade plastic, such as virgin polytetrafluoroethylene (PTFE) which is

FDA-approved and adheres to FDA (CFR 21-177.2600). PTFE is a fluoropolymer, classified among thermoplastics. It was invented by Roy J. Plunkett in 1938 at the DuPont company and it goes by the brand name Teflon in the commerce. Tetrafluoroethylene, the monomer, is used to polymerize PTFE (TFE). It has the C-F bond and the chemical formula $[(CF_2-CF_2)_n]$ in the PTFE formulation (Dhanumalayan and Joshi, 2018).

PTFE is chemically inert & non-reactive to almost all known chemicals, excellent thermal resistance up to 260°C, compatible with all manufacturing practices without leaching its contents during operation in the wide range of pH 0 to 14, anti-stick properties, exceptionally low coefficient of friction, outstanding electrical insulation properties, excellent weathering resistance, high resistance to radiation, non-absorbent of water, self-lubricant, non-toxic material, anti-microbial, FDA approved and resistant to magnetic field (Puts et al. 2019).

The application of virgin PTFE in dairy and food industries includes PTFE gasketing material: Gaskets, O/D/V/U rings, Universal rope, Expansion joints, Ball valves seats and Seals, Diaphragms of diaphragm valves and pumps, Sleeves of plug valves, Mechanical seals of pumps, Impellers / body of pumps, Tubing and hoses, Liners of reactors, Storage vessels, pipes and flanges, Thread seal tapes, Liners of butterfly valves. The use of PTFE in the food processing industry helps to prevent biofilm formation, food contamination, and fouling (Puts et al. 2019)

Legg et al. (2017) reported that pressing and moulding are crucial process in the production of paneer, cheese and tofu which involves using a hoop or mould, a special container which are basically cylindrical or rectangular in shape which is fabricated to hold and shape the curd into the desired shape while allowing for the application of pressure at the desired level which helps in removal of whey and further gives the curd into the desired mould shape. For whey drainage, holes were precisely drilled through

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the sides, base, and lid or pressure plate of these moulds. In conventional method which uses muslin cloth and stainless steel mould for production of paneer, cheese and tofu. The usage of muslin cloth is unhygienic and prone to microbial contamination which leads to deterioration, reduced yield and loss of pleasing appearance.

An innovative research was taken up to address the aforementioned drawbacks in traditional paneer production. In this research FDA-approved virgin PTFE has been successfully utilized to develop innovative new generation paneer, cheese and tofu press hoop or mould. In this new technology by the adoption of microholes, with a diameter of 300 μm , for the efficient drainage of whey without any loss of curd mass during pressing, thereby eliminating the need for cheese or muslin cloth.

In recent numerical methods such as Finite element analysis (FEA) are popularly employed in design and evaluation of processing equipment's including pressure vessel. The mould will be exposed to atmospheric pressure on the outside surface during working while inside applied pressure was on curd mass for moulding and dewheying.

Von-Mises stress (Von Mises, 1913; Mahesh and Ravindra, 2017) have used FEA concept to ascertain their design to verify the stability at given load conditions. By knowing Von Mises stress induced in material designer can make changes in materials of construction, wall thickness, stiffeners etc. so that Von - Mises stress (also known as equivalent stress) induced shall be less than yield stress of the material selection (for virgin PTFE material the induced stress should less than yield stress i.e., 30 MPa). FEA is a computational technique used to obtain Von Mises stress, deformation and factor of safety by providing loads or pressure, boundary conditions, material properties and meshing to ANSYS computational software (Ramachandra et al. 2021).

Material and Methods

The micro-perforated PTFE mould was fabricated from virgin PTFE material. The mould comprises of 3 components flanged micro-perforated cylindrical body, flat top micro-perforated circular disc which acts as pressure plate and flat bottom micro-perforated circular disc. Top disc was designed such a way that during pressing it will move inside the cylindrical body and further hot coagulum will be moulded under the action of pneumatic press, while bottom disc is fixed and it supports cylindrical body.

FDA-approved PTFE material of suitable size of bush and sheets were selected for development of mould on which micro holes of size 300 μm were drilled on the surface using flute type drill bits having tip size of 300 μm and length of 7 mm.

In pursuit of the major objective of the study, the first stage was to develop the major conceptualized micro-perforated PTFE mould. The standard engineering design procedures were adopted.

Mechanical strength of conceptualized mould has been analyzed using ANSYS software by FEA method. Based on the optimized design, fabrication was done using 3 and 4-axis CNC machines. The newly developed mould was then tested with actual production of product. Following design data viz., mechanical properties of material, required for FEA for stress analysis are shown in table 1. The design boundary conditions required for stress analysis by FEA are given in the table 2. The actual dimensions of micro-perforated PTFE mould in terms of diameter and height were derived based on desired processing capacity as given in table 2. A wall thickness of 6 mm was considered for developing the micro-perforated mould based on FEA and ASME results. The inside temperature was assumed to similar with coagulum temperature which is ranges 65 to 110°C and table 1 describes all other parameters. The size of the unit was designed based on capacity of processing and the major dimensions are shown in figure 1 a (micro-perforated bottom insert), 1 b (micro-perforated top insert or pressure plate) and 1c (micro-perforated cylindrical body) which also describes the other constructional features of mould.

Determination of actual pressure acting inside the developed micro-perforated PTFE mould

Pressure acting inside the pneumatic cylinder which is equipped in the automatic paneer pressing machine - maximum 5 bar (g) or 0.5 N/mm². Bore diameter of pneumatic cylinder (measured using Vernier caliper) is 80 mm. Cross- Sectional area and pressure acting inside mould was calculated using eq1 and 2.

$$A = \pi r^2 \text{ ————— Eq-1}$$

$$F = P \times A \text{ ————— Eq-2}$$

Stress analysis cycle (FEA)

In order to optimize the wall thickness of micro-perforated PTFE mould based on FEA stress analysis which includes induced Von-mises stress or equivalent stress, total deformation and Factor of safety was performed using ANSYS 22 software as procedure detailed by Mahesh and Ravindra (2017). The stress analysis cycle followed as shown in flowchart.

Validation of Shell thickness

To validate shell wall thickness obtained by FEA (ANSYS-22), results were compared with wall thickness of mould calculated by ASME design equation (Mahesh and Ravindra, 2017). The calculation done by adopting ASME norms which is basic strength calculation and doesn't account for stress induced while operation.

Calculation code as per ASME norms shown in equation 3.

Wall thickness of the cylindrical shell was calculated using

$$t = \frac{P \times R}{(2SE - 0.6P)} \quad \text{Eq -3}$$

Where,

t = Cylinder thickness (mm)

P = Design pressure (MPa)

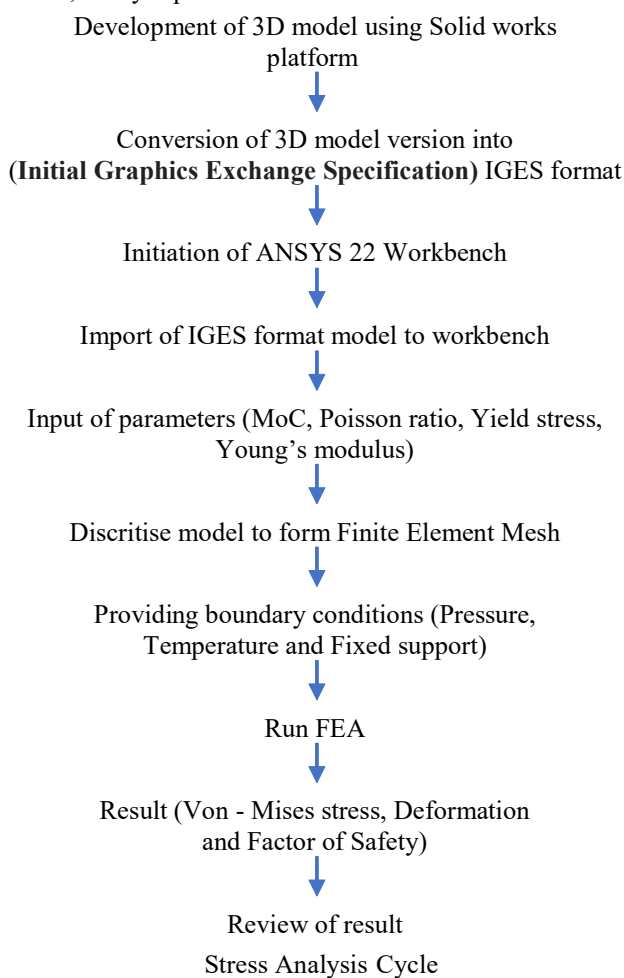
R = Cylinder Inside radius (mm)

S = Maximum Allowable Stress at design temperature (MPa)

E = Joint Efficiency

Procedure to run software programme for calculation of shell thickness (ASME 2011)

The calculation also requires the user to enter dimensions of model, pressure, operating temperature, mechanical of Virgin PTFE as shown in figure 2 (Virgin PTFE is resistant to corrosion, however, it may experience wear and tear over time. ASME design



equation-based software does not provide an option to ascertain values for wear and tear allowances. To account for this, a corrosion allowance of 1 mm was included).

Results and Discussion

The results obtained by FEA for the optimization of wall thickness of

Micro-perforated PTFE mould and comparison between the paneer made from micro-perforated PTFE mould and conventional stainless steel paneer mould were also depicted. The FEA result were presented in terms of Von-Mises (equivalent) stress, total deformation and factor of safety (FoS).

Determination of actual pressure acting inside the developed micro-perforated PTFE mould

The result obtained for calculating cross-sectional area of pneumatic cylinder of OD 80 mm was found to be 5024 mm² by using eq-1. The force exerted by pneumatic cylinder while operating at inside pressure of 5 bar(g) was found to be 2512 N using eq-2. Similarly, cross-sectional area of micro-perforated PTFE mould of ID was found to be 12,265.7 mm² using eq-1 and pressure acting inside the mould is 200 kPa or 0.2 N/mm² using eq-2. Therefore, the mould was designed to withstand pressure of 2 bar.

Stress analysis of the Micro-perforated PTFE mould

The results of FEA stress analysis are presented in terms of Von Mises (Equivalent) stress. The obtained simulation of the stress analysis by FEA using ANSYS 22 is shown in the figure 3a. Comparing the color scheme of the domain with the color legend panel. It depicts the magnified picture of the highest and lowest stress regions developed during the application of pressure during Pressing operation. The highest stress (red and yellow color) 1.47×10⁰⁷ Pa was induced in some regions of bottom perforated plate and around its circumference. The lowest stress (dark blue) 1.08×10⁰⁶ Pa while average stress experienced was 4.2004×10⁰⁶ Pa.

According to the Von Mises criterion, fracture or failure occurs when the energy or stress developed surpasses the yield stress, in this case 30 MPa (Virgin PTFE) (Von-Mises, 1913; Mahesh and Ravindra, 2017). The FEA analysis carried out using ANSYS 22 software, the stress induced during simulation was greater than the yield stress of virgin PTFE material when the envisioned mould was modelled and examined at 2, 3 and 4 mm wall thickness and hence it was decided to use 6mm thick virgin PTFE material to fabricate the micro-perforated PTFE mould. The maximum stress induced in the micro-perforated PTFE mould was 1.47×10⁰⁷ Pa (14.7 MPa) which is much less than the tensile yield strength of virgin PTFE material (30 MPa) and hence stress resulted from

Table 1: Mechanical properties of PTFE

Mechanical Properties	Value	Unit
VIRGIN PTFE		
Color	Milky white	
Density	2.1-2.2	gm/cc
Poisson ratio	0.42-0.44	
Modulus of elasticity (tensile test)	0.4-0.75	GPa
Tensile strength	22 - 30	MPa
Tensile strength at break	-	MPa
Elongation at break	220 -300	%
Shore hardness	55	-
Compression strength	5	MPa
Ball indentation hardness	30	MPa
Coefficient of static friction	0.08- 0.10	-
Thermal Properties	Value	Unit
Crystalline melting point	327	°C
Service temperature (max)	260	°C
Service temperature (min)	-200	°C
Service pH	0-14	
Thermal expansion (CLTE)	9.5	10^{-5} K^{-1}
Specific heat	1000	J/(g*K)
Thermal conductivity	0.20	W/(K*m)
Water absorption	0	%
This material is FDA Approved (FDA CFR 21-177.2600 Compliant)		
PTFE is chemically inert & unaffected by all known chemicals except molten or dissolved alkali metals–Sodium; Potassium; Rubidium; Cesium; Francium & Fluorine gas, certain fluorine compounds & complexes at elevated temperatures.		

Table 2: Design Data and Boundary conditions

Description	Unit	Value
Material of construction	FDA (CFR 21-177.2600 Compliant)	PTFE
Micro-perforated PTFE mould		
Inner diameter of mould	125	mm
Wall thickness of mould	6	mm
Length of mould	100	mm
Outer diameter of top disc and its thickness	125 and 6	mm
Outer diameter of Bottom disc and its thickness	150 and 6	mm
Actual pressure acting (Inside)	200	kPa
Operating pressure (outside)	101.325	kPa
Operating temperature (inside)	60-80	°C
Operating temperature (outside)	25-30	°C
Fixed support	Micro-perforated PTFE mould will be mounted on SS/ Wooden support to prevent mould from movement	

simulations were considered within in the acceptable limits for operating conditions designed for the study.

Deformations from the FEA stress analysis

To complete the picture of the stress analysis, the total deformation generated in

Micro-perforated PTFE mould from stress analysis was evaluated and the result presented in figure 3b. FEA numerical analysis was also extended to the evaluation of anticipated deformation on the mould as result of stress generated. A close visual of results of FEA and the color legend panel indicated that the total deformation induced ranges from 0 to 2.1535×10^{-10} m which seems

Fig. 1a Dimensional drawing of conceptualized Micro-perforated bottom insert

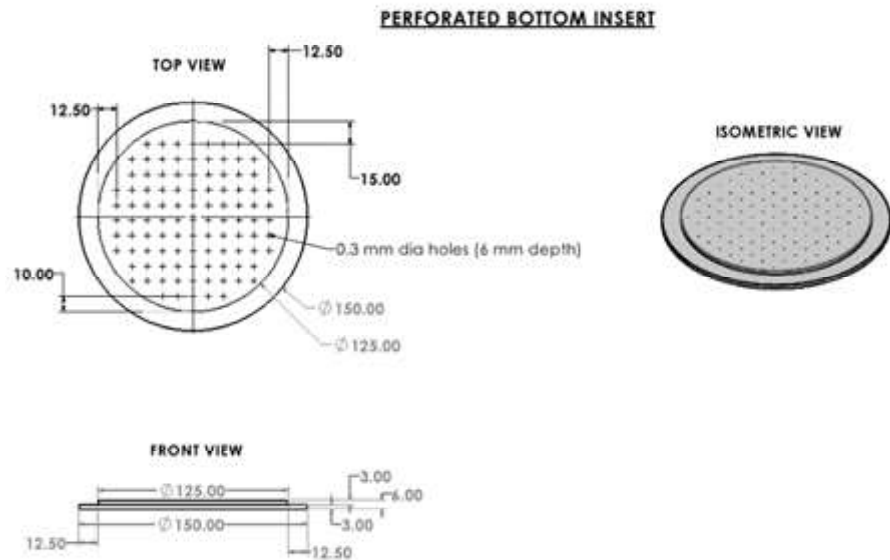
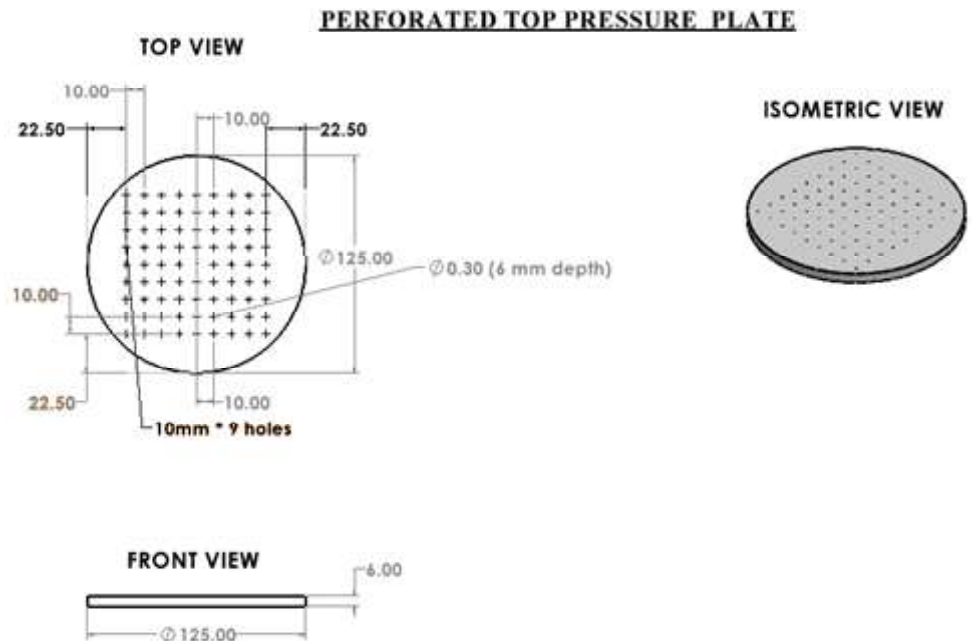


Fig 1b: Dimensional drawing of conceptualized Micro-perforated top pressure plate



to more acceptable due to rigid construction. Dutt (2016) developed the knuckle pin for tractor made of PTFE material, at max load of 50 KN, the deformations occurred was 3.96×10^{-6} mm and the present results was less than the reported and hence FEA analysis is deemed to be appropriate.

Factor of safety

The numerical stress analysis was also carried out to determine the factor of safety for the designed unit. The result of FEA analysis is presented. It is evident from the results shown in figure 3c. The minimum factor of safety obtained was 2.04 (yellow color region) obtained peak induced stress area which greater

than 1.0 and hence the design can be deemed to be safe (Krann et al. 2004), maximum FoS was 15 (Blue color region).

Jebelli et al. (2018) suggested that the minimum factor of safety (1.5 to 3.5) should be considered for fabrication of processing equipment's operating under pressure whose MoC was PTFE (23 to 30 MPa yield strength) and the present result of FoS falls in safe side and hence the FEA analysis value was appropriate.

Determination of shell wall thickness by using ASME approved design equation software

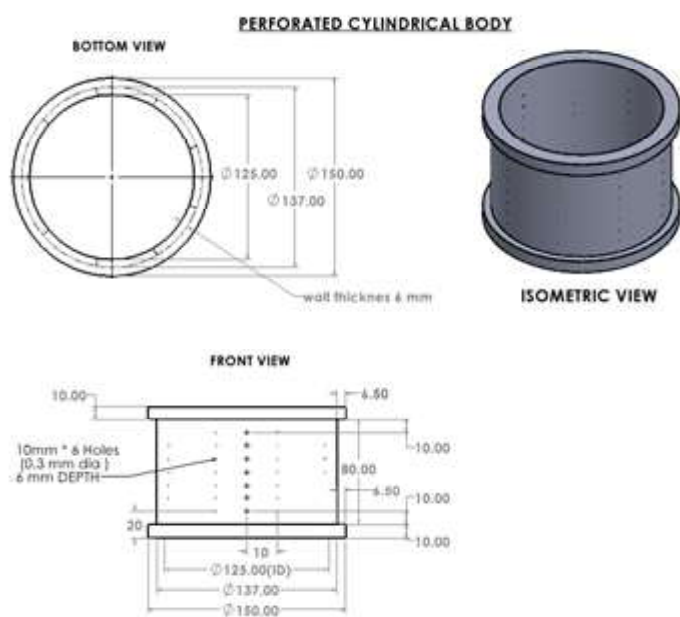


Fig 1c: Dimensional drawing of conceptualized Micro-perforated cylindrical body

To validate wall thickness of Micro-perforated PTFE mould, the design data were fed to ASME design equation based software. The results obtained shown in figure 3d. The wall thickness resulted from the ASME calculation is 2.46 mm. Above calculation doesn't took account of stress concentration, stress developed during fabrication and stress around holes. To overcome above drawbacks, 6 mm wall thickness was considered for the fabrication. It is evident that the FEA analysis and ASME results were appropriate. The developed micro-perforated PTFE mould shown in figure 3e.

Comparison of some selected quality parameters and processing conditions of paneer made from micro-perforated PTFE mould and conventional stainless steel paneer mould.

The developed micro-perforated PTFE mould utilized successfully in production of paneer and it can also implemented in cheese and tofu production. The pressing pressure (3, 3.5 and 4 bar) and time (10, 15 and 20 min) were adopted for research study. The pressure and time combinations were optimized to pressure (3.45 bar) and time (20 min) using RSM (Response surface Methodology) statistical analysis tool.

Quality parameters such as moisture content (MC), porosity (PR), bulk density (BD), hardness, chewiness and overall acceptability (OA) of different paneer samples (optimized paneer made from developed micro-perforated PTFE mould and paneer made using SS mould) are shown in Table 3.

Type of shell	Cylinder	
Design pressure P	0.2	N/mm ² (= 1 MPa = 10 Bar)
Design temperature T	60	°C
Material description	PTFE	
Select yield stress and specific gravity from material database		
Yield stress, design temp. S	30	N/mm ²
Specific gravity ρ	2200	kg/m ³
Outside diameter D _o	137	mm
Length tangent to tangent L	100	mm (If not a sphere)
Nominal wall thickness t	6	mm
Corrosion allowance Ca	1	mm
Tolerance tol	1.03	mm
Joint efficiency E	1	-
Semi angle at apex cone α	0	degree (For cone only)
Design Code	ASME	(ASME, Dutch R., PED)
Calculate		

Fig 2: Data input for thickness calculation

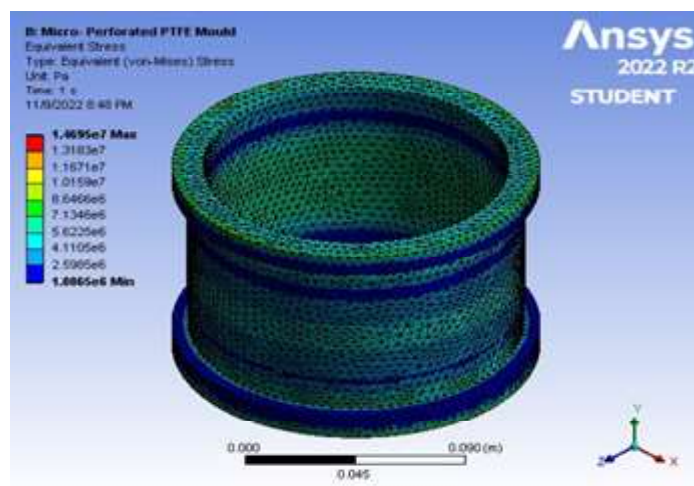


Fig 3a: Von Mises stress Induced in Micro-perforated PTFE mould

The optimized paneer made from developed micro-perforated

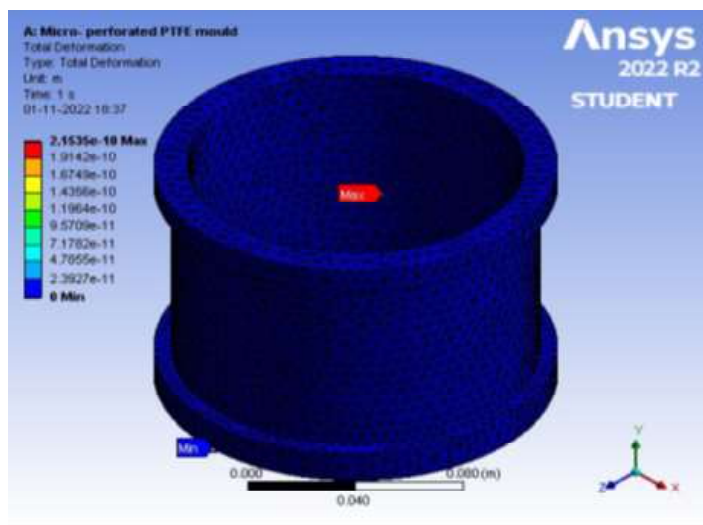


Fig 3b: Total deformation due to induced stress in micro-perforated PTFE mould

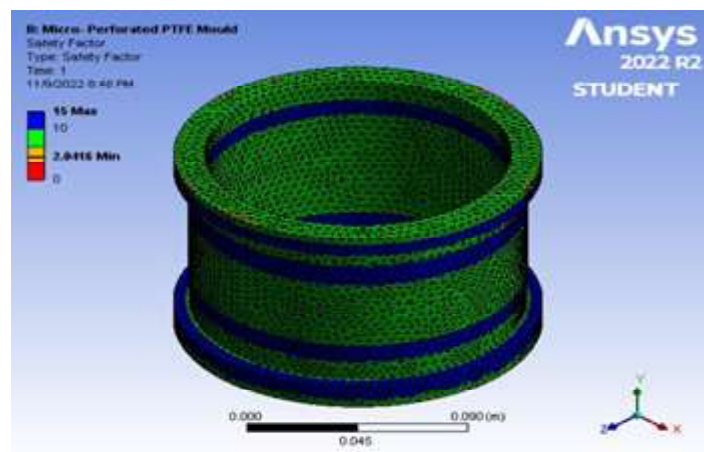


Fig 3c: Factor of Safety in micro-perforated PTFE mould

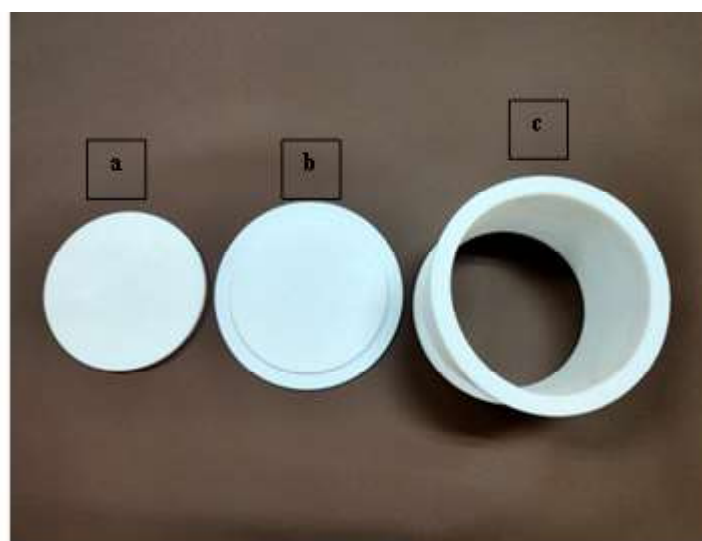
Wall thickness calculation of Cylinder according ASME			
Allowable stress	$S =$	$30 =$	30.00 N/mm^2
Corroded thickness	$t_c = t - Ca - tol =$	$6 - 1 - 1.03 =$	3.97 mm
Cylinder:			
Corroded inside radius	$R = \frac{D_0}{2} - t_c =$	$\frac{137}{2} - 3.97 =$	64.53 mm
Required wall thickness	$t_r = \frac{P \cdot R}{S \cdot E - 0.6 \cdot P} =$	$\frac{0.2 \cdot 64.53}{30 \cdot 1 - 0.6 \cdot 0.2} =$	0.43 mm
Nominal required thickness	$t_{rn} = t_c + Ca + tol =$	$0.432 + 1 + 1.03 =$	2.46 mm
Max. Allowable Working Press.	$MAWP = \frac{S \cdot E \cdot t_c}{R + 0.6 \cdot t_c} =$	$\frac{30 \cdot 1 \cdot 3.97}{64.53 + 0.6 \cdot 3.97} =$	1.78 N/mm^2
Thickness analysis, $t > t_{rn}$?	$t = 6 \text{ mm}$ is OK		
Weight			0.54 kg
Enclosed volume			0.001 m^3

Fig 3d: ASME design software result

PTFE mould got higher overall acceptability scores due to its uniform firm and compact body, pleasing appearance. Paneer made from developed micro-perforated PTFE had uniform color and appearance, dryness and rind formation in the surface is nil, while the control sample (paneer made from conventional stainless-steel paneer mould was highly porous, loose and open body, surface of the paneer doesn't appeared as uniform color and rind structure was noticed and hence paneer made from developed micro-perforated PTFE mould at optimized pressure and time combination is highly acceptable and appreciated than the conventional paneer.

Conclusion

The Von mises failure criterion states that failure occurs when the energy of distortion reaches the yield stress (explosion due to high hoop stress developed in mould). The max Von mises (equivalent) stress $1.47 \times 10^7 \text{ Pa}$ obtained by FEA, which is less than yield stress of virgin PTFE, the total deformation induced is also very negligible i.e., 0 to $2.1535 \times 10^{-10} \text{ m}$ which depicts the rigidity of construction of mould. Minimum FoS obtained 2.04 and maximum of 15. The result obtained from ASME shell thickness analysis confirms that the wall thickness well within



a: Perforated Top Pressure plate; b: Perforated bottom plate;
c: Perforated cylindrical body

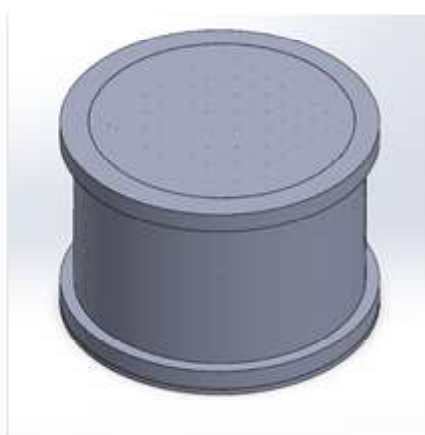


Fig 3e: Developed micro-perforated PTFE mould for paneer pressing

the selected wall thickness (6mm) which was used for stress analysis in ANSYS-22. The designed and required wall thickness was validated to be safe at this thickness. The results from FEA of Stress and stimulation carried out using ANSYS 22 and ASME design equation software confirms correctness of design and development of micro-perforated PTFE mould. Designed Micro-perforated PTFE mould fabricated from 6mm Virgin PTFE bush and 6 mm Virgin PTFE molded sheet. The working drawings of micro-perforated PTFE mould was developed and actual fabrication of designed mould was developed using advanced 3 and 4 axis CNC machines. Newly fabricated mould can withstand operating pressure of 5 bar(g) and even elevated temperature up

to 110°C. The developed PTFE mould performed satisfactorily without any deformation during pressing. Paneer manufactured using newly designed and developed micro-perforated PTFE mould resulted in a excellent quality paneer.

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RESEARCH ARTICLE

Anti-oxidant activity of functional yoghurt incorporated with *Hibiscus rosa sinensis* flower extract

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Abstract: Functional herbal yoghurt was developed by incorporating *Hibiscus rosa sinensis* flower extract at one and two per cent levels. The developed yoghurt was evaluated for physico-chemical parameters, anti-oxidant activity and sensory qualities. Incorporation of hibiscus has significantly increased the anti-oxidant activity. The antioxidant activity of control yoghurt and yoghurt incorporated with one and two per cent hibiscus flower extract were 14.4 ± 0.66 , 19.78 ± 0.66 and 36.32 ± 0.66 per cent respectively. The titratable acidity increased with respect to the increase in concentration of hibiscus extract and period of storage. There was a significant increase in total solids content in yoghurt incorporated with two per cent hibiscus extract. Hibiscus incorporation had also increased the syneresis percentage of yoghurt. Calcium and Iron content of yoghurt increased significantly in hibiscus incorporated yoghurt. Control yoghurt had 88.97 ± 1.56 and 1.14 ± 0.01 mg/100 g of calcium and of iron respectively. Yoghurt incorporated with one and two per cent hibiscus extract had 124.88 ± 1.67 and 224.8 ± 1.93 mg/100 g of calcium and 1.43 ± 0.01 and 1.92 ± 0.01 mg/100 g of iron respectively. No significant difference was observed in the fat percentage of yoghurt incorporated with hibiscus. Sensory quality of yoghurt

was not altered significantly due to the incorporation of hibiscus flower extract. The study revealed that hibiscus flower extract can be incorporated into yoghurt to improve the anti-oxidant property, calcium and iron content of yoghurt without affecting the sensory qualities.

Keywords: Hibiscus, herbal yoghurt, anti-oxidant activity, calcium enriched yoghurt, functional yoghurt

Introduction

Fermented dairy products are well known for their health benefits and are consumed traditionally from time immemorial. Yoghurt is the most popular fermented dairy product throughout the world. A significant growth in the consumption of yoghurt has been reported in many countries due to its health promoting properties. Functional additives such as plant extracts, probiotics and prebiotics are nowadays incorporated into yoghurt to improve the health benefits.

