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INVITED REVIEW

Role of fermented dairy foods in human health

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Abstract: Unhealthy lifestyle and unbalanced diet are directly associated with diseases like inflammatory bowel diseases (IBD), diarrhea, hypersensitivity, irritable bowel syndrome, *Helicobacter pylori* infection, lactose intolerance, and gastroenteritis etc.The malfunction of the mediators of inflammation affect intestinal epithelial barrier functions by disruption of the physiological pathways. Fermented dairy foods influence human health through secretion of bioactive compounds and interaction of their inherent beneficial microbes with gut. Among all foods, fermented products have also been used as the most efficient vehicle for delivery of beneficial or probiotic microbes as they not only preferred by consumer being superior in sensory attributes during oral intake but also provide buffered media for survival of bacteria in harsh gastric environment. *In-vitro* and *in-vivo* studies have shown that fermented dairy products (fermented milk, curd, yogurt, cheese, koumiss, paneer and kefir) assisted in the maintenance of normal mucosal homeostasis and modulate the immune response in a positive fashion which provides protection against various metabolic and pathogen mediated diseases by anti-oxidative, anti-microbial, anti-fungal, anti-inûammatory, anti-diabetic and anti-atherosclerotic activities. Cumulatively, this review focuses mainly on the health effects of fermented dairy foods.

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Introduction

Amongst the fermented foods, fermented dairy products play a key beneficial role in human diet since ancient times and recently attracted scientific interest due to their health promoting effects. The primary aim of fermentation is to increase the shelf life of highly perishable foods and improve the organoleptic properties, digestibility and bioavailability of nutrients. Fermentation of milk is a natural phenomenon shown by lactic acid producing microbes which acidify and coagulate the milk besides degrading complex bioorganic molecules into simpler and easily digestible substances. Milk being rich nutrient source is produced by almost all domesticated mammals in every part of the world. Buffalo and cow milk are ubiquitous, whereas milk and milk products of camel and yak are also consumed by people of Himalaya, Mid-west Asia and Northern Africa respectively. Robinson and Tamime (1990) categorized fermented milks on the basis of kind of microorganism dominating and majority of sensory metabolites of fermented products. Class I**:** Lactic fermentation where Lactic acid bacteria (LAB) species lead to the fermentation changes in the products. These can be further classified into three sub-classes on the basis of microbial type driving the fermentation process: (a) mesophilic type (e.g. natural acidified milk, cultured milk, cultured cream and cultured buttermilk); (b) thermophilic type (e.g.dahi, yogurt, bulgarian buttermilk, zabadi);(c) probiotic type (e.g. yakult, bifido milk, acidophilus milk).Class II: Fungal lactic fermentation where LAB and yeast both in assistance generate the fermented product. This fermentation further can be classified into two subclasses: (a) alcoholic milk (e.g. koumiss, kefir, acidophilus yeast milk) and (b) moldy milks (e.g.villi) (Mayo et al. 2010). Lactic acid bacteria (LAB) have been used as dominant starter culture for preparation of fermented dairy foods and beverages (e.g. kefir, cheese, buttermilk, koumiss, and yogurt) worldwide due to their symbiotic relationship with milk and their indigenous nature to human intestine. Fermented dairy products most commonly associated with microorganism belong to genera *Lactobacillus, Lactococcus, Pediococcus, Bifidobacterium, Leuconostoc, Bacillus* and *Propinobacterium*. Yeast, generally used for

fermentation belong to the species of *Saccharomyces*, *Kluyeromyces* and *Debaryomyces* while moulds used in cheese and milk fermentation include, *Penicillium, Mucor, Geotrichium* and *Rhizopus* species (Liu et al. 2020). Recently, special interest has been generated in fermented dairy foods because plethora of scientific information established its direct or indirect correlation with lactose intolerance, milk allergy, intestinal health improvement, and immune related disorders. Fermented dairy products play predominant role as a probiotic carrier also. Fermented dairy foods containing probiotic further enhance various health attributes by prevention of inflammatory disorder, cardiovascular diseases, dysbiosis, diabetes, osteoporosis, carcinogenesis, *Helicobacter pylori* infections and gastrointestinal tract disorders (Silva et al. 2016). The microbes present in these products interact with mucosal layer of intestinal epithelial cells (IEC) and are reported to enhance the selectivity of epithelial barrier functions by protecting the barrier disruption which generally occur due to pathogens exposure (Wells et al. 2008 and Ulluwishewa et al. 2011). The function and composition of the intestinal bacterial community can be transformed selectively with the use of bacteria and their metabolites used in fermentation as gut modulators. The health enhancing components associated with fermented dairy foods are proteins, peptides, minerals, vitamins, fats, oligosaccharides and most vital biologically active bioactive peptides (BAPs). Furthermore, unique sensory profile along with associated health attributes make functional fermented dairy foods as products of choice for consumer, nutritionists and clinicians. Thus linkage between fermentation, nutrition and health is important and has been popularized with the best known fermented dairy foods such as fermented milk, yogurt and cheese, etc. (Yoda et al. 2014). This review provides an overview of scientific literature related to the recent advancements taking place in dairy industry for the preparation of fermented products including beneficial microbes which directly or indirectly target various life-style associated human diseases.

Vehicle for beneficial organisms

Milk is the richest source of nutrients coupled with bioactive functional properties that helps in maintenance of growth and nourishment of infants. Among total milk produced, only 7% of is utilized in preparation of fermented milk products in India (Aneja et al. 1990). Three most common fermented dairy products are Indian dahi, shrikhand (sweetened concentrated curd), and lassi (stirred curd). Milk comprehends omega-3 fatty acids, phytosterols, isoflavins, conjugated linoleic acid, minerals and vitamins, so has great importance in the development of functional foods. Dairy products such as cream, cheese, yogurt, *Acidophilus-bifidus* milk, koumiss, kefir and fermented beverages have long been considered as best matrix for the probiotic microbes with increased content of lactic, butyric, citric and acetic acids which enhance not only the organoleptic properties but also bio-functional efficacy of products. Rezac et al. (2018) surveyed many fermented foods and observed that mostly they contained 10^{5-7} lactic acid bacteria per mL or gram, although there was considerable variation based on geographical region and sampling time. In general, cultured dairy products consistently contained higher levels, up to 10^{9}/mL or g. Fermented dairy products continue to be the primary delivery vehicle for food and beverage products featuring beneficial or probiotic microbes. Firstly fermented dairy products such as yogurt are generally understood to contain live microbes because unlike many other fermented foods, dairy products are typically not pasteurized or heat treated at the end of production, which ensures microbial viability (Marco et al. 2017). Moreover microbes have been well studied for their growth and survival in dairy products. Davidson et al. (2000) evaluated the viability of probiotic strains in low-fat yogurt using *Streptococcus salivarius ssp. thermophiles, L. delbrueckii ssp. bulgaricus, B. longum* and *L. acidophilus*, and verified that culture bacteria did not decrease during frozen storage without altering its sensory characteristics. Further, the yogurt matrix appeared good vehicle for microbes due to its composition, which includes milk proteins, fat and lactose, as well as other compounds. Besides, its frozen state contributes to its efficiency. Moreover, yogurt should have relatively high pH values between 5.5 to 6.5, in order to favor an increased survival of lactic cultures during storage. The lower acidity also results in increased consumer acceptance, especially among consumers who prefer milder products (Cruz et al. 2009). Similarly, *Saccharomyces boulardii*, had the ability to grow in bio-yogurt and reached maximum counts exceeding 10^{7}CFU g^{-1}. The number of yeast populations was substantially higher in the fruit-based yogurt, mainly due to the presence of sucrose and fructose derived from the fruit. Despite the inability of *S. boulardii* to utilize lactose, the yeast species utilized available organic acids, galactose and glucose derived from bacterial metabolism of the milk sugar lactose present in the dairy products (Lorens-Hattingh and Viljoen, 2001). Similarly fermented milks supplemented with lemon and orange fibers increased the counts of *L. acidophilus* and during cold storage compared to the control set (Sendra et al. 2008). Likewise cheeses are fermented dairy products which have a strong potential for delivering beneficial microorganisms into the human intestine, due to their specific chemical and physical characteristics. Cheeses have higher pH levels, lower titratable acidity, higher buffering capacity, more solid consistency, relatively higher fat content, higher nutrient availability and lower oxygen content than yogurts. These qualities protect probiotic bacteria during storage and passage through the gastrointestinal tract (Karimi et al. 2011 and Ong et al. 2006).Whey is also a by-product of cheese industry produced in millions of tons having proteins which represent approximately 20% of total protein content in bovine milk. Whey could also be used as media for beneficial bacteria due to the presence of sufficient amount of lactose as source of energy (Koller et al. 2012).According to Karimi et al. (2012), recommendations for the minimum viable counts of each probiotic strain in gram or millilitre of probiotic products vary when it comes to providing health

benefits related to probiotic organisms. However, populations of 10^6 -10^7 CFU/g in the final product have been shown to be more acceptable as efficient levels of probiotic cultures in processed foods (Talwalkar et al. 2004), with numbers attaining 10^8 - 10^9 CFU when provided by a daily consumption of 100 g or 100 mL of probiotic food, and hence benefiting human health (Jayamanne and Adams, 2006).

Physiological impact on health

Firstly, live microorganisms present in fermented product, particularly LAB are able to produce huge amounts of secondary metabolites with excellent health benefits and preservative properties (i.e. antimicrobial activity). Indeed, some microorganisms can increase the levels of several bioactive compounds (e.g. vitamins, antioxidant compounds, peptides, etc). Secondly, fermented foods contain living organisms which contribute in the modulation of the host's physiological balance and gut microbiota, enriching, at the same time, the host's diet with new bioactive molecules. Thus stimulation of useful bacteria in the gut, maintain gut homeostasis, improves cardiovascular health, anti-bacterial, inflammatory and anti-cancerous effects (Ranadheera et al. 2017). Yu et al. (2016) reported a specific dose of whey protein extract induced the beneficial microbiota profile in gut of rats with the increase in relative abundance of *Lactobacillus spp., Bifidobacterium spp*. and *Bacteroidetes* and also a probiotic growth enhancer with the quicker generation of metabolic products. Emerging studies have also shown that the highly selective intestinal barrier may be compromised during dysbiosis and gut related diseases (Genser et al. 2018). Psychological stress and exhaustive exercise have also been shown to increase the permeability of the intestinal barrier (van Wijck et al. 2012 and Li et al. 2013). The first formal investigation of a probiotic and human mental outlook involved 132 otherwise healthy adults consuming *L. casei* fermented milk beverage for three weeks; vs. placebo, significant improvement in mood scores were noted among those with the higher baseline depressive symptoms (Benton et al. 2007) . Ingestion of vibrant probiotics, especially in fermented foods, is found to cause significant positive improvements in balancing intestinal permeability and barrier function (Hiippala et al. 2018), with direct effects on metabolic syndrome, atherosclerosis, inflammatory bowel diseases, and colon cancer (Wang et al. 2011) and indirect effects on depression, anger, anxiety, and levels of stress hormones (Wallace et al. 2017). Fermented foods also influence the bioavailability and activity of the chemical constituents. Fermented dairy products are reported to be more potent than other milk products in increasing absorption of nutrients (Biver et al. 2018). This is likely due to their pre- and probiotic content and their beneficial effect on gut microflora, calciotrophic growth hormones and intestinal inflammation, which consequently lead to inhibition of bone resorption and stimulation of bone formation. Moreover, microorganisms involved in fermentation produce enzymes such as lactase, lipase, protease and amylase. These enzymes of microorganisms improve the nutritional value of anti-nutritive and inedible substrates, transforming them into edible products with many health benefits for consumers (Tamang et al. 2016). On the other hand, lactate, ethanol, pyruvate and succinate produced by some lactic acid bacteria during dairy fermentation are utilized by species other than their producers in intestine which generate short-chain fatty acids (SCFA) in the intestinal content. Therefore, the major products of sugar catabolism are acetate, propionate and butyrate, which represent 85–95% of total SCFA in all the colon regions (Cummings and Macfarlane, 1997). Although SCFA come mainly from the breakdown of carbohydrates, protein, peptide and glycoprotein precursors. These are involved in a wide range of physiological functions such as the transport of electrolytes and water, growth and differentiation of colonic mucosa, apoptosis of aged or altered colonocytes, metabolism of lipids in the liver and energy supply for different tissues. Among these SCFA, butyrate is most important which can affect the microbiome diversity and interfere with host immune system via multiple pathways (Castro et al. 2019).

Bioactive compounds released during dairy fermentation with health attributes

Major bioactive compounds synthesized by plethora of microbes during fermentation of dairy products are depicted in Table 1. During dairy fermentation, microorganisms not only degrade fermentable carbohydrates into end products such as organic acid, carbon dioxide, and alcohol (Kim et al.2016) but also produce anti-microbial metabolites such as bacteriocins that increase food safety by inhibiting food-borne pathogens (Nout et al. 2014). Besides, fermented dairy foods are often attributed to the release of bioactive peptides from milk proteins by the microbial degradation using proteinases in the cell wall of lactic acid bacteria (Otag and Hayta 2013 and Martinez Villaluenga et al. 2017). The most significant subgroup of bioactive peptides present in fermented dairy foods include anti-hypertensive (Beltran-Barrientos et al. 2016), osteogenic (Reddi et al. 2018), anti-oxidative and anti-inflammatory (Sowmya et al. 2018 and 2019), mineral binding peptides (Mann et al. 2019) and the angiotensin-1-converting enzyme (ACE) inhibitor peptides (Nejati et al.2013). Exo-polysaccharides, composed of repeating mono- or oligosaccharide subunits bound by various glycosidic linkages (Fanning et al. 2012), are also produced by various microorganisms during fermentation (Deepak et al. 2016). Recently certain strains of LAB have become a focus of interest because of various potential health benefits of exopolysaccharides (Patel and Prajapat, 2013). The mechanism by which bacterial exopolysaccharides could reduce total serum cholesterol involves binding cholesterol, reducing cholesterol absorption, and inducing the release of bile acids (Tok and Aslim, 2010). Tofalo et al. (2019) studied the effect of traditional cheese fermentation on the accumulation of healthy (γ-aminobutyric acid or GABA) and toxic biogenic amines (BA) compounds and

confirmed a greater BA formation and proteolytic activity in cheese made by pig rennet than those made by calf rennet. Conjugated linoleic acid (CLA) is a generic term used for describing the geometrical and positional isomers of linoleic acid (C18:2 *cis*9, *cis*12) with a conjugated double bond system (Jones et al. 2005) which are biologically active molecules due to their protective effects against various common diseases such as obesity (Kennedy et al. 2010), atherosclerosis, diabetes (Dilzer and Park,2012), some chronic inflammatory diseases and cancer (Evans et al. 2010). Gutierrez et al(2016) reported the effects of the milk fermentation on the CLA concentration of fermented dairy products and suggested that the increase in CLA concentration by milk fermentation was strain-dependent, because of the different linoleate isomerase activity of the species. Although several studies have reported increases in the concentration of CLA in milk and fermented milks in a natural manner but they were fairly moderate and the obtained levels of CLA are significantly lower than those recommended to achieve therapeutic effects.

Health attributes of fermented dairy products

In-vitro and *in-vivo* studies have shown that fermented dairy products (fermented milk, curd, yogurt, cheese, koumiss, paneer and kefir) assisted in the maintenance of health through various biological activities such as anti-oxidative, anti-microbial, anti-fungal, anti-inûammatory, anti-diabetic, anti-atherosclerotic and immune-modulation etc. which provide protection against various metabolic and pathogen mediated diseases. The list of various health attributes shown by various fermented dairy products have been summarized in Table 2**.**

Fermented milk

Fermented milks have always played an important role in the nutrition of people worldwide. Consumption of fermented milks has a long tradition. Historically, fermented milks, often referred to as sour milks, were made and consumed at home. Most of these products have a characteristic thick consistency and ropiness and could be kept for weeks or even months in a cool room. Fermented milk products are created when milk ferments with specific kinds of bacteria called *Lactobacilli* or *Bifidobacteria*. Kapila et al. (2006) described the cholesterol lowering and antioxidative potential of *L. casei* ssp *casei* fermented milk in wistar rats. Widodo et al. (2013) examined the viability of LAB in cow and goat milk using different starter cultures and found that LAB viability is 10.83% in cow milk and 11.40% in goat milk after 28 days storage period. Thus fermented milk quality could be affected by different factors such as starter culture, milk source and storage period (Taufiq et al. 2013). Fermentation means the milk is partially digested by the bacteria. This makes the milk product easier to digest, especially for people who have milk allergies or are lactose-intolerant. Ishikawa et al. (2003) reported that *Bifidobacteria* fermented milk reduced clinical activity index, histological score and *Bacteroids vulgates* in patients suffering from active ulcerative colitis in a clinical trial. Yadav et al. (2019) showed that oral consumption of *L. rhamnosus* (LR MTCC 5897 and MTCC 5957) fermented milk improves the diet induced hypercholesterolemia and helped to keep liver and kidney healthy by reducing inflammatory mediators in wistar rats. The same authors (Yadav et al. 2018) also found that consumption of probiotic fermented milk decreases the blood glucose level and improved the oxidative stress and serum inflammation in diabetic wistar rats. Sharma et al. (2014) observed that *L. rhamnosus* (MTCC 5897) fermented milk intervention potentially diminished the age associated imbalance in Th1/Th2 response. Similarly, supplementation of *L. fermentum* (MTCC 5898) fermented milk alleviated the severity of *E. coli* induced infection in aging mice with enhanced *E. coli* specific antibodies and anti-oxidative enzyme activities along with anti-immuno-senescence potential. Various type of foods are responsible for the cause of food allergies during early infancy because it is the

Table1 Biologically active components in fermented dairy products

Bioactive compound	Fermented dairy food	Microorganism	Reference
CLA	Fermented milk	*L. plantarum*	Pandit et al. (2012)
	Cheddar cheese	*Lactococcus lactis* CI4b	Mohan et al. (2013)
GABA	Mozzarella cheese	*S. thermophiles* MZZ1	Siragusa et al. (2007)
	Fermented milk	*Streptococcus salivarius* fmb5	Chen et al. (2016)
Bioactive peptides	Fermented cow milk	*L. casei* HZ1	Han et al. (2012)
	Munster cheese	*Lactobacillus plantarum* WHE92	Arques et al. (2015)
Exo-polysaccharides	Kefir	*Leuconostocpseudomesenteroids*	Chen et al. (2016)
	Yogurt	*Bifidobacterium longum* CCUG52486	Parassanna et al. (2013)
Butyric acid /SCFA	Yogurt	ABY1 & YO-MIX™ 211	Vaseji et al. (2012)
Bacteriocins	Goats's milk cheese	*Lactococcus lactis* IFPL359	Martinez-Cuesta et al. (2001)
	Yogurt	*Lactobacillus acidophilus* CH5	Ahmed et al. (2010)
Organic acids	Yogurt	*S. thermophiles* and *L. bulgaricus*	Venica et al. (2014)
Vitamins (B_9)	Fermented milk	LAB and *Bifidobacteria* species	Revuelta et al. (2018) Saubade et al. (2018)
Folic acid (B_{12})	Fermented milk	*Bifidobacterium animalis* Bb12	Patel et al. (2013)

Table2 Health promoting effects of fermented dairy products

Fermented dairy food	Microorganism	Associated action	Disorder	References
Fermented milk	*Bifidobacterium breve*, *B. bifidum,* and *L. acidophilus* YIT0168	Preventive effects in ulcerative colitis relapse, reduced *B. vulgatus* and luminal butyrate with concomitant reduction in albumin in murine model	Ulcerative colitis	Ishikawa et al. (2003
	L. paracasei CNCM I 1518	Decreased pro-inflammatory mediators, oxidative damage and improve gut dysbiosis in rats	Cirrhosis	Sanchez et al. (2017)
	L. rhamnosus MTCC 5897	Increased antioxidative activities,	Hypercholestrolemia	Yadav et al. (2019)
	L. rhamnosus MTCC 5957	decreased lipid peroxidation in wistar rats, anti-allergic and protect against salmonella infection in mice	Food allergy Diarrhea	Saliganti et al. (2015) Tanedjeu et al. (2016)
	L. fermentum MTCC 5898	Upregulation in *E. coli* specific antibodies and phagocytic activity in murine model	Immunosenescence	Sharma et al. (2014)
	L. reuteri LR6	Adjuvant effect	Protein energy malnutrition	Garg et al. (2017)
	L. helveticus LH511	Regulated tight junction protein expression and modulate TLR signaling pathway with decrease in inflammatory markers *in-vivo* and *in-vitro* milieu	Barrier dysfunction	Ho et al. (2020)
Goat milk	*L. rhamnosus* CRL1505	Sufficient dose (10^8 cfu/g) stimulates mucosal immune system and increases defense action to limit the dissemination of pathogen in immune-competent mice model	Intestinal and respiratory infections	Salva et al. (2011)
Camel milk (Shubat drink)	0.1% direct vat set (DVS) yogurt culture	Anti-microbial agents in camel milk showed antiviral activity against diarrhea causing viruses in rats	Diarrhea	Mona, (2010)
Dahi	*Lactococcuslactis, L. lactis ceromonis, Leuconostac mesenteroids cremoris*	Mixture of probiotic culture of 10^8 count reduced diarrheal symptoms in children	Diarrhea	Agarwal and Bhasin, (2002)
	Lactobacillus acidophilus *Lactobacillus casei*	Reduces hyperglycemia, dyslipidemia, oxidative stress	High fructose induced Diabetes	Yadav et al. (2007)
	Lactobacillus acidophilus LaVK2 and *B. bifidum* BbVK3	Decreases inflammatory mediators involved and reduces histopathological damage in colonic mucosa in swiss mice	Ulcerative colitis	Jadhav et al. (2012) Shandilya et al. (2016)
	Lactobacillus acidophilus LaVK2 and *Bifidobactrium bifidum* BbVK3	Reduces IgE and skewed Th2 immune response towards Th1, suppresses allergic response in mice	Hypersensitivity Gastrointestinal health	Sivasankari et al. (2017)
Shrikhand	*L. acidophilus* NCDC14	Improved gut health and valuable for lactose intolerant individuals as good probiotic carrier		
Koumiss	*L. helveticus* NS8	Protective against colitis and associated with immunomodulatory activity *in-vitro* condition	Ulcerative colitis	Rong et al. (2015)
	Lactobacillus casei Zhang	LcZhang modulate immune response, with increase in sIgA	Immunomodulation	Ya et al. (2008)
Kefir	*Lactobacillus lactis* subs., *Leuconostoc*subs., *Streptococcus thermophilus, Lactobacillus* subs., and yeast of kefir	Fermented kefir ameliorates the colitis induced symptoms in dose dependent manner, relieves from disease induced diarrhea and macroscopic damages caused in mucosal wall	Inflammatory bowel disease (IBD)	Sevencan et al. (2019)
Yogurt	*L. rhamnosus* GG	Symbiotic mixture of probiotic with prebiotic promote beneficial microbiota and also helps in alleviation of IBS symptoms	Inflammatory bowel syndrome (IBS)	Lee et al. (2013)

	L. acidophilus, *B. lactis, L. bulgaricus*, and *S. thermophilus*	Probiotic yogurt intake increased IgA, reduced in IL-6 by suppressing the *H. pylori* and helps in immune protection	*H. pylori* infection	Yang et al. (2012)
Cream cheese	*L. chungangensis* CAU28	Maintain Th1 and Th2 balance as well as reduces IgE immunoglobulin associated with immune homeostasis	Atopic dermatitis	Kim et al. (2019)
Squacquerone cheese	*L. crispatus* BC4	Cheese promoted women health and prevented gynaecological infections by modulating the microbial ecosystem	Vaginal infections	Patrignani et al. (2019)
Probiotic cheese	*L. plantarum* TENSIA	Decreased TAG, LDL and total cholesterol lowers blood pressure that reduced the symptoms of metabolic syndrome	Hypertension	Sharafedtinov et al. (2013)
Cheddar cheese	*L. plantarum* K25	Decreased total cholesterol, glucose and LDL improves cardiovascular health	Cardiovascular health	Zhang et al. (2013)

critical period for the development of immunological memory (Saliganti et al, 2016). Saliganti et al. (2015) indicated that fermented milk prepared with *L.rhamnosus* and fed to mothers and their offspring separately or successively during suckling and weaning transition has protective effects in ovalbumin induced mice allergic model. Santiago-López et al. (2018) proved that fermented milk (*L. fermentum*) potentially reduced Th17 response in intestinal mucosa and responsible for anti-inflammatory effects because of the metabolites and cell components released during fermentation process. Moreover, it is well known that intestinal barrier consist of IEC regulated by tight junctional proteins and fermented milk showed a maintenance of gut barrier which helps in alleviation of various gastrointestinal disorders. Adhesion of pathogen in gut is an initial act of the inflammatory processes. Probiotic drink developed with *L. plantarum* helped in ameliorating the *Salmonella* infection which helped in reinforcement of epithelial barrier function (Rokana et al. 2016). Moreover, it has also been proved that metabolic products released during milk fermentation (*L. paracasei* CBAL 74) impart protective effects in colitis induced damage with a tendency to increase in IL-33 and cyclooxygenase (Cox)-2 production which in-turn reduced the inflammatory cytokines (IFN-γ and IL-17a). Fermented milk formula containing *L. paracasei* CBA L-74 also created new perspective of infant nutrition by providing immune benefits to formula-fed infants (Zagato et al. 2014). Furthermore, feeding of *L.paracasei* subsp.*paracasei* CNCM I-1518 fermented milk in carbon tetrachloride (CCl_4) induced cirrhosis rats reduced the bacterial translocation, TNF-α level, malondialdehyde (MDA) and increased β-defensin-1 expression which reduced gut dysbiosis and enhanced the gut barrier function (Sanchez et al. 2017). In an another study, it is reported that oral administration of *Lactobacillus reuteri* LR6, as probiotic fermented milk act as an adjuvant to combat protein energy malnourishment induced gut disturbances in mice (Garg et al. 2017).A recent study by Ho et al. (2020) reported that combination of fermented milk (LH511) with citrulline showed positive effects *in-vitro* and *in-vivo* maintenance of tight junctional proteins (ZO-1, occludin, claudin-1) expression with restored epithelial permeability through decreasing inflammation via up-regulation of TLR-2 expression. In northwest Argentina, goat milk interestingly has been used in large quantity for the preparation of fermented dairy products which has great importance as media for LAB culture with probiotic properties. "Labneh" is concentrated yogurt prepared from goat milk with high short and medium chain fatty acids. ''Kishk" is also a traditional drink of Lebanon prepared from goat milk along with yogurt and bulghur (Naagar et al. 2019). Salva et al. (2011) showed that consumption of fermented goat milk (*L. rhamnosus* CRL 1505) by immune-compromised malnourished mice resulted in recovery of nutritional status, biochemical parameters and offer resistance against intestinal and respiratory infections. Interestingly, fermented camel milk also has bio-functional properties which were established as health stimulating antioxidative, anti-proliferative and anti-diarrheal under *in-vitro* conditions. 'Shubat' naturally fermented camel drink was mainly dominated by *Enterococcus* and *Lactococcus* genus which showed virucidal properties against ortho and paramoxyvirus (Solanki et al.2018).

