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Comprehensive review on *Rabri* -A traditional heat desiccated product

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Abstract: *Rabri* is a traditional Indian dairy product, manufactured by the concentration of sugar-containing milk via evaporation, maintaining slow heating conditions. It contains several layers of *malai* (clotted cream), formed via air cooling the surface of milk and continuous removal of formed layer which represents its typical attribute as well. It possesses a sweet taste and cooked flavor still, it may be infused with additional flavors, color, fruits and nuts for garnishing to enhance its sensory attributes. The physico-chemical, sensory and textural properties of *rabri* vary with the locality, type of milk used and the method employed for its preparation. This review briefly discusses the effect of different types of milks and manufacturing processes on chemical composition and typical sensory attributes of *rabri*. Recent innovations and advances in mechanization in *rabri* manufacturing, microbial quality, the problem of adulteration, shelf-life enhancement through the use of different packaging materials and fortification of *rabri* with different non-dairy ingredients and calorie reduction strategies have also been emphasized.

Keywords: *Basundi*, *Rabri*, Shelf life, Sugar, Sweetened condensed milk, Traditional dairy products.

Introduction

Indigenous dairy products have a great socio-economic significance for Indians. Traditional Indian dairy products (TIDPs) can be elaborated as all such milk products that are native of the India and were evolved over ages utilizing locally available fuels and cooking wares. The widespread consumption and distribution of traditional milk-based sweets on festivals and other auspicious occasions represents a signatory feature of Indian culture.

Rabri is a heat desiccated dairy based sweet, quite popular in Indian subcontinent (Ghayal et al. 2015). It is prepared from sweetened condensed milk and had a pale yellow to light caramel colored appearance (Bandypadhyey and Mathur, 1987). It contains multiple layers of clotted cream (or *malai*) formed during *rabri* manufacturing process (De, 1977). It is consumed as an authentic milk dessert in many (specially eastern and northern) parts of India. The word ‘rabdi’ mentioned in “Chnadimangala” means a thickened sweetened milk that was consumed with other sweets in the early 1400s. Ancient literature revealed that, ‘*Rabri*’ was created in Mathura (City of lord Krishna), and perfected in Varanasi (known as Holi city), Uttar Pradesh. Probably, Parsis developed *rabri* by condensing milk and consumed it in the form of *rabdi falooda* (Bora, 2015).

Traditionally, small vendors (*halwais*) prepare *rabri* in conventional shallow pans at a smaller scale. *Rabri* manufacturing process is considered to be very time consuming and tedious. The product is usually prepared under poor hygienic conditions and stored in open environment, which favors microbial proliferation and had a detrimental effect on its shelf life. Very little attention is so far paid towards the sanitary production, handling and packaging of *rabri* by *halwais* which still remains a matter of concern. Figure 1 shows traditional practices employed in preparation of *rabri*, typical texture of *laccha rabri* and traditional way of its serving.

Undoubtedly, *rabri* is a highly nutritious product owing to concentration of milk components and sugar during its manufacturing. Sugar when added in *rabri* plays a vital role as it promotes sweetness in the product as well as increases its shelf

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life. Buffalo milk is usually preferred for *rabri* preparation. Buffalo milk is subjected to simmering over a long span i.e. continuous heating or simmering of milk in stainless steel (SS) kettle or *karahi* on wooden fire/gas results in gradual evaporation of moisture and the layer of cream formed (clotted cream) on the surface is continuously removed and adhered to the upper (usually cooler) portion of *karahi*. Thereafter, after achieving the desirable consistency of milk (5 folds concentration based on initial milk volume or weight), sugar is added @ rate of 6%. The layer of clotted cream is mixed in concentrated milk sugar mixer before packaging (Aneja et al. 2002). *Rabri* is mostly prepared by small sweet-maker. Hence, in lack of standardized scientific method of production, lot of inconsistencies in terms of variation in chemical composition and quality of *rabri* is inevitable. However, some of the Indian milk brands have developed *rabri* at commercial level by incorporating technological interventions in manufacturing process. Sensory quality of *rabri* prepared from different methods is shown in Table 1.

Various fruits and nuts such as grapes, apples, *kaju*, *pista*, etc. can be infused in *rabri* during its preparation and, named as fruit

rabri. In addition to fruit and nuts; colors and flavors may also be incorporated in *rabri* to enhance its appealing properties (Dhumal et al. 2018).

Some of the functional varieties of *rabri* have also been developed through the incorporation of custard apple, date syrup or crush, mango pulp, fig pulp and sapota pulp to improve its functionality and palatability. Another development includes preparation of dietetic *rabri* using artificial sweetener (aspartame) to replace refined and processed sugars. Manufacturing of *rabri* employing in-line and mechanized system improves the hygienic terms in *rabri* making process and also ensures proper quality control. In-line production units for commercial production of *rabri* have also been developed using different process vats (Chopde et al. 2016). Innovative and safe packaging of product hikes domestic demand and also favors export of hygienic, wholesome quality product to global consumers including Indian diaspora. Starting from the raw material, production methods used for *rabri* production, its composition, recent innovations and mechanization; packaging material and methods used for *rabri* have been reviewed and discussed in following sections.

Table 1: Sensory quality of *rabri* prepared from different methods

Type of Rabri	Flavour	Body and texture	Colour and appearance	Reference
Rabri prepared from traditional method	Pleasant caramelized flavour	Creamy consistency and viscous body containing several layers of clotted cream with a chewy texture	Creamy white to light caramel in colour	FSSAI, (2020)
Dietetic Apple Rabri	Pleasant flavour, sweetish caramel and pleasant aroma	Viscous body and creamy consistency, containing several layers of clotted cream with a soft, chewy texture	Creamy white to light brown colour	Bharti et al. (2018)
Rabri production by mechanized method	Pleasant slightly caramelized	Plastic consistency with uniformly dispersed but slightly firm clotted cream layer	Pale yellow with tinge of brown	Chopde et al. (2013)
Rabri blended with Khajur crush	Enhanced flavour due to Khajur crush	Superior body and texture obtained with 6 per cent khajur pulp	Better colour and appearance with increased sensory score due to incorporation of khajur pulp	Kahandal et al. (2020)



Figure 1: a. *Rabri* being prepared by traditional method in medium heat at simmering temperature in iron shallow pan in Varanasi locality near Shri Kashi Vishwanath temple; b. Traditional *rabri* garnished with chopped dry fruits; c. *Rabri* with typical Lachha texture *rabri*, and d. *Rabri* served in earthen cup topped with extra *malai* (clotted cream)

Preparation of *rabri*

Type of milk

Composition of milk varies with species and breed of animal. Final yield of *rabri* depends upon the composition of milk used for *rabri* making. Different types of milks have been used for the preparation of *rabri* (Chatterjee et al. 1994). Fresh milk is filtered through muslin or nylon cloth to remove foreign particles and then analyzed for fat and SNF content. Adulteration, acidity, clot-on-boiling (COB) and alcohol test are performed for quality assessment of milk. Sweet-makers do not perform these tests due to lack of testing facilities and awareness which leads to subsequent repercussions.

From buffalo milk

Conventionally, *rabri* is prepared from buffalo milk as it comprises of higher TS and results in more yield. Higher fat content in milk results in a better body and texture of the final product owing to the formation of more clotted cream or *malai* during simmering. Standardized milk (6% fat and 9% SNF) is primarily practiced at commercial scale for *rabri* making as it ensures a consistent product quality (Chopde et al. 2016; Singh and Gupta, 2007). Figure 2 depicts different methods used for the manufacturing of *rabri* from standardized milk (6% fat and 9% SNF).

Gayen (1989) prepared *rabri* from standardized buffalo milk (6% fat) via heating at 90-95°C followed by constant removal of clotted cream (10% of the initial milk volume). Thereafter, milk was concentrated up to 3-folds concentration. Sugar was added @ rate of 6% of initial milk. *Rabri* thus prepared exhibited excellent sensory properties. The acidity and percent TS, fat, protein, total carbohydrate (lactose plus sucrose) and ash contents and yield of this *rabri* were 0.31% LA, 50.80, 16.10, 21.80 (lactose-10.80% plus sucrose-11.90%), 10.01, and 1.88, ~50%, respectively.

Singh et al. (2009) prepared *rabri* using milk of Bhadwari breed; standardized to 5, 6 and 7% fat level and concentrated up to 3, 3.5 and 4 folds concentration. Sugar was added @ of 6%, 7% and 8%. The prepared *rabri* samples were stored at 5 ± 1 °C, analyzed for different microbiological parameters up to 20 days storage. Fresh *rabri* sample did not showed any coliform and yeast and mold counts, but exhibited standard plant count (SPC). The counts of SPC, coliform and yeast and mold increased with the advancement in the duration of storage. The researchers concluded that *rabri* samples were found suitable for consumption up to 15 days of storage at 5 ± 1 °C.

Cow milk, mixed milk and skimmed milk

Chauhan et al. (2014a) evaluated the physico-chemical attributes of *rabri* prepared from different (cow milk, buffalo milk, skim milk and mixed milk) milks at various levels (6, 8 and 10% of initial milk quantity) of sugar addition. They determined chemical constituents, analyzed physical properties and recorded sensorial attributes of prepared *rabri* samples. Physical attributes and chemical (fat and sucrose) constituents were influenced by different levels of sugar addition while other chemical constituents (such as moisture, protein, lactose and ash content) remained unaltered even by varying levels of sugar addition. Physical attributes and chemical quality attributes (except protein and ash) of *rabri* were significantly ($p \leq 0.05$) affected by the use of different types of milk. However, the flavor, sweetness and overall acceptability of *rabri* were affected by combined effect of milk and sugar while physical attributes (body texture, color and appearance) and chemical constituents did not change.

Chauhan et al. (2014) prepared *rabri* using buffalo milk, cow milk, mixed milk and skimmed milk and stored under refrigeration conditions (5 ± 1 °C). The prepared *rabri* samples were then evaluated for shelf-life and microbial count i.e., standard plate count (SPC), yeast and mold and coliform count during the 15 days storage period at an interval of every 5 days. Microbial

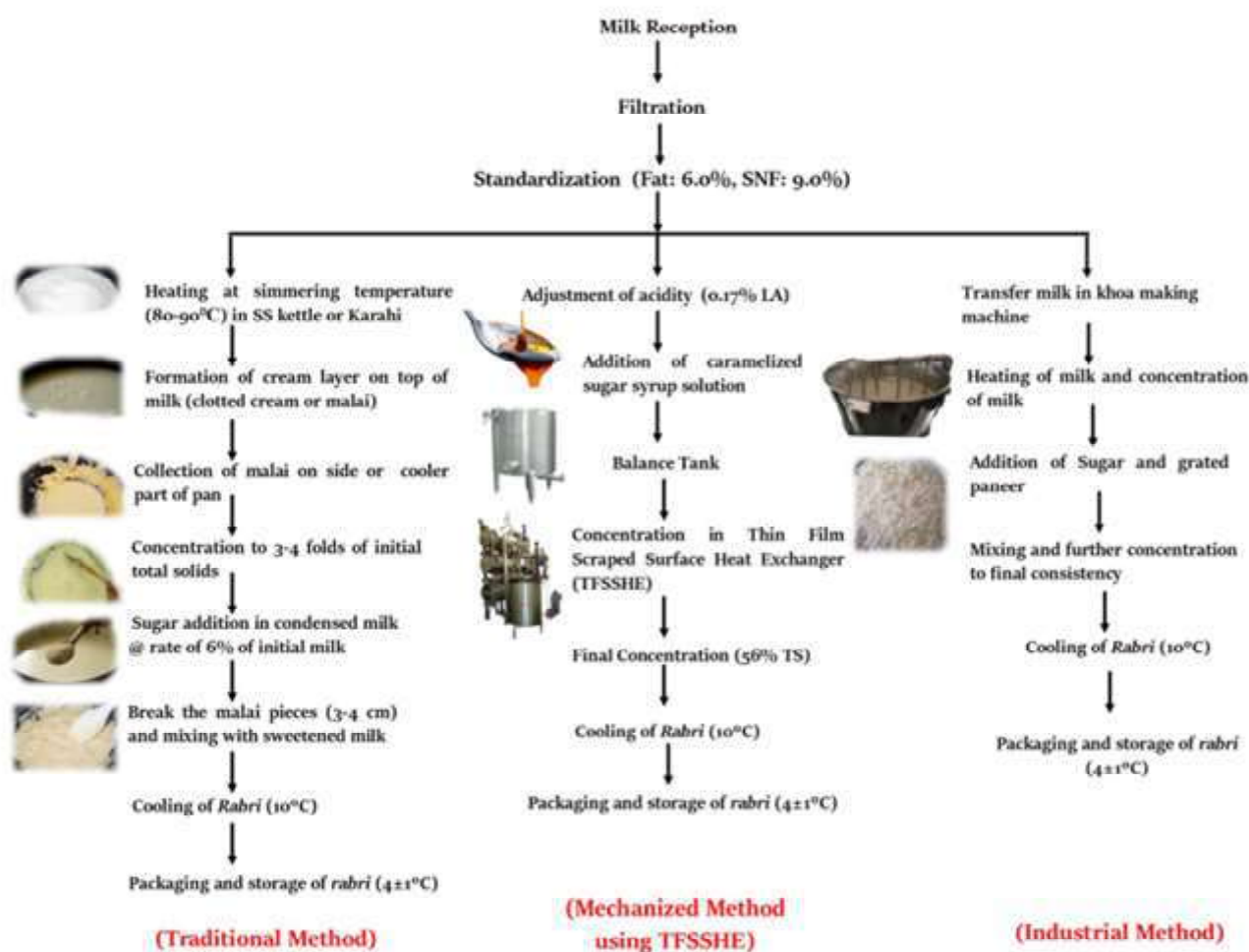


Fig. 2 Flow diagram of rabri manufacturing by different methods (i) Traditional Method, (ii) Mechanized method using TFSSHE, and (iii) Industrial Method

counts in *rabri* samples were not found to be affected significantly ($p \leq 0.05$). The increment in contamination primarily depends on milk type and storage temperatures. Studies recommend that buffalo milk is the most suitable for preparation of optimum quality *rabri*. Samples remained fit for human consumption up to 15 days at 5°C.

Yield of *rabri*

Yield of *rabri* mostly depends on milk type and amount of sugar added. Average yield of *rabri* was found to be 28.07, 28.93 and 29.77% for cow, mixed and buffalo milk, respectively. Furthermore, with the addition of 4, 6 and 8% sugar levels, the yield of *rabri* was 27.27, 28.9 and 30.6%, respectively (Pandey et al. 2004).

Ghayal et al. (2015) prepared dietetic *rabri* using 10 kg standardized milk (4 % fat and 8.5 % SNF). This milk was strained followed by Inulin (50 g or @ 0.5 % of initial milk) addition. Thereafter, it was heated at 85-90 °C (simmering temperature), kept undisturbed employing controlled heating and fanned to

accelerate the formation of skin over its surface which was subsequently removed and collected. After achieving the desired concentration of milk (i.e. 2 kg concentrated milk), the collected skin layers were mixed and cooled at 37-42 °C followed by the addition and mixing of 32 g aspartame. This process results in 2.13 kg final product with 21.30% yield.

Chemical composition of *rabri*

The chemical composition of dairy products is governed by the chemical makeup of the raw material and other ingredients used and the additives added during their preparation. Hence, chemical composition of *rabri* is governed by the constituents present in milk intended for its preparation, degree of concentration achieved and the level of sugar and other ingredients added. *Rabri* composition widely differs with its type, region, however, milk used for product manufacturing had the major effect. Gayen and Pal (1991b) prepared *rabri* from buffalo milk and reported a gross composition containing 49.8% moisture, 15.5% fat, 9.50% protein, 11.3% lactose, 12.0% sugar and 2.0% ash. Further, Pandya and

Khan (2008) reported that percent TS, lactose, fat, protein, sucrose and ash contents of their experimental *rabri* sample were 51, 11, 16, 10, 12% 1.9, respectively.

Khaskheli et al. (2008) evaluated *rabri* samples and observed variation. The range of percent moisture, fat, protein, total carbohydrates and ash contents were 24.33 - 38.85, 16.23 - 22.55, 9.94 - 12.01, 27.08 - 43.72% and 2.09 - 2.84, respectively. The energy values of *rabri* samples ranged between 315.59 - 400.15 Kcal/100g.

As per Rai et al. (2017) *rabri* manufactured from buffalo milk with 6% added sugar comprised of 63.28% TS, 9.87% protein, 20.33%

fat, 30.39 % total carbohydrates (lactose plus sugar), and 2.14% ash content. Furthermore, *rabri* prepared from cow milk and mixed milk (buffalo and cow milk mixed in 1:1 ratio) had 56.78% and 62.68% moisture, 14.74% and 17.83% fat, 9.70% and 9.83% protein, 30.20% and 32.80% total carbohydrates and 2.14% and 2.22% ash content, respectively. Based on sensory evaluation, researchers concluded that *rabri* prepared from buffalo milk fetched better scores for flavor, consistency, color, sweetness and overall consumer acceptability.

Table 2 shows the detailed chemical composition of different *rabri* samples either prepared from different milks or collected from market.

Table 2: Chemical composition of *rabri* samples prepared from different types of milks, manufactured on commercial scale and collected from different markets

Rabri samples	TS (%)	Fat (%)	Protein (%)	Sucrose (%)	Total carbohydrates (%)	Ash (%)	Reference
Prepared from buffalo milk							
<i>Rabri</i> (Buffalo Milk)	50.2	15.5	9.50		23.3	2.0	Gayen and Pal (1991)
BM (%fat/ % SNF)	63.28	20.33	9.87		30.39	2.14	Rai et al. (2017)
Prepared from cow milk							
<i>Rabri</i> (Cow Milk) with 6% sugar	56.78	14.74	9.70		30.20	2.14	Rai et al. (2017)
<i>Rabri</i>	51	16	10		23.0	1.9	Pandya and Khan (2008)
<i>Rabri</i>	70	20	10		27	2.0	Srinivasan and Anantkrishnan, (1964)
<i>Rabri</i>	65	20	13		31	3.5	Bandyopadhyay and Mathur, (1964)
Prepared from mixed milk							
<i>Rabri</i> (Mixed Milk, buffalo and cow milk in 1:1 ratio) with 6% sugar	62.68	17.83	9.83		32.80	2.22	Rai et al. (2017)
Industrial/ commercial sample							
<i>Rabri</i> from Amul		18	6.2	11	22.2	-	https://amul.com/products/amul_rabri_info.php
<i>Rabri</i> from Ananda Dairy		16	7.0	8.5	22.5	-	https://www.jiomart.com/p/groceries/gopaljee-ananda-royal-rabri-80-g/590087607
Market samples							
<i>Rabri</i> market samples	61.15 - 75.67	16.23 - 22.55	9.94 - 12.01		27.08 - 43.72	2.09 - 2.84	Khaskheli et al. (2008)

Ghayal et al. (2015) prepared dietetic *rabri* adopting the process flow chart depicted in Figure 3. This contained 38.7 % moisture, 25.1 % lactose, 20.8 % protein, 12 % fat and 2.9 % ash. The calorific value of the dietetic *rabri* was 291.60 Kcal/100g. Conventional *rabri* prepared by De (1980) contained 30 % moisture, 17 % lactose, 10 % protein, 20 % fat, 3 % ash and 20 % sugar and 368 kcal/100 g.

Innovations in *rabri* manufacturing

Rabri formulated using different fruits

Beside conventional *rabri*, researchers have incorporated different other ingredients and prepared *rabri* variants for the supplementation and complementation of benefits.

Pawar et al. (2011) prepared *rabri* incorporating mango pulp @ 2, 4, 6 and 8% of initial weight of buffalo milk. Based on physicochemical and sensory characteristics, *rabri* prepared with 6% mango pulp incorporation was had maximum acceptability. Chemical composition of optimized *rabri* was 16.90% fat, 58.49% total solids, 11.67% protein, 13.17% reducing sugar, 15.48% non-reducing sugar, 0.32% LA acidity and 2.28% ash.

Gite et al. (2017) prepared *rabri* samples via incorporating custard apple (*Annona squamosa* L.) pulp at different (100:0, 80:20, 70:30, 60:40 and 50:50) levels. Sugar was added @ 6% of initial milk during *rabri* preparation. Researchers concluded that 30% level of custard apple pulp incorporated *rabri* indicated the most preferred sensory attributes. Chemical composition of optimized custard apple *rabri* contained 1.96% ash, 14.60% crude fat, 8.70% crude protein, 39.18% carbohydrate, and 0.4% LA acidity.

Kumar et al. (2017) used sapota/*Chikoo* (*Achras zapota*) pulp for *rabri* preparation. During preparation of *rabri*, the level of sapota pulp (3.5–10.5%), sugar (2.5–5%), milk fat (3.5–6.5%) and simmering temperatures (85–90°C) were varied. Product prepared using 8.50% sapota pulp, 3% sugar and 4% milk fat and 89.5°C simmering temperature fetched maximum sensory scores. The yield of this product was calculated as 27.52%.

Dhumal et al. (2018) prepared *rabri* from concentrated Fig (*Ficus carica* L.) pulp and sweetened condensed milk (SCM). Fig incorporated *rabri* was prepared by SCM mixing with concentrated fig pulp (35° brix). *Rabri* prepared from combination of 150 g of concentrated fig pulp and 1 L of SCM was referred as the best based on sensory evaluation with overall acceptability scores of 8.65 amount different combination. The cost of *rabri* thus prepared was calculated to be 237.81/kg.

Kahandal et al. (2020) prepared *rabri* from Khajur (*Phoenix dactylifera*) crush and the same was incorporated at 3%, 6%, 9% and 12% levels. It was observed that 6% khajur crush containing *rabri* samples exhibited the most acceptable sensory score of

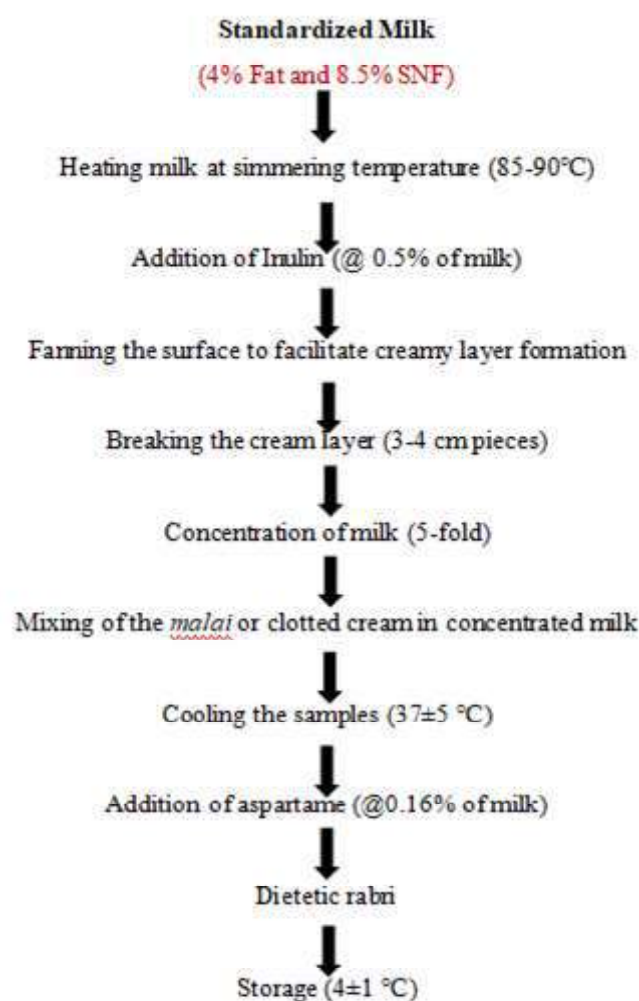


Fig. 3 Manufacturing Protocol for Dietetic Rabri by using Inulin and Aspartame (adopted from Ghayal et al. (2015))

7.81, 8.06, 7.89 and 7.89, colour and appearance, flavour, body and texture and overall acceptability, respectively.

Rabri formulated using sugar alternatives

Kumar (2012) developed a dietetic *rabri* from cow milk in which refined sugar was replaced with artificial sweetening agent such as sucralose, aspartame and acesulfame -k. Addition of 350 ppm sucralose, 1400 ppm acesulfame-k and 1600 ppm aspartame resulted in equivalent sweetness perception in *rabri* compared to control product prepared with 6% sugar addition. Dietetic *rabri* prepared with sucralose as sweetener (100% replacement) fetched maximum sensory scores based on physicochemical and sensory characteristics. Shelf life of dietetic *rabri* was ~10 days at 5±1 °C. Addition of artificial sweetener was within in the limits approved in Food Safety Standards Authority of India (FSSAI).

Table 3: Compositional variation in *rabri* prepared using different ingredients

Type of <i>Rabri</i>		Chemical composition (%)						Ash	% LA acidity	Calorific value	Reference
		TS	Moisture	Fat	Protein	Sugar	Total carbohydrate				
<i>Rabri</i> with custard apple pulp (30%)		64.44	35.56	14.6	8.7	-	39.18	1.96	-	-	Gite et al. (2017)
Mango incorporated <i>rabri</i> (6%)	pulp	58.49	41.51	16.90	11.67	15.48	13.17	2.28	0.32	-	Pawar et al. (2011)
Dietetic <i>rabri</i> with sugar replacer (Aspartame) and partial fat replacer (Inulin)		61.3	38.7	12	28.0	-	25.1 (Lactose)	2.9	-	291.6 kcal/100g	Ghayal et al. (2015)
<i>Rabri</i> by using date (<i>Phoenix dactylifera</i>) syrup as sugar substitute		60.80	39.20	23.5	13	-	-	2.84	0.08	-	Kaushik et al. (2016)

Acceptability of *rabri* decreased with increasing the level of artificial sweetener that was mainly attributed to its poor flavour. It was concluded that 100% replacement of sugar with artificial sweetener leads to several defects in *rabri* major includes cooling sensation in mouth and bitter after taste.

Bharti et al. (2018) prepared dietetic apple *rabri* via incorporation of 20% apple crush and 0.3% stevia using in buffalo milk followed by its 2.5- 3 folds concentration. Based on sensory evaluation and microbial analysis, the product was reported to be stable at 5 °C up to 15 days.

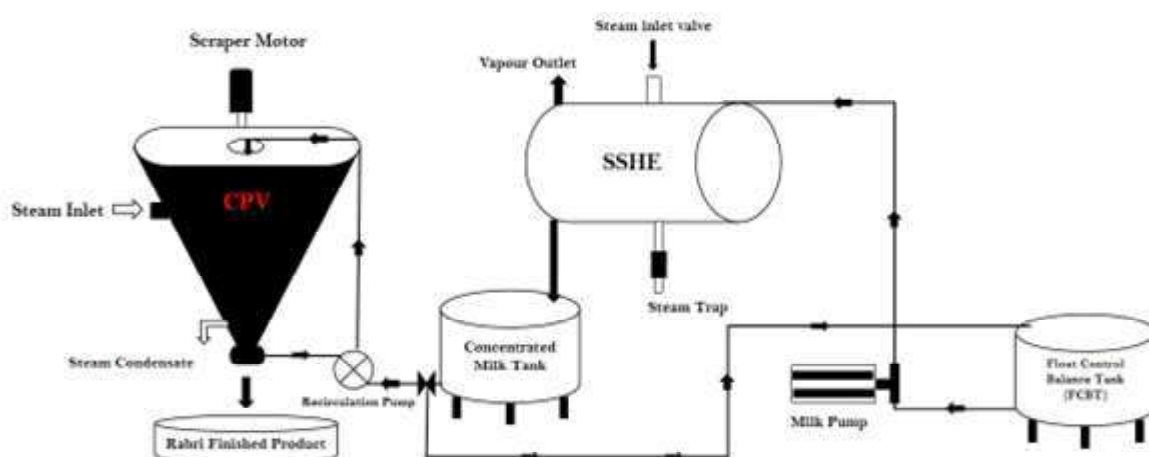
Kaushik et al. (2016) prepared *rabri* incorporating date (*Phoenix dactylifera*) syrup as sugar substitute. *Rabri* prepared with ratio of date syrup and milk (6.8:93.20) contained 39.20% moisture, 0.08% LA acidity, 2.84% ash, 13% protein and 23.5% fat. Date syrup *rabri* was found to be stable up to 16 days under refrigeration temperature. However, the sensory qualities decreased with storage; and pH, acidity, fat and protein and moisture showed an increasing trend with the advancement of the storage period.

Kaur (2002) prepared *rabri* using aspartame as sugar replacer. It was added in different levels (0.025%, 0.030% and 0.035% of initial level of milk) in standardized milk samples containing 3%, 4.5% and 6% fat contents. Product was packed in Polypropylene (PP) cups. Researchers concluded that addition of aspartame at the rate of 0.03% level in standardized milk (4.5% fat) resulted in best quality *rabri*. Furthermore, microwave treatment at different power levels such as 150W/240 s, 300W/120 s and 400 W/75 s were also studied for shelf life extension. Shelf life of untreated *rabri* enhanced from 2 days to 7 days (for microwave treated sample) at room temperature (14-18!)

Ghayal et al. (2015) prepared dietetic *rabri* from standardized milk (Fat 4.0%, SNF 8.5%) in which sugar and fat replacement was targeted using Aspartame and Inulin. Chemical composition of the formulated dietetic *rabri* was determined. It had 38.70 % moisture, 25.10% lactose, 28.0% protein, 12% fat and 2.9 % ash contents with 291.6 kcal/100g calorific value. Table 4 shows compositional variation of *rabri* prepared using different ingredients.

Ghayal et al. (2015) studied the enhancement in shelf-life of prepared *rabri* samples using different packaging conditions. During storage, the change in 5-hydroxymethylfurfural (HMF), thiobarbituric acid (TBA) and free fatty acid (FFA) contents of *rabri* samples packed in different conditions were determined. It was observed that the HMF content of fresh *rabri* sample was $3.02 \pm 0.04 \mu \text{ moles}/100 \text{ g}$ which increased to $3.37 \pm 0.05 \mu \text{ moles}/100 \text{ g}$ in modified atmosphere packaging (MAP) filled with 50 % CO_2 :50 % N_2 and also increased to $3.33 \pm 0.05 \mu \text{ moles}/100 \text{ g}$ in 100% N_2 MAP filled. The MAP filled with 100% atmospheric air showed highest increased in HMF ($3.38 \pm 0.04 \mu \text{ moles}/100 \text{ g}$) content after 9 days of storage. MAP packed with inert gaseous environment was less prone to Maillard reaction compared to control conditions. Higher TBA value of sample packed in MAP (0.23-0.25) over that of control sample (0.22), clearly indicated that during storage, oxidative changes occurred in MAP in dietetic *rabri*. Free fatty acid formed due to lipid hydrolysis. FFA content of *rabri* sample packed in MAP with 50 % CO_2 :50 % N_2 increased from 1.24 ± 0.19 to $36.7 \pm 2.56 \mu \text{ eq/g}$ while it increased to $29.31 \pm 2.15 \mu \text{ eq/g}$ in MAP with 100% N_2 FFA value during 30 days of storage period. Increased in FFA content of sample packed with 100 atmospheric air (control sample) was highest, with FFA content; $28.2 \pm 2.90 \mu \text{ eq/g}$ after 9 days of storage period. Sample packed with MAP was devoid of rancidity during storage. It was

Fig. 4 Design of in-line *rabri* production unit (adopted from Chopde et al. 2016).



concluded that shelf life of the developed dietetic *rabri* could be extended up to 30 days at 10 °C by MAP packaging (N₂, CO₂ and their combination).

Preparation of *rabri* powder

Pandey et al. (2009) formulated freeze-dried *rabri* powder employing RSM. Initially, milk was concentrated up to 34% TS using a steam kettle. Further, subjected to freeze drying up to 2.40% moisture. Almond and pistachio nuts were also added into *rabri* for functional enrichment. The total fatty acid profiling of the prepared powder was achieved by gas chromatography. It was observed that linoleic and linolenic acids content were decreased while saturated fatty acid varied slightly. Value of TBA and FFA showed an increasing trend during storage. Microbial count such as SPC, yeast and mold counts did not increased during storage and pathogens (such as *E. coli*, *Salmonella* and *Staphylococcus aureus*) remained absent throughout the storage span. The developed product had a calorific value of 494 Kcal/100 g. The prepared powder was reported to be stable up to 6 months at studied (28±5 °C) temperature.

Mechanization in *rabri* manufacturing

Acharya (2003) developed commercial method for production of *rabri* utilizing thin film scrapped surface heat exchanger (TSSHE) and vacuum evaporator. Buffalo milk (6% fat) containing 6% sugar was concentrated in TSSHE at 2.0 kg/min flow rate with 3.0 and 0.8 kg/cm² steam pressure in 1st and 2nd cylinders, respectively. Milk was concentrated up to 51% TS followed by addition of 5% shredded *chhana* and 5% *khoa*. Prepared product held a shelf-life of ~2 days at 30±1°C and 16 days 5±1°C.

Kumawat (2013) also designed a system for in-line *rabri* production. In reported design, clotted cream layer for *rabri* production was collected by means of forced convective air flow that resulted in production of better quality *rabri* in terms of

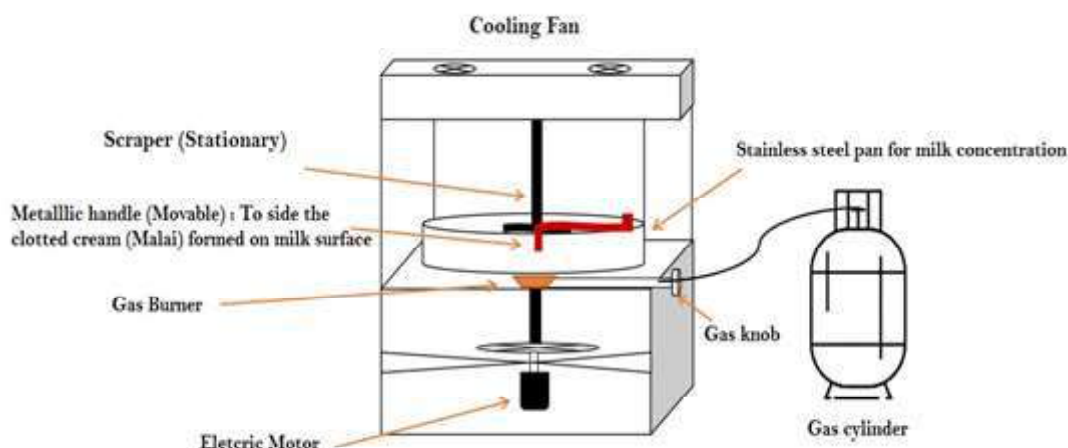
sensory attributes, textural parameters, color and chemical composition. Optimized parameters for this in-line production of *rabri* constituted air velocity 4.5 m/s, fan angle 30° and 71°C milk temperature in CPV.

Three staged thin film SSHE was used for continuous manufacturing of *rabri* via optimization of the processing parameters. Speed of scraper in first, second and third stage of SSHE were optimized at 127, 121 and 15 rpm, respectively with 151 kg/h milk flow rate and 0.17% LA milk acidity. Prime quality *rabri* was obtained with TS of 29.9% in SSHE and 39% TS final concentration of milk in CPV before the addition of sugar (Chopde et al. 2013).

Singh (2016) developed semi-automatic *rabri* filling system. The 45% TS containing *rabri* containing was selected for filling operation out of 40%, 45%, 50% and 55% on the basis of engineering properties of *rabri* such as density, rheological characteristics and color. Different variables of filling system were optimized by RSM methodology such as filling temperature 40 °C, agitator speed 15 rpm, speed of flow regulator screw 35 rpm and inclination angle 0°. Filling speed was kept highest in PLC based system (15 cups/min, 100 g/cups) followed by pedal system (13 cups/min) and in manual (10 cups/min).

Chopde et al. (2016) designed an in-line *rabri* production system which consisted a scraped surface heat exchanger (SSHE) and conical process vat (CPV). In-line manufacturing of *rabri* from buffalo milk (6% fat and 9% SNF) and processing parameters such as initial milk concentration in SSHE, final concentration in CPV, ratio of clotted cream layer (CCL) to SCM and steam pressure during CCL removal from CPV were subsequently optimized at 30%, 40%, 0.167 and 0.8 kg/cm², respectively. Firmness of *rabri* prepared from designed machine ranged from 0.263 N to 0.551 N. Design of in-line production unit of *rabri* is shown in Figure 4.

Fig. 5 Design of semi-automatic *rabri* making unit operated on LPG and electricity.



Kumawat (2017) designed milk flake formation system for *rabri* production. The system consists vertical SSHE, scraper blade assembly, drive motor, milk distributing unit and control unit. Buffalo milk with initial 26% TS and 1.5 kg/cm² steam pressure, 130 rpm scraper speed and 400 L per hour milk flow rate led to better physico-chemical properties and sensory scores. Study concluded that designed milk flake formation system can be used in commercial production of *rabri* with typical sensory attributes.

A semi-automated *rabri* making machine was also used that was operated on liquefy petroleum gas (LPG) and scraper rotated by the electric motor. This machine consists of a movable and static part that gives typical body and texture to *rabri* (also known as *laccha rabri*). About 12 L milk was poured into stainless steel pan and boiled by LPG gas burner. The SS pan continuously rotated (17-20 rpm) by electric motor, static scraper avoid burning and sticking of milk to the pan surface. *Rabri* making machine equipped with movable SS handle that continuously aside the *malai* or cream formed during concentration of milk. Sugar was added in concentrated milk @ of 6% of initial milk volume and clotted cream (*malai*) was cut into small pieces before packaging. Fan placed on top of the machine facilitated fast cooling of *rabri*. This machine is available in the market and used by several manufacturers for *rabri* making. Its design is depicted in Figure 5.

Microbiology of *rabri*

Pooja et al. (2020) reported that microbial count of street market sample collected from Ramnagar, Varanasi was ranged from Log₁₀ CFU/ml for coliform count, 8.30 - 9.30 Log₁₀ CFU/ml for total plate count (TPC) and 6.69 to 7.20 Log₁₀ CFU/ml for yeast and mold count.

Ghayal et al. (2015) evaluated microbial quality of dietetic *rabri* prepared using aspartame and inulin as sugar and fat replacers and packed in MAP with different combination of N₂ and CO₂ at 10! of storage temperature. TPC of sample packed in MAP with

50% N₂ and 50% CO₂ and MAP with 100% N₂ were ranged from 5.2 to 6.2 Log₁₀ CFU/ml and 5.2 to 5.9 Log₁₀ CFU/ml during 30 days of storage, while increase in TPC of control sample ranged from 5.4 to 6.4 Log₁₀ CFU/ml during 9 days of storage period. MAP containing 50% N₂ and 50% CO₂ and MAP with 100% N₂ had yeast and mold count from 5.6 to 6.3 Log₁₀ CFU/ml and 5.6 to 5.9 Log₁₀ CFU/ml during 30 days of storage. Yeast and mold count of air filled *rabri* sample was highest and ranged from 5.6 to 6.4 Log₁₀ CFU/ml during 9 days of storage.

Common adulterants and their adulteration in *rabri*

Quality of raw milk governs the final quality of dairy products prepared from it. Hence, raw milk should be tested for organoleptic, chemical, physical and microbiological quality to ensure its suitability in formulation of fine quality dairy products. Use of adulterated milk lead to adulteration of final *rabri* as well.

Most common adulteration in *rabri* includes the replacement of milk fat with cheap source of fats such as vegetables fat or oils. Adulteration of vegetables fat has been detected by various methods such as via estimation of Butyro Refractometer (BR) reading of ghee or Boudouins Test. Other adulterant such as blotting paper as thickening agent to increase consistency of *rabri* at low total solids contents. The blotting paper adulteration can be detect by simple method, in which a teaspoon of *rabri* sample taken into test tube followed by addition 3 ml hydrochloric (HCl) acid and 3 ml of distilled water. Uniformly mix the content with glass rod and remove the rod and examine. Presence of fine fiber on glass rod will indicate the presence of blotting paper in *rabri*. So far FSSAI (2020), has not defined any legal standard pertaining to chemical composition and microbial quality of *rabri*. In lack of legal standards, huge variation in quality attributes of *rabri* samples is inevitable.



Fig. 6 Different packaging material used for packaging of *rabri*

Packaging material and shelf life of *rabri*

Traditionally, indigenous dairy products have been packed in leaves, paper cartons or paper-board boxes. These materials do not provide sufficient protection to *rabri* from manual handling practices and atmospheric contamination's and also absorb the undesirable foreign odors. Consequently, this product soon lose its typical body and texture and, also lose their typical aroma attributes. This also favor unwanted mold growth.

Rabri is generally sold in earthen containers and loose polystyrene cups of 50-100 g capacity by small vendors. Commercially manufactured *rabri* is packed into polystyrene cups with a covering of aluminium foil. A well reputed commercial brand sample had 10 days shelf life at 4°C. Products similar to *rabri* like *basundi* are also packed in polystyrene cups apart from tetrapaks (UHT treated) and retort packs for extending its shelf-life. Different packaging material used for different form of *rabri* packaging were shows in Figure 6. Flexible packaging materials like metallic pouches can also be utilized for *rabri* packaging. Novel packaging techniques like intelligent and active packaging have fulfilled the requirement of current market as consumers demand with real time monitoring of product (Wyrwa and Barska 2017). Intelligent packaging indicates the customers about the running quality and shelf life of products by placing sensor and indicator inside the packages (Lee and Rahman 2014). Advanced packaging techniques when employed have the potential to serve as a vehicle not only for packaging of traditional sweets like *rabri*, *basundi* and other sweets but to capture more customers in domestic and international market by delivering quality and safe product.

Conclusions

Presently, the production of *rabri* is scattered and mainly governed by *halwais* using traditional methods. Although, several commercial models of *rabri* have been developed but very few mechanizations could be successfully employed for

rabri making owing to its sensitive quality and sensory parameters. Some milk brands have attempted to commercialize *rabri* in selected markets on commercial scale such as Amul and Ananada. The wide array of indigenous milk products are poised to take strong industrial footing in the years ahead with the development and application of mechanized manufacturing technologies and unit operations. However, further emphasis needs to prioritize in scheduling and standards process along with the appropriate production line facilities for preparation, storage and testing of *rabri* so as to minimize the errors in quality attributes and also to enhance its consistency in terms of sensory and quality parameters.

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RESEARCH ARTICLE

Checking the feasibility of utilizing pectin as a stabilizer in ‘*Bhapa dahi*’

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Abstract: The investigation was carried out to check the feasibility of utilizing the pectin as stabilizer in *Bhapa dahi*. *Bhapa dahi* was prepared adopting the process standardized by using thermophilic starter culture. A blend of *chakka* and sweetened condensed milk (SCM) [1:1 w/w] served as the base mix; optimum steaming period was 15 min. The mango pulp was added at the rate of 18% of the base mix. The moisture, fat on dry matter (FDM), protein and pH of mango *Bhapa dahi* were 52.48-52.72%, 15.69-15.72%, 7.83-7.89% and 5.18-5.19 respectively. *Bhapa dahi* samples had compositional values that were similar to values laid down by FSSAI for fruit *shrikhand*. On the basis of the findings of the experiment, it was decided not to utilize pectin in the formulation of *Bhapa dahi*, since it exerted some adverse impact on the flavour and body and texture of product. For sensory evaluation 100 point scorecard was used and *Bhapa dahi* made without pectin had highest sensory score of 92.68 (0.0% pectin), 89.93 (0.1% pectin) and 88.65 (0.2%).