Hibiscus rosa sinensis is a shrub that belongs to Malvaceae family. It is commonly known as China rose or Shoe-flower. It is considered to be a medicinal herb due to the fact that it possesses anti-oxidant, anti-tumor, anti-inflammatory and anti-microbial activity. Petals of hibiscus flowers are edible, non-toxic and contain valuable nutrients. Several studies have proved the presence of anti-oxidant, anti-fungal and antimicrobial properties in flowers of *Hibiscus* (Khan et al., 2017 and Singh et al., 2019)

It is important to prevent the peroxidation of lipids and formation of free radicals in order to increase the shelf life and to preserve the quality characteristics of fat rich dairy products. Lipid oxidation is inhibited by anti-oxidant agents. Anti-oxidants are compounds that can stop or limit free radicals. Synthetic anti-oxidants such as Butylated hydroxy anisole, Butylated hydroxy toluene and Propyl gallate exhibit strong anti-oxidant activity against several oxidation systems. However, the use of synthetic anti-oxidants in foodstuffs is restricted because of the potential risks to human health (Jeong et al., 2005). Currently anti-oxidants from natural sources are receiving increased attention. Medicinal plants rich in natural anti-oxidants are increasingly used in dairy foods to improve nutritional and therapeutic properties. The

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extract of hibiscus flower contains bioflavonoids, tannins and anthocyanins responsible for the anti-oxidant property (Rengarajan et al., 2020). The research work was designed to develop functional yoghurt incorporated with *Hibiscus rosa sinensis* flower extract and to determine the antioxidant activity and physico- chemical properties of functional yoghurt.

Material and Methods

Preparation of the aqueous extract of *Hibiscus rosa-sinensis* flower

Fresh *Hibiscus rosa sinensis* flowers were collected from Mannuthy. The petals were dried at 30°C in a hot air oven. Powdered form of *Hibiscus rosa sinensis* flowers (5 g) were extracted under reflux condenser with sterile water (350 ml) by using accelerated solvent extractor at 100°C for 30 to 40 minutes as per the procedure described by Iwalokun (2007). Hibiscus flower extract was lyophilized in a freeze drier (Operon, -70°C). Lyophilized extract was stored in refrigerated temperature for further use.

Preparation of yoghurt

Yoghurt was prepared as per Tamime and Robinson (2007). Skim milk was standardized to 0.5 per cent fat and skim milk powder (3 per cent) was added to increase the solids-not-fat content. Then it was heated at 90°C for 10 minutes and six per cent sugar was added. The contents were mixed properly and cooled to 42°C. Two percent yoghurt culture (NCDC-145) obtained from National Collection of Dairy Cultures, NDRI, Karnal was added. Two experimental yoghurt samples were prepared by adding one and two per cent of aqueous extract of *Hibiscus rosa sinensis*.

Physico- Chemical analysis of functional yoghurt

Titrateable acidity of yoghurt samples was determined according to the procedure laid out in the FSSAI (2015). The fat content of yoghurt was determined by the Rose Gottlieb method outlined by FSSAI (2015). Total solids content of yoghurt was determined by the procedure prescribed by Bureau of Indian Standards (IS: 12333, 1997). The syneresis percentage was determined as per the procedure prescribed by Doleyres and Lacroix (2005). Syneresis is expressed as percentage weight of drained whey over the initial weight of the yoghurt sample.

Anti-oxidant activity of yoghurt samples was determined as the ability of each extract to scavenge 1,1 – diphenyl 2 – picrylhydrazyl (DPPH) radicals by the procedure laid out by Rasdhari et al. (2008). Anti-oxidant activity of yoghurt is expressed as inhibition percentage of DPPH free radicals.

Calcium and iron content of hibiscus incorporated yoghurt was determined by the procedure laid by Arslaner et al. (2021) with some modifications. The yoghurt samples were acid digested in

microwave digester by taking 2 grams of yoghurt sample in a digestion vessel. Then eight ml of 69% HNO₃ solution was added and then kept for digestion at 200°C and a pressure of 30 bar. After digestion, the vessels were allowed to cool to room temperature.

Atomic absorption spectroscopy

A hollow cathode lamp with the cathode made of calcium and iron were used as a source of light at 422.8 nm and 248.33 nm respectively. Samples were placed one by one for the mineral analysis. Two percentage HNO₃ was used as the blank solution

Sensory evaluation

Sensory evaluation of yoghurt samples was carried out by six expert panelists. Yoghurt samples were evaluated for their sensory characteristics such as color and appearance, body and texture, flavor and overall acceptability as per the score card suggested by IDF (1987).

Results and Discussion

Titrateable Acidity

The mean titrateable acidity values of control yoghurt (C) were 0.805 ± 0.02, 0.91 ± 0.01, 1.032 ± 0.01 and 1.082 ± 0.13 per cent lactic acid respectively on the first, third, fifth and seventh days of storage. The corresponding values of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 0.798 ± 0.02, 0.977 ± 0.01, 1.08 ± 0.01 and 1.127 ± 0.13 per cent lactic acid respectively (Table 2). The mean values for yoghurt with two per cent hibiscus flower extract (T2) were 0.828 ± 0.02, 1.03 ± 0.01, 1.103 ± 0.01 and 1.148 ± 0.13 per cent lactic acid respectively. There was a significant increase ($p < 0.05$) in titrateable acidity from the first day to the seventh day of storage. Higher titrateable acidity values were observed in yoghurt samples incorporated with two per cent hibiscus extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one percent hibiscus flower extract (T1). Kim et al. (2019) reported that the titrateable acidity of yoghurt incorporated with one per cent *Hibiscus sabdariffa* extract reached up to 1.0 percent lactic acid. The results obtained are in agreement with the above findings.

Total Solids Content

The mean total solids per cent of control yoghurt (C) were 13.477 ± 0.007, 13.608 ± 0.004, 13.832 ± 0.008 and 13.938 ± 0.008 per cent respectively on the first, third, fifth and seventh day of storage. The respective values for yoghurt incorporated with one percent hibiscus flower extract (T1) were 13.408 ± 0.007, 13.423 ± 0.004, 13.608 ± 0.008 and 13.823 ± 0.008 per cent. The total solids content of yoghurt added with two per cent hibiscus flower extract (T2) were 13.463 ± 0.007, 13.618 ± 0.004, 14.117 ± 0.008 and 14.133 ± 0.008 per cent respectively. A significant increase ($p < 0.05$)

in total solids content during storage was noticed. Highest total solid content was observed in yoghurt incorporated with two per cent hibiscus extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one per cent extract (T1). Similar findings were reported by Arslaner et al. (2021)

Syneresis Percentage

The mean syneresis values of control yoghurt (C) were 2.18 ± 0.06 , 2.05 ± 0.03 , 2.03 ± 0.02 and 2 ± 0.03 per cent respectively on the first, third, fifth and seventh day of storage. The syneresis values of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 3.53 ± 0.06 , 2.42 ± 0.03 , 2.47 ± 0.02 and 2.3 ± 0.03 per cent respectively. The corresponding values for yoghurt with two per cent hibiscus flower extract (T2) were 3.08 ± 0.06 , 2.43 ± 0.03 , 2.45 ± 0.02 and 2.37 ± 0.03 per cent respectively. A significant difference in the mean syneresis value was noticed in all yoghurt samples ($p < 0.05$) during storage. Highest syneresis value was observed in yoghurt sample incorporated with one per cent of hibiscus extract (T1) and it was significantly different from the yoghurt incorporated with two per cent extract (T2) and that of control. Nguyen (2021) found that yoghurt incorporated with *Hibiscus sabdariffa* had higher syneresis value than control.

Fat Percentage

The mean fat percentage of control yoghurt were 0.17 ± 0.03 , 0.2 ± 0.03 , 0.2 ± 0.03 and 0.2 ± 0.03 per cent respectively on the first, third, fifth and seventh day of storage. The fat percentage of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 0.23 ± 0.03 , 0.22 ± 0.03 , 0.22 ± 0.03 and 0.22 ± 0.03 per cent. The respective values for yoghurt prepared with two per cent hibiscus flower extract (T2) were 0.2 ± 0.03 , 0.2 ± 0.03 , 0.22 ± 0.03 and 0.2 ± 0.03 per cent. No significant difference in fat percentage was observed between yoghurt samples. Kavas et al. (2003) had also reported no appreciable change in the fat content of yoghurt incorporated with hibiscus.

Antioxidant Activity

The mean antioxidant activities of control yoghurt (C) were 10.73 ± 0.71 , 14.4 ± 0.66 , 8.38 ± 0.46 and 7.24 ± 0.46 per cent respectively on the first, third, fifth and seventh day of storage. The mean values for yoghurt incorporated with one per cent hibiscus flower extract (T1) were 17.76 ± 0.71 , 19.78 ± 0.66 , 11.85 ± 0.46 and 10.71 ± 0.46 per cent respectively. The corresponding mean values obtained for yoghurt with two percent hibiscus flower

Table 1: Antioxidant activity (Mean $\pm$ S.E) of yoghurt (percentage)

Sample	1 st day	3 rd day	5 th day	7 th day	Overall
C	10.73 ± 0.71^{bc}	14.4 ± 0.66^{ac}	8.38 ± 0.46^{cc}	7.24 ± 0.46^{cc}	10.19 ± 0.31^c
T1	17.76 ± 0.71^{bb}	19.78 ± 0.66^{ab}	11.85 ± 0.46^{cb}	10.71 ± 0.46^{cb}	15.02 ± 0.31^b
T2	25.5 ± 0.71^{ba}	36.32 ± 0.66^{aA}	19.71 ± 0.46^{cA}	17.73 ± 0.46^{dA}	24.82 ± 0.31^A
Overall	18 ± 0.41^b	23.5 ± 0.38^a	13.31 ± 0.26^c	11.89 ± 0.26^d	

Table 2: Physico-chemical parameters of yoghurt (Mean $\pm$ S.E)

Parameters	Samples	Storage Days			
		1st Day	3rd Day	5th Day	7th Day
Titratable acidity (in per cent lactic acid)	C	0.805 ± 0.02^{db}	0.91 ± 0.01^{cc}	1.032 ± 0.01^{bc}	1.082 ± 0.13^{ac}
	T1	0.798 ± 0.02^{db}	0.977 ± 0.01^{cb}	1.08 ± 0.01^{bb}	1.127 ± 0.13^{ab}
	T2	0.828 ± 0.02^{dA}	1.03 ± 0.01^{cA}	1.103 ± 0.01^{bA}	1.148 ± 0.13^{aA}
Total solids content (%)	C	13.477 ± 0.007^{Da}	13.608 ± 0.004^{cA}	13.832 ± 0.008^{bc}	13.938 ± 0.008^{ab}
	T1	13.408 ± 0.007^{Cb}	13.423 ± 0.004^{cB}	13.608 ± 0.008^{ab}	13.823 ± 0.008^{bc}
	T2	13.463 ± 0.007^{cA}	13.618 ± 0.004^{bA}	14.117 ± 0.008^{aA}	14.133 ± 0.008^{aA}
Syneresis (%)	C	2.18 ± 0.06^{ab}	2.05 ± 0.03^{abB}	2.03 ± 0.02^{bB}	2 ± 0.03^{Bb}
	T1	3.53 ± 0.06^{aA}	2.42 ± 0.03^{bA}	2.47 ± 0.02^{bA}	2.3 ± 0.03^{cA}
	T2	3.08 ± 0.06^{aC}	2.43 ± 0.03^{bA}	2.45 ± 0.02^{bA}	2.37 ± 0.03^{bA}
Fat (%)	C	0.17 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.03
	T1	0.23 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.03
	T2	0.20 ± 0.03	0.20 ± 0.03	0.22 ± 0.03	0.22 ± 0.03

Table 3: Calcium and Iron content (Mean $\pm$ S.E) mg/100g of yoghurt

Sample	Calcium	Iron
C	88.97 ± 1.56^c	1.14 ± 0.01^c
T1	124.88 ± 1.67^b	1.43 ± 0.01^b
T2	224.8 ± 1.93^a	1.92 ± 0.01^a

Table 4: Sensory scores (Mean $\pm$ S.E) of yoghurt

Sample	Appearance and colour	Body and texture	Flavor	Overall acceptance
C	4.44 $\pm$ 0.29	4.56 $\pm$ 0.24	9.34 $\pm$ 0.17 ^A	18.34 $\pm$ 0.8
T1	4.56 $\pm$ 0.18	4.66 $\pm$ 17	9.34 $\pm$ 0.17 ^A	18.56 $\pm$ 0.52
T2	4.44 $\pm$ 0.24	4.44 $\pm$ 0.24	8.22 $\pm$ 0.35 ^B	17.11 $\pm$ 1.16

extract (T2) were 25.5 $\pm$ 0.71, 36.32 $\pm$ 0.66, 19.71 $\pm$ 0.46 and 17.73 $\pm$ 0.46 per cent (Table 1). A significant decrease in the antioxidant activity from the third day was noticed in all yoghurt samples ($p < 0.05$). Higher antioxidant activity was observed in yoghurt containing two percent hibiscus flower extract (T2) and it was significantly different from that of control and from yoghurt incorporated with one per cent hibiscus flower extract (T1). Higher anti-oxidant activity in hibiscus incorporated yoghurt was also reported by Biomy et al. (2017) and Hamwenye et al. (2020).

Calcium and iron content

The mean calcium content of control yoghurt (C) was 88.97 $\pm$ 1.56 mg/100g. The corresponding values of yoghurt prepared with one and two per cent hibiscus flower extract were 124.88 $\pm$ 1.67 and 224.8 $\pm$ 1.93 mg/100g respectively. The mean iron content of control yoghurt (C) was 1.14 $\pm$ 0.01 mg/100g. The corresponding values for yoghurt incorporated with one and two percent hibiscus flower extract (T1) were 1.43 $\pm$ 0.01 and 1.92 $\pm$ 0.01 mg/100g respectively (Table 3). A significant increase in mean calcium and iron content was noticed in treatment groups of yoghurt samples ($p < 0.05$). Highest value was observed in yoghurt containing two percent hibiscus flower extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one per cent hibiscus flower extract (T1). Similar findings were reported by Bahuguna et al. (2018).

Sensory Evaluation

The mean values of appearance and color, body and texture, flavor and overall acceptability score of control yoghurt (C) were 4.44 $\pm$ 0.29, 4.56 $\pm$ 0.24, 9.34 $\pm$ 0.17 and 18.34 $\pm$ 0.8 respectively. The respective values for yoghurt incorporated with one percent hibiscus flower extract (T1) were 4.56 $\pm$ 0.18, 4.66 $\pm$ 17, 9.34 $\pm$ 0.17 and 18.56 $\pm$ 0.52 (Table 4). The values for yoghurt incorporated with two percent hibiscus flower extract (T2) were 4.44 $\pm$ 0.24, 4.44 $\pm$ 0.24, 8.22 $\pm$ 0.35 and 17.11 $\pm$ 1.16 respectively. A significant decrease in flavor score was observed in yoghurt sample incorporated with two per cent hibiscus extract when compared to control yoghurt. However, no significant difference was observed in the overall acceptance of yoghurt samples. Tomar et al. (2020) had also reported positive effect of hibiscus on sensory and functional properties of yoghurt.

Conclusion

In the present study yoghurt incorporated with *Hibiscus rosa sinensis* flower extract showed higher antioxidant activity than

the control yoghurt. The incorporation of hibiscus significantly increased the titratable acidity, total solids, syneresis, calcium and iron content. No significant difference was observed in the fat percentage. Sensory quality of yoghurt was not altered significantly by the incorporation of hibiscus. The study revealed that hibiscus flower extract can be incorporated into yoghurt to improve the antioxidant activity, calcium and iron content of yoghurt without affecting the sensory qualities.

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RESEARCH ARTICLE

Applying sensory and instrumental techniques to evaluate the texture of Paneer (an Indian variety of cheese)

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Abstract: The aim of the work is to investigate the relationship between the instrument and sensory data. Paneer samples from thirty-five manufacturers were evaluated for quality of paneer using texture analyser and perceived sensations through sensory evaluation. The modified sensory scorecard can distinguish the samples well. The correlation between sensory attributes and instrumental parameters was obtained by using Principal component analysis and Pearson correlation analysis. The correlation data showed poor correlation between instrumental parameters and sensory attributes (flavour, body and texture, colour and appearance, and overall acceptability). Instrumental techniques have potential to replace sensory analysis.

Keywords: Paneer, cheese, texture analysis, sensory evaluation, PCA

Introduction

Paneer is a type of soft cheese, usually made from cow or buffalo milk. It is made by heat coagulation of milk with an edible acid such as lemon juice, vinegar, or yogurt. India is the largest producer of *paneer* and the second largest consumer in the world (Kapoor R. et al. 2021). The quality of *paneer* is characterized by fat content, moisture content, colour, flavour, body and texture. A marble white colour, sweetish, slightly acidic taste, nutty flavour, spongy body, and closely knit, smooth texture are all

characteristics of good grade *paneer* (Khan and Pal, 2011). The texture can be characterized as a sensory experience which can only be perceived, described, and quantified by humans. It's a multi-parameter trait that's commonly related to mechanical, geometrical, and other qualities (mostly composition of food) that may be recognised through the senses of vision, hearing, and touch (Szczesniak, 2002).

Sensory (subjective) and instrumental (objective) methods are commonly used to assess the *paneer* texture. Although, sensory analysis is a time consuming method but it is a methodical, scientifically-driven process for evaluating characteristics of *paneer* such as extensibility, chewiness, juiciness, flavor, body & texture, and appearance etc. Sensory technique also implies specialized equipment, trained personnel, and calibration standards to guarantee accuracy and consistency. Researchers have invested a lot of time and effort into developing various instrumental approaches and attempting to build a link or model between sensory qualities and instrumental factors for different food products like beef, meat (Pematilleke et al. 2021) and French fries (Li et al. 2020). As far literature does not report the correlation between sensory attributes and instrumental parameters of *paneer*. Therefore, there are some reasons to identify the key drivers for developing correlations between sensory and instrumental measurements to provide valuable insights in: (a) determining a suitable instrument for monitoring changes in food quality; (b) forecasting customer behaviour; (c) comprehending what is experienced in sensory texture assessment; and (d) developing and enhancing instrumental test procedures. Instrumental techniques have been utilised in several investigations to assess the texture of *paneer* samples under various test conditions and probes. On an addition, literature reported the use of different size probes is 75mm (Ahmed and Bajwa, 2018, Kumar et al. 2019, Dey et al. 2019), 25mm (Kapoor R. et al. 2021, Kapoor S. et al. 2021), and 20 mm (Amini et al. 2019) but the most preferred for assessing *paneer* texture is still lacking.

Despite of the widespread usage of the typical instrumental texture analysis of *paneer*, but there is still no clear scientific studies/ agreement on the types of probes to be preferred and their relationship between probe types and with the sensory parameters texture of *paneer*. It is therefore, the major thrust area

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for the present investigation was to (a) examine the instrumental and sensory texture characteristics of *paneer* samples collected from different manufacturers, and (b) determine the relationship between perceived experiences and instrumental measurements.

Materials and Methods

Sample collection & preparation

The present investigation was carried out on the top 35 different *paneer* manufacturers from two different cities, Moga and Ludhiana of Punjab state. In the coding, alphabet 'M' implies Moga and 'L' implies Ludhiana. These two districts are selected because both are located in centre of Punjab state. The sample numbered from '01' to '17' were collected from Moga and from '18' to '35' were collected from Ludhiana city. The fresh samples, after collection from the manufacturing units were coded for references and delivered to Dairy Engineering Department Laboratory, Guru Angad Dev Veterinary and Animal Sciences University for experimental analysis. The samples were collected on the same day of preparation i.e. freshly prepared and in triplicates but on different days of preparation from same unit of manufacturing. After collecting fresh samples of early morning lot from each manufacturing unit were immediately stored in an insulated boxes with ice packs/gels at the company site to maintain refrigerated conditions (4 °C) during transportation. Further, each sample was preserved in laboratory refrigerator (4 °C) and immediate analysis on same day were preferred by authors keeping delay time (about 5 hours) constant. These samples were immediately tested for both, sensory evaluation and instrumental assessment. Each sample was cut with the dimensions of 20 x 20 x 20 mm (L x B x H) to maintain the uniformity for the experimental purpose.

Sensory Evaluation

The sensory evaluation was conducted by ten semi-trained panellists (males and females, five each), who were dairy professionals having cheese or paneer as their major research field in dairy department of university. Panellists were mostly faculty members selected on the base of their interest, sensitivity towards product parameters and familiarity (knew the production process of *paneer*). The sensory testing was carried out in the sensory analysis laboratory of college having almost five separate seating arrangements with separate, properly focused light, having white coloured table tops and walls along with separate glass water. The samples were presented in disposable cups (one sample in one cup) labelled as three-digit random numbers and evaluated by the ten semi-trained panellists. The sensory evaluation was divided into seven phases and presented randomly.

Sensory evaluation was performed following the suggested scorecard given by Kumar et al (2011) with slight modification as

shown in Table 1. The packaging attribute was replaced with the overall acceptability attribute. To each panellist, the separate sensory score card with proper description for scoring pattern against each parameter was provided (Table 1). The different sensory aspects of *paneer* were judged using a 100-point scorecard: flavour (50), body and texture (35), colour and appearance (10) and overall acceptability (5). After evaluation, the scoring sheets were retrieved, and the mean values of ten assessors for all descriptors were used for statistical analysis.

Instrument analysis

The texture was examined in the paneer samples with TA-XT Plus texture analyser from stable micro systems at $20 \pm 1^\circ\text{C}$ using Texture Exponent software (version 6.1.1.0, Stable Micro Systems Co. Ltd., UK). The texture evaluation comprised of a texture profile analysis (TPA) test using flat, an aluminium cylinder probe P/75 (75 mm diameter). The tests were carried out by measuring compression force, using a 5 kg load cell and a trigger force of 5.0 g, a test speed of 5 mm/s, and a compression 50% of the sample's original height, as per literature (Kumar et al. 2019). Two cycles of axial compression were applied to the samples in a succession. The typical texture parameters were obtained: hardness (g, peak force of the 1st compression); adhesiveness (g-s, negative force area during the 1st compression); gumminess (product of hardness and cohesiveness); resilience (dimensionless, the ratio of the area before and after deformation at the 1st compression); cohesiveness (dimensionless, the positive force area during the 2nd compression divided by that in the 1st compression); chewiness (g, product of gumminess and springiness); and springiness (dimensionless, the ratio of the height on the 2nd compression to the original height). The samples before analysis were stored at a temperature of 5°C before analysis and removed just before the textural measurements.

Statistical Analysis

The SPSS statistical programme was used to analyse the sensory and instrumental data of *paneer* samples (Version 26.0, SPSS Inc., Chicago, Illinois, USA). The data was checked for significant differences ($\alpha=0.05$) using a one-way analysis of variance. Using the SPSS statistics software, Pearson's correlation coefficients between the averaged value of sensory attributes and instrumental parameters were obtained to investigate the linear relationship of variables. The Origin-Pro software was used to perform Principal Component Analysis (PCA) (Version 2021b, Origin-Lab Corporation, Northampton, Massachusetts, USA). All variables were centred and scaled to unit variance. The spatial distribution of data and the association between sensory qualities were also determined using PCA. Furthermore, PCA was used to identify the most essential characteristics of the data. The data can be compressed by PCA by restricting the number of dimensions and defining the number of principal components without losing much information (Rodriguez-Campos et al. 2011).

Results and Discussion

Sensory Analysis of texture

The sensory of paneer depends on characteristics, such as its texture (firm, chewy, crumbly, creamy), flavor (nutty, salty, sour, sweet), aroma (grassy, earthy, milky), and appearance (white, yellow, orange), were found to be important in determining sample variability. After analysis, among all the samples ML16 and ML26 have maximum whereas ML07 samples have minimum overall acceptability along with body & texture scores (Table 2).

The ML23 sample had the highest intensity of flavour, while ML03 had the highest intensity of colour and appearance, while other samples, had moderate intensity values for their respective descriptive sensory parameters. The *paneer* processing conditions of different manufacturing units might be responsible for their sensory variances.

Fig. 1(a, b) shows the correlation between loadings and scores of PCA. The first (Principal Component 1, x-axis) and second (Principal Component 2, y-axis) dimensions of the principal component analysis explained 48.54 and 31.59 per cent of the variation in the sensory data, respectively. The two principal components were able to capture the majority of the variance in the data, as they contributed to 80.13% of the total variance. This indicates that the first two principal components were able

to explain all the major features of the data, and that any additional components would likely contribute very little additional information. The loading plot is used to identify and visualize patterns in the data, while the scoring plot is used to evaluate the similarity of the samples and separate them into meaningful clusters (Shin et al. 2010). The sensory attributes of PC1, ranging from colour and appearance to overall acceptability correlation loadings, were visualized in Fig. 1a, while PC2 was characterized by body and texture to flavour. On PC1, colour and appearance were found to be inversely related to overall acceptability. The study on PC2 showed that the more cohesive the sample was, the rougher it felt, suggesting a strong positive correlation between the two. This indicates that the two factors are inextricably linked, creating a unique and fascinating relationship. Furthermore, the score plot of the first two components (Fig. 1b) revealed that the 35 samples were projected into four quadrants, demonstrating the differences in sensory texture features between the samples. The samples ML01, ML03, ML06, ML09, ML10, ML11, ML12, ML16, ML19, ML23, ML25, ML26, ML27, ML28, ML29, ML30, and ML31 found in the positive PC1 revealed a stark contrast, with other samples located in the negative region of PC1. The ML09, ML11, ML16, and ML29 samples were located closer to the body & texture, and overall acceptability, indicating respective dominant sensory characteristics of these samples. Similarly, ML01, ML10, ML23, ML26, and ML30 had dominant flavour and overall acceptability. Colour and appearance were major characteristics of ML13, ML18, ML21, and ML35. The

Table 1: Suggested scores of *paneer* on the basis of degree of defects

Attribute	Defect	Slight	Definite	Pronounced
Flavour (50)	Sour/Acid	42	37	29
	Flat	42	37	29
	Stale	42	37	29
	Smoky/Burnt	42	37	29
	Bitter	42	37	29
	Feed/Weed	42	37	29
	Foreign	42	37	29
	Musty	42	37	29
	Putrid	42	37	29
	Rancid	42	37	29
	Unclean	42	37	29
	Yeasty	42	37	29
Body and texture (35)	Crumbly	32	30	26
	Hard	32	30	26
	Rubbery/chewy	32	30	26
	Weak	32	30	26
	Pasty	32	30	26
Colour and appearance (10)	Dull	9	8	7
	Dry	9	8	7
	Visible dirt	9	8	7
	Uneven surface	9	8	7
	Mouldy	9	8	7
Overall Acceptability (5)	Mean value of other attributes			

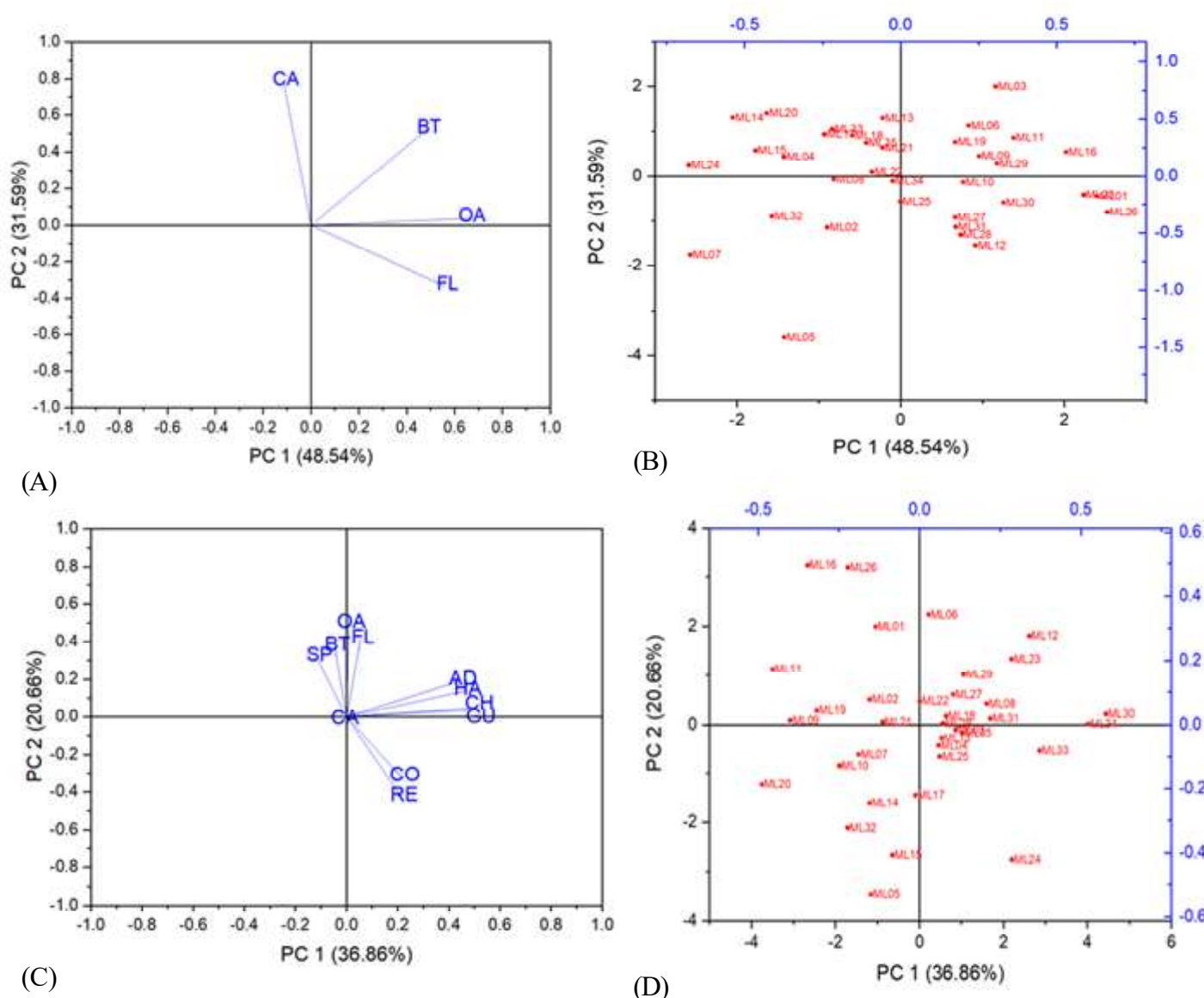


Fig. 1 Principal component analysis of sensory description analysis (a, b) and fused data from sensory and instrument (c, d). Where, FL= Flavour; BT= Body and Texture; CA= Colour and Appearance; OA= Overall Acceptability; HA= Hardness; AD= Adhesiveness; SP= Springiness; CO= Cohesiveness; GU= Gumminess; CH= Chewiness; RE= Resilience.

samples in the IIIrd quadrant (ML02, ML05, ML07, ML08, and ML32) were not found near any of the sensory qualities, indicating they lacked part of a prominent sensory texture characteristic (Meullenet and Gross 1999). Furthermore, ML13, ML21, ML22, ML25, and ML34 exhibited near-zero loadings on PC1, indicating that their sensory intensity scores were lower than other samples, as seen in Table 2.

Instrumental texture

P/75 aluminium probe and adopted test settings generated different average values and the *paneer* samples were differentiated based on instrumental parameters (Table 3 and Table 4). However, in several textural parameters, such as

hardness, gumminess, and chewiness, ML12, ML30, and ML34 showed greater textural values. Other samples' instrumental texture values were found to be in the middle range (Table 3 and Table 4). The hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of 35 *paneer* samples were measured at 1959.96–3511.23 g, -8.17 - (-0.62) g.sec, 0.83-0.89, 0.59-0.69, 1268.21-2448.49, 1066.92-2378.39 g, and 0.32-0.43, respectively. The results of hardness, adhesiveness, springiness, cohesiveness, and chewiness were consistent with previous studies (Ahmed and Bajwa, 2018, Kumar et al. 2019). However, the results of gumminess and resilience were lesser than those reported in the previous studies (Ahmed and Bajwa, 2018). These differences were probably caused by different processing and testing conditions.