Indian Dahi /Curd

Dahi is the oldest Indian dairy product obtained by milk fermentation, generally prepared by using lactobacilli culture. Indian dahi consumption has significance in diet is due to its nutritional and beneficial effect on health. Dahi contain large amount of folic acid, riboflavin and thiamine and also contain β-galactosidase enzyme essential for lactose hydrolysis in lactose intolerant infants. Additionally, it also contains CLA and other essential aminoacids that show anti-carcinogenic activity and other therapeutic benefits (Aneja and Murthi, 1990). Dahi is used in formation of other Indian products includes shrikhand, lassi and chhach etc. Shrikhand comprises minerals including calcium, magnesium, phosphorus, copper, iron and zinc which impart valuable effects (Sarkar, 2008). A probiotic dahi prepared using *L.acidophilus* and *L.casei* delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia and oxidative stress in high fructose-induced diabetic rats, indicating a lower risk of diabetes and its complications (Yadav et al. 2007). In an another study, dahi containing *L. acidophilus* and *B. bifidum* consumption reduced colitis symptoms and disease activity score with decrease in IL-6, TNF-α, interferon and MPO activity in Dextran sulfate sodium (DSS)induced mouse model (Jadhav et al. 2012). Dahi containing 10^8 count of *Lactococcus*

lactis, Lactococcus lactis ceromonis, Leuconosta cmesenteroids cremoris ameliorated the diarrhea severity in children (0-5years) within three days (Agarwal and Bhasin, 2002). Likewise, dahi mixture of *Leuconostoc citrovorum* and three species of *Lactococcus lactis* (*lactis, diacetylactis, and cremoris*) activated non-specific immune system and decreased the colonization of *Shigella dysenteria*e in liver and spleen of swiss mice (Singh and Kansal, 2003). Shandilya et al. (2016) demonstrated that probiotic dahi (*L. acidophilus* and *B.bifidum*) intervention prevented the whey protein induced hypersensitivity with decrease in IgE and shifts Th2 immune response towards Th1 specific immune response. Probiotic dahi also maintained the immune homeostasis and imparted notable beneficial effects on aging immune system (Kaushal and Kansal,2011) who showed that probiotic fermented dahi reversed the age related decline in immune functions through stimulating lymphocytes and down regulating the IL-6 and TNF-α production in ageing mice. On the other hand, shrikhand also served as a carrier of different probiotics microbial species (*L. acidophilus* and *L.rhamnosus*) which may promote the gastrointestinal health and health benefits in lactose-intolerant individuals (Sivasankari et al. 2017). Consumption of shrikhand improves the immunity and increases the IgG in albino mice (Subramanian et al. 2005).

Yogurt

Yogurt is the utmost prevalent fermented dairy product recognized from centuries and has global acceptance due to its health promoting effects. In general, yogurt considered as a healthy fermented food due to its good digestibility and nutrient availability of proteins with higher biological value, energy, and potential source of essential amino acids, micro and macro nutrients that are required for good health. According to, National Yogurt Association, yogurt defined as the final product that contains live (LAB) in amount $>10^8$ cells/g at the end of manufacture (Chandan et al. 1993). Yogurt is the most documented instance of best known food containing probiotics and defined as a coagulated milk product that resulted from the fermentation by *L.bulgaricus* and *Streptococcus thermophiles* in milk which has been traditionally consumed for several health benefits (Bourlioux et al. 1988 and Yoda et al. 2014). To date, number of studies designated to explain the positive effects of yogurt consumption on human health. Daily yogurt intake in healthy women boosted the cellular immune system (Meyer et al. 2006). Similarly, yogurt consumption improved the risk factor for number of diseases like cardiovascular disease, anti-diabetic properties, boost host immunity, and maintained microbial balance and lower renal disorders risk (El-Abbadi et al. 2014). Probiotic (*L. rhamnosus* GG) and prebiotic (dietary fiber) yoghurt was not simply a food gradient but also its consumption help in improvement of inflammatory bowel syndrome (IBS) and maintenance of fecal microflora in patients with reduction in putrefactive bacteria *Clostridium difficile* and *E. coli*. Thus probiotic yogurt was related with alleviation of IBS symptoms like constipation, abdominal pain, bowel movement frequency and flatulence after 6 weeks of consumption (Lee et al. 2013). Uyeno et al. (2008) also showed that lactobacilli containing yogurt intake for 20 days in healthy individuals restored the gut microbiota composition of two major bacterial populations such as *Bacteroids* with *Prevotella* and the *C. coccoides* with *E. rectalei* group. *H. pylori* infected children restored beneficial microbiota (*Bifidobacterium spp)* with the rise in IgA and reduction in inflammatory mediators (IL-6) in serum in 4 weeks of yogurt ingestion besides it helped in reducing gastric bacterial load and enhanced systemic and innate immunity (Yang et al. 2012). Moreover, Shadnoush et al. (2013) showed administration of probiotic-yogurt to IBD patients for 8 weeks resulted in decreased level of pro-inflammatory cytokines (TNF-α, IL-1β, CRP) with enhanced levels of anti-inflammatory cytokine (IL-6 and IL-10) in sera which was proved to be beneficial in the maintenance of homeostatic conditions and prevention of diseases. Natural yogurt consumers exhibited healthier metabolic profile with rise in fecal *Akkermansia* level in intestine which was associated with lower inflammation and reduced serum levels of CRP and MDA (Gonzalez et al. 2019). Additionally, clinical trials showed that yogurt intake was related with immunity and reduced the level of inflammatory mediators in pregnant women (Asemi et al. 2011). In this regard, it has been reported that yogurt modulates humoral immunity with enhancement in stimulated release of pro-inflammatory cytokine (Meyer et al. 2007). Chaves et al. (2011) demonstrated that probiotic yogurt activates anti-inflammatory cytokine (IL-10) and cellular immunity against acute intestinal TNBS-induced inflammation via regulating CD4 and CD8 T cells with the increase in TLR9 and decrease in TLR-4 expression in mice.

Koumiss

Koumiss is fermented dairy beverage of ancient origin known by several names koumiss, kumiss, kumis, kymis, kymmyz. It is traditionally made milk beverage of the horse milk originated from the nomads of Central Asia. Extensively formed in Russia, Turkic, Bulgar, Kazakhstan in Western Asia predominantly for its health promoting potential. Mangolia considered it as a national drink and called as Airagand distilled koumiss called as Araka. Koumiss has mild alcoholic and sour taste. Koumiss and kefir are almost related as both are prepared from yeast and lactic acid fermentation except it is manufactured from liquid starter in contrast to kefir grains (Ørskov et al. 1995). Thus Koumiss undergoes two main fermentations, namely lactic acid fermentation and alcohol fermentation (Chen et al. 2010), and these changes produce a distinctive sour, alcoholic favor. Mare milk has strong laxative effect due to this it is not consumed raw, instead fermented in koumiss. Mare's milk contains more lactose as compared to cow's milk and lactic acid bacteria in koumiss ferment lactose to lactic acid, ethanol, and carbon dioxide. Koumiss microflora contain lactic acid bacteria (*L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus*), lactose-fermenting yeast (*Saccharomyces* spp. *K. Marxianus* var. *marxianus* and *Candida koumiss*), non-lactose-

fermenting yeast (*Saccharomyces cartilaginous*), and non-carbohydrate-fermenting yeast (*Mycoderma* spp.) (Wszolek et al. 2006). Hence, the milk becomes well tolerated by the people with lactose intolerance as compared to raw milk products. Based on lactic acid and ethanol content, it is characterized into mild, medium and strong. Koumiss due to higher alcoholic content is stated as milk wine (Dhewa et al. 2015).

Probiotic bacteria contain various surface markers their cross talk with host intestinal epithelial cells initiates the immunological response (Sanders et al. 2018). Koumiss containing probiotic strains have also been shown to have good nutritional value impart beneficial and health endorsing effects. For example, Rong and colleagues(2015) showed the beneficial effects of *L. helveticus* NS8 as a neutraceutical product in amelioration of severe ulceration in mucosa and supported in keeping intestine healthy. Moreover, NS8 up-regulated the anti-inflammatory cytokine IL-10 in PBMC (peripheral blood mono-nuclear cells) and in LPS induced murine macrophage cell line (RAW 264.7). Another study also focused on gastrointestinal resistance towards infections, as novel *Lactobacillus casei Zhang* (LcZhang) in koumiss enhanced gut local immune response with increased sIgA, it inhibited the attachment of virus and bacteria to intestine. Additionally, *L.casei Zhang* administration also influenced systemic immunity with an increased IFN-γ and decreased TNF-α in circulating blood of mice which showed immune enhancing effects (Ya et al. 2008). Traditional koumiss described to contain dominant LAB strains i.e. *L. helveticus* and *L. fermentum* which also showed antibacterial and antifungal activities (Ispirli et al. 2017). Koumiss being used as a functional food also had anti-hypertensive activity of peptides which helped in promotion of cardiovascular health enhancing effects (Chen et al. 2010). In a clinical study it has been found that 60 days koumiss consumption by patients alleviated the signs of chronic atrophic gastritis as verified with a reduction in blood cholesterol and platelet levels via modulating gut microbiota (Li et al. 2017). Koumiss is an utmost digestible food due to the presence of high percentage of whey protein: casein ratio as well as presence of spongiform structure of protein that enhance digestibility and its consumption has beneficial effects in treating atopic dermatitis, and chronic diseases of GIT (Barreto et al. 2019). Thus koumiss have shown positive effects on the kidneys, liver, endocrine glands, blood formation organs, and the digestive, nervous, immune and cardiovascular systems in addition to healing effects on disorders such as anemia, a-vitaminosis, gastric and intestinal diseases (Sanlier et al. 2019)

Kefir

Kefir 'fermented dairy drink' originated from Northern Caucasus, Mongolian, Tibetan mountains thousands of years back and is different and less popular than other fermented milk products such as yogurt, cheese. It is manufactured through traditional fermentation of milk based on starter-kefir grains that contain various bacteria and yeast which live in symbiosis that influence organoleptic and sensory characteristics of drink. Kefir is made from raw milk of cow, goat, sheep and buffalo containing kefir grains and has raised attention in the scientific community due to its beneficial effects on health (Rosa et al. 2017). Kefir created from the Slavic word keif which means 'wellbeing' for those who consume it. Kefir grains contain starter culture mainly *Lactobacillus kefiri*, species of genera *Leuconostoc, Lactococcus* and *Acetobacter* and also constitute lactose fermenting (*Kluveromyces marxianus*) and non-fermenting (*Saccharomyces unisporus, Saccharomyces cerevisiae* and *Saccharomyces exiguous*) yeast. According to codex standard (243-2003) kefir contain milk protein 2.7%, milk fat <10%, lactic acid 0.6% and total microorganism present $<10^7$ in cfu/ml, yeast should be present not less than 10^4 cfu/ml (Codex Alimentarius Commission, 2011) and having acidic and yeasty flavor. Yeast produces vitamins, amino acids and other essential growth factors that help in promotion of bacterial growth. Kefir components have multiple bio-therapeutic properties that help in maintenance of immune response and several health related parameters. Vinderola et al. (2006) proved that kefir activated immune system with down-regulation of Th2 response that promoted cell mediated immune response against tumor and pathogenic infection in mice. Previous studies performed by Bellikci-Koyu et al. (2019) showed that consumption of kefir frequently for 12 weeks in patients having metabolic syndrome displayed microbiota alteration with the increase in *Actinobacteria* without any changes in *Bacteroidetes* and *Proteobacteria* population. Kefir possessed antioxidative, antibacterial and hepato-protective activity against CCl_4 induced liver toxicity in mice (AbdEl-Mogheith et al. 2017). Kefir also has antimicrobial activity against foodborne pathogens (Kim et al. 2016). Oral administration of kefir in young rats daily improved the intestinal mucosal immune response against cholera toxin but not in senescent rats (Horeux et al. 2001). Kefir isolated strain *L. kefir* and its surface layer protein (S-layer) *in-vitro* confirmed to be safe that antagonize the invasion of *Salmonella enterica* in Caco-2/TC-7 cells(Golowczyc et al. 2007). Furthermore, S-layer protein from probiotic kefir lactobacilli also proved to have cytotoxic effect against clostridial toxins on Vero cells (Carasi et al. 2012). Likewise, *L. kefiri* CIDCA 8348 derived from kefir established as a good candidate for the gut related disorders and helped in the maintenance of intestinal homeostasis with the up-regulation of IgA and anti-inflammatory cytokine (IL-10) and mucin-6 gene (Carasi et al. 2015). Vinderola et al. (2005) described the immunomodulatory capacity of kefir and pasteurized kefir and found that both had similar effects in modulating gut mucosal immunity in dose-dependent manner through the metabolites released during fermentation and achieving balance between Th1 and Th2 response. Likewise *L. lactis* subs., *Leuconostoc* subs., *Streptococcus thermophilus, Lactobacillus* subs., and yeast of kefir involved in its fermentation showed dose dependent effects, where 10% kefir on 14 days feeding reduced diarrhea and bloody stool symptoms in TNBS-induced colitis rats in contrast to 30%

kefir which aggravated the TNBS-induced response (Sevencan et al. 2019). Additionally, *L. kefiranofaciens* M1strain of kefir acted through TLR-2 receptor and decreased pro-inflammatory cytokines leading to strengthening of the epithelial barrier in DSS induced colitis (Chen et al. 2012).Similarly, *L. delbruekii* CYC 10047 strain from kefir displayed strong adhesion ability to Caco-2 cells and strongly prevented the *Salmonella typhimurium* attachment to the cells (Santos et al. 2003).

Cheese

Cheeses have higher shelf life amongst fermented dairy foods and commonly available in the market such as other fermented milk and yogurt products. Presently, functional cheese is popular as a better alternative food matrix for probiotic microbes where required viable counts can be maintained to provide therapeutic benefits (Boylston et al. 2004). Mostly probiotic cheese have *Bifidobacteria* and *Lactobacillus.* Cheeses exhibit higher pH as compared to fermented milk, also provide good ripening environment which is almost anaerobic. The high fat content of cheese helps in passage of probiotic bacteria across the gut peptic environment (Corbo et al. 2001). Boylston et al. (2004) explained that cheese offers an environment for long-term survival of *Bifidobacterium* that helped in the maintenance of sensory characteristics. Cheese processing involves addition of rennet, lactic acid bacteria, proteases and peptidases from secondary microbial ûora to break down casein and produce bioactive compounds that are responsible for a wide range of biological activities (López-Expósito et al. 2017). Cheddar cheese has been considered as a matrix for various commercial probiotic cultures *L. rhamnosus, L. casei, L. paracasei* and all *Bifidobacterium* species which remained viable at 10^6-10^7 CFU/g after 32 weeks of storage (Phillips et al. 2006). Production of probiotic cheese is not the problem but survival of probiotics with required number is the main issue. Numerous types of cheeses are prepared by incorporating the probiotic bacteria such as cheddar, Gouda, fresco, white cheese etc. (Karimi et al. 2012).

Cheese's vitamins, minerals together with peptides and other biologically active compounds such as CLA are mainly responsible for its effects in preventing and treating diseases (Hur et al. 2017). Cheese's anti-carcinogenic characteristics originate from the CLA and sphingolipids it contains (Walther et al. 2008).Consumption of probiotic cheese attenuates the immune suppression in rats and infection illness (Lollo et al. 2012) along with reinforcement of intestinal health (Medici et al. 2004). Several probiotic LAB were isolated from artisanal Cocido Mexican cheese may be used as a good candidate in milk fermentation that modulates the immune system with the up-regulation in IgA, IL-10, IL-6 in rats (Santiago-López et al. 2018).Among all fermented milk products cheese has the utmost variability in market, most prominently increases probiotic survival during passage through gastrointestinal tract with a reduction in highly acidic gut environment (Da Cruz et al. 2009). Kim et al. (2019) evidenced that *L. chungangensis* CAU28 cream cheese modulate gut microbiota with the rise in short chain fatty acid level along with an increase in butyrate producing bacteria. Moreover, suppression of T cell mediated Th2 response along with reduction in IgE level in the atopic dermatitis mouse model was observed with the CAU28 cream cheese administration (Kim et al. 2019). Consumption of cheese prepared with *L. rhamnosus* HN001 and *L. acidophilus* NCFM having 10^9 CFU/day significantly enhanced the innate immunity in the elderly population with increased cytotoxicity of natural killer cells to kill the tumor cells. Moreover, increased phagocytic activity in addition to immune-stimulation was also observed in cheese fed to elders due to presence of starter culture (*L. rhamnosus* HN001 and *L. acidophilus* NCFM)(Ibrahim et al. 2010). Sharafedtinov et al. (2013) found in clinical study that protein rich full fat probiotic cheese (*L. plantarum* Tensia) supplemented in a hypo-caloric diet lowered the blood glucose levels and also reduced the total cholesterol, TAG (triglyceride) or LDL (low density lipoprotein). Thus, it was concluded that consumption of cheese along with hypo-caloric diet had the potential to reduce the symptoms of metabolic syndrome in obese patients with hypertension and aided in reduction of body mass index and arterial blood pressure. Furthermore, Cheddar cheese also used as a matrix for probiotic (*L. plantarum* K25) delivery and suggested to diminish the risk for cardiovascular diseases by lowering the total cholesterol, triglycerides, LDL in high fat mouse model (Zhang et al. 2013). Patrignani et al. (2019) also reported that Squacquerone probiotic cheese (Italian fresh cream cheese) containing *L.crispatus*BC4 is good source of calcium, vitamins with antimicrobial properties for food borne pathogens, which was found valuable for women well-being because it's consumption modulated or simulated gastrointestinal and genitourinary microbial ecosystem because of its ability to survive in gastrointestinal tract.

Conclusions

Fermented dairy foods have long been showed beneficial effects on gastrointestinal health that strengthen the immune system. Diseases related to gut have been growing due to western lifestyle, dietary habits of commercial foodstuffs which not only increases intestinal permeability, transcytosis of antigens but also leads to inflammation, ulceration and apoptosis. Food play a major role in modulation of body physiological function because of continuous interaction of dietary antigens with an immune system. Fermented dairy foods have potential health promoting effects on body in various conditions like diarrhea, obesity, high cholesterol, cardiovascular health etc. Fermentation process increases the bioactive peptides, level of vitamins and also other compounds produced by bacteria which imparts the beneficial effects to thebody. Pre-clinical and clinical trials in support to health has proved that administration of fermented dairy foods with or without probiotic bacteria have a pivotal role in human health by preventing or ameliorating the inflammatory conditions which was evidenced through a wide range of strains and their

doses. Fermented products increase the intestinal tight junction barrier functions in IEC with increase in mucus, sIgA secretion and antimicrobial peptides. Thus fermented dairy foods promise to be, 'functional food package' with increased shelf life and sensory attributes for the benefit of mankind.

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RESEARCH ARTICLE

Development of synbiotic ice cream from goat milk

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Abstract: Synbiotic ice cream was developed from goat milk by using inulin as a prebiotic and *Lactobacillus plantarum* (UBLP-40) as a probiotic. The treatment mix was formulated to contain more than 10% fat, 8.6% milk solids not fat, 2% inulin, 15% sugar, 0.4% stabilizer-emulsifier combination and probiotic *Lactobacillus plantarum* culture. Physico-chemical, microbiological and sensory qualities of control and synbiotic ice cream were compared. Synbiotic ice cream showed higher whipping ability ($p<0.05$) than control. Synbiotic ice cream was superior in sensory qualities than control during 30 days of storage. There was no significant difference in fat, protein, meltdown time and weight per litre.

Keywords: Functional ice cream, Goat milk ice cream, Inulin, *Lactobacillus plantarum*, Synbiotic ice cream

Introduction

Ice cream is the most popular dairy product widely consumed by all age groups of people. Probiotics are live microorganisms when administered in sufficient amounts give a number of health benefits to the host. The important criterion for the successful development of probiotic products is selection of resistant probiotic strains which can maintain their survivability during commercial production and storage. Prebiotics are non-digestible food ingredients that stimulate the growth of probiotic bacteria (Bindels et al. 2015). Inulin and inulin-type fructans are soluble fibers that can delay absorption of glucose and enhance glucose metabolism and capable of modulating gastric evacuating and intestinal transit time (Wilson and Whelan, 2017). Synbiotics are appropriate combinations of probiotics and prebiotics (Cencic and Chingwaru 2010). Incorporation of probiotic cultures in goat milk products can also mask the unpleasant goaty flavor due to the production of flavor compounds and can improve the rheological properties of goat milk products (Ranadheera et al. 2019). Ice cream is a better carrier for probiotic bacteria when compared to fermented dairy products. The pH of ice cream is higher than that of regular fermented milk, which will facilitate the survival of probiotic bacteria (Ahmadi et al. 2014).

Approximately 12.2 million metric tons of goat milk is produced annually which contributes to 2% of all world production by the dairy industry. India ranks first in goat milk production. Goat milk has many advantages such as better digestibility, lower allergenic properties and stronger antimicrobial characteristics when compared to cow milk. However, no significant effort has been made in India for the production of functional dairy products from goat milk. Development of commercially viable functional dairy product such as synbiotic ice cream from goat milk will improve the utilization of goat milk. *Lactobacillus plantarum* was found to be a versatile species with many beneficial properties (Behera et al. 2018). Synbiotic ice cream prepared by using probiotic *Lactobacillus plantarum* was found to have superior viscoelastic properties and slower melting rate (Siti Radhiah Omar and Siti Nazirah Omar 2018). Therefore, this study was designed with an objective to develop synbiotic ice cream using inulin as prebiotic and *Lactobacillus plantarum* as probiotic and evaluating the physico-chemical, microbiological and sensory qualities of the product.

Materials and Methods

Fresh goat milk was procured from University Goat and Sheep farm, Mannuthy. Cream was separated from goat milk by using a centrifugal cream separator. Skimmed milk powder (sagar®), sugar

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and flavour (bush®) were purchased from local market. The stabilizer and emulsifier used was cremodan sampoorna® of Danisco. Inulin was procured from Amrutha Herbals private limited, Indore. Probiotic *Lactobacillus plantarum* UBLP-40 (DVS) culture was provided by Unique Biotech, Hyderabad.

Analysis of dairy ingredients

The fat per cent in milk, skim milk and skimmed milk powder were determined by the procedure described by AOAC (2016). The fat content of cream, total solids content of milk, cream, skim milk and skimmed milk powder were estimated by the procedure described by BIS (1981).

Preparation of ice cream

Ice cream was prepared as per the procedure suggested by Akin et al. (2007). The proportionate quantity of different ingredients to meet the minimum standards for fat and total solids as per Food Safety and Standards Act was calculated and the mix was prepared. It was pasteurized at a temperature of 83ºC for 25 seconds. Homogenization of the mix was done at a temperature of 65ºC by using a pressure of 2500 PSI at first stage and 500 PSI at the second stage. The mix after homogenization was immediately cooled to 4ºC and later transferred to a cold storage maintained at a temperature of 4 ± 1ºC for ageing. Vanilla (bush®) flavor at the rate of 2ml per kg of the mix was added to the mix and mixed well. Probiotic culture *Lactobacillus plantarum* was added just before freezing. The ice cream mixes in each treatment group were frozen individually using a softy ice cream freezer (Technogel HMT®). The ice cream was collected in 500 ml poly propylene containers. Then it was stored at -20±1ºC.

Standardization of level of inulin for the preparation of synbiotic ice cream

Three treatment formulations were evaluated during the preliminary trials in which inulin was incorporated at 2(T1), 2.5(T2) and 3(T3) per cent levels. The results of sensory evaluation of different product formulations are presented in Table 1. From the results, two per cent inulin (T1) was selected as it had shown better sensory acceptability with minimum level of inulin.

Synbiotic ice cream mix

Synbiotic ice cream was prepared by using selected level of inulin as prebiotic along with *Lactobacillus plantarum* UBLP-40 (10^{13}cells/g) DVS culture. Inulin was added along with other dry ingredients during the preparation of mix as shown in Table 2.

Physico- chemical properties of ice cream

The titratable acidity of control and treatment groups of ice cream was determined by the procedure outlined by BIS (1981). The pH of the ice cream samples were analysed by digital pH meter (Hanna HI2020). The percentages of total solids, protein and fat were determined by the procedure outlined by AOAC (2000). While the mix was being frozen in a softy ice cream freezer, mix was drawn at five minutes intervals up to ten minutes and weighed. The loss of weight of the mix due to air incorporation was recorded. Hundred grams of ice cream was carefully placed on a four square inch glass plate rested on the brim of glass funnel, fitted on a metal stand with its tail end leading into a 100 ml graduated cylinder. The time taken for complete meltdown was recorded. Weight per litre of ice cream was estimated using the procedure outlined in BIS (1986). The probiotic count of ice cream during 0, 15 and 30 days of storage was performed as per the procedure described by Inoue et al. (1998). Serial dilutions of the ice cream sample was done and one milliliter of diluted inoculum was transferred to sterile Petri dish. Then 20 ml of sterile molten MRS agar maintained at 45 ºC was poured and mixed with inoculum. The plates were incubated at 37 ºC for 48 hours. After incubation the plates showing 30-300 yellow coloured oval to round colonies were selected and counts were taken with the help of the colony counter. The coliform count of ice cream during 0, 15 and 30 days of storage was performed as per BIS (1981). The sensory evaluation was done by using the score card as per Homayouni et al. (2008). The data obtained were subjected to statistical analysis using the software SPSS version 24 as per the procedure suggested by Snedecor and Cochran (1994).

Results and Discussion

Synbiotic ice cream was prepared by using 2 per cent inulin and *Lactobacillus plantarum*. Control ice cream was prepared without these functional ingredients. The control and synbiotic ice cream were compared based on physico- chemical, microbial and sensory qualities.

The mean pH, titratable acidity, total solids, fat, protein, weight per litre, whipping ability and meltdown time values are presented in Table. 3 and 4. The mean pH of control and synbiotic ice creams was 6.383 ± 0.035 and 6.416 ± 0.043 respectively for 0^{th} day of storage. The pH on 30^{th} day of storage was 6.35 ± 0.055 and 6.416 ± 0.056 respectively. There was no significant difference in pH between control and synbiotic ice cream. The mean titratable acidity of control and synbiotic ice cream was 0.137 ± 0.012 and 0.126 ± 0.018 per cent lactic acid on 0^{th} day and 0.156 ± 0.007 and 0.127 ± 0.005 per cent lactic acid on 30^{th} day of storage respectively. There was no significant difference in titratable acidity between control and synbiotic ice cream. The mean total solids for control and synbiotic ice creams were 40.689 ± 0.987 and 40.99 ± 2.32 per cent respectively on 0^{th} day of storage. The total solids on 30^{th} day of storage were 37.917 ± 1.434 and 37.9 ± 0.905 per cent respectively. Even though there was a decrease in total solids content during storage it was not statistically significant. Similar results were reported by Turgut and Cakamakci (2009). In their study, the total solids content of ice cream samples was not influenced by the presence of the probiotic strains and there was

no significant change in total solids content during storage. The mean fat content of control and synbiotic ice creams were 10.1 ± 0.57 and 10.1 ± 0.16 per cent respectively on 0th day of storage. The fat content on 30th day of storage were 10 ± 0.13 and 10.2 ± 0.25 per cent respectively. No significant difference in fat per cent was observed between control and synbiotic ice cream. The mean protein content of control and synbiotic ice creams were 3.94 ± 0.18 and 3.71 ± 0.36 per cent respectively on 0th day of storage. The protein content on 30th day of storage were 3.95 ± 0.203 and 3.73 ± 0.152 per cent respectively. Protein content showed no significant difference between control and synbiotic ice cream. Turgut and Cakamakci (2009) also observed that fat and protein content in ice cream samples were not affected by the presence of probiotic strains. The mean values of weight per litre of control and synbiotic ice creams were 727.66 ± 18.76 and 707.39 ± 15.63 g/l respectively. There was no significant difference in weight per litre between control and synbiotic ice creams ($p>0.05$). The mean values of whipping ability of control and synbiotic ice creams were 31.43 ± 0.98 and 37.65 ± 0.63 per cent respectively. The whipping ability of synbiotic ice cream was significantly higher ($p<0.05$) than that of control ice cream. Akalin and Erisir (2008) had also reported higher overrun in synbiotic ice cream than control. This may be due to the ability of inulin to incorporate air. The mean meltdown time for control and synbiotic ice cream was 50.83 ± 2.89 and 54.83 ± 3.02 minutes respectively on 0th day of storage. The meltdown time on 30th day of storage was 53.17 ± 1.9 and 50.5 ± 3.71 minutes respectively. No significant difference in meltdown time was observed between control and synbiotic ice cream. Silva et al. (2015) had also reported no significant difference in the melting behavior of the probiotic goat milk ice cream when compared to control. However, several earlier researchers have reported increase in meltdown time in synbiotic ice cream. Akin (2005) reported that addition of inulin caused an improvement in meltdown characteristics in synbiotic ice cream.