Keywords: Pectin, Thermophilic starter culture *Chakka* and Sweetened Condensed Milk

Introduction

‘*Bhapa dahi*’ is a famous Bengali delicacy prepared domestically in West Bengal, India. It is basically a steamed sweetened *dahi* (similar to concentrated Yogurt) and is also known as the Indian cheese cake’. The conventional method for manufacture of ‘*Bhapa dahi*’ is time consuming as it involves concentrating of the milk by slowly condensing it, setting the *dahi* through the incubation with starter culture, then steaming it to form a firm gel structure like mass. The gelled structure and sweet-acidic taste are main characteristics of such product.

The scientific research on ‘*Bhapa dahi*’ is very limited. Bhattacharya et al. (1979) and Bhattacharya et al. (1980) had worked on *Bhapa dahi*. Then after a long period of time, Patel et al. (2016) had standardized the formulation and technology for preparation of *Bhapa dahi* utilizing a blend of *chakka* and sweetened condensed milk (SCM) (*chakka*: SCM, 1:1 w/w – served as base mix) as the base mix to which mango pulp (@ 18% by weight) was incorporated for flavouring and value addition. In the present investigation attempt had been made to check the feasibility of pectin (@ 0.0, 0.1 and 0.2% by wt of base mix) as stabilizer in manufacturing of *Bhapa dahi*. The base mix was subjected to steaming for 15 min. to obtain the set product after which the product was cooled and kept under refrigeration. Fruits are perceived as part of healthy diet by the consumers. The association of fruit with *dahi* would further support its health image. As consumers connect both these foods with health and wellness, the two categories of fruits and cultured milk products

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are typical example of fusion dairy products offering health, flavour and convenience. Value addition to fermented milk foods can be accomplished through use of natural fruit or vegetable juices/pulp/particulates incorporated into milk solids. Such practice will not only enhance the palatability of product, but also enrich them with phytochemical i.e. β -carotene (a precursor of Vitamin A) and mineral content too. Mango (pulp form) as well as carrot (cooked and sweetened) are well established natural flavourings for a variety of food products viz., stirred yogurt, frozen yogurt, etc. (Agawam and Prasad 2013; Mbaeyi-Nwaoha et al. 2017). Patel et al. (2017) reported that using mango pulp as flavouring in *Bhapa dahi* got higher sensory scored compared to carrot shreds.

Stabilizers have been used in food products for a variety of reasons; including thickening, aiding stability and improving mouth feel (Phillips and Williams, 2000). In stirred yogurt, the particles of fragmented acid-casein gel remain in contact with one another, giving a self-supporting structure. In case of over-incubation, mistreatment during handling of product or use of lower milk solids in yoghurt/dahi, 'wheyng-off' may occur which leads to separation into a casein-rich lower layer and an upper layer of clear 'serum'. Hydrocolloid stabilizers are commonly used to retard separation in acidic milk drinks (Kiani et al. 2010). Stabilizers, such as pectin or gelatin are often added to the milk base (~ 0.4 %) to enhance or maintain appropriate yogurt properties including texture, mouth feel, appearance, viscosity and to minimize wheyng-off (Tamime and Robinson, 1999)

Pectin is widely used as a functional ingredient in the food industry due to its ability to form aqueous gels and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products (Everett and McLeod, 2005). High methoxyl pectin (HMP) is best known as the gelling agent in jams and marmalade, but its use in acid milk drinks is of comparable commercial importance. In commercial HMP, 65.0-75.0 % of the galacturonate residues occur as methyl ester. The ester content can be reduced by pectin methyl esterase enzymes, or by hydrolysis with alkali or acid, to give low-methoxyl pectin (LMP), which typically has a degree of esterification (DE) of 30.0-35.0 % (Kiani et al. 2010). In food industry it is a common practice to add functional hydrocolloids as stabilizers, such high methoxyl pectin (Laurent and Boulenger, 2003) and carboxy methyl cellulose (Ntazinda et al. 2014), to avoid excessive aggregation of milk proteins in acidified milk beverage products. Pectin molecules interact with casein through calcium ions and prevent protein aggregation, sedimentation. Hence, presence of pectin prevents serum separation through ionic and steric stabilization in acidic milk beverages (Foley and Mulcahy 1989; Atamer et al. 1999; Lucey et al. 1999 and Jensen et al. 2010).

Bhattarai et al. 2015 reported that gelatin was added at the rate of 0.2 % showed less syneresis compared to control yoghurt. Yoghurt made with pectin (0.05%, w/v) and WPC-80 (1%, w/v)

exhibited 15% less susceptibility to syneresis and also had significantly higher water holding capacity (~56%) than the control (33%) yoghurt samples (Gyawali and Ibrahim, 2018). The current study was attempted to add-value to '*Bhapa dahi*' by incorporating fruit (mango pulp) in line with the 'Fruit Yoghurt' and the influence of food additive like stabilizer i.e. pectin was studied.

Materials and Methods

The ingredients used in the manufacture of *Bhapa dahi* is as detailed below.

Dairy ingredients

Tea special milk (Amul brand – homogenized milk having 4.5% fat, 8.5% SNF) was procured from Amul Shoppe, Anand, India to prepare the product. Partly skimmed Sweetened Condensed Milk (SCM) (M/s. Nestle Co., brand Milkmaid) packed in tin can was procured from Granary, Anand, India.

Non-dairy ingredients

Lyofast Y170F, Sacco yoghurt culture (freeze dried lactic cultures; Cadorago Co., Italy) was used for the preparation of curd. For the preparation of curd, 1 UC (UC is standard unit) was sufficient to convert 100 l. of milk into yogurt. Alphonso tinned mango pulp (M/s. Vadilal Co., Ahmedabad, Gujarat, India) was purchased from local market in Anand, Gujarat, India. Pectin (Poly-D-galacturonic acid methyl ester) was used as a stabilizer. It was procured from M/s. Himedia Laboratories Pvt. Ltd., Mumbai having 65.0-70.0 % degree of esterification.

Preparation of Bhapa dahi

The method standardized for preparation of *Bhapa dahi* by Patel et al. (2016) was followed. All the selected ingredients (i.e. *chakka*, SCM, mango pulp and HMP) were weighed accurately as per the calculations based on the formulation. Hobart mixer (M/s. Hobart, Corporation, Ontario, Canada, Model No. N 50) was used to prepare the base mix. The flow diagram for preparation of *Bhapa dahi* is shown in Figure 1. The experiment was replicated five times.

Results and Discussion

The approximate composition of mango pulp and partly skimmed sweetened condensed milk is shown in Table 1. The *Bhapa dahi* should be seen clean when it to be cut, custard-like, firm and preferably without whey separation. The consistency of *Bhapa dahi* can be enhanced through use of suitable type and quantity of stabilizer. Since the current experiment entails use of mango pulp as one of the flavouring, it was anticipated that the citric acid contained in it might destabilize the milk protein present in sweetened condensed milk and maybe in *chakka* too. Hence, it

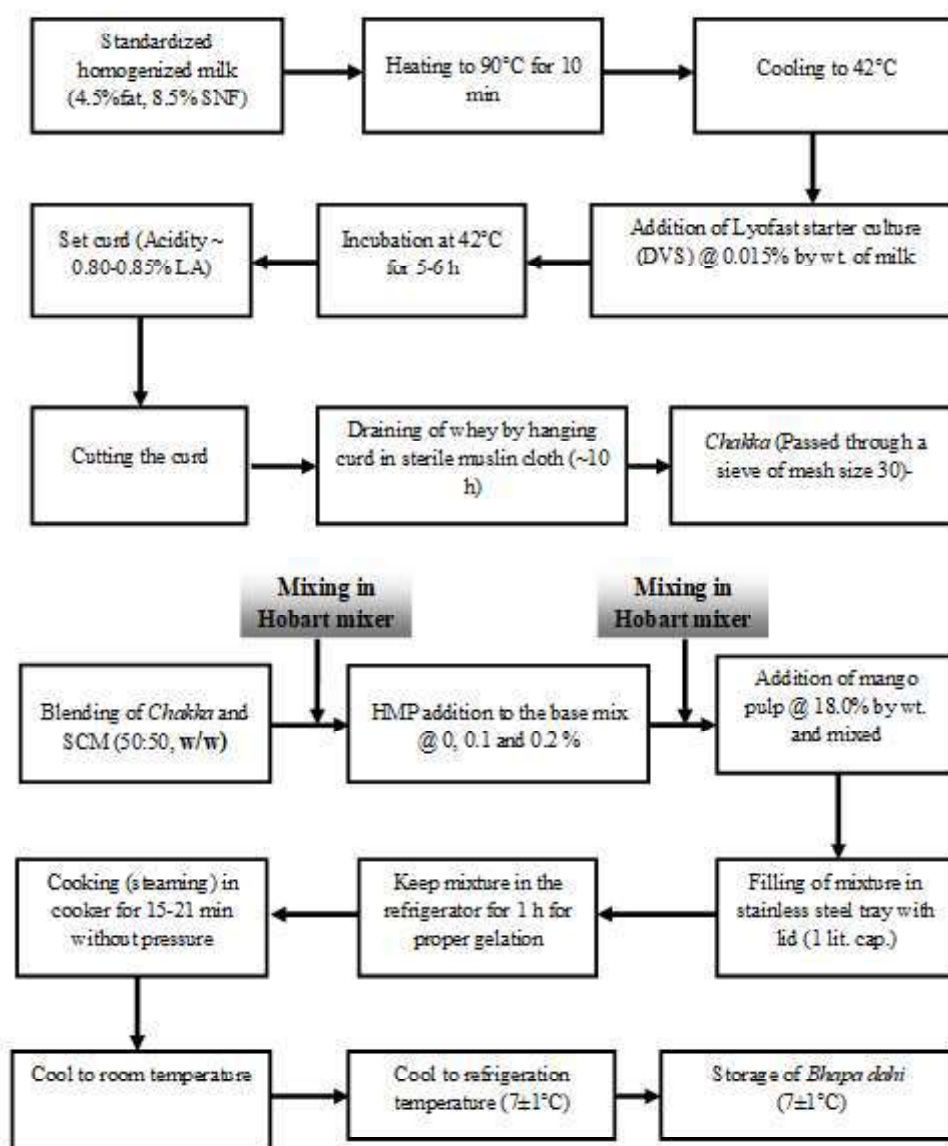


Fig. 1 Preparation of *Bhapa Dahi* using pectin as stabilizer

Table 1: Proximate composition of *Chakka*, Partly Skimmed Condensed milk and Mango pulp used as intermediate material in the preparation of *Bhapa dahi*

Parameters	<i>Chakka</i>	Sweetened Condensed Milk	Parameters	Mango pulp
Fat	12.50 %	3.90 %	Colour of pulp	Orange
Protein	6.50 %	8.20 %	TSS	24%
Total Carbohydrates*	6.40 %	56.50 %	TS	26.15%
Ash	0.90 %	1.95 %	pH	3.80
Total solids	26.30 %	70.55 %	Acidity (% citric acid)	0.65
Acidity (% LA)	1.75 %	-		

*carbohydrate was obtained by difference; LA: Lactic Acid

was planned to utilize pectin as a stabilizer in manufacture of *Bhapa dahi* since pectin is reported to exert protective effect on milk protein in acidic food systems (Tromp et al. 2004). In acidified milk systems pectin is reported to adsorb onto the casein micelles

as a result of electrostatic interaction (Janhoj et al. 2008). Pectin has been successfully used to stabilize acidic protein drinks, such as drinking yogurt at levels ranging from 0.5 to 1.0 % (Srivastava and Malviya, 2011). Pectin added at the rate of 0.6%

significantly improved the rheological quality of the yogurt and also seems to prevent the exudation of whey during the conservation (Arioui et al. 2017). The use of HMP in yoghurt drinks and yoghurt fruit drinks prevents the aggregation of proteins; in thermized products the proteins are protected against dehydration and sedimentation (Rolin, 2005). Incorporation of HMP in drinking yoghurt led to a product that exhibited good mouth feel characteristics; the product does not become chalky, and there is no sedimentation (Endre and Christensen, 2000).

The stability of Yoghurt may be reduced by addition of fruit concentrates (as flavouring), which therefore requires some stabilizers like pectin or starch in its formulation (Ramaswamy and Basak, 1992). In preparation of *Bhapa dahi* two factors might go against the stability of milk protein viz., (i) addition of mango pulp (acidic in nature) to the base mix, and (ii) steaming of the base mix containing added mango pulp. Since, pectin is reported to interact favourably with milk protein, conferring stability to it, it was envisaged to see whether inclusion of pectin can afford protection against 'syneresis' (during cutting and storage of *Bhapa dahi*) or even improve the mouth feel of product. High Methoxyl Pectin (HMP) was added to the base mix at levels of 0 (control), 0.1 and 0.2 % by weight of base mix (i.e. *chakka* + sweetened condensed milk) in the preparation of '*Bhapa dahi*'.

Each treatment was replicated five times. The photographs of *Bhapa dahi* made using two levels of pectin and control product are shown in Figure 2.

Composition of *Bhapa dahi* as affected by incorporation of pectin

The average values for different parameters depicted in Table 2 show that incorporation of HMP at levels of 0.1 and 0.2 % did not have any significant influence on most of the chemical constituents; the only parameter affected was the moisture content of *Bhapa dahi*.

The moisture content of *Bhapa dahi* made using 0.2 % HMP was significantly ($P < 0.05$) higher than the one containing zero (i.e. control) and 0.1 % level. The latter two *Bhapa dahi* samples were at par with each other with regard to the moisture content. Pectin is known to be a good stabilizer suited in acidic and sugar containing media (Thakur et al. 1997). The stabilizers are known to hydrate water in the food system and hence, even after steaming, the amount of moisture retained in *Bhapa dahi* containing 0.2 % HMP was significantly ($P < 0.05$) higher than the other two samples (i.e. control and other containing 0.1 % pectin). In case of mango soy fortified set yoghurt, as the level of pectin was raised from 0.2 to 0.6 % level, the initial moisture content of

Fig. 2 Effect of addition of pectin @ 0.0, 0.1 and 0.2% w/w on *Bhapa dahi*



Table 2: Physico-chemical properties of *Bhapa dahi* as affected by the level of pectin

Constituents [#]	<i>Bhapa dahi</i> * made using pectin at levels (% w/w)			SEm	CD (0.05)	CV%
	0.0	0.1	0.2			
Moisture	52.48±0.09	52.47±0.08	52.72±0.15	0.049	0.15	0.21
Fat	7.46±0.05	7.45±0.04	7.43±0.02	0.018	NS	0.55
FDM ¹	15.69±0.14	15.67±0.12	15.72±0.07	0.050	NS	0.71
Protein	7.89±0.15	7.86±0.03	7.83±0.04	0.053	NS	1.51
Protein (52% moisture)	7.97±0.16	7.94±0.03	7.95±0.04	0.044	NS	1.22
Carbohydrate ²	31.14±0.26	31.20±0.10	31.01±0.12	0.078	NS	0.56
Ash	1.03±0.01	1.02±0.00	1.01±0.02	0.006	NS	1.33
Acidity	1.04±0.1	1.03±0.01	1.03±0.01	0.003	NS	0.74
pH	5.18±0.01	5.19±0.01	5.19±0.01	0.003	NS	0.14

Values placed after ± indicates standard deviation, 1-Fat-on-dry matter, 2-Total carbohydrate taken as by difference, *- product made using *chakka* + sweetened condensed milk (50:50, w/w); # - all values are in % except for pH, acidity is expressed as % lactic acid.

87.74 % marginally decreased to 87.69 % (Kumar and Mishra, 2004).

Textural characteristics of *Bhapa dahi* as affected by the levels of pectin

The data depicted in Table 3 clearly shows that HMP addition had a significant ($P<0.05$) influence on the cohesiveness and adhesiveness of *Bhapa dahi*; hardness remained unaffected (Figure 3).

The cohesiveness of *Bhapa dahi* decreased as the level of HMP was increased from 0.0 to 0.2 % level. Such effect was significantly ($P<0.05$) different, when cohesiveness values was compared between each of the three samples. The protein matrix in yogurt has been reported to be responsible for the cohesiveness in yoghurt (Tunick, 2000). Likewise, the adhesiveness was also significantly affected by the addition of HMP at two levels. Use of 0.1 % of pectin led to *Bhapa dahi* having significantly ($P<0.05$) lower value of adhesiveness when compared with *Bhapa dahi* devoid of pectin. However, when pectin was used at 0.2 % level, it yielded adhesiveness value that was significantly ($P<0.05$) higher than the values associated with samples made using 0 and 0.1 % of pectin. Kumar and Mishra (2004) reported that the hardness, cohesiveness and adhesiveness of mango soymilk fortified yoghurt increased when pectin stabilizer was used up to 0.4 % level. Karaca et al. (2013) reported that use of pectin and guar gum at levels of 5 g/lit. of milk led to increased and decreased firmness of resultant yoghurt respectively. The values of firmness of product being 73.5 and 19.43 g respectively when using pectin and guar gum. They also found that inclusion of such stabilizers

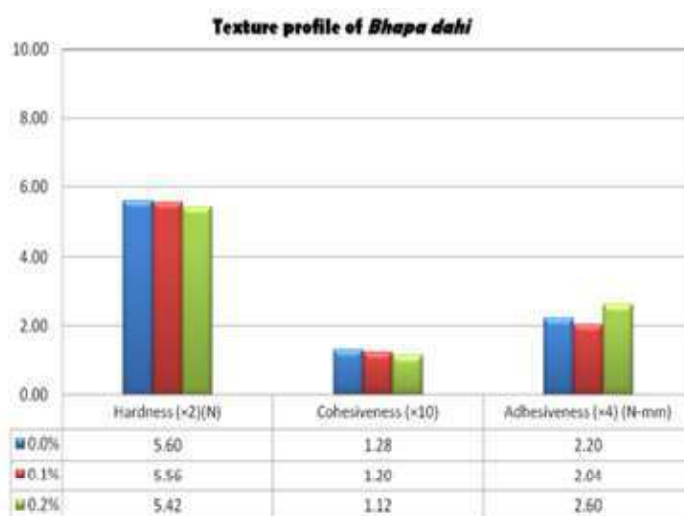


Fig. 3 Influence of pectin addition on the texture profile of *Bhapa dahi*

decreased the cohesiveness of product, the values being 19.37 and 10.20 g respectively for pectin and guar gum containing yoghurt.

Effect of pectin on the sensory properties of *Bhapa dahi*

The data furnished in Table 4 indicates that use of HMP at varying level had a significant influence on most of the sensory properties of *Bhapa dahi*, the exception being for colour and appearance. The photographs of *Bhapa dahi* cut into portion sized forms are

Table 3: Influence of pectin addition on the texture profile of *Bhapa dahi*

Parameters	<i>Bhapa dahi</i> * made using pectin at levels (% w/w)SEm				CD(0.05)	CV%
	0.0	0.1	0.2			
Hardness (N)	2.80 $\pm$ 0.03	2.83 $\pm$ 0.04	2.77 $\pm$ 0.07	0.023	NS	1.86
Cohesiveness	0.128 $\pm$ 0.003	0.120 $\pm$ 0.004	0.112 $\pm$ 0.001	0.001	0.004	2.39
Adhesiveness (N-mm)	0.55 $\pm$ 0.02	0.51 $\pm$ 0.02	0.65 $\pm$ 0.03	0.010	0.03	3.90

Values placed after $\pm$ indicates standard deviation, *- product made using *chakka* + sweetened condensed milk (50:50, w/w)

Table 4: Effect of pectin addition on the sensory scores of *Bhapa dahi*

Sensory attributes	<i>Bhapa dahi</i> * made using pectin at levels (% w/w)SEm				CD (0.05)	CV%
	0.0	0.1	0.2			
Flavour (45)	42.95 $\pm$ 0.73	41.51 $\pm$ 0.33	41.15 $\pm$ 0.30	0.22	0.68	1.18
Body & Texture (30)	27.92 $\pm$ 0.48	26.77 $\pm$ 0.31	25.96 $\pm$ 0.23	0.16	0.49	1.32
Colour & Appearance (10)	8.62 $\pm$ 0.16	8.54 $\pm$ 0.16	8.54 $\pm$ 0.19	0.08	NS	1.99
Acidity (10)	8.21 $\pm$ 0.00	8.11 $\pm$ 0.08	8.00 $\pm$ 0.09	0.03	0.10	0.89
Package [#] (05)	5.00	5.00	5.00	-	-	-
Total score (100)	92.68 $\pm$ 1.11	89.93 $\pm$ 0.80	88.65 $\pm$ 0.30	0.36	1.12	0.90

Values in parentheses indicates maximum scores, # - Full score was given to package, * - product made using *chakka*+sweetened condensed milk (50:50, w/w)

shown in Figure 2. The impact of pectin addition on the sensory properties of *Bhapa dahi* has been discussed focusing on each sensory attribute individually hereunder.

Flavour

The control *Bhapa dahi* (i.e. devoid of pectin) had significantly ($P < 0.05$) greater score for flavour when compared to product made utilizing 0.1 and 0.2 % of HMP. However, the flavour score of both samples of *Bhapa dahi* containing pectin at two levels was at par with each other (Table 4). The *Bhapa dahi* made using 0.1 and 0.2 % HMP had slightly flat and bland flavour, the flavour perception was somewhat subdued. Use of higher or excess level of stabilizer is reported to slightly impair the flavour characteristics of fermented milk product. Pectin negatively affected the formation of carbonyl compounds in yoghurt (Decourcelle et al. 2004). The use of stabilizers like high methoxyl pectin and gelatine influenced the taste and the odor of Ayran (a yoghurt drink popular in Turkey) and found unacceptable in the sensory analysis (Koksoy and Kilic, 2004). Rega et al. (2002) and Lubbers and Guichard (2003) reported that in a pectin model gel with sugars, the 3- dimensional network of pectin chains was found to retain aroma compounds.

Body and texture

The control *Bhapa dahi* (i.e. devoid of pectin) had a uniform, soft to firm body, smooth texture and good slicing property. On the other hand, *Bhapa dahi* made using 0.1 and 0.2 % pectin had a firm body but was associated with slight gumminess/pastiness. There was tendency of control *Bhapa dahi* to 'syneresis' upon cutting; however *Bhapa dahi* containing any of the two pectin levels exhibited some resistance to 'syneresis' upon cutting.

Hence, the body and texture score of *Bhapa dahi* devoid of pectin was significantly ($P < 0.05$) higher than that associated with samples containing either 0.1 or 0.2% of pectin (Table 4). The product made using 0.2 % pectin had significantly ($P < 0.05$) lower body-texture score than the relevant score allotted to product made using 0.1 % pectin. Such inferior score of product made using higher level (i.e. 0.2 %) of pectin was due to the greater intensity of pastiness. The drinking yoghurt (50% yoghurt, 8% sugar and 42% water) made with addition of 0.2% stabilizers (mixture of 0.1% guar gum + 0.1% High Methoxyl Pectin) prevented serum separation with good viscosity (Sobhay et al. 2019).

HMP is known to prevent association of casein particles in acidic milk drinks by steric stabilization, forming a protective layer bound electrostatically to the surface of the particles. HMP and gelatin could prevent serum separation in Ayran at levels ≤ 0.50 % (Koksoy and Kilic, 2004). Overstabilization of yoghurt through use of higher level of stabilizer culminated in a gummy product (Hui, 2006).

Colour and appearance

All the three *Bhapa dahi* samples appeared uniform yellowish orange in color (see photograph in Figure 2). The *Bhapa dahi* made using 0.1 and 0.2 % pectin showed minute holes on its surface. The control product (devoid of pectin) however had a smooth surface. The difference in the colour and appearance score of the three products was however non-significant.

Acidity

The control *Bhapa dahi* was allotted the highest score for acidity when compared with the samples containing added pectin at two levels. Such difference in the acidity score was significant ($P < 0.05$) when each product was compared with the other one (Table 4). The perception of acidity in produced was higher in control than in pectin containing *Bhapa dahi* samples; this led to the judges preferring the former product.

The yogurt made using gelatin (0.5%) stabilizer led to increase of the pH value and decrease in the number of microbes (Saadi et al. 2022). Alakali et al. (2008) reported that use of 0.5-0.75 % carboxy methyl cellulose depressed the lactic acid production in yoghurt making. The yogurt sample added with Gelatin (0.2%) stabilizer showed slower the acid development (Bhattarai et al. 2015)

Total sensory score

The total sensory score of *Bhapa dahi* samples was in the decreasing order as follows: Control > product containing 0.1 % pectin > product containing 0.2 % pectin (Table 4). The maximum total sensory score was associated with control (devoid of pectin) which differed significantly ($P < 0.05$) from the said score of *Bhapa dahi* containing two levels of pectin. Even *Bhapa dahi* containing higher level of pectin (0.2 %) had significantly ($P < 0.05$) lower score than the one containing lower level (i.e. 0.1 %). Such observation is obvious since the control *Bhapa dahi* had the maximum scores of each individual sensory attribute (Table 4).

Peanut milk-based yoghurt (60% peanut milk and 40% cow milk) added with the stabilizers i.e. high methoxyl pectin, propylene glycole alginate, carboxy methyl cellulose, xanthan gum and guar gum formed weak gels with little or excessive whey separation at the top (Isang and Zhang, 2008).

On the basis of the findings of the experiment, it was decided not to utilize pectin in the formulation of *Bhapa dahi*, since it exerted some adverse impact on the flavour and body and texture of product. Even the appearance of the product was somewhat impaired due to visible minutes pits on the surface of the product.

Conclusion

The *Bhapa dahi* made using pectin at two levels did not have any significant influence on most of the chemical constituents,

except for moisture. The pectin addition had a significant ($P < 0.05$) influence on the cohesiveness and adhesiveness of *Bhapa dahi*. The control *Bhapa dahi* had significantly ($P < 0.05$) greater score for flavor, body-texture as well as total sensory score as compared to product containing 0.1 or 0.2 %pectin. On the basis of the findings of the investigation, it was decided to avoid use of pectin in the formulation of *Bhapa dahi*, since it exerted some adverse impact on the flavour and body and texture of product. Even the appearance of the product was somewhat impaired due to presence of visible minute pits on the surface of the product.

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RESEARCH ARTICLE

Composition and characteristics of flavoured milk prepared using tagatose as a substitute of sucrose

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Abstract: In a current era of reduction in consumption of sucrose, finding an ideal alternative of sucrose for application in sweetened food products serves as a boon. However, it remains distant dream to find an idea sucrose substitute. Fortunately, now tagatose is coming out as highly potential sucrose substitute to be used as a sweetener in food products. In this study tagatose suitability as a sweetener in flavoured milk was evaluated. The results of the study indicated that flavoured milk prepared by partial (50%) substitution of sucrose was well acceptable in sensory evaluation and that prepared by full replacement of sucrose using blend of tagatose along with fructose as an adjunct sweetener was highly acceptable. It was possible to use tagatose as sucrose substitute in flavoured milk without changing any process parameters. Flavoured milk prepared by sucrose substitution shows almost identical chemical composition, physicochemical properties and microbial counts to flavour milk prepared using sucrose as a sweetener. Shelf life of flavoured milk prepared by sucrose substituted was too similar to respective flavoured milk which was made using sucrose as a sweetener.

Keywords: Flavoured milk, Sweetener, Sugar substitute, Tagatose

Introduction

Sucrose is most liked by humans among the sweeteners, due to day to day consumption of sucrose in diet. Though sucrose elicit a typical sweet taste, high sucrose consumption leads to too much calorie intake, which in causes obesity and several lifestyle diseases. Thus, it becomes vital to target to reduce sucrose consumption. Replacement of sucrose with sweeteners having low-calorie or non-calorie appears pragmatic approach to reduce consumption of sucrose. However, in sensory arena, sucrose is regarded as a gold standard for sweetness for sweetening food products, (WHO 2017). None of the sucrose substitutes reported till now seems to be perfect in view of the requirements for an ideal alternative of sucrose, because together with typical sensory characteristic sucrose possesses unique combinations of physical, chemical and microbiological properties for its functions in the sweetened food products. Therefore, sucrose poses several challenges for its substitution in different types of food products (Davis 1995). Consequently, producing a high-quality food products using alternatives of sucrose is very difficult and highly challenging task. As a result total sucrose substitution appears to remain almost a pipe dream forever (Hutchings et al. 2019).

While selecting a suitable alternative sweetener for substitution of sucrose, its suitability is generally examined on basis of its source (natural or artificial), potency and sensory characteristics, calorie content, glycemic index and any possible health benefits/hazards on consumption (Martí et al. 2008; Streak 2015). In these context tagatose (technically D-tagatose) is regarded as naturally occurring rare sugar in fruits like apples, orange and pineapple (Skytte 2006). Its presence is also noticed in sterilized milk, milk powder, hot cocoa, yogurts, certain cheese varieties and other dairy foods in minute amounts (Troyano et al. 1996; Mendoza et al. 2005; Oh 2007). The tagatose sweetness potency is relatively higher (0.92 or 92%) compare most of other low calorie full bulk alternatives (Levin et al. 1995; Patel and Aparnathi 2021) and its sweetness characteristics are highly resembling to that of the sucrose (Fujimaru et al. 2012; Patel and Aparnathi 2021). At 1.5 kcal/g the caloric value of tagatose is low for humans, around 37.5% compared to the sucrose caloric value 4.0 kcal/g (Wanarska and Kur 2012). Similarly, glycemic index of tagatose is very low (3) compared to sucrose (65) (Whole Health Library 2014; Guerrero-Wyss et al. 2018). For today's health conscious

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consumers, unique health benefits of tagatose, make it highly attractive sweetener for sucrose replacement (Van Laar et al. 2020). For use in beverage and foods, tagatose has been generally recognized as safe (GRAS) by FDA (FDA 2011).

Chemically D-tagatose is a mirror image of D-fructose at the fourth carbon atom (Vastenavond et al. 2012). Although tagatose is naturally exists in some dairy products and fruits (Skytte 2006), commercially it is prepared from lactose by enzymatic and/or chemical processes (Vastenavond et al. 2012). Tagatose produced from lactose exhibit sweetness characteristics highly similar to standard sweetener sucrose (Fujimaru et al. 2012). Moreover, many physical and chemical characteristics of tagatose are also very similar to those of the sucrose (Levin et al. 1995; Levin 2002; Kim 2004; Lu et al. 2008). Bulk value for sweetener tagatose is very similar to sucrose and tagatose is considered as full-bulk sweetener in use as an alternative to sucrose (Vastenavond et al. 2012; Xu 2015). Hence, it can substitute sugar in 1:1 ratio (Levin 2002) without any significant change in the process parameters in production of sweetened food products (Toothfriendly International 2013).

In recent years tagatose has received increasing attention from food industry and scientific community. Though tagatose has been emerging as highly promising substitute of sucrose, information on its suitability as a substitute of sucrose in sweetened dairy products is extremely limited. In India, among dairy based beverages, flavoured milk is the most popular throughout the country. Therefore, work was undertaken to evaluate suitability of tagatose as a sucrose replacement sweetener in flavoured milk.

Materials and Methods

Local ingredient suppliers provided all ingredients used in this study. NuNatural brand tagatose was used in this study and was of 100 % purity. Sucrose used was product of “Shree Renuka Sugars Limited” and of “Madhur” brand having 99.4 % purity. Fructose used was of “Loba chemie” brand having purity of 99 %.

Preparation of flavoured milk

Method provided by Aneja et al. (2002) was used to prepare flavoured milk. For preparation of flavoured milk, Amul brand standardized homogenized milk having 8.5% SNF and 4.5% fat was used. The flavoured milk available in Indian market usually

contains 8.0% sucrose (De 2001; Aneja et al. 2002). Quantities of tagatose as lone sweetener, sucrose tagatose blend, fructose tagatose blend and sucrose used as lone sweetener used in flavoured milk preparation are provided Table 1. The selection of these sweeteners and their rate of addition were adopted from Patel et al. (2021b).

Analysis of flavoured milk for proximate composition

Total solids (TS), fat, protein and ash content of flavoured milk were determined by gravimetric method as described by BIS (1981). Total carbohydrates were estimated by difference method in which sum total of percentage of other constituents in flavoured milk was subtracted from 100.

Analysis of flavoured milk for physicochemical characteristics

Specific gravity of flavoured milk was estimated at 30°C using pycnometer using method as given by Atherton and Newlander (1977). Viscosity of flavoured milk was determined at 30°C using Ostwald's viscometer following the method reported by Atherton and Newlander (1977). Flavoured milk acidity was estimated by titration protocol as suggested by BIS (1981).

Analysis of flavoured milk for microbial counts

Flavoured milk was analyzed for standard plate counts (SPC), coliform counts as well as yeast and mold counts (YMC). For the microbial counts methods suggested by BIS (1981) were used.

Sensory evaluation of flavoured milk

Flavoured milk sensory testing was done for its overall acceptability employing 9 point hedonic scale (Wichchukit and O'Mahony 2015). The testing was done by experienced judge panel. Averaging out was done on scores provided by different judges. For statistical analysis, averaged out values from all judges were calculated and used.

Shelf life evaluation of flavoured milk

Samples of flavoured milk were filled in PET bottles and stored at $6 \pm 1^\circ\text{C}$ in refrigerator and analysed for changes in acceptability by sensory evaluation and acidity by titration method at an interval of every 24 h (i.e. every day) for shelf life evaluation of flavoured milk till 7 days.

Statistical analysis

Table 1: Sweeteners quantities used in making of flavoured milk

Code	Sweetener	Amount (g/100 ml flavoured milk)
T	Tagatose	9.0
T + S	Tagatose + Sucrose	4.5 + 4.0
T + F	Tagatose + Fructose	8.0 + 1.0
S	Sucrose	8.0

Completely Randomized Design (CRD) was used for statistical analysis for average values of each of the attributes in the study. Steel et al. (1997) provided statistical model was adopted which can be written using equations as given below:

$$m + T_i + E_{ij} = R_{ij}$$

Where,

m = Mean

T_i = Effect of i^{th} treatment,

E_{ij} = Error due to j^{th} observation in the i^{th} treatment and

R_{ij} = Response due to j^{th} observation in the i^{th} treatment

Results and Discussion

Flavoured milk prepared using different sweetener was analysed for proximate composition, relevant physicochemical properties, microbial counts and acceptance in sensory evaluation.

Acceptability of flavoured milk in sensory evaluation

For evaluating acceptability, samples of flavoured milk were prepared using tagatose alone, blend of tagatose and sucrose, blend of tagatose with fructose and sucrose alone as sweeteners. In industrial production of flavoured milk, addition of sucrose is reported at the rate of 8% (De 2001). Therefore, in this study for preparation of flavoured milk sucrose was used at rate of 8.0%. The samples were evaluated for overall acceptability by a panel of experienced judges. Average results of overall acceptability from four replications are presented in Table 2.

The overall acceptability score of flavoured milk made by using different sweetener was in the order of, only sucrose > tagatose + fructose > tagatose + sucrose > only tagatose. The overall acceptability scores of flavoured milk in all three treatments which do not involve just pure tagatose were highly acceptable (>8.0) and statistically not different though there was nominal difference

in scores. Tagatose as lone sweetener has scored significantly lower compared to other treatments.

The results indicated that use of tagatose as a lone sweetener for substitution of sucrose in flavoured milk was not able to provide the product acceptability at par with sucrose. The results further suggested that in preparation of flavoured milk 50% sucrose can be replaced by using blend of 4.5 tagatose 4.0 g sucrose per 100 ml product, without significant decrease in acceptability of the product. In flavoured milk preparation, similarly sucrose can be fully replaced by using mixture made of 8.0 g tagatose and 1.0 g fructose as components per 100 ml flavoured milk.

When work was carried to evaluate potency and sweetness characteristics of tagatose, it was found that sweetness of 9.0% tagatose solution in water was almost equivalent to that of the 8.0% sucrose solution in water (Patel and Aparnathi 2021). In the same line it is reported in literature that the acceptability score of all the samples of lassi containing 12.0, 13.2 and 14.4 g tagatose alone as a sweetener per 100 ml product was not significantly different from the lassi made by employing 12.0 g sucrose per 100 ml lassi (Patel et al. 2021a). However, when tagatose was tried as a sole sweetener in flavoured milk, its performance was not found as promising as it is reported in case of lassi in literature. The possible reason for such differences in performance of tagatose as a sweetener in different products might be attributed to differences in composition and characteristics of the products and same reasons were responsible for low score of tagatose as sweetener when used in flavour milk.

It has been pointed out that the intensity and quality of taste are not dependent only on a chemical structure and shape of molecules, but also on other parameters of sensory perception including pH, viscosity, temperature, and the presence of other sweet or non-sweet molecules (Belitz et al. 2009). It is also noted in literature that attributes such as temperature and food systems characteristics in which sweeteners are used also affect the sensory profile of sweeteners (Fujimaru et al. 2012). Studies by Paixão et al. (2014) further corroborate this theory. As per these scholars, the product consumption temperature and food matrix

Table 2: Overall acceptability score of flavoured milk prepared using different sweeteners

Sr. No.	Sucrose replacement rate (%)	Quantity (g/100 ml) of Sweetener Employed	Nine point Score (Overall acceptability)
1.	100	Tagatose (9.0)	7.32a
2.	50	Tagatose (4.5) + Sucrose (4.0)	8.07b
3.	100	Tagatose (8.0) + Fructose (1.0)	8.20b
4.	0	Sucrose (8.0)	8.32b
ANOVA			
	SEm.	0.09	
	CD	0.36	
	Test(0.05)	*	
	CV%	2.14	

can drastically affect the perception of sweeteners in non-predictable ways. Therefore, similar phenomena might be at play for differences in performance of tagatose as a sweetener in flavoured milk.

Proximate composition of flavoured milk

For proximate composition, flavoured milk prepared using different sweeteners was analysed for fat, total carbohydrates (CHOs), protein, ash amount and total solids (TS). Proximate composition of the flavoured milk and its statistical analysis are summarized in the Table 3.

The data on proximate composition of flavoured milk prepared using different sweeteners suggested that TS and carbohydrate contents were highest, whereas, fat protein and ash content were lowest in flavoured milk prepared using tagatose alone as a sweetener. In flavoured milk's case which was prepared using sucrose as a lone sweetener, TS and carbohydrate contents were lowest, whereas, fat protein and ash content were highest. The proximate composition of remaining two types of flavoured milk were intermediate to those of flavoured milk containing tagatose alone and sucrose alone as sweetener.

Statistical analysis of the data revealed that in TS, total carbohydrates, fat, protein and ash content of all treatments for preparation of flavoured milk were statistically at par. The minor

nominal differences observed the proximate composition of flavoured milk prepared using different sweeteners might be attributed to differences in rate of the sweeteners used in their preparation. The results obtained on proximate composition of flavoured milk prepared using different sweeteners are almost in line with results reported for proximate composition of lassi prepared using different sweeteners (Patel et al. 2021c).

According to FSSAI standards, flavoured milk should have same minimum percentage of milk fat and milk SNF (Solids Not Fat) as the category of milk from which flavoured milk is made (FSSAI 2022). As in present study standardized milk was used to make flavour milk, values of per cent fat and milk SNF (Solids Not Fat) content in all the samples of flavoured milk were within the legal standards prescribed for flavoured milk from standardized milk. It should be noted that data on SNF content is not presented here, it can be easily estimated by subtracting sum total of %fat content and amount of sweetener added/100 ml from the respective %TS content.

Physicochemical properties of flavoured milk

For physicochemical properties, flavoured milk made employing tagatose as lone sweetener, mixture of tagatose and sucrose, mixture of tagatose and fructose along with sucrose as lone sweetener (total four combinations) were analysed for specific

Table 3: Proximate composition of flavoured milk prepared using different sweeteners

Sr. No.	Sweetener used	% Sucrose substitution	Constituent (%)				
			TS	Fat	Protein	CHOs	Ash
1.	T	100	22.06	4.51	2.91	14.01	0.63
2.	T + S	50	21.62	4.55	2.95	13.57	0.64
3.	T + F	100	22.01	4.51	2.93	13.98	0.63
4.	S	0	21.15	4.58	2.96	12.95	0.64
		ANOVA					
	SEm.		0.29	0.03	0.06	0.34	0.01
	CD						
	Test(0.05)		NS	NS	NS	NS	NS
	CV%		2.68	1.13	4.20	5.04	3.16

Table 4: Physicochemical properties of flavoured milk prepared using different sweeteners

Sr. No.	Sweetener used	% Sucrose substitution	Specific gravity	Viscosity (mPa.s)	Acidity (% lactic acid)
1.	T	100	1.063b	2.29	0.13
2.	T + S	50	1.061a	2.26	0.13
3.	T + F	100	1.063b	2.28	0.13
4.	S	0	1.060a	2.20	0.14
		ANOVA			
	SEm.		0.0002	0.04	0.004
	CD		0.001		
	Test(0.05)		*	NS	NS
	CV%		0.04	3.52	6.35

gravity, viscosity, and acidity. Average results of specific gravity, viscosity, and acidity and results are given in Table 4.

The data on physicochemical properties of flavoured milk prepared using different sweeteners suggested that values of specific gravity and viscosity were highest in flavoured milk prepared using tagatose alone as a sweetener. In flavoured milk prepared employing sucrose as a lone sweetener, specific gravity and viscosity were lowest. The values of specific gravity and viscosity of remaining two types of flavoured milk were intermediate to those containing tagatose alone and sucrose alone as sweetener. The values of acidity in samples of flavoured milk from all the four sweeteners were almost similar and values are close to normal milk acidity which was expected. Analysing data statistically, made it evident that viscosity and acidity different flavoured milk were statistically at par. However, specific gravity of flavoured milk containing tagatose alone and blend of tagatose with fructose was significantly higher than the flavoured milk containing sucrose alone and blend of tagatose with sucrose. These differences in specific gravity of flavoured milk containing different sweetener might be attributed differences in their total solids content.

Pugazhenth and Jothilingam (2013) reported a specific gravity of control sample of flavoured milk in their study as 1.065. Thus, specific gravity values of flavoured milks prepared in the present study were much closed to the values reported by these authors. Pugazhenth and Jothilingam (2013) prepared herbal flavoured milk adding 5 per cent Aloe vera pulp extract. They reported a viscosity of 320.35 centipoise for this flavoured milk. Jadhav and Pawar (2016) reported a viscosity of 8.4 centipoise for chocolate flavoured milk. Thus, literature clearly shows that viscosity is highly affected by additives to milk. In this study viscosity of flavoured milk remained closed to pure milk because of nature of additive i.e. low molecular weight additives like sucrose, tagatose and fructose. The results obtained on physicochemical properties of flavoured milk prepared using different sweeteners in this study are also in harmony with the results reported for physicochemical properties of lassi prepared using different sweeteners (Patel et al. 2021c).

Microbial counts of flavoured milk

Microbial analysis of the flavoured milk was carried for standards plate counts (SPC) coliform count as well as yeast and mold count (YMC). In enumeration of microbial counts of flavoured milk, only flavoured milk prepared using blend of tagatose with fructose was selected as treatment, since among all sucrose substituted flavoured milks it was best performing treatment in

sensory. The flavoured milk containing sucrose alone as sweeteners was also included in microbial counts to serve as control.

Table 5

The results of the microbial counts of flavoured milk from both the types of sweeteners suggested that SPC, coliform and YMC in flavoured milk fulfilled the requirements laid down by FSSAI for fermented milk products under standards for microbiological quality of milk and milk products (FSSAI 2022).