Table 2 Sensory evaluation scores of various samples of *paneer*

Sample	Flavour	Body and texture	Colour And appearance	Overall acceptability
ML01	47.20 ^b ± 1.02	32.80 ^{cde} ± 0.76	8.80 ^{defghi} ± 0.51	4.55 ^a ± 0.21
ML02	44.50 ^{jk} ± 0.95	30.20 ^k ± 0.80	8.85 ^{bcddefghi} ± 0.38	3.95 ^{bcd} ± 0.26
ML03	44.40 ^{jk} ± 1.06	33.70 ^{ab} ± 0.73	9.60 ^a ± 0.20	4.30 ^{abcd} ± 0.34
ML04	42.30 ^e ± 1.36	31.50 ^{gh} ± 0.70	9.20 ^{abcde} ± 0.31	3.80 ^{bcd} ± 0.33
ML05	44.40 ^{jk} ± 1.31	29.20 ^l ± 0.64	7.90 ⁱ ± 0.4	3.80 ^{bcd} ± 0.36
ML06	44.80 ^{ij} ± 1.11	32.80 ^{cde} ± 0.67	9.40 ^{ab} ± 0.27	4.25 ^{abcd} ± 0.33
ML07	44.50 ^{jk} ± 1.41	28.70 ^l ± 0.80	8.90 ^{cdefghij} ± 0.42	3.55 ^e ± 0.34
ML08	43.90 ^{kl} ± 1.30	31.30 ^{gh} ± 0.58	9.10 ^{abcde} ± 0.54	3.90 ^{bcd} ± 0.19
ML09	44.70 ^{jk} ± 0.95	33.20 ^{bc} ± 0.70	9.00 ^{bcddefgh} ± 0.34	4.20 ^{abcd} ± 0.16
ML10	46.30 ^{ef} ± 0.93	32.40 ^{cde} ± 0.76	9.00 ^{bcddefgh} ± 0.37	4.10 ^{abcde} ± 0.09
ML11	45.90 ^{efg} ± 1.40	32.50 ^{cde} ± 0.86	9.40 ^{abc} ± 0.27	4.40 ^{ab} ± 0.28
ML12	47.10 ^{bc} ± 1.19	31.30 ^{hi} ± 0.92	8.60 ^{fghi} ± 0.25	4.20 ^{abcd} ± 0.24
ML13	42.60 ^{no} ± 1.04	32.50 ^{cde} ± 0.69	9.40 ^{abc} ± 0.21	4.10 ^{abcde} ± 0.12
ML14	39.20 ^r ± 1.32	32.50 ^{cde} ± 0.62	9.20 ^{abcde} ± 0.27	3.70 ^{de} ± 0.19
ML15	41.50 ^p ± 1.28	30.00 ^k ± 0.67	9.50 ^{ab} ± 0.34	4.00 ^{abcde} ± 0.08
ML16	46.30 ^{ef} ± 1.12	33.90 ^a ± 0.58	9.00 ^{abcdefg} ± 0.50	4.35 ^{abc} ± 0.42
ML17	43.00 ^{mno} ± 1.36	32.40 ^{cde} ± 0.61	9.30 ^{abcd} ± 0.37	3.80 ^{bcd} ± 0.16
ML18	43.30 ^{lmn} ± 1.14	32.40 ^{cde} ± 0.66	9.30 ^{abcd} ± 0.22	3.90 ^{bcd} ± 0.07
ML19	44.40 ^{jk} ± 1.14	33.10 ^{bc} ± 0.65	9.15 ^{abcde} ± 0.20	4.15 ^{abcde} ± 0.06
ML20	39.10 ^r ± 1.18	32.90 ^{cd} ± 0.73	9.15 ^{abcde} ± 0.41	3.80 ^{bcd} ± 0.14
ML21	43.00 ^{mno} ± 1.25	32.10 ^{def} ± 0.83	9.20 ^{abcde} ± 0.51	4.10 ^{abcde} ± 0.18
ML22	40.40 ^q ± 1.21	32.90 ^{cd} ± 0.85	8.60 ^{efghij} ± 0.32	4.10 ^{abcde} ± 0.35
ML23	48.30 ^a ± 1.42	32.80 ^{cde} ± 0.64	8.90 ^{bcddefghi} ± 0.47	4.40 ^{abc} ± 0.32
ML24	39.10 ^r ± 1.40	30.45 ^{jk} ± 0.78	9.10 ^{abcde} ± 0.34	3.80 ^{bcd} ± 0.25
ML25	46.40 ^{cde} ± 1.16	32.00 ^{efg} ± 0.59	8.90 ^{bcddefghi} ± 0.23	3.85 ^{bcd} ± 0.20
ML26	46.30 ^{ef} ± 0.98	33.10 ^{bc} ± 0.83	8.50 ^{ghi} ± 0.49	4.60 ^a ± 0.43
ML27	44.80 ^{ij} ± 1.00	30.90 ^{hij} ± 0.61	8.80 ^{cdefghi} ± 0.39	4.40 ^{ab} ± 0.22
ML28	45.20 ^{ghi} ± 1.36	32.20 ^{def} ± 0.80	8.40 ^{hij} ± 0.31	4.15 ^{abcde} ± 0.20
ML29	47.00 ^{bcd} ± 1.09	33.10 ^{bc} ± 0.68	9.10 ^{abcde} ± 0.48	4.10 ^{abcde} ± 0.09
ML30	45.60 ^{fghi} ± 1.13	32.50 ^{cde} ± 0.75	8.70 ^{defghi} ± 0.33	4.30 ^{abcd} ± 0.26
ML31	44.00 ^{kl} ± 1.26	32.70 ^{cde} ± 0.82	8.30 ^{ij} ± 0.27	4.15 ^{abcde} ± 0.13
ML32	42.80 ^{mno} ± 1.23	30.50 ^{jk} ± 0.58	8.80 ^{cdefghi} ± 0.54	3.80 ^{bcd} ± 0.22
ML33	42.90 ^{mno} ± 1.42	32.10 ^{def} ± 0.88	9.40 ^{abc} ± 0.29	3.90 ^{bcd} ± 0.30
ML34	44.90 ^{ghij} ± 1.11	31.50 ^{fgh} ± 0.69	9.10 ^{abcde} ± 0.36	4.05 ^{abcde} ± 0.16
ML35	43.40 ^{lm} ± 1.32	31.50 ^{fgh} ± 0.92	9.40 ^{abc} ± 0.44	4.10 ^{abcde} ± 0.24

(N=10, Results are expressed as Mean ± SE, with different small letters superscript (a,b,c) within row differ significantly (P< 0.05) among the samples. Where, M denotes Moga and L denotes Ludhiana. The sample numbered from ML01 to ML17 were collected from Moga and from ML18 to ML35 were collected from Ludhiana city)

Relation between sensory and instrumental texture

Pearson's correlation and Principal component analysis were utilised to see if sensory attributes were connected to instrumental parameters. With the application of Principal component analysis, the relation between attributes and significant knowledge of datasets can be acknowledged due to the reduction in the number of variables (Gilbert et al. 2013). Further, the linearity of the relationship among different variables can be quantified by Pearson's correlation analysis (Goldner et al. 2012).

Based on the sensory attributes and instrumental parameters shown in the PCA correlation loadings (Fig. 1c) it was revealed that there was a significant variance between *paneer* samples as presented in the score plot (Fig. 1d). The first two principal components explained 57.52 per cent of the variation, with 1st PC contributing 36.86 per cent and 2nd PC accounting for the rest 20.66 per cent. Correlation loadings projected in Fig. 1c can be used to derive the link between sensory and instrumental texture. Except for body and texture, springiness, and colour and appearance, all characteristics loaded on PC1 were positively associated. Springiness, overall acceptability, flavour, and body

Table 3 Instrumental texture analysis of various samples of *paneer*

Sample	Hardness	Adhesiveness	Springiness
ML01	2575.35 ^{jk} ±193.24	-5.17 ^{jk} ±1.07	0.88 ^{ab} ±0.02
ML02	2622.38 ^{hijk} ±254.76	-4.28 ^{gh} ±0.79	0.87 ^{ab} ±0.01
ML03	2767.70 ^{ghij} ±198.79	-4.35 ^{gh} ±0.87	0.84 ^{ab} ±0.02
ML04	2758.37 ^{ghij} ±296.04	-3.34 ^{de} ±0.61	0.88 ^{ab} ±0.02
ML05	2137.22 ^{nop} ±154.55	-6.61 ^m ±1.38	0.85 ^{ab} ±0.04
ML06	3034.07 ^{cdef} ±209.16	-3.16 ^{cde} ±0.51	0.89 ^a ±0.04
ML07	2481.10 ^{kl} ±230.18	-4.23 ^{gh} ±1.16	0.87 ^{ab} ±0.03
ML08	3166.56 ^{bcd} ±143.17	-2.13 ^b ±0.32	0.87 ^{ab} ±0.01
ML09	1959.96 ^p ±118.09	-7.28 ^m ±0.96	0.87 ^{ab} ±0.01
ML10	2049.71 ^{op} ±167.66	-6.50 ^m ±0.80	0.85 ^{ab} ±0.04
ML11	1964.96 ^p ±139.07	-6.46 ^m ±0.34	0.86 ^{ab} ±0.01
ML12	3175.31 ^{bcd} ±441.49	-0.91 ^a ±0.09	0.89 ^{ab} ±0.01
ML13	2735.78 ^{ghij} ±317.86	-3.28 ^{de} ±0.65	0.85 ^{ab} ±0.01
ML14	2599.85 ^{ijk} ±221.62	-5.98 ^l ±0.97	0.88 ^{ab} ±0.03
ML15	2393.22 ^{klm} ±415.99	-5.90 ^l ±1.10	0.85 ^{ab} ±0.03
ML16	2314.92 ^{lmn} ±233.39	-4.87 ^l ±0.72	0.88 ^{ab} ±0.02
ML17	2594.06 ^{ijk} ±139.61	-4.59 ^{hi} ±0.38	0.84 ^{ab} ±0.02
ML18	2973.89 ^{defg} ±218.89	-3.03 ^{cd} ±0.10	0.85 ^{ab} ±0.02
ML19	2235.68 ^{mno} ±97.08	-6.94 ^{mn} ±0.88	0.88 ^{ab} ±0.01
ML20	1984.66 ^p ±367.87	-8.17 ^o ±0.71	0.89 ^a ±0.01
ML21	2577.43 ^{jk} ±356.42	-4.60 ^{hi} ±0.63	0.86 ^{ab} ±0.02
ML22	2844.96 ^{efghi} ±330.67	-3.98 ^{fg} ±0.07	0.88 ^{ab} ±0.01
ML23	2974.55 ^{defg} ±330.67	-3.00 ^{cd} ±0.34	0.88 ^{ab} ±0.04
ML24	3054.02 ^{cde} ±385.04	-3.40 ^{de} ±0.35	0.86 ^{ab} ±0.01
ML25	2797.25 ^{fghij} ±147.53	-5.43 ^k ±0.49	0.88 ^{ab} ±0.01
ML26	2612.02 ^{hijk} ±188.34	-4.32 ^{gh} ±0.26	0.88 ^{ab} ±0.01
ML27	2865.33 ^{efgh} ±286.11	-2.94 ^{cd} ±0.33	0.86 ^{ab} ±0.03
ML28	2768.66 ^{ghij} ±356.69	-4.14 ^{gh} ±0.56	0.85 ^{ab} ±0.03
ML29	2979.22 ^{defg} ±215.86	-3.60 ^{ef} ±0.51	0.86 ^{ab} ±0.02
ML30	3511.23 ^a ±407.38	-1.04 ^a ±0.11	0.84 ^{ab} ±0.01
ML31	2799.11 ^{fghij} ±247.14	-2.66 ^c ±0.35	0.85 ^{ab} ±0.01
ML32	2184.44 ^{mno} ±98.10	-5.30 ^{jk} ±1.14	0.83 ^b ±0.03
ML33	3265.71 ^{bc} ±298.54	-2.07 ^b ±.61	0.87 ^{ab} ±0.04
ML34	3315.34 ^{ab} ±334.81	-0.62 ^a ±0.13	0.85 ^{ab} ±0.03
ML35	2954.76 ^{defg} ±302.67	-3.13 ^{cde} ±0.77	0.85 ^{ab} ±0.01

(N=10, Results are expressed as Mean ± SE, with different small letters superscript (a,b,c) within row differ significantly (P< 0.05) among the samples. Where, M denotes Moga and L denotes Ludhiana. The sample numbered from ML01 to ML17 were collected from Moga and from ML18 to ML35 were collected from Ludhiana city)

Table 4 Instrumental texture analysis of various samples of *paneer*

Sample	Cohesiveness	Gumminess	Chewiness	Resilience
ML01	0.65 ^{abcde} ±0.03	1603.19 ^j ±172.42	1494.44 ^{klmno} ±184.09	0.37 ^{bcdef} ±0.01
ML02	0.61 ^{cde} ±0.01	1589.35 ^{ijk} ±85.15	1577.18 ^{ghijklm} ±92.57	0.34 ^{ef} ±0.02
ML03	0.68 ^{ab} ±0.03	1974.93 ^{def} ±78.59	1679.37 ^{fghijkl} ±46.51	0.39 ^{abcde} ±0.02
ML04	0.65 ^{abcde} ±0.04	1969.99 ^{def} ±190.69	1746.85 ^{defghi} ±228.00	0.37 ^{bcdef} ±0.03
ML05	0.69 ^a ±0.02	1488.29 ^{ijkl} ±189.55	1344.86 ^{nopq} ±201.48	0.43 ^a ±0.01
ML06	0.63 ^{bcde} ±0.01	1874.35 ^{fg} ±237.12	1726.36 ^{efghij} ±124.21	0.35 ^{def} ±0.05
ML07	0.59 ^{de} ±0.01	1582.06 ^{ijk} ±99.94	1571.20 ^{ghijklm} ±164.23	0.34 ^{ef} ±0.02
ML08	0.63 ^{bcde} ±0.01	2139.21 ^{bcd} ±251.06	1878.56 ^{cde} ±167.32	0.36 ^{bcdef} ±0.01
ML09	0.64 ^{abcde} ±0.02	1375.37 ^{klm} ±172.98	1163.57 ^{qr} ±189.40	0.39 ^{abcde} ±0.01
ML10	0.68 ^{ab} ±0.02	1496.65 ^{ijkl} ±196.27	1253.33 ^{pqr} ±168.09	0.38 ^{abcde} ±0.01

ML11	0.63 ^{bcd} ± 0.03	1268.21 ^m ± 127.15	1066.92 ^r ± 115.83	0.35 ^{def} ± 0.01
ML12	0.65 ^{abcde} ± 0.06	2280.33 ^{abc} ± 278.72	2082.59 ^{bc} ± 215.82	0.37 ^{bcd} ± 0.05
ML13	0.66 ^{abc} ± 0.05	1877.86 ^{fg} ± 213.64	1698.36 ^{ghijk} ± 73.77	0.38 ^{abcde} ± 0.02
ML14	0.65 ^{abcde} ± 0.05	1642.56 ^{hij} ± 140.60	1503.48 ^{ijklmno} ± 135.37	0.39 ^{abcde} ± 0.02
ML15	0.67 ^{ab} ± 0.04	1626.4 ⁱ ± 174.39	1565.85 ^{hijklmn} ± 187.56	0.41 ^{abc} ± 0.04
ML16	0.59 ^e ± 0.02	1436.74 ^{ijklm} ± 134.03	1386.05 ^{mnpq} ± 122.85	0.32 ^f ± 0.01
ML17	0.64 ^{abcde} ± 0.02	1838.69 ^{fgh} ± 173.52	1553.35 ^{ijklmn} ± 169.35	0.40 ^{abcd} ± 0.01
ML18	0.61 ^{bcd} ± 0.02	1955.45 ^{ef} ± 202.95	1711.51 ^{fghijk} ± 229.72	0.36 ^{bcd} ± 0.01
ML19	0.64 ^{abcde} ± 0.01	1442.75 ^{ijklm} ± 104.12	1304.85 ^{opq} ± 52.5	0.39 ^{abcde} ± 0.04
ML20	0.63 ^{bcd} ± 0.01	1330.16 ^m ± 99.79	1173.23 ^{qr} ± 165.20	0.38 ^{abcde} ± 0.06
ML21	0.65 ^{abcde} ± 0.01	1698.89 ^{ghi} ± 173.59	1468.70 ^{lmnop} ± 44.30	0.35 ^{cdef} ± 0.02
ML22	0.64 ^{abcde} ± 0.01	1872.23 ^{fg} ± 62.34	1726.96 ^{efghij} ± 74.37	0.37 ^{bcd} ± 0.04
ML23	0.67 ^{ab} ± 0.03	2138.78 ^{bcd} ± 151.04	2046.27 ^{bc} ± 44.85	0.42 ^{ab} ± 0.04
ML24	0.67 ^{ab} ± 0.05	2188.34 ^{bcd} ± 289.19	1965.08 ^{bcd} ± 53.02	0.41 ^{ab} ± 0.01
ML25	0.67 ^{ab} ± 0.02	1857.30 ^{fg} ± 134.03	1754.92 ^{defghi} ± 161.42	0.41 ^{ab} ± 0.02
ML26	0.59 ^{de} ± 0.02	1564.59 ^{ijk} ± 96.60	1380.14 ^{mnpq} ± 130.43	0.34 ^{ef} ± 0.01
ML27	0.64 ^{abcde} ± 0.02	1933.78 ^{ef} ± 171.12	1676.40 ^{fghijkl} ± 52.14	0.38 ^{abcde} ± 0.01
ML28	0.65 ^{abcde} ± 0.02	1867.33 ^{fg} ± 87.23	1732.22 ^{efghij} ± 99.34	0.39 ^{abcde} ± 0.03
ML29	0.64 ^{abcde} ± 0.03	2011.16 ^{def} ± 168.83	1789.42 ^{defg} ± 98.92	0.38 ^{abcde} ± 0.03
ML30	0.69 ^a ± 0.03	2334.67 ^{ab} ± 315.50	2378.39 ^a ± 49.57	0.41 ^{abc} ± 0.04
ML31	0.66 ^{abc} ± 0.03	2106.60 ^{bcd} ± 258.20	1946.06 ^{bcd} ± 101.62	0.39 ^{abcde} ± 0.04
ML32	0.62 ^{bcd} ± 0.01	1486.08 ^{ijk} ± 190.11	1322.80 ^{opq} ± 193.50	0.39 ^{abcde} ± 0.02
ML33	0.65 ^{abcd} ± 0.01	2255.45 ^{abc} ± 168.83	2151.02 ^b ± 62.78	0.41 ^{ab} ± 0.01
ML34	0.64 ^{abcde} ± 0.01	2448.49 ^a ± 232.25	2369.73 ^a ± 55.82	0.41 ^{ab} ± 0.01
ML35	0.63 ^{bcd} ± 0.01	1976.42 ^{def} ± 214.86	1799.86 ^{defg} ± 175.68	0.38 ^{abcde} ± 0.01

(N=10, Results are expressed as Mean ± SE, with different small letters superscript (a,b,c) within row differ significantly (P< 0.05) among the samples. Where, M denotes Moga and L denotes Ludhiana. The sample numbered from ML01 to ML17 were collected from Moga and from ML18 to ML35 were collected from Ludhiana city)

and texture, on other hand, were strongly positively associated with 2nd principal component.

Pearson's correlation coefficient was utilised to comprehend the strong association between sensory attributes and instrumental parameters. The r value allows researchers to compare correlations of various attributes within a given set of data (Table 5; Philipp et al. 2017). The sensory attributes (flavour, colour and appearance, body and texture, and overall acceptability) had no significant correlation with hardness, springiness, adhesiveness, chewiness, gumminess, cohesiveness, and resilience as their Pearson correlation coefficient ranges between 0.01 and 0.26. The instrumental parameters may not be able to predict the sensory qualities listed above linearly due to the lower correlation value. This finding could be helpful for dairy industry since texture measuring with equipment offers a more objective and cost-effective technique than utilising sensory evaluation, which requires extensive panellist training and takes a lot of time (Mohammadi Moghaddam et al. 2015).

Conclusion

Thirty five different paneer samples from different manufactures were collected and analysed the texture profile using sensory and instrumental technique. The samples show a wide range of

texture value and some samples coded as ML06, ML25, ML29, and ML30 showed distinct dominant sensory attributes. Data showed a deprived correlation between sensory attributes and instrumental parameters. This investigation could be helpful for dairy industry as well as dairy entrepreneurs in selection between instrumental and sensory evaluation of paneer samples.

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Table 5 Pearson correlation matrix between sensory attributes and instrumental texture parameters of various samples of *paneer*

	INSTRUMENTAL PARAMETERS							SENSORY ATTRIBUTES			
	HARDNESS	ADHESIVENESS	SPRINGINESS	COHESIVENESS	GUMMINESS	CHEWINESS	RESILIENCE	FLAVOUR	BODY AND TEXTURE	COLOUR AND APPEARANCE	OVERALL ACCEPTABILITY
INSTRUMENTAL PARAMETERS	HARDNESS	1									
	ADHESIVENESS	.92**	1								
	SPRINGINESS	-	-	1							
	COHESIVENESS	0.01	0.11		1						
	GUMMINESS	.94**	.90**	.36*		1					
	CHEWINESS	.94**	.90**	0.15	.36*		1				
	RESILIENCE	0.17	0.02	0.09	0.33	.96**					
SENSORY ATTRIBUTES	FLAVOUR	0.09	0.02	.77**	.36*	.35*	1				
	BODY AND TEXTURE	0.03	0.03	.39*	0.06	0.09	-	1			
	COLOUR AND APPEARANCE	-	-	0.03	-	-	0.15	0.19	1		
	OVERALL ACCEPTABILITY	0.01	0.08	0.07	0.02	0.08	0.13	-	0.23	1	
		0.04	-	-	0.02	0.03	0.08	0.27	-	-	1
		0.06	0.12	0.07	-	0.01	-	.60**	.56**	-	0.10

** . Correlation is significant at the 0.01 level (2-tailed) & * . Correlation is significant at the 0.05 level (2-tailed).

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RESEARCH ARTICLE

Effect of cold plasma on the quality parameters of custard apple juice milk beverage

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Abstract: This study is aimed to investigate and compare the physicochemical and sensory quality parameters which are associated with the freshness of the developed Custard apple juice milk beverage (CAJMB) subjected to cold plasma (CP) technology. The developed beverage was subjected to CP generated from two different configurations of Dielectric Barrier Discharge plasma (DBD) viz., High Voltage Atmospheric Cold Barrier Discharge plasma system (HV ACBD plasma system) and High Voltage Cold Barrier Discharge (HVCBD) Plasma torch/ Gun using Argon gas. The treatments were performed for different processing times (2 & 3 min) at an applied voltage gradient of 35 kV and 30 kV respectively. The treatments did not have any potential change in the sensorial quality attributes evaluated immediately after processing compared to untreated control sample. However, a noteworthy difference was observed in pH and titratable acidity after processing, directly proportional to increase in treatment conditions concerning voltage and processing time. The pH and acidity at defined intervals during storage showed a slow rate of reduction as compared to the control. In the control sample levels of pH and acidity reached to an unacceptable range in 3 to 5 days of storage, due to the formation of lactic acid by subsequent microbial growth. Whereas, CP treated sample using HV ACBD plasma system at 35kV for 3min exhibited promising results among other treatments and remained stable at refrigerated condition, with pH and acidity levels within the expected acceptable range for 9 to 10 days of storage.

Keywords: Cold plasma, Dielectric Barrier Discharge (DBD), Plasma Torch/ Jet, Custard Apple Milk Beverage, Physicochemical properties, Shelf life

Introduction

The demand for healthier minimally processed ready-to-drink (RTD) fresh fruit beverages with new choices and flavours is constantly increasing in the market. This is especially for combinations of fruit juices and milk with natural and added vitamins, minerals and fiber, which are consumed as functional foods in recent times. Milk contains lactose, fatty acids, proteins and various micronutrients such as minerals and trace elements. Therefore, the hazard of contamination by spoilage-causing and infective microbes in milk and fruit-based RTD beverages is of apprehension because these are consumed without any further processing after purchase from retailers. Custard apple (*Annona squamosa*) is a climacteric and very perishable fruit with 2 or 3 days of shelf life when ripened (Solanke et al. 2019). But it is a great source of iron, phosphorous, potassium and vitamin C. Also, it has numerous medicinal and nutritional factors containing a pool of sodium, magnesium, ascorbic acid, fiber and sugars, etc., with excellent antioxidant activity (Solanke et al. 2019). Thus, there is a necessity to utilize the nutritional as well as relish its flavour in the form of value-added food products to reduce post-harvest losses. Various value-added products of custard apple such as jams, ice creams, squash, crush, nectars, etc., which are highly processed are on market (Solanke et al. 2019). Milk beverages are generally thermally processed by pasteurization, retort processing and sterilization which improves the product safety and shelf life by destroying the microbial population. But these heat treatments lead to denaturation of essential proteins, non-enzymatic browning, nutritional loss, and higher alterations in the quality attributes including physicochemical and sensorial parameters (Pan et al. 2019). For such reasons, there is an essential need for non-thermal processing interventions. In current times, many non-thermal techniques such as Pulsed Electric Field (PEF), High Pressure Processing (HPP), Ultraviolet (UV) and Ultrasonication (US), and Ozone treatments were highly explored to increase the keeping quality of the food product while

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maintaining the quality characteristics of the treated food (Coutinho et al. 2018).

Cold Plasma (CP) is a newly emerging technology that is explored in recent periods among the other novel nonthermal technologies for the destruction of microbes and as a complementary to thermal techniques (Liao et al. 2017). Plasma is often described as the fourth state of matter with increasing energy from the gaseous state to energized plasma state containing elements like Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), positive ions, negatively charged atoms, neutrals, UV, free radicals, etc., (Liao et al. 2017). The element generated from plasma is reported to be the cause of the microbial inactivation but which element is particularly responsible and also the principle behind the influence of CP on Physico-chemical properties, interaction with biomolecules, Etc. are still under study (Coutinho et al. 2018). Two types of plasma have been classified as thermal and non-thermal plasma differentiated by their thermal equilibrium between ions and electrons. In non-thermal plasma the ions and electrons are in thermal equilibrium hence it is maintained at almost room temperature making it practically cold, which we can touch with bare hands thus causing no damage to the subjected food products but affecting the membranes of micro-organisms due to their relatively smaller cell size and structure (Surowsky et al. 2015). CP is generated by applying very high voltage to gas molecules. This high power can be generated by different means viz., alternating or direct current, microwaves, radio frequency, pulsed power source, etc., (Montejano et al. 2019). Various configurations and device geometries have been proposed for cold plasma generation among which Atmospheric pressure plasma (ACP) by Dielectric Barrier Discharge (DBD) system with symmetric electrode geometry and plasma jet/ plasma torch are widely explored for processing food products because of their simple design (Bourke et al. 2018).

Thus, this study mainly aims to evaluate the effect of plasma on the physico-chemical and sensorial quality parameters and keeping quality of the developed RTD custard apple juice milk beverage (CAJMB) using different methods of plasma generation of DBD viz., High Voltage Atmospheric Cold Barrier Discharge plasma system (HV ACBD) parallel plate plasma and High Voltage Cold Barrier Discharge (HV CBD) plasma torch system.

Materials and Methods

Experimental setup and experimental design

Cold plasma treatment of the CAJMB sample was carried out in two different methods of novel CBD non-thermal plasma systems. The systems used in this study were High Voltage Atmospheric pressure CBD plasma system and HV CBD plasma torch/ Gun (Zeonics Systech Defence and Aerospace Engineers (P) Ltd.,). The former system setup consists of two parallel plate electrodes measuring 400×400mm, made of stainless steel. The bottom

electrode is grounded whereas the upper electrode is connected to a high voltage power supply (50kV, 50Hz) and can be adjusted according to the size of the sample. About 50 mL of the sample was poured into the sterile borosil Petri plates of 90mm diameter and 15.5 mm depth. The depth of the sample was 14.5 mm. the gap between the high voltage electrode and the surface of the sample was kept constant at 10mm and the total gap between the two electrodes was 25.5 mm. The treatments were conducted with an input voltage of 35kV at two different treatment times (2 min and 3 min). The later system setup consists of two electrodes made of stainless steel. The upper electrode or the power electrode is a rod-shaped electrode surrounded by a glass tube acting as the dielectric in this CBD system. The bottom electrode is a stainless-steel plate electrode that is connected to the ground. In this system, argon gas is used as the plasma medium. The samples were poured into the sterile borosil glass beaker of volume 150mL. The experiment was conducted at 30kV for 2 min and 3 min respectively. The treated samples from both systems were transferred to sterilized PET bottles using sterile syringes, labelled and stored at refrigerated conditions until further analysis and throughout the storage studies.

Preparation of Custard Apple Juice Milk Beverage (CAJMB)

Fresh custard apple was procured from the local vendors. Custard apple pulp was prepared according to the procedure of (Ramesh et al. 2017) with slight modifications. The mucilage and the seed were separated manually. The pulp was blended using a blender and was filtered using a muslin cloth and was stored in a separate sterile PET bottle. The custard apple milk beverage was prepared by mixing 65% pasteurized cow milk, 35 % custard apple pulp and 5% of powdered sugar, in a mixing jar and blended well using a blender (Bakane et al. 2016). The prepared beverage was stored in sterile PET bottles at 4°±2° C temperature for further non-thermal plasma processing within 24hrs.

pH

The pH of the treated and control CAJMB sample was analysed using a digital hand pH meter (Eco Testr pH 1, Eutech instrument, OAKTON) after calibrating with pH 4 and pH 7 buffer standards solutions.

Titrateable acidity

The treated and control CAJMB samples were evaluated for titrateable acidity, 10 ml of sample was taken in a conical flask or beaker and titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The endpoint was noted when a pink color was noticed and persisted for about 30 sec (AOAC International methods, 1999). the volume of NaOH consumed was noted and further, the acidity was calculated in terms of lactic acid and expressed as ml/ L. The titrateable acidity was computed using the relationship in the equation.

$$\text{Acidity} = \frac{\text{Base titrant mL} \times \text{Normality of base} \times \text{Acid factor}}{\text{Sample volume mL}}$$

Sensory evaluation

Sensory evaluation of control and treated samples was done by a subjective test based on a 9-point hedonic scale (Gupta, 1976). Instructions were given to the panellists to rinse their mouths with drinking water after tasting every sample. The panellists were instructed to examine the sample for the attributes of colour, mouthfeel and flavour.

Shelf life and storage studies

The pH and acidity of the stored samples were assayed at steady intervals of 2 days throughout the storage period.

Statistical analysis

SPSS version 20 statistical software (SPSS, Inc., United States) was used for data analysis. All the experiments were done in six replicates. The statistical differences in the analysed parameters of the plasma-treated CAJMB samples considering the varying voltage and treatment time were calculated using Analysis of variance (ANOVA). Multivariate Duncan's test ($p < 0.05$) was used to evaluate statistical differences between treatments and a significant difference was defined at $p < 0.05$. The developed CAJMB samples were treated with maximum treatment conditions from both the systems viz., T_2 - 35kV, 3min and T_4 - 30kV, 3min. These two treatments were statistically analysed using the results of the pH and TA obtained to compare the impact of CP generated from different configurations of the CBD system used in this study on CAJMB.

Results and Discussion

pH

The pH values of the control and treated samples processed using both the HV ACBD parallel plate system and the HV CBD plasma torch over the storage period are shown in Table 1 and Table 3. The table depicts a significant decrease in the pH of the treated samples with an increase in voltage and treatment time compared to the control untreated sample immediately after processing. The pH of the fresh control sample was 6.39 ± 0.018 and the pH of the HV ACBD plasma plate system treated samples immediately after processing was between the mean of 6.29 and 6.32 respectively. The pH of the HV CBD plasma torch treated samples ranged between mean values of 6.3 and 6.33. Despite the statistical difference, the values of pH ranged within the expected levels. However, there was no significant difference in the pH within the treatments after processing. The decline in pH in treated samples compared to the control was 0.07 and 0.1, 0.09 and 0.06 for the HV ACBD plate system and HV CBD plasma

torch respectively. The pH value showed a highly significant descending trend in both control and treated samples throughout the storage period, even though there was no significant difference observed between the control and treated samples of CAJMB in both the systems during the second day of storage.

Titrateable acidity

Titrateable acidity (TA) of the cold plasma processed CAJMB was highly significantly higher to the control sample. There was a significant increase in the titrateable acidity in the samples immediately after processing as shown in Table 2 and Table 4. The TA of the fresh control sample was 0.128 ± 0.002 whereas the treated samples T_1 and T_2 had TA of mean in the range of 0.148 and 0.162 at the immediate point of the assay. The titrateable acidity of each of the treated CAJMB also showed a considerable significant difference between each other. The TA of the samples processed in the HV CBD plasma torch system ranged between 0.151 and 0.144 for treatments T_3 and T_4 correspondingly. However, there was no significant difference within the treatments as it was observed within the treated samples processed in HV ACBD parallel plate plasma system. The observed titrateable acidity values at specified intervals showed a noteworthy and exceedingly significant increase in the control and treated samples across the storage period, although there was no substantial difference during the second day of storage.

Sensory

Sensory evaluation of the control and treated custard apple milk beverage (CAJMB) was carried out immediately after the processing (zeroth day). Though there was a significant increase in titrateable acidity and decrease in pH, the sensory scores for colour, mouthfeel and flavour of the developed cold plasma processed CAJMB showed no significant difference ($P > 0.05$, from Table 7.) compared to the control sample. The results obtained for the processed samples exhibit much liking and acceptable scores of more than 9.00 (liked extremely).

Statistical comparison of the physicochemical quality parameters of the cold plasma treated samples in the HVACP CBD plasma system and HV CBD plasma torch

From Table 5 and Table 6, it was noticed that the pH and TA of the treatments T_2 and T_4 were significantly different but in a very narrow range at the beginning of the storage period excepting the second day. Also, a trivial increase in temperature by 2° to 3° was observed in the HV CBD plasma-torch treated samples. However, there was a noteworthy difference in the decline of pH and increase in TA between the treated samples (T_2 and T_4) as the storage days increased (on the 10th day). It was noted that the pH and acidity of the T_2 treated samples at the end of the 10th day showed an acceptable level for the developed CAJMB compared to the T_4 treated sample and control sample. From Tables 5 and 6, a gradually decreasing drift and ascending level

of pH and TA could be observed as it moved towards the tenth day of storage. pH and TA as one of the primary quality attributes of a food product since the increase in TA and decrease in pH can be associated with the growth of lactic acid bacteria, especially in milk products (data not presented). Hence, from Tables 5 and 6 it is observed that the HV ACBD plasma system has shown a slow decline in the pH and TA and prolonged the acceptable level of the sample till the 10th day of storage. Whereas, the control sample and T₄ sample reached unacceptable levels the same level on the 2nd day and 6th day of storage respectively.