Table 1 Sensory attributes of synbiotic ice cream incorporated with different levels of inulin

Sensory scores	Control ice cream	Synbiotic ice cream incorporated with inulin		
		T1(2% inulin)	T2(2.5% inulin)	T3 (3% inulin)
Flavour system	9 ± 0.25[a]	9.17 ± 0.3[a]	9 ± 0.36[a]	6.5 ± 0.42[b]
Body and texture	4.17 ± 0.16[a]	4.5 ± 0.22[a]	4.33 ± 0.21[a]	4.5 ± 0.22[a]
Color and appearance	4.83 ± 0.16[a]	4.67 ± 0.21[a]	4.83 ± 0.16[a]	4.5 ± 0.22[a]
Total scores	17.83 ± 0.3[a]	18.33 ± 0.21[a]	18.17 ±0.3[a]	15.5 ±0.71[b]

Means bearing different superscripts within the same row differ significantly ($p<0.01$)

Table 2. Formulation of different ice cream mixes (2 litre)

Ingredients	Control	Synbiotic ice cream
Milk(ml)	1277	1351
Cream (g)	349	287
SMP (g)	74	22
Sugar (g)	290	290
Stabilizer (g)	8	8
Inulin (g)	-	40

Table 3 Physico-chemical properties of synbiotic ice cream

Type of ice cream	Storage days	pH	Titratable acidity(%)	Total solids (%)	Fat(%)	Protein (%)	Meltdown time (minutes)
Control	0	6.383 ± 0.035	0.137 ± 0.012	40.689 ± 0.987	10.1 ± 0.57	3.94 ± 0.180	50.83 ± 2.89
	15	6.383 ± 0.057	0.146 ± 0.008	37.976 ± 1.523	10.1 ± 0.45	3.94 ± 0.197	54.50 ± 3.99
	30	6.350 ± 0.055	0.156 ± 0.007	37.917 ± 1.115	10.0 ± 0.13	3.95 ± 0.203	53.17 ± 1.90
Synbiotic ice cream	0	6.416 ± 0.043	0.126 ± 0.018	40.990 ± 2.32	10.1 ± 0.16	3.71 ± 0.360	54.83 ± 3.02
	15	6.406 ± 0.082	0.132 ± 0.010	38.782 ± 0.620	10.2 ± 0.16	3.71 ± 0.159	51.33 ± 3.72
	30	6.416 ± 0.056	0.127 ± 0.005	37.900 ± 0.905	10.2 ± 0.25	3.73 ± 0.152	50.50 ± 3.71

Table 4 Physical properties of synbiotic ice cream

Type of ice cream	Weight per litre(g/l)	Whipping ability (%)
Control	727.66 ± 18.76	31.43 ± 0.98[b]
Synbiotic ice cream	707.39 ± 15.63	37.65 ± 0.63[a]

Table 5 Microbial quality of synbiotic ice cream

Type of ice cream	Probiotic count (Log cfu/g)		
	0th day	15th day	30th day
Synbiotic ice cream	8.917 ± 0.151	9.146 ± 0.233	8.431 ± 0.365

No significant difference ($p>0.05$)
Means are averages of six replications

Table 6 Coliform count (log cfu/g) of synbiotic ice cream during storage

Type of ice cream	Coliform count (Log cfu/g)		
	0th day	15th day	30th day
Control	1.330±0.470	1.558±0.611	1.418±0.516
Synbiotic ice cream	1.143±0.393	1.460±0.541	1.446±0.552

No significant difference between control and treatment ($p>0.05$)
Means are averages of six replications

Table 7. Sensory evaluation of synbiotic ice cream

Type of ice cream	Storage days	Flavour (1-10)	Body & texture (1-5)	Colour& appearance (1-5)	Total (1-20)
Control	0	9.07 ± 0.2	4 ± 0.13	4.67 ± 0.12	17.73 ± 0.3
	15	8.73 ± 0.2	4.07 ± 0.2	4.53 ± 0.13	17.73 ± 0.3
	30	8.87 ± 0.23	3.93 ± 0.15	4.47 ± 0.13	17.27 ± 0.41
Synbiotic ice cream	0	9.13 ± 0.19	4.33 ± 0.15	4.67 ± 0.12	18.13 ± 0.33
	15	9.2 ± 0.1	4.27 ± 0.15	4.53 ± 0.13	18 ± 0.21
	30	9.07 ± 0.26	4.53 ± 0.13	4.6 ± 0.13	18.2 ± 0.44

The mean probiotic count is presented in Table. 5. The mean values of probiotic count in synbiotic ice cream during 0th, 15th and 30th day of storage were 8.917 ± 0.151, 9.146 ± 0.233 and 8.431 ± 0.365 log cfu/g respectively. No significant difference was observed in probiotic count during the entire storage period of 30 days. The viability of probiotic organism was maintained above the therapeutic minimum throughout the storage period. This could be attributed to the ability of inulin to improve the survivability of probiotic organism. Pandiyan et al. (2012) also found that incorporation of inulin enhanced the growth of *Lactobacillus acidophilus* and it could maintain at a therapeutic minimum of 10^6cells/g for a storage period of 15 days at -18 to -23°C in ice cream.

The mean coliform count of control and synbiotic ice creams were1.33±0.47 and 1.143±0.393 log cfu/g respectively on 0th day of storage. The corresponding values on 15th day of storage were 1.558±0.611 and 1.46±0.541 log cfu/g respectively. The values on 30th day of storage were 1.418±0.516 and 1.446±0.552 log cfu/g respectively. There was no significant difference in coliform count between control and synbiotic ice cream. There was also no change in coliform count during storage. Modzelewska-Kapitula *et al.* (2007) studied the influence of inulin and probiotic *Lactobacillus plantarum* on the microbial quality of soft cheese. Numbers of coli forms were less than 10 cfu/g for the entire storage period. In the present study also coliform count could be maintained within the prescribed limit until the entire storage period of 30 days.

The mean sensory scores are presented in Table. 6. There was no significant difference in flavour, body and texture, colour and appearance and total scores between control and synbiotic ice cream. There was also no significant change in sensory scores during storage. According to Silva et al. (2015) the probiotic goat milk ice cream was highly accepted, and the viability of *B. animalis* was maintained during the storage period of 120 days at "18°C. According to Mituniewicz–Małek, Zielinska and Ziarno (2019), the fermented goat milk produced with *L. plantarum* presented the highest acceptability mainly because of the highest intensity in smell, milky fermentative taste, and smoothness. Similar findings were also reported by Akin et al. (2007). They had reported no adverse effect on the sensory properties of probiotic ice cream incorporated with inulin at 1 or 2 per cent concentrations.

Conclusions

Synbiotic ice cream was successfully developed from goat milk by using inulin as a prebiotic and *Lactobacillus plantarum* UBLP-40 as a probiotic. This ice cream showed higher whipping ability than control and good sensory acceptability. There was no significant difference in pH, titratable acidity, total solids, fat,

protein, meltdown time and weight per litre. The probiotic count could be maintained above the minimum recommended level until 30 days of storage.

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RESEARCH ARTICLE

Development of modified aseptic pouch form fill seal machine

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Abstract: Aseptic packaging consists of filling sterile products into a sterilized packaging material at room temperature followed by sealing the packaging material in a sterile environment. A small scale modified aseptic pouch form fill seal machine for UHT milk with all necessary instrumentation was developed and evaluated for performance. The sterile air temperature used was 105 °C. The designed length, width and height of the hydrogen peroxide bath were found to be 0.38 m, 0.28 m, and 0.34 m respectively. The length of folding section taken was 0.51 m and that of width was 0.15 m which has been bent from both sides. The developed machine is hand and pedal operated and the capacity of machine was 120 l/h. The UHT milk was packed in polypropylene. The aseptically packed UHT milk was tested for microbiological effectiveness and the milk was found to be microbiologically safe up to two weeks. From the organoleptic point of view, the aseptically packed milk was acceptable up to 15 days, as it was free from gelation and drastic color change.

Keywords: Air sterilizer, Aseptic packaging, form fill seal machine, Hydrogen peroxide bath, Sealer, UHT milk

Introduction

India is the largest milk producing country of the world with annual milk production of 187.7 million tonnes - 20% of world milk production. Milk is often described as a complete food as it contains proteins, carbohydrates, fats, vitamins and minerals. Milk preservation prior to distribution and sale is a major problem in India due to the high temperatures of tropics and lack of sufficient cold chain transportation system. Ultra High Temperature (UHT) sterilization of milk followed by aseptic packaging enhances the keeping quality of milk. Ultra high temperature (UHT) technology is widely used in food industries, mainly in Western countries (Poliseli-Scopel, et al.; 2014). Aseptic packaging of sterilized milk is a packaging technology in which pre-sterilized product is aseptically packed in a sterile environment designed to provide outstanding product protection. Aseptic or long-life milk was originally introduced in Sweden called the "Tetra-pack" system (Sanjana, et al 2019). Aseptically packed milk eliminates the need for expensive refrigeration methods, and milk can be stored at room temperature and transported to longer distances. (Ansari, 2011,2016 & 2017). Since aseptically packed milk does not need refrigeration, milk availability to consumers is increased, capital investment in cold storage and insulated vehicles for storage and transportation is eliminated and in the present day of energy crisis a large amount of electrical energy may be saved. Aseptic processing and packaging is of considerable interest as it involves the production of high quality liquid foods (Betta, et al.; 2011).

The need for aseptic packaging is rapidly increasing and the system has proved to be invaluable in the distribution of milk. The demands of high quality shelf stable, and safe food has created huge development of aseptic processing and packaging (Moruzzi et al. 2000; Ansari et al. 2004 and Marquis and Baldeck, 2007). As aseptic packaging considers the filling with of a commercially sterile food in a sterilized package under aseptic conditions, the sterility of the inner package surface is a major requirement to ensure a shelf-stable product (Delgado et al.; 2012).

The use of hydrogen peroxide for the sterilization of packaging surfaces was approved by the FDA in 1981. Hydrogen peroxide is an effective sterilant with potential applications in the decontamination of food packaging materials (Ansari, 2018).

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UHT processing and aseptic filling of milk and milk based products, and fruit juice are being practiced throughout the world particularly in the countries with warm climates.

Adoption of aseptic packaging is growing exponentially worldwide, especially in Europe and Asia, and is on the rise in the U.S. The demand for ultra-high-temperature (UHT) processed and aseptically packaged milk is increasing worldwide (Chavan et al. 2011). UHT dairy foods represent a large share of the market in Germany, France, Italy, and Spain. The market share of UHT milk consumed varies considerably by country: Australia 9%, France 88%, Spain 83%, Germany 63%, Italy 55%, and UK 5 to13% (Datta and Deeth, 2007). Form fill seal aseptic pouch packaging systems are commonly used around the globe (Datta and Deeth, 2007). In this system, packaging material is sterilized by dipping in hot hydrogen peroxide. The lower cost of flexible packaging materials compared with metal for can has stimulated increased interest in form fill seal aseptic packaging machine. The single layer pouch is the most effective packing format due to its design, layout and cost. Advantages of aseptic pouch packaging are in product quality, low package cost, consumer convenience in terms of package carrying weight, non-fragile containers, easy to open and dispose, less space requirement and better appearance, long unrefrigerated product shelf life at normal room temperatures without preservatives, contamination-free product and maintenance of natural color and taste. The main limitations of adoption of aseptic processing and packaging are the cost and complexity of the plant which arises from the necessity to sterilize packaging materials associated pipe work and tanks, the maintenance of sterile air and surfaces in filling machines and the higher skill levels required by operators and maintenance (Fellows, 2000). At present the aseptic packaging machines are imported and too expensive. High cost of machinery and use of laminated multilayer packaging materials are the root cause of higher price for sterilized milk as compared to that of pasteurized milk. From economy point of view, imported machines are not at all suitable for rural Indian conditions. The main objective of this study was to develop a small scale aseptic pouch form fill seal machine for UHT milk.

Materials and Methods

Design considerations of aseptic pouch form fill seal machine

The aseptic pouch form fill seal machine consists of film conveying rolls, hydrogen peroxide bath, UV tube, folding structure, vertical sealer, horizontal sealer, pedal, handle, rubber roll, gear, milk filling tube, air jacketed tube and funnel.

Film conveying roll

Feed roll was used to convey the film in forward direction to the shoulder through a hydrogen peroxide bath. The concentration used was 8 % w/w at a temperature of 77 °C. Three rolls are fitted in angle iron frame. One roller is attached just after feed roll and, one after bath and another one before shoulder. These rollers provide tension and smoothness in movement without wrinkling. The length of roll was selected based on film width with some extra margin for ease in fitting. The roller is made of Aluminum. Nine rollers are fitted inside hydrogen peroxide bath to get desired residence time of packaging material.

Hydrogen peroxide bath

The sterilization of packaging material is prerequisite of aseptic packaging machine. The film was sterilized using commercially available food grade hydrogen peroxide. The main function of hydrogen peroxide bath was to hold the hydrogen peroxide to sterilize the packaging material. The bath was made of stainless steel grade 316L to avoid any chemical reaction with hydrogen peroxide. The desired temperature of bath was maintained using immersion water heater to increase efficiency of sterilization. The bath was designed based on assumption that film width was of 0.32 m and optimum residence time of film which was obtained from sterilization of plastic film at various time, temperature and concentration combinations. The residence time of 90 second with linear speed of film of 0.025 m/s was taken to get travel length of 2.25 m. A spring-loaded squeezer is used at the end of bath to remove excess peroxide. A UV tube is used just after end of bath to enhance the effectiveness of sterilization. Length and width of bath were estimated using following equations:

$$L = W_f + 2M_l$$

Where,

L = Length of bath, m
W_f = Width of packaging material, 0.32 m
M_l = Margin in length of bath, 0.03 m

$$W = 4S_r + 5d_r + 2M_w$$

Where,

W = Width of bath, m
S_r = Spacing between roll along width of bath, 0.037 m
M_w = Margin in width of bath, 0.016 m
d_r = Diameter of conveying roll, 0.02 m

Folding structure

It is an important part of aseptic packaging machine where plastic film is folded into a tube. Folding structure was made of stainless steel grade 316 L of thickness 0.005 m. This part was rigidly fixed with frame. This part was so designed that there should not be any wrinkle while tube forming. The width was taken slightly less than half width of plastic film. A plate was embedded into it, which helped in vertical sealing. Milk filling tube and air jacketed tube were also fixed with this structure. It was designed based on film width.

Vertical sealer

The function of vertical sealer is to seal the film vertically of already formed film tube. A vertical nichrome plate was fixed with sealer body. The plate was covered with Teflon sheath. The length of sealer was decided based on dimension of 500 ml capacity pouch. The length of such pouch was normally 0.15 m. So 0.15 m length was selected and width of plate was taken as 0.002 m. For aseptic pouch packaging, polypropylene plastic film of thickness 80 µm was taken.

Horizontal sealer

The function of this sealer was to seal horizontally already formed film tube. The horizontal sealer acts as sealer and cutting unit. In horizontal sealer a nichrome wire was used. Based on suitability, wire of diameter 0.002 m was taken with length as 0.25 m. The effective width of sealer was 0.15 m. Rest of the length was used for fitting purpose. The wire was covered with Teflon sheath.

Rubber roll and gear

Four rubber rollers were used in aseptic packaging machine to pull the film. Two were fitted at the front side and two were at the back side. A handle was attached to the main shaft. For power transmission a pair of gear made of plastic with equal number of teeth were used. The diameter of both roll and gear was decided based on length of 500 ml milk pouch, which is 0.15 m, so that one revolution of roll would give one packet length. Based on this length, the designed diameters for roll and gears were selected. The length of pouch can be changed by varying number of revolution.

Milk filling tube, air jacketed tube and funnel

The filling tube, air jacketed tube and funnel were made of stainless steel of grade 316L. The length of both filling and air jacketed tube was decided based on length of folding structure with some margin. The length of both tubes was kept as 0.78 m as per design of machine dimension and other constraints. Inner tube was used for milk filling while outer tube was used for conveying sterile air to dry the film and maintain asepticity inside tube during filling. The dimension of funnel was taken based on suitability and availability in local market and attached with filling tube with the help of socket.

Fabrication of aseptic pouch form fill seal machine

To fabricate aseptic pouch form fill seal machine, first of all angle iron frame was made of desired dimension. Feed roll was fitted at rear end of frame and after that hydrogen peroxide bath was fitted by welding with frame. Horizontal and vertical sealer were fitted in a single frame. Folding structure was fitted at the top of the frame. Filling tube and air jacketed tube were fitted with folding structure. Funnel was fitted with filling tube with the help of socket joint. Pedal was fitted at the front side of machine and handle was fitted with the main shaft. Rubber roll and gear was mounted at both the shafts. Pedal was connected with lever arrangement for sealer movement. Two control panels were attached with machine to regulate current and voltage. A 12 V transformer was used for sealing. One immersion water heater of 1 kW was fitted in hydrogen peroxide bath. Nine rollers were fitted in bath so that desired residence time of packaging material could be achieved while aseptic packaging for perfect sterilization of film. A box was also fitted with removable attachment just in front of folding structure to create aseptic environment during aseptic packaging. Two UV tubes were fitted, one just after the end of bath and one inside the box.

Performance evaluation of aseptic pouch packaging machine

The machine was first cleaned and thirty litres of hydrogen peroxide solution was used for sterilization of packaging film. The concentration of hydrogen peroxide, residence time of film in bath and temperature of solution was taken as reported by Ansari and Rai (2017). Two 6 Watt lamps with the intensity of 0.5 Watt / cm^2 were used. The type of UV lamp used was UV-C lamp (Ansari and Datta, 2003), which is generally recommended for microbicidal use. Both the lamps worked at 240 V and one such lamp was located at the end of hydrogen peroxide bath to get completely rid of microorganisms and avoid any recontamination of the packaging material coming out of the bath. The lamp was kept above the plastic film fitted in aluminum box. The other lamp placed near the packaging material folding section to get the packaging material free of microorganisms to maintain asepticity near folding section. Air sterilizer developed by Ansari and Datta (2006) was used. The hot air temperature used was 105°C. Milk filling line and pouch forming assembly were sterilized using hot sterile air at 121 °C and UV lamp for 30 minutes as per recommendation. Asepticity was maintained by hot sterile air. After sterilization of all contact surfaces, the sterile air was supplied through an annular tube outside the product filling tube continuously to assure aseptic filling to a point immediately above the end of the filling tube, where it was deflected upwards to carry away the peroxide residues evaporated from the surface of the film which also prevents the entry of microorganisms and dried the film. One 1kW heater was wrapped around the funnel and kept ON during operation to maintain surface temperature of the funnel around 150^0C using temperature controller. This ensures the sterile environment above the neck of funnel. The product to be packed was introduced by a stainless steel filling tube. Nichrome wire covered with Teflon sheath was used in horizontal sealer, which work as bottom sealing for next pouch as well as cutting for already formed packet. One revolution of rubber roll gave a packet of capacity 500 ml. For testing purpose, in-container sterilized milk was used for packaging and packed samples were analyzed for bacterial count, colour change and viscosity change during storage.

Quality assessment of aseptic milk

Bacteriological study of aseptically packed milk samples was carried out during storage at 3 days interval up to 15 days to find presence of bacteria during storage at room temperature. Bacterial colony count was done by plate count method using dilution technique. Colour of milk changes during storage period due to browning reaction. To measure the extent of the colour change during storage period of aseptically packed milk, Hunter Lab Colorimeter was used for measuring yellowness index and whiteness index. The Whiteness Index measures the degree of whiteness of a product while the Yellowness Index measures the degree of yellowness of a product from degradable chemical reactions occurring during storage (Ansari et al. 2018). The viscosity of aseptically packed milk increases during storage, leading to gelation. This increase in viscosity is mainly due to presence of heat resistive enzymes. To estimate the shelf life of milk, viscosity was measured using Brookfield Dial Viscometer.

Results and Discussion

An aseptic pouch form fill seal machine was developed (Fig. 1) (Isometric view). The aseptic pouch form fill seal machine consists of film conveying rolls, hydrogen peroxide bath, UV tube, folding structure, vertical sealer, horizontal sealer, pedal, handle, rubber roll, gear, milk filling tube, air jacketed tube and funnel. Feed roll was used to convey the film in forward direction to the folding device via hydrogen peroxide bath. The length of roll selected was 0.37 m and diameter was 0.020 m. The length, width and height of hydrogen peroxide bath were found to be 0.38 m, 0.28 m and 0.34 m, respectively. The length of folding section was taken as 0.51 m while the width was 0.15 m which was bent from both sides. The length of sealer was decided based on dimension of 500 ml capacity pouch. The length of such pouch is normally 0.15

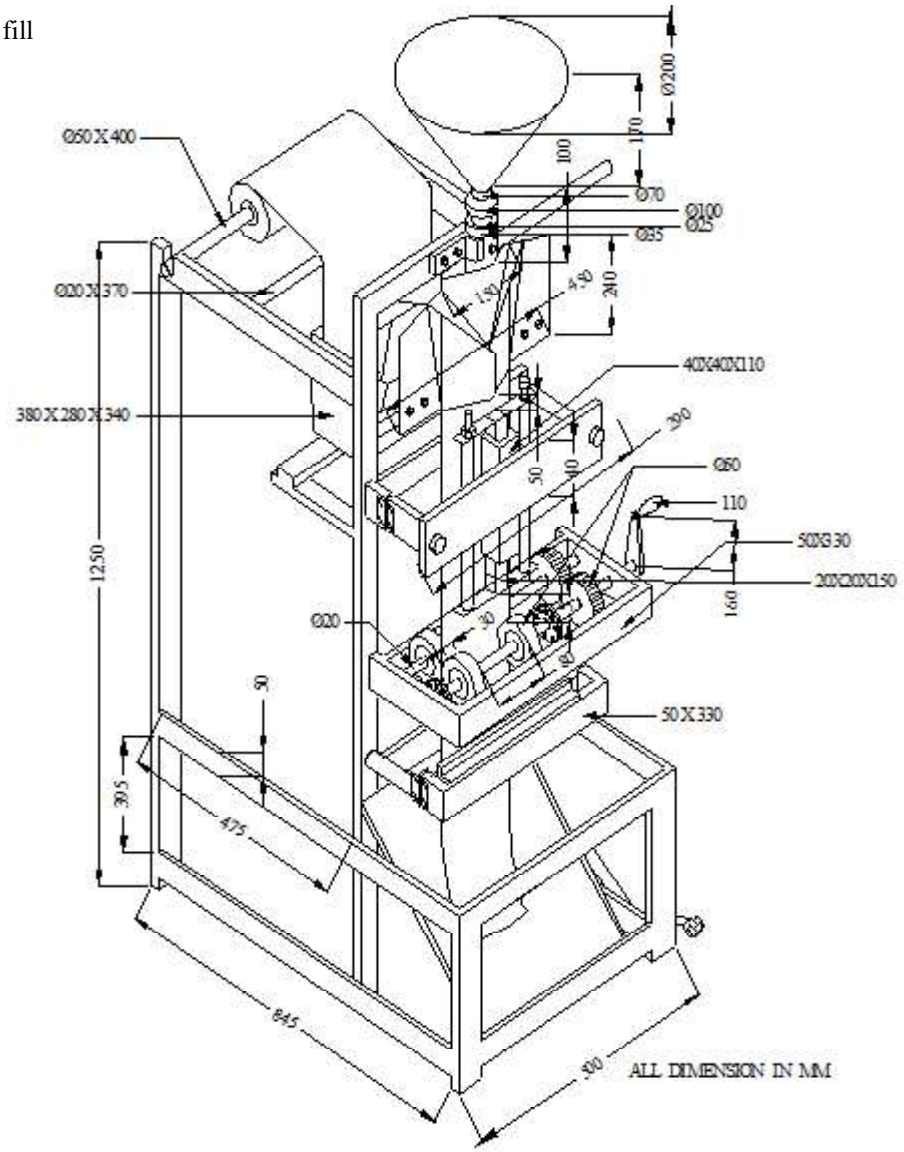


Fig. 1 Isometric view of aseptic pouch form fill seal machine

Fig. 2 Variation of Yellowness Index of aseptically packed milk with storage period

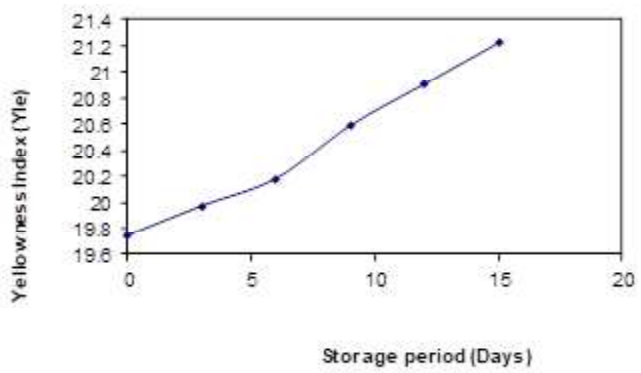


Table 1 Specification of the aseptic pouch form fill seal machine

S. No.	Parameters	Specification
1	Capacity	240 pouches/hour
2	Height	1.66 m
3	Length	0.845 m
4	Width	0.825 m
5	Thickness of polypropylene film	80 µm.
6	Width of packaging material	0.32 m
7	Length of conveying roller	0.37 m
8	Length of H_2O_2 bath	0.38 m
9	Width of H_2O_2 bath	0.28 m
10	Height of H_2O_2 bath	0.34 m
11	Length of milk filling tube	0.78 m
12	Diameter of milk filling tube	0.025 m
13	Diameter of jacketed tube	0.035 m
14	Length of folding section	0.51 m
15	Width of folding section	0.51 m
16	Diameter of pulling roll	0.05 m
17	Diameter of gear	0.05 m
18	No. of sealers	2(one horizontal and one vertical)
19	Length of sealer	0.15 m
20	Power required for each sealer	4.55 W
21	Current required for each sealer	0.4 A
22	Voltage required for each sealer	12 V
23	Resistance of electrode	30.20 Ohm

m. So 0.15 m length was selected and width of plate was taken as 0.002 m. For aseptic pouch packaging, polypropylene (PP) film of thickness 80 µm was taken. The sealing time was found to be 3 second for effective sealing. Heat required for sealing 0.15 m length, 0.002 m width and 0.00016 m thickness of poylethelene film was found to be 13.65 J. Power required for sealing time of 3 second was found to be 4.55 Watt. The current and voltage required were 0.4 Amp and 12 Volt respectively. The diameter of nichrome wire was taken as 0.002 m while length was taken as 0.25 m. The diameter of both roll and gear selected were 0.05 m. The length of both filling and air jacketed tube were decided based on the length of folding structure with some extra margin. The length of both tubes was kept as 0.78 m as per design of machine dimension and other constraints. The diameter of filling tube was taken as 0.025 m while diameter of jacketed tube was taken as 0.035 m. The upper diameter of funnel was taken as 0.20 m while bottom diameter was taken as 0.07 m. The time required for producing 500 ml milk pouch was found to be 15 sec. So the capacity of aseptic pouch form fill machine was found to be 120 litre/h. The bacterial counts were found to be zero up to 15 days of storage period which indicated the microbial effectiveness of aseptic filling.