Shelf life of flavoured milk

For shelf life evaluation, only flavoured milk prepared using blend of tagatose with fructose was selected, since among sucrose substituted flavoured milks it was best performing in sensory. The flavoured milk containing sucrose alone as sweeteners was also included in shelf life study to serve as control. Total three replications were conducted. Flavoured milk samples were prepared using 14.4 g tagatose per 100 ml product and using 12 g sucrose per 100 ml product. Prepared samples were filled in bottles made of PET and kept in refrigerator at $6^{\circ}\pm 1^{\circ}\text{C}$. Changes in overall acceptability and acidity value of flavoured milk were monitored on every day during storage, till their overall acceptability reached in vicinity of 6.0 score.

Changes in score for overall acceptability of flavoured milk during storage

During storage, the changes in score for overall acceptability of flavoured milk was determined using 9 point hedonic score by sensory panel of experience judges. Total three replications were conducted. The averages values of the acceptability scores of flavoured milk and statistical analysis values are provided in Table 6.

Comparison between the two types of sweeteners suggested that the acceptability scores of both the flavoured milks were statistically at par when fresh, as well as, during each interval of the storage and at the end of the storage also remained statistically remained at par. The results suggested that there was no statistically significant difference in overall acceptability score based on type of sweetener used to make flavoured milk during storage period i.e. same shelf life considering sensory score criteria.

On the other hand comparison between different intervals of storage revealed that overall acceptability score of flavoured milk

Table 5: Microbial counts of flavoured milk prepared using different sweeteners

Sr. No.	Sweetener used	SPC (log cfu/ml)	Coliform (cfu/ml)	YMC (cfu/ml)
1.	T + F	3.69 $\pm$ 0.123	Absent	9.67 $\pm$ 3.28
2.	S	3.81 $\pm$ 0.128	Absent	8.67 $\pm$ 1.76

Table 6: Overall acceptability score of flavoured milk containing different sweeteners during storage

Sr. No.	Storage period (days)	Acceptability score (out of 9)	
		Tagatose + Fructose	Sucrose
1.	0	8.60	8.67
2.	1	8.43	8.48
3.	2	8.31	8.40
4.	3	8.07	8.12
5.	4	8.05	7.95
6.	5	7.60	7.55
7.	6	7.10	7.10
8.	7	6.14	6.10
ANOVA			
	Type of sweetener	Storage period	Sweetener x Storage
SEm.	0.016	0.031	0.044
CD	—	0.143	—
Test(0.05)	NS	*	NS
CV%		0.98	

Table 7: Acidity of flavoured milk during storage prepared using different sweeteners

Sr. No.	Storage period (days)	Acidity (% lactic acid)	
		Tagatose + Fructose	Sucrose
1.	0	0.12	0.12
2.	1	0.12	0.12
3.	2	0.12	0.13
4.	3	0.12	0.13
5.	4	0.13	0.14
6.	5	0.13	0.14
7.	6	0.13	0.14
8.	7	0.13	0.14
ANOVA			
	Type of sweetener	Storage period	Sweetener x Storage
SEm.	0.001	0.003	0.004
CD		0.014	
Test(0.05)	NS	*	NS
CV%		5.67	

is significantly affected by the duration of storage period. The acceptability score of both the flavoured milk started decreasing significantly from initial period. The rate of decline was faster during the initial period up to 2 days, but became relatively slower at the latter stage.

In both the sweeteners the acceptability score of the flavoured milk decreased at almost at the similar rate during storage, irrespective of the sweetener used. Thus, stability of flavoured milk during the storage was not dependent on type of sweetener. This was also visible from the interaction effect between storage period and type of sweetener, which was non-significant.

Change in flavoured milk acidity during storage

Alteration in acidity of flavoured milk during storage period was estimated through method of titration. Three replications were

carried out in total. Statistical analysis and averages values of the acidity of flavoured milk are given in Table 7.

Comparison between acidity the two types of flavour milk using two different sweeteners during storage shows that, the acidity of both the flavoured milks was statistically at par when fresh, as well as, during each interval of the storage and at the completion of the storage. Results suggested that kind of sweetener employed in preparation had no significant effect on acidity both the flavoured milk.

On the other hand comparison between different intervals of storage has shown that the storage period significantly effects flavoured milk acidity. The acidity of both the flavoured milk increased during the storage. However, increase in acidity of flavoured milk from the blend of tagatose was not statistically significant. Whereas, there was statistically significant increase

in acidity of flavoured milk from sucrose was noticed during the storage.

In both the sweeteners the acidity of the flavoured milk increased at almost at the similar rate during storage, irrespective of the sweetener used. Thus, rise in acidity of flavoured milk during the storage was not dependent on type of sweetener. This was also evident from the fact that interaction effect between storage period and sweetener was non-significant.

No report was found in the literature regarding shelf life of planed pasteurised flavoured milk prepared using tagatose alone or its blend with sucrose as a sweetener. Therefore, this study is first to report this kind of positive results for feasibility of sucrose replacement by tagatose in flavoured milk i.e. indicating similar shelf-life.

Flavoured milk can be stored up to 7 days at 5°C without any significant change in its sensory qualities (M. Ranganadham et al. 2016). Pawar et al. (2010) found flavour milk shelf life of 5 days by storing products at 5°C. Whereas, Krishna et al. (2019) noted that during storage study, control sample of flavoured milk was good up to 12 days, at refrigerated temperature (4±1°C). Therefore, shelf life of flavoured milk obtained in this study was in accordance with that provided in literature. The results obtained on shelf life of flavoured milk prepared using different sweeteners are almost in line with the results reported on shelf life of lassi prepared using different sweeteners (Patel et al. 2021c).

The evaluation for shelf life of flavoured milk suggested that replacement of sucrose by a mixture of tagatose and fructose had no special effect of on its shelf life during storage at 6±1°C, when compare with the flavoured milk containing sucrose as a sweetening agent.

Conclusions

The results of the present study entailed to conclude that sucrose can be successfully substituted in flavoured milk by blend of 8.0 g tagatose with 1.0 g fructose as an adjunct sweeter, without significant decrease in its acceptability or shelf life. These findings ushers doors of successful sucrose replacement in flavour milk using tagatose.

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RESEARCH ARTICLE

Preparation of herbal *Shrikhand* using *Catharanthus roseus* powder

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Abstract: The purpose of this study was to develop herbal *Shrikhand* by incorporating *Catharanthus roseus* powder and adding stevia as sweetener. The analysis was carried out by adding *Catharanthus roseus* leaf powder to the product at the rate of 0%(T₀), 2%(T₁), 4%(T₂), 6%(T₃) and Stevia added at 4% to each. Prepared herbal *Shrikhand* was further analysed and the sensory evaluation revealed that T₁ combination was best accepted as compared to other samples. The T₁ combination was selected as the optimized product for further microbial analysis. Microbial activity of the product stored at 4°C was evaluated at intervals of 7 days. The cost of preparation was also estimated for the optimized product. Herbal *Shrikhand* was found to be superior in nutritional quality for diabetic population and the product was acceptable up to 28 days while control sample was acceptable up to 7 days only.

Keywords: *Catharanthus roseus*, Chakka, Herbal *Shrikhand*, Stevia, Shelf life

Introduction

Milk is an excellent vegetarian protein source in India, where bulk of the population follows a vegetarian diet. In India, the dairy industry is critical to the rural economy and contributes a

significant amount to the country's GDP every year. As estimated, around 50 to 55 percent of milk collected in India is converted into traditional dairy products. The production of traditional dairy products involves processing techniques such as coagulation, desiccation, and fermentation. Fermented milk products are an integral part of the human diet in many regions of the world (Swapna and Chavannavar, 2013; Devi et al. 2018). Fermentation is a process that increases the nutritional content and sensory qualities of food products. It is a cost-effective and scalable technology that enables the introduction of novel food products. The most popular indigenous fermented dairy products are *dahi* (curd), lassi and *Shrikhand*. They have been revered for their nutritional and therapeutic benefits since the dawn of time (Devi et al. 2018). Therapeutic, anticholesterolemic, and anticarcinogenic properties of fermented milk products have long been acknowledged in the scientific literature (David, 2015).

Shrikhand is generally made from lactic fermented whole milk curd and is a semi-solid product with a sweetish-sour taste. *Shrikhand* is a popular fermented milk product (Ojha et al. 2018) named after the Sanskrit word "Shikharani", which means a curd produced with fresh sugar, flavouring agents (Saffron), fruits, and nuts (Srinivas et al. 2017). It's particularly popular in the states of Maharashtra, Gujarat, and Karnataka. The curd (Dahi) is partially strained to remove the whey and induce a solid mass known as Chakka, which is the main component in *Shrikhand* (Singh et al. 2014; David 2015). *Shrikhand* is well-known for its excellent nutritive value, distinct flavour, taste, and affable quality, as well as its implicit remedial significance. It can be advised as a part of a healthy diet and is generally consumed during summer (Swapna and Chavannavar, 2013). As a fermented dairy product, *Shrikhand* has a high calorific value along with high protein content and is consumable by the lactose intolerant population (Dandile et al. 2014). As defined by FSSAI 2011, *Shrikhand* means the product obtained from chakka or Skimmed Milk Chakka to which milk fat is added. It may contain fruits, nuts, sugar, cardamom, saffron and other spices. Due to its higher sugar content and lower moisture level, *Shrikhand* has a longer shelf life than Dahi. The major determinant of the shelf life in *Shrikhand* is the native microflora present in it. It has a shelf life of 2-3 days at room temperature (30°C), although it can be kept in the refrigerator for up to 40 days without spoilage.

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Diabetes mellitus is a chronic disease marked by issues in glucose metabolism as well as fat and protein metabolism (Bisla et al. 2014). It appears that the supply of food for diabetics is not keeping pace with the rise in demand, which might lead to an increase in the disease's prevalence. In order to suit the needs of these customers, a robust market for processed items is growing. These products must fulfil the criteria that they must be locally sourced, flexible, easy to purchase, and are affordable.

Catharanthus roseus is a member of the Apocynaceae family. Periwinkle, Madagascar periwinkle, and *Sadabahar* are all the common names for *Catharanthus roseus*. It grows all over India and can be commonly found in waste spots and sandy areas. *Catharanthus roseus* (Periwinkle) is an evergreen sub herb plant that grows up to 1 m tall. Leaves of periwinkle are oval to oblong, 2.5- 9.5 cm long and 1-3.5 cm broad having glossy green colour with a pale midrib and a short petiole about 1- 1.8 cm long (Nisar et al. 2016). *Catharanthus roseus* leaves have a number of health benefits, including blood sugar control, decreasing high blood pressure, menstrual irregularities, and Hodgkin's disease prevention (Bisla et al. 2014). Phenolic acids, flavonoids, and alkaloids are the most active components in *Catharanthus roseus*. These active compounds have important anti-oxidative, anti-allergic, antibiotic, hypoglycaemic, and anticarcinogenic properties in the human body (Tolambiya and Mathur, 2016).

Stevia rebaudiana commonly known as Stevia belongs to the Asteraceae family of the plant kingdom. It is native to Paraguay's northeast regions, although it is grown in various parts of the world, including Europe, Asia, and North America. Stevia is well-known for its intense sweetness, which is roughly 250-300 times that of sucrose. The sweetness in stevia comes from the compound steviol glycosides, which are often used as a non-caloric sweetener and a sugar replacer (Masoumi et al. 2020). Stevia possesses hypoglycaemic and hypolipidemic effects along with various health benefits, including weight loss, diabetes management, and dental caries control. It also has antifungal and antibacterial qualities and heals blemishes and injuries. It helps with blood pressure management, improves immunological modulation and is non-toxic and harmless (Mathur and Johri, 2016).

In view of this, the present investigation was undertaken to prepare herbal *Shrikhand* by incorporating *Catharanthus roseus* powder and stevia. The antidiabetic property of *Catharanthus roseus* powder and stevia were taken into account in the preparation of herbal *Shrikhand* for patients suffering from diabetes.

Materials and Methods

The fresh *Catharanthus roseus* leaves were collected from the garden of the Department of Dairy Sciences and Food Technology, Institute of Agricultural Sciences (IAS), Banaras

Hindu University (BHU), Varanasi, Uttar Pradesh. Fresh, clean cow milk was procured from the *Gaushala* (Dairy Farm), Banaras Hindu University (BHU), Varanasi, U.P. Dahi was prepared using Amul *Dahi* containing starter culture strains of *Lactobacillus acidophilus* and *Bifidobacterium animalis*. Stevia was procured from the local market of Varanasi, Uttar Pradesh.

Preparation of *Catharanthus roseus* powder

Catharanthus roseus leaves were washed and tray dried at $35 \pm 2^\circ\text{C}$. After dehydration, the leaves were ground to powder form and sieved using a muslin cloth to obtain a fine powder. The powder thus obtained was vacuum packed in a pre-sterilised polyethene bag and stored at room temperature for further use. Fig.1 shows the preparation of *Catharanthus roseus* powder.

Processing of herbal *Shrikhand*

Fresh cow milk was filtered and heated to approximately 85°C and cooled down to $37 \pm 2^\circ\text{C}$. Milk was inoculated with starter culture and incubated at 37°C for 12-15 hours in an incubator for setting. After the incubation period, the curd was strained using a clean muslin cloth and hanged for about 18 hours to drain the whey off. The *chakka* obtained was mixed with *Catharanthus roseus* powder, stevia powder and cardamom powder. Finally, the prepared herbal *Shrikhand* was transferred to plastic cups and stored at a refrigeration temperature of $4 \pm 2^\circ\text{C}$ (Fig.2)

Incorporation details as shown in Fig.3:

T0 represents the control sample of *Shrikhand*

T1 herbal *Shrikhand* with 2% *Catharanthus roseus* powder

T2 herbal *Shrikhand* with 4% *Catharanthus roseus* powder

T3 herbal *Shrikhand* with 6% *Catharanthus roseus* powder

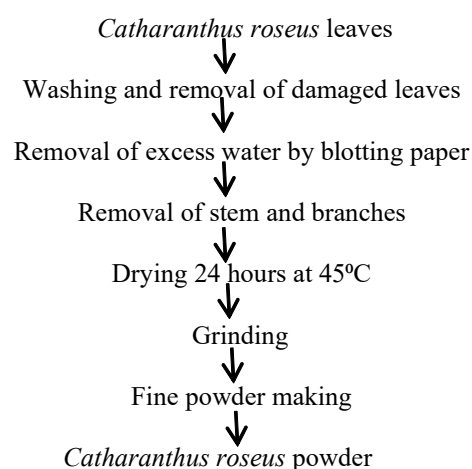


Fig.1 Flow chart for *Catharanthus roseus* powder preparation

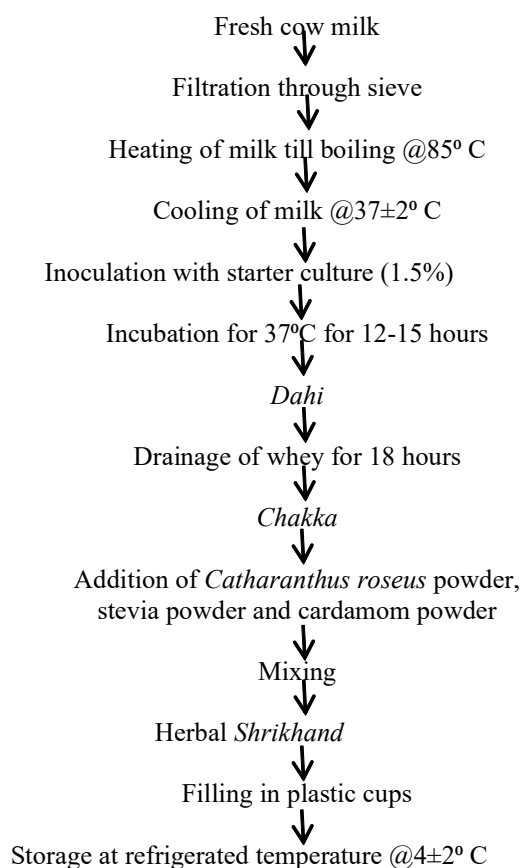


Fig.2 Flow chart for the processing of Herbal *Shrikhand*



Fig.3 Different variants of herbal *Shrikhand*

Physical analysis of herbal *Shrikhand*

Colour Flex, Hunter Lab (L^* , a^* and b^*) system was used to measure the colour of the product surface. Where L^* stands for lightness, a^* for greenness, and b^* for yellowness. The texture profile of the prepared herbal *Shrikhand* was examined using TexturePro CT V1.6 (Brookfield Engineering Labs. Inc. MA, USA) and gumminess, cohesion, adhesiveness, and hardness of the product were evaluated.

Proximate compositional analysis of herbal *Shrikhand*

Proximate analysis of herbal *Shrikhand* including moisture, total solids and ash content (AOAC, 2015), protein, fat (AOAC, 2000) along with carbohydrate content (on a dry weight basis) was determined using different methods suggested in ISI: SP: 18 part XI, (1989). pH was monitored by a digital pH meter and Titratable acidity was determined using the method mentioned by Rangana (2001).

Analysis of bioactive compounds in herbal *Shrikhand*

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) technique was used to estimate the antioxidant activity of herbal *Shrikhand*, according to Benzie and Strain (1996).

Using the Folin-Ciocalteu phenol reagent and the technique provided by Huang et al. (2005), the total phenolic content (TPC) of each herbal *Shrikhand* sample was determined. A standard curve was produced using gallic acid (5–60 mg/mL; $y = 0.00128x - 0.3079$; $r^2 = 0.989$; y is the absorbance; x is the solution concentration).

Determination of microbial population

Microbial analysis of the developed herbal *Shrikhand* were conducted for fresh samples as well as stored samples. The samples were stored in polystyrene (PS) cup type packaging material and stored at refrigerated temperatures. The analysis was conducted in every 7 days interval upto 28 days of storage. The analysis was determined by the method prescribed by (AOAC, 2000). Total plate count, yeast and mould count along with coliform count were determined using nutrient agar, potato dextrose agar (PDA) and VRBA (Violet Red Bile Agar) respectively to aid the growth of microbes.

Sensory evaluation of herbal *Shrikhand*

The prepared herbal *Shrikhand* samples of each trial were evaluated for sensory attributes viz. flavour, body and texture, colour and appearance, mouthfeel and overall acceptability with the help of a 9-point hedonic scale as suggested by Amerine et al. (1965).

Statistical analysis

All the data obtained were analysed with the help of ANOVA using MS EXCEL 2019 and presented as a mean with a standard deviation of the triplicate analysis.

Results and Discussion

Leaves of *Catharanthus roseus* were dried using the tray drying method and ground into fine powder. Herbal *Shrikhand* was prepared by adding *Catharanthus roseus* powder at different

rates of 0%, 2%, 4% and 6% along with 4% stevia in each sample. The best-accepted sample (on the basis of sensory score) was selected and studied for its sensory, physical, chemical and microbiological attributes.

Determination of the Physical & Chemical properties of the Herbal *Shrikhand*

Physical analysis

Colour:

The effect of *Catharanthus roseus* powder after drying on the colour of herbal *Shrikhand* is shown in Table 1. The colour was determined with the help of the Hunter colour lab instrument and values of L*, a* and b* were determined. As periwinkle leaves powder contains chlorophyll pigment and other coloured components the product obtained had green colour. The colour and appearance score on the 9-point hedonic scale of the prepared herbal *Shrikhand* along with control showed variation. The maximum score for the colour and appearance was given to T1 (8.14 ± 0.168) while the minimum score was given to T4 (5.92 ± 0.540).

Texture:

Back extrusion test examined the textural characteristics such as hardness, gumminess, cohesiveness, and adhesiveness of herbal *Shrikhand* which are listed in Table 2. The mean obtained for the firmness of the prepared herbal *Shrikhand* was 64.23g while for the control it was 66.36g. The mean obtained for the gumminess of the prepared herbal *Shrikhand* was 43.96 while for control it was 47.16. The mean obtained for the cohesiveness of the prepared herbal *Shrikhand* was 32.5 while for the control it was

37.86. The mean obtained for adhesiveness of the prepared herbal *Shrikhand* was 35.13 g sec while for the control was 28.23 g sec. Due to an increase in compactness on the microstructure of Herbal *Shrikhand* produced with *Catharanthus roseus* powder, the values of the textural parameters like stiffness, gumminess, cohesiveness, and adhesiveness decreased. This may have occurred as *Catharanthus roseus* powder reduced the moisture content of the herbal *Shrikhand* and the stevia reduced the gumminess, resulting in loosening and reduced binding capacity. According to Rai et al. (2018), the increase in compactness on the microstructure of *Shrikhand* prepared with Tulsi extract caused a decrease in firmness and consistency.

The body and texture score on the 9-point hedonic scale of the prepared herbal *Shrikhand* was estimated. The maximum score for the body and texture was given to T1 (8.06 ± 0.089) while the minimum score was given to T4 (5.96 ± 0.167).

Proximate compositional analysis of herbal *Shrikhand*:

Moisture content, total solids, ash, protein, fat, and carbohydrate content of the herbal *Shrikhand* made with addition of different level of *Catharanthus roseus* powder was analysed. The analysis of sample T₁ was carried out as *Catharanthus roseus* addition at the rate of 2% was found appealing in sensory analysis. The mean values for the parameters were: moisture 39.96%, total solids 60.03%, protein content 9.5%, fat content 10.01%, ash content 0.91% and carbohydrate content 38.49% (Table 3). More or less similar results for protein, fat, fibre, ash and carbohydrate were reported by David (2015) in the herbal *Shrikhand* by using basil extract and Dubey et al. (2018) in preparation of *Shrikhand* using moringa (*Moringa oleifera*) powder.

Table 1: Colour attributes of herbal *Shrikhand*

Treatment	L*	a*	b*
	Components		
T0	90.33 ± 0.04^a	-0.68 ± 0.05^a	10.02 ± 0.02^b
T1	75.63 ± 0.27^a	-2.25 ± 0.01^b	15.73 ± 0.02^a
T2	72.65 ± 0.02^a	-4.51 ± 0.09^b	17.73 ± 0.01^a
T3	70.84 ± 0.01^a	-6.57 ± 0.15^a	19.85 ± 0.01^b

The results are indicated as mean $\pm$ standard deviation (n = 6). Different letters in the same column indicate significant differences ($p < 0.05$).

Table 2: Values of textural property of herbal *Shrikhand*

Treatment	Firmness (g)	Gumminess (g)	Cohesiveness	Adhesive Force (g sec)
T0	66.36 ± 0.21^a	47.16 ± 0.55^a	37.86 ± 0.67^a	35.13 ± 0.31^a
T1	64.23 ± 0.45^a	43.96 ± 0.38^a	32.5 ± 0.4^a	28.23 ± 0.31^b
T2	62.83 ± 0.05^a	41.56 ± 0.15^a	30.03 ± 0.2^b	24.86 ± 0.15^b
T3	61.1 ± 0.1^a	38.73 ± 0.15^a	28.1 ± 0.2^b	21.87 ± 0.15^a

The results are indicated as mean $\pm$ standard deviation (n = 6). Different letters in the same column indicate significant differences ($p < 0.05$).

The Proximate analysis of the sample revealed a diversified result, but the sample seemed to be nutritionally richer than the control. The higher protein content and lower fat content can make a significant contribution to dietary intake, especially in diets for diabetics.

pH and Titratable acidity:

The mean value for pH in herbal *Shrikhand* and its titratable acidity were examined and the pH and titratable acidity of herbal *Shrikhand* was found to be higher than the control sample. David (2015) had observed an increased pH and titratable acidity in herbal *Shrikhand* prepared by addition of basil extract than in the control sample.

The mean value for titratable acidity in herbal *Shrikhand* was 0.89% obtained from the treatment of addition of 2% periwinkle powder while titratable acidity in the control was found to be 0.74%.

Analysis of bioactive compound in herbal *Shrikhand*

Antioxidant activity of herbal *Shrikhand*

The inhibitor color of the free radical compound (DPPH) determined the antioxidant activity of the herbal *Shrikhand*. The mean value for antioxidant activity obtained by the DPPH inhibition method in the herbal *Shrikhand* T₁ sample was 0.91%.

The results obtained were in agreement with the Pugazenthi et al. (2020) for the antioxidant activity by the DPPH inhibition method in which they performed the test on functional *Shrikhand* prepared with pomegranate (*Punica granatum*) fruit peel extract.

Total phenol content of herbal *Shrikhand*

The total phenol content of the produced herbal *Shrikhand* was determined and is depicted in the Table 3. Phenols are an important component of plants and phenolic molecules are active hydrogen donors and effective antioxidants, proving that there is a clear link between total phenols and antioxidant activity (Tyagi et al. 2020). According to Nisar (2017), the antioxidant activity and total phenolic content in the leaves and various plant parts of *Catharanthus roseus* are high. The herbal *Shrikhand* is made from *Catharanthus roseus* it is also high in phenolic content, making it a valuable source of natural antioxidants. The mean value for total phenolic content in herbal *Shrikhand* sample T₁ was found to be 4.73 mg/100g.

Sensory evaluation

The sensory evaluation scores obtained after the sensory analysis showed that the T₁ scored the highest for the sensory attributes such as colour and appearance (8.14 ± 0.168), mouth feels (8.36 ± 0.230), flavour (8.12 ± 0.178), body, and texture (8.06 ± 0.089) compared to the other samples and was selected for the further analysis. The sensory evaluation scores are shown in the Table 4.

Table 3: Proximate composition of control and herbal *Shrikhand*

Parameters	Treatments			
	T0	T1	T2	T3
Carbohydrate (%)	38.49 ± 0.1 ^a	39.61 ± 0.1 ^a	42.07 ± 0.2 ^b	43.99 ± 0.2 ^b
Moisture (%)	41.1 ± 0.16 ^a	39.96 ± 0.20 ^b	37.36 ± 0.11 ^a	35.6 ± 0.17 ^a
Total Solids (%)	58.9 ± 0.2 ^a	60.03 ± 0.2 ^a	62.63 ± 0.11 ^b	64.4 ± 0.17 ^b
Fat (%)	10.43 ± 0.16 ^a	10.01 ± 0.12 ^a	9.7 ± 0.1 ^b	9.2 ± 0.1 ^a
Protein (%)	9.11 ± 0.13 ^a	9.5 ± 0.08 ^b	9.83 ± 0.15 ^a	10.23 ± 0.15 ^b
Ash (%)	0.86 ± 0.15 ^a	0.91 ± 0.02 ^a	0.95 ± 0.01 ^b	0.97 ± 0.01 ^b
pH	4.28 ± 0.08 ^a	4.54 ± 0.06 ^a	4.83 ± 0.05 ^b	5.16 ± 0.15 ^a
Titratable acidity (% lactic acid)	0.74 ± 0.01 ^a	0.89 ± 0.02 ^a	0.94 ± 0.15 ^b	0.97 ± 0.15 ^a
Antioxidant activity (% DPPH inhibition)	0	28.66 ± 0.3 ^a	61.76 ± 0.15 ^a	77.73 ± 0.15 ^b
TPC (mg/100g)	0	4.73 ± 0.22 ^a	5.8 ± 0.11 ^a	7.66 ± 0.2 ^a

The results are indicated as mean ± standard deviation (n = 6). Different letters in the same column indicate significant difference (p < 0.05).

Table 4: Sensory attributes of herbal *Shrikhand* and control sample

Trial No.	Colour and Appearance	Body and Texture	Flavour	Mouthfeel	Overall Acceptability
T0	7.22 ± 0.23 ^a	7.5 ± 0.39 ^a	7.46 ± 0.22 ^a	7.6 ± 0.22 ^a	7.43 ± 0.14 ^a
T1	8.14 ± 0.17 ^a	8.06 ± 0.09 ^a	8.12 ± 0.18 ^a	8.36 ± 0.23 ^b	8.17 ± 0.01 ^b
T2	7.02 ± 0.04 ^a	6.86 ± 0.11 ^b	7.1 ± 0.17 ^a	7.06 ± 0.09 ^b	6.98 ± 0.04 ^a
T3	6.82 ± 0.20 ^a	6.64 ± 0.25 ^a	6.86 ± 0.18 ^b	6.76 ± 0.29 ^a	6.77 ± 0.08 ^b

The results are indicated as mean ± standard deviation (n = 6). Different letters in the same column indicate significant differences (p < 0.05).

Table 5: Microbial attributes of herbal *Shrikhand*

Days Intervals	Treatment	TPC (log cfu/g)	Yeast and Mould count (log cfu/g)	Coliform Count (log cfu/g)
0 th day	Control	7.26 ± 0.15 ^a	ND	ND
	Sample (T1)	4.36 ± 0.15 ^a	ND	ND
7 th day	Control	7.70 ± 0.17 ^a	5.4 ± 0.42 ^a	ND
	Sample (T1)	4.63 ± 0.06 ^a	2.33 ± 0.21 ^b	ND
14 th day	Control	8.52 ± 0.06 ^a	6.28 ± 0.02 ^a	ND
	Sample (T1)	4.8 ± 0.12 ^a	3.36 ± 0.15 ^a	ND
21 st day	Control	—	—	ND
	Sample (T1)	5.03 ± 0.57 ^a	8.36 ± 0.32 ^b	ND
28 th day	Control	—	—	ND
	Sample (T1)	5.33 ± 0.42 ^a	—	ND

ND= Not Detected; — indicates spoilage. The results are indicated as mean ± standard deviation (n = 6). Different letters in the same column indicate significant differences (p < 0.05).

Table 6: Cost analysis of control and herbal *Shrikhand*

Quantity of ingredients	Cost of ingredients (Rs.)	Control	Herbal <i>Shrikhand</i> (T1)
Cow milk	45/ lit.	144	144
Starter culture	3.30/ lit.	10	10
<i>Catharanthus roseus</i> powder	Rs.140 / 100g	0	28
Stevia	Rs.53.5 / 100g pouch	0	22
Sugar	Rs.45 / Kg	18	-
Cardamom	Rs.273 / 100g	3	3
Processing cost (labour, fuel, miscellaneous)	5	9	9
Plastic cups	8	8	8
Cost per Kg		192	224

Microbial analysis of herbal *Shrikhand*

As per the sensory evaluation score, T1 was selected as the best-accepted sample and further subjected to microbiological analysis. According to the microbiological analysis, it was found that the microbiological load in the herbal *Shrikhand* sample T₁ was less than the control. The control was acceptable for up to 20 days, as after 21 days of storage control sample was spoiled and no further microbial analysis was carried out (Table 5). It was observed that after every 7-day interval, the microbial load had slightly increased in the experimental sample (T1) but it was far less than that observed in the control sample. The greater shelf-life of herbal *Shrikhand* might be due to antimicrobial activity of herb extract added. The same trend of the result was reported by Ojha et al. (2018) in the herbal *Shrikhand* prepared from the incorporation of basil and turmeric powder whose acceptability was 10 days and after 15 days product got spoilage.

Cost analysis of herbal *Shrikhand*

Table 6 depicts the cost of production per Kg of herbal *Shrikhand* compared with the control sample. According to calculations, prepared herbal *Shrikhand* cost around Rs. 224/ Kg and Rs. 192/

kg for the control sample. The prepared herbal *Shrikhand* had a higher cost than the control sample because of the high cost of stevia and the *Catharanthus roseus* powder used. Due to higher antioxidant activity, total phenolic content, lower cost of production and added functional attributes, *Catharanthus roseus* powder-based herbal *Shrikhand* could be introduced as a value-added functional dairy product in the human diet.

Conclusions

This study has explored the research possibilities of *Catharanthus roseus* in the fermented dairy product. It may be concluded that herbal *Shrikhand* can be successfully prepared from cow milk with the addition of *Catharanthus roseus* leaf powder and stevia. It was found that herbal *Shrikhand* in treatment T1 was best in terms of sensory characteristics. Herbal *Shrikhand* made with the addition of *Catharanthus roseus* leaf powder had a shelf life of 28 days in polystyrene cups at refrigeration temperature, comparatively higher than the control sample. As per the economic analysis of the optimized herbal *Shrikhand*, the cost was found to be Rs.224/ Kg. The experimental herbal *Shrikhand* is more costly than the control sample

Shrikhand but due to its therapeutic attributes, a great market potential lies for the developed product.

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RESEARCH ARTICLE

Protease activity as a marker of *Bacillus* spore germination and its utility for spore eradication

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Abstract: *Bacillus* spores are of major concern to the food and dairy industry due to their ability to survive during processing and their potential to germinate, subsequently decreasing the safety and shelf-life of food and dairy products. The execution of spore germination preceding to elimination step is a potential strategy to eradicate the various spores. Biomarkers of spore germination possess a high level of specificity for differentiation in dormant and germinating spores. In present study, the activity of protease enzyme released during germinant induced germination of *Bacillus megaterium* spores was determined in terms of casein hydrolysis using petriplate and microtitre plate assay. The expression of protease was observed positive for germinating spores as determined by appearance of clear zone (12 mm) due to casein hydrolysis on skim milk agar and enhanced fluorescence [$3.63\text{--}97.98 (\times 10^3)$ FU] upon cleavage of FITC-labeled casein as protease enzyme act on the fluorogenic substrate. Protease enzyme expression by germinating spores were further validated by phase contrast microscopy as spore loss their refractility due to change of their refractive index during germination. The research outcome demonstrates the scope of caseinolytic principle for determination of germination mediated protease in order to explore the significant aptitude of protease as keynote marker of spore's germination. The developed germination method can be helpful for reducing aerial and food contact surfaces contamination of *Bacillus* spores in food and dairy industry subsequently minimizing the potential risks of food poisoning outbreaks.

Keywords: Biomarker, Casein, Eradication, Germination, Protease, Spore

Introduction

Several species of *Bacillus* and *Clostridium* have the inherent ability to sense harsh environmental conditions and undergo the process of spores formation. The spores are extremely resistant to adverse conditions of temperature, pressure, radiations, toxic chemicals and many other type of physical and chemical stresses (Sella et al. 2014; Tehri et al. 2018a). *Bacillus species* spores are ubiquitous in natural environment and their presence results unavoidably in their transmission into the food chain. The spores have unexpected resistance against common food preservation techniques and disinfection treatments commonly used in the food industry (Fan et al. 2019). Thus, the food contacted surfaces contaminated by spores can be source of cross-contamination into food processing, catering, and domestic environments (Alzubeidi et al. 2018). So, the implementation of spore germination prior to elimination step might be a potential strategy to eradicate the various spores (Alzubeidi et al. 2018; Udombijitkul, et al. 2013).

Dormant spores upon coming in contact with the appropriate germinant get convert into metabolically active vegetative cells form (Setlow, 2014; Tehri et al. 2018b). Germinants are generally of physiological and non-physiological types. Physiological germinants are most often low-molecular weight nutrients such as specific sugars, amino acids or purine derivatives etc. Non-physiological type mainly includes CaDPA, cationic surfactants and high hydrostatic pressure etc. (Setlow et al. 2017). Onset of germination in response to germinant has been found to be species and strain specific (Paredes-Sabja et al. 2011). Moreover, the triggering of germination in *Bacillus* spores has also been found to be dependent on stereospecific characteristic of nutrient germinants (Tehri et al. 2017).

Germinant induced germination of bacterial spores is a complex process involving different stages comprising of germinant recognition, release of CaDPA, cortex hydrolysis to core hydration. This process of germination resumes the metabolism and macromolecular synthesis leading to outgrowth of spores

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and their subsequent conversion into vegetative cells (Setlow et al. 2017; Setlow, 2003). The process of spore germination is of great significance for development of spore-based sensing and spores eradication techniques which will be very useful for food industries. In order to extend the concept of spore germination to facilitate these applications, detailed understanding of markers signaling the process of germination is required. Several such markers of bacterial spore germination including loss of refractility, decrease in OD, ATP, DPA, nucleic acid stainability, loss of heat resistance and enzymes have been discussed (Tehri et al. 2018c).

Keeping in view, the significance of biomarkers indicating the triggering of germination, the present study was aimed to investigate the scope of caseinolytic principle for assessment of protease, a germination marker. The activation of protease occurs during sporulation and initiation of spore germination. During sporulation, the hydrolyzed proteins produced as a result of protease action are used for the formation of spores (Setlow, 2003; Moir et al. 2002). On the other hand, during spore germination and outgrowth, the activation of protease leads to rapid degradation of small acid soluble proteins (SASP), the dormant protein reserves in the spore core. The activated form of protease inducing such complex changes including SASP degradation is a sequence specific protease referred as Gpr. Gpr has been found to be produced as a zymogen during late stages of sporulation. Prior to completion of sporulation, an autocleavage event allowing processing of N-terminus of Gpr occur for its activation. However, due to the low water activity of core region, Gpr remain unable in cleaving its substrates until hydration takes place during germination (Ponnuraj et al. 1999; Carroll and Setlow, 2005).

The rationale for the present study design and execution is the activation of proteases during germination. The activity of protease released during spores germination has been evaluated using caseinolytic approach employing chiefly skim milk and casein conjugated FITC as a source of casein (Fig.1). Spore germination can be detected by making the use of various fluorogenic substrates specific to germination mediated enzymes. One such fluorogenic substrate is FITC-labeled casein which has the advantages like low cost, resemblance to other natural substrate and stable at incubation conditions over other substrates such as labeled collagen, labeled gelatin or a labeled fibrinogen.

Materials and Methods

Materials

Nutrient media, potassium phosphate buffer constituents and fluorogenic substrates were procured from Himedia, India. D-dextrose was purchased from Sigma Aldrich India. Acetone from Fisher Scientific, India was used in this study. Distilled water was produced by Bioage-Labpure ultra plus from BIOAGE TM, Punjab, India. Centrifuge used during spores production was of

Eppendorf North America, Inc. USA. Incubation was carried out in Innova 42 Incubator Shaker Series, Eppendorf, USA. VictorX3 2030 microbiological plate reader from Perkin Elmer, USA was used to measure absorbance and fluorescence. pH meter from Century, India was used for buffers and growth medium preparation. Black bottomed 96-well black bottom microtitre plates were obtained from Greiner bio-one, Germany. Petriplates were purchased from Genaxy, India. Phase contrast microscope: Olympus BX 51 photomicroscope equipped with UPLAPO PH series of objectives, phase-contrast condenser U-PCD2 and digital camera DP70 was used for spore's refractility examination.

Microbial culture and spores production:

Culture of *Bacillus megaterium* MTCC 2949 was procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. After revival on nutrient medium, the culture was stored by refrigeration at 4°C till further use.

Spores of *B. megaterium* were prepared as per the procedure used in our previous study (Singh et al. 2013; Tehri et al. 2017). Briefly, single colony of culture streaked on nutrient agar medium was inoculated in a tryptone, glucose and yeast extract-based propagation medium. Inoculum from this medium was added to a sporulation medium of defined composition providing nutritionally starved conditions to favor spore's formation. Prepared spore's suspension (equivalent 10^7 spores/mL) was stored by refrigeration at 2–4 °C.

Protease assay

The protease activity was measured using two assays. Each assay worked on basis of protease catalyzed caseinolytic principle. The methodology used to perform each assay is explained below.

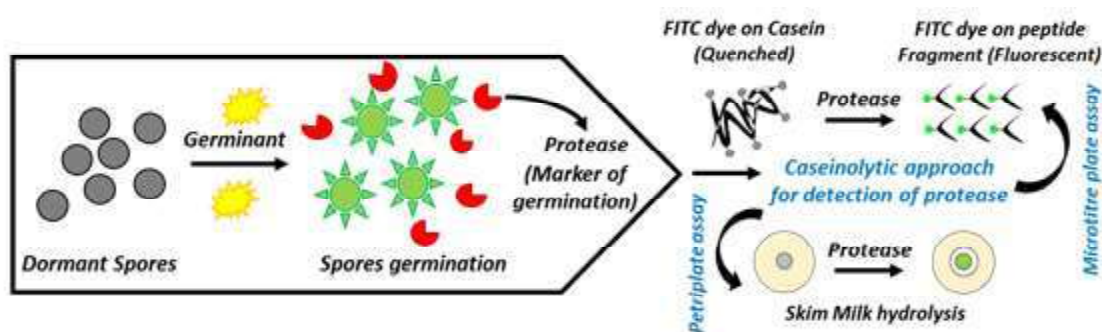
Petriplate assay

This method employed skim milk agar, which was prepared by aseptic addition of sterile nonfat dry milk (10%) to autoclaved nutrient agar medium. The prepared medium (15 mL) was poured into petriplates (90×15 mm) and allowed to solidify. Evenly spaced wells were formed in the agar with a flame-sterilized borer. One hundred microliter of spore suspension and D-dextrose in a ratio of 1:1 was added to test well while 50 µl each of spore suspension and sterile distilled water were taken in control well. The plate was incubated upright at 37°C for 12-16 h. Following incubation, activity of protease was determined by observing the plate for appearance of clearing zones of casein hydrolysis and diameters were measured in millimeter (mm).

Microtitre plate assay

This assay was carried out to study the kinetics of protease expression during spore germination in a 96-well black-bottom microtitre plate. A stock solution (1.0 mg/mL) of FITC labeled

Fig1. Schematic diagram illustrating the principle of using protease as marker of spore germination



casein was prepared in 10 mM potassium phosphate buffer (pH 6.8). Stock solution (10 μ L) of FITC labeled casein was added to 490 μ L potassium phosphate buffer to prepare a working solution. Fifty microliter each of spore suspension and germinant solution i.e. D-dextrose were added in test wells while control well contains spore suspension with potassium phosphate buffer replacing germinant. To each of these wells i.e. test and control, 50 μ L working solution of FITC labeled casein was added and incubation was carried out at 37°C. Fluorescence was measured using microbiological plate reader at an excitation-emission wavelength of 485/535 nm. Readings were recorded after every 10 min. for a period of 60 min and all assays were performed in triplicate.

Refractility examination of spores

This technique was also used to study dormant and germinating spores. Smear preparation was carried out as per the protocol used in our previous study (Tehri et al. 2017) with slight modification. Briefly, pre incubated (37°C, 10-15 min.) mixture of spore suspension with and without dextrose were spread as thin as possible on test and control clean glass slides respectively to make the smear. Smears were allowed to air dry. Prepared slides were observed for refractility to study dextrose mediated germination in spores of *B. megaterium* by phase contrast microscope.

Results and Discussion

Current work was carried out with the objective to explore the use of caseinolytic approach for detection of protease activity as marker of spore germination. The activity of protease was assessed for spores undergone the process of germinant induced germination and also in dormant spores taken as control. The findings obtained from protease assay were further confirmed by refractility microscopy technique.

Measurement of protease activity by petriplate assay

As per the procedure given under methodology section, spores of *B. megaterium* and germinant D-dextrose were added in test well on agar plate. Following incubation, activity of germination mediated protease was observed in terms of a clear zone of hydrolysis on skim milk agar (Fig.2). The zone of 12 mm in diameter



Fig 2. Petriplate assay-based observations on protease activity of spores - a) with germinant; b) without germinant

was observed within 6.0 h of incubation while no clear zone was observed for control well containing spores without germinant. Milk protein casein has also earlier been exploited as substrate to measure protease and peptidase activity in *B. megaterium* spores. Hence, clear zones formed in agar depict the areas where casein is hydrolyzed by the proteolytic activity of proteases released upon germinant induced triggering of germination.