Effect of the generated High Voltage Cold Barrier Discharge plasma on the parameters analysed (pH, TA and sensorial attributes) in the developed CAJMB

The results found in this study, from Tables 1, 2, 3, and 4 showed a significant decline in pH and an increase in TA after CP processing. These two parameters viz., pH and TA are the primary

factors that contribute to the freshness of food samples. This decrease in PH and increase in TA immediately after treatment might be potentially due to the reaction of plasma-generated reactive species like reactive oxygen species (ROS) and Reactive nitrogen species (RNS) with the moisture (i.e., water molecules) present in the food sample. (Muhammad et al.2018). These reactions lead to the formation of acidic compounds such as nitric acid, H₂O₂, and nitrous acids formed by the hydrolysis of NO and NO₂ reactive species and other elements in plasma-treated liquid samples (Zheng et al. 2019) resulting in a lessening of pH (Liu et al. 2015). Increased reduction in pH and increase in TA has been largely noted in the cases where nitrogen is used as the gas medium (Misra et al. 2016). The surge in acidity may also be owing to the split-up of amino acids (AA) that exists in the sample by plasma reactive species and diffusion of the same in the sample solution (Bußler et al. 2015) attributed to the formation of carboxylic groups from AA disintegration. The increase in the intensity of the voltage applied, gas flow rate and the treatment

Table 1: pH of HV ACBD plasma processed CAJMB at refrigerated condition during storage period

Treatment	pH						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₁ – 35 kV, 2min	6.32±0.021 ^{Ae}	6.30±0.017 ^{Ade}	6.25±0.030 ^{Bd}	6.14±0.018 ^{Bc}	6.07±0.017 ^{Bb}	5.72±0.018 ^{Ba}	118.054**
T ₂ – 35kV, 3 min	6.29±0.017 ^{Ae}	6.25±0.030 ^{Ade}	6.20±0.018 ^{Bcd}	6.17±0.017 ^{Bbc}	6.12±0.018 ^{Bb}	6.02±0.007 ^{Ca}	26.040**
C	6.39±0.018 ^{Bf}	6.24±0.017 ^{Ae}	6.10±0.018 ^{Ad}	5.81±0.017 ^{Ac}	5.20±0.021 ^{Ab}	5.00±0.017 ^{Aa}	1134.616**
F VALUE	7.406 *	2.143 ^{NS}	11.467**	189.259**	753.142**	1203.152**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p < .05).

Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); ^{NS} – Non significant

Table 2: Acidity of HV ACBD plasma processed cajmb at refrigerated condition during storage period

Treatments	ACIDITY						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₁ –35 kV, 2min	0.148±0.002 ^{Ba}	0.181±0.030 ^{Aab}	0.206±0.002 ^{Bbc}	0.211±0.018 ^{Abc}	0.224±0.017 ^{Abc}	0.258±0.018 ^{Ac}	4.690**
T ₂ –35kV, 3 min	0.162±0.001 ^{Ca}	0.175±0.021 ^{Aab}	0.173±0.014 ^{Aab}	0.207±0.017 ^{Aabc}	0.219±0.018 ^{Abc}	0.225±0.007 ^{Ac}	3.260*
C	0.128±0.002 ^{Aa}	0.213±0.002 ^{Ab}	0.237±0.002 ^{Bb}	0.287±0.018 ^{Bc}	0.312±0.007 ^{Bcd}	0.340±0.021 ^{Bc}	41.860**
F VALUE	88.371**	0.923 ^{NS}	14.037**	6.583*	12.466**	12.545**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p < .05).

Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); ^{NS} – Non significant

Table 3: pH of HV CBD plasma processed CAJMB at refrigerated condition during storage period

Treatments	pH						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₃ –30 kV,2min	6.33±0.014 ^{Ad}	6.27±0.017 ^{Ac}	6.23±0.030 ^{Bc}	6.15±0.017 ^{Bb}	6.10±0.018 ^{Bb}	5.70±0.007 ^{Ba}	150.475**
T ₄ –30 kV,3min	6.30±0.017 ^{Ad}	6.26±0.019 ^{Ac}	6.21±0.030 ^{Bc}	6.12±0.018 ^{Bb}	6.07±0.017 ^{Bb}	5.76±0.018 ^{Ca}	92.893**
C	6.39±0.018 ^{Bf}	6.24 ±0.017 ^{Ae}	6.10±0.018 ^{Ad}	5.81±0.007 ^{Ac}	5.20±0.021 ^{Ab}	5.00±0.017 ^{Aa}	1134.616**
F VALUE	8.700**	0.728 ^{NS}	7.047*	168.072**	735.320**	781.670**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p < .05).

Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); ^{NS} – Non significant

time results in the amplified concentration of different plasma-produced elements in the liquid products (Yong et al. 2015), such as ROS, RNS, free radicals, ions, energized and non-energized atoms (Pankaj et al. 2017a). This might lead to the production of higher acidogenic molecules, influencing the pH of the sample (Yong et al. 2015; Helmke et al. 2011). The increase in acidity in the sample (CAJMB) distinguished after CP processing might be caused by consecutive reactions such as oxidation of aldehydes ignited by the plasma species such as O_3 , OH and NO with water molecules present in the aqueous-air interphase (Liu et al. 2010). It could also be due to the breakdown of hydroxyl radical produced in the plasma discharge process as reported by Muhammad et al. (2019) for the CP-treated Tiger Nut Milk (TNM) and other researchers in various studies (Coutinho et al. 2019). The impact of plasma on the physico-chemical quality parameters such as the difference in pH of the food sample is also attributable to its buffering capacity, protein and phosphate content as in

milk (Ponraj et al. 2017) and acid content (Kim et al. 2015) which has been observed in cases cold plasma treated Guava Fruit juice Whey Beverage (GFWB) and orange juice and milk (M. R. Silveira et al. 2019; Xu et al. 2017; Manoharan et al. 2021). Though there was a decrease in pH and acidity, it was only in a narrow range, causing no effect on the major biomolecule dissolving power in the treated food samples of CAJMB. pH and acidity are considered important quality traits in processed food samples whose sweeping alteration can impact the sensorial parameters and shelf life (Pankaj et al. 2018). Noteworthy slight changes in those parameters did not affect the quality of the developed beverage in this study (CAJMB), which was practically shown by the non-significant change in the sensorial parameters examined after CP treatment which has been reported by various workers (Li and Xiong, 2021; Eazhumalai et al. 2021).

Table 4: Acidity of HVCBD plasma processed CAJMB at refrigerated condition during storage period

Treatments	ACIDITY						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₃ -30 kV, 2min	0.151±0.003 ^{Ba}	0.170±0.017 ^{Aab}	0.209±0.003 ^{Abc}	0.224±0.017 ^{ABbc}	0.231±0.018 ^{Ac}	0.242±0.007 ^{Ac}	8.085**
T ₄ -30 kV, 3min	0.144±0.002 ^{Ba}	0.196±0.017 ^{Aab}	0.212±0.006 ^{Ab}	0.222±0.018 ^{Ab}	0.237±0.017 ^{Ab}	0.245±0.018 ^{Ab}	6.310**
C	0.128±0.002 ^{Aa}	0.212±0.005 ^{Ab}	0.237±0.002 ^{Bbc}	0.287±0.018 ^{Bcd}	0.312±0.007 ^{Bdc}	0.340±0.021 ^{Bc}	40.971**
F VALUE	23.436**	2.174 ^{NS}	14.932**	4.426*	9.280**	11.113**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).

Superscripts in the F value indicates: ** - Highly significant ($p < 0.01$); * - significant ($p < 0.05$); ^{NS} - Non significant

Table 5: Comparative analysis of pH of effective treatments of each configuration of the plasma systems

Treatments	pH						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₂ - 35kV, 3min	6.29 ± 0.017 ^{Ac}	6.25 ± 0.030 ^{Ade}	6.20 ± 0.018 ^{Bcd}	6.17 ± 0.017 ^{Cbc}	6.12 ± 0.018 ^{Bb}	6.02 ± 0.007 ^{Ca}	26.040**
T ₄ - 30kV, 3min	6.30 ± 0.017 ^{Ad}	6.26 ± 0.019 ^{Ac}	6.21 ± 0.030 ^{Bc}	6.12 ± 0.018 ^{Bb}	6.07±0.017 ^{Bb}	5.76 ± 0.018 ^{Ba}	92.893**
C	6.39 ± 0.018 ^{Bf}	6.24 ± 0.017 ^{Ac}	6.10 ± 0.018 ^{Ad}	5.81 ± 0.007 ^{Ac}	5.20 ± 0.021 ^{Ab}	5.00 ± 0.017 ^{Aa}	1134.616**
F VALUE	10.603**	0.167 ^{NS}	7.274*	180.405**	753.142**	1230.006**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).

Superscripts in the F value indicates: ** - Highly significant ($p < 0.01$); * - significant ($p < 0.05$); ^{NS} - Non significant

Table 6: Comparative analysis of Titratable Acidity of effective treatments of each configuration of plasma systems

Treatments	ACIDITY						F VALUE
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	
T ₂ -35kV, 3min	0.162±0.001 ^{Ca}	0.175±0.021 ^{Aab}	0.173±0.014 ^{Aab}	0.207±0.017 ^{Aabc}	0.219±0.018 ^{Abc}	0.225±0.007 ^{Ac}	3.260*
T ₄ -30kV, 3min	0.144±0.002 ^{Ba}	0.196±0.017 ^{Ab}	0.212±0.006 ^{Bbc}	0.222±0.018 ^{Abc}	0.237±0.017 ^{Abc}	0.245±0.018 ^{Ac}	6.310**
C	0.128±0.002 ^{Aa}	0.212 ±0.005 ^{Ab}	0.237 ±0.002 ^{Bb}	0.287 ± 0.018 ^{Bc}	0.312±0.007 ^{Bcd}	0.340±0.021 ^{Bd}	40.971**
F VALUE	78.234**	1.410 ^{NS}	12.380**	5.858*	11.084**	13.506**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).

Superscripts in the F value indicates: ** - Highly significant ($p < 0.01$); * - significant ($p < 0.05$); ^{NS} - Non significant

Table 7: Sensory analysis of custard apple juice milk beverage before and after CP processing

CBD - Configuratio	Treatments	SENSORY – 0 th d		
		COLOUR	MOUTHFEEL	FLAVOUR
HV CBD Parallel	T ₁ – 35 kV, 2min	9.92 ± 0.083	9.83 ± 0.105	9.42 ± 0.083
Plate plasma	T ₂ – 35kV, 3 min	9.92 ± 0.083	9.75 ± 0.112	9.50 ± 0.129
HV CBD Plasma	T ₃ - 30 kV, 2min	9.92 ± 0.083	9.83 ± 0.105	9.42 ± 0.154
Torch using Argon gas	T ₄ - 30 kV, 3min	9.92 ± 0.083	9.67 ± 0.167	9.50 ± 0.183
Control	C	9.92 ± 0.083	9.83 ± 0.105	9.67 ± 0.167
	F VALUE	0 ^{NS}	0.377 ^{NS}	0.481 ^{NS}

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);

a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).

NS – Non significant

The significant decline in pH and a significantly slight increase in titratable acidity (TA) post CP processing observed in this study with CAJMB was similar to the results of Eazhumalai et al. (2021) in the treatment of oat milk. In the research, where cashew apple juice was treated with a spark DBD plasma, similar results of a slight descending level in pH were observed which was maintained during storage (Illera et al. 2019) supporting the discoveries of Dasan & Boyaci (2018) in the CP processed sour cherry nectar. In their study, Eazhumalai et al. (2021) reported that, decrease in pH and an increase in TA were directly proportional to significant increase in treatment time and no substantial difference has been observed due to voltage. It was also substantiated by the outcomes reported in the non-thermal Plasma treatment of chocolate milk (Coutinho et al. 2019), Tiger Nut Milk (Muhammad et al. 2019), plasma processed milk (Kim et al. 2015), Pea protein extract (Bubler et al. 2015), guava flavoured whey beverage (GFWB) (M. R. Silveira et al. 2019), High Voltage Atmospheric Cold Plasma (HVACP) treated orange juice (OJ) (Xu et al. 2017), Apple juice treated in DBD ACP (Liao et al. 2018; Xiang et al. 2018), where the pH decreased gradually with the increase in the treatment time, gas flow rate and applied voltage accordingly. But, when compared to thermal treatment (pasteurization, retort, etc.,) of milk drinks and beverages, the pH reduction during cold plasma processing was significantly less. Hence, the CP processed beverages had relatively higher pH value than in the heat-processed samples (M. R. Silveira et al. 2019). This is because, during heat treatment, the lactose present in the milk through various reactions form acids such as formic acid, acetic acid, etc and precipitation of calcium phosphate releasing H⁺ ions, which consequently increases the acidity of the beverage (Dursun et al. 2017; Fox et al. 1981). Similarly, a slight significant reduction in pH in the treatment of prebiotic orange juice (OJ) at 20 kV with direct and indirect exposure to plasma compared to untreated samples has been reported by Almeida et al. (2015). In their study, they also stated that there was no effect of treatment time on the decrease in pH and there was no change in pH has been observed between the ozone-treated and control samples (Almeida et al. 2015). This study was in corroboration with the findings of Manoharan et al. (2021) and Kim et al. (2015) in the cold plasma treatment of milk, and Ponraj et al. (2017) in the Argon gas CP treated milk. Also, tomato juice

after Cold plasma processing showing statistically no drastic or noteworthy decrease in pH among the treatments but a very narrow reduction was observed between treatment and control (Ali et al. 2021). Whereas, Xu et al. (2017) reported no significant change in the CP processed orange juice at 90kV. These results were supported by the data found in the cold plasma treatment of tender coconut water beverage with ascorbic acid and tomato-based beverage and White grape juice (Chutia and Mahanta, 2021; Mehta et al. 2019; Pankaj et al. 2017b). A similar report for no variations among control and treatments was submitted for the nonthermal processing like Ultra Sonication (US) (Caminiti et al. 2012) and Pulsed Electric Field (PEF) treatment of orange juice (Tiwari et al. 2008), Ultra Violet treatment (UV) of Apple juice (Tiwari et al. 2008), UV & US processed tomato beverage. But in the case of PEF processed TNM, a mild decrease in pH was observed at 20kV/ cm for 300µs. Also, in another study, no decrease or increase in pH of PEF processed orange juice milk beverage (OJMB) has been noted after treatment but a slow decline was observed across storage due to lactic acid formation by microbial growth (Sampedro et al. 2009). This result was in accordance with Rivas et al. (2006), in the PEF treatment of carrot juice which was comparable to the immediate observations after the cold plasma processing of carrot juice with respect to both voltage and processing time (Muhammad Umair et al. 2019).

Conclusion

The developed CAJMB was subjected to cold plasma with varying treatment time and voltage in two different configurations of Dielectric Barrier Discharge plasma (DBD) and was analyzed for physico-chemical quality parameters viz., pH and acidity. The data obtained were compared with the data of the control sample statistically. The observed results showed a slight variation of decrease in pH and increase in acidity due to the reaction between the components present in the food matrix of the sample and plasma reactive species. But, during the storage studies, the quality attributes were within the acceptable level for more days in the treated samples compared to control samples respectively. This exhibits the decontamination effect of plasma species on microbes slowing down their growth in the treated samples. The key finding in this study was that the CAJMB processed in

HVACBD parallel plate plasma system at 35kV for 3min (T_2) revealed promising results among other treatments, keeping pH and acidity within control limits for 9 to 10 days of storage in refrigeration temperature. Whereas, the control sample crossed the acceptable range in 3 to 5 days of storage. Thus, cold plasma shows to be potentially effective in extending the shelf life of milk-based beverages.

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RESEARCH ARTICLE

Effect of vitamin E and zinc supplementation in pre and post partum period in crossbred cows on anti-oxidant, immunity status and performance of new born calves

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Abstract: The present study was undertaken to investigate the effect of zinc (Zn) supplementation alone and its combination with vitamin E on growth, anti oxidant status and immunity status of new born calves of crossbred cows. The cows were supplemented with Zn @ 0 ppm (T₀), Zn @ 80 ppm (T₁) and Zn @ 80 ppm + 1000 IU α -tocopheryl acetate/d (T₂) from 60 days prepartum up to 90 days postpartum. The fat, SNF, total solid, protein, Zn and Cu contents content of colostrum showed no effect of dietary treatments but α -tocopherol content was higher (P<0.01) in group T₂ as compared to other groups. Total immunoglobulin in colostrum of T₂ group was 25.87% higher than control. There was no effect of dietary treatments on plasma Zn and Cu levels of calves, however α -tocopherol concentration at day 5 was higher (P<0.05) in group T₂ as compared to other groups. Status of antioxidant enzymes, lymphocyte proliferation index, total and differential leukocyte count was not affected by the treatments. Higher birth weight (P<0.05) was obtained in group T₂ group as compared to other group. Calves from treatment groups showed lower incidence of morbidity and mortality due to calf scour and other infectious diseases compared to control.

Keywords: α -tocopherol, Body weight, Calf morbidity, Colostrum, Crossbred cow, Immunity

Introduction

Colostrum serves as the first source of nutrients that a calf consumes after birth. It contains large amount of energy, protein, minerals and fat soluble vitamins which are required by the calves for normal metabolic functions, growth and establishment of immune system. At birth, calves exhibit a poorly developed (physiologically immature) immune system which renders them more susceptible to infectious diseases (Teixeira et al. 2014). Dairy replacement success or failure is dependent on newborn calf health and growth which can be impaired by poor maternal health, colostrum deprivation and poor calf nutrition (Teixeira et al. 2014). Maternal nutrition during late gestation and after parturition can have lasting impacts on calf health, growth and performance (Abuelo et al. 2019; Dunn et al. 2017). Calves supplemented with Zn shows greater average daily gain and lower mortality (Kegley et al. 2001; Ahola et al. 2004). Feldman et al. (2019) found that Zn supplementation was beneficial for prevention of diarrhea in dairy calves, thus, minimizing the use of antimicrobial. Calf diarrhea is a major cause of high mortality and morbidity in the dairy industry and it has been found that Zn status in animals declined in gastrointestinal disorders (Ranjan et al. 2006). Holstein cows supplemented with combination of inorganic and organic minerals (Zn, Cu, Mn and Co) from 21 day prepartum showed greater concentration of IgG in the colostrum (Kincaid and Socha, 2004; Jaff et al. 2020; Putman et al. 2018). The α -tocopherol does not cross the placenta in appreciable amounts, and calf is dependent on colostrum to obtain vitamin E after birth. Weiss et al. (1998) supplemented vitamin E and found higher vitamin E content in colostrum compared to control cows. Supplementation of fat soluble vitamins E and D₃ in advance pregnant buffaloes during 30 days prepartum resulted in higher birth weight of calves (Sikka et al. 2002). The vitamin E (α -tocopherol) status of dairy cows is one the important components of immune system because of its antioxidant effects at parturition, plasma concentrations of vitamin E were found to decrease by 47% because of secretion of the vitamin into the udder during colostrogenesis, decreased DM intake at calving, and an increased need for antioxidants during this time (Kafilzadeh et al. 2014) showing that supplementation of vitamin E had positive effect on mother and young ones. Minerals and vitamin

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supplementation in pre and post partum period affect the immunoglobulin levels as well as the antioxidant enzyme activity of the calves (Kincaid, 2008; Gaal et al. 2006). So supplementation of certain minerals and vitamins might affect the composition of colostrum ultimately the health and performance of the calves. Therefore, the objectives of present study was to determine the effects of zinc supplementation alone and its combination with vitamin E in pre and post partum period on growth, anti oxidant status and immunity status of new born calves of crossbred cows.

Materials and Methods

Selection of animals and feeding management

Twenty four crossbred (Karan Fries) pregnant cows in their late lactation (before drying) were selected from institute herd. All the cows were free from anatomical, physiological and infectious disorders, which are ascertained by physical examination as well as from records. They were divided into three groups of eight animals each on the basis of their expected producing ability (EPA; Lush, 1945). All the experimental procedure were in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2012, India) for the care and use of animal for scientific purposes. Experimental animals were fed iso-nitrogenous and iso-caloric diet as per NRC (1989). In addition to the basal level Zn was not supplemented (T_0), while cows of groups T_1 and T_2 were supplemented with Zinc @ 80 mg/kg DMI and Zn @ 80 mg/kg DM intake along with vitamin E @ 1000 IU/ cow/ d respectively, in the dry period (60 days before parturition) which was continued up to 90 days of lactation. Zinc was supplied in the form of $ZnSO_4 \cdot 7H_2O$ (Thomas Baker Pvt. Ltd.). The cows were moved to calving pen 15 days before the expected date of calving and provided with soft bedding during this time. Colostrum samples on day 1 and 5 were collected from cows and milk composition (Fat, protein, SNF and total solids), Zn and Cu Copper content, α -tocopherol content and total immunoglobulins estimations were carried out. Blood samples were collected from individual calf on day 1 and 5 after birth for the estimation of plasma zinc and copper, α -tocopherol, total antioxidant activity, enzymes (Superoxide dismutase, glutathione peroxidase and alkaline phosphatase), lymphocyte proliferation, Plasma total immunoglobulin, total leukocyte count and differential leukocyte count. The incidence of morbidity and mortality in calves were recorded up to the age of three months.

Chemical analysis of samples

The roughage and concentrate were ground individually, labeled and analyzed for proximate composition as per AOAC (2005) and cell wall constituents as per Goering and Van Soest (1970). Concentration of Zn and Cu in colostrum and blood samples were analysed by atomic absorption spectrophotometer (Hitachi

Z-5000, Hitachi Ltd., Japan). An HPLC method for simultaneous estimation of retinol and α -tocopherol in feed, fodders, plasma and milk samples was adopted (Chawla and Harjit Kaur, 2001; Harjit Kaur et. al. 2004). In blood samples the haemoglobin (Drabkin, 1944), total leukocyte count was made by the Haemocytometer method by (Schalm, 1961), total antioxidant activity in term of Ferric Reducing Antioxidant Power assay by Benzie and Strain (1999). The activity of superoxide dismutase (Marklund and Marklund, 1974), glutathione peroxidase (Hafeman et al., 1974), Alkaline phosphatase activity (Klin, 1970). Immunoglobulins in the plasma sample were estimated by Zn turbidity method (McEwan and Fisher, 1970). Colostrum samples were analysed for percentage of fat (AOAC 2005), crude protein (BIS, 1981) and total solids (AOAC, 2005). The SNF content of colostrum was calculated by subtracting percentage of fat from total solids. Fat, protein, SNF and total solids contents of milk were determined by Funke-Gerber Lactostar (Benny Impex Pvt. Ltd.).

Statistical analysis: Statistical analysis of the data was by ANOVA as per Snedecor and Cochran (1994) with the help of software package (SPSS 1998). The effect of treatments was analysed by two-way ANOVA.

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad (i = 1 \dots I; j = 1 \dots J)$$

where μ , Overall mean; α_i , Row effect; β_j , Column effect and ε_{ij} , Random error for observation X_{ij}

Results and Discussion

Effect of supplementation of Zn and vitamin E on composition of colostrum

The fat, SNF, total solid and protein content of colostrum decreased on the day of parturition to day 5 (Table 1) and was similar ($P > 0.05$) between different groups from 1st to 5th day showing no effect of dietary treatments. The contents of fat, protein, SNF and total solids of colostrum were within the range reported earlier in crossbred cows (Panda et al. 2006; Patoo et al. 2016) and were not affected by the dietary treatment of cows.

The Zn, Cu and α -tocopherol contents of colostrum

There was no effect of dietary treatments on Zn and Cu content of colostrum on both day 1 and day 5 samples in three respective groups ($P > 0.05$; Table 2). The α -Tocopherol content of colostrum on day 1 averaged 4.68, 4.87 and 5.52 $\mu\text{g/ml}$ and decreased to 3.29, 3.38 and 4.09 $\mu\text{g/ml}$ in groups T_0 , T_1 and T_2 , respectively and was significantly ($P < 0.01$) higher in group T_2 on both days ($P < 0.01$; Table 2). Higher α -tocopherol content in group T_2 might be due to supplementation of Vitamin E along with Zn in this group which affect the absorption and secretion of α -tocopherol in the colostrum (Weiss et al. 1997; Panda et al. 2006; Micinski et al. 2017).

Influence of supplementation of Zn and Vitamin E on total immunoglobulin concentration of colostrum

Cows supplemented with combination of Zn and vitamin E produced higher ($P<0.05$) total Ig (25.87%) in colostrum than control (Table 2). At the end of colostrum period the total Ig concentration in colostrum declined in all the groups. The values were similar in all the groups. The total immunoglobulin was significantly ($P<0.05$) higher in group T_2 indicating that cows supplemented with combination of Zn and vitamin E produced more colostral immunoglobulins and have better immunity status over other two groups (Panigrahi et al. 2005; Sikka and Lal, 2006; Dang et al. 2009; Immler et al. 2022).

Plasma Zn, Cu and α -tocopherol Status of Calves

There was no effect of dietary treatments given to cows on plasma Zn and Cu levels of calves on first 5 days of their age (Table 3). The plasma α -tocopherol concentration at day 5 was higher ($P<0.05$) in group T_2 than other two groups. At the end of colostrum feeding period, plasma α -tocopherol concentration in calves increased in group T_2 which might be due to consumption of vitamin E rich colostrum from vitamin E supplemented cows (Pavlata et al. 2004; Kincaid et al. 2004; Sikka and Lal, 2006)

Antioxidant status of Calves

The activity of FRAP and erythrocytic antioxidants like SOD and GPx was similar ($P>0.05$) in different groups on day 1 and 5. The activity of ALP was also not affected by treatments (Cusack et al. 2005; Gaal et al. 2006).

Immunity status of calves

Cell mediated immune response in calves was assessed by *in vitro* lymphocyte proliferation by using ConA as mitogen. The

Table 1: Composition of colostrum in cows

Day	T_0	T_1	T_2	SEM
Fat (%)				
1	6.46	6.40	6.39	0.08
5	4.88	4.78	4.83	0.08
SNF (%)				
1	16.79	15.64	16.65	0.30
5	8.50	8.40	8.23	0.13
Total solids (%)				
1	23.25	22.04	23.04	0.30
5	13.38	13.18	13.05	0.10
Protein (%)				
1	11.08	11.33	11.24	0.12
5	4.19	4.18	3.99	0.06

stimulation index (SI) after birth averaged 0.73, 0.70 and 0.75 and increased to 0.93, 0.95 and 0.94 at the end of 5 days of colostrum feeding (Table 5). However, there was no difference in lymphocyte proliferation index of calves on both days in three respective groups. Though supplementation of Zn at different levels ranging from 15-360 ppm in calves resulted in improved lymphocyte proliferation response (Kegley et al. 2001; Nagalakshmi et al. 2009) but feeding of colostrum from Zn supplemented cows did not improve the cell mediated immunity of their calves (Table 5) because there was no increase in the Zn content of colostrum (Table 2) which could have stimulated the T-cell proliferation. Buffalo calves fed on vitamin E supplemented milk (1500 and 2000 IU/d) for 60 days resulted in improvement in cell mediated immune response (2.06 vs. 1.89) compared to control (Panda, 2006), but no such effect was seen in the present experiment in calves born to T_2 group of cows supplemented with Zn and vitamin E probably because the experiment was restricted to only 5 days and moreover the amount of Zn and vitamin E had not increased at such extent to produce effect.

Table 2: The Zn, Cu, α -tocopherol and total immunoglobulin concentration in colostrum of cows

Day	T_0	T_1	T_2	SEM
Zinc (ppm)				
1	12.82	13.63	13.55	0.30
5	10.00	10.49	10.92	0.28
Copper (ppm)				
1	0.62	0.60	0.63	0.02
5	0.49	0.53	0.51	0.03
α -tocopherol (μ g/mL)				
1	4.68 ^a	4.87 ^a	5.52 ^b	0.11
5	3.29 ^a	3.38 ^a	4.09 ^b	0.10
α -tocopherol (μ g/g) of fat				
1	72.94 ^a	76.37 ^a	86.97 ^b	2.29
5	67.80 ^a	71.20 ^a	85.18 ^b	2.34
Total Immunoglobulin (mg/mL)				
1	23.38 ^a	27.18 ^{ab}	29.43 ^b	1.07
5	3.93	3.77	3.89	0.51

^{a,b} Means having different superscripts within a row differ significantly ($P<0.05$).

The total Ig concentration at birth of calves was not affected by dietary treatments (Table 5). At the end of colostrum period, the total Ig concentration was higher ($P<0.05$) in group T_1 and T_2 calves than control group. Sikka et al. (2002) also observed that calves born from vitamin E supplemented buffaloes showed higher absorbed Ig levels (51 vs. 30 mg/10 mL) in blood serum up to 45 days after birth. In a similar study, secretion of immunoglobulins in colostrum was enhanced by 80% in fat soluble vitamins injected buffaloes (Sikka and Lal, 2006).

Total and differential leukocyte count of calves

There was no difference in the TLC count at birth and at day 5 in T_0 , T_1 and T_2 groups of calves (Table 6). The TLC and DLC values

in the study were in normal physiological range (Mishra et al. 2005; Kapale et al. 2008).

Birth weight of calves

The birth weight of calves averaged 27.0, 23.0 and 30.2 kg in three respective groups (Table 7). Higher ($P<0.05$) birth weight was observed in group T_2 compared to other groups. Supplementation of two doses of fat soluble vitamins (vitamin A and D_3 @ 2,500,000 IU and vitamin E @ 1000 IU) in advance pregnant buffaloes during 30 days prepartum resulted in higher birth weight (29.0 vs. 27.5 kg) of calves (Sikka et al. 2002). Injection of either 1000 IU vitamin E and 10 mg Se or 2000 IU vitamin E and 20 mg Se at 4th and 2nd week prepartum to heifers resulted in

Table 3. Plasma Zn, Cu and α -tocopherol status of calves

Day	T_0	T_1	T_2	SEM
		Zinc (ppm)		
1	0.79	0.83	0.81	0.027
5	1.00	0.99	1.02	0.029
		Copper (ppm)		
1	0.61	0.58	0.59	0.026
5	0.70	0.71	0.69	0.030
		α -tocopherol (μ g/mL)		
1	0.41	0.38	0.47	0.033
5	0.52 ^a	0.59 ^a	0.88 ^b	0.058

^{a,b} Means having different superscripts within a row differ significantly ($P<0.05$).

Table 4. Antioxidant status of calves

Days	T_0	T_1	T_2	SEM
		FRAP (μ mol/L)		
01	2614.3	2571.4	2652.3	45.73
05	2847.6	2909.5	2957.1	46.47
		SOD (Units/g Hb)		
01	1848.2	1928.2	1804.1	44.11
05	1943.1	2129.0	2034.4	52.06
		GPx (Units/g Hb)		
01	119.87	121.65	123.39	1.34
05	122.39	122.27	125.18	0.90
		ALP (U/L)		
01	67.34	70.45	72.93	2.45
05	40.14	39.70	37.58	1.35

Table 5: Immunity status of calves

Days	T_0	T_1	T_2	SEM
		Lymphocyte Stimulation Index		
1	0.73	0.70	0.75	0.020
5	0.93	0.95	0.94	0.016
		Total Ig (mg/mL)		
1	7.20	8.40	7.86	0.40
5	18.95 ^a	22.08 ^b	21.59 ^b	0.56

^{a,b} Means having different superscripts within a row differ significantly ($P<0.05$).

Table 6. Total and differential leukocyte count of calves

Days	T ₀	T ₁	T ₂	SEM
		Total leukocyte Count (10 ³ cumm)		
1	7.81	8.02	7.77	0.18
5	7.62	7.91	7.69	0.14
		Neutrophils (%)		
1	22.40	22.20	23.60	0.77
5	22.20	21.80	22.40	0.62
		Lymphocytes (%)		
1	73.60	74.20	73.40	0.84
5	75.00	75.00	74.80	0.52
		Monocytes (%)		
1	2.00	1.40	1.20	0.23
5	1.20	1.40	1.00	0.22
		Eosinophils (%)		
1	1.60	1.80	1.40	0.34
5	1.20	1.40	1.20	0.26
		Basophils (%)		
1	0.40	0.40	0.40	0.13
5	0.40	0.40	0.60	0.13

Table 7: Birth weight, morbidity and mortality rate of calves in different groups

	T ₀	T ₁	T ₂	SEM
	Body weights (kg)			
Birth weight (kg)	27.0 ^{ab}	23.0 ^a	30.2 ^b	1.40
	Morbidity and mortality			
Morbidity (%)	50.0	37.5	25.0	
Mortality (%)	37.5	25.0	25.0	

^{a,b} Means having different superscripts within a row differ significantly (P<0.05).

tendency towards higher birth weight (41.10 and 42.03 vs. 39.84 kg) and ADG (516 and 529 vs. 512 g) of their calves compared to unsupplemented control (Moeini et al. 2008). Calves fed on milk from vitamin E supplemented buffaloes showed higher average body weights (47.10 vs. 43.23 kg) between 2-8 weeks of age (Panda et al. 2006). The higher birth weight of calves in the vitamin E and Zn supplemented group might be due to effect on growth and immunity status of calves.