Sequence of operations and time requirements for packaging

Vertical and horizontal sealing	3 s
Pulling the film down for the length of 500 ml pouch	6 s
Pouch filling time (500 ml volume)	6 s

Fig.3 Variation of whiteness index of aseptically packed milk with storage period

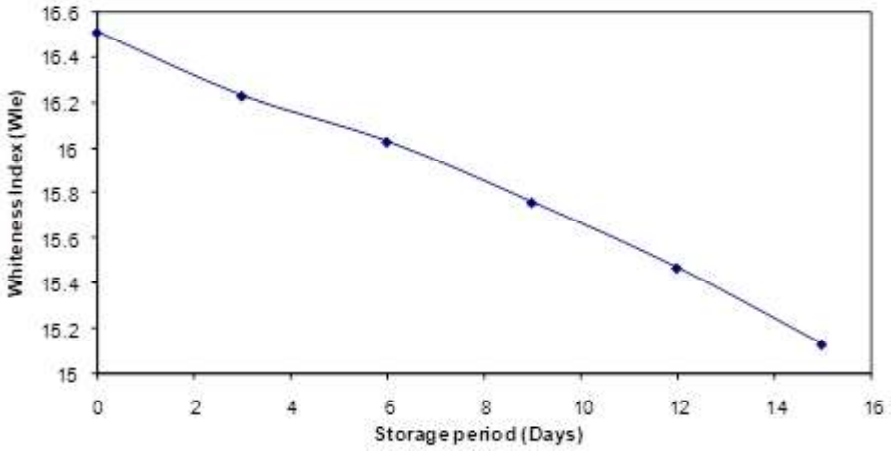
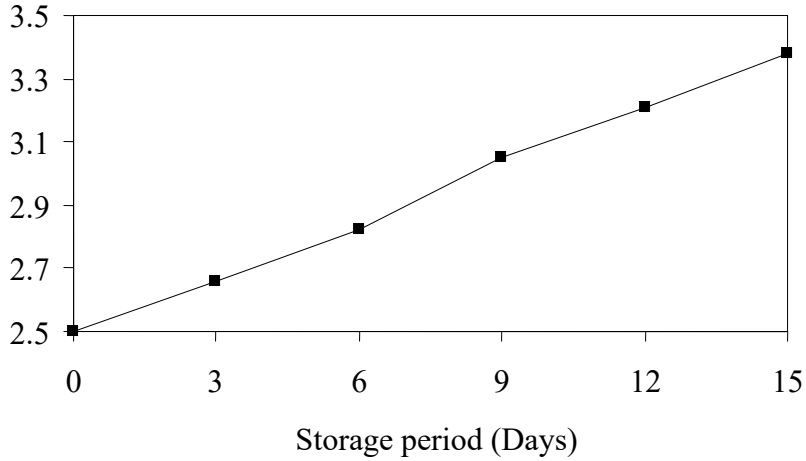


Fig. 4 Variation of viscosity of aseptically packed milk with storage period



Total time 15 s

So, time required for producing 500ml milk pouch is 15 s.

Hence the machine can produce 3600/ 15 = 240 pouch of 500 ml.

Capacity of aseptic packaging machine

The machine can produce 240 pouches of 500ml per hour, so its capacity is 120 litre/h.

The specification of the aseptic pouch form fill seal machine is given in Table 1.

Colour change in aseptically packaged milk

The variation of Yellowness Index of aseptically packaged milk with storage period (Fig.2) showed slow increase throughout the storage period and is due to increase in browning reaction prevalent during storage period.

The variation of Whiteness Index of aseptically packed milk over storage period (Fig. 3) showed slow decrease throughout the storage period. Similar finding was reported by Sahoo et al. (2002). The decrease of Whiteness Index during storage period is due to browning reaction. Over all, no marked change in color indices was seen during the storage period, which would render the product to be unaccepted.

Changes in viscosity of aseptically packed milk

The variation of viscosity of aseptically packed milk with storage period (Fig. 4) showed the slight increase in viscosity with storage period up to 15 days could be due to presence of heat resistive bacterial enzyme or casein -serum protein complex formation (Ansari et al. 2018). The progressive rise in viscosity during storage period leads to gelation. Usually gelling in aseptically packaged milk starts when viscosity reaches above 200 cp. Since viscosity of aseptically packaged milk after 15 days of storage period was far below the value of 200 cp, it was free from gelation. Milk was completely stable after two weeks.

Conclusions

A semi automatic hand and pedal operated small aseptic pouch form fill seal machine, was developed and evaluated. The time required for producing 500 ml milk pouch is 15 s and milk filling capacity of the machine is 120 l/h. The developed machine was

found satisfactory and simple in operation. The aseptically packaged milk was found microbiologically and organoleptically safe and acceptable as it was free from gelation and drastic color change and viscosity change up to 15 days of storage period. The developed machine could be used by small scale dairy and food processing industry for any liquid foods with slight modifications.

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RESEARCH ARTICLE

Incidence and preliminary characterization of Lactic acid bacteria as potential probiotic strains from an artisanal milk product, Chilika curd of Odisha

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Abstract: A traditional fermented milk product prepared from buffalo milk in Chilika, situated in the eastern coast of India, is known for its unique organoleptic properties, an exceptionally extended shelf-life and a special method of preparation by the ethnic community of that region. In order to study the potential probiotic lactic acid bacteria associated with this indigenous curd, a total of five isolates were identified and characterized for their probiotic properties. The isolates were identified as *Lactobacillus plantarum* SSU1, *Lactobacillus delbreuckii* subsp. *bulgaricus* SSU2, *Lactobacillus rhamnosus* SSU3, *Lactobacillus casei* SSU4 and *Lactobacillus rhamnosus* SSU5 by 16S rDNA gene sequencing. All the isolates showed good acid and bile tolerance ability, antibacterial activity against wide range of pathogens and presence of bacteriocin producing genes. Such strains with the probiotic attributes could be potential novel starter cultures for producing natural probiotic and various fermented functional food.

Keywords: Chilika curd, fermented buffalo milk, Lactic acid bacteria, probiotic, Plantaricin

Introduction

Milk and milk products are considered to be wholesome food due to the presence of whole array of nutrients such as proteins, fats, carbohydrates, vitamins and minerals. Milk being a good substrate provides a perfect atmosphere for many microorganisms of which Lactic acid bacteria (LAB) are the predominant. LAB are a group of Gram positive, rod shaped, non- spore forming bacteria, mostly associated with various fermented food such as dairy products, beverages, meat and vegetables (Grosu-Tudor and Zamfir, 2012). They are known for the rapid acidification of substrate and production of acetic acid, ethanol, aromatic compounds, exo-polysaccharides and enzymes (Vuyst and Leroy, 2007). LAB and their food products are thought to confer a variety of important nutritional and therapeutic benefits and have many documented health promoting or probiotic effects in human (Parvez et al. 2006). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Joint FAO/WHO Expert Consultation 2002). LAB for use as probiotic cultures must be tolerant to acid and bile as these are the primary factors affecting strain selection (Hyronimus et al. 2000). LAB also exhibits vast antimicrobial property due to production of various organic acids, hydrogen peroxide and certain proteinaceous compounds called bacteriocins (Jamuna and Jeevaratnam, 2004). Bacteriocins produced by LAB have high thermal stress, wide pH range with no reported development of resistant bacteria (Perez et al. 2014) thus, making it highly useful for probiotic purpose.

Curd is one of the most popular milk products in India and forms a part of the regular diet of the common population. It is produced using the fermentation process in which LAB plays a key role. As curd is prepared in nearly every household, each region of the country has its own unique combinations of LAB flora used as starter culture in making of the indigenous curd of that region. Chilika curd is one such dairy food traditionally prepared with milk produced from buffaloes of Chilika lake area of Odisha state by the ethnic community of this region. Situated in the eastern coast of India and being the largest brackish water lagoon, the sea weeds of high salinity content serves as a special diet for the buffaloes of this region (Nanda et al. 2013). This makes the curd produced from the milk of these buffaloes very unique in its organoleptic properties. It is also known to have an exceptionally extended shelf life (Nanda et al. 2013).Thus, it is essential to study the consortium of LAB flora present in the Chilika curd responsible for its uniqueness and to recognize the probiotic properties of these LAB.

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Materials and Methods

Preparation of Chilika curd

The farmers of Chilika Buffalo Society follow a unique traditional technique for preparation of Chilika curd which is made in baskets of bamboo mats. The inner surface of these baskets is layered with inoculum of previously made curd and is sun dried. This process is repeated with multiple curd layers and intermittent sun treatment. Finally, boiled concentrated buffalo milk cooled to room temperature is added to these baskets covered with leaves and left undisturbed for the fermentation process to be completed.

Sampling and isolation of LAB

Random curd samples (n = 3) were collected aseptically from the local sellers covering regions in and around Chilika, Odisha. The samples were then transported to the laboratory in aseptic refrigerated condition. Each of these samples was homogenized. 1 ml of each sample was added to 9 ml of MRS (de Man, Rogosa and Sharpe) broth (Himedia) and incubated at 37° C for 48 h. Subsequently, dilution series of each enriched culture was prepared by using sterile normal saline solution (1:10; wt:vol) and from appropriate dilution, 0.1 ml was spread plated evenly on MRS plates. The plates were incubated anaerobically at 37°C for 48 h. Individual colonies were selected on the basis of morphology, Gram staining and catalase activity. The Gram positive and catalase negative isolates were transferred into MRS broth and incubated at 37°C for 48 h. The isolates were kept in MRS broth containing 20% (v/v) glycerol at - 80°C. Further analysis was carried out from the stored cultures.

Phenotypic characterization

The isolates were initially characterized based on Gram reaction, catalase activity, motility, carbohydrate fermentation and arginine hydrolysis (Marroki et al. 2011; Yu et al. 2012). The results are shown in Table 1.

Genotypic identification using 16S rDNA

Isolates showing varying phenotypic properties were selected for genotypic identification. Genomic DNA was extracted using overnight cultures of the selected isolates as described previously by Abed (2013). Amplification of the 16S rDNA was carried out using universal bacterial 16S rDNA primers; forward primer (5'-AAGAGTTTGATCCTGGCTCAG-3') and reverse primer (5'-GGTTACCTTGTTACGACTT-3') amplifying 1500 bp amplicons as per Stanley et al. (1995). The PCR reaction mixture consisted of 100 ng of DNA template, 20 pmol of both forward and reverse primer, 2.5 mM of dNTPS, 1xPCR buffer, 0.75 units of Taq DNA polymerase and PCR water. The PCR was carried out under following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 7 min. Partial sequencing of the purified PCR products was done. The sequences of the isolates were deposited in GenBank database. Sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST) program. The partial 16S rDNA sequences determined in the present study and those available in GenBank database were analyzed. Isolates were identified with e" 97% identity.

Screening for probiotic properties

Acid and bile tolerance activity

The acid and bile tolerance of each isolate was tested according to the method described by Hyronimus et al. (2000) with some modifications. Active cultures of LAB isolates (1% v/v) with initial concentration of 10^6 cfu ml^{-1} were used for both the tests. For acid tolerance test, the isolates were inoculated in 10 ml MRS broth with the pH adjusted to 2, 3 and 4 by using 1N HCL. Similarly, for bile tolerance tests, isolates were inoculated in MRS broth with 0.1%, 0.2% and 0.3% Ox bile (Himedia). All the test broths were incubated at 37°C for 24 h. The viable number of LAB at different pH and bile concentrations were enumerated by pour plate count on MRS agar plate incubated at 37°C for 48 h, compared to initial bacterial concentration and the percentage of survivability was calculated.

Antibacterial activity

The antibacterial activities of the strains were confirmed using agar well diffusion assay as described by Yang et al. (2012) with slight modifications. The isolates were tested against five food spoilage and pathogenic bacteria, *Vibrio parahaemolyticus* JF966211*, Bacillus cereus* ATCC 11778 (Himedia), *Aeromonas hydrophila* CAHH14*, Salmonella enteric* ATCC 35640 (Himedia) and *Staphylococcus aureus* ATCC 6538 (Himedia). Strains *Vibrio parahaemolyticus* JF966211 and *Aeromonas hydrophila* CAHH14 were obtained from Fish Health Management Division, ICAR- Central Institute of Fresh water Aquaculture, Bhubaneswar, India. Overnight cultures of the indicator strains were grown in nutrient broth (Himedia) and were lawn cultured in sterile Tryptic Soya Agar (TSA) (Himedia) plates with 6mm diameter wells. 50 ìl of cell free supernatant of the isolates (10^8 cfu ml^{-1}) was placed in the wells in TSA plates. After 24 h of incubation at 37°C, the diameter of the zone of inhibition surrounding each agar well was measured.

To further test the proteinaceous nature of the substance responsible for inhibition, 10 ìl of the trypsin enzyme (Sigma, Madrid) solution (10 mg ml^{-1} in distilled water) was added to the cell free supernatant of the isolates which was then subjected to well diffusion assay (Ben Omar et al. 2008). Absence of zone of inhibition indicated the proteinaceous nature of inhibitory substances.

Screening for known plantaricin gene

All the isolates were tested for the presence of plantaricin genes (plnA, plnB, plnC, plnD, plnEF, plnI, plnJ, plnK, plnG and plnN) as described by Ben Omar et al. (2008). PCR amplification was carried out under following conditions: initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 1 min, annealing at appropriate temperatures (Table 2) for 1 min, extension at 72°C for 30 s and final extension at 72°C for 5 min. The PCR products were electrophoresed in 2% agarose gel and 1× TBE buffer (Sambrook et al. 1989).

Statistical analysis

Statistical analysis was conducted using the computerized software Statistical Package for Social Sciences (SPSS) version 18.0. Data are reported as means ± SE. Statistical significant difference was determined using Tukey's Method with One-Way ANOVA. Differences were considered significant at $P < 0.05$.

Results and Discussion

Phenotypic characterization and genotypic identification

In the present study, the isolates were phenotypically characterized using 21 biochemical tests including Gram's reaction, catalase activity, motility and carbohydrate utilization tests. Almost all the strains isolated from curd in MRS agar were considered to be LAB based on their positive Grams reaction, absence of motility, absence of spore formation and absence of catalase activity. The fermentation profile revealed that the isolates assimilated variously a panel of carbohydrates that

Table 1 Phenotypic characteristics of the LAB isolates

Characteristics	Isolates				
	L. plantarum SSU1	*L. delbreuckii bulgaricus* SSU2	*L. rhamnosus* SSU3	*L. casei* SSU4	*L. rhamnosus* SSU5
Gram's Reaction	+	+	+	+	+
Shape	R	R	R	R	R
Catalase	-	-	-	-	-
Motility	-	-	-	-	-
Arginine hydrolysis	-	-	-	-	-
L-arabinose	-	-	-	-	-
D - glucose	+	+	+	+	+
Esculin	+	+	+	+	+
Galactose	+	-	-	+	-
Inositol	-	-	-	-	-
Inulin	+	+	+	-	+
Lactose	+	-	+	+	+
Maltose	+	-	-	+	+
Mannitol	+	-	-	-	-
Melezitose	+	-	-	+	-
Melibiose	-	-	-	-	-
Raffinose	-	-	-	-	-
Ribose	+	-	+	+	-
Salicin	+	+	+	+	-
Sorbitol	-	-	-	-	-
Sucrose	+	-	+	+	+
Trehalose	-	-	-	+	+

reflected their enzymatic and genetic potentials along with their phenotypic heterogeneity and diversity. The LAB genomes are predicted to carry a large number of carbohydrate transport and utilization genes that display substantial variations among strains (Ceapa et al. 2015; Maji et al. 2016).

Of the phenotypically characterised strains, 5 rod shaped isolates showing varying phenotypic characters were presumptively determined as derivatives of the genus *Lactobacillus* and subjected to genotypic identification. The partial sequencing of 16S rDNA gene identified the isolates as *Lactobacillus plantarum* SSU1, *Lactobacillus delbreuckii* subsp. *bulgaricus* SSU2, *Lactobacillus rhamnosus* SSU3, *Lactobacillus casei* SSU4 and *Lactobacillus rhamnosus* SSU5 with accession numbers as KF971888, KF971889, KF971890, KF971891 and KF971892 respectively. Nanda et al. (2013) have also reported the isolation of *Lactobacillus, Lactococcus, Streptococcus* and *Leuconostoc* from this indigenous curd. Every region has its typical sets of lactoflora in the indigenous fermented product of that region and *Lactobacillus* is considered to be the predominant genus in almost all dairy products. A study on naturally fermented yak milk found *L. delbreukii* subsp. *bulgaricus* and *S. thermophilus* as the predominant microflora in the product (Sun et al. 2010). Bettache et al. (2012) also reported the dominance of *Lactobacilli* in the microflora of Dhan, a traditional butter. Angmo et al. (2016) identified and characterized *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus fermentum* as probiotic lactic acid bacteria from chhurpe, a dried cottage cheese and chhang, a barley based beer of Ladakh. Also, *Lactobacillus* was found to be the dominant lactic acid bacteria isolated from various traditional fermented dairy products of Turkey (Erginkaya et al. 2018).

Acid and bile tolerance activity

LAB are known for their probiotic characteristics and their use in probiotic products. Acid and bile tolerance are important traits in characterizing the probiotic property of a strain. In order to reach the hindgut in an active and functional form and exert their beneficial properties, the strains should tolerate and survive in extremely stressed conditions like low pH and high bile concentration in the upper parts of gastrointestinal tract (Maji et al. 2016; Zhang et al. 2016). In the present study, most of the isolates showed good survivability even after 24 h of exposure to pH 3 and pH 4 (Table 3) which is in consistent with earlier findings (Grosu-Tudor and Zamfir, 2012). However, not all strains showed similar tolerance capability as survivability at low pH is strain specific (Tokatl et al. 2015) and is an adaptation to environmental conditions (Grosu-Tudor and Zamfir, 2012). *L. casei* SSU4 showed maximum significant ($P < 0.05$) viability at pH 3 and pH 4 compared to other isolates. Also, none of the isolates survived at pH 2 for such a long time whereas *L. delbreuckii* subsp. *bulgaricus* SSU2 did not survive at pH 3 but showed 100% survivability at pH 4. Similarly, in bile tolerance test, all the isolates showed 100% survival at 0.1%, 0.2% and 0.3% bile after 24h of exposure except *L. delbreuckii* subsp. *bulgaricus* SSU2 which showed no survival rate at 0.2% and 0.3% bile concentration (Table 3). Earlier studies have reported high survivability rate of

Table 2 PCR primers used for detection of Plantaricin genes

Target Genes	Primers	Annealing Temperature(°C)	Size(bp)
plnA	F: GTA CAG TAC TAA TGG GAG R: CTT ACG CCA ATC TAT ACG	53	450
plnB	F: TTC AGA GCA AGC CTA AAT GAC R: GCC ACT GTA ACA CCA TGA C	51.5	165
plnC	F: AGC AGA TGA AAT TCG GCA G R: ATA ATC CAA CGG TGC AAT CC	49.5	108
plnD	F: TGA GGA CAA ACA GAC TGG AC R: GCA TCG GAA AAA TTG CGG ATA C	53	414
plnEF	F: GGC ATA GTT AAA ATT CCC CCC R: CAG GTT GCC GCA AAA AAA G	53.2	428
plnI	F: CTC GAC GGT GAA ATT AGG TGT AAG R: CGT TTA TCC TAT CCT CTA AGC ATT GG	52.5	450
plnG	F: TGC GGT TAT CAG TAT GTC AAA G R: CCT CGA AAC AAT TTC CCC C	52.8	453
plnN	F: ATT GCC GGG TTA GGT ATC G R: CCT AAA CCA TGC CAT GCA C	51.9	146
plnJ	F: TAA CGA CGG ATT GCT CTG R:AAT CAA GGA ATT ATC ACA TTA GTC	51	475
plnK	F: CTG TAA GCA TTG CTA ACC AAT C R: ACT GCT GAC GCT GAA AAG	52.9	246

L. plantarum and *L. brevis* in 0.3% and 0.5% bile concentrations (Grosu-Tudor and Zamfir, 2012; Tokatl et al. 2015).

Antibacterial activity

All the isolates showed antibacterial activity against the food spoilage and pathogenic bacteria. The inhibitory zones varied between the ranges of 11 mm to 17 mm in diameter (Table 4) with *L. casei* SSU4 showing the best results. These findings are in agreement with earlier studies where similar *in vitro* antagonisms against several food spoilage pathogens have been reported by using *Lactobacillus* spp (Jamuna and Jeevaratnam, 2004; Yang et al. 2012; Zhang et al. 2016). The antimicrobial activity of the LAB isolates is thought to be multi factorial and is due to the

Table 3 Acid and bile tolerance capability of the isolates

Isolates	*Initial viable count*	*Viable count at pH 2 after 24h (CFU mL^{-1})*	*Viable count at pH 3 after 24h (CFU mL^{-1})*	*Viable count at pH 4 after 24h (CFU mL^{-1})*	*Viable count at 0.1% bile after 24h (CFU mL^{-1})*	*Viable count at 0.2% bile after 24h (CFU mL^{-1})*	*Viable count at 0.3% bile after 24h (CFU mL^{-1})*
L. plantarum SSU1	7.87 ± 0.01	Nil	4.43 ± 0.23^{a}	5.38 ± 0.1^{a}	6.26 ± 0.13^{a}	7.31 ± 0.16^{a}	7.14 ± 0.18^{a}
L. delbreuckii subsp. *bulgaricus* SSU2	7.81 ± 0.05	Nil	Nil	6.06 ± 0.17^{a}	7.68 ± 0.04^{b}	Nil	Nil
L. rhamnosus SSU3	7.86 ± 0.02	Nil	4.9 ± 0.15^{a}	5.66 ± 0.08^{a}	7.61 ± 0.06^{b}	7.45 ± 0.08^{a}	7.41 ± 0.06^{a}
L. casei SSU4	7.9 ± 0.05	Nil	5.71 ± 0.05^{b}	7.6 ± 0.11^{b}	7.78 ± 0.04^{b}	7.71 ± 0.05^{a}	7.56 ± 0.08^{a}
L. rhamnosus SSU5	7.76 ± 0.08	Nil	4.59 ± 0.1^{a}	6.48 ± 0.1^{a}	7.5 ± 0.1^{b}	7.47 ± 0.09^{a}	7.4 ± 0.1^{a}

Values are mean of triplicate and presented as means ± S.E; In columns, parameters of isolates with different letters (a, b) are significantly different ($P < 0.05$)

Table 4 Antibacterial activity of the isolates

Isolates	Zone of inhibition (in mm) ± SEM against *V. parahaemolyticus*	Zone of inhibition (in mm) ± SEM against *B. cereus*	Zone of inhibition (in mm) ± SEM against *A. hydrophila*	Zone of inhibition (in mm) ± SEM against *S. enterica*	Zone of inhibition (in mm) ± SEM against *S. aureus*
L. plantarum SSU1	12.2 ± 0.12	12.8 ± 0.30	10.3 ± 0.27	9.6 ± 0.27	11 ± 0.19
L. delbreuckii subsp. *bulgaricus* SSU2	14 ± 0.11	11.1 ± 0.16	15 ± 0.23	13.1 ± 0.13	10.3 ± 0.27
L. rhamnosus SSU3	15.3 ±0.13	13.1 ± 0.22	11.6 ± 0.36	10.5 ± 0.23	11.1 ± 0.36
L. casei SSU4	16 ± 0.09	15 ± 0.19	10.6 ± 0.27	11.8 ± 0.36	13.7 ± 0.32
L. rhamnosus SSU5	17 ± 0.23	13 ± 0.09	12.8 ± 0.13	12.4 ± 0.28	9.5 ± 0.23

SEM : standard error of mean

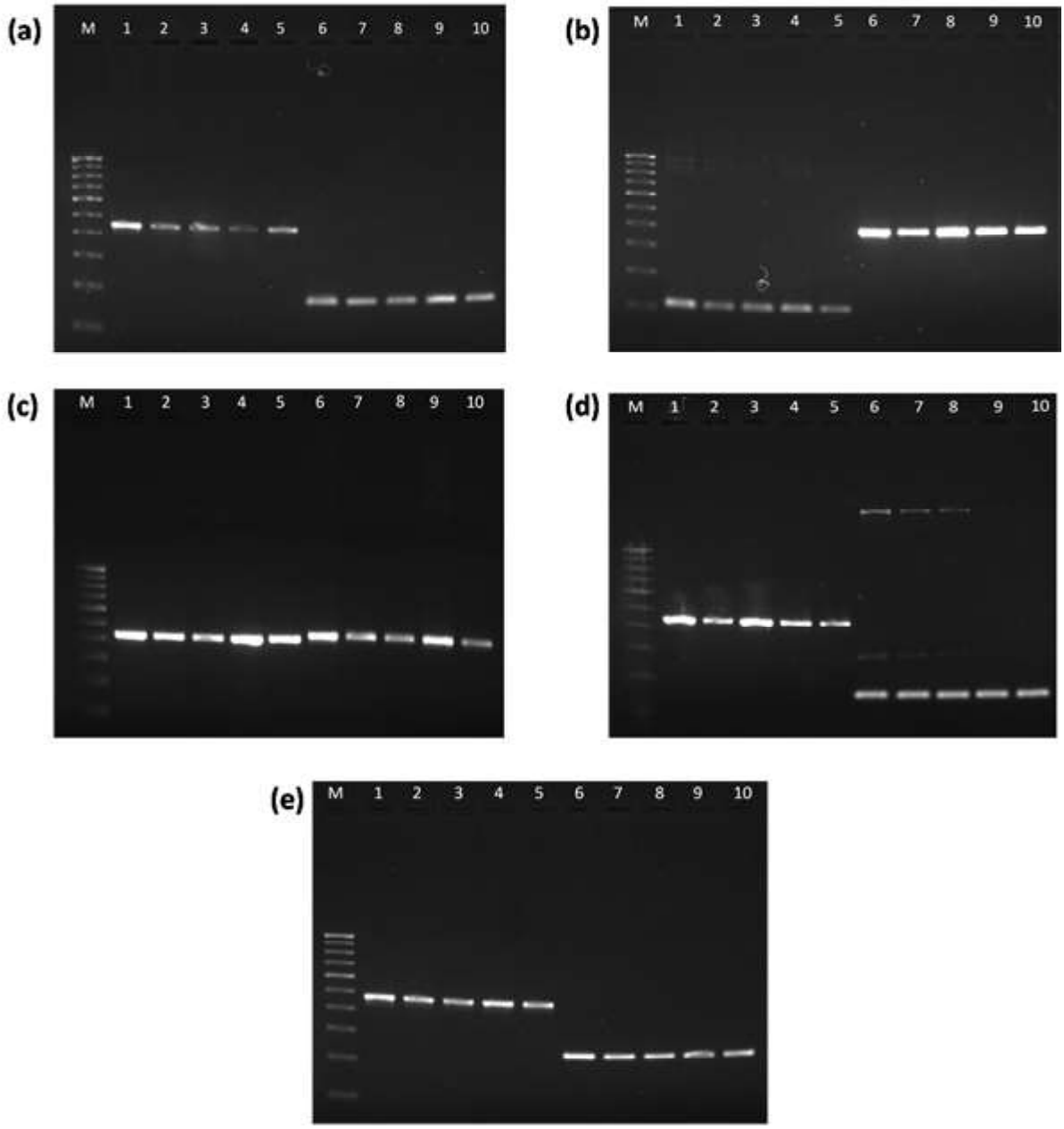

Fig. 1 Agarose gel electrophoresis of PCR amplification products of bacteriocin, Plantaricin genes; (**a**) plnA, 450 bp, Lane 1 to 5 & plnB, 165 bp, Lane 6 to 10 (**b**) plnC, 108 bp, Lane 1 to Lane 5 & plnD, 414 bp, Lane 6 to 10 **(c)** plnEF, 428 bp, Lane 1 to 5 & plnI, 450 bp, Lane 6 to 10 **(d)** plnG, 453 bp, Lane 1 to 5 & plnN, 146 bp, Lane 6 to 10 **(e)** plnJ, 475 bp Lane 1 to 5 & plnK, 246 bp, Lane 6 to 10 in isolates; *L. plantarum* SSU1 (Lanes 1 & 6); *L. delbreuckii subsp. bulgaricus* SSU2 (Lanes 2 & 7); *L. rhamnosus* SSU3 (Lanes 3 & 8); *L. casei* SSU4 (Lanes 4 & 9); *L. rhamnosus* SSU5 (Lanes 5 & 10); M: Marker

synergistic effect of production of organic acids (acetic acid or lactic acid) and strain specific metabolites or non-lactic acid molecules, bacteriocins, etc (Maji et al. 2016).

During preliminary screening for bacteriocin production, all the strains showed no zone of inhibition after addition of trypsin indicating the proteinaceous nature of the inhibitor. Bacteriocin, the proteinaceous compound produced by LAB usually inhibits closely related species (Ben Omar et al. 2006). In the present study, however, the isolates showed inhibitory activity against a wide range of Gram positive as well as Gram negative bacteria, which is a very desirable probiotic property. A few earlier reports have also shown antagonistic activity of *Lactobacillus* against Gram negative bacteria such as *V. parahaemolyticus*, *E. coli, S. typhi, S. enterica, P. fluorescens*, and *P. putida* (Gong et al. 2010; Chowdhury et al. 2012; Zhang et al. 2016).