Activation and release of an array of enzymes during initial stages of spore germination has been formerly used as a parameter to detect spore germination (Ferencko et al. 2004). The activity of protease is well known to be checked by using caseinolytic principle in vegetative cells (Vijayaraghavan and Vincent, 2013) which supported our study. The skim milk contains casein, a milk protein that undergo proteolytic cleavage by proteases to smaller polypeptides, peptides, and amino acids resulting in formation of clear and halo zones. Owing to the simplicity, accuracy and ease of performing, we have used a petriplate assay based on the skim milk based agar diffusion approach to detect protease activity in bacterial spores. D-dextrose which is well-known to trigger germination in *B. megaterium* spores was used as germinant in current work (Tehri et al. 2017).

Fig 3. Exponential kinetics of protease expression during germinant mediated germination of spores (n=3; Error bars represents SE of the mean FU value)

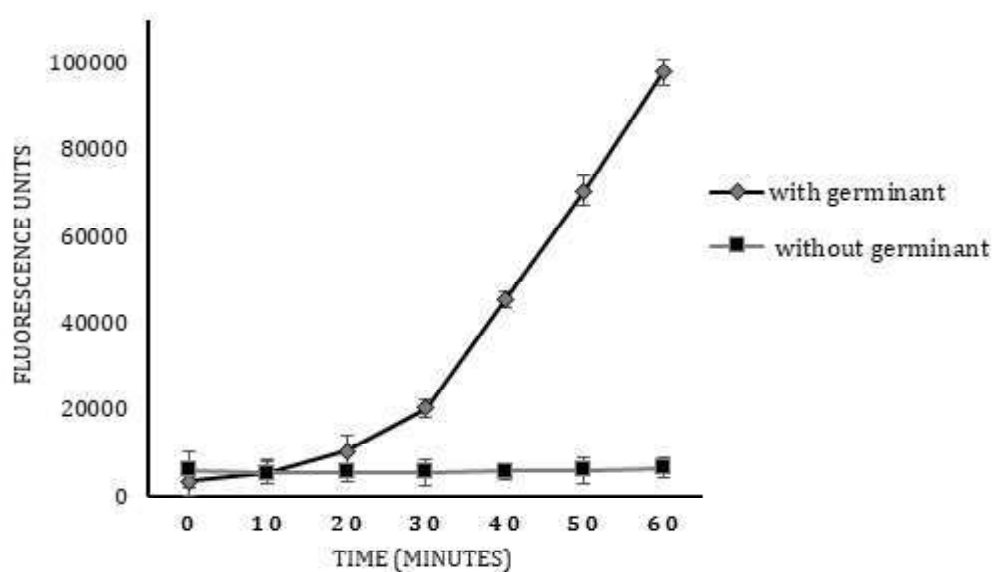


Fig 4. Phase contrast microscopic observations of spores

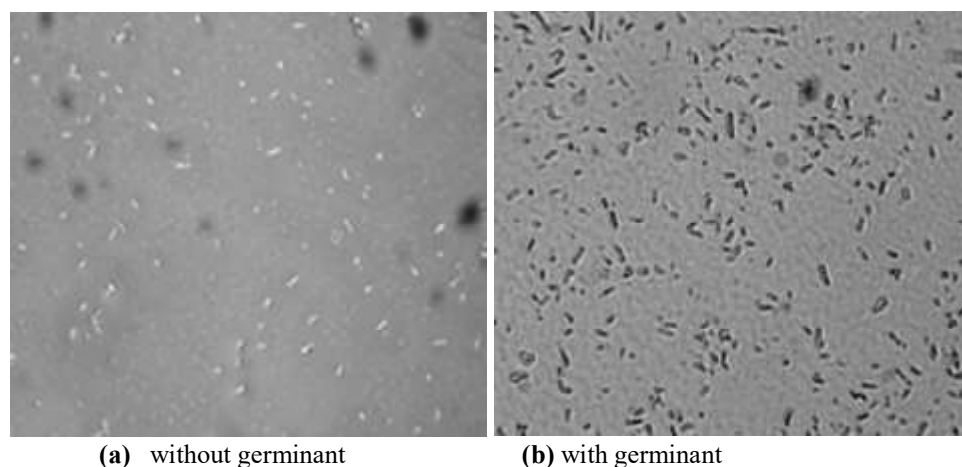


Table 1. Summary of observations recorded on expression of protease during germinant induced spore germination

^a Reaction	^b Diameter of zone of casein hydrolysis(mm)	^c Fluorescence Units (FU) ($\times 10^3$)	^d Phase bright/ Dark
Spores with germinant	12	97.98	Phase dark
Spores without germinant	Nil	6.76	Phase bright

^a Dextrose was used as a germinant; ^b Represents findings of petriplate assay ; ^c Represents findings of microtitre plate assay

^d Observations with phase contrast microscopy

Measurement of protease activity by microtitre plate assay

The fluorogenic substrate i.e. FITC-labeled casein was cleaved by protease to yield the fluorescein thiocarbamoyl (FTC) derivative resulting in increased fluorescence which can be measured in a time dependent manner using a basic fluorometer (Cupp-Enyard 2009). This approach has been used in our work to optimize a microtitre plate method for studying the kinetics of protease released during spores germination. The results obtained on kinetic experiment are shown in Fig.3. At time zero, D-dextrose

and FITC labeled casein was added to the spore suspension and fluorescence was measured at an interval of every 5 min. for a period of 0 min. After a lag period of 5–10 min, a rapid increase in protease activity showing exponential kinetics was observed. On the other hand, during the same time period there was no significant activity of protease when dextrose was omitted. Thus, activity of protease was measured in terms of increase in fluorescence due to cleavage of FITC-casein over time. Significant increase in activity of protease ranging from 3.63 to 97.98 ($\times 10^3$) FU was observed within 0 to 60 min. of exposure of

spores to nutrient germinant. In contrast, control containing spores incubated without germinant expressed baseline protease activity with FU values of 5.67 to 6.76 ($\times 10^3$) FU. Thus the findings obtained with this assay clearly depicted activation of protease during germination of spores only upon incubation with germinant. Similar principle for measurement of protease activity has also been reported for *G. stearothermophilus* to determine the sterility of biological articles (Chandrapati et al. 2011). Using fluorogenic approach, like protease expression of esterase during germination has also been studied as a parameter to quantify germination in spores of *B. anthracis* (Ferencko et al. 2004).

Spores refractility examination

Refractility of dormant and germinating spores was studied using phase contrast microscopy. Observations taken from the images grabbed by the phase contrast confirmed that the spores expressing protease have undergone germination triggering by D-dextrose. The spores incubated without germinant were not germinated as they were found refractile (phase bright) in appearance (Fig.4a). On the other hand, the germinated spores were accompanied by rapid loss of refractility (phase dark) due to change of their refractive index (Fig.4b). This change in refractive index of spores during germination has been reported to occur vividly due to release of core's DPA, water uptake, and subsequent hydrolysis of cortex leading to further swelling of core and more uptake of water (Setlow, 2003; Zhang et al. 2010). The above findings are well supported by earlier studies showing germinated spores to lose their characteristic refractility and appear dark when examined by phase-contrast microscopy (Tehri et al. 2017).

The aforementioned findings (Table 1) reported in this communication demonstrates: (i) simple, easy to perform skim milk and agar diffusion based petriplate assay and; (ii) casein conjugated FITC based fluorimetric microtitre plate assay for detection of germination by measuring protease activity; (iii) protease release as an early event occurring during nutrient induced spore germination and; (iv) expression of protease correlates with loss of spore refractility as measured by phase-contrast microscopy.

Conclusions

The activation of protease during germinant induced germination of spores has found to be under strict regulatory control as it remains inactive in dormant spores. Hence, expression of protease represents a significant biochemical marker activity, particularly suitable for easy and fast detection of spore's germination as measured by the protease catalyzed hydrolysis of skim milk and FITC labeled casein-based approach attempted in this study. The advent of assaying markers like protease can further open the door to explore the efficient use of spore germination process for development and evaluation of spore eradication systems

from the air and food contact surfaces from the food as well as dairy industries.

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RESEARCH ARTICLE

Isolation of *Escherichia coli* from raw milk and detection of antibiotic resistance genes by blaTEM PCR

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Abstract: *Escherichia coli* is an important pathogen that causes urinary, gastrointestinal infections and septicaemia. The present study aimed to identify the prevalence of *E. coli* in raw milk samples and relate the results with public health implications of the infection. A total of 106 raw milk samples of cattle and buffalo were collected from the local vendors and organized as well as unorganized dairy farms in and around the Mumbai region. Total coliform count and isolation of *E. coli* from milk samples was done on MacConkey and Eosin Methylene blue agar. The recovered isolates were subjected to a blaTEM PCR assay. The results showed that the total coliform count range was from 3.7×10^5 to 1.2×10^6 , 2.6×10^5 to 1.8×10^6 , 3.6×10^5 to 1.48×10^7 , 4.4×10^5 to 6.2×10^6 , and 4.9×10^5 . It can be concluded that the rate of isolation of *E. coli* from raw milk samples in our study was 39.62 %. In vitro antimicrobial sensitivity testing of *E. coli* showed that 92.85% of the isolates were sensitive to Ciprofloxacin, followed by 69.04% to Imipenem, 66.66% to Amikacin, 59.52% to Ceftriaxone, 57.14% to Tetracycline and 11.90% to Amoxicillin + clavulanic acid. Whereas 88.09% of the isolates were resistant to Amoxicillin + clavulanic acid followed by 42.85% to Tetracycline, 40.47% to Ceftriaxone, 33.33% to Amikacin, 30.95% to Imipenem and 7.14% to Ciprofloxacin. Out of 42 isolates, ten isolates (23.8%) showed a blaTEM gene-specific amplicon of 1215 bp. There is a need to reduce the contaminants by implementing appropriate measures as resistant *E. coli* contaminated raw milk samples were found in the Mumbai region.

Keywords: Raw milk, *E. coli*, blaTEM PCR assay, Total coliform count

Introduction

Milk is a highly nutritious medium and many bacteria, including spoilage and pathogenic bacteria, can grow and propagate in it. Raw milk may be contaminated by a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp., *Brucella* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Corynebacterium* spp., as well as various yeasts and moulds. Thus, raw milk containing a high bacterial count, including food borne pathogens, likely increases the probability of contamination of dairy products to be consumed by humans. Raw milk with pathogenic bacteria may pose a significant public health concern, especially for those individuals who still drink raw milk in rural areas (Ryser, 1998). Good milk hygiene practices must be followed to improve the quality of raw milk. The practices like maintaining clean environment at the farm, hygienic habits of milk handler will reduce contamination of raw milk (De Silva et al. 2016).

E. coli is also an important pathogen that causes various illnesses, including urinary and gastrointestinal infections and septicaemia. The most dangerous ones associated with raw milk and milk products are enterotoxigenic *E. coli* (ETEC) and verotoxigenic *E. coli* (VTEC) (Kaper et al. 2004). The contamination with one of these bacteria might cause watery diarrhoea, nausea, abdominal cramps, and fever. Beta-lactams, including cephalosporins, are generally used in human and veterinary medicine to treat these infections. The primary mechanisms of resistance to oxyimino-cephalosporins in *E. coli* rely on the production of extended-spectrum beta-lactamases i.e. ESBLs. Antimicrobial resistance has emerged as a significant global problem in the past years, and many programs have been set up for its surveillance in human and veterinary medicine (Lanz et al. 2003; Perez et al. 2007).

Traditional methods like culture, bacterial count are used for bacterial detection from raw milk samples. Nowadays molecular techniques like PCR are used for diagnostic purpose. In the present study, we planned to isolate *E. coli* from raw milk samples

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and detect antibiotic resistance genes by conventional and molecular techniques. The antimicrobial susceptibility patterns of isolated bacteria were also studied.

Materials and Methods

Milk samples

A total of 106 raw milk samples of cattle and buffalo were collected from the local vendors and organized and unorganized dairy farms during this study. The samples were collected from various locations in and around the Mumbai region and other areas of the Thane district. All the milk samples were processed for the total coliform count, isolation and identification of *E. coli*, antimicrobial susceptibility test of recovered isolates by conventional methods and further subjected to PCR to detect the antibiotic resistance genes.

Processing of milk samples for bacterial count and Isolation and identification of *E. coli*

The milk samples were centrifuged at 12000 rpm for 15 minutes. The supernatant and fat layer were discarded with the help of micropipette and sterile tips. Around 100 µl of sample was taken to make serial ten-fold dilutions by transferring to 900 µl of NSS. This procedure is carried out till the desired number of dilutions has been obtained. From this, 100 µl of three dilutions of each sample were taken on MacConkey and Eosin Methylene blue agar using an L-shaped sterile spreader by spread plate method. Duplicates of each plate were made to minimize error. The inoculated plates were incubated at 37°C for 24 hr and examined for the appearance of bacterial colonies. After the incubation period, bacterial colonies on the culture plates were counted manually. Two necessary dilutions per sample were counted. A plate was divided into quarters using a marker pen and colony-forming units were counted on at least two critical dilution plates by the colony counter. Two consecutive plates with less than 300 colonies were considered for record

Expression of results

Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor. The CFU/ml can be calculated using the formula:

$$\text{cfu/ml} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

The milk samples were processed for isolation of *E. coli* using standard bacteriological procedures (OIE, 2004). After centrifugation of milk samples, the pellet was inoculated aseptically on the plates of MacConkey and Eosin Methylene blue agar and incubated at 37°C for 24 hrs in the incubator. The cultures were observed regularly for the appearance of growth. The colonies suggestive of *E. coli* were examined for the

morphology and staining characters employing Gram's staining method. The colony characters suggestive of *E. coli* were processed further to identify the organism using standard bacteriological procedures (Quinn *et al.* 1994; Edward and Ewing, 1972). The isolates showing gram-negative rod-shaped forms and producing lactose fermenting pink, smooth, round colonies on MacConkey agar and green metallic sheen on EMB agar were tentatively considered as *E. coli* and subjected further for identification of the organism by biochemical studies.

The isolates suspected to be of *E. coli* confirmed by gram's staining, morphology, colony characteristics were subjected further to different identification tests recommended by OIE (2009), Cruickshank *et al.* (1975) as described below.

Oxidase test

Standard oxidase discs (Hi Media Laboratories Ltd., Mumbai) were used to perform the test. The loopful of culture from single colony was touched on the disc. Immediate development of blue color was considered as positive test whereas in negative test no color change was observed.

Catalase test

This test was performed with the help of slide method in which the loop was used to transfer a small amount of colony growth on the surface of a clean, dry glass slide. Then the drop of 3% H₂O₂ was poured on the glass slide. Immediate production of gas bubbles was considered as positive test, where as in negative test no gas bubbles were observed.

Indole test

The test was done by inoculating the organisms in tryptone broth (Himedia) and incubating at 37°C for 24 hrs followed by addition of 0.2 mL of Kovac's reagent from side of the test tube (Hi-media). Appearance of pink colored ring was considered as positive and yellow colored ring at the interface was considered as a negative test.

Methyl-Red (MR) test

The test was performed using Methyl red (MR) broth in which pure culture was inoculated. The tubes were incubated at 37°C for 48 hours. Thereafter 5 drops of methyl red indicator solution was added to the test tube. Positive results shows bright red colour however yellow colour indicates negative results.

Voges-Proskauer (VP) test

The test was performed using Voges-Proskauer (VP) broth in which pure culture was inoculated. The tubes were incubated for 48 hours at 37°C. After incubation 3 mL of Barrit B reagent (alpha naphthol) and 1 mL of Barrit A (40% KOH) reagent was added.

The tube was exposed to atmospheric oxygen for 10-15 minutes. Positive results shows pink colour in 2-5 mins and negative result shows yellow or copper colour at the surface.

Citrate test

Simmon's citrate agar slants were inoculated from 18–24 hours old culture colony and incubated at 37°C and observed upto 7 days. A positive reaction was observed by color change from green to blue in the slant along with growth and negative showed no color change and no growth.

Urease test

Christensen's urea agar slants were inoculated with 18–24 hours old culture and incubated at 37°C and observed upto 7 days. A positive reaction was observed by development of pink color in the slant and no color change of the slant in negative reaction.

In vitro antimicrobial sensitivity test

Antibiotic sensitivity test of *E. coli* was carried out according to Bauer et al. (1966) disc diffusion method on Muller Hinton Agar (MHA) medium. Antibiotic disc of Imipenem (IPM 10 mcg), Ciprofloxacin (CIP 5 mcg), Amoxicillin/clavulanic acid (AMC 10 mcg), Ceftriaxone (CTR 30 mcg), Tetracycline (TE 30 mcg) and Amikacin (AK 30 mcg) from Hi media Mumbai, were used. Antibiotic discs were stored at 4°C and allowed to reach room temperature before being used. The inhibition zones developed around the discs measured in millimetre (mm) were interpreted as susceptible (S), intermediate (I) or resistant (R) to a particular antimicrobial agent according to Clinical Laboratory Standards Institute/ CLSI (2018).

Molecular characterization of *E. coli*

DNA extraction

DNA was extracted from cultures as per the protocol described by Sambrook and Russell (2006).

Two to three loops of growth from freshly grown cultures was transferred with the help of wire loop (5mm diameter) to a micro centrifuge tube containing 400 µl of 1X TE buffer. 20 µl of lysozyme (20mg/ml) was then added to the suspension, the tube was vortexed briefly and incubated at 37°C for 1 hr in water bath. To disrupt the cell membrane 200µl of 10 % SDS and 20 µl of Proteinase K (20mg/ml) was added, vortexed briefly and incubated at 65°C for 2 hrs in water bath. Following incubation, 200 µl each of 5M NaCl and pre-warmed 200 µl CTAB- NaCl solution was added. The mixture was vortexed and incubated at 65°C for 20 mins. Subsequently, same amount of P:C:I (25: 24: 1) was added and vortexed for 10 sec and centrifuged at 10,000 rpm for 10 mins. The aqueous phase at top containing DNA was carefully transferred to a fresh micro centrifuge tube and DNA was

Table 1: Oligonucleotide primers for blaTEM PCR

F	5' –TTC-TTG-AAG-ACG-AAA-GGG-C'3
R	5' -ATG-GTG-AGT-GGA-ACG-AAA-AC-'3

precipitated by addition of 0.6 volume of isopropanol. The tubes were then kept at -20°C for 3 hours or overnight followed by centrifugation of 12,000 rpm for 15 mins. The supernatant was discarded, leaving about 20µl above the pellet which was washed with 1ml of 70% Ethanol and centrifuged at 11,000 rpm for 10 min. Pellet was dried at room temperature for 30 min. or until evaporation of all ethanol, dissolved in 50 µl of sterile distilled water and kept at 65°C in water bath for 1 hr. The DNA so extracted was stored at -20°C until use.

Detection of *E. coli* by blaTEM PCR assay

The amplification of 1215 bp region of blaTEM genetic element of *E. coli* was carried out using published oligonucleotide primer sequences (Table 1) as per (Dewangan *et al.* 2015). The oligos were manufactured and supplied by M/s Bangalore Genei, Bangalore (India)

The PCR standardized in a total reaction volume of 25 µl, containing 10X PCR buffer, MgCl₂ (25 mM), dNTPs mix (10 mM), Primers F (10pM/ µl) and Primer R (010pM/ µl), Template DNA and Taq DNA polymerase (5 U/µl). The reaction was standardized in a thermal cycler (Master Cycler, Eppendorf) with initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 49°C for 45 s and 72°C for 1 min 30 sec. Final extension was carried out at 72°C for 7 min. The amplified product was electrophoresed on 2% agarose gel stained with ethidium bromide (EtBr) and the products were visualized and documented using Automatic Computerized Gel Documentation and Analysis System (Gel Doc EZ Imager, Bio-Rad) using UV light source. The size of PCR product was estimated with the help of Image Lab (Version 4.1) software available with the gel documentation system.

Results and Discussion

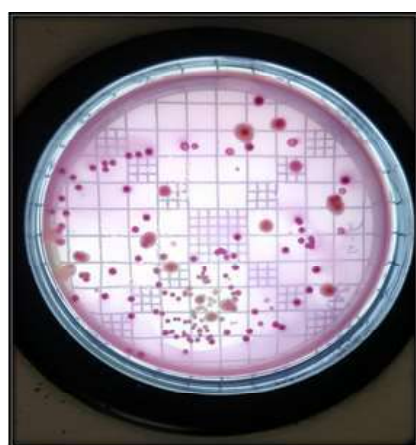
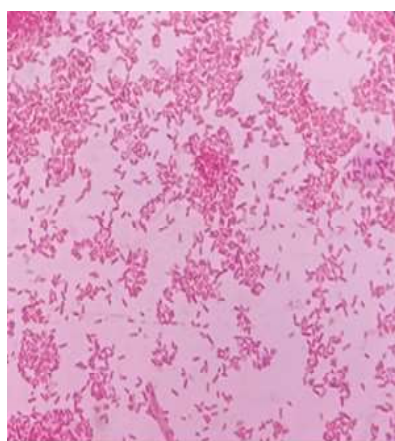
A total of 106 raw milk samples of cattle and buffalo collected from various dairy farms were used in the present study. Out of 106 milk samples 54 samples were randomly selected and processed for total coliform count. TCC of milk samples was carried out to assess the microbial quality of raw milk samples of Mumbai region.

Total Coliform Counts

A total of 54 milk samples were cultured for Total Coliform Count (TCC). The results showed that the TCC range of 10 milk samples of farm-I was from 3.7x10⁵ to 1.2x10⁶. Farm II TCC range was from 2.6x10⁵ to 1.8x10⁶ while TCC range of 15 milk samples of farm-III was from 3.6x10⁵ to 1.48x10⁷. Farm IV and VI showed no TCC

Table 2: TCC in raw milk samples

Sources of Milk samples	No. of Tested samples	TVC cfu/ml
Dairy Farm (I)	10	3.7×10^5 to 1.2×10^6
Dairy Farm (II)	04	2.6×10^5 to 1.8×10^6
Dairy Farm (III)	15	3.6×10^5 to 1.48×10^7
Dairy Farm (IV)	10	—
Dairy Farm (V)	05	4.4×10^5 to 6.2×10^6
Dairy Farm (VI)	05	—
Kalyan Tabela (VII)	05	4.9×10^5
Total	54	

**Fig. 1:** Total Coliform Count (TCC) on MacConkey Agar**Fig. 2** Gram staining of *E. coli***Fig. 3** Lactose Fermenting Colonies of *E. coli* on MacConkey Agar

count. The TCC range of 05 milk samples of farm V was from 4.4×10^5 to 6.2×10^6 . Farm VII showed TCC was 4.9×10^5 (Table 2 and Fig.1).

The acceptable limits of coliform counts in milk should be less than 100 cells/ml (Shojaei and Yadollahi, 2008; Douglas, 2003; Boor *et al.* 1998). The overall results indicated that the TCC of milk samples were higher than the maximum recommended level. The findings of the present study are in accordance with the results of Lee *et al.* 1983; Ogot *et al.* 2015 and Wanjala *et al.* 2017.

Wanjala *et al.* (2017) reported higher coliform counts i.e. 4.70 and 1.0 log₁₀cfu/ml for raw and pasteurized milk in 77.8% and 4.8%, respectively of raw and pasteurized milk samples. Lee *et al.* (1983) found that the bacterial count in raw milk ranged from 4×10^6 to 2.7×10^7 per ml. Ogot *et al.* (2015) found that the total bacterial count was significantly higher than the coliform count in all the samples. In addition, the coliform count in 60% of the raw milk samples was more than 50000 cfu/ml.

High counts are attributed to poor hygienic conditions (Saeed *et al.* 2009), lack of cooling facilities (Murphy and Boor, 2000) and duration of milk storage at the farms before marketing. Moreover, Gran *et al.* (2002) mentioned that unhygienic practices during the milking process affect raw milk's microbiological quality. Moreover, inadequate sanitization of the post milking process

that leaves dung in contact with udder may also contribute to the increased bacterial load of raw milk.

Isolation of *E. coli*

A total of 106 raw milk samples of cattle and buffaloes were processed for isolation of *E. coli* resulting in the recovery of 42 (39.62 %) isolates of *E. coli*. The rate of isolation of *E. coli* from raw milk samples in our study was 39.62 %.

All the isolates recovered were tentatively identified as *E. coli* on the basis of morphology, staining reaction in Gram's staining method and growth characteristics on MacConkey agar and Eosin Methylene Blue agar. The isolates showing gram negative rod shaped forms (Fig. 2), motility and produced lactose fermenting smooth, round opaque colonies on MacConkey agar (Fig. 3) and green metallic sheen on EMB agar (Fig. 4) were tentatively identified as *E. coli* and further confirmed by biochemical tests. All isolates showed IMViC pattern (Fig. 5) as depicted in Table 3 wherein catalase (Fig. 6) test was positive and urease (Fig. 7) and oxidase tests were negative.

Several workers studied the raw milk samples for isolation of *E. coli* like Soomro *et al.* (2002), Altalhi and Hassan (2009), Kumar *et al.* (2010), Thaker *et al.* (2012), Yohannes, (2018) and Megersa *et al.* (2019). The prevalence of *E. coli* in raw milk samples is

presumably because it is the commonest environmental contaminant, which is closely associated with poor farm hygienic conditions and poor environment. Its pathogenicity is associated with poor animal hygiene and environmental conditions on the farm premises. Moreover, the existence of high counts of *E. coli* in milk also indicates the relatively poor quality of milk, related to substandard hygiene of the farm, poor management during milk collection and processing. The isolation of *E. coli* is of public health significance as this bacterium is known to cause severe gastrointestinal disorders in both young and adult humans (FAO and WHO, 2004).

Results of AST showed that 39 *E. coli* isolates (92.85%) were sensitive to Ciprofloxacin. Twenty nine isolates (69.04%) were sensitive to Imipenem while 28 (66.66%) isolates were sensitive to Amikacin. Of 42 *E. coli* isolates 25 (59.52%) were sensitive to Ceftriaxone, 24 (57.14%) to Tetracycline and 05 (11.90%) to Amoxicillin + clavulanic acid. However 37 (88.09%) isolates were resistant to Amoxicillin + clavulanic acid, 18 (42.85%) were

Table 3: Identification of *E. coli* isolates by biochemical tests

S.No.	Name of the test	<i>E. coli</i>
1	Catalase test	Positive
2	Urease test	Negative
3	Oxidase test	Negative
4	MR(Methyl Red)	Positive
5	VP(Voges Proskaur test)	Negative
6	Indole test	Positive
7	Nitrate Reduction test	Positive
8	Citrate	Negative

resistant to Tetracycline, 17 (40.47%) were resistant to Ceftriaxone, 14 (33.33%) were resistant to Amikacin, 13 (30.95%) to Imipenem and 03 (7.14%) to Ciprofloxacin. The most effective antibiotics were ciprofloxacin and Imipenem while the less effective antibiotics were Amoxicillin + clavulanic acid and Tetracycline (Fig. 8, Table 4 and Fig. 10).

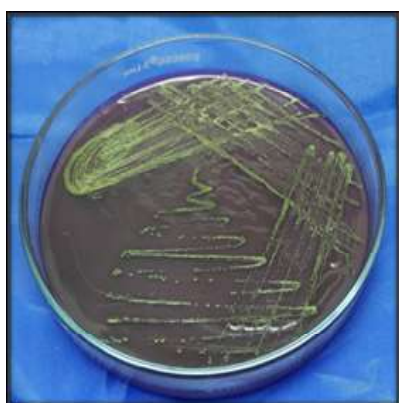


Fig. 4 Green Metallic Sheen of *E. coli* on Eosin Methylene Blue (EMB) agar

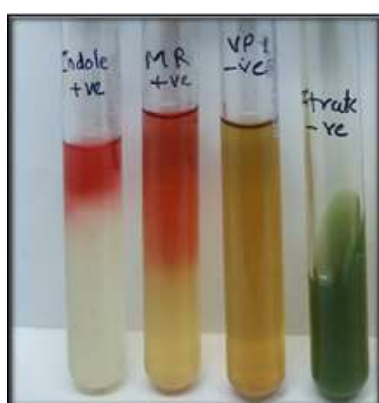


Fig. 5 Biochemical tests (IMViC pattern) of *E. coli*

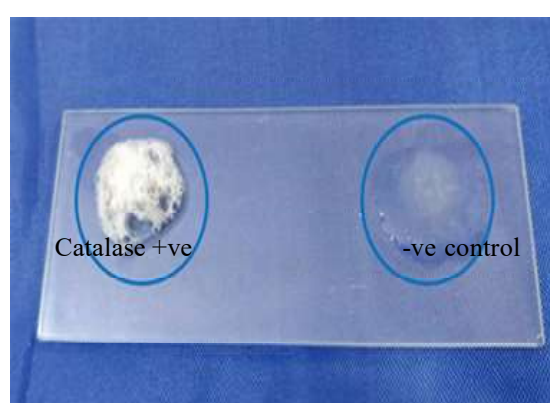


Fig. 6 Catalase Test

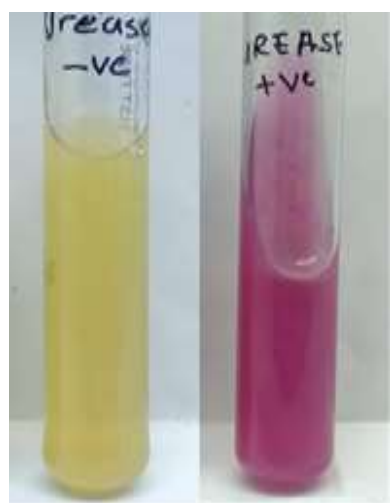
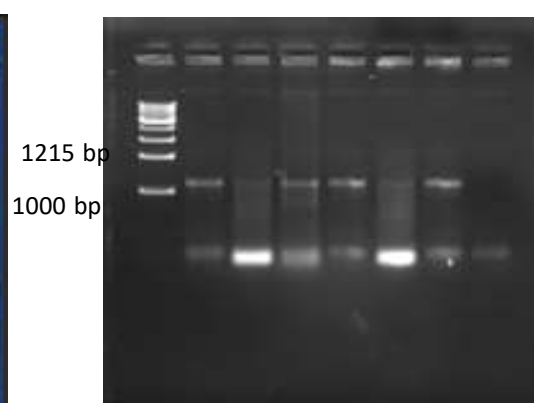


Fig. 7 Urease Test

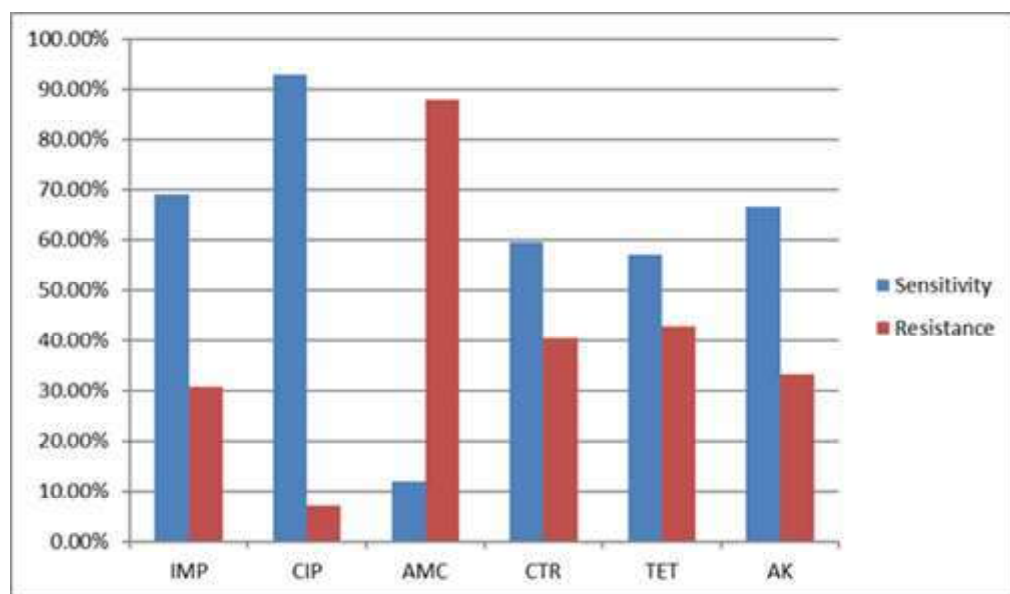


Fig. 8 Antibiotic Sensitivity Test Results



Lane – 1Kb Ladder, 1 - 6- *E. coli* isolates, 7-Negative Control

Fig. 9 Identification of *E. coli* isolates by *bla*TEMPC

Fig. 10 *In-vitro* antimicrobial sensitivity test results of *E. coli*

1. IMP- Imipenem 2. CIP- Ciprofloxacin 3. AMC- Amoxicillin + clavulanic acid 4. CTR- Ceftriaxone 5. TET- Tetracycline 6. AK- Amikacin

Table 4: *In-vitro* antimicrobial sensitivity test results of *E. coli*

Antibiotics used	IMP	CIP	AMC	CTR	TET	AK
Resistance	13(30.95%)	03(7.14%)	37(88.09%)	17(40.47%)	18(42.85%)	14(33.33%)
Sensitive	11(26.19%)	27(64.28%)	00	14(33.33%)	18(42.85%)	7(16.66%)
Intermediate	18(42.85%)	12(28.57%)	05(11.90%)	11(26.19%)	06(14.28%)	21(50%)
Total	42	42	42	42	42	42

Our findings are in agreement with Akarsh et al. (2019), Bindayna et al. (2009), Ombarak et al. (2018) and Paghdar et al. (2020). Akarsh et al. (2019) found that 76.66% of *E. coli* isolates were susceptible to antibiotics Imipenem and 46.66% to Ceftriaxone. While Bindayna et al. (2009) showed that both Imipenem and meropenem remain the most effective class of drugs for *E. coli*. Ombarak et al. (2018) observed the resistance traits of 27.5% *E. coli* to tetracycline (27.5%), 1.4% to ciprofloxacin and 100% sensitivity to Imipenem. Paghdar et al. (2020) observed that 56(59%) of *E. coli* isolates were resistant to Ceftriaxone, 34(44.73%) to Cefpodoxime and 8(8.5%) to Ceftazidime and Clavulanic acid.

These findings indicate that the antibiotic sensitivity and resistance pattern of bacteria isolated from raw milk samples is recommended whenever *E. coli* is suspected.

The blaTEM PCR assay

The blaTEM PCR assay using blaTEM F/ blaTEM R primers was carried out to detect antibiotic resistance genes. A total of 42 isolates that were identified as *E. coli* by conventional methods were included in the PCR assay. Out of 42 isolates, ten isolates (23.8%) showed a blaTEM gene-specific amplification product of 1215 bp (Plate 9).

Similar studies were carried out by Bajpai et al. (2017), Ombarak et al. (2018), Akarsh et al. (2019) and Paghdar et al. (2020). Bajpai et al. (2017) found that blaTEM was the most important gene, with a prevalence rate of 48.7 %. Ombarak et al. (2018) detected the blaTEM gene in 40 *E. coli* isolates recovered from raw milk. Akarsh et al. (2019) found that the blaTEM gene was present in 17(56.66%) *E. coli* isolates. Paghdar et al. (2020) isolated extended-spectrum β -lactamase (ESBL) producing *E. coli* from 150 milk samples from Anand and around villages and subjected them to PCR targeting the blaTEM gene. They found 5(14.70%) isolates positive for the blaTEM gene. Dewangan et al. (2015) isolated *E. coli* from 104 raw milk samples. Further, they found that the 44 (42.3%) isolates were identified as presumptive ESBL producers, and out of them, 39.4% isolates were found to harbour the blaTEM gene on their plasmid DNA, showing the presence of multidrug-resistant ESBL producing *E. coli* in raw milk samples. Ansharieta et al. (2020) characterized *Escherichia coli* producing ESBL encoding genes isolated from 200 raw cow milk samples in East Java, Indonesia. They found that the double blaCTX-M and blaTEM gene was harboured by 2 ESBL isolates and one blaTEM gene by 1 ESBL isolate.

Raw milk samples were found to be contaminated by bacteria from the family Enterobacteriaceae with a diversity of extended-

spectrum beta-lactamase (ESBL) encoding genes. The finding of Extended-Spectrum Beta-Lactamase (ESBL) carriers in milk reveal the risk posed to food safety, particularly to communities that consume raw milk.

In the present study, it was observed that the raw milk was contaminated with resistant strains of *E. coli*. There is, therefore, a need to detect the sources of this resistance and implement interventions to reduce the emergence and spread of these resistant strains of *E. coli*. Therefore, implementing measures that could reduce the presence of antimicrobial-resistant *E. coli* strains in the raw milk food chain is essential.

Conclusions

Total coliform count (TCC) was higher than the maximum recommended level so raw milk is not suitable for drinking purpose. Strict hygienic practices during milking process must be followed to improve the microbiological quality of raw milk. The isolation rate of *E. coli* was of 39.62 % from raw milk samples. Imipenem and Ciprofloxacin were more effective antibiotics for treatment of *E. coli* infection in raw milk samples while Amoxicillin + clavulanic acid was the least effective antibiotic. The blaTEM PCR assay proved to be effective in detecting the presence of antibiotic resistant gene in *E. coli* isolates. Further elaborate studies are needed to confirm the role of environmental contamination as a source of *E. coli* in raw milk.

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Optical Butyrometric reading tube: An approach towards Gerber automation

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Abstract: The Gerber Test is among the oldest and most widely used laboratory test with significant applications that include determination of Fat of different dairy products. A major limitation in automation of Gerber test which can be used as reference method is handling of its complex test equipment Butyrometer and its manual reading procedure. To make the device auto readable detail understanding of butyrometer design and its operation is required. In this research paper, the design of newly developed butyrometer reading tube and measurement reading with it is discussed. The developed butyrometer reading tube can provide real time sample analysis after centrifugation and is compatible to several commercial butyrometers. In the prototype of automatic butyrometer reading system four type of milk sample of different fat were used for analysis. The collected data are valuable for analytical purposes (e.g., quality control, milk price estimation), or as feedback to the user. The system adds new functionality to existing manual Gerber test, saving users time and providing useful feedback. This concept enables new analytical applications for an instrument that has remained largely unchanged for decades. Successful application of the equipment would be very helpful to all types of Dairy vendors.

Keywords: Gerber, Butyrometer, Optical Butyrometer reading, Regression model

Introduction

Milk fat is valuable nutrient that help support a growing body, including calcium and protein (Żywica et al. 2012). The fat content defines the milk quality, both economically and physiologically. Many quick measurement tests are used to determine its value. Reference methods are rigorously studied and elucidate measurement procedures. These methods are used to evaluate the reliability and calibration of other measurement procedures. Specific instruments may be used to produce “reference values” for other instruments and for other laboratories in case of a system with centralized calibration (Badertscher et al. 2007). Famous technique used worldwide is Gerber test which was developed by Niklaus Gerber, patented in 1891 under the name “Acid-Butyrometer” (Niklaus, 1891; IS, 1977; Shrestha, 2021). The Gerber test is an elementary rapid, and economical method for determination of the fat content of various milk and milk products. Gerber test is unique due to its requirement of several specialized apparatus like butyrometers, pipette, centrifuge, and water bath, to carry out the test (James, 1995). Butyrometer reading is manual which is drawback of the test and in this paper a smart tube is develop to make the reading automatic (Pankti Desai, 2022). This will help the manual Gerber method to record readings automatically and feed to remote central control system which was not possible before. Low-cost approach of automatic butyrometer reading tube can be extremely helpful in data acquisition and quality control for all type of industries. Additionally, Auto cost estimation system can be developed for small milk vendors once IOT based system transmit the fat value from small milk vendors to collection centres.

Materials and Methods

Gerber Process

In procedure of this test, portion of milk is pipetted into a Gerber milk butyrometer containing sulfuric acid. Isoamyl alcoholic is added, and the contents of the butyrometer are mixed to dissolve the milk and release the fat. The released fat is isolated in the

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neck of the butyrometer by centrifugation. The percentage of fat in milk (g/100 g) is determined by reading the calibrated scale on the neck of the butyrometer. This method due to its inexpensive and rapid process result is famous in India and Europe's dairy industry. Different butyrometer are designed for different dairy products are used such as whole milk, skim milk, and cream. Due to use of concentrated sulfuric acid, it is difficult to automate the Gerber test. The handling of butyrometer requires practical experience, which has an adverse effect on the robustness of the method. Many experiments are done to establish relation between the fixed butyrometer scale with the fat values. Also, extraction of fat is not possible using butyrometer. Routine method on other hand are fully automatic methods and faster in operations but drawback is its high price. Automatic butyrometer reading tube and transmission of fat value to remote station can be great revolution. Few literatures for Gerber automation were found in which they had tried to develop rotating disc based automatic butyrometer filling system but the fat reading in that case was also manual (Phade et al. 2018). Automatic measurement of butyrometer fat was not attempted due to complex relation of fat and butyrometer graduation tube. For automatic butyrometer the first step was to understand butyrometer design, its scale and fat relation.

Butyrometer Design

The butyrometers are made of clear glass which is as free as possible from visible defects. The stress in the glass shall be reduced by annealing to minimize the possibility of fracture by thermal or mechanical shock.

Butyrometer hardware is divided in to three main parts namely Neck, Large bulb & Graduation tube. Depending on the range of fat available in dairy product the different graduation tube design in butyrometer is selected for example 6%, 8% and 10% scale for whole milk. 1% and 4% for skimmed milk, 70% scale – for testing cream, 40% scale – for testing cheese etc. For our research we have done experiments for 10% butyrometer. Fig 1 shows 10% butyrometer with the detail dimensions.

The length of graduation tube for the total the scale from the 0 percent mark to the graduation nearest the bulb, shall be not less than 65 mm. 10% butyrometer has corrugated neck, with the corrugations at right angles to the axis of the butyrometer and not in the form of a spiral producing a screw thread. The large bulb capacity is measured between the end of the neck and the 0 % graduation line as shown in Fig 1 between levels A and B. for 10% butyrometer standard capacity of large bulb in ml is 21.0 ± 0.4 . The graduated tube is the flat-bore type, The length of the scale between the extreme graduation lines is measured between levels B and C. The position of the scale on the flat-bore tube shall be such that the scale is approximately central with respect to the length of the tube and that the tube is internally uniform in cross-section for at least 3 mm beyond each end of the scale

(ISO, 2008). And the capacity of the flat-bore tube between any two graduation lines covering a range of 1 % shall be 0.125 ml. When material is pipeted in butyrometer it is first hold in inverted side by holding large bulb upward and small neck downward position. After centrifugation the butyrometer is hold in position as shown in fig and then zero is adjusted with stopper and fat is read on the graduation scale. Table 1 below shows the 10% butyrometer design dimension details.

Challenge in this conventional method is error caused during reading fat, adjusting zero after centrifugation. If zero is not adjusted properly it may cause false reading. Some times in case of weak acid used it produces residue in pure fat and in case of manual reading this trial is discarded and rerun of test is done. In current scenario readings feed manually to the control system and SNF is calculated. This daily practical problem was addressed by one of the biggest dairy industries in south Gujrat, India which was motivation to develop smart Butyrometric reading tube.

Approach of automatic reading tube

To calculate fat from butyrometer automatically, understanding of the relation between graduation mark and fat is required. A handful of researches were found that tried to established the relation between milk fat and butyrometer graduation mark, one of them discover that the volume at 15° Centigrade of the graduation (x) for a containing x per cent of fat by weight will be sample as shown in Equation1 (Day et al. 1918)

$$\text{Volume of scale division } x = \frac{x}{100} * \frac{W}{DT} \{1 - 0.000027(T-15)\} - M c.c$$

Equation 1

Where W = weight of sample taken, in grams

D_T = apparent density of fat in glass at T° . gram/l

T = temperature of reading. in $^\circ\text{C}$

M = volume of fat in the meniscus, c.c.