Morbidity and mortality rate

The morbidity (due to pneumonia and calf scour) recorded in different groups of calves was 50.0, 37.5 and 25.0% (Table 7) which indicated that, the calves born from cows supplemented combination of Zn and vitamin E were in better immune status than other two groups of calves. The mortality of calves recorded in groups T₀, T₁ and T₂ was 37.5, 25.0 and 25.0%, respectively. The role of vitamin E in bovine neonatal morbidity has been established through relationships between vitamin E status and higher morbidity. It has been observed that there has been strong relationship between neonatal morbidity and vitamin E status (Panousis et al. 2001 Mee 2014). Improved humoral or cellular immunity have been reported after supplementation of vitamin E

and Zn (Gaal et al. 2006; Moeini et al. 2008). Thus, supplementation of both Zn and its combination with vitamin E to cows were responsible for better immune response and performance of calves.

Conclusion

The supplementation of combination of Zn and vitamin E from 60 days prepartum up to 90 days postpartum produced more colostral immunoglobulins higher plasma α -tocopherol level and higher birth weight, lower the incidence of calf morbidity and mortality. Hence, supplementation of Zn @ 80 ppm along with vitamin E @ 1000 IU/d from 60 days drying off period to early lactation of 90 days proved beneficial for cows and calf growth performance. However more work need to be required to different levels, duration of supplementation as well as on the combination required to study the effect on growth and health status of calves.

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Effect of supplementation of phyto-pharmaceutical product on the health and productivity of crossbred cows during transition period

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Abstract: The present study was conducted to investigate the efficacy of supplementation of phyto-pharmaceutical product on the immune status of cows during transition period. Twelve crossbred cows in their late gestation were selected and randomly divided into two groups as control group (CON) and supplemented group (SG). The cows in the CON were fed as per the NRC standards while SG were supplemented additionally with 20g of poly-herbal formulation containing *Boswellia serrata* (Burseraceae) and 20g of *Berginia ciliata* (Saxifragaceae) for 21 days prepartum to 7 days postpartum. Immune status viz., total leucocyte count (TLC), neutrophil percentage (%), lymphocyte percentage (%), neutrophil: lymphocyte ratio (N:L), *in-vitro* phagocytic activity (PA) of blood neutrophils, plasma cortisol level, interleukin-2 (IL-2) and interleukin-8 (IL-8) were estimated as well as effects were studied on milk for somatic cell count (SCC) and its constituents (fat, protein and lactose) too. Results showed that supplementation mixture significantly ($P<0.05$) reduced cortisol levels, IL-2, IL-8, TLC, neutrophil % and neutrophil: lymphocyte ratio whereas lymphocyte % increased. PA also increased significantly ($P<0.05$) on day of parturition. Milk SCC levels were higher ($P<0.05$) and milk lactose was lower ($P<0.05$) in the CON as compared to the SG. It was inferred that feeding of the phyto-pharmaceutical product effectively reduces stress and enhance immunity during transition period.

Keywords: Crossbred cows, Poly-herbal formulation, immune status, Phagocytic activity, Transition period

Introduction

The metabolism of cow shifts from pregnancy to lactation during transition period. Reactive oxygen species (ROS) results due to the physiological and biochemical reactions because of this shift (Sordillo, 2009). Immune cells are most sensitive to ROS production, because their membranes contain higher concentration of poly-unsaturated fatty acids which are very susceptible to peroxidation. During the periparturient period animals are more prone to the infections and diseases due to stress and several physiological changes that have been well treated by antibiotics. Although antibiotic use have been an essential part of disease control but it adversely affected the stimulation of growth and influenced the prevalence of resistance in animal bacteria (Newman, 2002). In evidence of high disease incidences, development of antibiotic resistance, drug residue in milk and heavy economic losses worldwide. Herbal feed additives are a very good alternative to antibiotics and they act by affecting the feeding pattern or effect the growth of microorganisms in the rumen, or stimulate the secretion of different digestive enzymes, which in turn may improve the efficiency of nutrients utilization or stimulate the milk secreting tissue in the mammary glands, resulting in improved productive and reproductive performance of dairy animals. The benefit of using herb is its affordability, ready accessibility and safety for health. Phyto-therapy is a traditional remedy for different diseases where plants mainly herbs and their products are used. Secondary metabolism in plants led to the production of an extensive array of organic compounds and owing to their chemical structure these compounds are beneficial nutritively for animals. In ruminant nutrition the so called phytonutrients or phyto-compounds which are derived from plants are bioactive in nature and have wide range of antimicrobial activities against several pathogens and proves to be efficient rumen modifiers (Oh et al. 2017). Their mechanism of action involves the binding to particular receptor present on a intestine, neuron and other cells which led to various physiological changes in non-ruminants viz.; immune responses, oxidative stress, and insulin secretion and activity. Certain phyto-compound follows the similar mode of action as in non-ruminant species. They are more resistant to microbial degradation in the rumen due to their phenolic makeup and may express post ruminal activities. However to the best of author's knowledge the

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information on beneficial effect of phyto-compounds on health and productivity during transition period in crossbred cows is negligible. Thus, it is the aim of present study to investigate the effects of phyto-pharmaceutical product on health and productivity in transition cows.

Materials and Methods

A total of twelve cross-bred cows (Karan-Fries; Holstein-Friesian × Tharparkar) in their advanced gestation were selected at 30 days pre-partum from the Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana. Institute Animal Ethic Committee (IAEC) duly permitted the experimental protocol. These cows were randomly allotted to SG and CON with six cows in each group on the basis of parity (2-5) and 305 day milk yield (4523.36 ± 248.84) kg. Both groups were fed as per NRC (2001). CON group was fed according to their nutritional requirements as per NRC standards while SG was supplemented additionally with 20g of poly-herbal formulation containing *Boswellia serrata* (Burseraceae) and 20g of *Berginia ciliata* (Saxifragaceae) for 21 days pre-partum to up to 21 days postpartum. The poly-herbal powder was prepared by CSIR-Indian Institute of Integrative Medicine, Jammu under the Department of Biotechnology approved research project entitled “Development of Phyto-pharmaceutical product for Bovine Mastitis”. This product was tested in Jammu on immunosuppressant mice and then handed over to ICAR-NDRI, Karnal for testing on crossbred cows around the immunosuppressed period of parturition. To analyse the health status of cows blood samples were collected by puncture in jugular vein in the heparinised vacutainer tubes at -21, -14, -7, -3, 0, 3, 7, 14 and 21 days of parturition. TLC was estimated in fresh blood by haemocytometer. Mehrzad et al. (2004) method was followed to estimate the blood neutrophils in plasma samples. At -20°C plasma samples were stored for further study. Concentrations of viable neutrophils concentration were adjusted to 5.0×10^6 viable neutrophils/ml of PBS. Modified colorimetric nitro blue tetrazolium (NBT) reduction assay method was used to determine the PA of blood neutrophils (Chai et al. 2005). Blood plasma cortisol, IL-2 and IL-8 were estimated by bovine ELISA test kit (Endocrine Technologies, USA). The milk constituents such as fat, protein and lactose were analysed by Automatic Lactoscan milk analyser. Milk somatic cells were estimated by Lactoscan milk SCC counter (Milkotronic Ltd. Stara Zagora, Bulgaria).

Statistical analysis

Data were analysed statistically by two-way ANOVA with interaction to compare the effect of supplementation as well as week wise variation during transition period. Data are presented as Mean $\pm$ S.E. The analysis was considered significant at P values less than 0.05.

Results and Discussion

The present experiment was conducted to determine the effect of phyto-pharmaceutical product on health and production of dairy cows. Results of this study are presented in Table 1. The mean total leukocyte counts (TLC) value between SG and CON up to 14 days pre-partum did not differ significantly ($P < 0.05$), but from 7 day pre-partum to day of parturition mean TLC value decreased significantly ($P < 0.05$) in SG. At 7 day postpartum TLC decreased in SG, whereas, from 14 day postpartum and onward mean TLC value was significantly ($P < 0.05$) lower in SG.

The mean TLC values at 21st day prepartum were about 7.6 to 7.9 m/mm^3 , respectively in the blood of CON and SG group of cows. A significant ($P < 0.05$) increase was observed in the neutrophils % on the day of parturition as compared to 7th day pre-partum in both the groups as shown in Table 1. On comparison between the two groups, neutrophils increased significantly ($P < 0.05$) in the CON as compared to the SG. Neutrophils remained higher in the CON during the postpartum days, whereas it decreased in SG during the postpartum days. Blood lymphocyte percent ranged from 67.5% to 68.25% in both the CON and SG of cows, respectively (Table 1). Although, lymphocytes decreased significantly ($P < 0.05$) at the time of parturition in both groups, it was lower ($P < 0.05$) in the CON as compared to the SG. Percent (%) lymphocytes remained lower in the CON throughout the course of experimentation as compared to the SG cows. Comparison of N: L values has been presented in Table 1. N: L ratio was lower at 21st day pre-partum but increased ($P < 0.05$) on the day of parturition in both the group of cows. Moreover, it was seen that the N: L ratio increased ($P < 0.05$) in the CON on the day of parturition. Both the group of cows then showed a decline in N: L ratio postpartum. In Table 1, the result of PA has been presented. There was no significant ($P < 0.05$) difference at 28 days pre-partum in both the groups. PA of neutrophils further decreased significantly around parturition ($P < 0.05$) in both the groups. There was significantly ($P < 0.05$) more PA at 14 day postpartum in SG as compared to CON. This trend of increased PA was continued till 28 days postpartum. In Table 1, it has been shown that IL-2 and IL-8 concentrations were significantly ($P < 0.05$) higher in CON on the day of parturition and during postpartum days as compared to SG. The result pertaining to cortisol levels has been depicted in Table 1. Cortisol level was increased significantly ($P < 0.05$) in both the groups as cows approached to parturition then decreased and reached to basal level at 7 weeks postpartum. On the day of parturition cortisol level was significantly ($P < 0.05$) less in SG as compared to CON. Table 2 depicted the change in the percentage of milk fat which did not follow specific pattern and fluctuated during different days of postpartum period. It was numerically higher in the CON as compared to SG. However, it showed higher ($P < 0.05$) values at day 14 and 42 postpartum in the CON as compared to the SG. In Table 2, milk protein % was significantly ($P < 0.05$) higher in the CON at the first three weeks postpartum, it was lower around

Table 1: Effect of feeding phyto-pharmaceutical product during transition period on the blood cell counts, phagocytic activity, plasma cortisol and cytokine levels of crossbred cows (Mean±S.E.)*

Parameters	Group	Days peri-partum						
		-21 day	-14 day	-7 day	0 day	7 day	14 day	21 day
TLC (m/mm ³)	Control	7.98 ^a ±0.16	8.48 ^{ab} ±0.44	8.80 ^a ±0.37	9.56 ^c ±0.30	9.74 ^a ±0.85	8.12 ^a ±0.47	8.06 ^a ±0.39
	Supplemented	7.60 ^a ±0.36	8.00 ^{ab} ±0.36	8.16 ^a ±0.35	8.45 ^b ±0.28	7.91 ^a ±0.42	7.71 ^a ±1.29	7.70 ^a ±1.12
Neutrophil (%)	Control	28.75 ^a ±0.25	31.53 ^b ±0.26	32.24 ^b ±0.44	39.97 ^d ±0.55	34.52 ^c ±0.86	32.21 ^b ±0.63	29.12 ^a ±0.28
	Supplemented	28.75 ^a ±0.25	30.17 ^a ±0.27	30.92 ^a ±0.30	34.25 ^b ±0.20	31.79 ^a ±0.36	30.65 ^a ±0.36	26.76 ^a ±0.42
Lymphocyte (%)	Control	67.51 ^a ±0.53	64.91 ^b ±0.65	62.73 ^b ±0.63	54.15 ^a ±0.69	60.15 ^b ±0.52	61.87 ^b ±0.64	66.83 ^c ±0.87
	Supplemented	68.25 ^a ±0.49	66.14 ^a ±0.50	64.86 ^a ±0.48	59.77 ^a ±0.62	63.12 ^b ±0.36	64.53 ^b ±0.45	69.25 ^d ±0.35
NEL	Control	0.42 ^a ±0.01	0.48 ^a ±0.01	0.51 ^b ±0.01	0.73 ^c ±0.02	0.55 ^b ±0.02	0.52 ^b ±0.01	0.43 ^a ±0.01
	Supplemented	0.41 ^a ±0.01	0.45 ^b ±0.01	0.47 ^b ±0.01	0.57 ^c ±0.01	0.50 ^b ±0.01	0.47 ^b ±0.01	0.38 ^a ±0.01
P.A. (OD at 450nm)	Control	0.24 ^a ±0.01	0.23 ^a ±0.02	0.19 ^a ±0.01	0.17 ^{ab} ±0.01	0.16 ^a ±0.01	0.19 ^b ±0.03	0.17 ^{ab} ±0.01
	Supplemented	0.23 ^a ±0.01	0.22 ^a ±0.01	0.19 ^a ±0.01	0.19 ^{ab} ±0.01	0.17 ^a ±0.01	0.25 ^c ±0.01	0.25 ^c ±0.01
IL-2 (pg/ml)	Control	301.75 ^a ±27.52	354.25 ^b ±24.97	374 ^b ±45.18	437.75 ^c ±10.17	389 ^b ±11.27	352.50 ^b ±21.12	311.75 ^a ±11.92
	Supplemented	305.25 ^a ±19.35	372 ^{bc} ±36.82	394.5 ^b ±27.02	407 ^b ±11.07	353.25 ^b ±14.57	301.00 ^a ±12.96	270.75 ^a ±13.09
IL-8 (pg/ml)	Control	459.5 ^a ±33.96	468 ^a ±17.73	483.5 ^a ±36.71	527.25 ^b ±23.08	536 ^b ±8.25	472.75 ^a ±16.70	454.75 ^a ±11.95
	Supplemented	451 ^a ±14.39	443 ^a ±4.91	448.25 ^a ±20.08	503.25 ^b ±23.08	453.75 ^b ±22.40	450.25 ^b ±32.32	426.75 ^b ±35.16
Cortisol (ng/ml)	Control	3.19 ^{ab} ±0.21	3.37 ^b ±0.31	4.65 ^a ±0.29	8.58 ^c ±0.35	6.18 ^{ab} ±0.57	3.83 ^b ±0.57	2.71 ^a ±0.39
	Supplemented	3.04 ^{ab} ±0.37	3.22 ^b ±0.14	5.08 ^a ±0.21	6.99 ^c ±0.53	4.38 ^c ±0.35	2.93 ^{ab} ±0.41	2.46 ^a ±0.23

^{abc} superscripts within a row are significantly different ($p < 0.05$) among the days within the group; asterisk (*) within the column indicates the significant difference ($p < 0.05$) within same day between the groups.

peak lactation as compared to the SG. The milk lactose was always higher in the SG as compared to CON and showed significant ($P < 0.05$) difference in the postpartum period. In Table 3, the milk SCC was lower ($P < 0.05$) in the SG as compared to the CON. Results of SCC indicated that mammary health status of animals in SG were better and significantly ($P < 0.05$) lower as compared to CON.

An increase in TLC around parturition is mediated by pre-partum rise in cortisol levels (Hussain and Daniel, 1992). It substantiates the fact that stress in different species leads to increase in TLC (Kumari et al. 2018; Mc-Glone et al. 1993). Prior to late gestation high concentrations of cortisol have been reported that facilitate parturition, thereby producing immunosuppressive and anti-inflammatory properties in cows (Nagel et al. 2019; Mordak and Anthony, 2015; Dang et al. 2013). Positive effect of supplementation of mixture in lowering plasma cortisol values was also observed in 28 days postpartum. Phytochemicals have excellent (%) DPPH radical scavenging activity (Al-Rehaily et al. 2002; Mishra et al. 2005; Riddhi and Yogesh, 2012); which is helpful in reducing stress. The previous studies (Kimura et al. 1999) indicated that peak neutrophil numbers are observed at parturition, but level declines shortly after and reach basal conditions within 2 weeks. The results of this study followed similar patterns. Around parturition increased neutrophil count was physiologically associated with high peri-parturient blood concentrations of glucocorticoids (Lee and Kehrl, 1998). PA is reduced during parturition as cortisol binds to the receptors of the neutrophils (Burton et al. 2005). Intracellular rise of reactive oxidative species (ROS) occurs during phagocytosis process that mediates inflammation, however, adversely affects the cell and surrounding tissue (Sharma et al. 2014). Due to this intracellular rise of reactive oxidative species (ROS), PA is potentially reduced owing to its low antioxidant status. Most of the fat soluble antioxidant vitamins such as retinol, α -tocopherol and β -carotene concentration decreased at the time of parturition leading to depression in PA (Weiss, 1998). Cellular and humoral immune function is improved by these vitamins due to chain breaking lipid soluble tissue antioxidant properties (Halliwell, 1987). Chatterjee (1994) observed increased microbicidal activity of neutrophils and elevated antibody titre in both primary and secondary immunity assay at the dose rate of 20mg/kg body weight herbal preparation (Immu-21) containing *Ocimum sanctum*, *Embelia officinalis*, *Withania somnifera*, *Tinospora cordifolia* on immunological properties in rats. The present study reported low levels of cytokine expression in SG on day of parturition as compared to CON which indicates the immunomodulatory properties of poly-herbal product. Tian et al. (2015) reported that the cytokine production, the rise in the surface receptors for other molecules or the inhibition of their own effect by feedback inhibition results due to the up and down regulation of various genes and their transcription factors due to the cytokine interaction with its cell surface receptors. Cellular immune system acts through

Table 2: Effect of feeding phyto-pharmaceutical product during transition period on the milk composition of crossbred cows (Mean±S.E.)*

Parameters	Group	Days of lactation								
		0 day	7 day	14 day	21 day	28 day	35 day	42 day	49 day	56 day
Fat (%)	Control	4.40 ^{ab} ±0.07	4.68 ^{ab} ±0.47	4.43 ^{ab} ±0.15	4.46 ^{ab} ±0.18	4.13±0.18	4.94 ^b ±0.84	4.84 ^b ±0.47	4.52 ^{ab} ±0.26	4.19±0.32
	Supplemented	4.44 ^b ±0.23	4.36 ^{ab} ±0.16	4.13±0.04	4.38 ^{ab} ±0.19	4.14±0.06	4.17 ^a ±0.07	4.18±0.05	4.33 ^{ab} ±0.15	4.00±0.04
Protein (%)	Control	3.68 ^b ±0.26	3.93 ^a ±0.14	3.80 ^{cd} ±0.18	3.58 ^{bc} ±0.26	3.47 ^{bc} ±0.33	3.44 ^b ±0.29	3.42 ^b ±0.13	3.20 ^a ±0.04	3.27 ^{ab} ±0.10
	Supplemented	3.53 ^{ab} ±0.24	3.45 ^{ab} ±0.16	3.61 ^b ±0.19	3.56 ^{ab} ±0.17	3.51 ^{ab} ±0.13	3.52 ^{ab} ±0.13	3.68 ^b ±0.10	3.48 ^{ab} ±0.10	3.43±0.08
Lactose (%)	Control	4.51±0.27	4.45±0.32	4.29 [±] 0.17	4.33±0.41	4.20 [±] 0.22	4.12 [±] 0.14	4.18±0.04	4.42±0.12	4.32±0.12
	Supplemented	4.75 ^{bc} ±0.13	4.66 ^b ±0.05	4.80 [±] 0.08	4.69 [±] 0.21	4.69 [±] 0.11	4.51 ^b ±0.14	4.28±0.19	4.62 ^b ±0.20	4.56 ^b ±0.13

^{abc} superscripts within a row are significantly different ($p < 0.05$) among the days within the group; asterisk (*) within the column indicates the significant difference ($p < 0.05$) within same day between the groups.

Table 3: Effect of feeding phyto-pharmaceutical product during transition period on the somatic cell counts $\times 10^5$ /mln colostrum and milk of crossbred cows (Mean±S.E.)*

Parameter	Group	Days of lactatio								
		0 day	7 day	14 day	21 day	28 day	35 day	42 day	49 day	56 day
Somatic cell counts $\times 10^5$ /m	Control	5.07 ^c $\pm$ 0.12	2.92 ^b $\pm$ 0.13	2.45 ^a $\pm$ 0.32	2.38 ^a $\pm$ 0.28	2.35 ^a $\pm$ 0.17	2.42 ^a $\pm$ 0.2	2.45 ^a $\pm$ 0.25	2.55 ^a $\pm$ 0.18	2.65 ^a $\pm$ 0.13
	Supplemented	4.55 ^a $\pm$ 0.14	2.29 ^b $\pm$ 0.2	2.25 ^b $\pm$ 0.13	2.20 ^b $\pm$ 0.15	1.98 ^{ab} $\pm$ 0.11	1.85 ^a $\pm$ 0.19	1.88 ^{ab} $\pm$ 0.22	2.04 ^{ab} $\pm$ 0.13	2.22 ^b $\pm$ 0.15

^{abc} superscripts within a row are significantly different ($p < 0.05$) among the days within the group; asterisk (*) within the column indicates the significant difference ($p < 0.05$) within same day between the groups.

their inflammatory cytokines which are multi-potential mediators and exhibits various biological activities. Barak (1995) reported that very low or high concentrations of these cytokines may lead to unfavourable effects. The duration of the immune response creates a balance between the production of inflammatory and anti-inflammatory cytokines (Bhatt et al. 2014). Kholef and Khorshed (2006) studied milk composition analysis and showed that milk protein was higher ($P < 0.05$) in animals fed experimental additives than the control. In other studies, milk fat, protein and lactose contents were not affected by poly-herbal supplementation ration fed lactating goats (Campanile et al. 2008; Erasmus et al. 2005). This study suggested the anti-inflammatory effects of the supplementation. The study also reported lower SCC in SG as compared to CON. Bhatt et al. (2014) studied that supplementation resulted in low bacterial count which may be indicative of the enhanced phagocytosis in the tissues due to the cytokines in SG. Hillerton (1999) studied that somatic cell was closely related with inflammation, udder health and milk quality as well as it reflects the herd health status. Also Chandra et al. (2016) reported that poly-herbal mixture and butyric acid supplementation during transition period has beneficial effect in improving udder health status in the supplemented group. This study indicated that inflammatory cytokine expression and function of neutrophil was regulated by butyrate especially in the presence of inflammatory stimuli.

Conclusion

Thus, feeding of extracts of *Berginia ciliate* and *Boswellia serrata* during the peri-partum period may reduce postpartum stress and supplements boost the activity of immune cells around the parturition period of high milk producing cows.

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RESEARCH ARTICLE

Socio-economic profile and constraints of farmers rearing Haryana cattle

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Abstract: The study was conducted in Hisar, Bhiwani, Rohtak and Jhajjar districts of Haryana state in India to assess the socioeconomic profile, technology adoption and constraints of the farmers rearing Haryana cattle in its breeding tract. A total of 240 respondents (60 respondents from each district) were purposively selected and interviewed individually. 84.58 % of the farmers belong to joint family. Occupation of majority of the respondents was agriculture (53.33 %) along with dairying. Most of the farmers were small (27.50 %), marginal (28.34 %) and landless (29.58 %) with an average land holding of 2.6 acres. 50 % of them belong to medium (40000-150000 lakhs) annual family income group. The average herd size and lactation yield of Haryana cattle in study area was 1.56 and 1014.21 L respectively. Majority of the farmers have adopted all the technologies only few of them have not adopted due to lack of knowledge. The major constraints in Haryana cattle rearing is low productivity compared to Murrah buffalo and CB cattle. The other prevailing constraints of farmers in the study areas were aggressive behaviour of Haryana cattle, sale of male calves, reduction in grazing land, lack of timely veterinary and AI services, dilution of breed arise from indiscriminate breeding and scarcity of feed and fodder.

Keywords: Constraints, Dairying, Feeding, Haryana cattle, Socio-economic, Technology

Introduction

The milk produced from cattle and buffalo is the largest agricultural commodity and plays a major role in Indian economy (Panchbhai et al. 2017). In order to achieve progress, dairy farmers should be modernized in knowledge, adoption and their personal, social and economic characteristics should be improved. Among the livestock farming systems, dairy farming plays significant role in sustaining the rural livelihoods (Shinde, 2011) by reducing the longstanding problems of unemployment and underemployment (Rachna et al. 2017). Adoption of technical recommendations at the farm level is dependent on the social, cultural, economic (Gopi et al. 2017) and environmental conditions facing by the farmer who own the animals (Solano et al. 2000; Ayenew et al. 2011).

India is having a wide reservoir of genetic diversity and possesses some of the best breeds of cattle and buffaloes in the world. Indigenous cattle breed have evolved over generations to adapt to local agro-climatic and socioeconomic need of people (Singh et al. 2021). Indigenous cattle, are robust, resilient and are suited to the climate and environment of their respective breeding tracts, and the ability to thrive under extreme climatic stress and sub-optimal nutrition (Singh et al. 2019). Each breed is known for its own characteristic features and utility, whether specific for milk production, draught power or both (Kumar et al. 2019).

Haryana has the pride of being the place of origin for famous breeds of Haryana Cows and Murrah buffaloes (Raj and Gupta, 2015). Haryana or Haryana cattle's home tract is in Haryana state but the breed is distributed in Uttar Pradesh, Bihar and parts of Rajasthan. Cows are good milkers and bullocks are useful for ploughing and transport. The milk yield of from 809 to 1,731 kg and bullocks are good for agricultural operations (Mishra et al. 1980; Upadhyay and Madan 1985; Kumar et al. 2019). The indigenous cattle rearing is declining due to low productivity, lesser income and reduced usage of draught power by the farmers (Raj and Gupta, 2015). The total estimated number of Haryana cattle during 2013 was 16,39,181 (Report, 2013). The quinquennial livestock census (2007, 2012 and 2019) have revealed a declining trend of cattle population in Haryana state (20th Livestock census, 2019). Therefore it is appropriate to assess the relevance of rearing

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native dairy animals in rural livelihood and to understand opportunities or challenges faced by smallholder dairy farmers. However, information on the type and level of socio-economic factors affecting dairy production systems is limited (Ayenew et al. 2011) especially for Haryana cattle. Therefore, this study was conducted with an objective to assess the socio-economic characteristics of the dairy production systems and to suggest appropriate intervention for Haryana cattle rearing systems in its breeding tract.

Materials and Methods

Study area and animals

The native breeding tract of Haryana cattle lies between 28°30' and 30° North latitude and 75° 45' and 76° 80' East longitude. The native breeding tract of Haryana cattle encompasses large part of Rohtak, Hisar and Gurgaon districts of Haryana state. Purebred Haryana cattle were abundant in Jhajjar, Beri and Jahajgarh locations of Rohtak district and the region was a leading trading centre particularly for Haryana bullocks. The home tract of this breed is the areas covered by the districts of Rohtak, Hisar, Gurgaon and part of Karnal in the Haryana State, and the Union Territory of Delhi.

Multistage stratified sampling procedure was used to select the districts, villages and respondents. Four districts (Rohtak, Jhajjar, Hisar and Bhiwani) were selected purposively and three tehsils were selected randomly from each identified district. From each selected tehsil, two villages were selected randomly. A total of 24 villages were selected for undertaking the study in the native haryana cattle breeding tract.

Distribution/classification of the respondents

A structured questionnaire was prepared and was divided into two sections. The first section was on socio economic condition of the Haryana cattle rearing farmers including age, gender, family size and education. Household and cattle rearing related characteristics included family labor availability, total acres of land holding, occupation, cows in production, daily milk output, family income and employment generation.

Section two was focused on the constraints of Haryana cattle rearing and were ranked based on the perception of the farmers. After the selection of the villages, a preliminary survey was conducted in the selected villages to know the total number of farmers practicing dairy farming.

Among these selected villages, 10 dairy farmers were selected by proportionate random sampling from each village by considering the herd size of the animals. Only those farmers were selected who were owning atleast one Haryana cattle. Thus, the total respondents of the study was 240 dairy farmers including 141 males and 99 female respondents (for management practices,

performance and economic viability). All farmers agreed to answer for the questionnaire and gave their consent prior to data collection during October and December 2017. Interview schedule was conducted at the convenience of dairy farmer either at his or her home or at farmstead.

Statistical analysis

The data collected from the dairy farmers were scored, tabulated and analyzed using descriptive statistics. The data collected was analysed using simple statistical tools such as averages, frequencies and percentage.

Results and Discussion

Socio economic profile of farmers

The results obtained indicated that majority of respondents were in middle age category, i.e. 35-50 years, followed by young age category and rest belonged to old age category (Table 1). It was observed that minimum age was 20 years and highest age was 75 years. Similarly, Gautam et al. (2007) observed that 73.30 % of dairy farmers were middle aged and 15 % and 11.70 % were in each old and young age group with a mean age of about 43 years among the dairy farmers in Haryana. The dairy farming activities are held largely by middle age group.

It is evident from the Table 1 that, majority (58.75 %) of the respondents are male and rest (41.25) were female. Similarly, Gabalebatse et al. (2013) and Singh (2012) also reported that majority of the respondents were male in the study area.

The study indicated that majority (84.58%) of the respondents belonged to joint family (>4 members) followed by, 15.42 % that belonged to nuclear (< or = 4 members) (Table 1). This might be due to the fact that, most of the respondents belonged to joint family in the study area reflecting the existence of joint family socio-cultural system in the rural areas of Haryana state. The results of the study are in agreement with Gour et al. (2015). They reported that majority of the respondents belonged to medium family size.

The district wise education level of farmers has been depicted in Table 1 and it is clear from the that 38.33 % of the respondents were educated up to secondary and senior secondary level, 20.42 % who had upper primary level of education, 16.25 % illiterate, 15.00 % up to primary school and 10.00 % graduation and above, respectively Muriithi et al. (2014) reported that most of dairy farmers (60.4 %) had above secondary education and further 2.3 % had gone up to university level education. Whereas, Gopi et al. (2017) found that nearly half (45.00 %) of the respondents were illiterate followed by high school education (19.17 %), primary school and middle school education (each 13.33 %), can read and write (5.00 %) and can read only (2.50 %). A negligible 1.67 % of the respondents had collegiate education in study area of Tamil Nadu.

Whereas Rachna et al. (2017) have reported that majority of the dairy farmers had high level of education in Hisar region of Haryana.

It could be observed from the table 1 that a majority (53.33%) of the respondents had agriculture and dairy as their primary occupation followed by agricultural labour (27.08%). The respondents raised cattle as a secondary source of income in

Table 1: Socio-economic characteristics of farmers rearing Haryana cattle

Parameters		Districts				Total (n=240)
		Bhiwani (n=60)	Hisar (n=60)	Rohtak (n=60)	Jhajhar (n=60)	
Age (%)	Young (<35)	43.33 (26)	36.67 (22)	35.00 (21)	25.00 (15)	35.00 (84)
	Middle (35-50)	41.67 (25)	48.33 (29)	51.67 (31)	55.00 (33)	49.17 (118)
	Old (>50)	15 (9)	15 (9)	13.33 (8)	20.00 (12)	15.83 (38)
Gender (%)	Male	65.00 (39)	68.33 (41)	48.33 (29)	53.33 (32)	58.75 (141)
	Female	35.00 (21)	31.67 (19)	49.67 (31)	46.67 (28)	41.25 (99)
Family size (%)	< 4 and = 4	11.66 (7)	20.00 (12)	15.00 (9)	15.00 (9)	15.42 (37)
	>4	53 (8)	80.00 (48)	85.00 (51)	85.00 (51)	84.58 (203)
Education (%)	Illiterate	25.00 (15)	18.33 (11)	10.00 (6)	11.67 (7)	16.25 (39)
	Primary	13.33 (8)	26.67 (16)	15.00 (9)	5.00 (3)	15.00 (36)
	Upper Primary	20.00 (12)	25.00 (15)	26.67 (16)	10.00 (6)	20.42 (49)
	Secondary and Senior Secondary	41.67 (25)	16.67 (10)	35.00 (21)	60.00 (36)	38.33 (92)
	Graduation and Above	0 (0)	13.33 (8)	13.33 (8)	13.33 (8)	10.00 (24)
Occupation (%)	Agricultural (Agriculture + Dairy)	48.33 (29)	56.67 (34)	55.00 (33)	53.33 (32)	53.33 (128)
	Labour	3.33 (2)	13.33 (8)	8.33 (5)	6.67 (4)	7.92 (19)
	Agricultural + Labour	41.67 (25)	16.67 (10)	25.00 (15)	25.00 (15)	27.08 (65)
	Job	6.67 (4)	13.33 (8)	11.67 (7)	15.00 (9)	11.67 (28)
Farm characteristics						
Land (%)	Landless	31.66 (19)	35.00 (21)	23.33 (14)	28.33 (17)	29.58 (71)
	Marginal	18.33 (11)	18.33 (11)	33.33 (20)	43.33 (26)	28.34 (68)
	Small	21.67 (13)	35.00 (21)	35.00 (21)	18.34 (11)	27.50 (66)
	Medium	20.00 (12)	11.66 (7)	6.67 (4)	10.00 (6)	12.08 (29)
	Large	8.33 (5)	0 (0)	1.67 (1)	0 (0)	2.50 (6)
Average land holding (acres)		3.58	2.43	2.42	2.0	2.61
Annual family income (Rs.)	< 40000	28.33 (17)	35.00 (21)	25.00 (15)	38.33 (23)	31.67 (76)
	40000-150000	48.34 (29)	45.00 (27)	56.67 (34)	50.00 (30)	50.00 (120)
	>150000	23.33 (14)	20.00 (12)	18.33 (11)	11.67 (7)	18.33 (44)
Herd size (heads)		1.65	1.7	1.35	1.55	1.56
Average Lactation Yield (L)		1043.83	1141.83	999.5	871.67	1014.21

addition to using the milk and milk products for their own consumption. Bashir and Kumar (2013) and Solanki et al. (2011) also found that most of the respondents practiced crop farming as a major occupation. Sah (2005) reported that dairying was the main occupation among majority of the respondents.