Detection of known plantaricin gene

The entire plantaricin gene cluster consisting of plnA, plnB, plnC, plnD, plnEF, plnI, plnJ, plnK, plnG and plnN genes as described in *L. plantarum* C11 (Diep et al. 1996) was detected in *L. plantarum* SSU1. Also the isolates *L. delbreuckii* subsp. *bulgaricus* SSU2, *L. rhamnosus* SSU3, *L. casei* SSU4 and *L. rhamnosus* SSU5 showed the presence of all plantaricin genes (Fig. 1). Ben Omar et al. (2008) reported plantaricin genes in *L. fermentum* isolated from the same source as *L. plantarum*. Hurtado et al. (2011) also reported the presence of plantaricin in closely related species *L. plantarum* and *L. pentosus*. Bacteriocin production is frequently associated with mobile genetic elements such as plasmids which are common in LAB that may facilitate the transfer of genes between species and strain sharing the same niche. Also, some closely related species may have similar characteristic for survival in the same environmental conditions (Hurtado et al. 2011). Further confirmatory test are needed to be able to select the above mentioned strains of *Lactobacillus* as probiotic organisms.

Conclusions

The present study demonstrates the presence of diverse *Lactobacillus* species with promising probiotic potential in the indigenous curd unique to the Chilika lake area of the state of Odisha, India. It is to mention that, the Chilika Lake happens to be the largest brackish water lake of Asia. Further elucidated study of this artisanal product shall fully explore it as a potential source for such probiotic bacteriocinogenic strains which can contribute towards formulation of functional foods with health beneficial properties.

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RESEARCH ARTICLE

Assessment of milk adulteration in the commercially available milk for the consumers in Cauvery delta region of Tamil Nadu, India

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Abstract: Milk, has not been a exception for the pull strings of millennium developments. In recent years, milk available to the consumer is deviated from its inherent nutritional composition because of rampant adulteration. This study was designed to assess the quality with regards to adulteration in milk which is supplied to consumers in and around the Cauvery delta regions of Tamil Nadu. A total of 75 milk samples were randomly collected from various sources like street vendors, tea shops, restaurants, hotels and commercially available pasteurized milk outlets and tested for the presence of common adulterants like starch, sugar, glucose, detergents, neutralizers, salt and skim milk powder. This study revealed that the presence of detergent (48%), sodium chloride (36%), skim milk powder (29.3%), neutralizer (18%), sugar (12%), starch (8%) and urea (8%). It was found that tea shops happened to be a major source followed by restaurant and hotels. It is concluded that awareness needs to be created among the public about the health hazards of adulterants.

Keywords: Adulterants, Cauvery delta region, Milk, Quality assessment

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Introduction

Milk is an essential and confirmed source of nutrition (Medhammer et al. 2011; FAO, 2013). Advances in science and technology pillared milk economy with credible preservation and processing techniques which enhanced the availability of milk in remote places which in turn increased the demand for milk in India, even though India ranks first in milk production (Longvah et al. 2013). The inability of the farmers to meet this high demand as well as expectation of more profit out of this high demand paved way for adulteration (Spink and Moyer, 2011). Of all the food ingredients, milk happens to be the second top most food subjected to adulteration (USPC, 2015). Adulteration of milk has become rampant, in entire chain from the level of farmer, middle man, processor and vendor. As per the survey conducted by FSSAI, 20% of milk sold in Tamil Nadu does not meet the standards (FSSAI, 2012). As part of Veterinary Public Health mandate, this study was conducted to assess the quality of milk for its adulteration in the Cauvery delta region of Tamil Nadu.

Materials and Methods

Study area

Three districts *viz*, Thanjavur, Thiruvarur and Nagappattinam in the Cauvery delta region (CDR) were selected for this study. Before framing the sample collection process, complete analysis about the flow of fluid milk in the CDR was made. (Fig. 1). The debasing activity can be done at any point in the flow of fluid milk from production to consumption. Whatever may be the point of adulteration, samples from street vendors, local tea shops, restaurant and commercial milk outlets would be the true representative of the status of adulteration.

Sampling of milk

A total of 75 milk samples were randomly collected in the study area from the animal owners, milk vendors, tea shops, hotels, restaurants and commercial milk outlets (Table 1). The samples were collected with much care to maintain their original quality. Boiled milk samples from tea shops, hotels and restaurants were collected without sugar. Local tea shops near bus terminals in

district headquarters, town and village panchayat were given preference in the sampling of milk.

Detection of adulterants in milk

Milk adulteration kit (K088A HIMEDIA) was used for detection of adulterants such as starch, urea, neutralizer, detergent, sodium chloride, skim milk powder and sugar as per the manufacturer's instructions.

Results and Discussion

The fraudulent, intentional substitution/addition of a substance in a product for the purpose of increasing apparent value of the product/reducing the cost of its production is termed as adulteration (FDA, 2009) Adulterants were discussed under three separate headings. Table 2 shows milk (samples) available to the consumers in the delta region was highly contaminated with detergent. 48% of milk samples tested was found to be contaminated with detergents. Various levels of detergent adulteration in milk were reported by many authors. Sanjita et al. (2017) reported 8.1% detergent adulteration in Rajasthan, 8.4 % across India (FSSAI, 2012), 32% in Badin (Soomro et al. 2014) and 44% in Hyderabad (Singuluri and Sukumaran, 2015) and 100% in Dehradun (Kandpal et al. 2012).

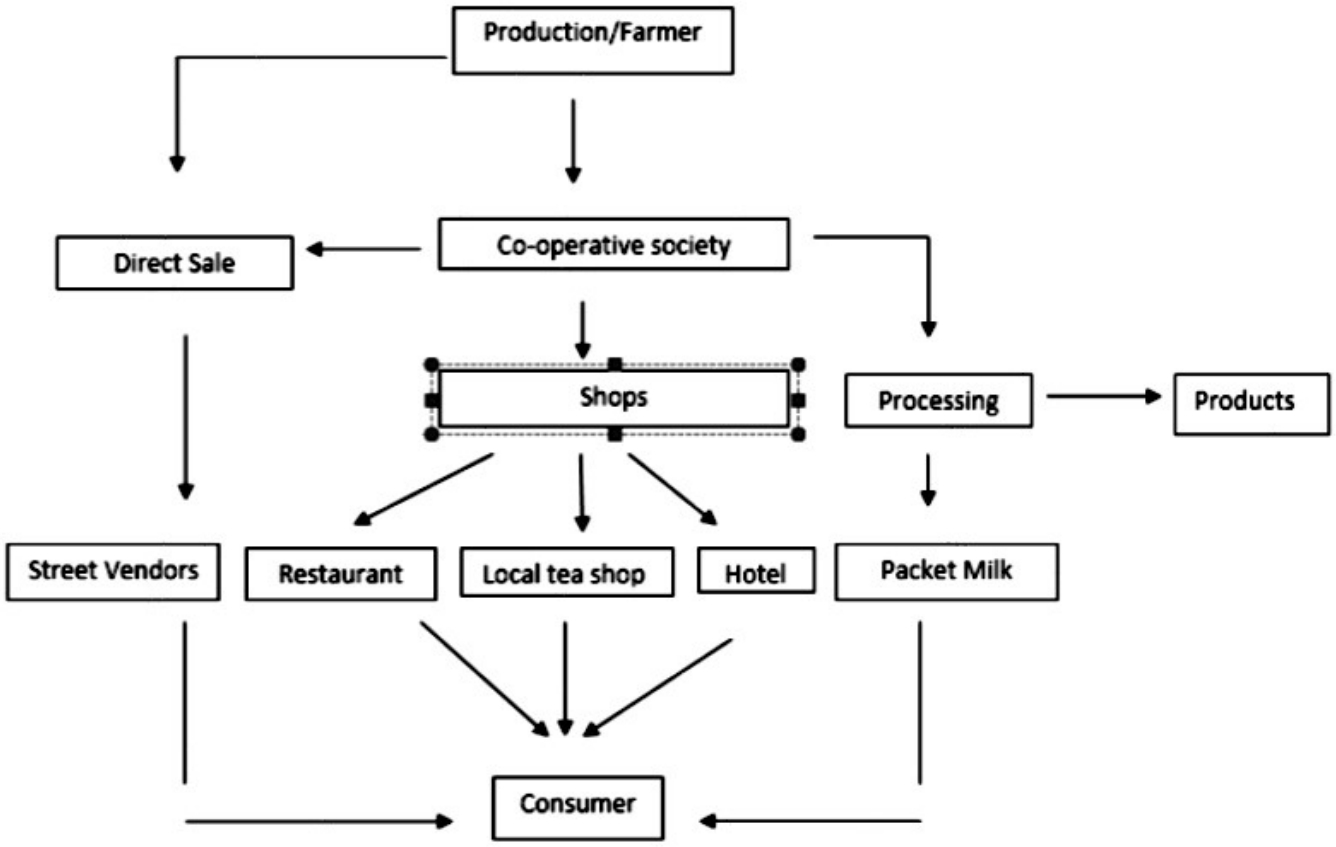
ICMR reported that consumption of milk adulterated with detergents cause food poisoning and gastero- intestinal complications (ICMR, 1993). FSSAI notes that consumption of milk with detergents is hazardous to health.

Bhatt et al. 2009 reported that the wide spread availability of synthetic milk in Utter Pradesh was the cause for the prevalence of headache, retarded eyesight and diarrhea among young children.

Detergent adulteration may either be intentional or unintentional. Detergent is a common ingredient in synthetic milk to emulsify and dissolve the oil in water to give frothy nature and characteristic white colour of milk (Kandpal et al. 2012). Also used to give intense frothiness in tea or coffee made with skim milk which gives little or no frothiness. Unintentionally, detergents used for cleaning remains in the vessel due to improper washing (more common in Tea shop) due to lack of awareness on importance of proper washing and the ill effects of detergents on health.

Samples from hotels, shops and restaurant were ordered in glass/ silver containers so as to avoid plastic cup (to minimize the cross reaction of plastic with hot milk which may interfere in results). Of the found positive (48%) the order of contamination of

Fig 1. Flow chart depicting flow of fluid milk from production place to consumers



detergents was found to be 83.3% from restaurant, 75.86% from tea shops 66.66% from hotels. Whereas the presence of detergent among loose milk sold in streets and packet milk were low 18.18% and 8.3%.

Neutralizers are added to prevent early spoilage thereby extending the shelf life of milk without refrigeration (Handford et al. 2016) and to mask improper refrigeration due to lack of cold chain (Singuluri and Sukumaran, 2014). In many regions of India milk has been reported to be adulterated with caustic soda, sodium carbonate and sodium bicarbonate to neutralize the developed acidity due to bacterial activity by milk traders. Unhygienic containers, improper transport temperatures also reasons for bacterial growth which cause early raise in acidity. Milk having greater acidity will be positive for clot on boiling test and will be rejected by consumers and also in milk processing plant. To avoid this loss and to mask the acidity, neutralizers are deliberately added to milk (Beall and Scofield, 1995). Some of the deleterious effects of neutralizers are disruption of hormone signal that regulates development and reproduction, Vomition, burn on lips, tongue and sloughing of esophagus mucosa (Mordjikian, 2001; Ryan et al. 2006).

Urea was detected in 8% of the samples analysed. (Table 2). Urea is added for increasing profit out of high demand for milk by increasing protein content and it is the major ingredient of synthetic milk (Mudgil and Barak, 2013). Indiscriminate use of urea containing fertilizer / herbicide / pesticide is also a possible reason of urea in milk (Kandpal et al. 2012). It affects infants and young children especially girls as it hastens up the puberty (Trivedi et al. 2009). Elevated urea levels may be associated with urinary obstruction, Gastro – intestinal disorder and renal diseases (EPA, 2011)

Next most common adulterant found was Sodium Chloride (36%) (Table 3). Whereas prevalence of 54% and 82% sodium chloride among the milk sample in Andhra Pradesh and Hyderabad has already been reported (Ramya et al. 2015). An expected reason behind the addition of NaCl would be to adjust the SNF content after the dilution with water, NaCl significantly elevate the Freezing Point Depression (FDP) hence masking the measurement of extraneous water (Harding, 1995). Samples collected from street vendors were found to have greater level of NaCl followed by restaurant, tea shops, packet milk and hotels. Though NaCl

Table 1 Number of milk samples collected and their source

Source of milk samples	Thanjavur	Thiruvarur	Nagapattinam	Total
Street vendor	12	5	5	22
Restaurants	2	2	2	6
Tea shops	15	8	6	29
Hotels	2	2	2	6
Commercial milk outlets	4	4	4	12
Total	35	21	19	75

Table 2 Percentage of adulteration with chemicals

Category	Adulterant	Source of samples	No. of Positive	% of adulteration	Overall %
I	Detergent	Tea shops	22	75.86	
		Hotels	4	66.66	
		Street vendors	4	18.18	48%
		Restaurants	5	83.3	
		Commercial milk outlets	1	8.3	
	Neutralizer	Tea shops	7	24.13	
		Hotels	1	16.66	
		Street vendors	2	9.09	18.6%
		Restaurants	1	16.66	
		Commercial milk outlets	3	25.00	
	Urea	Tea shops	4	13.79	
		Hotels	-	-	
		Street vendors	1	4.54	8%
		Restaurants	-	-	
		Commercial milk outlets	1	8.33	

Table 3 Percentage of adulteration with fat and SNF levelers

Category	Adulterant	Source of samples	No. of Positive	% of adulteration	Overall %
II	Sodium chloride	Tea shops	10	34.48	
		Hotels	1	16.66	
		Street vendors	12	54.54	36%
		Restaurants	3	50	
		Commercial milk outlets	3	25	
	Sugar	Tea shops	3	10.34	
		Hotels	-	-	
		Street vendors	-	-	12%
		Restaurants	1	16.66	
		Commercial milk outlets	5	41.66	

Table 4 Percentage of adulteration with thickening agents

Category	Adulterant	Source of samples	No. of Positive	% of adulteration	Overall %
III	Starch	Tea shops	4	13.79	
		Hotels	-	-	
		Street vendors	1	4.54	8%
		Restaurants	1	16.66	
		Commercial milk outlets	-	-	
	Skim milk Powder	Tea shops	11	37.93	
		Hotels	-	-	
		Street vendors	6	27.27	29.3%
		Restaurants	-	-	
		Commercial milk outlets	12	41.66	

doesn't have any health implication, it would be deleterious to people who are hypertensive.

In Table 4, 29.3% samples were adulterated with skim milk powder. Skim milk is a product prepared from milk after removing the essential nutrient components. Even though SMP have milk base, addition of SMP to pure milk reduces the nutritional content of the milk. More recent national snapshot survey on milk adulteration by FSSAI reported 44.7% of milk samples in India are adulterated with SMP (FSSAI, 2012) 80% adulteration of SMP was reported by other authors (Singuluri and Sukumaran, 2014). Apart from this SMP has also gained much popularity among teashop, hotel and restaurant owners to give the desirable thickness in the coffee/tea prepared with diluted milk.

Table 4 shows Starch is another common carbohydrate adulterant that has been reported in milk samples from different places. In this survey only 8% of samples were found to be adulterated with starch. Swathi and Kauser (2015) reported 60% of milk samples from Hyderabad city were adulterated with Starch.

Conclusions

The study evident that practice of milk adulteration is prevalent in cauvery delta region of Tamilnadu with substances that either reduce the nutritional value or it may have toxic effect on consumer . Detergent, Sodium chloride, Neutralizer and Skim milk powder were found to be in the top. Intentional and unintentional sources of detergent in milk must be studied in detail to control its adulteration. Neutralizers are added to mask early stage spoilage of milk which is either poorly stored or unhygienically handled or adulterated with poor quality adulterants. Providing training on hygienic milk production strategies among producers and vendors with necessary chilling facilities will definitely reduce the adulteration with harmful substance like detergents, neutralizers.. Empowering farmers with techniques and skills to face the increasing demand for milk in right way will sound good results. To curb the menace of milk adulteration, stringent legal protection and consumer awareness on the safety and quality must be promoted by administrators.

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RESEARCH ARTICLE

Effect of polyherbal supplementation on milk production and postpartum reproduction in crossbred cattle

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Abstract: The present study was conducted at farmer's field in Muzaffarnagar district of Uttar Pradesh to assess the response of polyherbal mixture supplementation on milk production and postpartum reproduction in crossbred cattle. The polyherbal mixture was prepared by mixing 25 g each of *Foeniculum vulgare* (Saunf), *Trachy spermumammi* (Ajwain), *Trigonella foenum-graecum* (Methi), *Zingiber officinale* (Sundh), *Anethum graveolens* (Sowa) and *Elettaria cardamomum* (Cardamom). Sixteen crossbred cattle were divided into two groups on eight each as T_0: control and T_1: treatment and the animals of T_1 were supplemented ployherbal mixture from the day of calving till day 10 of postpartum. The data was recorded for 2 months duration. The animals were managed as per the standard feeding practices followed by the farmers. Significant increase in milk yield ($P<0.05$) was recorded due to supplementation of polyherbal mixture as compared to control group. From 0 to 60th day, an average increase of 21.53 % in milk yield was recorded due to polyherbal mixture supplementation as compared to 6.91% in unsupplemented group. No case of foetal membrane retention was recorded in treatment group, whereas in control group, two cases were observed. Time required for expulsion of foetal membranes was reduced significantly ($P<0.05$) in supplemented animal as compared to control (4.38 vs 7.38 hours). Supplementation has also reduced number of insemination per conception in cattle (2.38) as compared un-supplemented group (2.88). Considering the present cost of feed supplement and the market price of milk, polyherbal mixture supplementation appears to be both economical and cost effective, and had a positive effect on milk production and postpartum reproduction in crossbred cattle maintained under small holder conditions.

Keywords: Crossbred cattle, Milk production, Polyherbal, Reproduction

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Introduction

The demand of milk and milk products is increasing, especially in developing countries due to increase in the human population, income and urbanisation (Herrero and Thornton, 2013). However, the average productivity of dairy cattle in India is far below as compared to the productivity levels of dairy animals in developed nations (Misra et al, 2019). The measure reason for low productivity is poor nutrition during transition period. Postpartum period is very crucial phase of the dairy animals, owing to high demand of nutrition due to physiological changes and stress related to last trimester of fetal growth, parturition and lactation along with dietary change that make the animal prone to metabolic and productive disorders (Chanderasekhar et al. 2019). Reduction in milk production and weight losses of postpartum animals is a common feature, which culminates in substantial economic losses to the farmers. Singhal (1995) reported that herbal supplementation showed galactopoetic activity and can be considered as an alternative for lactogenic hormones for inducing and enhancing milk yield in crossbred cows. Several researchers have tried various herbal feed supplements like polyherbal galactogogue biscuits (Patel et al. 2013) and *Asparagus racemosus* (Behera et al. 2013), poly-herbal mixture (Chandra et al. 2017, Chandrasekhar, et al. 2019) to improve the productive and reproductive performance, and health status of dairy animals. However, very limited studies have evaluated effects of polyherbal supplements at farmers' field. Therefore, the present study was conducted to assess the effect of polyherbal supplementation on milk production and postpartum

reproduction in crossbred cattle at farmer's field in Muzaffarnagar district of western Uttar Pradesh.

Materials and Methods

Description of study area

The present study was conducted in Lalukheri and Salahkehri villages of Baghara block, Muzaffarnagar, Uttar Pradesh, India. The study area are located at 29°47'N latitude, 77°71'E longitude and 233m altitude from mean sea level. June is the warmest month with average temperature of 30.2°C, and January is the coolest month with average temperature of 12.5°C. The average rainfall is 929 mm, and highest precipitation falls in July with an average of 261.4 mm.

Preparation of polyherbal mixture

The polyherbal mixture was prepared using six herbs: i) *Foeniculum vulgare* (Saunf), ii) *Trachy spermumammi* (Ajwain), iii) *Trigonella foenum-graecum* (Methi), iv) *Zingiber officinale* (Sundh), v) *Anethum graveolens* (Sowa) and vi) *Elettaria cardamomum* (Cardamom). Each herb was procured from local market after assessing their quality and grounded thoroughly in desired quantity to ensure proper mixing (Table 1). Polyherbal mixture along with 25g black salt was mixed in one litre of water, and this mixture was boiled for about 30 minutes till half of water remained. To this, 250 grams of jaggery (gur) was added and heated for another 5-10 minutes. The herbal mixture, thus, prepared was mixed with concentrate and fed to the cattle from the day of calving till day 10 of postpartum in the morning hours.

Experimental design and supplementation of polyherbal

Sixteen healthy crossbred cattle, mainly Holstein Friesian crosses of 2nd to 4th parity, were selected and divided into two groups (eight each) viz. T_0: control and $T_{1:}$ treatment. The sugarcane tops and wheat straw was the major source of roughage used for feeding of dairy cattle along with some amount of berseem fodder and homemade concentrate. Animals of T_1 group were supplemented ployherbal mixture. The cattle were managed as per the standard feeding practices followed by the farmers. The animals were provided both dry and green roughage, and homemade concentrate, and were housed in semi loose housing system. Fresh water was made available to the animals at all the time of the day. Hand milking was done twice a day, at 5 to 6 AM and 5 to 6 PM and milk yield of individual cattle was recorded by the farmers on milk recording sheet provided to them. Feeding of polyherbal mixture was done from the day of calving till day 10 of postpartum and data was recorded for 2 months during December 2018 to February 2019.

Partial budget analysis

The partial budget analysis was done to estimate the likely economic impact of supplementation of technology (Stemmer et al 1998) and Cost-benefit ratio was calculated to assess the economical profitability of supplements (Amir and Knipscheer 1989). In partial budget analysis only those items of income and expenses were considered that change (Peso 2002). Therefore, the costs of ingredients used in preparation of polyherbal mixture only have been considered, since all other variable costs are the same for both the groups.

Statistical Analysis

The data collected was analysed using statistical software SPSS 22 (SPSS version 22, SPSS Inc. Chicago, Illinois). One-way ANOVA was used to test the level of significance.

Results and Discussion

Effect of polyherbal supplements on milk yield and reproduction

The response of polyherbal supplementation in crossbred cattle is presented in Table 2. Average milk yield at the start of experiment was 9.52 lire /day which increased to 11.57 litre /day in cattle over a period of 60 days. The cows supplemented with polyherbal mixture produced more milk than those of without supplementation, and it was significantly ($P<0.05$) higher in T_1 as compared to control group T_0 . Higher milk production in polyherbal group may be due to galactopoietic activity of some of the herbs like *Anethum graveolens*, *Foeniculum vulgare* and *Trachy spermumammi* (Chandra et al. 2017). It is well know that

Table 1 Ingredients of polyherbal mixture and their cost (Rs.)

Ingredients	Quantity	Cost/kg	Cost (Rs./d)
Methi : *Trigonellafoeenum-graecum*	25 g	140	3.50
Ajwain: *Trachyspermum ammi*	25 g	250	6.25
Saunf: Foenicum vulgare	25 g	210	5.25
Sowa: *Anethum graveolens*	25 g	140	3.50
Sundh: Zingiber officinale	25 g	320	8.00
Bari elaichi*: Elettaria cardamomun*	15 g	1000	15.00
Black salt	25 g	34	0.85
Jaggary (Gur), kg	250 g	25	6.25
Total	415 g	-	48.60

Table 2 Effect of polyherbal mixture on milk production and reproduction

Details	Control(n=8)	Treatment(n=8)
Milk yield, l/cattle/day		
0 day	7.37±0.31	9.52±1.19
15th day	9.10±0.39	10.56±1.40
30th day	9.17±0.50	11.85±1.75
45th day	7.82^{y}±0.36	11.93^{x}±1.76
60th day	7.76^{Y}±0.32	11.93^{X}±1.44
Mean ± SE	8.46^{Y}±0.19	11.57^{X}±0.77
Retention of foetal membrane, Number	0	2
Time taken for expulsion of placenta, hour	7.38^{b}±0.89	4.38^{a}±0.67
Number of insemination per conception	2.88^{b}±0.23	2.38^{a} ±0.18

Means bearing different superscripts in a row differ significantly (P<0.05).

Table 3 Economic evaluation of poly-herbal mixture on milk production

Details	Control	Experimental
Av. Milk yield/h/d	8.46^{b}±0.19	11.57^{a}±0.77
Total milk yield (L)	507.8	694.2
Cost of feeding polyherbal mixture (Rs.)	-	486
Gross return from sale of milk (Rs.)	14980.1	20478.9
Gross return over control (Rs.)	-	5498.8
Net return over control (Rs.)	-	5012.8
B:C	-	10.31

*Sale price of milk @29.5/litre

Anethum graveolens acts as a galactogogue (Jana and Shekhawat, 2010), where as *Foeniculum vulgare* plays an important role in promoting milk ejection, stimulating milk flow and increasing milk production (Abascal and Yarnell, 2008) and *Trachys permumammi* acts as galactogogue, hypotensive, oxytocic, stimulate milk ducts of mammary gland tissue as well as promote milk ejection (Zuppa et al. 2010; Ghedira et al. 2010). The present findings are in consonance with the finding of Patel et al, 2017 and Japheth et al. (2019) who reported that feeding of polyherbal mixture in crossbred cattle significantly (P<0.05) improved milk yield. Thakur et al (2006) also reported that dietary supplementation of commercial herbal feed additive to lactating crossbred cows increased the milk yield.

No case of foetal membrane retention was recorded in treatment group, whereas in control group, out of eight animals, two cases were observed (Table 2). Time required for expulsion of foetal membranes was reduced significantly (P<0.05) in supplemented animal as compared to control, which indicates advantage of using poly-herbal mixture just after parturition. Supplementation of polyherbal mixture has also reduced number of insemination per conception in cattle (2.38) as compared un-supplemented group (2.88). Herbal therapy is beneficial for the uterine recovery process following delivery (Chandra et al, 2015)

Partial budget analysis

The net benefit due to polyherbal supplementation on per animal basis was worked out to be Rs. 83.54/day with B:C ratio of 10.31 (Table 3). High returns are required from any farm innovations to offset the risks associated with its adoption (Peso 2002). Patel et al. (2017) also reported that supplementation of Shatawari, Jivanti and Fenugreek in equal proportion @ 60 g/cow/day resulted in significant increase in milk yield and daily return in lactating Kankrej cattle. Apart from increase in milk production, significant improvement in reproductive parameters was also recorded in supplemented animals that have economic importance for life time productivity of dairy animals.

Conclusions

Considering the present cost of feed supplement and the market price of milk, polyherbal mixture supplementation appears to be both economical and cost effective. The cost of polyherbal supplementation per animal per day worked out to be Rs. 48.60 with net return of Rs. 83.55/day. Supplementation had a positive effect on milk production and postpartum reproduction of crossbred cattle maintained under small holder conditions.

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RESEARCH ARTICLE

A comparative study on the expression profile of aquaporin 3(AQP3) gene in the skin fibroblast cells of Barbari and Sirohi breeds of goat

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Abstract: Lack of availability of good quality and quantity of water affects the physiology of goats. Goats may adapt to thrive well in water deficit areas by making alterations in the expression of aquaporin (AQP) genes for their efficient water use. The current study was carried out in skin fibroblast cells of Barbari and Sirohi goats. Under *in vitro* conditions, during different temperatures and osmotic treatments, variations in relative expression of AQP3 genes were observed. At higher temperatures the relative expression of AQP3 genes in Sirohi breed was found to be significantly higher ($p<0.01$) than Barbari breed. However, during hyperosmotic concentration at normal temperatures the relative expression of AQP3 mRNA was significantly (($p<0.05$) increased in both the breeds. During a high temperature combination with hyperosmotic concentration in cell culture of skin fibroblast cells the relative expression of AQP3 mRNA was increased in Sirohi breed ($p<0.01$) whereas it decreased in Barbari goat's breed. The variations in AQP3 gene expression during water stress condition and during hyperosmotic concentrations of skin fibroblasts cell culture suggests the positive involvement of AQP3 gene in maintaining the water balance in the body.