0.000027 = coefficient of expansion of glass

From the detail study of butyrometer design, for automatic fat measurement, keeping above equation as reference and based on that prediction of % fat height (in mm) and its equivalent volume (in c.c) for 10% graduation tube is calculated. Spectrometric routine methods are very popular which uses optical properties (such as size and shape of particles suspended and Its relation with wavelength of incident light) for measurement of different substance. Same concept is utilized for making automatic reading tube. From Equation 1 volume of each graduation mark (from zero) and equivalent height of fat in 10% butyrometer is calculate which is shown in Table 2 .For 0 to 1% the volume is 0.119451 which is 0.006 less than the volume defined

Fig 1.10% butyrometer with corrugated neck (ISO, 2008), 1. small bulb, 2 matt surface, 3 graduated tube, 4 large bulb ,5 neck ,6 strengthening rim.

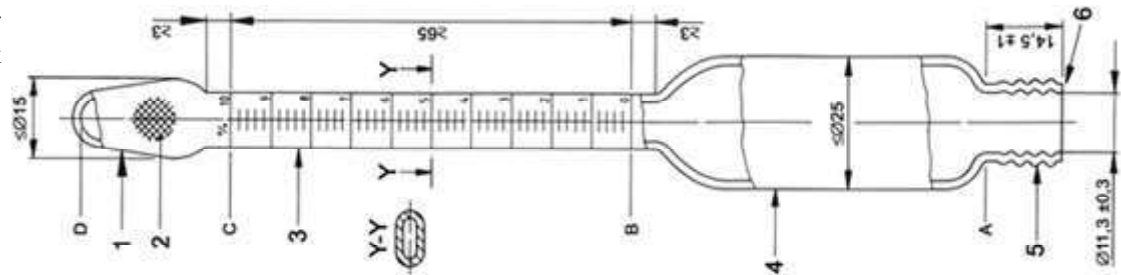


Table 1: Capacities, Scales and Tolerances for 10% Butyrometer(IS, 1977)

Sr no	Percent scale	Quantity of Material Tested	Basis of Graduation in ml	Capacity of Body in ml	Length of Scale, Min(mm)	Graduated at each Percent	Graduation of Intermediate Length at Each Percent	Numbered at each percent	Error Parentage of fat for 100gof sample Max
1	10	10.75 ml	0.125 corresponds to 1%fat	20.5±0. 5	75	0.1	0.5	1	0.05

Table 2: Fat Height (in mm) & % graduation relation for 10% Butyrometer calculated from Equation 1

Scale Graduation (x) Per Cent.	Volume, 0 to x. C C.	EquivalentHeight mm
1	0.119451	4.925
2	0.244902	6.257
3	0.370353	7.181
4	0.495804	7.915
5	0.621255	8.533
6	0.746706	9.072
7	0.872157	9.554
8	0.997608	9.992
9	1.123059	10.39
10	1.24851	10.77

by standards that is 0.125 (IS, 1977) . However, the difference of volume for successive graduations remains constant that is 0.125 which is as per the defined standard. These details are used for the placement of sensor and light source while designing automatic reading tube.

Concepts of merging spectrometric techniques to butyrometric reading is stepping stone towards Gerber automation.

Optical characteristic of milk fat

The light–milk interaction will be governed by the relationship between the size of particles suspended and the wavelength of incident light (Gastéllum-Barrios et al. 2020). According to the Beer-Lambert Law is a relationship between the attenuation of light through a substance and the properties of that substance. Consider monochromatic light transmitted through a solution; with an incident intensity of I_0 and a transmitted intensity of the transmittance, T , of the solution is defined as the ratio of the

transmitted intensity, I , over the incident intensity, I_0 and takes values between 0 and 1. The relation of transmittance is as shown in equation 2. Different fat molecules have unique relation with transmission of incident light and based on this principal the new instrument was developed the volume and fat height calculated from equation 2.

$$T = \frac{I}{I_0}$$

Equation 2

And in percentage transmittance:

$$T(\%) = 100 \frac{I}{I_0}$$

Equation 3

This optical spectrometric principle was used to detect the fat of butyrometer and special optical encloser was designed to measure optical transmission and special algorithm was designed to calculate fat. Various studies were found that attempted to find

Fig 2 . a) S m a r t
butyrometric reading
tube (b)Operating
mode view of tube(c)
Different fat in 10%
butyrometer

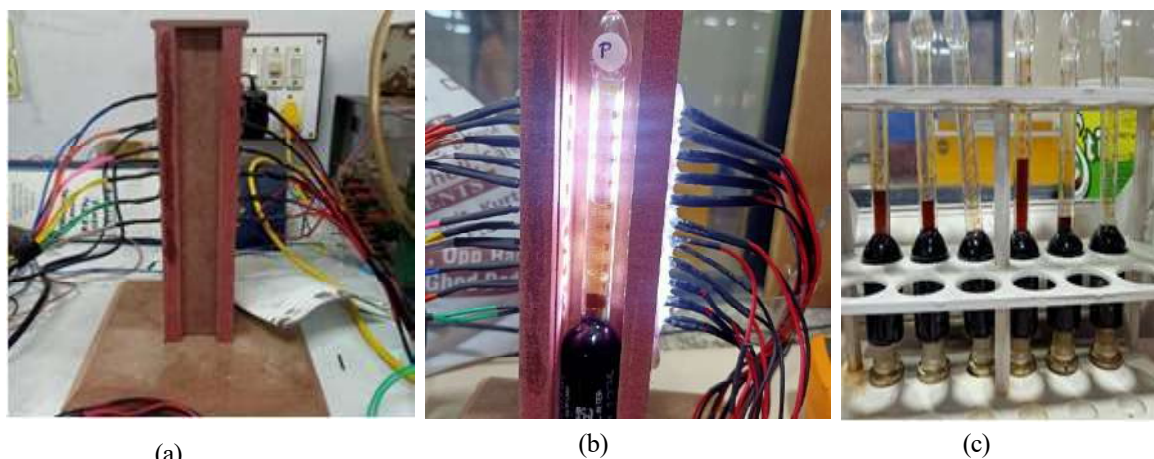


Fig 3.(a)Different milk sample
(b)Standard fat range of milk
sample



milk fat by using different electrical properties like the conductance and capacitance of milk (Żywiec et al. 2012) .

Smart Encloser Design

Tube enclosure is designed to hold 10% butyrometer such that graduation tube gets the fix position every time placed in enclosure for accurate measurement. Special stopper cap and bottom holder holds the butyrometer tightly so that it remains steady (in fix position) at the time of measurement. Encloser was designed such a way that after closing the tube it provides complete blackout for accurate optical measurement. In any butyrometer the position of separated fat is not fixed, it can take any position in graduation tube as shown Fig 2c so conventionally zero adjustment plays a very important role for calculating milk fat manually.

According to dimension of the 10% butyrometer design and calculation of Table 2 for optical measurement identical holes for led and ldr are made on both side of the tube such that it will illuminate whole graduation tube. At the time of calibration initial reading, it was taken care that optical path is clear without any obstacle for accurate led and ldr measurement. After centrifugation butyrometer is placed in the measuring device and measuring tube is covered to have full blackout. Reading of all ldr are taken and fat is calculated.

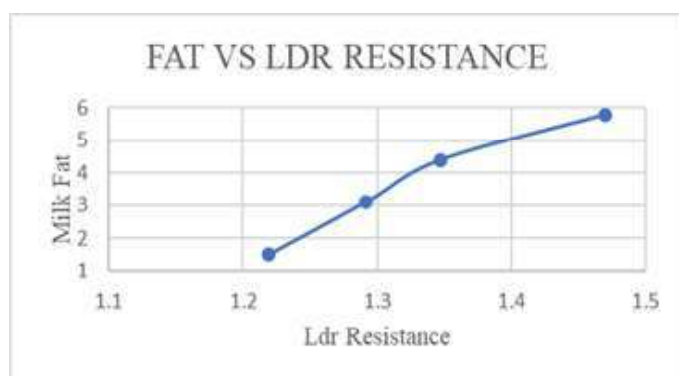


Fig. 4 (a) Milk fat (in %)/v/s LDR resistance ($K\Omega$) relationship

Milk Sample

To define proper optical and fat relation four different types of milk having different fat ranges are taken for sample named Amul shakti (4.55 to 4.65), Amul Gold (6.05 to 6.15), Amul Taaza (3.05 to 3.15), Amul SNT/DLITE (1.55 to 1.65). Pasteurized milk is used in experiment, samples directly collected from the dairy. In Fig 3 standard range of fat and SNF is given.

Instrument operation

Gerber test is performed according to standard procedure as described above. No alteration in chemical process is done. After centrifugation the tube directly is placed in the smart measuring tube. Readings of LDR are taken keeping all LEDs on & enclosure is closed so that no other light enters the tube. Readings are taken keeping all led source on at a time. Calibration procedure to

adjust initial LDR reading without butyrometer is done. After measurement fat is calculated.

Results and Discussion

LDR are very sensitive to light, with the increase of illumination intensity, the resistance value of the LDR decreases rapidly. After taking hundreds of results for each milk sample it was observed that as the Gerber fat increases transmission decreases and LDR

Table 3: Performance parameters of Linear Regression analysis for four milk samples

	Regression Statistics			
	SNT	TAAZA	SHAKTI	GOLD
R Square	0.835	0.738	0.478	0.8217
Standard Error	0.0131	0.016	0.024	0.0136

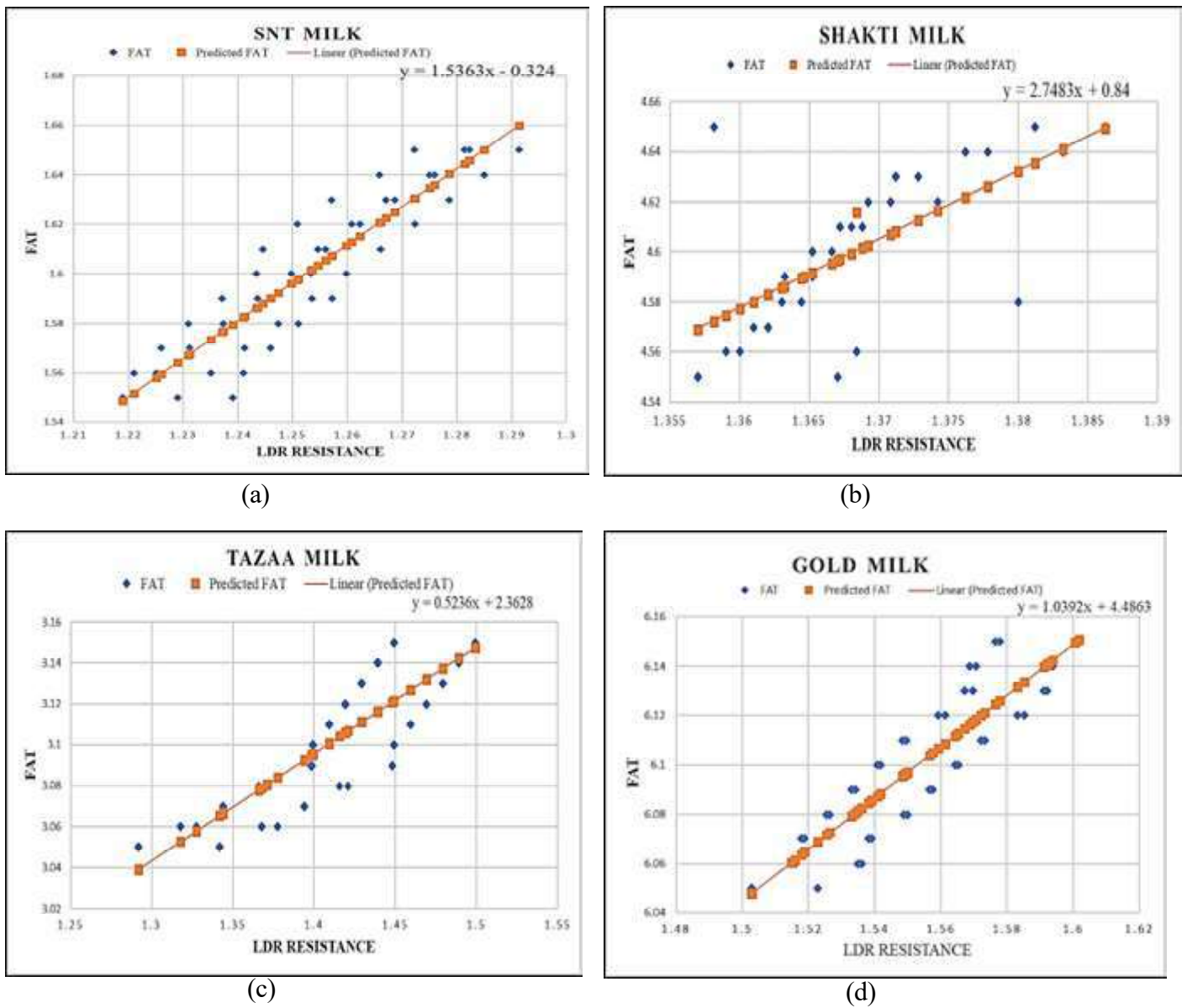


Fig. 5 Regression result of milk fat(%) v/s LDR resistance relationship (KΩ) of (a)TAZZA(b)SHAKTI(c)SNT (d) GOLD MILK

Table 4: Calculation of Fat from the Regression Model

Milk	LDR Average(K Ω)	Calculated Fat (%)	Actual Fat (%)	Error
SNT	1.219	1.5487497	1.5	-0.0325
TAAZA	1.291682	3.039124695	3.1	0.019637
SHAKTI	1.347015	4.542001325	4.4	-0.03227
GOLD	1.46968	6.013591456	5.8	-0.03683

resistance increases. Based on collection of multiple data for different milk fat it was found that the resistance and transmission have nearly linear relationship.

For higher fat less light is transmitted through sample and LDR gave high resistance value. Fig 4a gives idea of relation of fat and LDR resistance. For more accurate and precise relation between fat and LDR linear regression method was applied between collected data.

Aim of the data collected was to model the relationship between LDR resistance (input) and milk fat (Actual fat value). The performance metric used for evaluating a regression model was R-Squared. R-squared also known as the Coefficient of Determination explains the degree to which the LDR Resistance explain the variation of the output / predicted variable that is milk fat. Four different milk fat sample were taken so individual linear regression was applied to each group of milk samples and relationship was derived. Different Regression results are shown in Fig 5 for four milk type.

Table 3 shows the Performance parameters of four samples. R-squared value of 0.835 for SNT described that the input variables explain 83.5% of the variation in the output variable.

The higher the R squared, the more variation is explained by the input variables and better is the model. For TAAZA, Gold, Shakti the R-square values were 0.738, 0.478, 0.8217 respectively. Analysis explains that linear regression fits best for the above technology and results are nearly matching with manual readings. To check the model accuracy algorithm was applied to collected LDR data and fats were calculated which is shown in Table 4

Conclusion

Smart reading tube for butyrometer is a unique low-cost concept to automate Gerber reading for remote transmission. It can be observed by the results that proposed design calculates the fat from optical transmission with very less error. Proposed design eliminates the requirement of zero adjustment, water bath requirements of conventional methods. Smart tube not only establishes automatic reading of fat in butyrometer but in future by adding wireless transmission capability, it can be a part of centralized monitoring system of dairy plant. This device is beneficial for small scale milk vendors as well as large dairy industry where Gerber test is used as reference. Construction of tube, installation of sensor and source require high accuracy

while mounting it. Results for low fat sample is not up to the mark as small difference of resistor change is noted which is area of concern. Also, Residues generated due to use of weak acid obstruct the path of optical sensor, and cause false reading. This problem can be resolved by developing a combine fat detection model based on level and optical intensity measurement which is future work of research. The proposed combined method will also increase the accuracy of reading. So innovative butyrometer automation can bring historical change in dairy automation. However multiple trials are required to establish single butyrometer system for all type of milks as graduation mark will have no role in calculating fat.

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Antioxidant and free radical scavenging profile of milk in various indigenous cattle breeds

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Abstract: The present study was undertaken to investigate the total antioxidant and free radical scavenging activity in milk of Zebu cattle viz. Kankrej, Sahiwal and Rathi during different lactation and parity in hot arid region of India. The study included 150 indigenous milking cattle (50 from each breed), viz. Kankrej, Sahiwal and Rathi, and 100 ml of milk sample was collected as a pool sample from different livestock research stations of the Rajasthan University of Veterinary and Animal Science, Bikaner. Milk samples were analyzed by one way ANOVA and DMRT (SPSS 24) to assess total antioxidant capacity and per cent scavenging activity in milk. The results showed significant variation of antioxidant capacity ($p < 0.05$) and percent scavenging activity ($p < 0.01$) among three cattle breeds. Whereas, Kankrej milk had the lowest total antioxidant capacity and percent scavenging activity. Total antioxidant capacity varies significantly ($p < 0.01$) at different stages of lactation. Late stage of lactation accounted for significantly ($P < 0.05$) higher total antioxidant capacity as compared to early stage of lactation. Total antioxidant capacity and percent scavenging activity differed significantly ($p < 0.01$) across parities. The results of the present study concluded that milk from the indigenous cattle breeds possesses varied type and amount of antioxidants, which can be harnessed for the promotion of different functional foods and further pharmaceutical preparations can be promoted for the benefit of entrepreneurs and society.

Keywords: Antioxidant, Free radical scavenging, Indigenous cattle, Parity

Introduction

Antioxidants are substances that can inhibit oxidation and reactions that produce free radicals in a cell or organism. Free radicals harm the membrane of different cellular structures viz. protein, lipids and DNA (Alyaqoubi et al. 2014) in human body which is answerable for the introduction of various diseases together with diabetes, cancer, cardiovascular and Alzheimer's (Valko et al. 2007) aging, arteriosclerosis and stroke (Gupta et al. 2009). Antioxidants play crucial function to prevent the creation of free radical in human cellular and worried many protecting role of different ailment (Valko et al. 2007). An efficient amount of antioxidants are required to guard cells against free radicals to balance the disruption caused by these radicals (Vasundhara et al. 2008) because excessive formation of free radicals is responsible for reducing distinctive protective enzyme viz. catalase, peroxidase and superoxide dismutase and cause lethal and degenerative impact on cells (apoptosis) by oxidizing the cell membrane resulting in decreased cellular respiration. Milk is an essential source of nutrients such as protein, minerals and vitamins in the human diet. In dairy industries, composition of milk is important which also affect the product quality, quantity and price of the finished product (Singh et al. 2022). Antioxidant properties are governing by various milk components viz. fatty acids, Peptides, vitamins, enzymes etc. Milk peptides play a crucial function in maintenance of antioxidant defence structures by

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means of warding off the formation of free radicals or by scavenging free radicals and active oxygen species (Gupta et al. 2009). It is worth emphasizing that regular consumption of natural dairy antioxidants minimizes the risk of development of civilization diseases (e.g., cardiovascular disease, cancer, or diabetes) (Stobiecka et al. 2022). It also slows down the aging process in the organism. Total antioxidant activity and per cent scavenging activity are governed by milk constituents, fat soluble components, bioactive substances and enzymes. Different factors such as genetic factors viz. breeds of animals and non-genetic factors viz. stage of lactation, parity of animals, somatic cell count, season, and geography affect the concentration of antioxidants in milk (Bernabucci et al. 2002; Hanna et al. 2004; Alyaqoubi et al. 2014; Ramos et al. 2015). Milk antioxidants are more influenced by breed, lactation stage, and parity. As per available literature, relatively less information is available on the antioxidant and free radical scavenging profiles in the milk of various indigenous cattle breeds.

Material and Methods

The present study was performed at different cattle farms of Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner, Rajasthan, India. The Bikaner city is situated in middle of the Thar Desert and hot semi-arid region of Rajasthan. During summer, the temperatures can rise up to 48°C and in winter comes below the freezing point.

Ethical Approval

Research work on “Study on Quality and Bioactive Components in Milk of various Indigenous Cattle Breeds in Hot-Arid Region of Rajasthan” has been approved by Institutional Animal Ethical Committee (Approval No: PGIVER/IAEC/19-14).

Experimental Animals

For this study, a total of 150 indigenous milch cattle of three indigenous breeds (50 from each breed) viz. Kankrej, Sahiwal and Rath i were selected randomly and were reared under similar feeding and housing system. All the animals were housed in a loose housing system with a shared feeder through a fenceline feed barrier, and a water trough and provided standard covered and open floor area. All cows were offered a similar ration and standard feeding schedule. Animals were provided with seasonal farm-grown green fodder and dry roughage, on an *ad libitum*

basis. All the experimental animals were hand milked; full hand method, twice a day at 4:00 to 5:00 in the morning and evening at equal twelve-hour intervals.

Milk Sample Collection

Milk samples were collected from all the four quarters of lactating cows as pooled sample. About 100 ml of pooled milk samples of all individual cows have been collected aseptically in the sterilized sampling bottle after discarding the first 4-5 streaks of foremilk. The milk composition was tested by an electrically operated automatic milk analyzer (Lacto-scan SL20, Rajasthan Electronic and Instruments Limited). Estimation of the TAC of milk by ferric reducing/antioxidant power (FRAP) assay and PSA by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay was done according to Musa et al. (2011) with minor modification. In present study, the levels of antioxidants were measured using the FRAP assay which measures the TAC and DPPH assay that which determines the PSA. These methods were used to evaluate the level of antioxidants in milk of three different indigenous cattle breeds during different stages of lactation and parity. The total antioxidant capacity (FRAP assay) and free radical scavenging activity (DPPH assay) of milk samples of indigenous cow breeds viz. Kankrej, Sahiwal and Rath i, were evaluated across early (<100 days), mid (101-200 days), and late (>200 days) stages of lactation and different parity viz. 1, 2, 3, 4 and above 4.

Statistical Analysis

The data with respect to breeds, parity and stage of lactation were analyzed by one way analysis of variance (ANOVA) of SPSS software statistical package (version 24.0) and the means were compared by Duncan's multiple range test (DMRT). Differences were considered statistically significant when $p < 0.05$. Results are presented as LS means $\pm$ SEM.

Results and Discussion

Total antioxidant capacity and percent scavenging activity

The data pertaining to antioxidant capacity ($\mu\text{Mol/ml}$) and per cent free radical scavenging activity in Kankrej, Sahiwal and Rath i cows are presented in the table 1.

Table 1 Total Antioxidant Capacity and Per cent Scavenging Activity of milk from different cattle breeds (N=50 of each breeds)

Breeds	Assay	
	Total antioxidant capacity ($\mu\text{Mol/ml}$)	Percent scavenging activity (%)
Kankrej	326.51 $\pm$ 3.11 ^a	39.33 $\pm$ 0.78 ^a
Rath i	366.96 $\pm$ 2.98 ^b	41.14 $\pm$ 0.84 ^a
Sahiwal	366.75 $\pm$ 3.43 ^b	48.80 $\pm$ 0.08 ^b
Level of significance	P<0.01	P<0.01

The total antioxidant capacity was significantly greater ($P<0.05$) in Sahiwal and Rathi cows than in Kankrej cows, however, the per cent free radical scavenging activity was similar among three breeds of indigenous cows.

The results showed (table: 2) the highly significant variation ($p<0.01$) of TAC in different stages of lactation and the total antioxidant capacity was significantly greater ($P<0.05$) in the late stage of lactation as compared to the early stage of lactation, whereas between the mid and late stage of lactation the TAC in milk samples was found to be non-significant. However, the percentage of free radical scavenging activity was similar among different stages of lactation.

The data pertaining to antioxidant capacity ($\mu\text{Mol/ml}$) and per cent free radical scavenging activity in different parities are presented in table 3. The present study revealed that total antioxidant capacity was significantly greater ($P<0.05$) in first parity than above fourth parity, whereas among first, second, third and fourth parities, the TAC was found to be non-significant. The PSA significantly differed ($p<0.000$) in different parities and was higher in third parity than in fourth.

Relationship between TAC, PSA and Quality Traits

The results pertaining to correlation coefficient in TAC and milk fat are depicted in Table 4.

The fat percentage was significantly positive correlated with the TAC. Protein was positively weak correlated with TAC.

The results pertaining to correlation coefficient in TAC and PSA are depicted in Table 5. A significant positive correlation was measured between the results of TAC by FRAP methods and per cent scavenging activity using DPPH methods in milk of cattle. The capacity of the milk to reduce ferric ions was evaluated by performing the FRAP assay. FRAP assays depends on the mechanism that involves oxidation and reduction reactions, in which ferric ion reduced to ferrous ion. This mechanism can be correlated with the redox properties of antioxidant compounds in milk samples. Milk contains several enzymatic and non-enzymatic antioxidants which are crucial to preventing the production of reactive oxygen species and help in strengthening the body antioxidant defence mechanism against oxidative stress. Reactive oxygen species are produced or their level is increased during different physiological processes like parturition, exercise, etc. Animal bodies employ antioxidants to reduce these free radicals which neutralize the free radicals produced by neutrophils during phagocytosis (Jackson et al. 2002; Chang et al. 2007). Further, bovine whole milk with higher fat content showed high antioxidant potential as compared to semi-skimmed and skimmed milk (Ryan and Petit 2010). Our results are consistent with the previous studies in goat milk (Alyaqoubi et al. 2014) where they reported the significant effect of breeds on TAC. Comparably, Jamnupari

Table 2 Total Antioxidant Capacity and Per cent Scavenging Activity of milk from different lactation stages of cows

Assay	Total Antioxidant Capacity ($\mu\text{Mol/ml}$)	Percent Scavenging Activity (%)
Stage of lactation Early (<100 days)	342.51 $\pm$ 3.92 ^a	42.33 $\pm$ 0.88
Mid (101-200 days)	355.26 $\pm$ 3.89 ^b	42.63 $\pm$ 0.88
Late (>200 days)	366.02 $\pm$ 4.01 ^b	44.74 $\pm$ 0.83

Table 3 Total Antioxidant Capacity and Per cent Scavenging Activity of milk from different parity of cows

Assay	Total Antioxidant Capacity ($\mu\text{Mol/ml}$)	Percent Scavenging Activity (%)
Parity First	368.38 $\pm$ 5.25 ^b	42.99 $\pm$ 1.85 ^{ab}
Second	358.02 $\pm$ 4.22 ^b	43.89 $\pm$ 0.92 ^{bc}
Third	359.96 $\pm$ 6.74 ^b	47.11 $\pm$ 0.70 ^c
Fourth	358.41 $\pm$ 5.67 ^b	44.38 $\pm$ 1.12 ^{bc}
Above Fourth	340.34 $\pm$ 4.12 ^a	40.25 $\pm$ 0.88 ^a

Table 4 Pearson correlations of Total Antioxidant Capacity with milk quality traits

	Total Antioxidant Capacity ($\mu\text{Mol/ml}$)	Fat %	Protein %
Total Antioxidant Capacity (%) ($\mu\text{Mol/ml}$)	1	0.279	0.112
Pearson correlation		.001	.172
Sig. (2-tailed)	150	150	150
N			

Table 5 Pearson correlations of Total Antioxidant Capacity with Per cent Scavenging Activity

Pearson correlations	Total Antioxidant Capacity ($\mu\text{Mol/ml}$)	Percent Scavenging Activity (%)
Pearson correlation	1	0.272
Sig. (2- tailed)		.001
N	150	150
Pearson correlation	0.272	1
Sig. (2- tailed)	.001	
N	150	150

milk had higher antioxidant capacity than crossbred throughout all the different location. Throughout the lactation period the antioxidant capacity of Vechur milk was higher than that of goat milk ($p < 0.01$) (Annie et al. 2019). According to the findings of the present study, there was a significant higher level of TAC measured by FRAP assay in milk samples from later stages of lactation compared to milk samples from mid and early stages of lactation. This could be due to high fat content in milk samples during the late stage of lactation causing reactivity of lipid soluble antioxidants. Also, a correlation between milk fat content and the antioxidant activity of milk was reported by Chen et al. 2003. The results are consistent with the fat per cent and increase fat in milk gradually with stage of lactation where the total antioxidant capacity of milk had significantly higher values for the late stage of lactation as compared to the early and mid stages of lactation. During late stage of lactation, milk could help in improving the antioxidant system for newborns. Stage of lactation has significant ($p < 0.05$) effect on fat content among crossbred cows and found higher fat value in late lactation (Shuiep et al. 2016). The concentration of milk fat was observed to increase during advanced lactation as positively affected by the lower milk yield (Auldist et al. 1998). The stage of lactation had a significant effect on fat percentage, with the highest fat percentage recorded in the late stage of lactation (Yogi et al. 2016). Annie et al. 2019 observed the significant variation of total antioxidant activity in different stage of lactation. Shuiep et al. 2016 reported that the fourth parity revealed significantly ($p < 0.05$) lower milk fat content in local breeds. A decrease in milk fat content in cows of later parity could be attributed to an age factor, as local cows give birth to their first calves between the ages of 5 and 7 year (Abdel-Aziz et al. 2005). The result obtained from present study was supported by Nyamushamba et al. (2014) as they reported a linear relationship between age at calving and milk fat. These results were in complete agreement with Agnihotri and Rajkumar (2007) who found that fat content steadily increase with parity in goat. DPPH assays are often used to determine the capacity of primary antioxidants in milk samples, in which these primary antioxidants react to scavenge free radicals from DPPH solution. Therefore, the formation of an initiation chain of free radicals is inhibited and the propagation chain is destroyed through the donation of a hydrogen atom or an electron. Accordingly, free radicals can be modified to a more stable form of products (Nurliyana et al. 2010;

Yan et al. 2006). The results elucidate the discoloration of a milk sample from purple to yellow in DPPH solution. DPPH is another very popular method to determine free radical scavenging activity in milk and is based on the acceptance of hydrogen atoms or electrons donated by antioxidants. Our results are consistent with the previous studies in goat milk (Alyaqoubi et al. 2014) where they have reported that the significant effect of breeds on PSA and the milk sample from Jamunapari collected from Bander Baru Bangi exhibited the highest percent scavenging activity. Whereas, crossbred from Johor Bahra had the lowest values. Comparably, Jamnupari milk had higher antioxidant capacity than crossbred throughout all the different locations. Hazra et al. 2022 reported the significant variation of DPPH radical inhibition assay activity in sheep and cow milk and sheep milk have higher activity compared to cow milk. El-Fattah et al. 2020 reported the significant genetic effect on DPPH radical scavenging activity in milk of different species and sheep milk significantly exhibited the highest DPPH radical scavenging activity compared to buffalo, cow and camel milk. Mann et al. (2016) observed that PSA showed no difference across lactation stages in Sahiwal and KF cows. Milk fat and protein were positive correlated with the TAC. It was noted that with increasing fat content in milk TAC values rose significantly, which might be due to an interference of lipids as well as the reactivity of lipid soluble antioxidants and the fat globule membrane proteins (Chen et al. 2003). Milk fat contains several free fatty acids, liposoluble vitamins and antioxidant compounds, which contribute to milk TAC. Small amounts of retinol, α - and γ -tocopherol and β -carotene in milk fat have been observed by Calderón et al. (2007) and Chauveau-Duriot et al. (2010). Similar finding were observed by Niero et al. 2019 who found a significant positive correlation of total antioxidant activity with fat in Holstein Friesian cows.

The present study revealed a significant positive correlation between TAC and PSA in milk of cattle. Similar results were observed by Zarban et al. (2009) who found a significant correlation between the results of FRAP and DPPH methods for total antioxidant capacity and radical scavenging activity of the samples of breast milk ($r = 0.562$, $p < 0.001$). Mann et al. 2016 observed a positive correlation between the results of TAC by the FRAP method and radical scavenging activity using DPPH radicals in the milk of Murrah buffalo ($r = 0.512$), KF cow ($r = 0.293$), Sahiwal cow ($r = 0.557$) and HF cow ($r = 0.812$).

Conclusion

It was concluded that the breeds, stage of lactation and parity affect the concentration of total antioxidants and free radical scavenging activity in milk. Milk from the indigenous cattle breeds viz. Kankrej, Sahiwal, and Rathi has a distinct composition and possesses a varied type and amount of antioxidants, which can be harnessed for the promotion of different functional foods based on indigenous cattle milk and further pharmaceutical preparation, can be promoted for the benefit of farmers and entrepreneurs. However, looking at the rapidly emerging demand and interest of society in indigenous cattle, those preliminary data also suggest more in-depth investigations in different agroclimatic zone of India to obtain a sizeable volume of information on the milk of indigenous breed of cattle.

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RESEARCH ARTICLE

Prediction of lifetime milk production on the basis of early economic traits in Deoni cattle

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Abstract: The present study was conducted to estimate lifetime milk production in Deoni cows based on early expressed traits viz., age at first calving, first and second lactation traits. Data on milk yield of Deoni cows maintained at ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru (India) during the period of 2002 to 2019 revealed overall mean lactation milk yield, lactation length, dry period and calving interval were estimated as 996.96 ± 56.84 kg, 221.35 ± 9.41 days, 211.67 ± 13.40 days and 432.15 ± 13.65 days, respectively. Data standardization and normalization were carried out before analysis. Lifetime lactation milk yield comprising of the first five and more lactations was recorded as 5090.24 ± 247.14 kg with a range of 2101.25 to 8988.24 kg. There was a significant correlation between lactation milk yield and lactation length during different lactations. Second lactation milk yield was found to be highly associated with lifetime milk production. When age at first calving and lactation milk yield, lactation length, and calving interval of first and second parities were included independently in the regression equation, only second lactation milk yield significantly contributed with a R^2 value of 83.7 percent against first lactation traits with a R^2 value of 53.8 percent ($P < 0.001$). Therefore, it is concluded that culling in this dual purpose breed based on first lactation milk yield is not encouraged and second lactation milk yield could be

the basis for the selection of superior animals for lifetime milk production performance.

Keywords: Age at first calving; Calving interval; Deoni; Lactation milk yield; Lifetime milk production

Introduction

Deoni is one of the most prime dual purpose breed found mainly in drought prone and dry regions of India. These cattle are well known for better disease resistance; endure better in local environment, suitable for draught work and moderate yielder with low input management system. The name of the breed Deoni has derived from the habitat *i.e.* Deoni taluk of Latur district in Maharashtra. This breed is also known as Surti, Dongarpati and Dongri. The breed has been evolved from crossbreeding of Gir breed of the Kathiawar region of Gujarat with the Dangi breed of Marathwada and local desi cattle of Nizam state from Bidar and Osmanabad. This breed has massive and upstanding body with considerable depth, prominent broad and slightly bulged forehead (Singh et al., 2006). The cow have bowl shaped udder having fairly well developed quarters with squarely placed black teats. It has straight powerful legs with broad steadily placed strong black colour hooves and well suited to the local condition of cultivation and transportation. Phenotypically, this breed is classified into three strains *i.e.*, Balankya (complete white), Wannera (complete white with partial black face) and Waghya or Shevera (black and white spotted). There is a great similarity in general conformation and hardiness to the Dangi cattle which is not far from the Deoni cattle breeding area. This breed contributes and plays a significant role towards milk production, mainly in Karnataka, Maharashtra and parts of Telengana.

There is a need to improve the lifetime productivity of dairy animals. Lifetime milk production reflects true performance of a herd as the dairy animal's overall productivity depends on their lifetime performance rather than a single lactation performance. Selection of dairy animals on the basis of early expressed productive and reproductive traits is the primary choice of the breeders as it is not desirable to wait for an animal to complete its life span for selection purpose (Kumar and Hooda, 2013; Singh et al., 2013). Selection of dairy animals needs to be carried out

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based on the early expressed productive and reproductive traits at an early age so that the expenses are reduced and dairy enterprise will be a profitable venture. Usually, Deoni breed of cattle is maintained in dry arid region with available feeds and fodders. Farmers face challenges to maintain cows due to lack of prior knowledge on total lactation milk yield during its lifetime. Keeping this in view, the study was taken up to estimate the regression of age at first calving, and other early expressed traits such as lactation milk yield, lactation length, calving interval of both first and second parities on total lactation milk yield comprising first five and above lactations. Also, the aim of this study was to estimate lifetime milk production in Deoni cows based on early expressed traits (age at first calving, first and second lactation traits).

Materials and Methods

Data on milk yield from Deoni cows maintained at ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru (India) were collected during 2002 to 2019 to predict lifetime milk production. Animals had less than 300 kg of milk yield and lactation length less than 100 days were excluded from the study. Abnormal lactation records due to unambiguous causes such as abortion, still birth, repeat breeding and pathological conditions which affect lactation yield were excluded. In this study, first five and above lactation were taken into one group. Due to less number of observations a total number animals is 52 with normal lactation records up to five and above lactations. Due to limitation of sample size was not possible to calculate the Least Square Means. However, the unadjusted mean lactation milk yield was estimated.

Economic traits, viz. age at first calving, lactation milk yield, lactation length and calving interval during different lactation stages, were considered. The lifetime milk production (LTP) was defined as total milk production up to five and above lactations. Economic traits were expressed as Mean $\pm$ Standard Error (SE). Pearson correlation was performed to find the relationships among the early economic traits and with LTP. Statistical significance of the correlation coefficient was tested using t-test. The significant correlation coefficients were graded as weak (upto 0.3), moderate (0.3 - 0.7), and strong (> 0.7).

Multiple regression analysis was used to predict lifetime production based on early lactation traits. First prediction was attempted by developing a regression model with 3 independent predictors (first lactation milk yield, age at first calving, and calving interval). Secondly, Second lactation milk yield alone was used to predict LTP. Economic traits such as lactation length, calving interval etc. during second parity were not significant. The significance of regression coefficients were tested using t-test and model (R^2) performed by F-test. Statistical analysis was performed using SPSS 16.0 software for windows.

Results and Discussion

Lactation milk yield across different lactations ranged from 956 kg in first lactation to 1056 kg in five and above lactations (Table 1). The mean lactation milk yield, lactation length, dry period and calving interval were estimated as 996.96 ± 56.84 kg, 221.35 ± 9.41 days, 211.67 ± 13.40 days and 432.15 ± 13.65 days, respectively. Basak and Das (2018) reported overall mean lactation milk yield, lactation length and calving interval as 819.98 ± 16.5 kg, 195.23 ± 2.62 days and 445.97 ± 3.67 days respectively. In Deoni cows average lactation milk yield varied from 238.86 ± 76.0 kg (Chakravarthi et al., 2002) to 868.24 ± 49.56 kg (Singh et al., 2002), average lactation length varied from 149.43 ± 33.52 days (Chakravarthi et al., 2002) to 293.30 ± 2.9 days (Deshpande and Singh, 1977), dry period varied from 103.66 ± 19.78 days (Chakravarthi et al., 2002) to 282.77 ± 12.85 days (Kumar et al., 2006). Das et al. (2011) reported overall dry period and calving interval of Deoni cattle as 256.87 ± 7.34 days and 447.22 ± 6.64 days, respectively. In the same study, lifetime lactation milk yield comprising of first five and above lactations varied from 2101.25 to 8988.24 kg and the mean lifetime yield was 5090.24 ± 247.14 kg. In Tharparkar cattle, Gupta and Gurnani (1984) reported that the six years, eight years and ten years aged animals produced lifetime milk yield of 5585.77 ± 93.23 kg, 9589.30 ± 153.34 kg and 14029.62 ± 220.34 kg, respectively.

Correlation among economic traits

In the present study, the correlation coefficients between milk yield during different lactations were significant at $P < 0.001$ (Table 2). These correlations ranged from medium to strong. Second

Table 1: Means and Standard Errors for Lactation Milk Yield, Lactation Length, Dry Period and Calving Interval in Deoni cattle ($n=52$)

Parity	Lactation milk yield (kg) (Mean $\pm$ SE)	Lactation length (days) (Mean $\pm$ SE)	Dry period (days) (Mean $\pm$ SE)	Calving interval (days) (Mean $\pm$ SE)
1	956.77 ± 50.54	229.46 ± 8.57	216.12 ± 15.39	445.65 ± 13.79
2	1009.21 ± 53.56	214.40 ± 9.37	214.50 ± 13.03	428.63 ± 13.11
3	1007.00 ± 56.01	216.17 ± 9.71	221.63 ± 14.67	436.06 ± 15.01
4	1062.00 ± 60.70	225.52 ± 9.07	211.48 ± 11.88	432.69 ± 13.83
5 and above	1056.00 ± 63.43	221.21 ± 10.37	194.63 ± 12.04	417.73 ± 12.51
Overall	996.96 ± 56.84	221.35 ± 9.41	211.67 ± 13.40	432.15 ± 13.65

Unadjusted means

lactation milk yield in Deoni cow was highly associated with LTP (0.917; $P < 0.001$). There was a significant correlation between lactation length and lactation milk yield during different lactations at $P < 0.001$ (Table 3). Correlation between calving interval and milk yield was low (Table 4). Dong and Van Vleck (1989) estimated correlation between first and second lactation milk yield in Holstein cows as 0.82. Amasaib et al. (2008) revealed the correlation

between milk yield and lactation length for the first three lactations in Holstein-Friesian cows of Butana farm and University farm (Sudan) as 0.669 and 0.746, respectively. In the present study, the correlation of lifetime milk production with first lactation milk yield, age at first calving and first calving interval in Deoni cow was found to be 0.752. Singh et al. (1964) reported multiple correlation coefficients as 0.782 in Haryana cattle.