In the present study, a considerable size (29.58%) of the respondent's belonged to landless category, followed by 28.34, 27.50, 12.08 and 2.50 % of respondents belonged to marginal, small, medium and large category respectively (Table 1). Our findings are in agreement with the findings of Verma et al. (2016) who also reported that majority of respondents were small and marginal. In contrary to our study, Khode et al. (2009) also reported that most of the respondents belonged to large farmers category. Also, Muriithi et al. (2014) concluded that majority of respondents had less than 2 acres of land and 5.8 % of respondents had more than five acres of land these are finding contraindicates to above findings. Also, Panchbhai et al. (2017) reported that 35.00 % of respondents' belonged to small farmer category, 22.50 % belonged to the category of marginal and 19.50 % to the category of medium farmers. Only 19.00 % farmers belonged to large category and 4.00 % farmers were landless, respectively.

Income is a crucial variable, which influences the farmer's investment in farming activities. The income obtained from various sources viz., crop, livestock and others as reported by the respondents were considered in order to calculate the gross annual income per family. The Table 1 shows that the annual income of 50.00 % of the respondent was Rs. 40000 to Rs. 150000, followed by 31.67 % of the respondent had less than Rs 40000 annual income and 18.33 % respondent had more than Rs. 150000 annual income. It might be due to the fact that farms and livestock give more annual income for dairy farmers as compare to other sources like labor, job etc. Similar to findings of our study, Panchbhai et al. (2017) also reported that the majority of the

respondents (46.50 %) were in medium income group followed by low income (27.00 %) and high income (26.50 %), respectively.

Employment generation and technology adoption

Since milk production is a continuous process, it has greater ability of employment generation as compared to crop sector. Also, dairy farming is considered to be a tool of women empowerment since many dairy operations are performed by women. Table 2 indicates employment potential per household in dairy farming. The results indicated that in terms of hours, women are the highest contributors of labour in dairy farming operations in Haryana but in terms of man-hours, adult males are highest contributors. On an average, each households spent nearly two man-hours in dairy farming operations.

The majority of farmers (90 %) fed concentrates to Haryana cattle (Table 3). Jarial, (2006) also reported that majority of the respondents fed concentrate feed. In respect to calf management practices, all the farmers fed colostrum to the calves. Mahla et al. (2015) and also reported that majority of the respondents fed colostrum to calf after the placenta was shed. The present study revealed that most of the respondents did not have complete knowledge about good dairy farming practices including colostrum feeding to young ones.

The practices of dehorning and deworming were adopted by 24.16 % and 60 % of the farmers, respectively. Similarly to our findings, Yogendra (2010) reported that 80 % of the farmers followed deworming schedule in Haryana cattle. Some of the respondents used indigenous methods to prevent ecto-parasites. In contrary to our findings, Mahla et al. (2015) found that none of the respondents had resorted to dehorning in indigenous cattle.

Table 2. Employment generation per household from Dairy Farming in Haryana

Labour Source	Time (in hours)/ day	Time (in man-hours)/ day
Adult male	1.02	1.02
Adult female	1.07	0.80
Children	0.33	0.17
Total	2.42	1.99

Table 3. Technologies adopted by farmers rearing Haryana cattle

S.No.	Technologies	Users (%)	Non users
1	Concentrate feeding	216(90)	24
2	Colostrum feeding to calf	240(100)	0
3	Dehorning	58(24.16)	182
4	Deworming	144(60)	96
5	Vaccination	235(97.91)	05
6	Artificial insemination	72(30)	168
7	Mineral mixture feeding	24(10)	216
8	Clean milk production	48(20)	192

Table 4: Constraints on rearing of Haryana cattle faced by respondents

S. No.	Constraints of Haryana cattle rearing	N	Per cent	Rank
1	Farmers inclination towards rearing of Murrah buffalo and CB cattle owing to their better profitability	61	76.25	I
2	Specific behavioural problems such as aggressive behaviour, Allowing milking to an individual family member, Milk holding habit that results in incomplete milking.	52	65	II
3	Sale of male calves in particular and dry animals in general because of governmental ban on slaughter	50	62.5	III
4	Reduction in grazing land/ Panchayat Charagah	47	58.75	IV
5	Lack of timely veterinary and AI services	43	53.75	V
6	Dilution of breed arise from indiscriminate breeding	41	51.25	VI
7	Scarcity of fodder during summer	38	47.5	VII
8	Diversification of Agriculture	36	45	VIII
9	Non- availability of pedigree bull	35	43.75	IX
10	Short lactation length	31	38.75	X
11	Poor market value of bullocks	24	30	XI

Nearly 97.91 % (235) farmers were following the vaccination against contagious diseases which is provide by state government whereas 5 farmers did not followed vaccination schedule (Table 3.). Similar to our study, Yogendra (2010) found that most of the farmers were using the veterinary health care services for the animals in Haryana state. In contrary to our findings Kumar et al. (2014) found that most of the respondents were not able to access the animal health care services.

Majority (70 %) of the respondents preferred to natural services and remaining (30 %) adopted artificial insemination (AI) for breeding purpose of their cattle. The findings of study were in agreement with Sheikh and Parmar (2015), Kumar et al. (2014) and Yadav et al. (2009). The results of the study showed that, respondents of the study area did not prefer the AI over natural service to Haryana cattle because of their furious nature, AI cost is high, and hospitals are far away from home. In the case of natural mating, majority of cattle are being mated indiscriminately with any approachable bull, while some of the respondents took care of their cows by mating with best selected bull. In the case of heat detection practices, most of the respondents used all the indigenous techniques of heat detection viz. bellowing, restlessness, frequent urination, allowing other animal to mount and vaginal discharges. Similarly, Jarial (2006) also reported that majority of the respondent identified heat by indigenous methods. Majority (90 %) of the respondents did not add any mineral mixture in animals diet due to high cost and less awareness about it. The

lack of mineral mixture and common salt in the diet lead to various reproductive disorders like repeat breeding and anestrus.

In the case of clean milk production, only 20 % of the respondents followed washing of hands before milking because majority of the farmers were not aware about the drawbacks caused by the unhygienic milk handling which clearly indicated the lack of knowledge about the clean milk production practices at field level. Majority of the farmers were not maintaining cleanliness in their house and milking premises. All people follow washing of udder with normal water before milking. Further, most of the respondents were not aware of removal of hair from the udder and the practice of discarding the first two streams of milk from each teat. Further, after milking large majority of the respondents were not following the practice of not allowing the animal to sit soon after milking at least for twenty minutes which is very helpful in prevention of mastitis. Properly cleaned milk utensils- an important practice in hygienic milking practices is adopted by very less percentage of farmers. Instead of using separate utensils for milking, most of them were habitual in using of utensils which were commonly used in their kitchen and most of the time they used only normal water for cleaning of milking utensils.

Constraints of Haryana cattle rearing

Livestock production system particularly the rearing of Haryana cattle is facing various challenges/limitations in the surveyed

region. Few of them were of social or personal origin and their elimination to some extent was possible, while others were related to climatic and geographical factors which were largely beyond the control of the farmers. Response of several respondents have revealed that the major constraints faced by farmers during rearing of Haryana cattle as shown in Table 4. which includes farmers inclination towards rearing of Murrah buffalo and CB cattle owing to their better profitability (76.25 %), specific behavioral problems such as aggressive behavior, allowing milking to an individual family member, milk holding habit that results in incomplete milking (65 %), problem in sale of male calves in particular and dry animals in general because of governmental ban on slaughter (62.50 %), reduction in grazing land/ Panchayat Charagah (58.75 %), lack of timely veterinary and AI services (53.75 %), dilution of breed arise from indiscriminate breeding (51.25 %), scarcity of fodder during summer (47.50 %), diversification of agriculture (45 %), non-availability of pedigree bull (43.75 %), short lactation length (38.75 %) and poor market value of bullocks (30 %). Some of the constraints as discussed above were also reported earlier by Yogendra (2010). Constraints faced by farmers might be identical to the location and species of livestock. However, some of the problems are constant in our country and the same are referred as the common problems of livestock management and practices (Jangid and Rohilla, 2004).

Conclusions

It can be concluded that the farmers tend to specialize in dairy production along with agriculture which forms important source of family income. The middle aged farmers were more among the Haryana cattle rearing farmers with more number of secondary and senior secondary education. The technology adaptation can be improved by the access to services such as the transfer of technical knowledge, deworming, dehorning, mineral mixture feeding, artificial insemination, knowledge of clean milk production and other veterinary services. The productivity and the non-feasibility of rearing Haryana cattle are its aggressive behaviour, problems of rearing male calves, reduction in grazing land, scarcity of fodder, short lactation length, poor market value of bullocks are likely to hamper the rearing of this breed and need to be addressed by specific policy schemes. Therefore, this work can be helpful to design any programmes or schemes meant for improvement of Haryana cattle breed in its breeding tract.

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RESEARCH ARTICLE

Estimation of feed costs and feed efficiency in typical dairy Farms of Bangladesh during Coronavirus (Covid-19) emergency: implications toward feed support policy

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Abstract: Feed cost is the highest cost item to the total costs of dairy farming all over the world including India, Pakistan and Bangladesh. Reducing feed costs and increasing feed efficiency are two promising ways to decrease the cost of milk production and increase the competitiveness nationally and internationally (Hemme et al. 2014). Feed price in Bangladesh dairy farm is 51% higher than global feed price which has increased further due to the sudden infection of Coronavirus (Covid-19) (IDRN, 2020). Therefore, the objective of this paper is to estimate the impact of pandemic novel Coronavirus (COVID-19) prevalence on feed costs and feed efficiency in order to find a suitable feed supporting policy to the dairy farmers. The International Farm Comparison Network (IFCN) Feed Simulation Approach and Technology Impact Policy Impact Calculations (TIPICAL) model was used for estimating feed costs and feed efficiency of the dairy farm with and without Corona situation (This model is highly relevant to this study due to the fact that this model can produce results even with the scarcity of the data and it is highly scientific and produce the results that are simultaneously used both Academia (University and Research organization) and Industry (for business strategic decisions). The Integrated Dairy Research Network (IDRN) dairy sector and dairy farm database (January 2019 to March 2020) was used where 2019 is considered as without Corona (WOC) and March 2020 is considered as with Corona (WC). Two typical farms: 2cow farm (BD-2) which is representative of 42% household farm (Household Farm (HF) is defined as one the income source for livelihoods, mainly consumed at household level and sells the surplus milk. The average size of the household

farm (1-3 cows/farm) as per the International Farm Comparison Network (IFCN) methodology) and fourteen cow (BD-14) which is representative of 48% family farms in Bangladesh were selected. The share of feed cost to the total costs of milk production is increased by 9.5% and 8.9% for BD-2/19-WC and BD-14/20-WC, respectively. The purchased feed costs before corona was 22.1 USD/100 kg milk for BD-2/19-WOC and 32.2 USD/100 kg milk for BD-14/19-WOC which is increased to 23.8 USD/100 kg and 35.0 USD/100 kg for BD-2/20-WC and BD-14/20-WC, respectively (Author's own results). The purchased feed costs increased by 7.6% and 8.7% for BD-2/20-WC and BD-14/20-WC, respectively while for the homegrown feed, it is increased even higher which is 14.1% and 9.7%, respectively. The results revealed that decreasing milk price and increasing feed price has direct impact on increasing feed costs of 8.6% and decreasing margin over compound feed costs by 4.5% due to Coronavirus. As a result, an increase in feed cost, decrease in the margin over total feed cost and the feed efficiency (total, energy, and protein efficiency) was observed due to coronavirus (Covid-19) infection. The finding of this study revealed that for short-term and medium-term, farmers might need feed incentives either in the form of reduced feed price or liberalized concentrate feed input market. The outcome of this study is expected to be beneficial for policy makers, feed suppliers and farmers in Bangladesh and similar other countries like India, Pakistan, Sri Lanka and Nepal.

Keywords: Covid-19, Feed Cost, Feed efficiency, Feed Policy, South Asia

Introduction

The novel Coronavirus infection, since its first identification in Wuhan province in China during the end of December 2019, its subsequent infection globally were highly pandemic with four peak time (Covid-19 wave) with different variants, Covid-19, Delta and Omicron, in all over the world including Bangladesh. This has made tremendous challenge for general wellbeing and health risk for millions of dairy farmers in Bangladesh and lead the dairy farmers under financial stress (Uddin et al. 2020). Realizing the overall impact of Coronavirus on human health, the government of Bangladesh responded with an immediate action by taking all possible options and preventive measures for the safety of human

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health. However, there was an impact on dairy farm and its costs and profitability due to Covid-19 (Uddin et al. 2020). Within the total cost of the milk production, feed cost represents the highest cost item in milk production. Therefore, it is highly interesting and motivating to estimate the impact of the Covid-19 on the feed costs and feed efficiency in Bangladesh dairy farms.

Considering this, dairy farming activity was taken as unique as it required special attention for 24 hours in a day and 7 days in a week and it is not possible to stop suddenly. Therefore, the dairy farming, was quite challenging during Corona period not only in Bangladesh but also in the neighboring country (India, Pakistan, Nepal and Sri Lanka). Apart from the economic loss, the dairy farmers fall under other three-dimensional problems: i) taking safety measures for their own health against Coronavirus, ii) managing the dairy cattle and preventing them, and iii) selling their milk regularly with normal price. Against this, milk price has decreased substantially which was 17% lower than the previous month in one hand and on the other hand feed price was increased by 3.7% (IDRN, 2020). This had further been aggravated by distorting the regular milk market channel causing limited access to milk market for selling their milk. This had negative consequences on the demand for milk. This implies that Corona had not only affected the human health but was continued its effect in the short and long-run on the economics of the dairy sector.

Feed and feeding management cost are the highest cost items in the dairy farming ranging from 18 to 82% of the total costs globally wherein that is from 65-72% in Bangladesh (Hemme et al. 2014; Uddin et al. 2010). The Bangladesh feed price was 51% higher than global feed price in which was further increased to 68% in March 2020 (IDRN, 2020). Apart from this, feed is the top agenda both at farm level, supply chain level, policy as well as international level because feed is the major input for increasing productivity, influential drivers for environmental sustainability, greenhouse gas emission and water footprint estimation (Hagemann et al. 2011; Sultana et al. 2014; Uddin and Akter, 2019). Against this, the pandemic Coronavirus infection (Covid-19) had strong impacted on increasing the cost of milk production of typical dairy farms with an average increase by 15% due to Corona (Uddin et al. 2020). Therefore, it is of high interest how did Coronavirus (Covid-19) affect to the feed costs and feed efficiency, which in turn, might be helpful for defining the strategy for supporting the dairy farmers by developing a conducive feed policy addressing the corona.

Methodology

Selection of model

This study utilizes the concept of International Farm Comparison Network (IFCN) developed by Hemme (2000) which is further refined and validated each year to update the model and increase

its ability to take the real time changes in the model (IFCN, 2019). This method consists of three pillars: i) Typical Farm Approach (TFA), ii) Technology Impact Policy Impact Calculations (TIPICAL) model and iii) the Concept of dairy networking. This method is based on the principle of Farm Level Income and Policy Simulation Model (FLIPSIM) which was developed Texas A&M (Richardson, 1986). The IFCN is superior in simulation the sector data (macro level) to produce the changes in the farm output (micro level). The data and results in IFCN method reflected the real time (most up to date) than the FLIPSIM (Uddin et al. 2020).

Selection of the simulation variables

For estimating the impact of Coronavirus (Covid-19), five simulating variables which are considered most influential on the simulation model are selected which are i) Milk yield (-3%), ii) Milk price (-17%), iii) Marketable milk (-4%), iv) Milk wastage (-5%) and v) Feed price (+3.7%) where the (-) indicates decrease and (+) indicates increase. The effect of Corona on the selected variables were taken from the IDRN database where the changes were taken compared with 2019 (without corona) March 2020 (with Corona).

Selection of typical farms

The changes of simulating variables were applied to the typical farms. To do this, two typical farms using the TFA approach of IFCN (Uddin et al. 2010; Hemme et al. 2014 and Sultana et al. 2014) were selected. The first typical farm is BD-2 cow, that represents the mode farm (the most frequently occurring farm in a normal distribution curve) with herd size ranges from 1-3 cows. This is called household farm (small farm) and the second one BD-14 cow is the family farms (medium farms). The selection of BD-2 cow farm is based on the Transect survey of 616 dairy farms with herd size ranges from 1-3 and BD-14 is based on the transect survey of 723 dairy farms with herd size ranges from 4-16. The selected household farm for this study represents the 42% of the farm and family farm represents 48% of the total farms in Bangladesh (IDRN, 2020). For further analysis and interpretation easily, it is worth to mention that IFCN farm classification approach has defined three types of dairy farms: i) Household Farms, ii) Family Farms and iii) Business Farms were selected which were defined as below:

Household farm (HF) is defined as small farms, dairy is the one the income source for livelihoods, mainly consumed at household level and sell the surpluses milk. The herd size ranges from 1-3 cows (global standard: 1-30 cows).

Family farm (FF) is defined as the medium farm, work is done mainly by family members, with a herd size from 4-16 (global standard: 31-300 cows).

Business farm (BF) is defined as the large farm who operates their business-based Return on Investment (ROI) and work is

done by mainly hired employee. The herd size is >16 (global standard >300).

Based on this, our farms represent household and family farms which are described below:

BD-2 is typical household farm which has 2 lactating and dry cows with 0.4 ha of land (10% for dairy) with a milk production of 945 kg (natural content without any correction for fat and protein) -2.59 kg/day/cow with mostly family labour and no hired labour.

BD-14 is typical family farms which as 14 lactating and dry cows with 2.3 ha land (35% for dairy) with a milk production of 1227 kg (natural content without any correction for fat and protein) -3.47 kg/day/cow with combination of both family and hired labour.

Typical farm- a typical farm represents the most common farm production system which produce significant proportions of milk in a country or region.

In the cross-farm comparison, milk is standardized to Solid corrected milk (SCM) (IFCN 2019). The SCM is standardized to 4% fat and 3.3% protein which is calculated as

Estimation of output variables

The output variables are estimated as below:

1. Activity based costing for total feed costs in dairy farms (USD/100 kg SCM)

$$AB_c = \sum (CF_p + CF_{hg} + F\&M_c + M_c + C_{ch} + Mn_c)$$

Where,

AB_c = Activity Based Costing (USD/100 kg SCM)

CF_p = Cost for purchased feed (C/100 kg SCM), CF_{hg} = Cost for Home Grown Feed Production (USD/100 kg SCM), $F\&M_c$ = Feeding and Manure handling cost (USD/100 kg SCM), M_c = Milking Cost (USD/100 kg SCM), C_{ch} = Cow handling cost (USD/100 kg SCM), Mn_c = Management Cost (USD/100 kg SCM), In = Infrastructure (USD/100 kg SCM)

2. Cost for purchased feed (USD/100 kg SCM)

$$CF_p = \frac{(PF_c \times \%C_d) + (PF_{nc} \times \%NC_d)}{MM_p}$$

Where,

CF_p = Cost for purchased feed (C/100 kg SCM)

PF_c = Purchased Feed Concentrate (USD/year), $\%C_d$ = % of concentrate feed used for dairy (%), PF_{nc} = Purchased Feed Non-Concentrate (USD/year), $\%NC_d$ = % of non-concentrate feed used for dairy (%)

3. Cost for Home Grown Feed Production (C/100 kg SCM)

This estimation is varied based on whether the pasture land and some part of arable land is used for dairy or not.

Home-grown cost: If the pasture and arable land is not used for dairy:

$$CF_{hg} = \sum FC - CF_p$$

Home grown cost: In case, where pasture and arable land is used for dairy

$$CF_{hg} = (OC_{a\&p} + RC_{a\&p}) + (\sum FC - CF_p)$$

Where,

CF_{hg} = Cost for Home Grown Feed Production (USD/100 kg SCM)

$\sum FC$ = Total feed cost (USD/100 kg SCM), CF_p = Cost for purchased feed (USD/100 kg SCM), $OC_{a\&p}$ = Opportunity cost for own arable land and pasture land (USD/100 kg SCM), $RC_{a\&p}$ = Cost of rented arable land and pasture land (USD/100 kg SCM)

4. Share of feed cost to the total costs (%)

$$FC_{s-Tc} = \frac{\sum FC}{\sum AB_c + \sum AC}$$

Where,

FC_{s-Tc} = Share of feed cost on total cost (%)

$\sum FC$ = Sum of total feed cost (USD/100 kg SCM), $\sum AB_c$ = Sum of Activity based costing (USD/100 kg SCM), $\sum AC$ = Sum of additional Cost (USD/100 kg) where, Additional cost includes opportunity cost for own land, own land-alternative use, own labour, own capital and own quota

5. Margin over feed costs (USD/100 kg SCM)

$$Mo_{FC} = MP - CF_p - CF_{hg}$$

Where,

Mo_{FC} = Margin Over Feed Cost (USD/100 kg SCM)

MP = Milk Price (USD/100 kg SCM), CF_p = Cost for purchased feed (C/100 kg SCM), CF_{hg} = Cost for Home Grown Feed Production (USD/100 kg SCM)

6. Total feed efficiency (kg SCM/kg DM intake)

$$FE_t = \frac{MP_{c\&d}}{DMI_t}$$

Where,

FE_t = Total Feed Efficiency (kg SCM/kg DM intake)

$MP_{c\&d}$ = Milk Production per cow and day in SCM (kg/cow/day), DMI_t = Total Dry Matter intake (kg/cow/day)

Data and analysis

The data was taken from five sources: i) IFCN dairy sector data, ii) IDRN dairy sector and dairy farm database, iii) DLS 2019 annual data on livestock) online source of worldometer.info. and v) Conversion factor for BDT to USD from Bangladesh Bank and adjusted from www.oanda.com (conversation date: March 31, 2020) Data was analyzed using MS Excel 365 and TYPICAL software version 5.6

Results and Discussions

Effect on milk and feed prices across the country due to Corona (Covid-19)

Effect on milk price and Feed Price

The monthly development for Bangladesh dairy market price for milk and feed is depicted in Table 1. The milk price is shown at three levels: National, farmgate and consumer level. National feed is the average of formal and informal milk price. The feed price is the weighted average of mostly used feed ingredients in dairy farms, wheat bran (35%), rice polish (20%), mustard oil cake (25%) and corn (20%) which is used as national feed indicator.

The average milk price was increasing compared to 2019 in January 2020 from 43.79 to 45.09 BDT/kg, respectively which is 3% higher. As the Corona started globally especially in China and started to spread widely, this has already started to make impact on Bangladesh price which is decreased by 3% in February and in first half of March 1-5% and second half of the March it is 10%. This implies that Coronavirus infection is negatively correlated with milk price.

At the same pace, the feed price is increased 3.7% which cause imbalance in farm accounting system. The larger farm who is dependent mostly on purchased concentrate has been affected mostly. However, the overall decrease in milk price by 17% can be exemplified with deeper analysis on the individual regional decrease which will show the reality than the aggregate decrease at national level. Out of 64 districts, we have analyzed 43 districts among which, milk price is decreased in 37 districts (86%) while the milk price is increased for 4 districts (9.3%) and stable for 2 districts (4.7%).

The dairy farmers fall in critical situation as the decrease in milk price is combined with main feed input price (feed price) is increased by 3.7% (IDRN, 2020). However, the price increase for the different feed ingredient is shown in Table 2. Among four important feed ingredients price, the price of the wheat bran and rice polish, which together is used 55% of the weighted average diet, are increased following by corn price.

Effect of Coronavirus (Covid-19) on feed costs in typical dairy farms

Effect of Corona on the total activity costs

The IFCN farm simulation model have three different types of costs: i) Cost of milk production only (COMPO) which is the unique aspect of cost calculation in dairy farms. The COMPO is the real costs which are explicitly described the cost only to produce kg milk which is possible to compare with milk price, ii) The total cost of the dairy enterprise and iii) Activity based costing (ABC) which shows the feed costs and feeding and manure handling costs. The major concern for this study is the cost for all types of feed costs used in the dairy ration. The ABC is depicted in Figure 1. The figure shows that total costs have been increased by 15.12% and 10.18% for BD-2/19-WOC and BD-14/20-WC, respectively. The higher cost is mainly arisen from higher feed price. The feed cost is also increased due to Coronavirus as the share of feed cost to the total costs increased by 9.5% and 8.9% for BD-2/19-WC and BD-14/20-WC, respectively.

The higher feed cost is associated with lower profit as the input cost directly depress the output (Uddin et al 2010). To be profitable

Table 1 Dairy market development in Bangladesh: 2019 to March-2020

Parameter	Unit	Without Corona			With Corona	
		2019	*Jan/20	*Feb/20	15-Mar/20	31-Mar/20
Milk price						
National milk price	BDT/kg	43.79	45.09	44.21	42.91	40.49
Farmgate	Informal	BDT/kg	48.4	50.6	48.9	41.43
	Formal	BDT/kg	39.17	39.55	39.55	39.55
Consumer (unpasteurized)	BDT/kg	59.40	59.27	55.72	54.50	47.35
Consumer (pasteurized)	BDT/kg	71.0	71.0	71.0	71.0	73.33
UHT milk price	BDT/kg	90	90	90	90	90

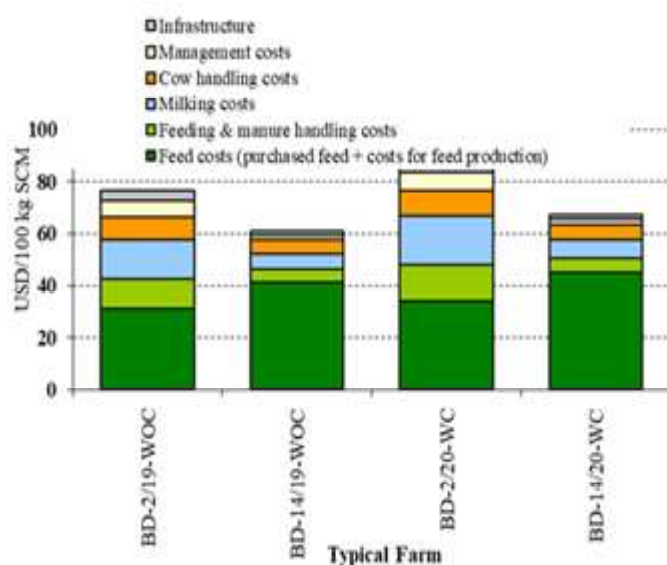


Fig. 1 Activity based costing (ABC) for dairy enterprise

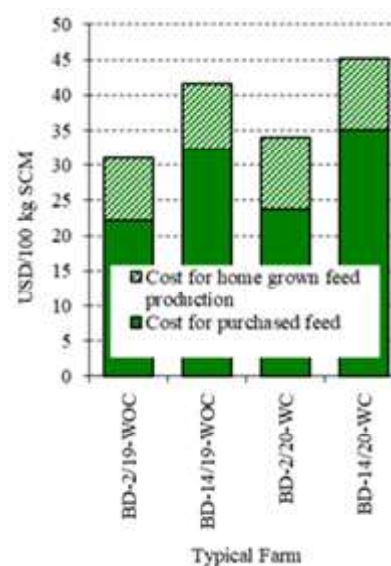


Fig. 2a Purchased and Home grown feed cost

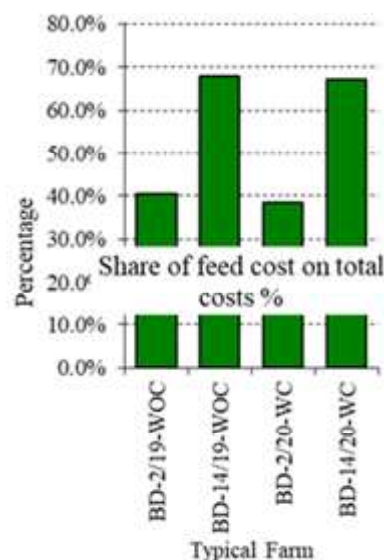


Fig. 2b Share of feed cost on total cost

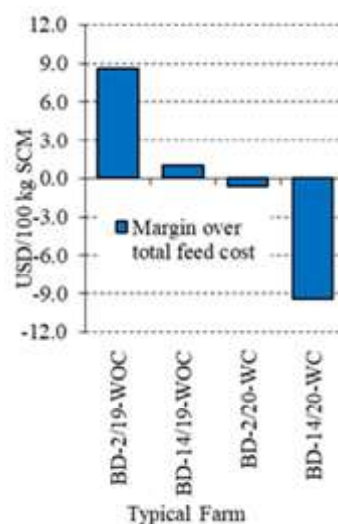


Fig. 2c Margin over total feed cost

Table 2 Feed Ingredient price development in Bangladesh: 2019 to March-2020

Indicator	Unit	Without Corona				With Corona	
		2019	*Jan/20	*Feb/20	15-Mar/20	31-Mar/20	
Concentrate feed price	BDT/kg	27.17	28.39	29.45	29.16	30.05	
Rice Straw: Basal feed for dairy	BDT/kg	8.04	7.47	7.29	7.46	7.91	
Wheat Bran	BDT/kg	32.95	35.01	36.24	36.13	37.33	
Mustard Oil cake	BDT/kg	34.19	35.33	36.13	36.12	36.15	
Rice Polish	BDT/kg	12.46	12.65	13.35	13.05	13.86	
Corn/Maize	BDT/kg	23.02	23.88	25.35	24.36	25.88	
Milk price and feed /price ratio (MP:FP)	Ratio		1.61	1.59	1.50	1.47	1.35

Source: IDRN, 2020; Profitable dairy = Milk price and feed price ratio ≥ 1.5 (IFCN, 2019)

in dairy farming, it is necessary to decrease the costs. In order to decrease the feed costs, it is necessary to understand different share of the feed costs and its components.

Effect of Corona on the various cost components

The total cost of the typical dairy farm is composed of purchased

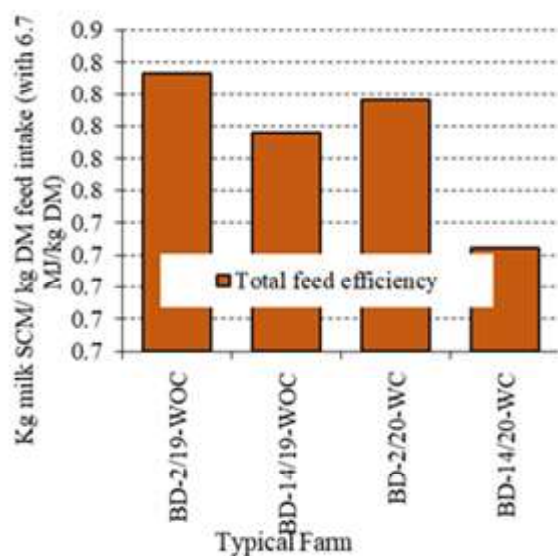


Fig. 3a Total feed efficiency

feed costs and home-grown feed costs which are depicted in Figure 4a. Both purchased feed costs and homegrown feeds are increased due to Corona. The purchased feed costs before corona was 22.1 USD/100 kg milk for BD-2/19-WOC and 32.2 USD/100 kg milk for BD-WOC which is increased to 23.8 USD/100 kg and 35.0 USD/100 kg for BD-2/20-WC and BD-14/20-WC, respectively. The purchased feed costs increased by 7.6% and 8.7% for BD-2/20-WC and BD-14/20-WC, respectively while for the homegrown feed, it is increased even higher which is 14.1% and 9.7%, respectively.

The higher purchased feed cost is due to the fact that increase in concentrate feed price which is the major share to the purchased feed. The higher home-grown might be due to the fact that the milk production is decreased which is reflected when the cost is expressed per 100 kg milk. However, interesting to note that share of the total feed costs the total costs remain stable which implies that farmers might have some adaptation to the cost reduction strategy where although the feed costs are increased but might take necessary precautions to control the other input costs.