Keywords: AQP3, Barbari, Sirohi, Hyperosmotic,Skin fibroblasts

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Introduction

Global water scarcity is expected to be intensified in the future by a number of emerging threats such as growing world population and climate change. Many areas of the world that have already experienced shortage of water resources will further worsen their water issues, causing hardships for sustaining the livelihood. According to the United Nations World Water Development report 4, by 2025, about 1.8 billion people will live in areas affected by water scarcity, with two-thirds of the world's population living in water-stressed regions (UNESCO, 2012). Livestock production is widely considered as an intensive water consuming activity (Molden et al. 2010). Compared to the total water usage in livestock production systems, water for livestock consumption is proportionately small in amount but is an important requirement for health and productivity of animals (Amenu et al. 2013). In addition, lack of sufficient source of water can be a critically limiting factor in affecting the normal physiology and productivity (Alamer M, 2010).

Goats are known as poor man's cow because of its vast contribution to the rural economy. With 115.3 million goat population, India stands second in goat production in the world in which a major proportion of goat (>70%) thrives in arid and semiarid areas of western region and southern peninsular region of India (Sejian et al. 2012). Arid zones are characterized by excessive ambient temperatures, low humidity, low soil moisture and insufficient and erratic pattern of precipitation. In many countries like India with arid zones, high environmental temperatures during summer seasons may last up to 6 months, with average temperatures over 30°C (Avendano-Reyes, 2012). This is important because a high proportion of the goat population lives in these areas where water requirements are not easily met. Breeds of goats in arid and semi- arid zones are well adapted to survive in these water deficit areas. It has been well established fact that even though livestock including goats require large quantities of water for maintaining their body functions, some of the breeds which have evolved and naturally developed in arid regions, survive and remain to be productive with less water intake at higher temperatures.

Aquaporin proteins (AQP) are known as the plumbing system of the cell and act as water channels across the cell. Aquaporin proteins have been identified in different plants (Johansson et al. 2000; Schaffner 1998) and animals (Agre et al. 2002; Agre and Kozono, 2003; King et al. 2004). So far, thirteen isoforms of Aquaporin (AQP0- AQP12) have been discovered. Aquaporin proteins are broadly classified into two groups known as orthodox aquaporins (AQP 1, 2, 4, 5 & 8) that selectively transport water and the aquaglyceroporins (AQP 3, 7, 9 & 10) that transports small solutes along with water. Among all the aquaporin proteins, AQP1, AQP3 and AQP5 are widely expressed in skin tissues and found to play an important role in normal skin physiology (Boury-Jamot et al. 2006).

Even though several studies have been carried out on different functions of AQPs in different tissues, very few or scanty studies have been conducted in goats on the expression of AQP3 gene and its osmoregulatory role in skin. The variations in the gene expression of the native breeds thriving at arid and semiarid regions need to be studied to understand the mechanism involved for less water consumption as compared to other breeds. This leads to a better understanding and exploitation of full genetic potential of native goat breeds of India to develop new strains or breeds through the introduction of genes responsible for temperature resistance and higher water efficiency, thus sustaining the livestock productivity in coming decades of climate change. Sirohi and Barbari are the most adapted breeds in arid and semiarid, respectively. Keeping all in consideration, the present study was designed to investigate the expression of AQP3 in the skin fibroblast cells of the Barbari and Sirohi goat and its variation in the expression during hyperosmotic stress conditions in different temperatures.

Materials and Methods

Fibroblast cell culture and treatment

Skin explants were collected from the three adult Barbari and Sirohi goats and the skin fibroblasts culture were established in the laboratory. The fibroblasts were sub-cultured in Dulbecco's Modified Eagle's medium (Sigma Aldrich, USA) supplemented with 20% Fetal Bovine Serum (FBS) (Sigma Aldrich, USA) and 1% Penicillin- Streptomycin antibiotic solution (Himedia, Mumbai) at 37 °C and 5 % CO_2. Upon confluency, the fibroblast cells were passaged using 0.25% trypsin – EDTA solution (Thermofischer scientific, USA) at a split ratio of 1:3. The skin fibroblasts obtained after 4th passage were used for the different treatments. These fibroblast cell samples were treated at 3 different temperatures at two different osmotic concentrations. Briefly, samples of two breed was divided into 5 groups and treated as follows:

Treatment 1

The cell culture flask was incubated at 25°C in the CO_2 incubator for 3 hours. These cells were grown in cell culture media containing 20% FBS (Sigma Aldrich, USA) and 1 % penicillin-streptomycin- amphotericin B solution (Himedia, Mumbai).

Treatment 2

The cell culture flask was incubated at 37°C in the CO_2 incubator for 3 hours. These cells were grown in cell culture media containing 20% FBS (Sigma Aldrich, USA) and 1 % penicillin-streptomycin- amphotericin B solution (Himedia, Mumbai).

Treatment 3

The cell culture flask was incubated at 42°C in the CO_2 incubator for 3 hours. These cells were grown in cell culture media containing 20% FBS (Sigma Aldrich, USA) and 1 % penicillin-streptomycin- amphotericin B solution (Himedia, Mumbai).

Treatment 4

The cell culture flask was incubated at 37°C in the CO_2 incubator for 3 hours. These cells were grown in Hyperosmotic cell culture media containing 20% FBS (Sigma Aldrich, USA) and 1 % penicillin- streptomycin- amphotericin B solution (Himedia, Mumbai).

Treatment 5

The cell culture flask was incubated at 42°C in the CO_2 incubator for 3 hours. These cells were grown in Hyperosmotic cell culture media containing 20% FBS (Sigma Aldrich, USA) and 1 % penicillin- streptomycin- amphotericin B solution (Himedia, Mumbai).

100 mM NaCl was added to the normal culture medium to simulate hyperosmotic (dehydration) condition in cells. The samples (three each) for two breed were treated in triplicate at each treatment.The skin fibroblast cells in normal culture medium at 37°C were taken as the control sample.

RNA isolation and RT- PCR

Total RNA was isolated from the treated skin fibroblast cells by miRNeasy kit (Qiagen,U.S). RNA concentrations were measured by Nanoquant and those RNA samples isolated from the fibroblast cells having purity between 1.9 and 2.0 using Tecan's i-control software were only used further for cDNA synthesis. The quality and integrity of the obtained RNA was analysed by 1.5% agarose gel electrophoresis. First strand cDNA was prepared by using Thermoscientific RevertAid First Strand cDNA Synthesis kit.

Relative expression of mRNA of AQP3 gene was measured by quantitative real time-PCR (qPCR) using SYBR® Green qPCR kit. A master mix was prepared as per the following components: Maxima SYBR Green qPCR Master Mix(2X) - 5 µl, reverse primer - 0.5 µl, forward primer - 0.5 µl, cDNA sample -2 µl and nuclease free water - 2 µl. Before performing Real time PCR, optimum annealing temperatures for AQP3 gene and GAPDH (housekeeping gene) were determined by carrying out gradient PCR of 55°C to 65°C temperature range. The sequence information of gene was retrieved from NCBI database and suitable primers were designed using primer 3 web interfaces. The primer sequence and annealing temperature of the genes are given in Table 1.

PCR reactions were performed using following protocol

(50°C X 2 min; 95°C X 10 min-Initial denaturation) X 1 cycle; (95°C X 30 s - denaturation; T_m of gene X 30 s - annealing; 72°C X 30 s – elongation) X 25 cycles; (95°C X 1 min; 55°C X 30 s; 72°C X 30 s) X 1 cycle. Amplification Plots and Dissociation Curve for all reactions were analyzed for the presence of primer dimers or secondary structures or non-specific amplification. The relative expression of AQP3 was analyzed using $2^{-\Delta\Delta}$Ct method by keeping GAPDH as the reference gene and 37°C in normal culture medium as the control sample (Farhadi *et al.*, 2018; Livak & Schmittgen, 2001).

Statistical analysis

The statistical significance of variations in mRNA expression of the AQP3 gene in different breeds during different treatments was assessed by two-way ANOVA. A difference with value $P<0.05$ was considered statistically significant.

Results and Discussion

To the best of our knowledge, no study has been undertaken to give an insight about the expression of AQP3 gene in skin fibroblast cells of goats and their variation in relative expression during hyperosmotic stress. The current *in-vitro* study was performed to know the relative mRNA expression of AQP3 gene in different breeds. This will give an insight about the molecular mechanism by which the body reacts to it at various temperature and hyperosmotic conditions.The present *in-vitro* study was performed in skin fibroblast cells of Barbari and Sirohi breeds and the conditions in treatment 2 was considered as the optimum conditions for the normal cell expression.

RNA integrity and purity

The RNA isolated from the fibroblast cells has purity between 1.9 and 2.0 indicative of good quality RNA as optical density (OD) 260/280 ratio is greater than 1.8.The integrity of RNA sample from fibroblasts was checked by running on 1.5% agarose gel. The integrity of the isolated RNA samples illustrated two distinct bands, one for 18S and other for 28 S of RNA.

Gene expression analysis

The relative expression of mRNA for AQP3 gene of Barbari and Sirohi breeds during different treatments is presented in Table 2 and Fig 1. Relative expression of AQP3 gene in treatment 2 was taken as the calibrator.

Expression of mRNA of AQP3 gene

The relative mRNA expression of AQP3 gene in Barbari breed was observed to be lower meanwhile in Sirohi breed the relative expression of AQP3 mRNA was slightly increased. However in both the breeds of goat, the variations in the relative expression of AQP3 mRNA during treatment 1 were observed to be non-significant ($P>0.05$).

The relative mRNA expression of AQP3 gene was found to be lower in Barbari breed. The relative mRNA expression of AQP3 gene in Sirohi breed was observed to be higher and statistically significant ($P<0.01$).

In both the breeds, the relative expression of AQP3 mRNA was found to be increased significantly ($P<0.05$) during treatment 4 (Table 2). However, the mRNA expression value was significantly higher ($P<0.01$) in Sirohi breed compared to the Barbari breed. The relative expression of AQP3 mRNA in Barbari breed and Sirohi breed are presented in Table 2 and Figure 1 respectively. The relative expression of AQP3 mRNA in Sirohi breed was found to have increased significantly ($P<0.01$) though a decreased mRNA expression of AQP3 gene was seen in Barbari breed.

During treatment 1, 3, 4 and 5, the skin fibroblast cells of goat experienced stress as they were altered from the normal cell conditions for their optimum expression. The relative mRNA expression of the AQP3 gene in Barbari breed was found to be lower as compared to the Sirohi breed. Both Barbari and Sirohi breed showed a similar trend of increased relative mRNA

Table 1 Gene transcripts, primer sequence (5' to 3') and their product size (bp)

S. No	Primer	F/R	Sequence (5' to 3')	Tm (°C)	Product size. (bp)
1	AQP 3	F	CTTGGCTCAGACTCTGGGAGCCT	58	131
		R	GTGGCAAAGATGCCAGCTGTGCCATT		
2	GAPDH	F	CCAACGTGTCTGTTGTGGATCTGA	62	218
		R	GAGCTTGACAAAGTGGTCGTTGAG		

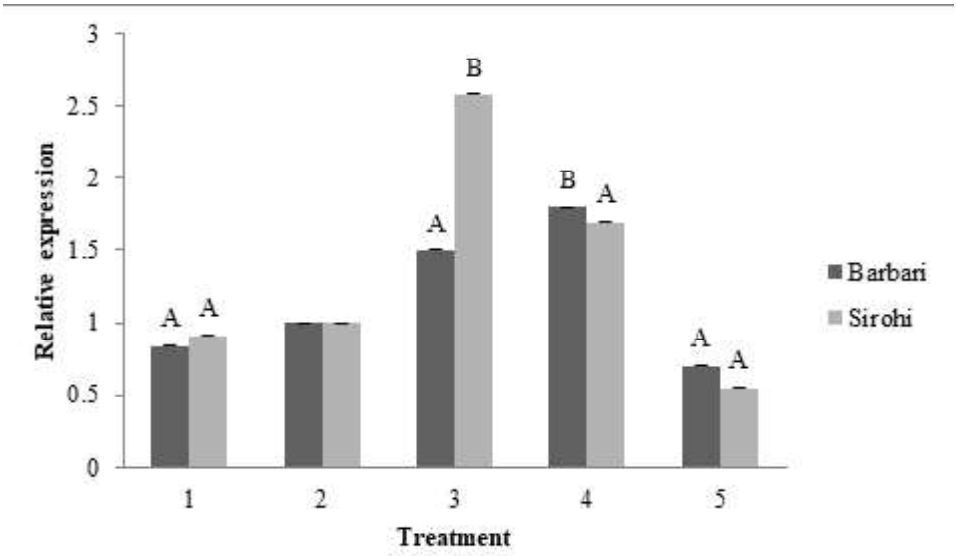

Fig. 1 The relative mRNA expression analysis of AQP5 gene by quantitative polymerase chain reaction (qPCR) in skin fibroblast cells of Barbari and Sirohi breeds of goat during different treatment conditions (1,2,3,4 and 5) in *in-vitro* studies. Different superscripts denote statistically different values (P<0.05) in comparison to treatment 2.

Table 2 Relative AQP3 gene expression in fibroblast cells during different treatment conditions

	AQP3 gene				
Treatment	1	2	3	4	5
Barbari	$0.37^{A}\pm0.06$	1.0±0	$0.59^{A}\pm0.04$	$2.23^{B}\pm0.90$	$0.71^{A}\pm0.04$
Sirohi	$1.08^{A}\pm0.04$	1.0±0	$8.77^{C}\pm0.87$	$11.72^{D}\pm0.99$	$3.50^{B}\pm0.75$

^{ABC}Bars with different superscripts are significantly different (p<0.05) between Treatments

expression during treatment 3 (normal temperature and hyperosmotic condition). A significantly (P<0.01) higher increase in relative mRNA expression of the AQP3 gene was observed in Sirohi breed with increase in temperature and osmotic concentration. But the increase in the AQP3 relative mRNA expression in treatment 5 (high temperature and hyperosmotic condition) was lower as compared to the treatment 3 (normal temperature) and treatment 4(normal temperature and hyperosmotic condition). During hyperosmotic treatment of Barbari skin fibroblast cells, there was a significant increase (P<0.05) in the relative expression of AQP3 mRNA at normal temperature and a decline in the relative expression at higher temperature.

At higher temperature, relative mRNA expression of AQP3 gene was observed to be increased in Sirohi breed as compared to Barbari. The increased relative mRNA expression of AQP3 in the Sirohi breed may be an adaptive mechanism developed by the animal to maintain its subcutaneous hydration during high temperature and hyperosmotic stress conditions. AQP3 results in the expression of membrane channel protein that selectively transports water and glycerol in epidermis (Sougrat et al. 2002). Subcutaneous skin hydration, glycerol and water permeabilities of epidermis were reported to be decreased in AQP3 null mice (Ma et al. 2002).

Increased relative mRNA expression of AQP3 may also play a role in early keratinocyte maturation in the Sirohi breed as AQP3 acts as glycerol transporter across the cell. The glycerol is utilized for the production of phosphatidyl glycerol which in turn excites an effector enzyme for the expression of early keratinocyte differentiation markers in rapidly dividing cells of the skin in epidermis (Bollag et al. 2007). Increased keratinocytes in the skin of Sirohi goat may act as a protective mechanism for Sirohi breed from heat stress in arid regions, thus indirectly reducing the water loss. There was an increased relative expression of AQP3 mRNA in both Sirohi and Barbari goats during dehydration (hyperosmotic stress) at normal body temperature meanwhile during higher temperature combined with hyperosmotic stress, the relative expression of AQP3 was found to be reduced. This may be due to the reason that with the upregulation of AQP3 along with the physiological mechanisms of the body, the animal may be able to overcome the hyperosmotic stress at normal body temperature. AQP3 expression was observed to be increased more than two fold times in keratinocyte cultures of humans during hyperosmolar conditions (Sugiyama et al. 2001). Previous studies have reported that the expression of AQP3 in non-keratinized embryonic skin helps in regulating the transepidermal water transport (Agren et al. 2003). AQP3 also transports small solutes such as urea along with water and glycerol across the cell. This characteristic of AQP3 gene may be playing a major role during hyperosmotic stress in the arid adapted breeds during dehydration resulting in their increased expression in Sirohi breed. But during hypertonic stress at higher temperature, AQP3 gene shows a decline in their relative expression. This may be due to

the fact that the cell may have adapted to stress or other mechanisms might have played a role.

Conclusions

The present study concluded that the mRNA expression of AQP3 gene is generally breed specific as there were variations in their relative mRNA expression patterns. This may be attributed to their difference in their adaptability to water stressed conditions. Among the two breeds studied, the Sirohi breed was found to have higher AQP3 mRNA expression indicative of high tolerance in the water deficit areas especially in arid areas. The relative expression of AQP3 mRNA was highly varying during different temperature treatments and osmotic treatments. And it may be due to its role played in transport of solutes especially glycerol along with water. Since experiments on AQP3 gene expression were done *in vitro*, more detailed studies (*in vivo*) are further required to explain more role of AQP gene physiology in the goat breeds.

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RESEARCH ARTICLE

Testing the efficacy of different lactation curve models for monthly test day milk yield

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Abstract: The test day milk yield data on 511 crossbred cattle were obtained and utilized according to monthly interval upto 305th day maintained at Instructional Dairy Farm, of G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). The different statistical models considered in this study for predicting the shape of lactation curve were Exponential Function, Parabolic Exponential Function, Inverse Polynomial Function and Gamma Type Function. Maximum coefficient of determination (R^2) value was observed for Inverse Polynomial function (R^2 = 99.97%) followed by Gamma function (R^2 = 88.28%), Parabolic function (R^2 = 80.95 %), and Exponential function (R^2 = 39.10%). The root mean square error (RMSE) was least for Inverse Polynomial function (0.07 kg) followed by Gamma Type function (0.10kg), Parabolic Exponential function (0.12 kg) and Exponential function (0.21 kg). The absolute mean deviation (AMD) was least for Inverse Polynomial (0.16 Kg) followed by Gamma Type function (0.24 Kg), Parabolic Exponential (0.31Kg) and Exponential function (0.48) confirming the same order of superiority.

Keywords: Exponential function, Gamma type function, Inverse polynomial function, Lactation curve, Parabolic exponential function, Test day yield**,**

Introduction

Lactation curve is the graphical depiction of milk production along with time in dairy cattle. Lactation curve starts with a certain level of milk production as initial milk yield and it increases with progression of time or advancement of lactation. This phase is influenced, to a certain extent, by physiological, nutritional and managemental factors. The peak phase symbolizes the maximum level of physiological activity and during this phase animal expresses milk production to the maxima of its genetic potential in an environment. Prediction of lactation yield at any point of time of lactation with least error is the main aim of lactation curve studies in case of irregular milk recordings. In the past, various models (linear and non-linear) have been used to explain the shape of lactation curves in cattle and buffalo. Among all models, the model of best fit is yet to achieve, because of the impact of several non-genetic factors on lactation (Sahoo et al. 2015). In the present study, lactation curves in crossbred cattle were obtained for Monthly Test Day Milk Yields (MTDMYs) using Exponential, Parabolic Exponential, Inverse Polynomial and Gamma Type functions. The coefficient of determination (R^2), the root mean square error (RMSE) and absolute mean deviation (AMD) for the four functions were calculated and the curves of predicted milk yield by these four functions along with the observed milk yields were presented.

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Materials and Methods

The test day (TD) milk yield data on 511 crossbred cattle were obtained and utilized according to monthly time interval upto 305th day distributed over a period of 28 years starting from 1990 to 2017 maintained at Instructional Dairy Farm, of G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Ut

Different statistical models considered in this study for predicting the shape of lactation curve were proposed by various workers such as Exponential Function, Parabolic Exponential Function (Sikka, 1950)**,** Inverse Polynomial Function **(**Nelder, 1966), and Gamma Type Function (Wood, 1967). These functions were analyzed by multiple regression method to estimate the model parameters (A, b, c, b_0, b_1, and b_2). The test day milk yield data on 511 crossbred cattle having complete lactation records were obtained and utilized according to monthly interval upto 305th day.

Exponential Function

$$Y_t = A\, e^{-ct}$$

The equation was modified by taking natural logarithms of Y_t thus

$\log_e Y_t = \log_e A + \log_e e^{-ct}$; or $\log_e Y_t = \log_e A - ct$; or $z = A + bt$

where, $z = \log_e$; $A = \log_e A$ and $b = -c$, the constant

Parabolic Exponential Function

$$Y_t = A.\, e^{bt+ct^2}$$

After taking natural logarithm and linear regression model the equation becomes :

$\log_e Y_t = \log_e A + bt + ct^2$; or $z = A + bt + ct^2$; or $z = A + b_1 t_1 + b_2 t_2$

where, $z = \log_e Y_t$; $A = \log_e A$; $b_1 = b$; $b_2 = c$; $t_1 = t$ and $t_2 = t^2$

Inverse Polynomial Function

Yt = t (b0 + b1t + b2t2)-1
After dividing both the sides by t and taking reciprocal the above equation can be written as :
t/ Yt = b0 + b1t + b2t2

Gamma type function

Yt= A tb e-ct
By taking natural logarithm on both sides the equation becomes:
loge Yt = loge A + loge tb - ct; or loge Yt = loge A + b loge t - ct; or z= A` + b1 X1 + b1 X2
where, z = loge Yt ; A` = loge A; b` = b; X1 = loge t; b2 = - c and X2 = t
Where, Yt = Yield in the tth month; A = constant, the value of initial theoretical rate of milk flow at the time of parturition; e = exponential constant (2.71828); b = ascending slope parameter upto the peak yield; c = descending slope parameter (persistency measure); b0 = theoretical value at the time of parturition; b1 = rising extremes of the curve; b2 = declining extremes of the curve and t = length of time since calving. The estimated values of coefficient of determination (R2) and the Root mean square error (RMSE) were computed as:
The coefficient of determination (R2) was calculated by following formula as:

$$R^2 = \left(\sum_i \left(Y_i - \overline{Y}\right)^2 \sum_i \left(Y_i - \hat{Y}_i\right)^2 / \sum_i \left(Y_i - \overline{Y}\right)^2\right)$$

Where, Y = observed value; ¯Y = mean of observed values and ?_i= estimated value
The Root mean square error (RMSE) was computed using the following formula:

$$\text{RMSE} = \frac{\left[\sum_{i=1}^{n} \left(Y_i - \hat{Y}\right)^2\right]^{0.5}}{n}$$

Where, n= number of observations; Yi = observed values and = predicted value
The adequacy of the goodness of fit of these different functions, used to explain the course of lactation curve, was judged by R2 values, RMSE and absolute mean deviations (AMD) of predicted milk yield computed according to these functions from observed milk yield.

The efficacy of all four functions was judged by the estimated values of coefficient of determination (R2) and the root mean square error (RMSE). The adequacy of the goodness of fit of these different functions, used to explain the course of lactation curve was judged by R2 values, RMSE and absolute mean deviations (AMD) of predicted milk yield computed according to these functions from observed milk yield.

Results and Discussion

The initial milk yield was observed as 8.50 kg (TD-1). The peak milk yield of 10.55 kg approached in the TD-2 and subsequently declined to 8.07 kg in TD-10. Similar findings were reported by Singh et al. (2016) in Karan Fries. Whereas, Kim et al. (2008) reported peak of monthly test day at 3rd month in Korean Holstein. Lactation curve parameters (A, b, c, b0, b1 and b2) were estimated by multiple regression analysis to fit into the mathematical models to obtain the prediction equation for FTDMYs (Table 2). The prediction equations for these mathematical functions are given in Table 3.

The predicted monthly test day milk yields (MTDMY) using Exponential function have been presented in Table 1 and the curve so obtained is shown in Figure-2. The peak yield was obtained on first test day (TD-1) and the curve indicated that milk production was linearly but inversely related to the advancement of lactation. The Exponential function described the descending phase of lactation and did not explain the ascending phase. The predicted graph followed closely the observed values only for 6, 7 and 8 test day (TD-6, TD-7 and TD-8). Therefore, the function can be used for prediction of sixth, seventh and eight monthly test day milk yield. The predicted monthly test day milk yield of Parabolic Exponential function is presented in Table 1 and the curve so obtained are presented in Figure-3. The peak yield was obtained on 4th test day. The function fits best to the 4th, 8th and 9 th test day milk yield i.e more accurate for descending phase. The predicted monthly test day milk yield using Inverse Polynomial

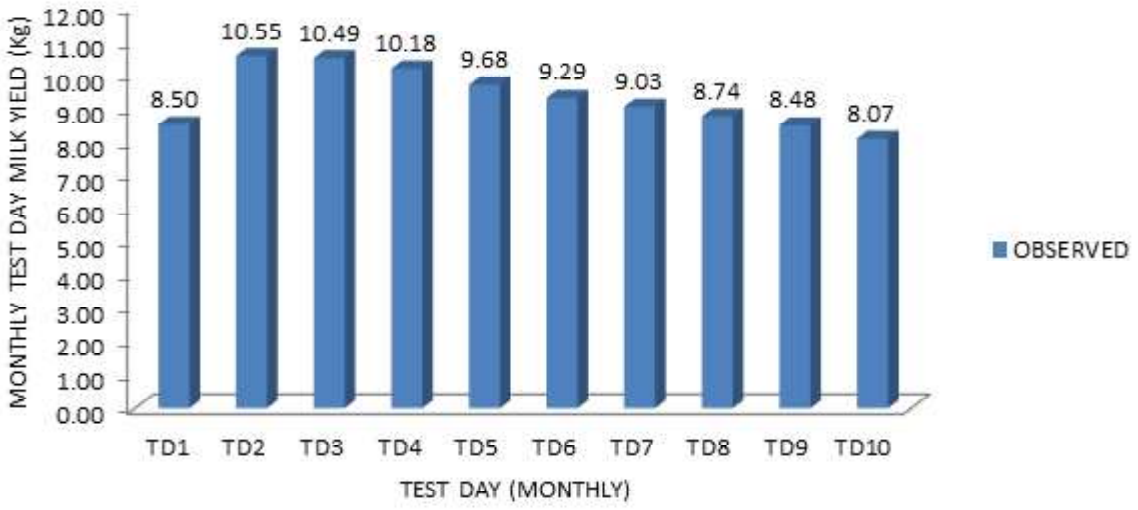

Fig. 1 Observed Least Squares Means of Monthly Test Day Milk Yields (MTDMYs)

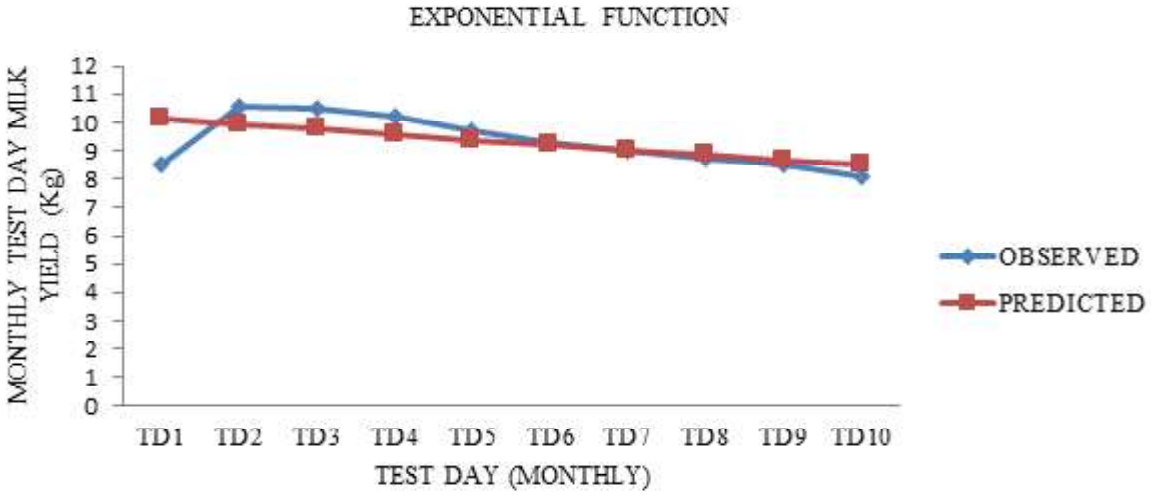

Fig. 2 Predicted Monthly Test Day Milk Yields (MTDMYs) by Exponential function

Table 1 Predicted Monthly Test Day Milk Yields (MTDMY) and Error of different Lactation Curve functions