Table 2: Correlation among different lactation yield and lifetime total production

Lactation	LMY1	LMY2	LMY3	LMY4	LMY5	LTP
LMY1	1	0.636	0.567	0.550	0.546	0.747
LMY2		1	0.805	0.775	0.762	0.917
LMY3			1	0.730	0.767	0.895
LMY4				1	0.730	0.880
LMY5					1	0.888
TMY						1

All the above correlation coefficients were highly significant at $P < 0.01$

LMY1: First lactation milk yield;

LMY2: Second lactation milk yield;

LMY3: Third lactation milk yield;

LMY4: Fourth lactation milk yield;

LMY5: Fifth and above lactation milk yield;

LTP: Lifetime total production

Table 3: Correlation between lactation length and milk yield during different lactations

Traits	LMY1	LMY2	LMY3	LMY4	LMY5	LTP
LL1	0.783	0.655	0.571	0.551	0.553	0.710
LL2	0.544	0.816	0.639	0.667	0.645	0.764
LL3	0.457	0.635	0.847	0.596	0.527	0.706
LL4	0.503	0.566	0.509	0.794	0.582	0.686
LL5	0.364	0.558	0.551	0.578	0.830	0.669

All the above correlations coefficients were highly significant at $P < 0.01$

LL1: First lactation length;

LL2: Second lactation length;

LL3: Third lactation length;

LL4: Fourth lactation length;

LL5: Fifth and above lactation length

Table 4: Correlation between milk yield and calving interval in different lactations

Traits	LMY1	LMY2	LMY3	LMY4	LMY5	LTP
CI1	0.119	0.199	0.083	0.189**	0.129	0.166
CI2	0.269	0.323	0.174	0.350*	0.188	0.299*
CI3	0.055	0.094	0.177	0.327*	0.039	0.162
CI4	0.162	0.123	0.146	0.138	0.016	0.131
CI5	0.132	0.190	0.111	0.267*	0.325	0.243

**Significant at $P < 0.01$; *Significant at $P < 0.05$

CI1: First calving interval;

CI2: Second calving interval;

CI3: Third calving interval;

CI4: Fourth calving interval;

CI5: Fifth and above calving interval

Table 5: Influence of age at first calving, first lactation milk yield and calving interval on Life time milk yield

Traits	Regression coefficients	Constant of regression(a)	Multiple correlation coefficient (R)	Adjusted R^2 Value (%)	F-value
AFC (b1)	4.791*				
LMY1 (b2)	3.591	824.75	0.752	53.8	20.79**
CI1 (b3)	1.429				

** Significant at $P < 0.001$

LMY1: First lactation milk yield;

AFC: Age at first calving;

CI1: First calving interval

Table 6: Prediction of total lifetime production using second lactation milk yield

Traits considered	Regression coefficient	Constant of regression(a)	Multiple correlation coefficient (R)	Adjusted R^2 Value (%)	F-value
LMY2	4.22**	829.03	0.917	83.7	262.67**

** Significant at $P < 0.001$

LMY2 – Second lactation milk yield

Prediction of lifetime milk production up to five and above lactations

An attempt was made to predict LTP from early expressed traits, viz., age at first calving, first lactation milk yield, first calving interval and second lactation traits. When age at first calving, first lactation milk yield and first calving interval were used in the regression analysis for prediction of LTP, R^2 value was only 53.8 per cent at $P < 0.001$ (Table 5). Age at first calving, first lactation milk yield, lactation length and calving interval were significant. When second lactation milk yield, lactation length and calving interval were included in the regression equation, only second lactation milk yield was significant ($P < 0.001$). Therefore, it was decided to develop a prediction equation only with second lactation milk yield (Table 6) which contributed R^2 value of 83.7 per cent ($P < 0.001$). Similar study was conducted by Singh et al. (2002) and Gandhi et al. (2009). Dalal et al. (2004) and Shinde et al. (2010) indicated in Haryana and Phule Triveni crossbred cattle, the R^2 values of 61 and 50.91 percent, respectively, for prediction of lifetime milk production considering the first three lactations.

Puri and Sharma (1965) predicted lifetime milk production based on first lactation milk yield and age at first calving in Red Sindhi and crossbred cows. Gandhi et al. (2009) and Shinde et al. (2010) estimated lifetime milk yield up to third lactation in Phule Triveni cattle. Prediction of lifetime milk production in Karan Fries cows with first and second lactation records; revealed that the trait entered at the first step was second lactation milk yield prediction was 44 percent. Gandhi and Bhattacharya (2001) reported that when first lactation entered at the second step the R^2 value decreased by 7 percent. The R^2 value recorded in different animals were: 0.697 in Murrah buffaloes (Dutt et al., 1965), 0.617 in Haryana cattle (Bhasin and Desai, 1967), 0.62 in Sahiwal cattle (Gopal and Bhatnagar, 1972), 0.15 in Holstein Friesian cattle (Lin and Alliaire, 1978), and 0.708 in Tharparkar cattle (Gupta and Bhatnagar, 1979).

In the present study, regression coefficients of age at first calving, first lactation milk yield and first calving interval were recorded as 4.79, 3.59 and 1.43, respectively.

Conclusions

The present study revealed that there is a significant correlation between lactation milk yield and lactation length during different lactations in Deoni cows. Further, second lactation milk yield was significantly associated with lifetime milk production. Prediction of regression model for estimation of lifetime milk production based on age at first calving, lactation milk yield, calving interval for both first and second parities revealed that second lactation milk yield could be the basis for selection of superior animals with respect to lifetime milk production. Therefore, second lactation milk yield may be considered towards culling of poor performing Deoni cows.

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Estimation of factors influencing the monthly test day milk yields in Mehsana buffaloes under field condition

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Abstract: The present research work was carried out using data of first lactation monthly test day milk yield records of 7113 Mehsana buffaloes sired by 173 bulls spread over a period of 20 years from 1993 to 2012, collected from the Dudhsagar Research and Development Association (DURDA), Dudhsagar Dairy, Mehsana. The data were classified according to season, period and age of calving groups to study the effect of genetic and non-genetic factors. The overall least square means of different monthly test day milk yields (MTDMY) obtained as 6.32 ± 0.32 , 7.84 ± 0.31 , 7.92 ± 0.31 , 7.57 ± 0.31 , 7.08 ± 0.30 , 6.54 ± 0.30 , 5.98 ± 0.30 , 5.33 ± 0.30 , 4.64 ± 0.30 , 3.99 ± 0.31 , 3.42 ± 0.33 liters for MTDMY1 to MTDMY11, respectively. There were significant effects of all the non genetic factors taken in the study on almost all the monthly test day milk yields in Mehsana buffaloes. The heritability estimates for monthly test day milk yields viz. MTDMY1 to MTDMY11 were 0.08 ± 0.02 , 0.12 ± 0.02 , 0.27 ± 0.04 , 0.18 ± 0.03 , 0.16 ± 0.03 , 0.44 ± 0.05 , 0.22 ± 0.03 , 0.25 ± 0.03 , 0.30 ± 0.04 , 0.28 ± 0.04 , 0.18 ± 0.04 , respectively. The low to moderate heritability estimate of MTDMYs indicates that these traits were influenced more by environmental factors than by genetic factors and could be improved by efficient management, proper care and feeding during pregnancy and lactation.

Keywords: Buffaloes, Genetic factors, Mehsana buffaloes, Monthly test day milk yield, Non-genetic factors

Introduction

India is the top most milk producer country in world since 1998, representing one of the world's largest and fastest growing markets for milk and milk products. It contributes about 20% of world milk production. In India, dairying has become an important secondary source of income for millions of rural families and the rearing of buffaloes has occupied a most important role in providing employment and income generating opportunity. Presently India is the largest milk producing (198.4 million tones with 407 gram/day per capita availability) country (DAHD, 2021). Keeping in view of the huge increase in human population in India, this much per capita availability of milk is great achievement. However, the estimated demand for 2030 at all India level is 266.5 million metric tonnes for milk and milk products (Economic Survey, 2021)

The buffaloes are the second largest source of milk supply in the world after cattle. The world population of water buffaloes is approximately 207 million and more than 95% buffaloes are present in the Asia. India has rich repository of well-known 17 recognized buffalobreeds like Murrah, Nili-Ravi, Mehsana, Banni, etc. Mehsana buffaloes are a breed of water buffalo from the state of Gujarat and are believed to have lineage of Surti and Murrah and evolved long time ago in the districts of Mehsana, Banaskantha and Sabarkantha of Gujarat state. A relatively smaller breed than the Murrah, Mehsana is reputed as persistent milker, regular breeder and even more economical.

Under field conditions the daily milk recording of dairy buffaloes is costly and time consuming. Therefore, the test day milk yield recording is an alternative form of record keeping, which is used to reduce the cost involved in daily milk recording and also time saving proposition for selection of sire in field condition. In genetic evaluation system of breeding bulls (especially, under field progeny testing) test day milk yield records are having their own importance. Test day milk yields are greatly affected by many environmental and hereditary factors in dairy animals. Non-genetic factors tend to suppress or inhibit the expressivity of the

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true genetic ability of the animals in various ratios according to climatic conditions. For more accurate genetic evaluation of dairy animals, better adjustment of non genetic factors influencing the milk yield can be used. Monthly test day records helps in early genetic evaluations, thereby reducing the generation interval, economize the genetic evaluation with better accuracy and minimize cost of maintaining cows and bulls (Bilal and Khan, 2009). The genetic parameters viz. heritability, genetic and phenotypic correlation of monthly test day milk yield can be used to choose the appropriate selection method to estimate response to selection and to assess the producing ability of buffaloes in the herd. Hence, the present study was carried out to know the effect of various non-genetic factors on monthly test day milk yield (MTDMY) and to estimate genetic parameters of MTDMYs in Mehsana buffaloes.

Materials and Methods

Sources of data

The first lactation monthly test day milk yield records pertaining to 7113 Mehsana buffaloes sired by 173 bulls spread over a period of 20 years from 1993 to 2012 under field progeny testing programme of Dudhsagar Research and Development Association (DURDA), Dudhsagar Dairy, Mehsana, Gujarat, were analysed to determine various factors affecting MTDMYs in Mehsana buffaloes under field progeny testing programme.

Standardization and classification of data

The information which was required for identification of buffaloes as well as for classification of data was collected as follows: Animal number, Sire number, Date of birth, Date of calving and Monthly test day milk yield (litre). Milk production records of 5th, 35th, 65th, 95th, 125th, 155th, 185th, 215th, 245th, 275th and 305th day were obtained. In the beginning, a total of 8222 first lactation records of Mehsana buffaloes were available. For present study, the lactations having five or less test day records were considered short and therefore eliminated from the data. Similarly, the sires with the records of less than 10 daughters were excluded from the study. Therefore, finally 7113 first lactations records of daughters of 173 sires distributed in different villages were considered for the study. Depending upon the calving of the animals, data were classified into five periods [1st (1993-1996), 2nd (1997-2000), 3rd (2001-2004), 4th (2005-2008) and 5th (2009-2012)]; and 2 seasons [Breeding season 1 (January to June) and Breeding season 2 (July to December)]. The three age group of first calving (AFC) were classified based on age at first calving of Mehsana buffaloes in order to examine the effect of various non-genetic factors on MTDMYs.

Statistical methods

The least squares model was used for obtaining least squares means of various MTDMYs (Harvey, 1990); $Y_{ijkl} = \mu + A_i + B_j + C_k$

+ e_{ijkl} . Where, Y_{ijkl} is the l^{th} record of buffalo calved in i^{th} period, j^{th} season and k^{th} age group of first calving, μ is Population mean, A_i is the fixed effect of i^{th} period of calving ($i = 1, 2, 3, 4$ and 5), B_j is fixed effect of j^{th} season of calving ($j = 1$ and 2), C_k is fixed effect of k^{th} age group of first calving ($k = 1, 2$ and 3) and e_{ijkl} is the random error (NID, 0, σ^2). Effect of non genetic factors on AFC was estimated using period and season of calving instead of birth. Further, Duncan's multiple range test by Kramer (1957) was used for estimation of differences among least squares means.

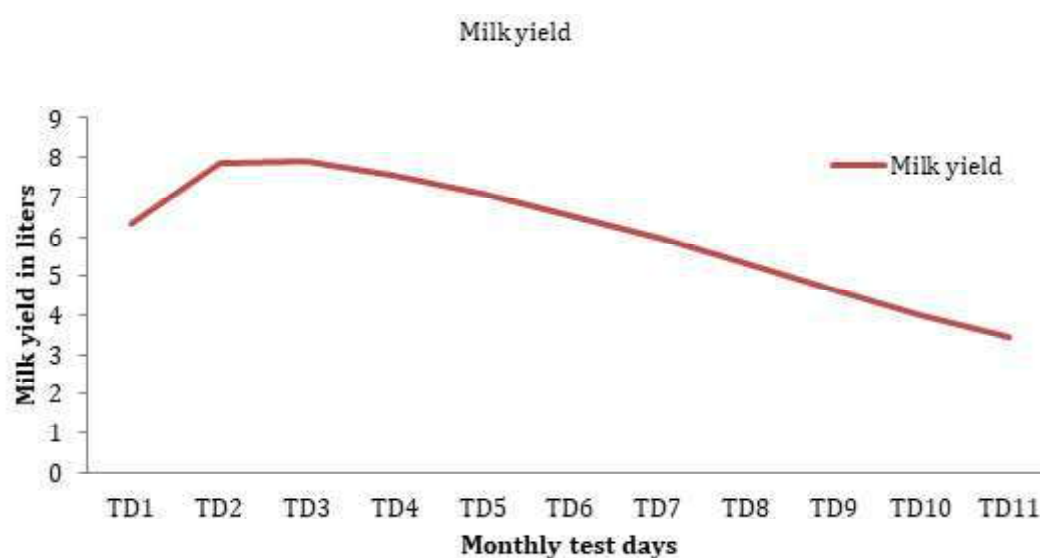
After the adjustment of data, paternal half-sib correlation method (Becker, 1975) was used to estimate heritability for the traits. The sires with ten or more progenies were only included for the estimation of heritability of traits. The model for estimation of heritability was, $Y_{ij} = \mu + S_i + e_{ij}$. Where, Y_{ij} is observation of the j^{th} progeny of the i^{th} sire, μ is population mean, S_i is effect of the i^{th} sire and e_{ij} is random error $\sim$ NID (0, $\sigma^2 e$). The genetic and phenotypic correlations were estimated from analysis of variance and covariance among sire groups as given by Becker (1975).

Results and Discussion

The least squares means of various MTDMYs along with their standard errors are presented in Table-1. The highest MTDMY was observed in MTDMY3 (7.92±0.31 liters) and lowest in MTDMY11 (3.42±0.33 liters). In general, MTDMY increased till MTDMY3 with subsequent gradual decline till the end of lactation (Figure-1). Galsar et al. (2016) also reported highest MTDMY4 (7.31±0.13 liters) and lowest MTDMY11 (3.09±0.11 liters) in Mehsana buffaloes. The highest MTDMY was observed in MTDMY5 (5.98±0.29 liters) and lowest was observed in MTDMY11 (4.35±0.26 liters) in Jaffarabadi buffalo by Sharma et al. 2017. The minimum least-squares means were obtained as 3.91±0.17 kg (Geetha et al. 2006) on MTDMY1 (5th day), 4.17±0.12 kg (Katneni, 2007) on MTDMY11 (305th day), 4.43±0.09 kg (Chakraborty et al. 2010^a) on TDY1 (6th day), 4.19±0.09 kg (Patil et al. 2012) on MTDMY11 (305th day) while, maximum least-squares means were obtained as 7.15±0.15 kg (Geetha et al. 2006) on MTDMY3 (65th day), 8.05±0.11 kg (Katneni, 2007) on MTDMY3 (65th day), 8.11±0.25 kg (Chakraborty et al. 2010^a) on MTDMY3 (65th day), 8.10±0.09 kg (Patil et al. 2012) on MTDMY3 (65th day) in first lactation of Murrah buffaloes. Similarly, Singh et al. 2020 reported the mean of MTDMY increased from 4.625±0.145 kg on MTDMY11 to a peak yield of 10.636±0.214 kg MTDMY3 for first lactation in Murrah buffaloes.

Effect of Period of Calving

The effect of period in the current study on all the MTDMYs was observed as significant ($P \leq 0.05$) to highly significant ($P \leq 0.01$) in Mehsana buffaloes (Table-1). In Murrah buffaloes, similar to present study, significant effect of period of calving was observed on the first lactation monthly test-day yield (Wakchure et al. 2008; Chakraborty et al. 2010^a; Kumar et al. 2012; Singh et al.

Fig. 1 Average monthly test day milk yields

2020). Tailor and Singh (2011) reported similar results as of present study in Surti buffalo. Significant effects were also reported on almost all first lactation MTDMYs except MTDMY5 and MTDMY9 in Mehsana buffaloes (Galsar et al. 2016). Contrary to these findings, Gupta et al. (2012) reported non-significant effect of period of calving on FLTDYs in Murrah buffaloes. Similarly, Sharma et al. (2017) also reported non-significant influence of period of calving in Jaffarabadi buffaloes. The significant to highly significant effect of periods of calving on MTDMYs in the present findings indicates overall variation in the management practices over the period and also the difference in prevailing climate condition in the region over the period.

Effect of Season of Calving

Buffaloes are known to be seasonal breeder and it was observed that only 18.08% of the calving occurred in least calving season (January to June) and in most calving season (July to December) 81.92% of the calving were accounted. Season of calving had highly significant ($P \leq 0.01$) effect on all the MTDMYs. Further, it was observed that the all the MTDMY traits found to be significantly higher in January to June (S1) in comparison of July to December (S2) season. The present finding of significant effect of season of calving on MTDMYs was in conformity with the reports of Kumar et al. (2012), Singh et al. (2016) and Singh et al. (2020) in Murrah buffaloes. Contrary to that, Galsar et al. (2016) reported non-significant effect of season of calving on MTDMYs in Mehsana buffaloes. A non-significant effect of season of calving on first lactation monthly test-day yield in Murrah buffaloes was earlier reported by Chakraborty et al. (2010^b), Penchev et al. (2011) and Gupta et al. (2012). Similarly, no significant differences between various MTDMYs were observed due to the effect of season of calving in Jaffarabadi buffaloes (Sharma et al. 2017). Better performance of buffaloes calved during January to June (S1) season might be due to the fact that buffaloes calved

in relatively fodder scarce season would have sufficient source of fodders in the subsequent monsoon and winter seasons.

Effect of Age at First Calving

In the present finding, age at first calving group had highly significant ($P \leq 0.01$) influence on all the MTDMYs in Mehsana buffaloes. The MTDMYs (up to TD5) were found to be the highest in age at first calving group-1 and in later stage these were noted to be highest in age at first calving group-2 (Table 1). The findings of present investigation were in agreement with Kumar et al. (2015) and Singh et al. (2016). However, Sharma et al. (2017) noted non-significant differences between various MTDMYs due to the effect of age at first calving in Jaffarabadi buffaloes. Singh et al. (2020) observed non-significant effect of season of calving on all the monthly test day milk yields except TD2 and TD3. Significant effect of age at first calving group on MTDMYs indicated that large variation in these traits is accounted due to different AFC groups and records must be adjusted for this non genetic influence before estimation of genetic parameters.

Effect of genetic parameters on monthly test day milk yield

Estimation of genetic parameters will be useful for improving accuracy in evaluation of animals and also appropriate breeding plans may be devised. The heritability (h^2) estimates of MTDMYs along with genetic and phenotypic correlations are presented in Table-2. In general heritability estimates in the present study were low to moderate for various first lactation monthly test-day milk yields. The heritability estimates of MTDMYs ranged from 0.08 ± 0.02 for MTDMY1 to 0.44 ± 0.05 for MTDMY6. Further, MTDMY3, MTDMY8 and MTDMY10 had moderate estimates of heritability, which were all significantly different from zero ($P \leq 0.01$). Madad et al. (2013) reported that the h^2 estimate for monthly TD milk yield ranged from 0.09 to 0.33 in Iranian buffaloes. Similar to present findings, low to moderate (0.13 to 0.38)

Table 1: Least squares means $\pm$ SE for monthly test day milk yields

Traits	MTDMY1	MTDMY2	MTDMY3	MTDMY4	MTDMY5	MTDMY6	MTDMY7	MTDMY8	MTDMY9	MTDMY10	MTDMY11
μ	6.32 $\pm$ 0.32	7.84 $\pm$ 0.31	7.92 $\pm$ 0.31	7.57 $\pm$ 0.31	7.08 $\pm$ 0.30	6.54 $\pm$ 0.30	5.98 $\pm$ 0.30	5.33 $\pm$ 0.30	4.64 $\pm$ 0.30	3.99 $\pm$ 0.31	3.42 $\pm$ 0.33
Period	*	**	**	**	**	**	**	**	**	**	**
1	6.35 $\pm$ 0.54 ^b	7.45 $\pm$ 0.53 ^c	7.69 $\pm$ 0.52 ^c	7.47 $\pm$ 0.52 ^b	7.03 $\pm$ 0.51 ^b	6.55 $\pm$ 0.51 ^b	6.00 $\pm$ 0.51 ^b	5.36 $\pm$ 0.51 ^b	4.69 $\pm$ 0.51 ^a	4.02 $\pm$ 0.52 ^{ab}	3.41 $\pm$ 0.58 ^b
2	6.15 $\pm$ 0.56 ^c	7.74 $\pm$ 0.55 ^d	7.89 $\pm$ 0.54 ^b	7.55 $\pm$ 0.53 ^b	7.04 $\pm$ 0.53 ^b	6.52 $\pm$ 0.52 ^{bc}	5.98 $\pm$ 0.52 ^b	5.35 $\pm$ 0.52 ^b	4.66 $\pm$ 0.53 ^{ab}	4.04 $\pm$ 0.55 ^{ab}	3.30 $\pm$ 0.57 ^b
3	6.11 $\pm$ 0.49 ^c	7.87 $\pm$ 0.48 ^c	7.88 $\pm$ 0.48 ^b	7.52 $\pm$ 0.47 ^b	7.01 $\pm$ 0.47 ^b	6.46 $\pm$ 0.46 ^{bc}	5.85 $\pm$ 0.46 ^c	5.25 $\pm$ 0.46 ^{bc}	4.55 $\pm$ 0.47 ^{bc}	3.92 $\pm$ 0.50 ^{bc}	3.40 $\pm$ 0.55 ^b
4	6.15 $\pm$ 0.49 ^c	8.00 $\pm$ 0.48 ^b	7.99 $\pm$ 0.48 ^b	7.56 $\pm$ 0.47 ^b	7.03 $\pm$ 0.47 ^b	6.41 $\pm$ 0.47 ^c	5.81 $\pm$ 0.46 ^c	5.17 $\pm$ 0.46 ^c	4.48 $\pm$ 0.48 ^c	3.81 $\pm$ 0.50 ^c	3.34 $\pm$ 0.55 ^b
5	6.82 $\pm$ 0.57 ^a	8.15 $\pm$ 0.56 ^a	8.14 $\pm$ 0.55 ^a	7.76 $\pm$ 0.55 ^a	7.26 $\pm$ 0.54 ^a	6.77 $\pm$ 0.54 ^a	6.23 $\pm$ 0.54 ^a	5.54 $\pm$ 0.53 ^a	4.79 $\pm$ 0.54 ^a	4.16 $\pm$ 0.57 ^a	3.65 $\pm$ 0.61 ^a
Season	**	**	**	**	**	**	**	**	**	**	**
1	6.37 $\pm$ 0.52 ^a	8.04 $\pm$ 0.51 ^a	8.10 $\pm$ 0.51 ^a	7.71 $\pm$ 0.50 ^a	7.17 $\pm$ 0.50 ^a	6.67 $\pm$ 0.49 ^a	6.16 $\pm$ 0.49 ^a	5.55 $\pm$ 0.49 ^a	4.84 $\pm$ 0.50 ^a	4.14 $\pm$ 0.50 ^a	3.53 $\pm$ 0.53 ^a
2	6.26 $\pm$ 0.28 ^b	7.64 $\pm$ 0.27 ^b	7.73 $\pm$ 0.27 ^b	7.44 $\pm$ 0.27 ^b	6.99 $\pm$ 0.26 ^b	6.42 $\pm$ 0.26 ^b	5.80 $\pm$ 0.26 ^b	5.12 $\pm$ 0.26 ^b	4.43 $\pm$ 0.27 ^b	3.84 $\pm$ 0.28 ^b	3.32 $\pm$ 0.31 ^b
AFC Group	**	**	**	**	**	**	**	**	**	**	**
1	6.40 $\pm$ 0.55 ^a	7.95 $\pm$ 0.54 ^a	8.00 $\pm$ 0.53 ^a	7.61 $\pm$ 0.53 ^{ab}	7.14 $\pm$ 0.52 ^a	6.60 $\pm$ 0.52 ^a	6.01 $\pm$ 0.52 ^a	5.31 $\pm$ 0.52 ^b	4.56 $\pm$ 0.53 ^b	3.90 $\pm$ 0.56 ^b	3.29 $\pm$ 0.62 ^b
2	6.31 $\pm$ 0.31 ^{ab}	7.89 $\pm$ 0.30 ^a	7.96 $\pm$ 0.30 ^a	7.63 $\pm$ 0.29 ^a	7.13 $\pm$ 0.29 ^a	6.60 $\pm$ 0.29 ^a	6.05 $\pm$ 0.29 ^a	5.42 $\pm$ 0.29 ^a	4.72 $\pm$ 0.29 ^a	4.05 $\pm$ 0.30 ^a	3.51 $\pm$ 0.32 ^a
3	6.24 $\pm$ 0.56 ^b	7.69 $\pm$ 0.55 ^b	7.78 $\pm$ 0.54 ^b	7.47 $\pm$ 0.54 ^b	6.96 $\pm$ 0.53 ^b	6.44 $\pm$ 0.53 ^b	5.87 $\pm$ 0.52 ^b	5.27 $\pm$ 0.52 ^b	4.63 $\pm$ 0.53 ^{ab}	4.02 $\pm$ 0.55 ^{ab}	3.46 $\pm$ 0.59 ^a

** = $P \leq 0.01$; * = $P \leq 0.05$; NS: non-significant, Superscripts may be read column wise for each effect for mean comparison. Similar superscript shows that the means do not differ significantly.

Table 2: Heritability estimates, genetic and phenotypic correlations between different monthly test day milk yields

Traits	MTDMY1	MTDMY2	MTDMY3	MTDMY4	MTDMY5	MTDMY6	MTDMY7	MTDMY8	MTDMY9	MTDMY10	MTDMY11
MTDMY1	0.08 $\pm$ 0.02**	0.70 $\pm$ 0.76	0.47 $\pm$ 0.97	0.62 $\pm$ 0.84	0.52 $\pm$ 0.10**	0.57 $\pm$ 0.77	0.59 $\pm$ 0.85	0.45 $\pm$ 0.10**	0.36 $\pm$ 0.11**	0.38 $\pm$ 0.11**	0.61 $\pm$ 0.11**
MTDMY2	0.58 $\pm$ 0.97	0.12 $\pm$ 0.02**	0.61 $\pm$ 0.70	0.69 $\pm$ 0.62	0.70 $\pm$ 0.64	0.30 $\pm$ 0.93	0.58 $\pm$ 0.77	0.34 $\pm$ 0.99	0.11 $\pm$ 0.11	0.15 $\pm$ 0.11	0.36 $\pm$ 0.13*
MTDMY3	0.50 $\pm$ 0.10**	0.77 $\pm$ 0.75	0.27 $\pm$ 0.04**	0.79 $\pm$ 0.37*	0.71 $\pm$ 0.51	0.54 $\pm$ 0.60	0.33 $\pm$ 0.86	0.59 $\pm$ 0.61	0.41 $\pm$ 0.78	0.40 $\pm$ 0.84	0.14 $\pm$ 0.12
MTDMY4	0.41 $\pm$ 0.11**	0.69 $\pm$ 0.86	0.82 $\pm$ 0.68	0.18 $\pm$ 0.03**	0.98 $\pm$ 0.34**	0.73 $\pm$ 0.43	0.68 $\pm$ 0.56	0.82 $\pm$ 0.33*	0.65 $\pm$ 0.59	0.66 $\pm$ 0.61	0.54 $\pm$ 0.93
MTDMY5	0.43 $\pm$ 0.11**	0.58 $\pm$ 0.96	0.69 $\pm$ 0.86	0.79 $\pm$ 0.73	0.16 $\pm$ 0.03**	0.66 $\pm$ 0.54	0.66 $\pm$ 0.61	0.80 $\pm$ 0.38*	0.61 $\pm$ 0.66	0.62 $\pm$ 0.69	0.56 $\pm$ 0.93
MTDMY6	0.35 $\pm$ 0.11**	0.53 $\pm$ 0.10**	0.65 $\pm$ 0.90	0.74 $\pm$ 0.80	0.78 $\pm$ 0.74	0.44 $\pm$ 0.05**	0.51 $\pm$ 0.66	0.84 $\pm$ 0.25**	0.88 $\pm$ 0.19**	0.75 $\pm$ 0.38*	0.39 $\pm$ 0.91
MTDMY7	0.30 $\pm$ 0.11**	0.51 $\pm$ 0.10**	0.56 $\pm$ 0.98	0.67 $\pm$ 0.88	0.71 $\pm$ 0.84	0.81 $\pm$ 0.70	0.22 $\pm$ 0.03**	0.75 $\pm$ 0.43	0.67 $\pm$ 0.53	0.79 $\pm$ 0.38*	0.75 $\pm$ 0.52
MTDMY8	0.22 $\pm$ 0.12	0.43 $\pm$ 0.11**	0.50 $\pm$ 0.10**	0.62 $\pm$ 0.94	0.61 $\pm$ 0.95	0.73 $\pm$ 0.82	0.82 $\pm$ 0.70	0.25 $\pm$ 0.03**	0.96 $\pm$ 0.72	0.95 $\pm$ 0.10**	0.64 $\pm$ 0.70
MTDMY9	0.16 $\pm$ 0.12	0.36 $\pm$ 0.12**	0.40 $\pm$ 0.11**	0.50 $\pm$ 0.11**	0.51 $\pm$ 0.11**	0.62 $\pm$ 0.97	0.68 $\pm$ 0.91	0.83 $\pm$ 0.69	0.30 $\pm$ 0.04**	0.96 $\pm$ 0.80	0.59 $\pm$ 0.73
MTDMY10	0.12 $\pm$ 0.13	0.28 $\pm$ 0.13*	0.32 $\pm$ 0.13*	0.39 $\pm$ 0.12**	0.41 $\pm$ 0.12**	0.49 $\pm$ 0.12**	0.56 $\pm$ 0.11**	0.66 $\pm$ 0.99	0.80 $\pm$ 0.79	0.28 $\pm$ 0.04**	0.62 $\pm$ 0.71
MTDMY11	0.15 $\pm$ 0.15	0.26 $\pm$ 0.15	0.24 $\pm$ 0.15	0.34 $\pm$ 0.15*	0.36 $\pm$ 0.14*	0.38 $\pm$ 0.14**	0.48 $\pm$ 0.14**	0.53 $\pm$ 0.13**	0.62 $\pm$ 0.12**	0.74 $\pm$ 0.10**	0.18 $\pm$ 0.04**

** = $P \leq 0.01$; * = $P \leq 0.05$; above diagonal = genetic correlations; below diagonal = phenotypic correlations; diagonal = heritability and S.E. estimates

heritability estimates for fortnightly test-day yields in Surti buffaloes reported by Tailor and Singh, 2011. Rana et al. (2021) also reported heritability estimates for monthly test day milk yields ranged from 0.22 ± 0.15 (MTDMY1) to 0.51 ± 0.19 (MTDMY9). Contrary to this, low to high (0.05 to 0.85) heritability estimates for various first lactation monthly test-day yields were observed by Galsar et al. (2016). In the present study, the genetic correlations between various MTDMYs varied from low to high. The present finding of low to moderate h^2 estimate for MTDMYs implies that larger portion of the variation in this trait may be due to environmental differences among individuals and due to sampling error, therefore, uniformity in management and feeding practices over the periods and seasons may help in improving the trait. Mass selection alone would not be effective in improving the trait.

A close perusal of estimates of genetic correlations (r_g) among various MTDMYs presented in table-2 revealed that the highest and highly significant r_g was obtained between MTDMY4 and MTDMY5 (0.98 ± 0.34), however, lower but non-significant r_g was obtained between MTDMY2 and MTDMY9 (0.11 ± 0.11). Similar to present finding low to high genetic correlations between various MTDMYs were reported by Galsar et al. (2016) in Mehsana buffaloes. However, Geetha et al. (2007) reported moderate to high genetic correlations between various first lactation monthly test-day yields in Murrah buffaloes. The phenotypic correlations (r_p) between all the MTDMYs in table-2 indicated that the highest but non-significant r_p was obtained between MTDMY8 and MTDMY9 (83 ± 0.69) and, lower r_p was obtained between MTDMY1 and MTDMY10 (12 ± 0.13). Similar to present finding (an increasing trend in phenotypic correlation as the test day progressed), Rana et al. (2021) reported phenotypic correlations among different monthly test day milk yields ranged from 0.14 (MTDMY1 and MTDMY11) to 0.83 (MTDMY9 and MTDMY10) in Murrah buffaloes. However, Singh et al. (2016) reported a decreasing trend in phenotypic correlation with late test day milk yields in same breed.

Conclusions

The present investigation showed that period of calving, season of calving and age at first calving group had significant effect on all the monthly test day milk yields which indicated that environmental factors influenced the different lactation phase due to change in climatic conditions. Moreover, these environmental factors were important to adjust the all the test day yield records for estimation of the genetic parameters. Low to moderate heritability estimate of MTDMYs along with its high phenotypic correlations with each other suggests that selection for early MTDMYs would be effective for prediction of total milk yield and thereby improvement in one test day yield would be associated with wanted improvement in total lactation milk yield.

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RESEARCH ARTICLE

Genetic analysis of reproductive traits in Karan Fries cattle

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Abstract: The present study was conducted on Karan Fries herd maintained at ICAR-NDRI, Karnal. In the present investigation on Age at first calving (AFC), Days to first service (DFS), Service period (SP), Number of services per first conception (NS/C), Pregnancy Rate (PR) and Conception Rate (CR) 624, 1793, 1921, 1933, 1921 and 1933 observations respectively were recorded. The effect of non-genetic factors on reproductive traits were studied by least-squares technique and the significant difference of means between subclasses of season, period and parity were tested. Genetic and phenotypic parameters of reproduction traits were estimated. Season of calving had significant effect on AFC, DFS, SP and NS/C. Further, period of calving had significant effect on AFC, DFS, SP, NS/C, PR and CR. Parity had significant effect on DFS, SP and PR in HF crossbred cattle. The data were adjusted for the significant non-genetic factors to remove the differential environmental effects. The heritability estimates of fertility traits viz. DFS, SP, PR, NS/C and CR were, in general, very low (0.04-0.13). These estimates indicated that these fertility traits are influenced more by managemental and environmental factors than by genetic factors.

Keywords: Conception rate, Heritability, Pregnancy rate, Repeatability

Introduction

Fertility of dairy animals is considered as an important economic trait, as it brings animals into lactation and maximizes the profitability by in-time calf crop. Although fertility of cattle is very important, the reproductive efficiency of dairy cattle has been neglected and decreased dramatically, which is a concern to cattle stock holders. As a consequence, several developed countries have included fertility traits in their breeding goals and selection programs, where they have used fertility traits like daughters pregnancy rate (DPR) and sire conception rate (SCR) for the genetic evaluation of bulls (Miglior et al. 2005; VanRaden et al. 2004; Weigel 2006; De Vries, 2011; USDA, 2009 and Cabrera, 2011). Several reports have shown that a significant percentage of reproductive failure in dairy animals is attributable to bull sub-fertility (Braundmeier and Miller 2001 and DeJarnette et al. 2004). Bull fertility is a major contributor in improving herd productive and reproductive performance, higher male fertility can help the selection of dairy bulls with a higher semen quality (Crepaldi et al. 2009). Age at first calving is an important trait because lower age at first calving in dairy animals leads to shorter generation interval and hence increases genetic gain of desired traits. Service period (SP) in HF crossbred cattle is one of the important reproduction traits. Minimizing service period helps to control the calving interval and maximum calf crop and milk yield can be harvested. Days to first service are the initial phase of lactation during which no inseminations occur. The lower the DFS shows higher the breeding efficiency in dairy animals. Pregnancy rate is defined as the percentage of non-pregnant cows that become pregnant during each 21 days period, because each oestrus cycle represents one chance for a cow to become pregnant. Number of services per first conception (NS/C) and Conception Rate (CR) are also important fertility traits. Therefore, present investigation on the assessment of fertility traits of Karan Fries cattle is very important.

Materials and Methods

The present study was conducted on Karan Fries herd maintained at ICAR-NDRI, Karnal. For investigation of Age at first calving (AFC), Days to first service (DFS), Service period (SP), Number

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of services per first conception (NS/C), Pregnancy Rate (PR), Conception Rate (CR) 624, 1793, 1921, 1933, 1921 and 1933 observations respectively were recorded for the present study.

Statistical analysis

The analysis of data was done with the help of Pentium based computer system under UNIX multi-user environment at the Computer Centre and SYSTAT-12 available at Animal Genetics and Breeding Division of the ICAR-National Dairy Research Institute, Karnal, India.

Least-Squares Analysis

The effect of non-genetic factors on reproduction traits were estimated using least-squares analysis for non-orthogonal data as suggested by Harvey (1990). The models were used with assumptions that different components being fitted into the model are linear, independent and additive.

The model for reproductive traits is described as follows:

$$Y_{ijkl} = \mu + S_i + P_j + L_k + e_{ijkl}$$

where,

Y_{ijkl} = Observation on the i^{th} animal calved in i^{th} season, j^{th} period and of k^{th} parity
 μ = Overall mean
 S_i = Effect of i^{th} season of calving
 P_j = Effect of j^{th} period of calving
 L_k = Effect of k^{th} parity of animals
 e_{ijkl} = Random error associated with observation, assumed to NID (0, $\sigma^2 e$)

The model for AFC is described as follows:

$$Y_{ijk} = \mu + S_i + P_j + e_{ijk}$$

where,

Y_{ijk} = Observation on the k^{th} animal calved in i^{th} season and j^{th} period
 μ = Overall mean
 S_i = Effect of i^{th} season of calving
 P_j = Effect of j^{th} period of calving
 e_{ijk} = Random error associated with observation, assumed to NID (0, $\sigma^2 e$)

Duncan's Multiple Range Test (DMRT)

The difference of means between subclasses of season, period and were tested for significance using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957).

Estimation of Heritability

Paternal half-sib correlation method was used to estimate the heritability of different characters (Becker, 1975). The following model was used to estimate the heritability:

$$Y_{ij} = m + S_i + e_{ij}$$

where,

Y_{ij} = Observation of the j^{th} progeny of the i^{th} sire
 m = Overall mean
 S_i = Effect of the i^{th} sire
 e_{ij} = Random error $\sim$ NID (0, $\sigma^2 e$)

Estimation of repeatability of reproduction traits

The following model was used for the estimation of repeatability:

$$Y_{ijk} = \mu + K_i + B_j + e_{ijk}$$

Where,

Y_{ijk} = random measurement of reproduction traits of j^{th} cow in i^{th} parity
 μ = overall mean of a trait
 K_i = the effect between i^{th} parity within cow ($i = 1$ to 4)
 B_j = the effect of j^{th} cow
 e_{ijk} = random error, NID (0 to $\sigma^2 e$)

Genetic and Phenotypic Correlations

The genetic and phenotypic correlations were calculated from the analysis of variance and covariance among sire groups as given by Becker (1975). The significance of phenotypic correlations were tested by 't' test as given by Snedecor and Cochran (1994).

Results and Discussion

Age at first calving (AFC)

The overall least-squares mean for AFC was 34.97 ± 0.24 months (table 1). Among the different factors affecting AFC; the season of calving and period of calving were found to have significant effect on AFC. The season of birth had significant effect on AFC in Karan Fries cattle. However, result was not agreement with those reported by Parmar et al. (1986); Jadhav et al. (1991) and Yazdani et al. (1993) in Friesian crosses. The animals which were calved during the rainy season were having highest (37.24 ± 0.54 months) AFC and the lowest (31.54 ± 0.50 months) AFC was observed during the autumn season (Table 1). The period of calving was found to have significant effect on AFC in Karan Fries cattle. The animals which were born during the period 2005-

2008 were having highest (35.31 ± 0.48 months) AFC and the lowest (34.34 ± 0.50 months) AFC was observed during the period of 2009-2012. However, Rana (1991) in Friesian crosses reported non-significant effect of period of birth on AFC.

Service period (SP)

Service Period was significantly influenced by period of calving and parity (Table 1). Season of calving was found to have significant effect on SP. The overall least-squares mean of SP was 129.43 ± 1.47 days (Table 1). The highest SP (135.65 days) was recorded for autumn calvers and least (126.31 ± 2.80 days) for winter calvers. Arora et al. (1993) in Frieswal, Mandal and Sachdeva (2001) in Karan Swiss reported significant effect of season on SP. The period of calving had significant effect on SP. The significant effect of period of calving obtained in the study was in agreement with that of Umrikar et al. (1990) in Jersey X Gir cross, Mandal and Sachdeva (2001) in Karan Swiss, however, reported non-significant effect of period of calving on SP. The period of calving had significant effect on SP.

Days to first service (DFS)

The days to first service reflects the ability of the cattle to come into estrus after calving and the ability to conceive successfully. For the interval from first insemination to conception, high producing cows tend to have more opportunities for re-insemination in the case of failure of conception. Waiting period is influenced by management decisions. To account for this bias in the present study information on culled cows was, also incorporated. Among the different factors considered in the study

season of calving, period of calving and parity found to be significant. The overall least-squares mean for waiting period was 93.49 ± 1.01 days (Table 1). The effect of season of calving on waiting period was statistically significant. The least-squares mean of waiting period was highest (97.22 ± 2.16 days) in autumn calvers and lowest (89.19 ± 2.05 days) in summer calvers (Table 1). The study revealed that period of calving had significant effect on waiting period in HF crossbred. The longest first to successful service period was in cows which had calved during 2005-2008 (102.73 ± 2.05 days) and lowest during 2001-2004 (84.84 ± 1.98 days). This finding can be correlated with increased milk production over the years as repeated chance for insemination was obtained by only those cows which were having a higher production record. Also the overall CTFS in the herd was 87 days; by this time the animal would have achieved high production level and there is a chance for negative energy balance, which may lead to failure of conception. Proper detection of estrus and timely insemination are also deciding factors for successful conception.

Pregnancy Rate (PR)

The overall least-squares mean for pregnancy rate was $0.38 \pm 0.80\%$ days (Table 2). The effect of season of calving on waiting period was statistically non significant. The least-squares mean of pregnancy rate was highest ($0.39 \pm 0.01\%$) in rainy calvers and lowest ($0.36 \pm 0.06\%$) in rainy calvers (Table 2). The study revealed that period of calving had significant effect on pregnancy rate in Karan Fries cattle. The longest pregnancy rate was in cows which had calved during 2007-2010 ($0.42 \pm 0.01\%$) and lowest during

Table 1: Least-squares means and standard errors of reproduction traits in HF crossbred cattle

Effects	AFC (months)	DFS (days)	SP (days)
Overall (μ)	34.97 ± 0.24 (624)	93.49 ± 1.01 (1793)	129.91 ± 1.59 (1921)
	Season of calving		
Winter (Dec-Mar)	37.16 ± 0.44^c	93.24 ± 1.83^{ab}	126.31 ± 2.80^a
Summer (Apr-Jun)	33.95 ± 0.49^b	89.19 ± 2.05^a	127.52 ± 3.04^{ab}
Rainy (Jul-Aug)	37.24 ± 0.54^c	94.32 ± 2.19^{ab}	130.17 ± 3.37^{ab}
Autumn (Sep-Nov)	31.54 ± 0.50^a	97.22 ± 2.16^b	135.65 ± 3.18^b
	Period of calving		
2001-2004	34.98 ± 0.48^{ab}	84.84 ± 1.98^a	133.30 ± 2.96^b
2005-2008	35.31 ± 0.48^b	102.73 ± 2.05^c	127.79 ± 3.18^{ab}
2009-2012	34.34 ± 0.50^a	90.07 ± 2.13^{ab}	123.88 ± 3.28^a
2013-2016	35.26 ± 0.50^b	96.34 ± 2.22^b	134.69 ± 3.24^b
	Parity		
First	-	107.82 ± 1.74^b	133.21 ± 2.54^b
Second	-	87.98 ± 2.29^a	130.23 ± 3.18^{ab}
Third	-	91.33 ± 2.36^a	132.94 ± 4.34^b
Four and above	-	86.84 ± 1.88^a	123.27 ± 2.70^a

Figures in parentheses indicates number of observations. Dissimilar superscript indicates significant ($p < 0.05$) difference of subclass of means.

2011-2015 ($0.35 \pm 0.06\%$). Parity found significant effect on pregnancy rate.