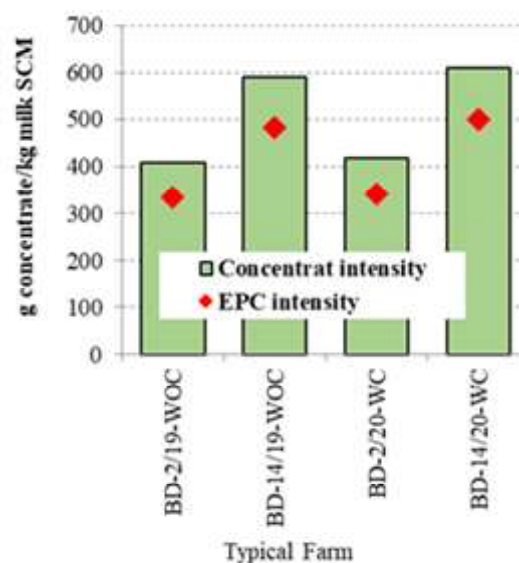


Fig. 3b Use of concentrate feed intensity

The feed price increase as shown in Table 1 (methodological section) and also observed in the Figure 2a have negative impact on the margin over total feed costs which is depicted in Figure 2c. The higher feed cost influences the profit margin which is calculated as margin over total feed costs which is decreased substantially in larger farm (BD-14/20-WC) due to Corona mainly due to higher concentrate feed price. Before Corona infection started, the margin over total feed costs were 9 USD/100 kg milk and 0.98 USD/100 kg for BD-2/19-WOC and BD-14/19-WOC, respectively which is decreased to negative margin (0.6 USD/100 kg milk for BD-2/20-WC and -9.5 USD/100 kg milk for BD-14/20-WC). This implies that feeding management for the large farm is prior needs during corona and even after corona crisis. Since the larger farms have to rely more purchased feed and also use hired labour for operation the feeding, which together cause higher costs and now suffering from negative margin from the feed input. This signifies the immediate support from the government either by providing feed incentives or intervention on the feed market.

Effect of Corona on Total feed efficiency and concentrate feed intensity

The overall feed efficiency measured as total feed efficiency and concentrate feed intensity are used as the direct impact on profitability and costs of feed which are depicted in 3a and 3b. The figure 3a shows that total feed efficiency is decreased from 0.83 to 0.82 for small farms but substantial decrease in large farm which is from 0.80 to 0.72.

Concentrate feed intake (g/kg milk) is another useful indicator to improve the feeding management. Since the concentrate price or in other word, purchased feed is the highest cost item, increase in concentrate directly affect to increase the total cost of feed. The figure 3b shows that concentrate requirement per kg milk

production increases from 409 g to 417 g per kg milk production while for the large farm 592 g to 611 g. The benchmark for profitable dairy is considered as 300 g per kg milk production (IFCN, 2019) which is not found in any of the farm even before the corona infection. The corona infection has direct impact on increase the feed price, decrease the overall feed efficiency, and increase the cost of milk production leading decrease in profit.

Strategies toward feed support policy and replication to South Asian Countries

Dairy farming and milk production is the major source of income and livelihood of the most South Asian Countries (India, Pakistan, Bangladesh, Sri Lanka and Nepal) where these three countries are producing 29% of the global milk production which is shown in Table 3 (Uddin et al. 2011 and IFCN, 2019).

As shown in table 6 that unlike Bangladesh, India and Pakistan are the biggest contributor to the total global milk production raking first and 3rd in the global milk production. In contrast, the However, the three countries are facing similar trend while taking into account the farm responses toward milk production and milk price during the Coronavirus (Covid-19) crisis as it is seen the table 4.

It is quite clear that the highest decrease in milk production is observed during Covid-19 crisis. This if combined with feed price increase which is observed particularly for Bangladesh. However, Bangladesh government has taken dynamic decision as soon as the onset of the Corona infection with a financial supporting package of 11301.1 million USD (95615 Crore BDT) for agricultural support which is 3.3% to the total GDP of the country. The question remains what the real feed costs is with and without Corona situation and which type of farm should be supported

for reducing feed costs and how much. The answer lies on the estimation of feed costs and feed efficiency considering with and without Corona. A synthesis of current research findings is presented in Table 5.

The results clearly revealed that purchased concentrate feed cost represents the highest cost which is accounted for 70% of the total cost of milk production (Tale 4) where the large farm has more concentrate costs than smaller one. In this regard, the findings of the present study clearly revealed that even the support for the small and marginal farmers are sought to be done but this results could guide that the feed support and price support is needed more for the large farmers compared with small farmers. The study done by Uddin et al. (2020) considering the effect of Corona on financial resilience, it was found that large farms were trouble due to decrease in operating capital and reduced cash flow.

The extent of the loss in the dairy industry will be further increased as a result of overall global and local economic loss at the macro level where feed costs and efficiency would play role to overcome the crisis. With immediate reactions from the government is to make short-term crisis management which has already been in implementation process while the medium to long-term strategies might need to be taken. To overcome the challenges arises from the crisis, all the stakeholders might have to take their own responsibility while the government can act as mediator and provide all kind of legal and regulatory actions. The multi-stakeholder's participation (e.g. dairy farmers, processors, input suppliers and companies, farmers association, university and research organization, policy makers and donor agencies in the development of strategic framework for addressing the impact of Coronavirus.

Table 3 Milk production in Bangladesh, India and Pakistan in relation to global milk production in 2019 (before Covid-19)*

Country	Milk production (m.ton SCM)	Comparison with Global (%)	Rank in Global
Bangladesh	8.36	1%	23
India	201.22	23%	1
Pakistan	48.36	5%	3

*Total milk production globally in 2019: 887 m. ton SCM

Source: IFCN, 2019 and IDRN, 2020 and adjusted to SCM (4.0% fat, 3.3% protein)

Table 4 Real time farm responses on milk production and milk price changes during COVID-19 time (March 2020)*

	February/20	March/20	February/20	March/20	February/20 Vs March/20	February/20 Vs March/20
Indicator	Milk production (m.ton SCM)		Milk price (USD/100 kg)		Milk prod. change (%)	Milk price Change (%)
Bangladesh	0.76	0.69	53.09	50.10	-8.0%	-5.6%
India	20.28	19.15	42.35	40.68	-5.6%	-3.9%
Pakistan	5.06	4.88	32.32	31.58	-3.7%	-2.3%

Source: Source: IFCN, 2020 and IDRN, 2020

*estimated based on IFCN real time monthly data in natural content and adjusted to SCM (4.0% fat, 3.3% protein)

Table 5 Different types of feed cost to produce 1 kg SCM and total feed efficiency

Parameter	Unit	BD-2/19- WOC	BD-14/19- WOC	BD-2/20- WC	BD-14/20- WC
Total feed costs (purchased + home-grown)	USD/kg SCM	0.31	0.42	0.34	0.45
Costs for purchased feed	BDT/kg SCM*	26.23	35.11	28.56	38.052
	USD/kg SCM	0.22	0.32	0.24	0.35
Concentrate	BDT/kg SCM	18.70	27.24	19.99	29.4
	USD/kg SCM	0.17	0.25	0.19	0.28
Non-concentrate	BDT/kg SCM	14.76	21.41	15.86	23.226
	USD/kg SCM	0.05	0.07	0.05	0.08
Cost for home-grown feed	BDT/kg SCM	3.95	5.86	4.25	6.3672
	USD/kg SCM	0.089	0.093	0.10	0.102
	BDT/kg SCM	7.53	7.87	8.57	8.568

*The results are also expressed in local currency for quick understanding for the local use; Currency conversion: 1 USD =84.61 BDT (2019) and 84.00 BDT (2020), SCM = Solid Corrected Milk.

The farmers have to make quick adaption to the changing farm and feeding management. As a first step is to change their feeding practices from purchased based concentrate feeding to locally available feed resources. Since the feed cost is the highest cost item for the dairy farms (Uddin et al. 2010, Hemme et al. 2014, Ndambi et al. 2008 and Alqaisi et al. 2019), the reduction in the concentrate feed would make trade off with cost of milk production. At the same time, the milk yield might be decreased which is quite meaningful to do since the market access become limited due to Corona. The other study done by Uddin et al. (2017) showed that cost reduction is the key strategy for increasing profit and in this case, feed cost reduction 'strategy can be taken by the famers where government support can be extended in the form of cash support per kg concentrate use or supporting the feed industry to reduce the compound feed cost for the dairy farmers. Hence altering the feeding management and rationalization of overall feed related activities are key areas that might be supported by the policy decisions.

At the same pace, the research organization and research networking should act as mediator among the key stakeholders between Government and famers as well as processors. The research organization should apply the suitable methods (either it is forecasting or forward modeling), networking among several stakeholders, analysis of the real time data and facts without any judgment, and visual the real status quo to the government for implement their strategic actions. The strategic action plan will be more effective once the data and facts are authentic. The government, in other way, also might use the research capacity and network for their use rather to rely on the annual published data.

Considering the negative impact of Coronavirus the Bangladesh Government initiatives to declare the emergency services for all the things related to dairy is highly appreciated to trade off the losses encountered by the farmers as well as to increase the farm level profitability from the negative to the positive trend. This

study results are, thus, expected to be beneficial for the dairy farmers and policy makers as well as input suppliers (e.g. feed suppliers) and processors to take their right decision to increase the dairy farm income.

At this current scenario of the dairy sector development which has passed turbulent situation in Bangladesh, India, Pakistan, Sri Lanka and Nepal, the implications of this study might be extrapolated. Bangladesh has already taken incentives policy for providing the direct cash subsidy for feeding support to the dairy cows considering the loss of the Covid-19 between 60 USD/ household farm (BD-2) and 235 USD/family farm (BD-14). (With the latest data on global milk price and feed price which are decreasing trend in January 2022 in one hand and on the other hand, the on-going fourth Wave of Covid-19 (Omicron variants), each of the country can take strong lessons on the defining the feed policy both for feed ingredients and compound feed price considering the unexpected shock on feed market. Due to the fact that feed price vis-à-vis feed costs directly influence the profitability of the dairy farms, strategic actions plan on ensuring the feed with affordable price in all South Asian Countries are highly recommended.

Conclusions

The application of the farm simulation model of IFCN has produced the output which are quite helpful to make policy decisions. The impact of corona infection has impacted on feed price increase by 3.7% which has been translated to have impact at farm level by increasing purchase feed cost by 8.6% and decrease the margin over feed cost from positive to extremely negative which is from +4.8 USD/kg SCM to -5.05 kg USD/kg SCM. Total feed efficiency is decreased from 81.5% to 77% as a result of the incorporation of the corona infection in the analysis with higher decrease in large farm than smaller farm. To combat the corona-induced crisis, both type of farmers (small and large)

needs feed support, however, the relatively higher support might be sought for the large farmers. The findings of this study are expected to be useful for other country South Asian countries like India and Pakistan as the Covid-19 has affected with the similar pace in neighboring countries like India, Pakistan, Nepal and Sri Lanka

Acknowledgement

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RESEARCH ARTICLE

Indigenous technical knowledge used in dairying by pastoralists of Jammu and Kashmir

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Abstract: The study was carried out in current Union Territory of Jammu & Kashmir (erstwhile state of Jammu & Kashmir). Two districts, namely Rajouri & Poonch from Jammu division were selected purposively because of being the districts of highest *Gujjar* (transhumants primarily rearing cattle and buffaloes) population; whereas, two districts, namely Anantnag & Baramulla from Kashmir division were selected purposively because of being the districts of highest *Bakarwal* (transhumants primarily rearing sheep and goats) population. Sixty respondents (*Gujjars*) from migratory tracts of district Rajouri & Poonch from Jammu division were selected randomly and sixty respondents (*Bakarwal*) from migratory tract of districts Anantnag & Baramulla from Kashmir division were selected randomly comprising a total of 120 respondents. Those pastoralists who were rearing at least 10 milch animals were selected for the study. Focus group discussion method was used for collection of data related to Indigenous Technical Knowledges (ITKs). The discussion revealed that the respondents were using ITKs primarily in breeding, feeding and health-care management practices. With regard to the induction of heat, thirty per cent of the respondents used chapatti of a mixture of *Gur* and *ghee* in the ratio of 2:1 in wheat flour and fed to animal once a day to induce heat. For retention of placenta, 34.17 per cent of the respondents fed extract obtained from tea leaves (*Camellia sinensis*) on boiling after sieving to animals. As far as ITKs regarding feeding practices are concerned 40.83 per cent of the

respondents fed boiled *Ber* leaves (*Ziziphus mauritiana*) once a day to increase milk production. The study reveals that 42.50 per cent of respondents fed 10-12 leaves of *Katori* (*Xylosnalongifolium*) plant to animals having diarrhoea and 15.83 per cent fed fruits of *bel* (*Aegle marmelos*) to animals having diarrhoea.

Keywords: *Bakarwal*, Dairying, *Gujjar*, Indigenous Technical Knowledge, Pastoralism

Introduction

Pastoralists are people who depend primarily on livestock for subsistence. They inhabit in those parts of the world where the potential for crop cultivation is limited. There are about 120 million pastoralists in the world (Rass, 2006). Pastoralism makes a significant contribution to the economy of developing countries in terms of providing employment and in supplying nutrition to the rural poor. There are so many pastoral communities in India but Pastoralism in a country like India is under-researched and poorly documented. The pastoral communities of Jammu and Kashmir have retained all the typical characteristics of pastoral communities, viz. migration, having no land for tilling, having livestock as the sole source of subsistence and have no permanent structures. So, pastoral communities of J & K are a typical model to study. Pastoral communities use different ITKs for breeding, feeding and health-care management of the dairy animals. To identify the ITKs used by these communities regarding dairying, this study was undertaken in Jammu and Kashmir Union Territory.

Research Methodology

In the study, two districts Rajouri & Poonch from Jammu division were selected purposively because of being the districts of highest *Gujjar* population. Two districts Anantnag & Baramulla from Kashmir division were selected, purposively, because of being the districts of highest *Bakarwal* population. Sixty respondents (*Gujjars*) from migratory tracts of district Rajouri & Poonch from Jammu division were selected randomly and 60 respondents (*Bakarwals*) from migratory tract of districts Anantnag & Baramulla from Kashmir division were selected

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randomly comprising a total of 120 respondents. Pastoralists, who were rearing at least 10 milch animals were selected for the study. Focus group discussion method was used for collection of data related to Indigenous Technical Knowledges (ITKs). The respondents were asked about ITKs they were using related to dairy farming.

Result and Discussion

The discussion revealed that the respondents were having considerably vast knowledge regarding use of ITKs in rearing of their animals.

Breeding Practices

Induction of heat

It is very important for the dairy farmers to identify heat detection at right time to make dairy farming profitable by overcoming the loss due to extra feeding and long dry period in animals. It has been estimated through National Dairy Research Institute, Karnal study that farmer loses Rs.4000 when he misses each heat in dairy animal (Srivastava et al. 2013). Results in table 1 revealed that thirty per cent of the respondents used chapatti of a mixture of *Gur* and *ghee* in the ratio of 2:1 in wheat flour and fed to animal once a day to induce heat. Forty per cent of the respondents fed boiled *methi*(*Trigonella foenum-graecum*) grains empty stomach to animals for 3-5 days for inducing heat.

Retention of Placenta (ROP)

The results indicate that 34.17 per cent of the respondents fed extract obtained from tea leaves (*Camellia sinensis*) on boiling after sieving to animals. It was observed that 25.83 per cent of the respondents fed extract obtained after boiling rice to cure ROP (retention of placenta), 27.50 per cent of the respondents believed that cold water feeding expedited the process of shedding of placenta and 25 per cent of the respondents fed Gulkand in cases of ROP (retention of placenta).Lanset al. (2003) reported that in northern region paddy (*Oryza sativa*) cultivation is predominant and paddy is also called as a “heated substance” which means that heat of the paddy would help to break down the uterine lining that helps in easy expulsion of placenta.

Feeding Practices

As far as ITKs regarding feeding practices are concerned 40.83 per cent of the respondents fed boiled *Ber* leaves (*Ziziphus mauritiana*) once a day to increase milk production, 43.33 per cent of the respondents fed Garna leaves (*Carissa opaca*) and shoots to increase milk yield in goats, 25.83 per cent of the respondents fed Massa (*Bulbostylis barbata*) (cut into pieces) mixed in wheat flour to increase milk production and 53.33 per cent of the respondents fed one *paav*(250 g) *Jaggery* (Red

Table 1: Indigenous Technical Knowledge related to breeding and feeding practices

S NO	Breeding Practices	F*	%
	Induction of Heat		
1.	<i>Gur</i> and <i>ghee</i> in the ratio of 2:1 were mixed in wheat flour and then chapatti of this mixture was fed to animal once a day to induce heat in animal.	36	30.00
2.	Boiled <i>methi</i> (<i>Trigonella foenum-graecum</i>) grains were fed empty stomach to animals for 3-5 days to induce heat.	48	40.00
3.	Retention of Placenta		
4.	Extract obtained from tea leaves (<i>Camellia sinensis</i>) on boiling after sieving was fed to animals in case of retention of placenta.	41	34.17
5.	Extract obtained after boiling rice was also fed to animals in case of retention of placenta.	31	25.83
6.	Cold water feeding also expedited the process of shedding the placenta.	33	27.50
7.	Gulkand feeding is also effective in case of retention of placenta.	30	25.00
	Feeding Practices		
8.	<i>Ber</i> leaves (<i>Ziziphus mauritiana</i>) were boiled and fed to animal once a day to increase milk production.	49	40.83
9.	Garna leaves (<i>Carissa opaca</i>) and shoots were fed to increase milk yield in goats.	52	43.33
10.	Massa (<i>Bulbostylis barbata</i>) (cut into pieces) mixed in wheat flour and fed to animal to increase milk production.	31	25.83
11.	One <i>paav</i> (250 g) <i>Jaggery</i> (Red sugar made of desi gur) mixed in dry flour and given to animal once a day helps in increasing milk production.	64	53.33

*F = frequency out of 120 respondents

sugar made of desi gur) mixed in dry flour to animal once a day to increase milk production.

Diarrhoea

The study reveals that 42.50 per cent of respondents fed 10-12 leaves of *Katori* (*Xylosoma longifolium*) plant to animals having diarrhoea and 15.83 per cent fed fruits of bill (*Aegle marmelos*) to animals having diarrhoea. The study on plants belonging to different plant families such as Anathaceae, Anacardiaceae, Ceasalpinniaceae, Clusiaceae, Punnicaceae and Verbenaceae indicated anti-diarrheal activity (Panda et al. 2012) against pathogens causing diarrhoea in human being was tested successfully.

Constipation

Results show that 28.33 per cent of the respondents fed Gulkhand in case of constipation, 15.83 per cent fed mango pickle to relieve animals of constipation and 37.50 per cent fed Saunf (*Foeniculum vulgare*) powder mixed in wheat flour in case of constipation.

Tympany

Table 2 indicates that 55.83 per cent of the respondents used to cut upper ear tip with a razor in cases of tympany, 25.83 per cent fed one *paav* (250 g) Hing (*Ferula asafetida*) once a day in cases of tympany, 66.67 per cent drenched animals suffering from tympany with one *paav* (250 g) mustard oil (*Brassica campestris*) and 35.83 per cent fed leaves of *Tulsi* (*Ocimum sanctum*) in case of tympany.

Fracture

It was observed that fifteen per cent of the respondents applied Til oil (*Sesamum indicum*) on the affected part after fixing it with bamboo splints in case of fracture and 9.17 per cent applied a cooled down suspension of Amla (*Emblica officinalis*) and piece of iron boiled in water.

FMD

As per the results obtained 15.00 per cent of the respondents applied the oil from the seeds of the mustard plant (*Brassica campestris*) after mixing with Haladi powder (*Curcuma longa*) and kerosene oil externally on the foot in case of animals suffering from FMD, 26.67 per cent of the forced animals suffering from FMD to walk on hot sand for 20 minutes and 36.67 per cent of applied extract from the stem of deodar tree (*Cedrus deodara*). The most frequently used plants for the treatment of foot and mouth disease were *Fagopyrum esculentum* and *Tamarindus indica*. *Fagopyrum esculentum* documented in the present study have not been documented for foot and mouth disease so far as no information regarding its use was available in the literature,

however, it has been documented for the treatment of anaemia by Ratan et al. (2011). Similarly *Tamarindus indica* has been reported for the treatment of black quarter after mixing with soil of ant hill and applying it on the back or thigh region by Deshmukh et al. (2011) in early studies. Feeding of fish and snail to animals in order to prevent and treat foot and mouth disease was also reported from the study area which has also been reported for the treatment of foot and mouth disease in Sargodha district of Pakistan by Dilshad et al. (2009).

Cough

It was found that 25.83 per cent of the respondents burnt blue colour cloth and its fumes were given to affected animals and 31.67 per cent fed *kali mirch* (*Piper nigrum*), *badielaichi* (Cardamom) and *ajwain* (*Trachyspermum ammi*) in equal proportions ground and mixed in water twice a day in case of cold and cough.

Ectoparasites

The results reveal that forty five per cent of the respondents applied salt added to mustard oil (*Brassica campestris*) over the body of affected animals for control of ectoparasites and 19.17 per cent of the respondents fed Shambar leaves (*Artemisia nilagirica*) to animals for removing internal as well as external parasites. A similar study found that Cow urine and black ash; cloth dipped in petrol; camphor application; red soil on legs (Subrahmanyeshwari and Chander, 2013) in cases of ectoparasites. The most frequently used plant for helminthiasis and external parasites was processed resin of *Cedrus deodara* used almost in all the species. *Cedrus deodara* has been reported to be used for external parasites in Jammu and Kashmir by Slathia et al. (2007) and in Pakistan by Sindhu et al. (2010). *Aconitum deionorrhizum* and *Sarcococcalignawere* other widely used plants in almost all the parasitic and other skin conditions in animals. Use of *Rabdosia rugosa* and *Gentianakurroo* as remedy of helminthiasis in livestock has also been reported in Jammu and Kashmir by Kumar et al. (2009). *Nicotiana tabacum* reported from the study area for treatment of maggots has been documented earlier in the literature by Nfi et al. (2001) for the same ailment. *Saussurea costus* reported for maggot infestation has also been reported for the same condition in Jammu and Kashmir by Khan et al. (2004) and in Uttaranchal by Bisht et al. (2006).

Mastitis

Majority (72.50 %) of the respondents applied common salt on teats in case of mastitis, 55.83 per cent of the respondents applied turmeric (*Curcuma longa*), alum and black pepper were ground and then applied on teats in case of mastitis and 62.50 per cent applied alum or phitkari on teats in case of mastitis. Similar study revealed that 100 gm turmeric (*Curcuma longa*) 10 gm alum or phitkari and 10 gm black pepper grind and massage on teats

Table 2: Indigenous Technical Knowledge related to health-care prac

S. No	Health Care Practices	F*	%
Diarrhoea			
1	10-12 leaves of <i>Katori</i> (<i>Xylosonalongifolium</i>) plant were fed to animal having diarrhoea.	51	42.50
2	Fruits of bill (<i>Aegle marmelos</i>) were fed to animals having diarrhoea.	19	15.83
Constipation			
1	Feeding of gulkand also was effective in cases of constipation.	34	28.33
2	Mango pickle was also fed to relieve constipation.	19	15.83
3	Saunf(<i>Foeniculum vulgare</i>) powder mixed in wheat flour was also used in treating constipation.	45	37.50
Tympany			
1	The upper ear tip is cut with a razor in case of treating tympany and this is one of the most widely used.	67	55.83
2	One <i>paav</i> (250 g) Hing (<i>Ferulaasafoetida</i>) fed once a day is also effective against tympany.	31	25.83
3	One <i>paav</i> (250 g) mustard oil (<i>Brassica compertris</i>) drench is very effective in cases of tympany.	80	66.67
4	Feeding leaves of <i>Tulsi</i> (<i>Ocimum sanctum</i>) is also effective in tympanitis.	43	35.83
Fracture			
1	Application of Til oil (<i>Sesamum indicum</i>) on the affected part after fixing it with bamboo splints in case of fracture.	18	15.00
2	Amla (<i>Embllica officinalis</i>) and piece of iron is boiled in water. When this suspension was cooled down, it was applied on the feet of affected animal in case of FMD.	11	9.17
Foot and Mouth Disease (FMD)			
1	The oil from the seeds of the mustardplant (<i>Brassica compestris</i>) after mixing with Haldi powder (<i>Curcuma longa</i>) and kerosene oil was applied externally on the foot in case of animals suffering from FMD.	18	15.00
2	Affected animals were forced to walk on hot sand for 20 minutes.	32	26.67
3	Extract from the stem of deodar tree (<i>Cedrus deodara</i>) was applied externally to cure FMD.	44	36.67
Cough			
1	Blue colour cloth was burnt and its fumes were given to affected animals in case of cough.	31	25.83
2	<i>Kali mirch</i> (<i>Piper nigrum</i>), <i>badielaichi</i> (Cardamom) and <i>ajwain</i> (<i>Trachyspermumammi</i>) in equal proportions is ground and mixed in water and fed to animals twice a day in case of cold and cough.	38	31.67
Ectoparasites			
1	Salt added to mustard oil (<i>Brassica compertris</i>) was applied all over the body for control of ectoparasites.	54	45.00
2	Kerosene oil or petrol applied all over the body in case of ectoparasites.	59	49.17
3	Shambar leaves (<i>Artemisia nilagirica</i>) were fed to animals for removing internal as well as external parasites.	23	19.17
Mastitis			
1	Application of common salt on teats helps in mastitis.	87	72.50
2	Turmeric (<i>Curcuma longa</i>), alum and black pepper were ground and applied on teats in case of mastitis.	67	55.83
3	Alum or phitkari was applied on teats in mastitis.	75	62.50

*F = frequency out of 120 respondents

once a day (Subrahmanyeshwari and Chander 2013) was applied in cases of mastitis.

Conclusion

ITKs amongst pastoral communities are treasures of knowledge maintained across generations which need to be documented on priority. Modernization has adversely hit these time tested

remedies and these ITKs have taken a back seat. In the times of changing dynamics about treatment of different diseases and management of dairy animals, these locale specific indigenous ways of management need using locally available resources need to be validated on priority so that this time tested institution doesn't wither away. The reconsideration of traditional medicinal systems in the industrialized world and the fact that modern medicine was too expensive for many developing countries, the

World Health Organization (WHO) in the 1970's decided to promote traditional medicinal systems by checking scientifically the efficacy of plants used in traditional medicine and identifying the principles responsible for genuine therapeutic effects (Bizimana, 1997). ITKs on Mastitis management are the key area where the ITKs can be tried, tested and adopted so that the economic loss met by the dairy farmers is prevented. Another aspect of ITKs that need further exploration is multiple uses of single ITK products especially the plants. Researches can be guided towards delineation of the roles played by different ingredients present in the plant parts used in treatment of different diseases.

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RESEARCH ARTICLE

Information utilization pattern among male and female dairy farmers of Punjab

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Abstract: The current research was carried out in rural Punjab to study gender desegregated information seeking and utilization behaviour as well as their role in the dairy venture. By using pretested interview schedule, the data were collected from 160 dairy farmers from three socio-cultural zones of Punjab. Gender desegregated role performance showed that male dairy farmers were more responsible for animal health care, feeding and breeding while female dairy farmers were majorly responsible for dairy finance and shed cleaning. Dairy farmers relied more upon the personal cosmopolite sources for the information followed by localite sources and impersonal cosmopolite channels. Male dairy farmer significantly sought more information from DDB, *Pashu Palan Mela*, State Extension Officer, PAU/KVK experts and progressive farmers as compared to females who significantly sought information more from family/relatives. In the utilization of information, female dairy farmers were ahead of their male counterparts as they significantly utilize more information sought from progressive farmers, family/relatives, localite organisations and co-operative societies. It is eye opening to note that though women sought less information, whatever they sought they utilize. Results offer food for thought to effectively transfer technologies, ideas, practices to grass root level. It is suggested to ensure equal participation of female in various extension programs so that new ideas are readily accepted and utilised by farm families. So, it can be concluded that if new technologies or innovations are transferred effectively to women then the technologies will be more readily acceptable among farm families.

Keywords: Gender desegregated; Dairy; Information seeking; Information utilization

Introduction

Dairying is an integral part of the diversified farming systems that have sustained Indian agriculture for centuries by providing farmers with a solid economic backbone. Dairy sector has a key role in delivering health benefits, supplementing family incomes, and creating employment opportunities for small-marginal farmers and women in rural and transitional areas (Shiva et al. 2019). The rural women play a significant role in dairy farming and are involved in practices like feeding, breeding, management and health care. In India, 75 million women work in the dairy industry, compared to 15 million men (Thakur and Chander 2006). However, the vital and significant role played by women in dairy has not received the recognition it deserves, and they continue to be invisible labourers (Chayal et al. 2009) while most of the males had land ownership, livestock and credit. These prevalent patriarchal gender differences are also found among different communication sources (Bhuyan and Ponnusamy 2017). The participation of women in extension programs is usually lower as compared to male counterparts. Women face numbers of constraints in accessing information sources as compared to the male dairy farmers. Looking into their high participation in dairy, it's of utmost importance that benefits of extension programmes reach the female as well. The strategies to overcome these constraints can be worked out if extension functionaries get recent updated information about the information seeking and utilization behaviour of male and female dairy farmers. This compels us to compare the information seeking and utilization behaviour of the male and female dairy farmers. This paper presents the gender desegregated information seeking and utilization behaviour, along with their role in dairy farming.

Materials and Methods

The study was conducted in three socio-cultural zones of Punjab. One district from each of the socio-cultural zone i.e. Ludhiana, Gurdaspur and Hoshiarpur were selected from Malwa, Majha and Doaba, respectively. As Malwa is bigger in area so two blocks from Malwa i.e. 'Raikot' and 'Doraha' and one block each from Majha and Doaba i.e. 'Batala' and 'Hoshiarpur' were selected. Thus, total four blocks were selected for the present study. Forty rural dairy farm families from each block who possessed at least

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4 milch animals were selected by using snowball sampling technique. From each of the selected family, the head of the dairy venture either male or female was considered as respondent. Thus, 160 dairy farmers comprised the sample for study. Out of which 116 families had male head of dairy while rest 44 families had female head. Data were collected personally using interview schedule prepared for the purpose. It comprised of their dairy profile, role in dairy farming besides information sources and frequency of utilization. Information sources were exhaustively listed under different heading namely personal cosmopolite sources, personal cosmopolite channels, impersonal cosmopolite channels, localite sources and localite channels. Frequency of seeking information from different information sources was measured on a three-point continuum as 'always', 'sometimes' and 'never' with score 2, 1 and 0 respectively. To find the extent of utilization of information, the sources from which information was sought were included in the interview schedule and respondents were asked how frequently they utilize the information. The frequency of information utilization was measured on the three point continuum 'fully', 'partially' and 'not at all' with scores 2, 1 and 0 respectively. The arithmetic mean for information seeking and utilization score for different sources and practices were computed and t-test was used to analyse the gender difference.

Results and Discussion

Table 1 represents the dairy profile of the respondents is discussed. It is evident that most of the farmers (96.3%) had comparatively small herd size (4-9 milch animals). Result is in line with Wani et al. (2016). Data shows that, few farmers (3.1%) had 10-15 milch animals and negligible number of farmers (0.6%) had large herd size of 16-21 animals. Gender comparison shows that majority of dairy households of both the male (95.7%) and female dairy farmers (97.7%) had small herd size whereas, very few of males (3.4%) and females (2.3%) had medium herd size. None of the female respondents possessed large herd size against 0.9 per cent of male respondents. There was no significant gender difference in possession of milch animals.

Further the Table 1 depicts the average milk production of respondent's dairy farm. Data revealed that majority of dairy farmers (78.8%) had daily milk production between 12-48 litres. Some of the respondents (19.4%) had the milk production of 49-85 litres, while only few farmers (1.9%) had milk production of 86-120 litres per day. Gender wise comparison depicts that majority of female (88.6%) as well as male dairy farmers (75.0%) also had milk production between 12-48 litres per day. Double males (22.4%) than female (11.4%) had average milk production

Table 1: Gender difference in dairy profile of dairy farmers

n=160

Characteristics	Total		Z - Test	Overall f(%)
	M(116) f(%)	F(44) f(%)		
Herd- Size (Number)				
Small (4-9)	111(95.7)	43(97.7)	0.54	154(96.3)
Medium(10-15)	4(3.4)	1(2.3)	0.70	5(3.1)
Large (16-21)	1(0.9)	-	0.53	1(0.6)
Daily Milk Production (litres/day)				
12-48	87(75.0)	39(88.6)	0.06	126(78.8)
49-85	26(22.4)	5(11.4)	0.11	31(19.4)
86-120	3(2.6)	-	0.28	3(1.9)
Daily Milk Sale (litres/day)				
10-43	82(70.7)	38(86.4)	2.04*	120(75.0)
44-77	31(26.7)	6(13.6)	0.08	37(23.1)
78-110	3(2.6)	-	0.28	3(1.9)

*p<0.05

Table: 2 Gender disaggregated role performance in dairy farm practices

Dairy Practices	Male	Female	Z Test
	f (%)	f (%)	f (%)
Feeding	119(74.4)	99(61.9)	2.39
Breeding	130(81.3)	90(56.3)	4.82**
Animal Health care	123(76.9)	102(63.8)	0.10
Finance	109(68.1)	132(82.5)	2.98**
Shed/ House Cleaning	85(53.1)	101(63.1)	1.81
Milk sale & produce records	104(65.0)	119(74.4%)	1.82

*Multiple response, **p<0.01

of 49-85 litres while only male dairy farmers (2.6%) had milk production between 86-120 litres.