MTDMY	Observed value {ls mean (kg)}	Exponential function		Parabolic exponential function		Inverse polynomial function		Gamma type function	
		Predicted value (kg)	Error (kg)	Predicted value (kg)	Error (kg)	Predicted value (kg)	Error (kg)	Predicted value (kg)	Error (kg)
TD1	8.50	10.13	1.63	8.93	0.44	9.07	0.58	8.83	0.33
TD2	10.55	9.94	-0.61	9.80	-0.75	10.20	-0.35	9.90	-0.65
TD3	10.49	9.75	-0.74	10.12	-0.37	10.28	-0.21	10.22	-0.27
TD4	10.18	9.56	-0.62	10.14	-0.04	10.07	-0.11	10.18	0.01
TD5	9.68	9.38	-0.30	9.98	0.30	9.76	0.08	9.96	0.28
TD6	9.29	9.20	-0.09	9.70	0.40	9.41	0.12	9.63	0.33
TD7	9.03	9.02	-0.01	9.33	0.29	9.07	0.03	9.23	0.20
TD8	8.74	8.85	0.11	8.88	0.14	8.73	-0.01	8.79	0.05
TD9	8.48	8.68	0.20	8.36	-0.12	8.41	-0.07	8.34	-0.14
TD10	8.07	8.51	0.44	7.78	-0.29	8.10	0.03	7.88	-0.19

function are presented in Table 1 and the curves so obtained is presented in Figure-4. The initial milk yield was 9.07 kg which then increased to a peak yield of 10.28 kg at 3rd test day and subsequently declined to 8.10 kg on the last test day. Thus, it indicated that Inverse Polynomial function estimated an initial ascending phase followed by peak and descending phase with

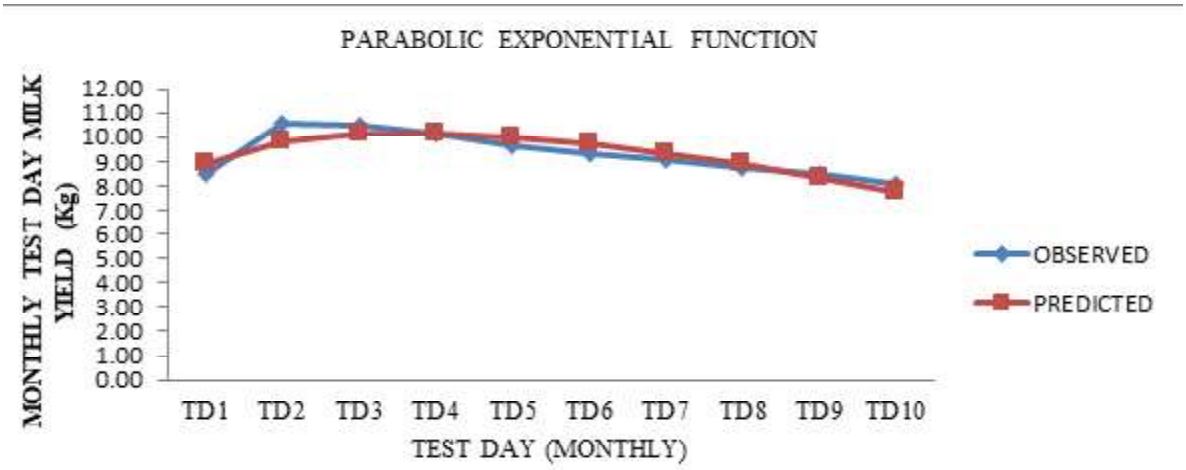

Fig. 3 Predicted Monthly Test Day Milk Yields (MTDMYs) by Parabolic Exponential function

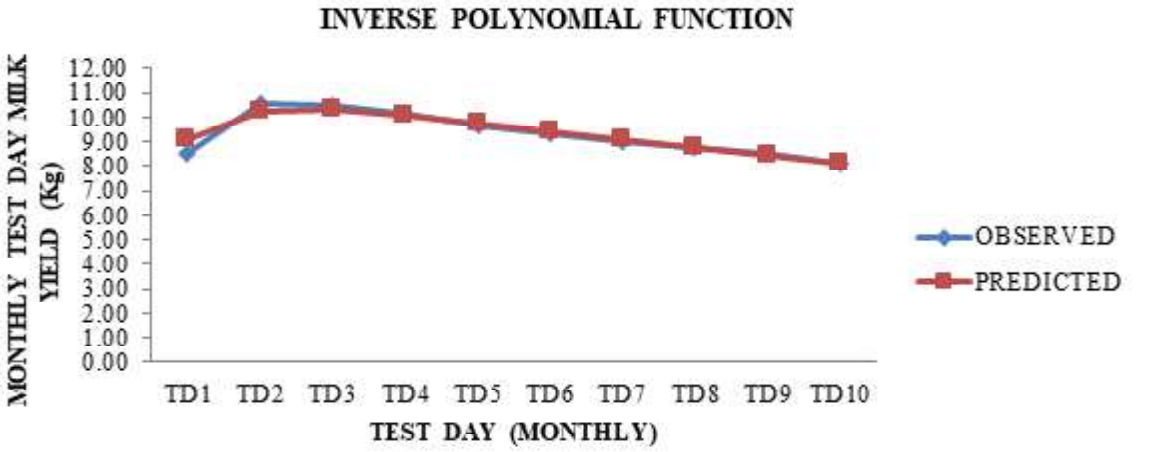

Fig. 4 Predicted Monthly Test Day Milk Yields (MTDMYs) by Inverse Polynomial function

Table 2 Estimated Lactation Curve parameters of different functions for prediction of Monthly Test Day Milk Yields (MTDMYs)

Lactation curve functions	Equations	Parameters					
		A	b	c	b_0	b_1	b_2
Exponential	$Y_t = A.e^{-ct}$	10.3280	-	0.0190	-	-	-
Parabolic exponential	$Y_t = A.e^{bt+ct^2}$	10.8640	-3.8283	-0.0308	-	-	-
Inverse polynomial	$Y_t = t.(b_0 + b_1.t + b_2.t^2)^{-1}$	-	-	-	0.0340	0.0713	0.0049
Gamma type	$Y_t = A.t^b.e^{-ct}$	9.6329	0.2915	0.0872	-	-	-

the advancement of lactation. The predicted monthly test day milk yields (MTDMYs) using Gamma Type function are presented in Table 1 and the curves so obtained is presented in Figure-5. The initial milk yield was 8.83 kg which then increased to a peak yield of 10.22 kg on 3rd test day and subsequently declined to 7.88 kg on the last test day. Thus, it indicates that Gamma function showed little lower value for the milk yield of ATD 2 nd,3 rd ,10th and lower values for the milk yield of ATD 5th and 6th. This indicates that the function best fit to ATD 4th, 7th, 8th, and 9th.

Comparison of four models

The coefficient of determination (R2), the root mean square error (RMSE) and absolute mean deviation (AMD) for the four functions are given in Table 3 and the curves of predicted milk yield by these four functions along with the observed milk yields are presented in Figure-6. Maximum R2 value was observed for Inverse Polynomial function (R2 = 99.97%) followed by Gamma function (R2 = 88.28%), Parabolic function (R2 = 80.95 %), and Exponential function (R2 = 39.10%). The root mean square error (RMSE) was least for Inverse Polynomial function (0.07 kg) followed by Gamma Type function (0.10kg), Parabolic Exponential function (0.12 kg)

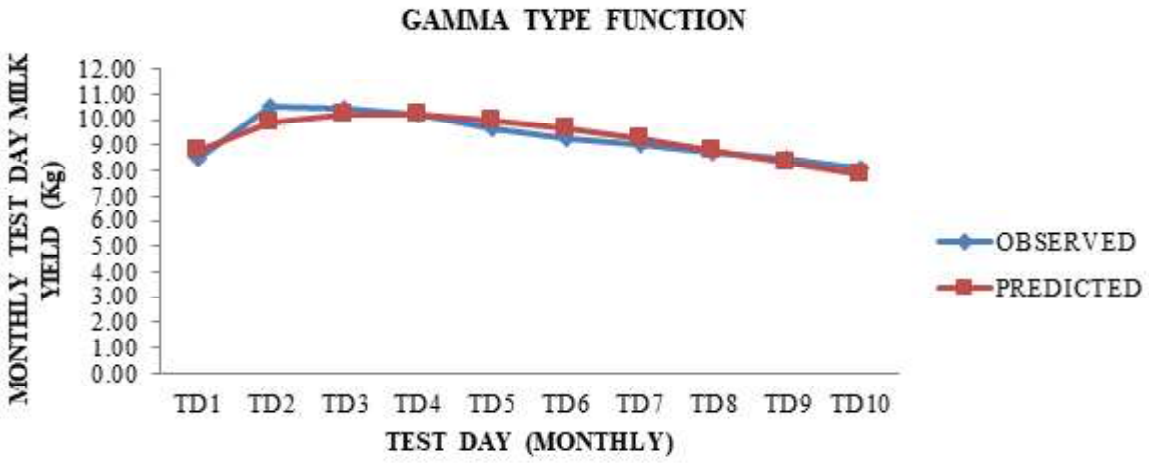

Fig. 5 Predicted Monthly Test Day Milk Yields (MTDMYs) by Gamma Type function

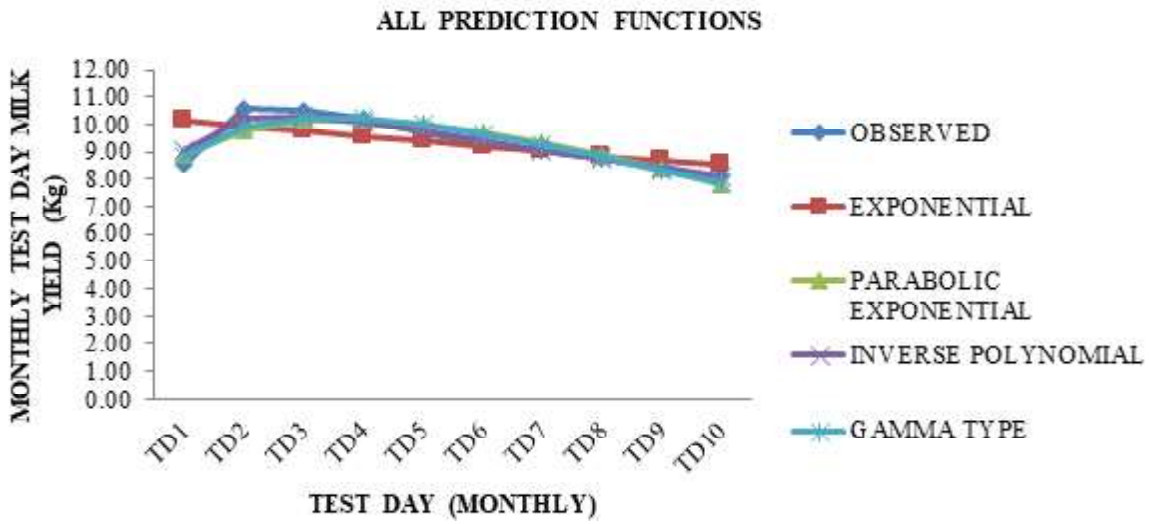

Fig. 6 Comparative evaluation of predicted Monthly Test Day Milk Yields (MTDMYs) by all four functions

Table 3 Different Lactation Curve functions with their prediction equations

Lactation curve functions	Equations	R^2 %	RMSE (Kg)	AMD (Kg)
Exponential	$Y_t = 10.3280.e^{-0.0190t}$	39.10	0.21	0.48
Parabolic exponential	$Y_t = (10.8640).e^{(-3.8283)\,t+(-0.0308)t^2}$	80.95	0.12	0.31
Inverse polynomial	$Y_t = t.(0.0340 + 0.0713\,.t + 0.0049.t^2)^{-1}$	99.97	0.07	0.16
Gamma type	$Y_t = 9.6329.t^{(0.2915)}.e^{-(0.0872)t}$	88.28	0.10	0.24

and Exponential function (0.21 kg). The absolute mean deviation (AMD) was least for Inverse Polynomial (0.16 Kg) followed by Gamma Type function (0.24 kg), Parabolic Exponential (0.31 kg) and Exponential function (0.48 kg) confirming the same order of superiority.

Graphical comparison, R2 value, RMSE and AMD value for the above models showed that Inverse Polynomial function explained better the different MTDMYs followed by Gamma Type, Parabolic Exponential and Exponential function. Hence, it can be concluded that Inverse Polynomial function is the best mathematical model for prediction of monthly test day milk yields in crossbred Cattle. Singh et al. (1998) in Jersey-Sahiwal F1 cows, Gandhi and Dongre (2013) in Sahiwal cattle found the same results as in this study. However, Singh et al. (1996) found Gamma type function

followed by Inverse Polynomial, Parabolic Exponential and Exponential function to be the best functions.

Conclusions

The relative effectiveness of four lactation curve models viz. Exponential Function, Parabolic Exponential function, Inverse Polynomial function and Gamma Type function were checked utilizing the first lactation monthly test day milk yields by computing coefficient of determination (R2), root mean square error (RMSE) and absolute mean deviation (AMD). The best fit lactation curve models were checked by computed highest R2 value with least values of RMSE and AMD. For monthly test day milk yield, the value of coefficient of determination was observed highest by Inverse Polynomial Function (R2 = 99.97%) followed by Gamma Type (88.28), Parabolic Exponential (80.95) and Exponential function (39.10) with least value of RMSE and AMD. Thus, these findings suggested that the Inverse Polynomial function was the best fit function for monthly test day milk yield records.

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RESEARCH ARTICLE

Economic analysis of informal dairy processing units in Karnal district of Haryana

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Abstract: Indian dairy sector is a blend of formal (cooperatives and private) and informal sector (small processing units, milk vendors etc.). Informal sector still dominates in terms of procurement of the sizeable milk surplus of the country. The present paper has examined the cost and return structure of 27 informal dairy processing units in Karnal district of Haryana for the year 2018-19. The net returns of these processing units are mainly attributed to varying level of milk handling capacity and diversification towards value added products. Dahi turned out to be most profitable product with 53 per cent of profit over cost followed by ghee (12.75 %). The costs of dairy products were found to be decreasing with rising milk processing capacity. Solvency status of dairy processing units was found to be satisfactory yet there is a need for further strengthening.

Keywords: Cost, Dairy Processing, Informal milk sector, Returns, Value addition

Introduction

The share of livestock in Indian economy is nearly 4.6 per cent of gross value added (GVA), 66 per cent of which is coming from dairy sectors (NDDB, 2018). Dairy production, processing and marketing employs 80-90 million people (Rao, 2017) which helped in poverty alleviation and rural employment in the country (Business Line, 2018). India has sustained its first position with record 176.4 million tonnes milk production in 2017-18 (NDDB, 2018). Milk per capita availability has improved and hovers around 375 gm per day. India surplus milk is divided largely into liquid milk sale (64 %), value added products (25 %), ghee (7 %) and milk powder (4 %) (Rabo Research, 2016-17). Margins in the liquid milk stood around 4-5 per cent and for the value-added products, it ranged from 12-18 per cent (CARE, 2017).

Indian dairy industry is characterised by the existence of formal/ organised and informal/ unorganised sectors. Formal dairy sector is under developed in local remote areas, milk sales in distant markets with higher transaction cost compels small dairy farmers for informal sectors (Birthal et al. 2005). Formal sector consist of dairy cooperatives and private processor which procures 23 per cent of total milk production and remaining 77 per cent is being procured by informal dairy sector which consists of small dairy processing units, milk vendors and halwais (Sharma, 2015; Birthal et al. 2017). There has been constant rise in expenditure on milk and milk products, 14 per cent (Urban) in 1970 to 20 per cent in 2011 (NSSO 68th round) on total food items. Rise in income, quality consciousness and nutritional benefits of dairy products has led toward the demand of value added products in future. Cooperatives has increased their attention towards value added products (around 20%) in recent years which is likely to rise upto 30 per cent by 2020-21 (Rural marketing, 2017). Current scenario of rising demand of value added products seems to be a big challenge for formal dairy sector as it has capacity and procurement constraints.

To meet the rising demand of value added dairy products, informal sector need to be supporting hand to the formal sector. From this view point present investigation carried out with following objectives: i) to estimate the cost and returns of value added products ii) to study the financial ratio of the different units.

Materials and Methods

The present study was carried out in the Karnal district of Haryana for the year 2018-19. Haryana ranks 2nd in per capita availability (1005 gm/day) of milk and highest productivity (8.39

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kg/day) of buffalo milk (NDDB, 2017-18). Annual income of household from animal rearing in Haryana is around 18 per cent as compared to the national average of 11 per cent. Karnal district and eastern part of Haryana is agriculturally advance region and earmarked as potential milk processing cluster by Government of Haryana. Presence of institutional support from ICAR- National Dairy Research Institute (NDRI) and ICAR-National Bureau of Animal Genetic Resources (NBAGR) has sent forth the adoption of milk production and processing activities to the small processing units in the study area.

Primary data were collected from 27 small scale milk processing units on the basis of random selection. Criteria for selection of the dairy units were daily procurement and processing of raw milk into value added dairy products (procurement up to 10,000 litres per day) in Karnal district of Haryana through survey method. Small processing units has an integrated structure where reception of milk, product manufacturing and sale of products takes place. Processing units were classified into three categories based on the daily average milk procurement for computing cost, returns and liquidity ratio. Both fixed cost and variable cost were included for estimation of cost of products. The details of these costs components are given as

Fixed costs

The elements of fixed cost are depreciation on fixed capital. To calculate fixed cost Capital Recovery Cost (CRC) method was used. Machineries in use and buildings were taken into account for fixed cost.

Variable costs

The major elements of variable cost were raw material cost, labour charges, fuel, electricity expenses, packaging cost, refrigeration charges and miscellaneous (water, watch and ward record keeping etc.). The details of variable costs included are given in Appendix-1

Financial ratio of dairy processing units

The financial ratios are very important for the measurement of the financial viability of the processing units. Different ratios calculated in order to find out the liquidity, solvency and efficiency of the different processing units were

a) Current ratio b) Quick ratio c) Return on investments

The current ratios were worked out to check the liquidity position with the help of formula given below.

a) Current ratio = Current assets /Current liabilities

The current ratio measures the extent to which current assets, if liquidated, would pay off all current liabilities. The higher the ratio, the greater the liquidity. A current ratio that is greater than one indicates satisfactory solvency of the processing units.

b) Quick ratio = Quick assets / Current liabilities

It is the ratio between quickly available or liquid assets and current liabilities. A normal liquid ratio is considered to be 1:1. A value with a quick ratio of less than 1 cannot currently fully pay back its current liabilities.

c) Return on capital employed (ROCE) = $\frac{\text{Profits}}{\text{Total capital employed}} \times 100$

Return on capital employed (ROCE) is a profitability ratio that measures how efficiently firms can generate profits from its capital employed by comparing net operating profit to capital employed. A higher ratio would be more favourable because it means that more rupees of profits are generated by each rupee of capital employed

Results and Discussion

The results are discussed under three different categories of the processing units. All the 27 milk processing units was post stratified into three categories on the basis of average daily milk handling by using Cumulative Square Root Frequency Method. (Appendix-II). After stratification, six processing units fall in Category-I (<400 LPD), 13 units in Category-II (400-700 LPD), and 8 processing units in Category-III (>700 LPD). The category-wise cost were calculated for different milk products, i.e., dahi, ghee, paneer and liquid milk (minimally processed), which contributes the major portion of the total revenue to the processing units.

Gross revenue from different products

Dairy products offered for sale by processing units were liquid milk, dahi, ghee, paneer, butter, cream, khoya and ice-cream. Lliquid milk, paneer, dahi and ghee shares the major portion on the gross revenue (Table 1). Liquid milk sale still dominates (39.20 %) the returns generated by the processing units followed by the dahi, paneer, ghee and other (butter, cream, khoya etc.).

Estimated cost of different dairy products

Cost of different dairy products were analysed and it was observed that for all the products, with rise in milk capacity, cost per unit decreases. The milk processing units under Category-III has slight advantage in terms of lower processing cost of milk (Rs. 39.58/ litre) as compared to Category-I (Rs. 40.59/ litre) and Category-II (Rs. 40.17/ litre). Overall, the average share of variable cost in total cost hovers around 98 per cent, and remaining is fixed cost

(Table 2). Among variable cost raw material share 85 per cent (Gurjar, 2017) followed by labour (3.77 per cent) and fuel charges (2.88 per cent). (Table 2)

The average cost involved in preparation of dahi was Rs. 39.30 per litre which uses (4 per cent milk Fat). The share of fixed cost and variable cost in total cost was 3.28 per cent and 96.72 per cent, respectively. Raw material contributes major portion of the cost (76.84 per cent) followed by labour, supervision and administration contributed 5.19 per cent and 2.36 per cent, respectively. (Table 2)

Average cost of production of Paneer turned out to be Rs. 217.78 per kg (Table 2). The contribution of total variable cost to the total cost was 97.74 per cent. Cost of raw material contributes 86 per cent followed by labour (4 per cent) and packaging (0.23 per cent) (Table 2). Cost of Paneer production was highest in case of Category-I (Rs. 220/kg) followed by Category-II (Rs. 219/kg) and lowest in Category-III (Rs. 215/kg), which reveals the economies of scale.

Average cost of manufacturing Ghee was found to be Rs. 425.24 per kg. The cost revealed a decreasing trend as the capacity increased from Category I to III. Share of variable cost in total cost was 98.18 per cent. Raw material shares major portion (88.19 per cent) of total cost in ghee manufacturing followed by labour (3.72 %) and fuel (3.22 %). (Table 2*)*

Profitability of different dairy products

Profitability of the milk and milk products was worked out by comparing the unit cost with the unit price received by the processing units for the sale of value added products. All the

Table 1 Gross revenue from different dairy value added products (Percentage)

S. No.	Products	Category I	Category II	Category III	Overall
1	Liquid milk (minimally processed)	34.7	37.1	45.8	39.20
2	Dahi	20.6	20.2	16.3	19.03
3	Paneer	21.2	24.3	22.8	22.77
4	Ghee	20.2	15.5	12.7	16.13
5	Other	3.3	2.9	2.4	2.87
	Total	100	100	100	100

Table 2 Component wise cost of dairy products (Rs/Unit)

Cost Components	Category											
	I	II	III	I	II	III	I	II	III	I	II	III
	Liquid	Milk		Dahi			Paneer			Ghee		
A.	Variable	cost	(Rupees)									
Raw material cost	34.50 (85.00)	34.50 (85.88)	34.50 (87.17)	30.20 (75.88)	30.20 (76.45)	30.20 (78.30)	188.00 (85.35)	188.00 (85.88)	188.00 (87.42)	375 (87.84)	375 (88.15)	375 (88.57)
Labour Charges	1.59 (3.92)	1.50 (3.73)	1.40 (3.54)	2.20 (5.53)	2.15 (5.44)	1.98 (5.13)	9.00 (4.09)	8.10 (3.70)	7.78 (3.62)	16.01 (3.75)	15.85 (3.73)	15.59 (3.68)
Electricity charges	0.10 (0.25)	0.08 (0.20)	0.06 (0.15)	0.05 (0.13)	0.04 (0.10)	0.04 (0.10)	1.90 (0.86)	1.65 (0.75)	1.59 (0.74)	9.2 (2.16)	8.95 (2.10)	8.5 (2.01)
Fuel	1.31 (3.23)	1.30 (3.24)	1.14 (2.88)	1.85 (4.65)	1.79 (4.53)	1.65 (4.28)	4.77 (2.17)	7.40 (3.38)	6.20 (2.88)	13.85 (3.24)	13.15 (3.09)	12.89 (3.04)
Refrigeration	0.70 (1.72)	0.60 (1.49)	0.96 (2.43)	1.20 (3.02)	1.11 (2.81)	0.99 (2.57)	3.72 (1.69)	3.16 (1.44)	4.20 (1.95)	2.51 (0.59)	2.35 (0.55)	2.20 (0.52)
Packaging	0.50 (1.23)	0.50 (1.24)	0.50 (1.26)	1.50 (3.77)	1.50 (3.80)	1.50 (3.89)	1.00 (0.45)	1.00 (0.46)	1.00 (0.46)	1.00 (0.23)	1.00 (0.24)	1.00 (0.24)
Supervision & Miscellaneous	0.79 (1.94)	0.74 (1.59)	0.42 (1.06)	1.38 (3.47)	1.31 (3.43)	1.08 (2.88)	6.09 (2.76)	4.05 (1.85)	2.88 (1.33)	1.18 (0.27)	1.16 (0.27)	1.11 (0.26)
Sub Total (A)	39.49 (97.29)	39.22 (97.64)	38.98 (98.48)	38.38 (96.43)	38.10 (96.45)	37.44 (97.07)	214.48 (97.37)	213.36 (97.46)	211.65 (98.41)	418.75 (98.09)	417.46 (98.13)	416.29 (98.32)
Fixed cost (B)	1.10 (2.71)	0.95 (2.36)	0.60 (1.52)	1.42 (3.57)	1.40 (3.55)	1.42 (3.57)	5.80 (2.63)	5.55 (2.54)	3.41 (1.59)	8.15 (1.91)	7.95 (1.87)	7.12 (1.68)
Total cost (A+B)	40.59	40.17	39.58	39.80	39.50	40.11	220.28	218.91	215.06	426.9	425.41	423.41
Average cost		40.11			39.30			217.78			425.24	

Figures within parentheses indicate percentage of total cost.

Table 3 Economics of different dairy products

S. No.	Products	Cost (Rs. /Unit)	Selling Price (Rs. /Unit)	Profit (Rs. /unit)	Per cent of unit profit to cost
1	Paneer (Kg)	217.78	245	27.22	12.50
2	Dahi (Kg)	39.30	60	20.70	52.67
3	Ghee (Kg)	425.24	480	54.24	12.73
4	Milk (Litre)	40.11	45	4.89	12.19

Table 4 Financial performance of dairy processing units (2017-18)

S. No.	Particulars	Category-I (<400 LPD)	Category-II (400-700LPD)	Category-III (>700LPD)	Overall
1.	Current Ratios	1.48	1.39	1.39	1.42
2.	Quick Ratio	1.39	1.39	1.38	1.38
3.	Return of capital Employed (ROCE)	10.83	13.25	14.59	12.89

dairy products manufactured by the processing units were found to be profitable in the present study.

It is evident from Table 3 that that dahi turned out to be the most profitable product with 53 per cent of profit over cost followed by ghee (12.73 %), paneer (12.50 %) and milk (12.19 %).

Financial performance of the dairy units

Financial performance of dairy processing units were analysed through liquidity ratio and return on capital employed to check the solvency and profitability of the firms.

Results (Table 4) shows that current ratio persisted just over 1 indicating a satisfactory liquidity position, considering the fact that dairy processing units were found to be engaged in major sale of liquid milk and dahi which is primarily a day to day business, the current ratio (1.42) as well as quick ratio (1.38) were satisfactory for the current year (2018-19). Return on capital employed (12.89 per cent) shows that the selected processing unit uses its available capital efficiently and produces additional profits.

Conclusions

Small milk processing units engaged in traditional value-added products were found to be an economical enterprise. The liquidity ratios were satisfactory (>1) for all the categories but need further strengthening by utilizing capacity efficiently and opting for scientific packaging which is rare in informal sectors. Among the dairy products paneer has been found least profitable (12.50 % profit to cost) which requires to explore the opportunities to enhance its profitability. Small enterprise needs to focus more on value added dairy products such as dahi (52.67% of unit profit to cost) as compared to the liquid milk sale (39.20% of gross revenue) to enhance profitability. There is imperative for the linking these dairy processing units to the institutions for further training in dairy entrepreneurship activities for improving their performance and boost local economy.