Number of services per first conception (NS/C)

In the present study the average number of services per first conception (NS/C) of Karan Fries cattle was estimated as 2.24 ± 0.08 and the coefficient of variation were obtained as 77.56% (Table 2). The effect of season of calving on number of services per first conception was statistically significant. The least-squares mean of number of services per first conception was highest (2.49 ± 0.11) in rainy calvers and lowest (2.29 ± 0.10) in autumn calvers (Table 2). The study revealed that period of calving had significant effect on number of services per first conception in HF crossbred cattle. The longest number of services per first conception was in Karan Fries cattle which had calved during 1999-2002 (2.44 ± 0.13) and lowest during 2003-2006 (2.19 ± 0.09). Parity found non-significant effect on number of services per first conception.

Conception Rate (CR)

In the present study the average conception rate of Karan Fries cattle was estimated as $61.76 \pm 1.08\%$ and the coefficient of variation was obtained as 51.94% (Table 2). The effect of season of calving on conception rate was statistically non-significant. The least-squares mean of conception rate was highest in summer calvers and lowest in autumn calvers (Table 2). The study revealed that period of calving had significant effect on conception rate in HF crossbred cattle. Parity found non-significant effect on conception rate.

Heritability of fertility traits

The heritability estimates of fertility traits viz. DFS, FSP, PR, NS/C and CR were, in general, very low (0.04-0.13) and suffered from high S.E. These estimates indicated that these fertility traits are influenced more by managemental and environmental factors than by genetic factors. However, the heritability of AFC was

Table 2: Least-squares means and standard errors of first lactation reproductive traits in HF crossbred cattle

Effects	NS/C (Number)	PR (%)	CR (%)
Overall (μ)	2.24 ± 0.08 (1933)	0.38 ± 0.80 (1921)	61.76 ± 1.08 (1933)
Season of calving			
Winter (Dec-Mar)	2.37 ± 0.09^{ab}	0.38 ± 0.01	60.65 ± 1.54
Summer (Apr-Jun)	2.23 ± 0.10^a	0.39 ± 0.01	62.75 ± 1.66
Rainy (Jul-Aug)	2.49 ± 0.11^b	0.39 ± 0.01	62.39 ± 1.87
Autumn (Sep-Nov)	2.29 ± 0.10^{ab}	0.36 ± 0.06	61.26 ± 1.80
Period of calving			
1999-2002	2.44 ± 0.13^b	0.37 ± 0.05^{ab}	62.54 ± 1.55^a
2003-2006	2.19 ± 0.09^a	0.39 ± 0.01^b	61.89 ± 1.58^a
2007-2010	2.23 ± 0.09^a	0.42 ± 0.01^b	61.35 ± 1.73^a
2011-2015	2.37 ± 0.10^b	0.35 ± 0.06^a	65.98 ± 1.77^b
Parity			
Heifer	2.22 ± 0.10	-	61.37 ± 2.21
First	2.34 ± 0.13	0.37 ± 0.05^a	60.71 ± 1.80
Second	2.47 ± 0.10	0.35 ± 0.06^a	64.37 ± 1.84
Third	2.25 ± 0.11	0.37 ± 0.05^a	60.70 ± 2.27
Four and above	2.32 ± 0.13	0.43 ± 0.01^b	61.64 ± 1.72

Figures in parentheses are the number of observations. Dissimilar superscript indicates significant ($P < 0.05$) difference of subclass of means.

Table 3: Estimates of heritability, genetic (below diagonal) and phenotypic correlations (above diagonal) of fertility traits in HF crossbred cattle

Traits	AFC	DFS	FSP	DPR	NS/C	CR
AFC	0.47 ± 0.17	-0.02 ± 0.04	0.01 ± 0.04	-0.17 ± 0.05	-0.06 ± 0.07	-0.13 ± 0.04
DFS	-0.45 ± 0.35	0.06 ± 0.12	-0.04 ± 0.10	0.20 ± 0.04	-0.03 ± 0.04	-0.12 ± 0.03
FSP	-0.17 ± 0.33	0.43 ± 0.56	0.05 ± 0.13	0.38 ± 0.07	0.72 ± 0.06	0.34 ± 0.08
DPR	0.05 ± 0.81	-0.17 ± 0.17	0.12 ± 0.13	0.04 ± 0.11	0.23 ± 0.09	-0.13 ± 0.07
NS/C	-0.20 ± 0.14	0.049 ± 0.13	-0.16 ± 0.18	0.22 ± 0.19	0.09 ± 0.17	0.49 ± 0.04
CR	-0.16 ± 0.23	-0.25 ± 0.17	0.46 ± 0.27	0.52 ± 0.13	0.019 ± 0.12	0.13 ± 0.11

Table 4: Repeatability of reproduction traits in HF crossbred cattle

Traits	Mean $\pm$ SE
Service Period	0.15 $\pm$ 0.03
Days to First Service	0.17 $\pm$ 0.03
Pregnancy Rate	0.09 $\pm$ 0.02
NS/C	0.10 $\pm$ 0.02
CR	0.16 $\pm$ 0.03

fairly high (0.47 ± 0.17) and this trait can be improved through selection. The heritability of DFS was 0.06 ± 0.12 (Table 3). Very small estimates of heritability of these traits were reported by Hailie-Mariam et al. (2003) and Zink et al. (2012) in HF also. Hailie-Mariam et al. (2003) reported the heritability of DFS 0.13 ± 0.02 , and Zink et al. (2012) reported 0.04 ± 0.01 . The heritability of FSP was 0.05 ± 0.13 . Similar estimates were also reported by Singh and Tomar (1991). They reported 0.036 ± 0.126 and 0.046 ± 0.143 in Karan Fries cattle. (Table 3)

Genetic (r_g) and the phenotypic (r_p) correlations of fertility traits among themselves

The genetic (r_g) and the phenotypic (r_p) correlations of fertility traits among themselves are presented in Table 3. The r_g among the fertility traits ranged between 0.019 ± 0.12 (CR with NS/C) to 0.52 ± 0.13 (CR with DPR). The r_p among the fertility traits ranged between 0.01 ± 0.04 (AFC with FSP) to 0.72 ± 0.06 (FSP with NS/C). The r_p between AFC and other fertility traits were small and ranging from -0.01 to -0.17 . The r_p among other fertility traits (FSP, DSP, PR, NS/C and CR) ranged from -0.01 to 0.72 . The lowest correlation was found between AFS and FSP (-0.01 ± 0.04) and the highest between FSP and NS/C (0.72 ± 0.06).

Repeatability of reproduction traits

Repeatability of reproduction traits are used to find out the effectiveness of selection and also to compare accurately the reproducing ability of animals with varying numbers of records. Repeatability estimates of reproduction traits in Karan Fries cattle were low presented in Table 4. The repeatability of fertility traits viz. FSP, day to first service, PR, NS/C and CR were estimated as 0.15 ± 0.03 , 0.17 ± 0.03 , 0.09 ± 0.02 , 0.10 ± 0.02 and 0.16 ± 0.03 respectively. The literature revealing repeatability estimates of pregnancy rate in buffalo was not available. Van Raden et al. 2004 reported the repeatability estimates of pregnancy rate in HF cows as 0.11, while Kuhn et al. 2004 reported the repeatability of pregnancy rate as 0.13 in HF cows. The low repeatability estimates suggest that fertility traits are strongly influenced by temporary environmental factors. It would be thus possible to improve fertility performance of dairy animals either through improvement in herd management or by incorporating fertility

traits in the genetic evaluation of cattle along with production traits.

Conclusions

Present investigation revealed that season of calving had significant effect on AFC, DFS and NS/C. Further, period of calving had significant effect on AFC, DFS, SP, NS/C, PR and CR and parity had significant effect on DFS, SP and PR in HF crossbred cattle. The heritability estimates of fertility traits viz. DFS, FSP, DPR, NSC and CR were, in general, very low (0.04 - 0.13) and suffered from high S.E. These estimates indicated that these fertility traits are influenced more by managerial and environmental factors than by genetic factors. The low repeatability estimates suggest that fertility traits are strongly influenced by temporary environmental factors. It would be thus possible to improve fertility performance of dairy animals either through improvement in herd management or by incorporating fertility traits in the genetic evaluation of cattle along with production traits. These findings will be very helpful in management of breeding cattle in dairy farm.

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RESEARCH ARTICLE

Assessment of area specific mineral mixture supplementation on growth and biochemical profile of Jersey crossbred heifers

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Abstract: The present experiment was conducted to assess the effect of area specific mineral mixture (ASMM) supplementation on growth, onset of puberty, blood biochemical and hormonal profiles in Jersey crossbred heifers. Crossbred heifers (n=12) were allocated into two groups (6 in each) namely, control (T0) and treatment (T1) and fed without ASMM and with 30 g of ASMM/heifer/day for a period of four months. In the present study, mean of total body weight gain (42 ± 4.82 vs. 58.08 ± 6.42 kg) and average daily gain (0.367 ± 0.07 vs. 0.480 ± 0.05 kg/d), plasma calcium (9.63 ± 0.57 vs. 9.85 ± 0.53 mg/dl), phosphorus (5.59 ± 0.33 vs. 5.88 ± 0.35 mg/dl), copper (0.56 ± 0.02 vs. 0.71 ± 0.02 ppm), zinc (0.59 ± 0.01 vs. 0.70 ± 0.02 ppm) and progesterone (0.67 ± 0.02 vs. 0.93 ± 0.06 ng/ml) were significantly lower ($P < 0.05$) in T0 group than T1, while the average values of glucose, NEFA, α -amino nitrogen level, serum SGOT, serum SGPT, and estradiol-17 β did not vary significantly ($P > 0.05$) between groups. Significantly ($P < 0.05$) lower age of puberty was observed in the ASMM fed group (20.10 ± 0.13 months) than control (22.12 ± 0.13 months). It can be concluded that supplementation of ASMM improved growth and lowered the age at puberty in crossbred heifers.

Keywords: Biochemical profiles, Growth, Heifers, Minerals, Puberty

Introduction

Macro- and micro-minerals play an important role in various metabolic functions and their deficiency leads to impairment in the production and reproductive performance of livestock. Generally, the mineral availability to livestock is dependent on the production system, feeding management, and the environment under which the animals are maintained (Singh and Bohra 2005). Soil plays a vital role among environmental factors as the concentration of nutrients including minerals in the feed and fodders fed to the livestock depend on the soil where they were cultivated. McDowell (1997) reported that the fodders macro-mineral status may be altered by stage of harvesting, season, plant species, fertilizer application rate, soil type and pH of the soil. It is important to determine the status of minerals in the soil, feed, forages and animals region-wise, to understand and meet the requirements of livestock to obtain optimum productivity (Pereira et al. 1997). Ghosh et al. (2013) observed that the soil, feed, fodder and blood of the animals in the lower Gangetic part of West Bengal were deficient with minerals such as Ca, P, Zn, Cu, Co, Mn, and subsequently they developed an area-specific mineral mixture (ASMM) for this region. Since no detailed study was conducted on the effect of this ASMM on growth performance in the livestock of the lower Gangetic region, the present experiment was undertaken to study the effect of ASMM on growth, age at puberty, blood biochemical and hormonal profile in Jersey crossbred heifers.

Materials and Methods

Experimental animals and feeding

The study was carried out at the Eastern Regional Station of the National Dairy Research Institute (ERS-NDRI), Kalyani, West Bengal, India. Jersey crossbred heifers of 12-18 months of age were taken for the study (n=12). The animals were allocated randomly into two groups as control (T0; n=6 heifers) and treatment (T1; n=6 heifers). The heifers were fed as per the ICAR recommendation (2013) and in addition, the heifers in the T1 group were supplied with ASMM @ 30 g/day/animal for a period of four months, while heifers in the control (T0) group were not provided with ASMM. The composition of ASMM is given in

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Table 1. All the experimental heifers were kept under observation for the appearance of first pubertal estrum, body weight gain and feed intake. The experiment was conducted from November 2016 to May 2017.

Chemical analysis of feed

Chemical analysis of feed and fodder was done as per AOAC (2005) and estimation of cell wall constituents was carried out as per the methods of Van Soest et al. (1991). The composition of feed that was fed to animals during the experimental period is presented in Table 2.

Collection of blood and analysis

Blood samples for the biochemical analysis were collected from the heifers at fortnightly intervals; and for the hormonal analysis, blood samples were collected at weekly intervals up to the onset of estrum and thereafter at alternate day intervals till the occurrence of the next estrum. Blood plasma was separated and stored at -20°C for further analysis. The copper soap extraction method as per Shipe et al. (1980) was followed for the estimation of plasma non-esterified fatty acids (NEFA) and blood α -amino nitrogen levels were estimated as described by Goodwin (1970). Commercially available glucose estimation kit (Span Cogent Diagnostics Ltd., India) was used for the estimation of blood glucose, while 2, 4-DNPH -Reitman and Frankel method was used for the estimation of blood serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels. The status of minerals in feed, fodder, and blood was analyzed using Atomic Absorption Spectrophotometer (AAS). Plasma progesterone (P4) and estradiol (E2) levels in blood samples were estimated from the blood samples collected from 42 days prior to the onset of the first estrus, and on the day of estrus and thereafter on every alternate day till the onset of the next estrus in the animals of both control and treatment groups. Plasma estradiol and progesterone levels in heifers were estimated by ELISA kit (Cusabio Biotech Co. Ltd).

Statistical analysis

Two-way ANOVA was employed considering the effects of treatments (animals fed with ASMM and control animals) and effects of the fortnight in the model to evaluate the effect of ASMM on the selected parameters in this study. Level of significance level was fixed at $P < 0.05$. Analysis of data was done by SPSS software (version 16).

Results and Discussion

Effect of ASMM on growth performance

Feeding of ASMM did not have any significant ($P > 0.05$) influence on total dry matter intake (TDMI) between the groups (Table 3). The average initial body weights (kg) between the control (T0) and treatment (T1) groups was non-significant (while the final body weight differed significantly ($P < 0.05$)). Average daily gain (ADG) in T0 (0.367 ± 0.07 kg/day) and T1 groups (0.480 ± 0.05 kg/day) differed significantly ($P < 0.05$) in the study. The present findings suggest that trace minerals had a positive effect on ADG during the growth phase of animals. Similar to the present findings, Godara et al. (2016) and Satapathy et al. (2016) also observed that ASMM supplementation significantly affected ADG and body weight gain in livestock species. Sawant et al. (2013) reported an increased growth rate and body weight in indigenous heifers. The increased body weight gain of crossbred heifers in the ASMM supplemented group in this study might be due to better feed utilization efficiency. Sharma et al. (2011) also suggested that supplementation of area-specific mineral mixture improved the feed utilization efficiency in crossbred heifers.

Effect of ASMM on age on puberty

Table 1: Composition (per kg) of area specific mineral mixture

Minerals	Quantity
Calcium	259.6 g
Phosphorus	200.8 g
Zinc	5.29 g
Copper	2.58 g
Cobalt	270mg
Manganese	110mg

Table 2: Chemical composition of concentrate, green and paddy straw fed to the experimental animals on % dry matter basis

Parameter	Concentrate	Green fodder	Paddy straw
Dry Matter (DM)	93.78	14.8	91.56
Organic Matter (OM)	93.23	87.45	86.74
Crude protein (CP)	18.34	11.09	3.33
Ether Extract (EE)	4.42	2.39	1.18
Crude Fibre (CF)	5.67	26.4	41.2
Total Ash (TA)	6.19	11.8	12.25
Acid Insoluble Ash (AIA)	1.72	3.59	6.23
Neutral Detergent Fibre (NDF)	34.21	66.22	78.18
Acid Detergent Fibre (ADF)	13.18	43	51.77

The mean age at first estrus in Jersey crossbred heifers in the present study was 22.12 ± 0.13 and 20.10 ± 0.12 months in the T0 and T1 groups, respectively and it differed significantly ($P < 0.05$). The early onset of puberty in T1 group might be due to the higher body weight gain of animals in this group and it is well established that age at puberty is related to the body weight of the animal. Our findings were similar to that of Agrawalla et al. (2017) and Choudhary and Patel (2019). The onset of puberty was delayed due to deficiency of Copper (Cu) along with Cobalt (Co) as reported by Nix (2002). Cromwell (1997) also found that delayed sexual maturity when phosphorus intakes were low. Underwood and Suttle, (2003) reported that heifers with deficient copper level suffered with low fertility, delayed or suppressed estrus.

Effect of ASMM on blood parameters

Overall mean values of NEFA and AAN in the heifers did not show any significant difference between the groups ($P < 0.05$; Table 4). Similar to current observations, Godara et al. (2015) also did not find any significant difference in AAN levels between treatment and control groups of black Bengal goats supplemented with an area-specific mineral mixture. Hornick et al. (1998) reported elevated plasma AAN levels during growth. Blood glucose level was similar in both the control and ASMM supplemented groups (Table 4). Likewise, Ashry et al. (2012) and Behera et al. (2012)

reported that feeding mineral mixture had no significant effect on blood glucose concentration.

Effect of the ASMM on serum enzymes and mineral profile

The levels of plasma SGOT (93.44 ± 2.56 vs. 95.22 ± 2.36) and SGPT (20.41 ± 2.45 vs. 24.75 ± 3.01) were similar in both control and treatment groups ($P > 0.05$; Table 4) and its concentrations were within the normal value. Similarly, Pandey (2018), and Chaudhary and Patel (2019) also observed non-significant changes in serum enzyme concentrations with the supplementation of either commercial or ASMM. However, Sharma et al. (2011) reported elevated levels of SGOT and SGPT in the mineral-supplemented group as compared to the control group. The overall mean values of Ca, P, Cu and Zn were higher ($P < 0.05$) in the T1 group than T0 (Table 4) and the findings were supported by Choudhary and Patel, (2019), Niaz et al. (2017) and Agrawalla et al. (2017). The higher levels of Ca, P, Cu and Zn in the blood of animals in the supplemented group in the current study indicated that the bioavailability of these minerals in ASMM was optimum.

Effect of ASMM on hormonal profile

In the present study, the concentrations of plasma progesterone were at a basal level during the pre-pubertal periods prior to the onset of the first estrus in control and treatment group animals

Table 3: Growth performances of experimental animals

Attributes	Control	Treatment
Mean TDMI (kg/day)	5.69 ± 0.569	5.93 ± 0.345
Initial BW (Kg; d 0)	183.08 ± 14.82	187.25 ± 13.17
Final BW (Kg; d 120)	$225.08^a \pm 14.38$	$245.33^b \pm 15.37$
Total BW gain (Kg)	$42.0^a \pm 4.82$	$58.08^b \pm 6.42$
ADG (Kg/d)	$0.367^a \pm 0.07$	$0.480^b \pm 0.05$

a, b Means bearing different superscripts within a row differ significantly ($p < 0.05$)

TDMI= Total dry matter intake; BW= body weight; ADG= Average Daily weight gain

Table 4: Overall mean ($\pm$ SEM) blood profile of the experimental animals

Attributes	Control	Treatment
NEFA (μ mol/L)	107.97 ± 4.37	105.67 ± 5.49
α -amino nitrogen (mg/dl)	41.01 ± 1.97	42.59 ± 2.08
Glucose level (mg/dl)	76.89 ± 1.78	78.40 ± 1.78
SGOT (IU/L)	93.44 ± 2.56	95.22 ± 2.36
SGPT (IU/L)	20.41 ± 2.45	24.75 ± 3.01
Phosphorus (mg/dl)	$5.59^a \pm 0.33$	$5.88^b \pm 0.35$
Calcium (mg/dl)	$9.63^a \pm 0.57$	$9.85^b \pm 0.53$
Copper (ppm)	$0.56^a \pm 0.02$	$0.71^b \pm 0.02$
Zinc (ppm)	$0.59^a \pm 0.01$	$0.70^b \pm 0.02$

a, b Means bearing different superscripts within a row differ significantly ($p < 0.05$)

Table 5: Progesterone and Estradiol-17 β concentration during pre-pubertal and different phases of oestrus cycle in experimental heifers

Day	Progesterone (ng/ml)		Estradiol-17 β (pg/ml)	
	Control	Treatment	Control	Treatment
-42	0.19 $\pm$ 0.00	0.33 $\pm$ 0.03	1.63 $\pm$ 0.10	1.71 $\pm$ 0.18
-27	0.21 $\pm$ 0.01	0.4 $\pm$ 0.07	1.6 $\pm$ 0.12	1.77 $\pm$ 0.08
-12	0.28 $\pm$ 0.04	0.53 $\pm$ 0.02	1.81 $\pm$ 0.00	1.94 $\pm$ 0.11
0	0.19 $\pm$ 0.00	0.26 $\pm$ 0.03	7.3 $\pm$ 0.33	8.33 $\pm$ 0.63
2	0.37 $\pm$ 0.01	0.5 $\pm$ 0.02	7.05 $\pm$ 0.37	8.44 $\pm$ 0.11
4	0.44 $\pm$ 0.00	0.76 $\pm$ 0.04	3.72 $\pm$ 0.50	3.19 $\pm$ 0.25
6	0.65 $\pm$ 0.03	0.91 $\pm$ 0.04	2.26 $\pm$ 0.38	2.55 $\pm$ 0.20
8	0.87 $\pm$ 0.01	1.36 $\pm$ 0.06	1.75 $\pm$ 0.11	2.36 $\pm$ 0.13
10	1.37 $\pm$ 0.03	1.77 $\pm$ 0.06	1.77 $\pm$ 0.03	1.63 $\pm$ 0.14
12	1.85 $\pm$ 0.04	2.14 $\pm$ 0.10	1.87 $\pm$ 0.27	1.91 $\pm$ 0.30
14	1.2 $\pm$ 0.01	2.11 $\pm$ 0.20	2.05 $\pm$ 0.13	2.16 $\pm$ 0.38
16	0.86 $\pm$ 0.10	1.49 $\pm$ 0.18	1.74 $\pm$ 0.04	1.94 $\pm$ 0.27
18	0.53 $\pm$ 0.01	0.86 $\pm$ 0.13	1.98 $\pm$ 0.12	1.84 $\pm$ 0.28
0	0.27 $\pm$ 0.03	0.6 $\pm$ 0.08	5.94 $\pm$ 0.40	5.15 $\pm$ 0.24
2	0.47 $\pm$ 0.03	0.46 $\pm$ 0.05	2.37 $\pm$ 0.14	4.99 $\pm$ 0.19
6	0.66 $\pm$ 0.02	0.75 $\pm$ 0.04	2.41 $\pm$ 0.11	2.38 $\pm$ 0.11
8	0.93 $\pm$ 0.05	0.92 $\pm$ 0.04	2.46 $\pm$ 0.12	1.87 $\pm$ 0.11
10	1.02 $\pm$ 0.05	1.23 $\pm$ 0.03	1.24 $\pm$ 0.03	1.44 $\pm$ 0.05

(0.19 $\pm$ 0 vs. 0.33 $\pm$ 0.03 ng/ml). The blood progesterone levels were recorded to be the lowest on the day of estrus (day 0) in all animals irrespective of supplementation of ASMM to the animals. The concentrations of plasma progesterone showed an increasing trend above the basal level from day 6 after the estrus and increased gradually to register the peak level on day 12 of the oestrous cycle in both control and ASMM treated animals (Table 5). The blood progesterone concentrations declined thereafter to the lowest level again on the day of the next estrus in both control and treatment groups. However, no significant differences in the level of progesterone between treatment and control groups during different phases of the oestrus cycle were observed (Table 5).

Plasma estradiol-17 β concentrations were at basal level in both control (1.63 $\pm$ 0.1 pg/ml) and treatment groups (1.71 $\pm$ 0.18 pg/ml) during the pre-pubertal periods prior to the onset of the first estrus (Table 5). In both the groups, the peak concentrations of plasma estradiol-17 β were observed to occur on the day of estrus and the level had been sustained for 2 days post-onset of estrus and then decreased gradually to reach the basal level on 8th day of the estrous cycle in control animals and on 10th day in ASMM treated animals. Irrespective of the groups, plasma estradiol-17 β concentrations were then maintained at the basal level till day 18 of the estrous cycle and thereafter started increasing steeply to register the peak levels on day 22 which is the onset of the next estrus. Godara et al. (2016) and Kedare et al. (2020) reported increased progesterone and estrogen levels in black Bengal goats on supplementation of ASMM. Some evidence suggests that the activity of certain endocrine organs is affected by

Manganese. In cholesterol synthesis, manganese is involved as a cofactor that is necessary for the synthesis of steroids like estrogen, progesterone and testosterone (Kappel and Zidenberg 1999). The corpus luteum has high manganese content and it may be affected by the concentration of manganese supplementation from ASMM. Dutta et al. (2001) reported that lower zinc level was directly related to the reduction in steroid hormone concentrations in anestrus heifers. Bearden et al. (2004) reported that minerals have an important role in hormone action at the cellular level and reproductive performance was affected by its deficiency.

Conclusion

The present study revealed that dietary supplementation of ASMM had increased the level of mineral concentration in the blood. Further, it had beneficial effects on growth performance, improved the average daily gain and decreased the age at puberty in growing Jersey crossbred heifers.

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Effect of pre- and post-partum challenge feeding on performance and serum mineral levels of crossbred dairy cattle

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Abstract: The present work was designed to evaluate the effects of pre- and post-partum challenge feeding on the production performance and serum mineral levels of the crossbred dairy cows under farm conditions. The work was scheduled for 21 days pre-partum to 60 days post-partum. Fourteen dry pregnant crossbred cows were divided into two equal groups and were maintained either on control diet (CN) or were challenge fed (CF) with concentrates from 21 days pre-partum to 45 days post-partum and thereafter maintained on the same diet as CN. The dry matter intake was significantly ($p < 0.01$) higher in CF group when compared to CN group both during pre- and post-partum period. No significant difference was noticed in average daily milk yield, peak yield, days to attain peak yield, 3.5 % fat corrected milk yield, energy corrected milk, yield of fat, protein and lactose between control and challenge fed groups. The efficiency of feed conversion into energy corrected milk was poorer in the CF group. Serum calcium and magnesium concentration was significantly higher in CF group ($p < 0.01$ and $p < 0.05$, respectively) however serum phosphorus concentration was similar. The results from this study indicated that high plane of nutrition during pre- and post-partum transition period, extending up to 45 days post-partum, did not yield any benefit over and above the group fed on par with the requirements.

Keywords: Birth weight, Crossbred cattle, Challenge feeding, Milk production, Serum mineral level

Introduction

Transition phase (three weeks before and after parturition) is viewed as the most stressful time in the production cycle of a dairy cow. The primary challenge faced by the cow at this time is a sudden and marked increase of nutrient requirements for milk production with concomitant decrease in dry matter intake. To make up this deficit, energy dense rations are generally provided in early lactation which pre-disposes the animal to sub-acute ruminal acidosis and lactic acidosis. Prior adaptation of the rumen to these energy dense rations can mitigate the above risks. Hence priming the rumen of the animal by feeding energy dense rations at a higher level starting from the pre-partum transition period itself will help in increasing the absorptive capacity of rumen by elongation of rumen papillae (Dirksen et al. 1985) and will also help the rumen microflora to adapt to diets rich in fermentable carbohydrates. Increasing energy density of the pre-calving rations by increasing the concentration of non fibre carbohydrates has been associated with increased milk production (Minor et al. 1998). Also, Grummer (1995) suggested that pre-partum dry matter intake (DMI) was positively correlated with post-partum DMI and that pre-partum DMI should be maximized to improve post-partum performance and health.

Post-calving challenge feeding is practised with a motive to elicit the maximum production potential of the animal, by gradual increments in the concentrates fed, over and above the requirement of the animal. Animals maintained on a higher plane of nutrition (20% above NRC) from 60 days pre-partum to 120 days post-partum have been reported to produce 17.75 % more milk when compared to animals fed as per NRC feeding standards (Singh et al. 2003).

Various works have been reported on challenge feeding either during pre-partum period alone or during both pre- and post-partum period, but the results are not consistent. There is a lack of consensus on the period to which the animals shall be subjected to the higher plane of nutrition. Also, studies reporting the effect of challenge feeding on serum mineral levels are sparse. Hence the present work was undertaken to study the effect of challenge feeding starting from 21 days pre-partum to 45 days

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post-partum on the production performance and serum mineral levels of crossbred cows.

Materials and Methods

Fourteen crossbred cows and heifers in their last trimester of pregnancy were selected from the dairy herd of Livestock Research Station, Thiruvazhamkunnu, Kerala Veterinary and Animal Sciences University. These animals were divided into two groups of seven each, as uniformly as possible with regard to body weight, parity and 305 days previous lactation yield and were assigned randomly to one of the two feeding systems viz challenge feeding (CF) and control feeding (CN). The trial was scheduled from 21 days pre-partum to 60 days post-partum. The animals under CN group were fed as per the Package of Practices Recommendations 2016, Kerala Veterinary and Animal Sciences University and the animals under CF group were fed over and above the CN group. During the pre-partum period the animals under CF group were fed additionally with 250g of concentrates (compounded cattle feed in form of pellets manufactured at feed plant under Department of Animal Nutrition, Kerala Veterinary and Animal Sciences, University) per day up to 1% body weight. During the first 45 days post-partum the animals under CF group were fed additionally with 500g of concentrates per day up to free choice level. After 45 days post calving animals in both the groups were fed similarly as per the Package of Practices Recommendations 2016, Kerala Veterinary and Animal Sciences University. The total concentrate allocation was divided and fed twice a day. Roughage was provided exclusively as green grass (Hybrid Napier and Guinea Grass) and was offered thrice daily with ad libitum water. The animals were maintained under intensive system with uniform managerial practices.

The animals, based upon their expected date of calving, were included in the trial as and when available and hence a duration of five months was required to complete the trial. Though the animals were included 21 days prior to their expected date of calving, due to deviations in the actual date of calving the average number of days to which the animals were exposed to pre-partum challenge feeding were 19 and 18 in the CF and CN groups, respectively. Two weeks prior to the expected date of calving animals were shifted to calving pens. Immediately after parturition, the calves were separated from the dam and birth weights of calves were recorded before being fed with colostrum.

The cows were milked twice daily using milking machines and milk yield of each crossbred cow was recorded daily from 7th day of calving to 60th days in milk and the daily values were averaged into weekly means for statistical analysis.

Morning and afternoon milk samples of each animal were analyzed for concentrations of fat, protein and lactose, at weekly intervals, using Ekomilk Ultra pro milk analyzer (Milkana KAM 98-2A, Bulteh 2000 Ltd., Europe). Milk components were adjusted for the yield

of milk in each milking to calculate the daily yields of fat, protein and lactose. The 3.5% FCM (Fat corrected milk) yield was calculated as $[(0.4324 \times \text{milk yield}) + (16.218 \times \text{milk fat yield})]$ (NRC, 2001). The ECM (Energy corrected milk) yield was calculated as $[(0.3246 \times \text{milk yield}) + (12.86 \times \text{fat yield}) + (7.04 \times \text{protein yield})]$ (NRC, 2001).

Blood was collected on days 0, 7, 15, 30, 45, 60 following parturition; serum was separated and preserved for further analysis. Serum calcium, phosphorus and magnesium concentrations were determined on semi-automated clinical chemistry analyzer (CHEM- 7, Erba Mannheim, Germany) using standard kits (Agappe Diagnostics Ltd., Kerala, India).

Compounded cattle feed and fodder were analyzed at Animal Feed Analytical and Quality Assurance Laboratory, Namakkal (AOAC, 2012). Ca and P estimation was done as per AOAC 927.02 and 965.17, respectively.

Data were expressed as mean $\pm$ standard error of the mean (SEM) for the measurements of cows in each group. Statistical differences between the mean of groups were assessed using t-test.

Result and Discussion

Chemical compositions of the different feedstuffs offered to the animals are presented in Table 1.

In the pre-partum period the animals in the CF group consumed significantly higher ($p < 0.01$) quantity of concentrate, as compared to those in the control group resulting in significantly higher total daily dry matter intake (Table 2). Similarly, during post-partum period, the DMI from concentrates in CF group was significantly higher ($p < 0.01$) than the control group which resulted in significantly higher total DMI in CF group as the quantity of roughage offered to both the groups were similar. The DMI in post-partum period was higher than the pre-partum period. Garnsworth and Topps (1982) also reported linear increase in DMI postpartum up to 45 to 50 days in order to meet the escalating demands of nutrients for milk production.

Table 1: Chemical composition (per cent on dry matter basis) of feed stuffs.

Parameters	Compounded cattle feed	Green grass
Crude Protein	21.13	6.06
Crude fibre	8.71	37.79
Ether extract	3.78	2.13
Total ash	8.96	6.87
Nitrogen free extract	57.43	47.16
Calcium	1.32	0.53
Phosphorus	1.14	0.30

The birth weights of the calves in both the groups were statistically similar (Table 2). The results were in accordance with the results of Khan et al. (2002); Singh et al. (2003); Panigrahi et al. (2005) and Raval et al. (2019) who observed that birth weight of calves were not significantly influenced by the pre-partum plane of nutrition. However, Singh et al. (2019) reported a significant increase in birth weight of calves in correspondence with an increased level of pre-partum feeding.

The average daily milk yield, throughout the experimental period, did not differ significantly in both the groups (Table 3). Neither the pre-partum nor the post-partum challenge feeding could produce benefits in terms of milk production in the trial. This was in agreement with the results of Lodge et al. (1975) and Keady et al. (2001) who concluded that higher plane of nutrition during late gestation did not have any significant positive impact on milk production. Khan et al. (2002) reported that ad libitum feeding of crossbred cows during late pregnancy resulted in more body weight gain in pre-calving period and produced less milk in the ensuing lactation when compared to animals on restricted diet. However, Singh et al. (2003); Bindal (2012) and Raval et al. (2019) observed a significant increase in milk production due to higher pre- and post-partum feeding levels.

The different feeding regimes also couldn't impart any effect on the peak milk yield of the animals and it was statistically similar in the both the control and CF group (Table 3). This was in

accordance with the results of Bindal (2012) and Raval et al. (2019), as they reported that higher feeding regimes both during pre- and post-partum period did not influence the peak yield of the animal. However, Singh et al. (2003) could establish a positive relationship between level of nutrition and peak yield. They observed that peak yield was significantly higher in animals fed 20% above NRC feeding standards both in pre- and post-partum period when compared to their counterparts which were fed either as per NRC feeding standards or 20% above NRC only during the pre-partum period.

The days to attain peak yield was longer in CF group when compared to control but the difference was statistically non-significant (Table 3). Similar results were reported by Kale (1984) and Bhat et al. (2000) who also couldn't observe any significant effect of feeding different concentrate regimes on days to attain the peak yield. However, Singh et al. (2003); Bindal (2012) and Raval et al. (2019) reported that the animals in higher plane of nutrition or the challenge fed group took significantly more time to achieve the peak yield.

Throughout the experimental period the yield of milk fat, protein and lactose were similar in both the groups (Table 3). The results were in congruence with the results of Das et al. (2007); Bindal (2012) and Raval et al. (2019) who also couldn't observe any significant effect of level of feeding on quality of milk. However, Bhat et al. (2000) and Singh et al. (2003) interpreted that higher

Table 2: Effect of challenge feeding on DM intake and calf birth wt.

Parameters	CF ² group	CN ¹ group
Pre-partum		
Total DMI (kg/day)	10.47 ^a ± 0.29	8.24 ^b ± 0.17
Concentrate DMI (kg/day)	5.22 ^a ± 0.29	2.99 ^b ± 0.17
Post-partum		
Total DMI (kg/day)	17.65 ^a ± 0.62	13.36 ^b ± 0.26
Concentrate DMI (kg/day)	11.35 ^a ± 0.62	7.06 ^b ± 0.26
Birth weight of calf (kg)	28.2 ± 0.70	28.8 ± 1.60

Means bearing different superscripts in the same row differ significantly ($p < 0.01$); ¹CN: control group, ²CF: challenge fed group

Table 3: Effect of challenge feeding on milk production

Parameters	CF ² group	CN ¹ group
Milk yield (kg/day)	12.86 ± 0.39	12.90 ± 0.41
Mean peak yield (kg)	15.14 ± 1.02	15.14 ± 1.24
Days to attain peak yield	28.14 ± 5.47	23.14 ± 4.03
Milk fat (kg/day)	0.45 ± 0.02	0.48 ± 0.04
Milk protein (kg/day)	0.41 ± 0.03	0.40 ± 0.04
Lactose (kg/day)	0.55 ± 0.04	0.55 ± 0.04
3.5% FCM ³ (kg/day)	12.77 ± 0.78	13.26 ± 1.19
ECM ⁴ (kg/day)	12.84 ± 0.82	12.98 ± 1.23
ECM/DMI	0.73 ^a ± 0.05	0.97 ^b ± 0.08

Means bearing different superscripts in the same row differ significantly ($p < 0.05$)

¹CN: control group, ²CF: challenge fed group, ³FCM: fat corrected milk,

⁴ECM: energy corrected milk.

Table 4: Effect of challenge feeding on serum mineral levels

Parameters	CF ² Group	CN ¹ Group
**Serum calcium (mg/dl)	10.27 ^a ±0.48	8.01 ^b ±0.21
Serum inorganic phosphorus (mg/dl)	7.34±0.50	6.39±0.24
*Serum magnesium (mg/dl)	2.78 ^a ±0.16	2.25 ^b ±0.09

Means bearing different superscripts in the same row differ significantly; ** (p<0.01), * (p<0.05); ¹CN: control group, ²CF: challenge fed group

level of feeding during pre-partum period alone or during both pre- and post-partum period improved the quality of milk significantly. The yield of 3.5 % fat corrected milk (FCM) and energy corrected milk (ECM) did not differ significantly among the groups as both the quantity and the quality of milk produced in both the groups were statistically similar. The efficiency of feed conversion into ECM was poorer in the CF group and the result was statistically significant (p<0.05). This was the sequel of significantly higher DMI in CF group when compared to control group.

Serum calcium concentrations (Table 4) in both the groups were in the normal range of 8-10 mg/dl, below which it leads to subclinical hypocalcemia (Houe et al. 2001). The calcium concentration was significantly higher (p<0.01) in CF group when compared to the control group which might be due to higher DMI in the CF group. Serum inorganic phosphorus concentrations were higher in CF group when compared to CN group but the difference was statistically insignificant. However, the serum phosphorus concentrations were in the normal range of 4 to 8 mg/dl (NRC, 2001) in both the groups. Serum magnesium concentration in the challenge fed group was slightly higher than the normal concentration of 1.8 to 2.4 mg/dl (NRC, 2001) and was significantly higher (p<0.05) than the control group. This might be due to the fact that the CF group had higher DMI from concentrates and availability of magnesium is reported to be more from concentrates when compared to forages as diets rich in concentrates reduces the pH below 6.5 which increases the solubility of magnesium and hence subsequently increases its absorption (NRC, 2001).

Conclusion

The results from this study conclusively revealed that high plane of nutrition during pre- and post-partum transition period extending up to 45 days post calving did not yield any benefit over and above the group fed on par with the requirement except for serum mineral levels which was on the higher side in CF group. Also, the feed conversion efficiency to energy corrected milk was poorer in the animals on high plane of nutrition. Hence, further research is warranted in this area to find out the appropriate period of exposure to pre-partum challenge feeding so that its benefit may be reflected in the post-partum period.

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RESEARCH ARTICLE

Export and import of Indian dairy products: An assessment

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Abstract: A paradigm shift has occurred in livestock sector after 1985 and India become a net export in livestock products. The economic policy reforms in India have further widened the market opportunities for the livestock sector. The study spans over a period from 1990-91 to 2020-21 on export and imports of livestock products to analyze the trend and variations. The results of the study infer that in milk and milk products India has some advantage at the farm level, but is not competitive in the export of milk and milk products at the prevailing world market situation. To sustain and tap the benefits of expanding global dairy trade, there is urgent need to broaden surplus milk base for efficient value addition. To put in place a dairy commodity market outlook mechanism to provide a continuum to the policy thrust to enable Indian dairy sector to compete in international market.

Keywords: Dairy products, Diversification, Indian Dairy Sector, Export, Import, Instability Analysis

JEL Classification: Q11, Q17

Introduction

The Indian livestock sector is on a rising spree with its current contribution of 25.6 per cent to the Agricultural Gross Domestic Product (Ag GDP), 4.11 per cent to the national Gross Domestic product in the year 2018-19 and providing employment to 8.8 per

cent of the Indian population, particularly to women folk, in principal or subsidiary status (Vikaspedia,2020). It possesses the biggest flock of cattle and buffaloes (16.1% and 57.9% of the world population, respectively), second largest flock of goats (16.7%), and third highest number of sheep (5.7%) in the world (GOI, 20th Livestock Census,2019). The fast growing economy coupled with ever increasing urbanization, changing food habits etc has resulted in changing pattern of food consumption, including creation of an unprecedented demand for livestock products, not only in the urban areas but among rural communities also. The economic policy reforms triggered in 1991 were reoriented towards liberalization and integration with the world economy and widened the market opportunities for the livestock sector. Sustained economic growth and rising incomes during the past two decades have been fuelling a rapid growth in the demand for livestock products. Consequently, the livestock sector has emerged as one of the important drivers of agricultural growth and income improvement in India. The rising global demand for livestock products, various global trade negotiations and domestic reforms in India, have improved the access to international markets substantially, particularly during the post-WTO period. Such developments offer an opportunity to India to increase its exports, especially for livestock products like bovine meat whose domestic demand is low. With improved domestic production and marketing efficiency, India has the potential to better access the expanding world market, and augment export of livestock products (Kumar, 2010). On the other side, apprehensions are being raised about the ability of Indian livestock farmers, a majority of whom are small and marginal, in taking the advantage of emerging opportunities, under the liberalized trade scenario. Also, non-tariff barriers like stringent sanitary and phyto-sanitary (SPS) standards, technical barriers to trade (TBT), anti-dumping duties, countervailing duties, etc. are emerging as the major challenges in tapping the benefits of export potential of the livestock.

To meet the challenges of globalization the key lies in continuous generation and dissemination of technologies that improve production efficiency of livestock and post harvest handling (Birthal et al. 2004). After signing the Uruguay Round of Agreement in, 1994, most of the restrictions on both exports and imports of agricultural produce were gradually relaxed.

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Agricultural exports and imports (which encompass livestock products also) were regulated through quantitative restrictions, such as quotas and licenses were channeled through a state trading organization or some combination of both till early-1990s (Nayyar and Sen, 1994).

Thus, a deeper understanding of the export and import performance of livestock sector in India would contribute towards building the development strategy of this sector. Further, the price trends of livestock products have significant implications on export competitiveness of livestock products and thus need a detailed study.

Methodology

The data has been compiled from various sources for the period from 1990-91 to 2020-21 so as to analyze the trend and variations. Basically two data sets have been used in the analysis. The data on National exports and imports of dairy products and total merchandise trade were compiled from the official website of Ministry of Commerce, Government of India. The data on GDP, Agricultural GDP and Livestock GDP were culled from the National Accounts Statistics, published by Central Statistics Organization (CSO), Government of India.

All the values of exports and imports in terms of US dollars were used to analyze the performance of exports and imports of various dairy products to net out the effect of fluctuations in exchange rates.

Diversification in Export and Import of Indian Dairy Products

The commodity diversification indices of dairy exports and imports have been calculated by the widely-used Gini-Hirschman Coefficient of concentration using the following formula:

$$C_{xt} = 100 - 100 * \left[\frac{(X_{it} / X_t)^2}{\sum (X_{it} / X_t)^2} \right]$$

where, C_{xt} is the diversification coefficient for exports and imports in the year t ; X_{it} is the value of exports and imports of commodity i in the year t ; and X_t is the total dairy exports/imports in the year t .