The data further represents the average sale of milk by the respondents. Three fourth of dairy farmers (75%) had milk sale between 10-43 litres per day. Gender wise also, majority of both female (86.4%) as well as male dairy farmers (70.7%) had milk sale between 10-43 litres. There was significant gender difference at five per cent level of significance. Some of the dairy farmers (23.1%) daily sold 44-47 litres of milk where males (26.7%) were ahead of females (13.6%). Only few dairy farmers (1.9%) sold 78-110 litres of milk all of them were male.

So, the result shows that comparatively milk production of male headed dairy farms was more than female headed farms.

Table 2 presents the gender desegregated performance in various dairy farm activities. According to the data, in the task of feeding animals, most of the households cited male (74.4%) member as more participative than female (61.9%). For breeding of animals also, participation of males (81.3%) was significantly higher as compared to females (56.3%). For animal health care, male dairy farmers (76.9%) were more participative than females (63.8%) however it was not statistically significant. These results are in line with study of Khare and Singh (2019) where men were found to be dominant in feeding and breeding of animals. It is interesting

to find that finance related to dairy is handled by females (82.5%) in majority of houses as compared to males (68.1%) and result was significant at one per cent level of significance. The task of shed-house cleaning is majorly performed by females (63.1%) than by males (53.1%). Similarly, milk sale and produce records were maintained majorly by females (74.4%) than males (65.0%).

Overall it can be concluded that, though male dairy farmers were more responsible for animal health care, feeding and breeding, female dairy farmers were majorly responsible for dairy finance, shed cleaning and milk produce records. In some activities performance of male was more while in others participation of female was more which shows that in dairy participation of woman is equal to male or it is a gender neutral occupation.

Table 3 represents data pertaining to use of personal cosmopolite sources by the respondents for dairy information. Interestingly, the overall mean for information seeking as well as utilization for both male and female was 2.23 which shows that dairy farmers sometimes sought and partially utilized the information gained from personal cosmopolite sources. Among all the sources, veterinary officers were most approachable as dairy farmers always (2.88) sought and fully utilized ($\bar{x} = 2.57$) the information from them. Their availability in the village/locality itself can be reason for this. The results are in line with Khuman et al. (2014)

Table 3: Gender comparison of respondents for use of personal cosmopolite sources

n=160

Sources	Information seeking				Information Utilization			
	M	F	t- test	Overall	M	F	t- test	Overall
Dairy Development Board experts	1.51	1.25	2.27*	1.44	2.13	2.33	0.32	2.16
Experts at Pashu Palan Mela/ animal welfare camp	2.10	1.68	3.38**	1.99	2.09	2.11	0.32	2.09
Kisan call centres	1.15	1.11	0.51	1.14	2.00	2.00	0.23	2.00
State extension officer	1.79	1.50	2.04*	1.71	2.49	2.31	1.00	2.45
PAU/KVK experts	1.70	1.43	2.15*	1.63	2.10	2.07	1.06	2.10
Vet officer/medical agent	2.89	2.86	0.47	2.88	2.58	2.55	0.50	2.57
$\bar{x}$	2.23	2.23	-	2.23	2.23	2.23	-	2.23

*p<0.05, **p<0.01, Mean range: 1-3

Table 4: Gender comparison of respondents for use of impersonal cosmopolite channels

Channels	Information seeking				Information utilization			
	M	F	t-test	Overall	M	F	t-test	Overall
Television	1.97	2.05	0.68	1.99	1.99	2.10	1.88	2.02
Radio	1.22	1.27	0.71	1.23	1.95	2.00	0.76	1.97
Newspaper	1.75	1.86	0.89	1.78	2.01	2.07	0.89	2.03
Magazines	1.64	1.55	0.74	1.61	2.08	2.11	0.37	2.08
Internet	2.36	2.48	0.88	2.39	2.11	2.13	0.27	2.12
$\bar{x}$	1.68	1.76	0.98	1.70	2.02	2.07	-	2.03

*p<0.05, Mean range: 1-3

where veterinary officers are found to be most approachable by dairy farmers in Asam. All of the rest personal cosmopolite sources were sometimes sought and partially utilized i.e. *Pashu Palan Mela* ($\bar{x}$ = 1.99, 2.09), State Extension Officer (= 1.71, 2.45) and PAU/KVK (= 1.63, 2.10) by dairy farmers. Dairy development board (= 1.44) and Kisan call centres (= 1.14) were never sought for information by dairy farmers, though respondents who visited partially utilized with respective mean score of 2.16 and 2.00.

Male dairy farmers significantly sought more information from DDB, *Pashu Palan Mela*, State Extension Officer and PAU/KVK experts as compared to females. The results are in line with Ganesan (2004), Nande et al. (2009), Sharma and Aparna (2021). No significant gender difference was observed in utilizing the information from personal cosmopolite sources.

Overall it can be concluded that dairy farmers relied more upon veterinary officers and the information gained from them. The results are in line with the findings of Sharma and Aparna (2021).

Table 4 shows the data pertaining to impersonal cosmopolite sources used by respondents. Overall mean score indicates that

dairy farmers sometimes seek (= 1.70) and partially (= 2.03) utilize the information gained information from impersonal cosmopolite sources. Among all the sources, internet (= 2.39, 2.21) was sought as well as utilized most frequently. Information gained from rest of the sources i.e television (= 1.99, 2.02), newspaper (= 1.78, 2.03) and magazines (= 1.61, 2.08) was sometimes sought and partially utilized. The radio (= 1.23) was never sought by dairy farmers for getting the information though dairy farmers who listens radio partially utilized the information with mean score of 1.97. The results are align with Aldosari et al. (2017), Saikia and Mittal (2022) and Raza et al. (2019) where internet was found to be used the most while contrary to Punitha et al. (2013) and Singh N et al. (2015) who reported television as most preferred channel whereas Chauhan and Kansal (2014) reported that extent of utilization of newspaper was higher among dairy farmers of Punjab. This can be concluded that in recent years internet has replaced T.V and Newspaper as a source of information.

The reason being that internet services can be explored anytime anywhere using handheld device i.e Mobile phones. Singh et al. (2015) and Saroj & Mittal (2016) recommended that mobile phones could be a suitable medium to reaching out to the users as

Table 5: Gender comparison of respondents for use of localite sources

n=160

Sources	Information seeking				Information utilization			
	M	F	t-test	Overall	M	F	t-test	Overall
Progressive Dairy Farmers	1.54	1.25	2.80**	1.46	1.98	2.22	2.55*	2.01
Local leader/ Sarpanch	1.89	1.66	1.90	1.83	2.04	2.00	0.72	2.03
Friends	2.46	2.61	1.73	2.50	2.28	2.14	1.88	2.24
Family / Relatives	2.66	2.89	2.87**	2.72	2.38	2.59	2.37*	2.44
Neighbours	2.28	2.50	1.76	2.34	2.09	2.17	1.06	2.11
$\bar{x}$	1.98	1.99	0.08	1.98	2.15	2.22	-	2.17

*p<0.05, **p<0.01, Mean range: 1-3

Table 6: Gender comparison of respondents for information seeking and utilization for use localite channels

n=160

Channels	Information seeking				Information utilization			
	M	F	t-test	Overall	M	F	t-test	Overall
Local organization / Club	1.05	1.07	0.40	1.06	1.83	2.00	2.55*	1.89
Dairy Co-operative	1.91	1.66	1.80	1.84	2.18	2.29	1.88	2.20
Local/Amul/Verka society	1.41	1.41	0.04	1.41	1.98	2.00	2.37*	1.98
$\bar{x}$	1.46	1.38	1.33	1.44	2.00	2.10	-	2.02

*p<0.05, Mean range: 1-3

Table 7: Comparison of different sources/ channels of information seeking and utilization

n=160

Sources/ Channels	Information seeking	Information utilization	Rank
Personal cosmopolite sources	2.23	2.23	I
Localite sources	1.98	2.17	II
Impersonal cosmopolite channels	1.70	2.03	III
Localite channels	1.44	2.02	IV
$\bar{x}$	1.84	2.11	

Mean range 1-3

everyone can access information from anywhere anytime. Though, there is need to create awareness among masses to differentiate between authentic and non-authentic information shared via different social networking sites.

All the impersonal cosmopolite sources were sometimes sought, there was no significant difference between female ($= 1.76$) and male ($= 1.68$).

Data in Table 5 shows that localite sources were sometimes ($= 1.98$) sought and partially ($= 2.17$) utilized by dairy farmers. Negligible difference was found in the overall mean score of female ($= 1.99$) and male ($= 1.98$). It is evident that among localite sources, family/relatives ($= 2.72, 2.44$) were always approached and most utilized by the dairy farmers followed by friends ($= 2.50, 2.24$). Similar findings were observed by Meena and Chauhan (2005) where family members were found to be most utilized among localite sources. Information was sometimes sought and partially utilized from the neighbours ($= 2.34, 2.11$) and local leaders ($= 1.83, 2.01$) were sometimes sought by dairy farmers.

Gender difference show that male ($= 1.54$) dairy farmers sought information significantly more ($t = 2.80$) from progressive farmers than females ($= 1.25$) while whosoever sought, females utilize the information significantly more ($t = 2.55^*$) than their male counterparts. From family/relatives, female dairy farmers significantly sought ($t = 2.87$) as well as utilized ($t = 2.37$) more information as compared to their male counterparts. So it can be concluded that male dairy farmers prefer seeking information from progressive farmers while female prefer getting information from family or relatives. This can be because females are confined to four walls of the house. The findings are in line with findings of Sharma and Aparna (2021).

Overall it can be interpreted from the Table that though localite sources were partially utilised, female were significantly ahead of male dairy farmers in utilising information sought from progressive dairy farmers as well as family or relatives.

Table 6 depicts that localite channels were rarely ($= 1.44$) approached by the dairy farmers, however who approached these channels, partially utilized ($= 2.02$) the information gained from them. There was gender difference in utilization of the information sought from local organization/club ($t = 2.55$) and local Amul/Verka society ($t = 2.37$) where female respondents were significantly ahead of male dairy farmers.

Table 7 depicts the preference of dairy farmers for sources of information seeking as well as utilization. Personal cosmopolite sources came out to be the most preferred one, followed by localite sources, impersonal cosmopolite channels and localite channels. The results are partially supported by the studies of Singh et al. (2014), Karthikeyan et al. (2018) and Kharmudai et al. (2018). Overall mean for information seeking was 1.84 which means dairy farmers

sometimes sought the information while overall mean for information utilization shows the partially utilize the information ($= 2.11$).

Conclusion

In performing various dairy farm activities, male dairy farmers were found to be more participative in the task of breeding, feeding and animal health care while in cleaning of shed house, handling finance and milk produce records females were more participative. Dairy farmers more relied upon the personal cosmopolite sources/channels for the information followed by localite sources and impersonal cosmopolite channels. Male dairy farmer significantly sought more information from DDB, *Pashu Palan Mela*, State Extension Officer, PAU/KVK experts and progressive farmers as compared to females while from family/relatives female dairy farmers significantly sought and utilize more information as compared to male dairy farmers. This is obvious as they are more introvert. In the utilization of information, female dairy farmers were ahead of their male counterparts as they significantly utilize more information sought from progressive farmers, family/relatives, localite organisations and co-operative societies. It shows that whatever they seek, they utilise the information. Therefore, it is inferred from the findings that if new technologies or innovations are to be transferred effectively to farm families then care should be taken that women also equally participate in various extension programs so that technologies may be more readily accepted among farm families.

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RESEARCH ARTICLE

Participatory evaluation of ethno-veterinary livestock health practices by farmers from surrounding villages in Ranthambore Tiger Reserve, India

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Abstract: The present study was conducted to document the ethno-veterinary practices, 360 livestock farmers from the 30 villages surrounding the tiger reserve were interviewed using an open-ended interview schedule to enlist all the practices against the ailments of livestock from the purposefully chosen zone namely Critical Tiger Habitat and buffer zone of the tiger reserve. For participatory assessment of the identified ethno-veterinary practices, Quantification of Indigenous Knowledge and to identify the best practices among overall practices one way variance followed by DMRT method was applied. Use of *Drimia indica* (Roxb.) Jessop was found most effective practice against mastitis and use of seeds of *Sorghum halepense* L. against the diarrhoea, stem bark of *Salodora persica* L. for fever, and fruits of *Areca catechu* for treatment of prolapse in the livestock were found most effective practices. The pharmacodynamics of these ethno-veterinary techniques could therefore be studied before further replication and implementation.

Keywords: Farmers, Indigenous knowledge, Livestock, Ranthambore Tiger Reserve

Introduction

HERBS and plant concoctions have been utilized to treat numerous ailments around the world since ancient times, and they have a special affinity with humans (Sharma et al. 2005). Because of higher cultural acceptance, compatibility with the human body, and fewer side effects, such formulations are still the mainstay of basic health care for roughly 75–80 percent of the world's population, particularly in third-world nations (Kamboj 2000). Ranthambore Tiger Reserve (RTR) is an abode to a plethora of plant species and homemade ingredients that have

been used time and again by the locals to cure many health problems in the livestock. The use of natural products of plants are not restricted to human but also helped to treat various diseases of livestock (Dzoyem et al. 2020). Most of the rural communities depend on livestock for their livelihood but the impact of livestock diseases severe for the rural people who have not able to access modern medicine therefore indigenous knowledge they highly depend on their traditional knowledge to treat various diseases (Eiki et al. 2021). The ethno-veterinary medicine is cheaper and more cost-effective than modern medicine (Masika et al. 2010). Farmers in the vicinity of RTR are unable to access modern veterinary facilities due to a lack of awareness and knowledge about modern medicine and solely depend on their traditional knowledge to treat their diseased animals. These knowledge treasures are poorly documented, and the knowledge base is in danger of extinction. Hence, the purpose of this study was to document and appraise ethnoveterinary treatments used to treat common livestock health problems.

Material and Methods

Study area

In India, there are 52 Tiger Reserves, covering 5.26 per cent of the country's geographical area.

Out of 52, Ranthambore Tiger Reserve was selected because the highest number of villages (306 villages) with one million human population, and these people primarily depend on agriculture and livestock for their livelihood and on their traditional knowledge to treat various livestock diseases. For the documentation of the ethno-veterinary practices, a study was conducted from September 2021 to November 2021, and for the assessment of the practices in January 2022.

Data Collection

Ranthambore Tiger Reserve is divided into two zones namely Critical Tiger Habitat also known as Core Zone (Ranthambore National Park, Sawai Madhopur Wildlife Sanctuary, Sawai Man Singh Wildlife Sanctuary) and buffer zone (Kaila Devi Wildlife Sanctuary) both zones were selected, and 30 villages were

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selected, randomly from both zone of RTR and 12 farmers who are engaged with livestock rearing was selected randomly, thus, a total of 360 livestock farmers were interviewed at their homes with help of an open-ended interview schedule for documentation of ethno-veterinary practices with the rationale used by them to treat different diseases of their livestock.

Data Analysis

Assessment of ethno-veterinary practices had been done through QuIK (Quantification of Indigenous Knowledge) method by key informants, using the method (QuIK) developed by De Villiers (1996) (De Villiers 1996). QuIK methodology represents a rapid and relatively cheap way to elicit ethno-veterinary practices. In QuIK, PRA tool, i.e. matrix ranking is combined with an interview schedule to elicit numerical data from key informants. Farmers were mainly using ethno-veterinary practices to cure four important diseases e.g. mastitis, diarrhoea, fever, and prolapse. Therefore these four diseases were selected for the participatory validation, and key informants were identified by socio-metric method (Moreno 1951) among the selected livestock farmers, and those were having depth understanding and knowledge of selected ethno-veterinary practices. Thus, 42 key informants were identified for participatory validation of mastitis. Accordingly, 38, 31, and 57 key informants were identified for the participatory validation of diarrhoea, fever, and prolapses, respectively. Four criteria or parameters, viz. availability, ease of preparation, healing effect, and lower side effect, were selected to appraise the comparative and relative effectiveness of the identified ethno-veterinary practices. Key informants were asked to put a required number of pieces of stone out of each block of the matrix as per their perception of each criteria/parameter of the identified ethno-veterinary practices. Data from each key informant were treated as an independent result. Data collected from the key informants on several criteria were subjected to a one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) modified by (Kramer 1957) was used to identify the most effective practices among the identified practices.

Results and Discussion

Ethno-veterinary practices used against various ailments in the livestock by the respondents

Mastitis

The respondents used a total of five practices (**Table 1**) to treat mastitis in animals. In the first practice, participants collected *Drimia indica* (Roxb.) Jessop leaves from the forest area, made a paste out of the plant leaves, and administered it to the animals' affected parts twice a day for two to three days. In the second practice, they prepared a paste of leaves of *Vitex negundo* L. and give it to animals orally for three to four days together with water and bread. As the third practice, a paste made from *Datura stramonium* L leaves was applied to the affected parts twice daily for three to four days. Due to its potent analgesic and sedative properties, datura was utilized by tribal in Rajasthan's Banswara district to cure mastitis in livestock (Yadav and Rajput 2015). The use of *Datura stramonium* L and *Chenopodium ambrosioides* L was found to be most effective against mastitis disease in livestock (Naseer et al. 2021). In case of the fourth practice, crushed *Capparis decidua* fruit was prepared into a paste and then applied to the animals' affected udder parts. And respondents used *Zingiber officinale* and ground it up, mixed it with sugar, and applied it to the affected udders of animals as the fifth practice. Mastitis is a common milking livestock like buffalo and cow disease that caused a tremendous economic loss to dairy farms. *Zingiber officinale* has antibacterial properties which helped to treat mastitis disease in dairy animals (Masniari 2011)

For the assessment of ethno-veterinary practices for the treatment of mastitis, a total of 42 key informants were interviewed. Matrix ranking for the relative performance of the options concerning each criterion, e.g. availability, ease in preparation, healing effect, and lower level of side effect was done with a score of 1 to 5 as presented in Table 2. The result shows discernible differences in different ethno-veterinary practices. The fifth practice used ginger as the most effective in terms of availability but in terms of ease of preparation, respondents perceived the use of Kair as the most effective significantly different from the first practice. And in the case of healing effects and lower levels of side effects, respondents perceived the use of Kolikanda most effective practice and has significant differences from all the practices and were finally adjudged as the most effective practice against the treatment of mastitis of livestock. The third practice use of Datura was least effective in terms of availability, ease of preparation as well as in healing effect. Forth practice use of Kair was most effective in ease of preparation as its less time in preparation but was perceived as least effective in healing effects and had a lower level of side effects. Fifth practice use of Adhrak was easily available at home and also in the market but was having least

Table 1: Ethno-veterinary practices used by the respondents for treatment of Mastitis in the livestock

Practice	Local name	Scientific name	Habitat	Part used	Form of product
First	Kolikanda	<i>Drimia indica</i> (Roxb.) Jessop	Herb	Leaves	Paste
Second	Nirgundi	<i>Vitex negundo</i> L.	Shrubs	Leaves	Paste
Third	Datura	<i>Datura stramonium</i> L.	Herb	Leaves	Paste
Fourth	Kair	<i>Capparis decidua</i>	Shrubs	Fruits	Paste
Fifth	Adhrak	<i>Zingiber officinale</i>	Rhizome	Rhizome	Paste

effective healing effect as it's taking much time to heal also least effective in the lower level of side effects so it was not found more efficient than other practices as per the perception of the respondents. Though they perceived that first practice use of *Drimia indica* (Roxb.) Jessop (Kolikanda) to the livestock against mastitis was more effective in terms of healing effect and lower level of side effects than other practices due to this reason first practice was the most effective practice.

Diarrhoea: A total of four ethno-veterinary practices were used by the framers for the treatment of diarrhoea (Table 3). In the case of the first practice, respondents collected seeds of *Sorghum halepense* (Linn.) and seed flour mixed with little water and given to animals orally for two-three days to cure diarrhoea. For the second practice, respondents collected roots of *Phoenix dactylifera* L and crushed then mixed them into the water and given to animals orally for two days. About 25-30 grams of leaves of *Phoenix dactylifera* L paste and given to animals with either help of water or bread or fodder or two-three times a day for three days in the third practice and case fourth practices respondents prepare leaves to paste of *Moringa oleifera* Lamk then given to animals. Mullukuma tribes of Kerla are knowing using of paste of *Moringa oleifera* Lamk for the treatment of diarrhoea (Silja et al. 2008) and used of *Moringa oleifera* also helped to not only decrease the incidence of diarrhoea but also helped to increase feed utilization and increased enzymatic antioxidants among the pre weaned calves (Kekana 2021). But Meena et al. (2023) found that A powder of sahjan (*Moringa oleifera*) root, nirgundi (*Vitex negundo*) leaves, desi ajwain (*Fumaria indica*), and a little salt was produced by Raika camel rearer of Rajasthan. Finally, this

powder mixed with ghee and administered to the camel twice daily for 4-5 days to cure *trypanosomiasis* (Surra) in the camel.

For the assessment of ethno-veterinary practices, 38 key informants were interviewed with four criteria viz. availability, ease of preparation, healing effect, and low level of side effects with a scoring pattern of 1-4 matrix of decision criteria for each practice for curing of diarrhoea in the livestock such as buffalo, cattle, and goat is presented in the Table 4. The first practice Jowar seed flour mixed with water was found to be the most effective practice against the curing of diarrhoea in comparison to the other three practices. With its easily available, preparation and lower level of side effects, therefore, this practice was perceived as the best effective one. There is no significance in the case of the healing effect of all practices but has a significant difference in the case of availability, ease of preparation, and lower level of side effects. Respondents perceived second practices roots of Khajuras as most effective in the lower level of side effects but these practices were not perceived best in case of availability and ease in preparation that's why this practice was judged second most effective practice in comparison to others. Third practice leaves of Sahjan were not easily available and also had a complexity to preparation due to this reason this practice was the least effective practice among others.

Fever

A total of four ethno-veterinary practices were used by the respondents for curing fever (Table 3). In case of first practice, first respondents take the bark of *Salvadora persica* L and mixed

Table 2 Participatory assessment of ethno-veterinary practices for treatment of Mastitis in the livestock by the livestock respondents (n=42)

Criteria	First Practice	Second Practice	Third Practice	Fourth Practice	Fifth Practice
Availability	2.10±0.906 ^d (IV)	1.88±0.145 ^d (V)	2.79±0.220 ^c (III)	3.64±0.148 ^b (II)	4.45±0.109 ^a (I)
Ease in preparation	2.67±0.225 ^b (IV)	2.67±0.220 ^b (V)	2.81±0.178 ^b (III)	3.57±0.190 ^a (I)	3.52±0.224 ^a (II)
Health effect	3.79±0.209 ^a (I)	3.31±0.217 ^a (II)	2.93±0.194 ^b (III)	2.86±0.185 ^b (IV)	2.10±0.204 ^c (V)
Lower level of side effect	4.45±0.128 ^a (I)	2.60±0.202 ^c (IV)	3.19±0.181 ^b (II)	2.64±0.228 ^c (III)	2.19±0.168 ^c (V)
Overall effect	13.00±0.375 ^a (I)	10.45±0.353 ^c (V)	11.71±0.377 ^b (IV)	12.71±0.418 ^a (II)	12.26±0.334 ^b (III)

(a,b,c,d means bearing different superscripts in a row under each criterion differ significantly (P<0.05). The multiple comparisons are based on the DMRT Post Hoc test. Values in parenthesis indicate the respective rank under each criterion).

Table 3: Ethno-veterinary practices used by the respondents for treatment of Diarrhoea in the livestock

Practice	Local name	Scientific name	Habitat	Part used	Form of product
First	Jowar	<i>Sorghum halepense</i> L.	Plant	Seeds	Liquid solution
Second	Khajur	<i>Phoenix Dactylifera</i> L.	Tree	Roots	Liquid solution
Third	Pudina	<i>Thymus vulgaris</i> L.	Shrub	Leaves	Paste
Fourth	Sahjan	<i>Moringa oleifera</i>	Tree	Leaves	Paste
Ethno veterinary practices used by the respondents for treatment of Fever in the livestock					
First	Pilu	<i>Salvadora persica</i> L.	Tree	Stem bark	Liquid Solution
Second	Kanthari	<i>Capparis sepiaria</i> L.	Shrub	Stem bark	Liquid Solution
Third	Isharmul	<i>Aristolochia indica</i> L.	Shrub	Roots	Powder
Fourth	Amrud	<i>Psidium guajava</i> L.	Tree	Leaves	Liquid Solution

it into juice form and give it to animals orally for two-three days however the paste of roots of *Salvadora persica* given to animals is effective against the fever (AI et al. 2020). In the second practice, respondents prepare the juice of the stem bark of *Capparis sepiaria* L. and give it to animals twice a day for three days to affected animals. *Capparis sepiaria* L. is good in giving relief to pain as well as fever and infection in animals (Meena et al. 2020). Prepare powder root of *Aristolochia indica* Linn. and given to animals orally with the help of water or chapati in the third practice. The study area is very famous for guava farming so they first collected leaves of *Psidium guajava* Linn and extracted them in the juice form of almost one liter and given to affected animals orally for at least three days. But paste of alum, given to animals was most effective practice against the fever in small ruminants (Chand et al. 2021).

For the assessment of ethno-veterinary practice, 31 key informants were interviewed with four criteria namely availability, ease of preparation, healing effect, and low level of side effects with a scoring pattern of 1-3. The matrix of decision criteria for each practice for curing fever in the livestock is presented in Table 4. The first practice, use of stem bark juice of *Salvadora persica* L. was found to be the most effective practice for treating fever when compared to the other three methods since it was the easiest to prepare, had a healing effect, and had less side effects. Fourth practice usage of *Psidium guajava* Linn was thought to be readily available but ranked second in other criteria. The use

of *Aristolochia indica* Linn was found to be the least effective since it was not easily available, difficult to prepare, and required a lot of time to heal.

Prolapse

A total of three practices were used by the respondents for curing prolapses as presented in Table 5. In the first practice, respondents crushed the fruits of *Areca catechu* which is available in the market, and make a paste of the fruits then given to animals along with a small quantity of ghee and sugar for two days. 15-20 pieces of *Areca catechu* also known as Supari first soaked in a small quantity of ghee then given to animals (Chopra et al. 1956). and in case of second practice respondents used to give orally raw fruits of *Lagenaria siceraria* for two days this helps to make body temperature level of the livestock. 250-gram desi ghee along with almost 2-3 kg *Cucurbita pepo*. And in the case of the last practice, the livestock keeper first brought roots of *Zezyphus sativa* then crushed it and made the paste, and gave it to animals twice a day for two days for curing prolapse in the livestock. Talukdar et al. 2015 found that almost 200 gram root of two different Rhamnaceae family plants namely *Ziziphus mauritiana* L. and *Chrysopogon zizanioides* mixed and decocted with three liters of water until the volume is reduced by one-third. The mixture is then filtered through muslin cloth and placed in a bottle for storage then 100 ml of the mixture is administered twice daily for 7–10 days to treat prolapse in cattle.

Table 4 Participatory assessment of ethno-veterinary practices for treatment of Diarrhoea in the livestock by the livestock respondents (n=38)

Criteria	First Practice	Second Practice	Third Practice	Fourth Practice
Availability	2.95±0.151 ^a (I)	2.63±0.148 ^b (II)	2.05±0.185 ^b (IV)	2.29±0.199 ^b (III)
Ease in preparation	2.92±0.194 ^a (I)	2.55±0.167 ^b (II)	2.05±0.141 ^c (IV)	2.47±0.199 ^c (III)
Health effect	2.66±0.197 ^a (I)	2.53±0.154 ^a (I)	2.47±0.176 ^a (I)	2.34±0.201 ^a (I)
Lower level of side effect	2.37±0.183 ^b (III)	3.05±0.130 ^a (I)	2.55±0.187 ^b (II)	2.08±0.190 ^b (IV)
Overall effect	10.89±0.331 ^a (I)	10.76±0.265 ^a (II)	9.13±0.307 ^b (IV)	9.18±0.377 ^b (III)

Participatory assessment of ethno-veterinary practices for treatment of Fever in the livestock by the livestock respondents (n=31)

Criteria	First Practice	Second Practice	Third Practice	Fourth Practice
Availability	2.13±0.190 ^b (III)	2.42±0.137 ^b (II)	1.94±0.185 ^b (IV)	3.32±0.176 ^a (I)
Ease in preparation	2.87±0.206 ^a (I)	2.35±0.200 ^b (III)	2.16±0.186 ^b (IV)	2.61±0.200 ^a (II)
Health effect	3.29±0.155 ^a (I)	2.19±0.182 ^b (III)	2.10±0.176 ^b (IV)	2.42±0.221 ^b (II)
Lower level of side effect	3.45±0.145 ^a (I)	1.90±0.199 ^c (IV)	2.29±0.148 ^b (III)	2.48±0.212 ^b (II)
Overall effect	11.74±0.328 ^a (I)	8.87±0.396 ^b (III)	8.48±0.347 ^b (IV)	10.84±0.412 ^a (II)

(a,b,c means bearing different superscripts in a row under each criterion differ significantly (P<0.05). The multiple comparisons are based on the DMRT Post Hoc test. Values in parenthesis indicate the respective rank under

Table 5 Ethno-veterinary practices used by the respondents for treatment of Prolapse in the livestock

Practice	Scientific name	Local name	Habitat	Part used	Form of product
First	<i>Areca catechu</i>	Supari	Tree	Fruits	Paste
Second	<i>Lagenaria siceraria</i>	Loki	Climber	Fruits	Fruits
Third	<i>Zezyphus sativa</i>	Ber	Shrub	Root	Paste

Table 6 Participatory assessment of ethno-veterinary practices for treatment of prolapses in the livestock by the livestock respondents (n=57)

Criteria	First Practice	Second Practice	Third Practice
Availability	2.26±0.121 ^a (I)	2.12±0.071 ^a (II)	1.61±0.108 ^b (III)
Ease in preparation	1.93±0.090 ^b (II)	2.60±0.096 ^a (I)	1.40±0.066 ^c (III)
Health effect	2.61±0.082 ^a (I)	1.44±0.066 ^c (III)	1.93±0.109 ^b (II)
Lower level of side effect	2.39±0.774 ^a (I)	1.72±0.102 ^b (III)	1.89±0.102 ^b (II)
Overall effect	9.19±0.196 ^a (I)	7.88±0.162 ^b (II)	6.84±0.200 ^c (III)

(a,b,c means bearing different superscripts in a row under each criterion differ significantly (P<0.05). The multiple comparisons are based on the DMRT Post Hoc test. Values in parenthesis indicate the respective rank under each criterion)

For the assessment of ethno-veterinary practices, 57 key informants were interviewed with four criteria e.g. availability, ease of preparation, healing effect, and lower level of side effect with a scoring pattern of 1-3 matrix of decision criteria with respect to each practice for curing prolapses of livestock are presented in Table 6. The first practice use of *Areca catechu* was found to be the most effective practice to control prolapses in the livestock in comparison to the other two practices. The first practice was perceived to rank first in availability, healing effect, and lower level of side effects due to this reason it was found to be the most effective practice, and in the case of the second practice it has no significant difference at <0.05 with the first practice in availability and this practice was perceived rank first in case of ease preparation. The third practice use of *Zezyphus sativaw* was not easily available as well as took much time in preparation so it was the least effective practice in comparison to other practices.

Conclusion

Livestock farmers in the vicinity of the Ranthambore Tiger Reserve are highly dependent on their traditional knowledge for the treatment of various ailments in the livestock. Participatory assessment appraised the use of *Drimia indica* (Roxb.) Jessop to treat mastitis, while *Sorghum halepense* L. seeds were shown to be the most effective treatment for diarrhoea. Concerns about efficacy, quality, safety, and dose standardization continue to exist. As a result, the pharmacodynamics of these ethno-veterinary methods must be evaluated urgently before they can be replicated and used.

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IDA MEMBERSHIP
(FOR INDIAN CITIZEN)

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SB a/c No.: 90562170000024

IFSC: CNRB0019009

Bank: Canara Bank

Branch Address: Delhi Tamil Sangam Building,
Sector-V, R.K. Puram, New Delhi.

Membership Form	Fee (in Rs.)	Admission Fee (in Rs.)	(GST@ 18%) (in Rs.)	Without Late Fee (in Rs.)	Late Fee After 31st May	Total Amount (in Rs.)	Period	Qr Code (Scan to Download Membership Form)
OM Form (1 Year)	1000	500	270	1770	Nil	1770	APR-MAR	
OM Renewal Form (1 Year)	1000	Nil	180	1180	118	1,298	APR-MAR	
LM Direct Form	10,000	500	1890	12,390	Nil	12,390	Life Time	
Convert OM To LM Form	10,000	Nil	1800	11,800	Nil	11,800	Life Time	
Student Membership Form	700	Nil	126	826	Nil	826	Per Course	
INSTITUTIONAL FORM Sustaining Membership (1 Year) Benefactor Membership (8 Year)	12,500 70,000	Nil Nil	2,250 12,600	14,750 82,600	Nil Nil	14,750 82,600	APR-MAR APR-MAR	

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