Measurement of Export and Import Instability

Various statistical measures have been suggested to calculate instability index in the literature on economics. Each of these methods has its relative strengths and weaknesses and there is no consensus as to what constitutes the most appropriate method for measurement of instability.

The present study has used the ratio method to assess the import and export instabilities.

Instability Index of export - import (I_X) = Standard Deviation of $\log (X_t/X_{t-1}) * 100$

Where, X_t is the export or import value in the year t and X_{t-1} is the export or import value in year $t-1$.

It is an unit free measure of instability and represents deviations from the trend growth line.

Harmonized System (HS) Codes for Dairy Products

The Harmonized System is a standardized numerical method of classifying traded products. It is used by customs authorities around the world to identify products when assessing duties and taxes and for gathering statistics. The HS assigns specific six-digit codes for varying classifications and commodities. Countries are allowed to add longer codes to the first six digits for further classification. The dairy products are broadly classified into six broad categories as specified below:

Results and Discussion

The destinations and status quo of international trade in dairy products

The destinations for trade are determined by several factors including geographical and political proximity, differences in comparative advantage, and degree of trade barriers. To identify major trading partners of India in the trade of livestock products, top ten importers and exporters of dairy products have been listed in Tables 2 and 3 respectively based on the size of their average trade for the past three years (2018-19, 2019-20, 2020-2021).

Export Destinations

The top ten countries accounted for nearly 83.42 per cent of total dairy exports of India in the year 2020-2021. The three year average reveals that about 62.79 per cent of total dairy exports of India is accounted by the top 10 importing countries. It has also been revealed that the share of top ten importing countries has increased in recent years. Table 2. shows that, on the three year average basis, UAE has been the largest importer of dairy products from India, accounting for about 16.34 per cent of the total value of exports followed by Bhutan (8.95 per cent) and Bangladesh (8.65 per cent). The United States of America, Singapore, Saudi Arabia, Malaysia,

Table1: HS Code classification dairy products in India

Product Code	Category of dairy product
0401	Milk and cream, not concentrated or sweetened
0402	Milk and cream, concentrated or sweetened
0403	Buttermilk, yogurt, kephir etc; flavored etc; or not
0404	Whey and milk products nesoi, flavored etc; or not
0405	Butter and other fats and oils derived from milk
0406	Cheese and Curd

Qatar, Oman and Australia have remained the major importers of dairy products from India with their varying shares in different time periods.

The diversification in export destinations seems to have been strengthened by the improved market access as a result of liberal trade policies adopted by several importing countries in the post-WTO period. The share of individual countries in total export of livestock products from India underwent a sea change. For instance UAE was the biggest importer of livestock products of India, accounting for 34 - 40 per cent till 1985, but thereafter its share started declining and in 2007 it reached a level of 9 per cent only. Similarly, Malaysia was not a significant importer of Indian livestock products during early-1980s, but emerged as its largest importer in the late-1980s and continued to retain its position till 2007, though its share too has declined in recent years (Kumar, 2009).

Import Destinations

A perusal of list of top ten countries exporting livestock products to India in the year 2020-2021 has been given in Table 3 which reveals that though their share has declined over the years, they still account for about 87.11 per cent of India's livestock import. It may be mentioned that import of livestock products has declined substantially over the years. From Table 3, it has also been revealed that, we imported about 31.55 per cent of total value of dairy products in 2020-2021 from France followed by New Zealand at 17.01 per cent and Netherlands at 10.71 per cent. These three countries together contribute to more than 50 per cent of India's total dairy product imports. The three year average reveals that about 74.78 per cent of total dairy imports to India is accounted by the top 10 importing countries. As few of the highest exporting countries to India in terms of value of dairy products, the share of France, Germany and Netherlands have been constant with reference

Table 2: Major Importing Countries of Indian dairy products

Importing Countries	2018-19 (US \$ Million)	2019-20 (US \$ Million)	2020-21 (US \$ Million)	Three Year Average (US \$ Million)
United Arab Emirates (UAE)	43.6(12.61)	36.94(19.78)	39.34(19.54)	39.96(16.34)
Bhutan	20.72(5.99)	22.46(12.03)	22.52(11.18)	21.9(8.95)
Bangladesh	38.19(11.05)	1.12(0.60)	24.13(11.98)	21.15(8.65)
United States of America	14.98(4.33)	14.29(7.65)	22.8(11.32)	17.36 (7.10)
Singapore	10.75(3.11)	11.44(6.13)	15.27(7.58)	12.49(5.10)
Saudi Arabia	13.55(3.92)	11.81(6.32)	11.47(5.70)	12.28(5.02)
Malaysia	13.39(3.87)	1.44(0.77)	8.67(4.31)	7.83(3.20)
Qatar	7.63(2.21)	5.62(3.01)	8.49(4.22)	7.25(2.96)
Oman	6.59(1.91)	6.09(3.26)	7.46(3.70)	6.71(2.74)
Australia	6.9(2.00)	5.26(2.82)	7.84(3.89)	6.67 (2.73)
Top 10 Total	176.3(50.99)	116.47(62.37)	167.99(83.42)	153.59(62.79)
Other Countries	169.43(49.01)	70.26(37.63)	33.38(16.58)	91.02(37.21)
Total	345.73(100.00)	186.73(100.00)	201.37(100.00)	244.61(100.00)

*Figures in the parentheses indicate percentages

So : APEDA, 2020

Table 3: Major Dairy Exporting Countries to India

Importing Countries	2018-19 (US \$ Million)	2019-20 (US \$ Million)	2020-21 (US \$ Million)	Three Year Average (US \$ Million)
France	10.39(28.52)	14.93(28.62)	15.43(31.55)	13.58(29.63)
New Zealand	0.99(2.72)	6.17(11.83)	8.32(17.01)	5.16(11.26)
Netherlands	3.46(9.50)	4.37(8.38)	5.24(10.71)	4.36(9.50)
Germany	2.48(6.81)	3.18(6.10)	2.83(5.79)	2.83(6.17)
United Kingdom	2.17(5.96)	2.01(3.85)	1.24(2.54)	1.81(3.94)
Denmark	2.00(5.49)	1.96(3.76)	1.30(2.66)	1.75(3.83)
Turkey	0.06(0.16)	1.97(3.78)	2.38(4.87)	1.47(3.21)
Uganda	0.07(0.19)	1.26(2.42)	2.10(4.29)	1.14(2.49)
Norway	1.25(3.43)	0.79(1.51)	1.32(2.70)	1.12(2.44)
Argentina	0.15(0.41)	0.57(1.09)	2.44(4.99)	1.05(2.30)
Top 10 Total	23.02(63.19)	37.21(71.32)	42.60(87.10)	34.28(74.78)
Other Countries	13.41(36.81)	14.96(28.68)	6.31(12.90)	11.56(25.22)
Total	36.43(100.00)	52.17(100.00)	48.91(100.00)	45.84(100.00)

*Figures in the parentheses indicate percentages

So : APEDA, 2020

to the past three years where as the share of New Zealand has shown considerable leaps.

Country-wise trade has revealed year-to-year variations in the volume of trade with India. One of the disquieting features of export destinations of livestock export is that India has not been able to make a significant dent in export to the developed countries, where it can realize a higher per unit value. Its exports have been confined largely to the neighboring South Asian, East Asian and Middle East countries. Its major trading partners for importing of dairy products have been changing from time to time but France, New Zealand, Bangladesh and Germany have remained the major exporters of dairy products to India with their varying shares in different time periods.

Size and composition of India's Dairy Trade

The dairy products include whole milk powder, skimmed milk powder, butter, cheese, curd and whey along with some other milk products. Earlier, butter, ghee and other fats and baby foods constituted the major share in exports of dairy products. Though, India was a net importer of dairy products till 2000, it has turned out to be a net exporter in the subsequent period. The export of dairy products gained momentum after 1991 due to economic liberalization and a significant rise in milk processing facilities which has resulted in higher export of dairy products. Dry milk has emerged as the largest constituent of dairy products exports. The processed cheese products are also slowly finding their way into the export markets. The major impetus to exports of dairy

products had come after the removal of quantitative restrictions, which motivated the exporters to tap the emerging opportunities in the global market. Sincere efforts by the government and exporters to comply with the SPS standards also seemed to have promoted the export of these commodities.

Looking to post-liberalization era, the dairy exports have registered a commendable rise during the entire study span (1990-91 to 2020-21). The average annual dairy exports have increased remarkably from US \$ 1.39 million in QE 1990-91 to US \$ 201.37 million in QE 2020-21. The product group 402, 405 and 406 have shown promising signs during this period. Whey and related products (product group 404) are a bit newer products which have shown their contribution since 2000-01. The total imports of dairy products over the period 1990-2020 have been depicted in Table 5. The imports have fallen drastically from US \$ 178.77 million in QE 2010-11 to US \$ 31.39 million in QE 2020-21. The import of product group 401 has started only after 2000-01 which is on a continuous rise. The import of dairy products of the group 402 has been on a decline during entire period of post-liberalization era where as that of group 406 has been on a continuous increase. The import of other dairy products has been erratic.

Diversification in export and import of Indian dairy products

The extent of diversification in the exports as commodity diversification and geographic diversification has shown a mixed trend. The commodity import diversification index fluctuated between low of 39.91 per cent (QE 1990-91) and high of 48.54 per cent in QE 2020-21. A moderate diversity among the imported dairy

Table 4: India's Dairy Exports (\$ million)

	Year -Quinquennium Ending(QE)						
HSCode	1990-91	1995-96	2000-01	2005-06	2010-11	2015-16	2020-2021
401	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.30(0.85)	2.25(1.87)	3.25(2.82)	8.60(4.27)
402	0.57(41.01)	5.83(74.17)	15.75(95.05)	126.82(82.98)	52.68(43.82)	48.91(42.44)	48.18(23.93)
403	0.02(1.44)	0.05(0.64)	0.02(0.12)	0.25(0.16)	0.88(0.73)	0.55(0.48)	1.77(0.88)
404	0.00(0.00)	0.00(0.00)	0.65(3.92)	3.73(2.44)	2.34(1.95)	0.06(0.05)	0.62(0.31)
405	0.74(53.24)	1.98(25.19)	0.00(0.00)	18.40(12.04)	53.95(44.88)	42.37(36.76)	102.17(50.74)
406	0.06(4.32)	0.00(0.00)	0.15(0.91)	2.33(1.52)	8.11(6.75)	20.11(17.45)	40.03(19.88)
Total	1.39	7.86	16.57	152.83	120.21	115.25	201.37

(Source : *agriexchange.apeda*) *Figures in the parentheses indicate percentages

Table 5: India's Dairy Imports (\$ million)

Year -Quinquennium Ending(QE)							
HS Code	1990-91	1995-96	2000-01	2005-06	2010-11	2015-16	2020-21
401	0.00(0.00)	0.00(0.00)	0.10(0.48)	0.02(0.13)	0.14(0.08)	0.53(1.08)	0.66(2.10)
402	2.03(96.67)	6.04(52.89)	9.36(45.11)	6.57(41.66)	93.67(52.40)	3.81(7.77)	1.54(4.91)
403	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.09(0.57)	0.53(0.30)	0.07(0.14)	0.12(0.38)
404	0.00(0.00)	0.04(0.35)	0.32(1.54)	1.82(11.54)	12.52(7.00)	21.63(44.11)	21.55(68.65)
405	0.04(1.90)	5.18(45.36)	10.39(50.07)	5.07(32.15)	64.56(36.11)	15.05(30.69)	3.1(9.88)
406	0.03(1.43)	0.16(1.40)	0.58(2.80)	2.2(13.95)	7.35(4.11)	7.95(16.21)	4.42(14.08)
Total	2.10	11.42	20.75	15.77	178.77	49.04	31.39

(Source : *agriexchange.apeda*) *Figures in the parentheses indicate percentages

Table 6: Diversification in export and import of dairy products: 1990-2020

Year(QE)	Commodity Diversification Index	
	Imports	Exports
1990-91	39.91	53.95
1995-96	44.98	43.65
2000-01	42.34	47.35
2005-06	45.53	41.08
2010-11	44.14	50.98
2015-16	45.86	42.49
2020-21	48.54	46.32

Source : Authors' calculations based on APEDA,2020

commodities has been observed though it shows consistent increasing tendency which indicates that a wider range of dairy products are being preferred among imported products and it is increasing over the years. On the other hand, clear trend has been observed in the exports of dairy products. The diversity coefficient is between 46.32 percent (2020-21) to 53.95 per cent in QE 1990-91. The imports have become more diversified after liberalization where as mixed trend was observed in the exports. It is evident from the analysis that the trade in dairy products is concentrated within a few commodities only and in case of exports, it has shown a tendency of getting specialized over time.

Export and Import Instability Analysis

In general, the exports of dairy products have been much volatile during the last many years. The exports of product group 403 had the highest instability followed by product group 404 (write name of product and in bracket group code)), 402,401 and 405. Product group 406 was found to have the least volatility in their exports. Again, no clear pattern of volatility emerged during either the post or pre-reform period with the only exception that exports of product group 406 were highly volatile before the liberalization period which has declined in subsequent years continuously.

The imports of product group 402 was found to have the highest instability followed by product groups 405,403,404 and 401. Product group 406 was having least volatility. The imports exhibit a pattern of volatility. In almost all the product groups, the volatility in their imports has increased post liberalization with the exception that imports of product group 406 were highly volatile before the liberalization period which has declined in subsequent years. It has least instability in the current decade. As inferred from Table 7 and Table 8, both the import and export instability of product group 406 has been declining over the years, it could imply that production of product group 406 needs to be specialized and strengthened in order to maximize revenue returns.

Conclusions

India from a net importer of livestock products till 1985, has become a net exporter post-1985, indicating the export potential of Indian livestock sector. In general, the prices of dairy products were suppressed during the 1990s and started

Table 7: Export Instability Indices for Dairy Products: 1991-2020

Product Group	Period						
	1991-1995	1996-2000	2001-2005	2006-2010	2010-2015	2016-2020	All 1991 - 2020
401					82.65	112.43	82.65
402	189.23	119.09	94.82	68.61	318.36	303.65	158.02
403				197.47	144.87	138.02	171.17
404				145.25	165.08	161.98	155.16
405	37.81	64.45	23.24	89.74	48.33	74.56	52.72
406			60.38	66.59	21.97	44.32	49.65

Source : Authors' calculations based on APEDA,2020

Table 8: Import Instability Indices for Dairy Products: 1990-2020

Product Group	Period						
	1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	All 1991-2020
401				20.44	35.47	37.49	31.13
402	126.37	192.51	240.88	33.68	253.59	147.35	165.73
403				45.22	53.13	61.44	53.26
404				28.04	39.01	33.97	33.67
405	358.93	186.22	65.03	183.12	123.50	49.12	161.00
406		57.62	13.46	24.58	16.94	18.58	22.84

Source : Authors' calculations based on APEDA,2020

firming up particularly after 2000. During the past two years, the dairy prices have witnessed a tremendous increase. In milk and milk products, India has some advantage at the farm level, but is not competitive in the export of milk and milk products at the prevailing world market situation. The improvement in efficiency of the processing of dairy products along with reduction in support to dairy industry in the developed countries only can increase the prospects of dairy exports from India. The long run desirable path for India dairy sector is that revenue of the product should be increasing and instability (risk) in revenue stream needs to be falling. By and large, India has become self-reliant in milk production and is able to generate some export surpluses. In the world trade of dairy products, it is still a very small player. Further, it seems that domestic policy initiatives and increased production and productivity are the important factors in increasing the export of dairy products. Strengthening of export supply capacity domestically holds the key for enhancing export of dairy products rather than expanding world market. The generation of adequate exportable surplus accompanied with demand creation for specific products would enable India to tap the benefits of expanding global dairy trade. A long-term outlook for export of dairy products should be developed, which can provide a continuum to the policy thrust.

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Knowledge and adoption of scientific dairy farming practices among the women beneficiaries of Self Help Group promoting institutes

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Abstract: The current study was conducted in Ludhiana district of Punjab to assess the knowledge and adoption level of scientific dairy farming practices (SLFPs) among the women beneficiaries of Self Help Group Promoting Institutes (SHGPIs) viz., dairy cooperative, government and Non-Government Organization (NGO). Women through dairy based Self-help Groups have involved themselves in different types of income generating activities. About 40 respondents from each SHGPI were selected randomly, thus total 120 respondents were subjected to structured interview schedule during the period of September 2018 to June 2019. The findings revealed that most of the beneficiaries of dairy cooperative SHGs had high level of knowledge (45.00%) and adoption (52.50%) about SLFPs followed by medium level among beneficiaries of NGO regarding knowledge (62.50%) and adoption (45.00%). The mean difference in knowledge and adoption between beneficiaries of different SHGPIs was found statistically significant ($P < 0.01$). In case of overall knowledge and adoption about SLFPs, significant difference was observed among the beneficiaries of dairy cooperative, government and NGO based SHGs. Beneficiaries of dairy cooperative SHGs had relatively better knowledge and adoption for scientific dairy farming practices with high mean value as compared to beneficiaries of

government and NGO based SHGs. This might be due to regular and more frequent contact of beneficiaries of dairy cooperative SHGs with veterinary officers as compared to the beneficiaries of government and NGO. The knowledge level of the women beneficiaries involved in dairy farming is of paramount importance in boosting productivity of their milch animals and generating income. SHGs promoting institutes (SHGPIs) had made a significant positive impact in promoting scientific dairy practices among the women beneficiaries of study area.

Keywords: Dairy cooperative, Non-Government Organization, Scientific dairy farming practices, Self help group promoting institutes, Women

Introduction

Women constitute 48.5 per cent (586.47 million) of total population (1210.19 million) in India (Government of India census, 2011). Most of the animals farming activities are performed by women. Their involvement in livestock sector does not get any consideration mainly due to existing socio-cultural perceptions in the society (Ponnusamy et al. 2017). Women accounted for 93.00 per cent of total employment in dairy production (Qureshi et al. 2016). About 69.00 per cent of the work force on livestock farms is provided by women (Patel et al. 2016). FAO's Report 2011 reveals that, if women farmers had the same access to productive resources as men, they could increase yields on their farms by 20 to 30 percent, in turn decreasing the number of hungry people in the world by 12 to 17 percent.

Women farmers could get better returns on developing value chain through Self Help Groups (SHGs). There are 1.02 crore groups under SHG-Bank Linkage Programme covering 12.4 crore households across India and in Punjab, there are around 28934 SHGs and more than 85 percent are exclusively women SHGs (Govt. of India NABARD 2019-20). Various income generating activities like dairy farming, poultry farming, piggery farming, goat farming, value addition etc. are carried out by women through dairy based Self-help Groups. Capacity building programmes organized by various Self Help Group Promoting Institutions (SHGPIs) involved in animal husbandry help in enhancing the knowledge and skills of the SHG beneficiaries. These groups act

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as resourceful means of technology transfer and considerably contribute in value addition of livestock products. To measure the extent to which different Self Help Group Promoting Institutions (SHGPIs) has helped the rural women in improving their knowledge and adoption regarding scientific dairy farming practices, the current study evaluated and compared the knowledge and adoption level of scientific dairy farming practices among the women beneficiaries of dairy based Self Help Groups promoted by Self Help Group Promoting Institutions.

Material and Methods

The current study was conducted in Ludhiana district of Punjab to assess the knowledge and adoption level of scientific dairy farming practices (SLFPs) among the women beneficiaries of Self Help Group Promoting Institutes (SHGPIs) viz., dairy cooperative, government and NGO. Ludhiana district was purposively selected based on large number of dairy based functional women Self Help Groups. A multistage random sampling procedure was applied. Twenty four (24) SHGs i.e. Eight (08) from each SHGPIs viz., dairy cooperative, government and NGO; were selected randomly which were successfully functioning for more than 3 years in dairy farming activities in various villages of different blocks of Ludhiana district of Punjab, India. Five (05) SHG women members were selected randomly from each SHG, which make the 120 respondents i.e. Forty (40) from each SHGPIs viz., dairy cooperative, government and NGO. The related information was collected through a set of structured interview schedule.

Measurements

To measure the knowledge level of the respondents, knowledge test already developed by Sah (2005) was used. The knowledge test was administered to the respondents and responses were obtained under multiple choice questions. The scores assigned to the responses were in the range of 0-3. Scoring was done

according to the correctness of the response against each item. Knowledge score of individuals in different aspects such as breeding (maximum score 15), feeding (maximum score 13), healthcare (maximum score 11) and management (maximum score 15) were obtained by summing up scores of each item under different aspects. The total score obtained by individual respondent for all the statements was calculated and analyzed with the help of mean and standard deviation and respondents were categorized as low (Below mean – Half S.D.), medium (mean $\pm$ Half S.D.) and high (Above mean + Half S.D.) with respect to their knowledge level. Mean differences for knowledge and adoption of scientific dairy farming practices (SLFPs) viz. breeding, feeding, health care and management; were calculated with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to differentiate between the beneficiaries of different Self Help Group Promoting Institutes (SHGPIs) viz., dairy cooperative, government and NGO.

Results and Discussion

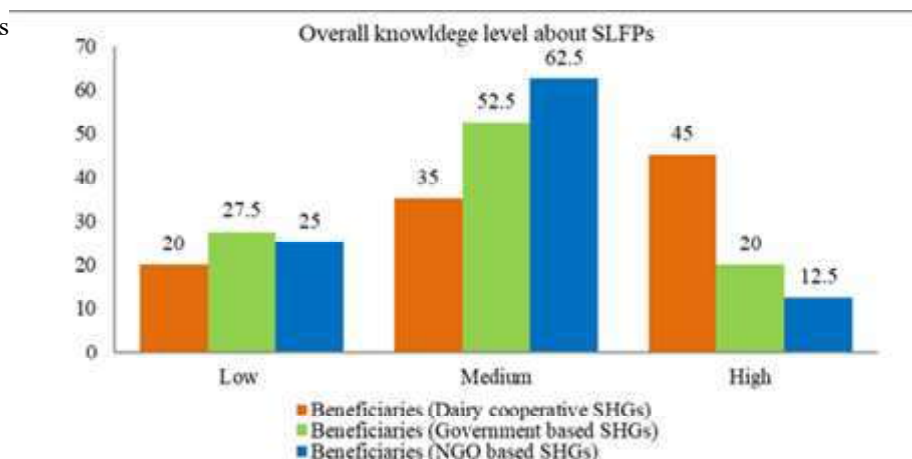
Knowledge of scientific dairy farming practices (SLFPs) among beneficiaries of SHGPIs

Most of the beneficiaries (45.00% and 47.50%) belonged to dairy cooperative SHGs possessed high level of knowledge for scientific breeding and health care practices respectively. However among government and NGO based SHGs, majority of beneficiaries were observed under medium category of knowledge (Table 1). The MILKFED Punjab came into existence in 1973 with a twin objective of providing remunerative milk market to the milk producers in the State and to provide technical inputs to the milk producers for enhancement of milk production on the other hand. High knowledge for scientific breeding and health care practices among beneficiaries of dairy cooperative SHGs pertains to the employment of veterinarians and trained personnel by dairy cooperative to ensure that every member obtains

Table 1: Distribution of beneficiaries on the basis of knowledge about SLFPs

Sr. No.	Aspects	Categories	Beneficiaries		
			Dairy cooperative SHGs (n=40)	Government SHGs (n=40)	NGO based SHGs (n=40)
1.	Breeding Practices	Low (<8)	05 (12.50)	06 (15.00)	11 (27.50)
		Medium (8-10)	17 (42.50)	30 (75.00)	23 (57.50)
		High (>10)	18 (45.00)	04 (10.00)	06 (15.00)
2.	Feeding Practices	Low (<8)	04 (10.00)	09 (22.50)	05 (12.50)
		Medium (8-10)	26 (65.00)	31 (77.50)	35 (87.50)
		High (>10)	10 (25.00)	0 (0.00)	0 (0.00)
3.	Health Care Practices	Low (<5)	07 (17.50)	13 (32.50)	15 (37.50)
		Medium (5-6)	14 (35.00)	22 (55.00)	20 (50.00)
		High (>6)	19 (47.50)	05 (12.50)	05 (12.50)
4.	Management Practices	Low (<11)	04 (10.00)	10 (25.00)	10 (25.00)
		Medium (11-12)	34 (85.00)	26 (65.00)	21 (52.50)
		High (>12)	02 (5.00)	04 (10.00)	09 (22.50)

(Figures in parenthesis indicate percentage)

Fig 1. Overall knowledge level of SLFPs among beneficiaries of different SHGPIs**Table 2.** Association of knowledge about scientific dairy farming practices among beneficiaries of SHGPIs (F Value)

Source	Character	Degree of freedom	Sum of Squares	Mean sum of Squares	F Value
Beneficiaries (Dairy Cooperative, government and NGO based SHGs)	Breeding Practices	2	8.87	4.43	1.55
	Feeding Practices	2	23.02	11.51	8.71**
	Health care Practices	2	27.95	13.98	7.35**
	Management Practices	2	0.87	0.43	0.19

veterinary aid/service at the doorstep for their animals. Another reason of high knowledge could be the activities such as animal health campaign, preventive vaccination, pregnancy diagnosis, treatment of infertility, timely artificial insemination, supply of inputs to their members, training opportunities etc. undertaken by dairy cooperatives officials on regular basis in the study area. Birchall and Simmons (2009); and Gasanga (2011) also reported the similar findings that cooperative helped members access services such as subsidized AI services, veterinary services, training opportunities and inputs supply, leading to improved productivity of the animals. On the other hand inadequate numbers of staff for regular follow up of SHGs and overburden of work with officials belonged to government based SHGs while problems such as inadequate trained personnel, lack of public participation and poor governance & networking faced by NGO based SHGs were the reasons for comparatively low knowledge among beneficiaries of government and NGO based SHGs. As far as overall knowledge about SLFPs was concerned, Fig 1 depicted that high level of knowledge possessed by majority of beneficiaries of dairy cooperative SHGs (45.00%) followed by medium level among beneficiaries of NGO (62.50%) and government based SHGs (52.50%). The present findings are in line with the results of Rahman and Gupta, 2015; Triveni 2018; Goswami and Biswas, 2021 who also reported that majority of the respondents possessed medium level of knowledge for scientific dairy farming practices.

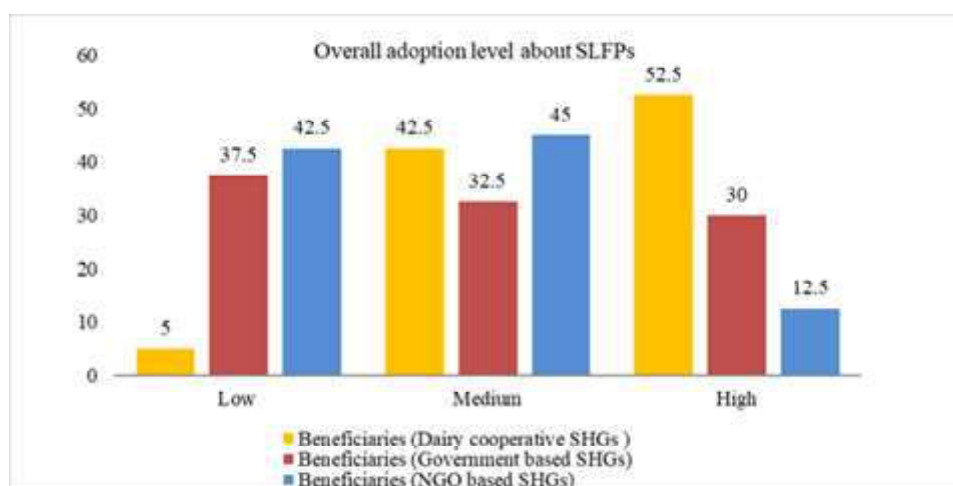
Association of knowledge about scientific dairy farming practices among beneficiaries of SHGPIs

Mean differences for knowledge about scientific dairy farming practices viz. breeding, feeding, health care and management; among the beneficiaries of different SHGPIs i.e. dairy cooperative, government and NGO were compared with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT), and presented in Table 2 while mean values are given in Table 3.

Table 2 depicted that overall model of knowledge about scientific dairy farming practices was found to be significant ($P < 0.01$) except for breeding and management practices. Table 3 demonstrated the estimated mean values and mean differences for knowledge among the beneficiaries of dairy cooperative, government and NGO based SHGs. No significant difference was observed among the beneficiaries of different SHGPIs with respect to knowledge of breeding and management practices. In case of feeding and health care practices significant difference was observed between the beneficiaries of dairy cooperative (higher mean value), government and NGO based SHGs.

Adoption of scientific dairy farming practices (SLFPs) among beneficiaries of SHGPIs

High level of adoption possessed by most of the beneficiaries of dairy cooperative SHGs for scientific breeding and management

Fig 2. Overall adoption level of SLFPs among beneficiaries of different SHGPIs**Table 3:** Association of knowledge about scientific dairy farming practices among beneficiaries of SHGPIs (Mean $\pm$ SE)

	Source	Character	Number	Mean $\pm$ SE
Beneficiaries	Dairy Cooperative SHGs	Breeding Practices	40	9.60 $\pm$ 0.26 ^a
	Government SHGs		40	9.05 $\pm$ 0.25 ^a
	NGO based SHGs		40	9.00 $\pm$ 0.29 ^a
Beneficiaries	Dairy Cooperative SHGs	Feeding Practices	40	9.18 $\pm$ 0.23 ^a
	Government SHGs		40	8.30 $\pm$ 0.16 ^b
	NGO based SHGs		40	8.20 $\pm$ 0.14 ^b
Beneficiaries	Dairy Cooperative SHGs	Health care Practices	40	6.00 $\pm$ 0.19 ^a
	Government SHGs		40	5.08 $\pm$ 0.23 ^b
	NGO based SHGs		40	4.90 $\pm$ 0.23 ^b
Beneficiaries	Dairy Cooperative SHGs	Management Practices	40	11.38 $\pm$ 0.12 ^a
	Government SHGs		40	11.43 $\pm$ 0.20 ^a
	NGO based SHGs		40	11.58 $\pm$ 0.34 ^a

Table 4: Distribution of beneficiaries on the basis of adoption about SLFPs

Sr. No.	Aspects	Category	Beneficiaries		
			Dairy cooperative SHGs (n=40)	Government SHGs (n=40)	NGO based SHGs (n=40)
1.	Breeding Practices	Low (<8)	0 (0.00)	07 (17.50)	14 (35.00)
		Medium (8-11)	19 (47.50)	22 (55.00)	23 (57.50)
		High (>11)	21 (52.50)	11 (27.50)	03 (7.50)
2.	Feeding Practices	Low (<9)	09 (22.50)	08 (20.00)	13 (32.50)
		Medium (9-11)	18 (45.00)	23 (57.50)	17 (42.50)
		High (>11)	13 (32.50)	09 (22.50)	10 (25.00)
3.	Health Care Practices	Low (<15)	01 (2.50)	18 (45.00)	13 (32.50)
		Medium (15-17)	22 (55.00)	09 (22.50)	19 (47.50)
		High (>17)	17 (42.50)	13 (32.50)	08 (20.00)
4.	Management Practices	Low (<10)	03 (7.50)	12 (30.00)	18 (45.00)
		Medium (10-13)	07 (17.50)	14 (35.00)	17 (42.50)
		High (>13)	30 (75.00)	14 (35.00)	05 (12.50)

(Figures in parenthesis indicate percentage)

practices followed by medium level among majority of beneficiaries belong to NGO and government based SHGs (Table 4). In case of overall adoption about SLFPs, Fig. 2 illustrated that high level of adoption was reported among most of the

beneficiaries (52.50%) belong to dairy cooperative SHGs followed by medium level among beneficiaries of NGO (45.00%) and low among government based SHGs (37.50%). The regular contact, awareness campaigns and trainings provided by various

Table 5: Association of adoption about scientific dairy farming practices among beneficiaries of SHGPIs (F Value)

Source	Character	Degree of freedom	Sum of Squares	Mean sum of Squares	F Value
Beneficiaries (Dairy Cooperative, government and NGO based SHGs)	Breeding Practices	2	124.52	62.26	13.26**
	Feeding Practices	2	13.27	6.63	1.73
	Health care Practices	2	62.82	31.41	5.85**
	Management Practices	2	324.45	162.23	25.62**

Table 6: Association of adoption about scientific dairy farming practices among beneficiaries of SHGPIs (Mean $\pm$ SE)

Source	Character	Number	Mean $\pm$ SE
Beneficiaries	Dairy Cooperative SHGs	40	10.43 $\pm$ 0.29 ^a
	Government SHGs	40	9.90 $\pm$ 0.34 ^a
	NGO based SHGs	40	8.05 $\pm$ 0.39 ^b
Beneficiaries	Dairy Cooperative SHGs	40	10.43 $\pm$ 0.28 ^a
	Government SHGs	40	9.78 $\pm$ 0.34 ^a
	NGO based SHGs	40	9.68 $\pm$ 0.31 ^a
Beneficiaries	Dairy Cooperative SHGs	40	16.85 $\pm$ 0.22 ^a
	Government SHGs	40	15.43 $\pm$ 0.40 ^b
	NGO based SHGs	40	15.23 $\pm$ 0.44 ^b
Beneficiaries	Dairy Cooperative SHGs	40	14.00 $\pm$ 0.36 ^a
	Government SHGs	40	11.45 $\pm$ 0.46 ^b
	NGO based SHGs	40	10.03 $\pm$ 0.36 ^c

concerned officials to the beneficiaries of dairy cooperative SHGs helped them in acquiring scientific knowledge and encouraged them to adopt improved practices. The present findings corroborate with the study conducted by Maousami et al. 2014; Sharma et al. 2015; Goswami and Biswas, 2021.

Association of adoption about scientific dairy farming practices among the beneficiaries of SHGPIs

Mean differences for adoption about scientific dairy farming practices viz. breeding, feeding, health care and management; among the beneficiaries of different SHGPIs i.e. dairy cooperative, government and NGO were compared with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

Overall model of adoption about scientific dairy farming practices was found to be significant at 1% ($P < 0.01$) level of significance except for feeding practices (Table 5).

Mean values for adoption among the beneficiaries (dairy cooperative, government and NGO based SHGs) were estimated and presented in Table 6. Regarding breeding practices beneficiaries of dairy cooperative (higher mean value) and government based SHGs had significant difference with the beneficiaries of NGO based SHGs. No significant difference was observed among the beneficiaries of different SHGPIs with respect to adoption of feeding practices. As far as adoption of health

care practices were concerned; beneficiaries of dairy cooperative SHGs having higher mean value were significantly different from the beneficiaries of government and NGO based SHGs. Beneficiaries of dairy cooperative based SHGs had better adoption level for management practices with high mean value and significantly differ from the beneficiaries of government and NGO based SHGs. Difference between various SHGPIs was due to the fact that beneficiaries of dairy cooperative SHGs were provided with services such as inputs, technology, advisory services on regular basis as compared to government and NGO based SHGs. Also inadequate staff, lack of skilled personnel, lack of dedicated leadership, absence of cohesive and strategic planning among the NGO personnel and improper follow-up mechanism was the reasons behind differences in adoption pattern and relatively low adoption compared to the dairy cooperative SHGs.

Conclusion

The process of sharing information by various SHG promoting institutes helped the rural women in gaining knowledge and adoption of improved dairy practices. Majority of the beneficiaries were found to have medium level of knowledge and adoption however beneficiaries of dairy cooperative promoted SHGs had relatively better and high knowledge and adoption regarding scientific dairy farming practices as compared to beneficiaries of

government and NGO based SHGs. To achieve visible changes in rural women position efforts have to be made by developmental workers and financial institutions to promote SHGs wholeheartedly as per their capacity to bring about speedy and desirable changes for overall development.

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Process optimization for the manufacture of Low-calorie yoghurt

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Abstract: The present study was carried out to prepare low-calorie yoghurt by adding inulin @1, 2, and 3% in skim milk as well as in double toned milk, and the effect of inulin as a fat replacer on the quality of low-calorie yoghurt preparation was investigated and compared with control yoghurt made from whole milk. The sensory, and physicochemical characteristics were examined at 0, 7, and 14 days of storage period. Based on sensory evaluation, T₄ (double toned milk + 1% inulin) was considered as best for flavor, color and appearance, body and texture, and overall acceptability scores were 8.30 ± 0.120 , 8.50 ± 0.115 , 7.96 ± 0.176 , and 8.03 ± 0.058 respectively. It was found that total fat%, protein%, ash%, pH, acidity%, total solid%, carbohydrate%, and calorific value of T₄ showed 1.50 ± 0.000 , 4.13 ± 0.012 , 1.032 ± 0.001 , 4.50 ± 0.012 , 0.984 ± 0.001 , 12.40 ± 0.011 , 5.74 ± 0.001 , 52.98 ± 0.077 Kcal/100g. The cost of the low-calorie yoghurt based on raw ingredients per 100g was Rs. 21.87 Therefore, the production of low-calorie yoghurt was economical.

Keywords: Double toned milk, Fat replacer, Inulin, Low-calorie Yoghurt, Skim milk

Yoghurt is a cultured product made from pasteurized, boiled, or toned milk through lactic acid fermentation (FSSAI, 2011) and the fermentation process increases the product's shelf life and

improves the digestibility of milk. It also acts as a vehicle to deliver probiotics and prebiotics and has an advantageous role in combination with inulin as a fat replacer (Chand et al. 2021). In this modern era, this low-calorie yoghurt will attract the consumer's attention because of its health benefits attributes. The reduction of fat in yoghurt showed a negative impact on characteristics but inulin is found to be a good fat substitute (Arango et al. 2020). Inulin promotes the growth of healthy bacteria and enhances calcium and magnesium absorption and immune functions and reduces the level of cholesterol and serum lipids (Anderson-Dekkers et al. 2021). Inulin contains polysaccharides, which make it a functional food additive with prebiotic properties and it has many technological properties, including acting as a bulking agent, low-calorie sweetener, and fat substitute as well as providing a smooth, creamy texture and mouthfeel while maintaining consumer acceptance. Thus, inulin served as a suitable ingredient for the preparation of low-calorie yoghurt (Soh et al. 2021). Even though much research on the effects of inulin addition on yoghurt quality has been conducted, to the best of the researcher, no specialized studies on the effect of inulin addition in skim and double-toned milk to prepare yoghurt and with their analysis by an expert panel and instrumentally have been performed. Hence, this work has been planned for process optimization for the manufacture of low-calorie yoghurt to examine the effect of added inulin on the physicochemical and sensory properties during a storage period of 14 days.

To formulate low-calorie yoghurt, milk (skim and double toned) was heated to 90 °C for 15 min. with continuous stirring, inulin added @ 1-3% and then cooled to $45 \pm 1^\circ\text{C}$ followed by inoculation with 2% starter culture and incubated at $42 \pm 1^\circ\text{C}$ until the milk coagulation and pH reached to 4.8 followed by rapidly cooling to $4 \pm 1^\circ\text{C}$.

The sensory characteristics such as flavor, color and appearance, body and texture, and overall acceptability were evaluated using the nine-point hedonic scale by 10 semi-trained member panelists, and data were analyzed by using a one-way analysis of variance test in Completely Randomized Design (CRD) using the OPSTAT statistical program.

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Table 1 Physicochemical evaluation of Low-Calorie Yoghurt

Parameters	Samples	Storage (days)		
		0	7	14
Fat (%)	T ₀	3.10±0.058	3.10±0.058	3.13±0.033
	T ₄	1.50±0.000	1.51±0.012	1.53±0.018
Protein (%)	T ₀	3.96±0.119	4.07±0.047	4.15±0.035
	T ₄	4.13±0.012	4.20±0.018	4.31±0.009
Ash (%)	T ₀	1.043±0.002	1.084±0.002	1.138±0.015
	T ₄	1.032±0.001	1.074±0.001	1.128±0.006
pH	T ₀	4.52±0.019	4.37±0.015	4.31±0.015
	T ₄	4.50±0.012	4.40±0.058	4.36±0.012
Titratable Acidity (%)	T ₀	1.286±0.002	1.376±0.013	1.424±0.002
	T ₄	0.984±0.001	1.150±0.012	1.280±0.012
Total solids (%)	T ₀	13.83±0.023	14.43±0.026	14.93±0.027
	T ₄	12.40±0.011	13.11±0.018	13.91±0.006
Moisture (%)	T ₀	86.16±0.022	85.56±0.030	85.06±0.018
	T ₄	87.60±0.019	86.88±0.031	86.09±0.016
Carbohydrate (%)	T ₀	5.74±0.260	6.18±0.007	6.52±0.039
	T ₄	5.74±0.004	6.32±0.005	6.94±0.142
Calorific value (Kcal/100g)	T ₀	66.69±0.270	68.90±0.267	70.85±0.125
	T ₄	52.98±0.077	55.67±0.186	58.77±0.052

Data represent mean ± standard deviation

The flavor, color and appearance, body and texture, and overall acceptability of six different formulations (T₁= skim milk+@1% inulin, T₂= skim milk+@2% inulin, T₃= skim milk+@3% inulin, T₄= double toned milk+@1% inulin, T₅= double toned +@2% inulin, T₆= double toned +@3% inulin) were analyzed and it was observed that treatment T₄ showed the highest score 8.30±0.120, 8.50±0.115, 7.96±0.176, 8.03±0.058, respectively. Thus, on the basis of sensory analysis, it was found that T₄ showed the best result and was considered an optimized product for further analysis. An increase in Inulin level and storage period significantly decreased the mean score of different characteristics of low-calorie yoghurt. Similar results from Guven et al. (2005); Srisuvor et al. (2013); Helal et al. (2018) showed that low-fat yogurt with 1 and 2% inulin received higher scores for sensory and overall acceptance than control yoghurt.

The physicochemical results (Table 1) showed that the fat% of optimized yoghurt (T₄) and control sample (T₀) had no significant (p>0.05) change by replacing milk fat with inulin and agreed with the work of Khodear et al. (2018). A significant increment in protein content of yoghurt was observed during storage, depending on the proteolytic action of lactic acid bacteria, which hydrolyses proteins into peptides and amino acids (Guyen et al. 2005; Khodear et al. 2018). The significant (p<0.05) increase in ash content during storage is analyzed due to the loss of carbon dioxide and water. The pH of products decreases during the storage period due to the production of lactic acid from lactose (Guyen et al. 2005; Kapitula and Klebukowska 2009; Mazloomi et al. 2011; Helal et al. 2018). But the pH and titratable acidity were not influenced by different concentrations of inulin (Guyen et al.

2005). The increase in acidity observed during the storage was due to the fermentation of carbohydrates into lactic acid, CO₂, and formic acid (Falah et al. 2021). The changes between the total solids content of optimized and control yoghurt were significant (p<0.05) and a positive relationship between the total solid content and inulin concentration was observed (Khodear et al. 2018; Bhaskar et al. 2018). The moisture% of yoghurt was decreased during the storage period on the increasing concentration of inulin (Khodear et al. 2018). The calorific value of optimized yoghurt was significantly (p<0.05) increased and the cost of 100g of optimized yoghurt was approximately Rs. 21.87.

Conclusion

Therefore, it is concluded that the use of inulin in the manufacture of low-fat yoghurt is recommended because of its health-beneficial attributes and the low-calorie yoghurt will attract a wide variety of consumers. Therefore, it may have the potential to increase sales in the market. This product was prepared with an aim to provide low-calorie to health-conscious consumers of society.

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