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RESEARCH ARTICLE

Economic analysis of milk production in peri-urban dairy farms of Odisha

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Abstract: East and South-eastern coastal plain zone of Odisha was purposively selected for conducting study. After complete enumeration, farms were categorized into small, medium and large categories on the basis of milch animals using cumulative square root frequency method. A total of 120 peri-urban dairy farms were selected from two towns namely Bhubaneswar town (Khorda district) and Cuttack town (Cuttack district). Budgeting technique was used to estimate costs and returns. Capital recovery cost method was used to evaluate fixed costs and variable costs were also evaluated for estimation. From the analysis it was concluded that crossbred cows were more profitable as compared to buffaloes. Milk productivity and returns from per litre milk of crossbred cow were more than buffalo. Concentrate feeding constitute major share in total feeding expenditure. Among different herd size categories of dairy farms, large farms were getting more profit per litre of milk than medium and small farms. Cost elasticity was estimated using Cobb-Douglas production function showed a negative relationship between per unit cost and milk yield.

Keywords: Budgeting, Capital recovery cost, Cobb-Douglas, Cumulative square root frequency

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Introduction

Milk production is playing an important role in the economy and socio-economic development of the country. Dairy farming has always been looked upon as a subsidiary occupation and not as a primary occupation. The share of livestock in agricultural gross domestic product has risen from 17 per cent to 25.6 per cent from 1970 to 2018 (NDDB). It shows a sustained growth in milk production for meeting growing demand of population. Increasing demand for milk in the urban area has led to market oriented dairy farming which is providing both profit and self-employment to the urban youth. So, dairy farming is gaining importance in and around urban centres. The reason for increasing demand for milk and milk products are due to increasing urbanization, rising per capita income and other related factors. Changing lifestyle and food habits of people in urban areas makes them more conscious towards more nutritious and healthy foods. Milk is an important constituent of most of these healthy diets. So, it is adding extra demand for milk in urban areas.

Profitability of dairy enterprises can be increased by either reducing cost or increasing milk production. When sale price of milk does not cover the total cost of milk production, farmers make loss. Economic analysis of dairy farming provides the basis for delineating the possibilities of controlling costs of milk production and increasing the returns to make it a potential dairy enterprise (Bhowmik and Sirohi, 2008).In order to evaluate and explore the possibilities of dairy farming as a profitable enterprise in Odisha, a study was undertaken in 2018-19 with focus on estimation of the cost and returns of milk production.

Materials and Methods

Sampling plan

The sampling design consisted of selecting the ultimate sampling unit, i.e., peri-urban dairy farms using stratified random sampling method. The study was based on the survey conducted in 2018-19 in East and South-eastern coastal plain zone of Odisha. Odisha has been selected purposively because despite of lowest dairy development among all the states (Kale et al. 2016), the per capita milk productivity is increasing over the years and currently it was 132 gm/day (NDDB, 2018-19). State is promoting dairy

through Dairy Entrepreneurship Development scheme. The east and south-eastern coastal plain zone was selected for the study because it is having highest milk producing potential (NDDB Odisha report,2016). East and south-eastern coastal plain zone of Odisha consists of 6 districts namely Kendrapara, Khordha, Jagatsinghapur, Cuttack, Puri and Nayagarh. Out of 6 districts 2 districts Cuttack and Khorda was selected randomly.

Data collection

Data was collected from two towns namely Cuttack town of Cuttack district and Bhubaneswar town of Khorda district. After complete enumeration, three potential areas of each town namely Nayabazar, Baxi Bazar and Chauliaganj (Cuttack town) and Saheed Nagar, Rental Colony and Kalinga Nagar (Bhubaneswar town) has been selected. These areas are having a total of 195 dairy farms out of which 120 dairy farms have been selected based on probability proportional to size. The selected 120 peri-urban dairies were stratified into three categories using Cumulative Square Root Frequency Method on the basis of number of milch animals. The peri-urban dairy farms were thus categorized into three herd size categories namely small (up to 18 milch animals), Medium (18-24 milch animals) and large (above 24 milch animals). The distribution of sampled peri-urban dairy farms in the small, medium and large herd size categories were found to be 49, 41 and 30, respectively. Primary data was collected from owners of the dairy farms using a well-structured pre-tested schedule.

Analytical framework

To achieve the objectives of the study, the data collected from 120 peri-urban dairy farms were scrutinized, tabulated and analyzed by employing various analytical tools. Budgetary technique was used to estimate cost and returns of milk production. The total cost was divided into fixed and variable costs. Components of fixed costs are depreciation and interest on fixed capital. Capital recovery cost (CRC) method was used to calculate the depreciation. The formula for estimation of CRC is given by:

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

Where,

R = Capital recovery cost

Z = Initial value of the capital asset

r = Interest rate

n = Useful life of the assets

When the asset is purchased from borrowed capital the actual interest rate charged by the bank will be taken as 'r', while in the case of owned funds, the interest on term deposit of 1-5 years will be taken. Equipment's for durable assets having productive life more than one year but not unlimited, depreciation was charged on the value of the assets by using straight line method.2,5,10,20 & 20 percent depreciation was considered for pucca building, semi pucca building, chaff cutter, milk can and bucket, respectively. The useful life of the animal or in other words its productive life was considered as 8 years for crossbred cow and 10 years for buffalo. The total CRC was then apportioned to individual animal in accordance with Standard Animal Units (SAUs).

Variable costs are those costs which are incurred on the variable factors of production and can be altered in the short run. It includes feed cost, labour cost, and miscellaneous cost. The information on the quantity of dry fodder, green fodder and concentrate fed to milch animals was recorded. Although the guess estimate provided by the farmers stems from his day to day experience in dairy activity, but to ensure maximum possible accuracy of data, as a counter check, personal observation was also made on size of the animal and total quantity of feed and fodder consumed. Labour cost included cost of family as well as paid labour (hired labour). The cost of hired labour was calculated considering type of work allotted and wages paid whereas, family labour costs were determined on the basis of existing wage rate of permanent farm labour. The labour utilized per standard animal unit in terms of adult male units (both hired and family) was converted into appropriate number of hours of adult male units for different categories of animals. The standard man hours of labour employed per animal per day was converted into monetary terms by multiplying with the corresponding wage rate. Miscellaneous cost included the cost of breeding for AI or service charge of bull as well as cost of vaccination and medicines, cost of repair, electricity, water charges, ropes, buckets etc. They were calculated on the basis of per milch animal per day for different types of milch animal kept by the sample households. Interest on working capital was not calculated as there is regular flow of income from sale of milk to the producer.

Considering the differences in regional endowments of animal wealth and species, the SAUs have been formulated by Sirohi et al. (2015) at regional level for five regions viz; Eastern (including north-east), Western, Southern, Northern plains and Hills. As the study area falls in the Eastern region so standard animal units for this region was given in Table 1. Other cost concepts used in the study were gross cost, net cost, cost per litre of milk, gross returns from sale of milk, net returns and net returns per litre of milk. These costs were calculated as

- Gross Cost = Total Fixed Cost + Total Variable Cost

- Net Cost = Gross Cost – Value of the Dung
- $\text{Cost per liter of milk} = \frac{\text{Net Cost Per Animal}}{\text{Milk yield of animal}}$
- Gross Returns from sale of milk = (Milk produced/ household /day) * Price of Milk
- Net returns = Gross returns – Net cost

$$\text{Net returns per liter of milk} = \frac{\text{Net returns per animal per day}}{\text{Milk produced per animal per day}}$$

To get direct estimates of cost elasticities average cost was regressed upon yield using Cobb-Douglas functional form.

$$C = \acute{\alpha} Y^{\beta}$$

Where C is the average cost of maintaining an animal in rupees per day and Y is the average milk yield of animal in Kilograms per day. β coefficient directly indicates the percentage change in average cost with one per cent change in yield.

Results and Discussion

To know whether the farm is getting remunerative price or not, it is important to calculate cost and returns from milk production of dairy enterprise. It provides a suitable basis for taking various decisions for making profit of farm business. The income flowing from the dairy enterprises is well spread over the entire year. There is desirability as well as scope for developing dairy enterprise both as a specialized or a supplementary enterprise. Keeping this in view, an effort was made to estimate cost and returns of different types of milch animals in this section. In order to draw better picture of the economic aspects of milk production for different species of milch animals based on per day milk production, cost and returns was worked out for different herd size categories.

Cost of milk production and returns from crossbred cow

The results provided in Table 2, revealed that total fixed cost accounted for 13.09 per cent of total cost. Out of total variable cost feed and fodder cost accounted for highest (51.56 %) followed by labour cost (45.72 %) and miscellaneous cost (2.70 %). So, out of total cost, total variable cost for crossbred cow constitute 86.90 per cent. Comparing farms of different herd size categories, it was found that total fixed cost was highest in case of large farms (17.51 %) followed by medium (13.94 %) and small farms (9.89 %). This is in conformity with the findings of earlier studies (Vishnoi,2014). Similarly, total variable costs were highest in case of small farms (90.10 %) followed by medium (86.05 %) and large farms (82.48%). It shows economies of scale was operating in these farms. Total variable cost constitutes feed and fodder cost, labour cost and miscellaneous cost. Labour cost was found to be minimum in case of large farms (40.94 %) followed by medium (46.89 %) and small farms (47.23 %). It means large farms were efficiently utilizing labour hour. Feed and fodder cost were the highest in case of large farms (55.58 %). This is in conformity with earlier studies carried out by Kumari, (2015) and Lal, (2016). Medium and small farms were found to have almost equal share for feed and fodder. Among green fodder, dry fodder and concentrate, concentrate was having highest share of costs. Costs for green fodder was low followed by dry fodder. Due to less availability of fodder farmers are mainly dependent on concentrate feeding of animals. Total net cost was found minimum for large farms (₹ 240.80) followed by small (₹ 266.97) and medium (₹ 271.64) farms. Due to higher fixed cost medium farms were having higher net costs. Economics of scale was observed for variable costs i.e., with increase in herd size costs were decreasing.

Overall average milk price of the study area was ₹ 38.13/litre and overall cost per litre of milk production was ₹ 28.29 /litre. Considering this the overall net return from per litre of milk was calculated as ₹ 9.83 /litre. The overall net return from crossbred cow was ₹91.83. Comparing across different herd size categories of peri-urban dairy farms, it was observed that highest net return from crossbred cow was obtained in case of large farms (₹ 129.12) followed by small (₹ 83.97) and medium farms (₹ 73.94).Similarly, net return from per litre of milk was highest in case of large farms (₹ 13.20) followed by small (₹ 9.22) and medium farms (₹ 8.09). It was found that per litre cost of milk production was lowest in case of large farms (₹ 24.62 /litre) which is due to operation of economies of scale. Farmers were getting total share in consumer rupees because peri-urban dairy farms are directly selling milk to consumer without involving middlemen.

Cost of milk production and returns from Buffalo

Table 1 Standard Animal Units for Eastern regions in India

	Cross bred cattle	Buffalo	Local Cow
Adult male (≥3 years)	1.07	1.02	0.92
Adult female (≥3 years)	1.20	0.86	1.00
Young stock male (<1 year)	0.25	0.25	0.27
Young stock female (<1 year)	0.24	0.23	0.24
Young stock male (>1 year)	0.51	0.42	0.41
Young stock female (>1 year)	0.38	0.38	0.37
Heifer	0.71	0.63	0.64

Perusal of the Table 3, it was observed that total fixed cost accounted for 12.76 per cent of total cost. Out of total variable cost, feed and fodder cost accounted for highest (53.36 %) followed by labour cost (44 %) and miscellaneous cost (2.62 %). So, out of total cost total variable cost for buffalo constitute 87.23 per cent. Comparing farms of different herd size categories, it was found that total fixed cost was highest in case of large farms (14.57%) followed by medium (13.25%) and small farms (10.91%). The reason for this may be due to more investment in infrastructure by large farms. Similarly, total variable costs were minimum in case of large farms (85.42%) followed by medium (86.74%) and small farms (89.08%). Total variable cost constitutes feed and fodder cost, labour cost and miscellaneous cost. It was found that miscellaneous cost is very low. Labour cost was found

Table 2 Cost and returns of milk production from crossbred cow (₹ /animal/day)

Cost component	Small	Medium	Large	Overall
Total fixed cost (TFC)	26.99(9.89)	39.00(13.94)	43.82(17.51)	35.30(13.09)
Green fodder*	9.03(7.29)	8.77(7.21)	8.18(7.13)	8.73(7.23)
Dry fodder*	27.03(21.83)	26.37(21.68)	25.00(21.79)	26.30(21.77)
Concentrate*	87.71(70.86)	86.44(71.09)	81.54(71.06)	85.74(70.99)
Feed & fodder**	123.78(50.34)	121.59(50.53)	114.74(55.58)	120.77(51.56)
Labour cost**	116.14(47.23)	112.83(46.89)	84.51(40.94)	107.10(45.72)
Misc cost**	5.96(2.42)	6.20(2.57)	7.17(3.47)	6.34(2.70)
Total variable cost (TVC)	245.89(90.10)	240.63(86.05)	206.43(82.48)	234.23(86.90)
Gross Cost (TVC+TFC)	272.88	279.64	250.25	269.53
Value of Dung	5.90	7.99	9.45	7.50
Net Cost (Gross cost-value of dung)	266.97	271.64	240.80	262.02
Price of milk (Rs/litre)	38.57	37.82	37.83	38.13
Avg milk/animal/day	9.09	9.13	9.77	9.28
Gross return	350.94	345.59	369.92	353.86
Net Return	83.97	73.94	129.12	91.83
Cost (₹ /litre)	29.34	29.73	24.62	28.29
Return (₹ /litre)	9.22	8.09	13.20	9.83

Figures in parentheses indicate percentage to the total, * indicate Figures in parentheses indicate percentage to total feed and fodder, ** indicate Figures in parentheses indicate percentage to total variable cost

Table 3 Cost and returns of milk production from Buffalo (₹ /animal/day)

Cost component	Small	Medium	Large	overall
Total fixed cost (TFC)	26.99(10.91)	39.00(13.25)	43.82(14.57)	35.30(12.76)
Green fodder*	6.98(7.09)	9.70(7.00)	12.13(7.34)	9.19(7.14)
Dry fodder*	22.14(22.51)	30.00(21.65)	35.98(21.80)	28.29(21.97)
Concentrate*	69.22(70.38)	98.87(71.34)	116.95(70.85)	91.28(70.88)
Feed & fodder**	98.35(44.67)	138.58(54.27)	165.07(64.28)	128.77(53.36)
Labour cost**	115.85(52.62)	110.53(43.29)	84.51(32.91)	106.20(44.00)
Misc cost**	5.96(2.70)	6.20(2.42)	7.17(2.79)	6.34(2.62)
Total variable cost (TVC)	220.17(89.08)	255.32(86.74)	256.76(85.42)	241.33(87.23)
Gross Cost (TVC+TFC)	247.16	294.32	300.58	276.63
Value of Dung	5.90	7.99	9.45	7.50
Net Cost (Gross cost-value of dung)	241.26	286.32	291.13	269.12
Price of milk (₹ /litre)	49.61	52.12	50.83	50.77
Avg milk/animal/day	5.59	6.01	6.97	6.08
Gross return	277.36	313.72	354.65	309.11
Net Return	36.10	27.39	63.51	39.98
Cost (₹ /litre)	43.15	47.56	41.72	44.30
Return (₹ /litre)	6.45	4.55	9.10	6.46

Figures in parentheses indicate percentage to the total, * indicate Figures in parentheses indicate percentage to total feed and fodder, ** indicate Figures in parentheses indicate percentage to total variable cost

Table 4 Cost elasticity of milk production

Dairy farm Category	Cost Elasticity
Small	-0.47
Medium	-0.66
Large	-0.68
Overall	-0.38

to be minimum in case of large farms (32.91%) followed by medium (43.29%) and small farms (52.62%). Feed and fodder cost were highest in case of large farms (64.28%) followed by medium (54.27 %) and small farms (44.67%). Among green fodder, dry fodder and concentrate, concentrate was having highest share of costs which is consistent with the earlier studies carried out by Vishnoi, (2014), Kumari, (2015) and Lal, (2016). Costs for green fodder was low followed by dry fodder. Total net cost was found highest for large farms (₹ 291.13) followed by medium (₹ 286.32) and small (₹ 241.26) farms.

Overall average milk price of the study area was ₹ 50.77 /lit and overall cost per litre of milk production was ₹ 44.04 /lit. Considering this the overall net return from per litre of milk was calculated as ₹ 6.46 /lit. The overall net return from buffalo was ₹ 39.98/lit. Comparing across different herd size categories of peri-urban dairy farms, it was observed that higher net return from buffalo was obtained in case of large farms (₹ 63.59) followed by small (₹ 36.10) and medium farms (₹ 27.39). In case of large farms higher milk productivity of the animals by performing better dairy management practices leads to higher net returns. Similarly, net return from per litre of milk was highest in case of large farms (₹ 9.10) followed by small (₹ 6.45) and medium farms (₹ 4.55). It was found that per litre cost of milk production was lowest in case of large farms (₹ 41.72 /litre) which is due to operation of economies of scale.

Cost elasticity of milk production for different herd size categories

The cost elasticity was estimated taking Cobb-Douglas functional form for average cost and yield. The result of cost elasticity of milk production is presented in the Table 4. From the Table 4 it was observed that overall cross elasticity of milk production was -0.38 which means that with one per cent increase in milk production, the average cost decreases by 0.38 per cent. The cost elasticity was found to be highest in case of large farms (-0.68) followed by medium farms (-0.66) and small farms (-0.47). The reason for this may be due to better dairy management practices followed by large farms and a greater number of crossbred cows. There was a negative relationship between per unit cost and milk yield. One per cent increase in yield reduced cost to the extent of 0.47, 0.66, and 0.68 per cent on small, medium, large farms, respectively.

Conclusions

The study shows that net return per litre of milk for crossbred cow (₹ 9.83) was more than buffalo (₹ 6.46). Therefore, it can be concluded that rearing of crossbred cow was beneficial for the farmers. Farms were mainly dependent on concentrate feeding which accounts for highest (about 70%) among feeding costs. So, dairy farmers should be encouraged for cultivation of green fodder and feeding proper nutrition for improving productivity of the animals. Labour cost was found to be higher in case of small and medium farms. It means there is a requirement of efficient utilization of labour hour for decreasing cost. Cost elasticity was found to be -0.47, -0.66 and -0.68 in case of small, medium and large farms, respectively. It was found that large farms were getting more returns on per litre of milk which may be due to better dairy management practices followed by these farms. Thus, there is a requirement of training for creating awareness about good dairy farming practices which will helpful in increasing net returns of the dairy farms.

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RESEARCH ARTICLE

Entrepreneurial behaviour of dairy farm women in Nainital district of Uttarakhand

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Abstract: The investigation was conducted in Nainital district of Uttarakhand to know the entrepreneurial behaviour of dairy farm women. A sample of total 120 respondents was selected from the five selected villages and pre-tested interview schedule was used for the data collection. Statistical techniques such as frequency, percentage, arithmetic mean, standard deviation, coefficient of correlation and t-test were used to analyze the data. The findings of the study revealed that majority of the respondents (72.5%) belonged to middle age category, 30.83 per cent had education up to high school, 69.17 per cent had medium family size, 59.16 per cent had medium dairy experience, 52.5 per cent respondents had medium herd size and milk production, 72.50 per cent had medium level of monthly income from dairy farming, 37.5 per cent had small land holding (1-2 hectares), 75 per cent had medium economic motivation and 77.5 per cent had medium scientific orientation. The study revealed that majority of the respondents (62.5%) had medium level of entrepreneurial behaviour with medium innovativeness (46.67%), achievement motivation (57.5%), decision making ability (70%), risk orientation (50%), coordinating ability (67.5%), planning ability (59.17%), information seeking behaviour (73.33%), cosmopoliteness (82.5%) and self-confidence (56.67%). It could be observed that family size, herd size, milk production, monthly income from dairy farming, land holding, economic motivation and scientific orientation showed positive and significant relationship with entrepreneurial behaviour at 1% level of significance, whereas education and dairy experience were positively and significantly related to entrepreneurial behaviour at 5% level of significance. On the other hand, age had positive and non-significant relationship with entrepreneurial behaviour of dairy farm women.

Keywords: Dairy farm women, Entrepreneurial behaviour

Introduction: In past few years, India has achieved major milestones in the field of milk production. Our country ranks first among the world's milk producing Nations since 1998. As per latest data, total milk production in India is 176.4 million tonnes and per capita availability of milk is 374 grams per day (DAHDF, 2017). India's has also the largest bovine population in the world and India's livestock holds 11.6 per cent of world total livestock population.

In rural India, dairy farming has been practicing for generating employment and steady income. Thus, entrepreneurial development in the field of dairy sector might be the best possible way to make people competent and self-reliant. Nowadays, entrepreneurs have been taken as a concept, not only for industries but also in the development of the agriculture and allied sectors. Dairy farming is considered as an entrepreneurial venture predominantly for women because most of the women are involved in dairy activities starting from caring of animals to marketing the milk products.

In Uttarakhand, about 90 per cent of dairy farmers operates dairy at small and medium scale. Milk production of Uttarakhand state is 1.565 million tonnes and the basic dairy species of state is cow and buffalo (DAHDF, 2015). Dairy farming is an income generating sector by which rural women can socially empowered and their self-actualization and psychological needs can be fulfilled. Dairy farming helps them to earn money and reduce poverty of the family.

Rural women play a significant role in livestock production. On an average, they work nearly 15 hours in a day. Along with their household chores, they work on the field and take care of their

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livestock as well. Their contribution cannot be measured in economic terms. Being effective manager of resources, they have potential to become a successful entrepreneur. Hence, there is a need to explore what entrepreneurial characteristics women possessed and to identify the areas and entrepreneurial skills which needs to be developed in dairy sector.

Materials and Methods

The present study was conducted in Nainital district of Uttarakhand state. Nainital district was selected purposively as it has the highest milk production. There are eight blocks in the selected district. From these blocks, Haldwani block was selected purposively for the study due to highest milk production and large number of dairy farm women among all the blocks. Five villages were selected purposively from the Haldwani block with higher number of dairy entrepreneurs. A list of 120 respondents who were practicing dairy and possessing five or more than five animals were selected from all the five selected villages through Proportional Allocation method. The pre-tested interview schedule was used to get information on profile characteristics and entrepreneurial behaviour of dairy farm women. Statistical tools such as frequency, percentage, arithmetic mean, standard deviation, co-efficient of correlation and test of significance were used for analyze of data. Scale developed by Chaudhary et al. (2007) was used to measure the entrepreneurial behaviour of dairy farm women. The scale is comprised of nine entrepreneurial components i.e. innovativeness, achievement motivation, decision making ability, risk orientation, coordinating ability, planning ability, information seeking behaviour, cosmopoliteness and self -confidence. To measure the entrepreneurial behaviour of dairy farm women an Entrepreneurial Behaviour Index was developed.

$$EBI = \frac{\sum_{n=1}^{9} \frac{Totalobtainedscoreofninecomponents}{Maximumobtainablescoreofninecomponents} \times Scalevalueofninecomponents}{\sum_{n=1}^{9} Scalevaluesofninecomponents}$$

Results and Discussion

Profile characteristics of dairy farm women

The findings related to profile characteristics of dairy farm women has been presented in Table 1. It shows that majority of the dairy farm women (72.50%) belonged to middle age category, whereas, 14.17 per cent belonged to old age and 13.33 per cent belonged to young age category. Thus, it can be concluded that middle age (37-51 years) dairy farm women have more responsibility towards the family and are more efficient and experienced than the younger ones. Usually, dairy farming needs good experience in the field therefore; women between 37 to 51 years are actively engaged in dairy farming.

The data regarding education of the dairy farm women were 30.83 per cent were having education up to high school, followed by 27.50 per cent had education up to middle level. Furthermore, 19.17 per cent dairy farm women had primary level of education, 11.67 per cent were intermediate, 6.67 per cent were illiterates and only 4.16 per cent were belonged to graduates and above category. Education helps the dairy farm women to collect new information required for dairy enterprise. Generally, in rural areas, women are not allowed to higher education and indulged more in household activities. Thus, it can be concluded that mostly dairy farm women were educated up to high school which enable them to develop entrepreneurial competencies.

Regarding family size, 69.17 per cent dairy farm women had medium family size, followed by large (18.33%) and small (12.5%) family size. This might be due to the fact that dairy farming has many activities which cannot be managed by a single person. There should be a cooperative and trustful family who helps to upgrade dairy farming skills and make it a profitable venture.

Nearly sixty per cent i.e. (59.16%) dairy farm women had medium level of dairy experience (16-31 years), whereas 21.67 per cent dairy farm women had low level of dairy experience (less than 16 years) and only 19.17 per cent had high level of dairy experience (above 29 years). It can be concluded that most of the dairy farm women had 16 to 29 years of dairy experience. It mainly depends upon the age of the dairy farm women. Those who have high experience in dairy sector must be in middle or old age group. Dairy experience provides better understanding in the field of dairy enterprise.

It is evident from Table 1 that nearly half of the dairy farm women (52.50%) had medium herd size (6-10), whereas 36.67 per cent had small herd size (less than 6) and only 10.83 per cent had large herd size (more than 10). It may be due to the fact that women were involved in most of the household chores so they can manage medium herd size efficiently. Medium herd size helped them to spend their valuable time with their family members and has long term economic impacts.

The data also indicates that 52.50 per cent respondents had medium level of milk production from dairy animals (less than 18 litres), followed by 29.17 per cent with low milk production from dairy animals (above 25 litres) and 18.33 per cent had (18-25 litres) high milk production from dairy animals. Milk production can be considered as a booster or motivation for dairy farm women because higher the milk production from animals, higher the income.

Majority of the dairy farm women (72.5%) had medium level of monthly income from dairy farming, followed by 15 per cent had high level and only 12.5 per cent had low level of monthly income from dairy farming.

37.50 per cent of dairy farm women had small size of land holding (1-2 ha), whereas 33.33 per cent had marginal category (less than 1 ha), 20.84 per cent had semi- medium category (2-4 ha), 8.33 per

cent had medium category of land holding (4-10 ha) and none of them had large land holding (above 10 ha). This might be due to the reason that most of the families in Uttarakhand had fragmented land due to division of joint families.

The data in Table 1 also shows that three-fourth of the dairy farm women (75%) had medium level of economic motivation, followed by high (16.67%) and low (8.33%) level of economic motivation and 77.50 per cent dairy farm women had medium level of scientific orientation, followed by 12.5 per cent low level of scientific orientation and only ten per cent had high level of scientific orientation.

These findings are similar with the findings of Rathod et al. (2012), Yadav (2013), Bhosle et al. (2014), Kaur (2015), Raina et al. (2016), Chaurasiya et al. (2017) and Adhikari (2018).

Entrepreneurial behaviour of dairy farm women

Entrepreneurial behaviour of dairy farm women is defined as the cumulative outcome of nine components viz. innovativeness, achievement motivation, decision making ability, risk orientation, coordinating ability, planning ability, cosmopoliteness, self-confidence and information seeking behaviour. Entrepreneurial behaviour of dairy farm women was assessed for different components and presented in Table 2.

Innovativeness

The data in Table 2 shows that maximum number of dairy farm women (46.67%) had medium level of innovativeness, followed by high (37.5%) and low (15.83%) level of innovativeness.

Table 1 Profile characteristics of dairy farm women (n = 120)

S. No.	Characteristics	Category	Mean	SD	Frequency	Percentage
1.	Age	Young (<37 years)	44.28	6.86	16	13.33
		Middle (37-51 years)			87	72.50
		Old (>51 years)			17	14.17
2.	Education	Illiterate			8	6.67
		Primary education			23	19.17
		Middle education			33	27.50
		High school			37	30.83
		Intermediate			14	11.67
		Graduation and above			5	4.16
3.	Family size	Small (<5)	6.52	1.82	15	12.50
		Medium (5-8)			83	69.17
		Large (>8)			22	18.33
4.	Dairy experience	Low (<16 years)	22.52	6.26	26	21.67
		Medium (16-29 years)			71	59.16
		High (>29 years)			23	19.17
5.	Herd size	Small (<6)	9.68	3.77	44	36.67
		Medium (6-10)			63	52.50
		Large (>10)			13	10.83
6.	Milk production	Low (<18 litres)	21	2.83	35	29.17
		Medium (18-25 litres)			63	52.50
		High (>25 litres)			22	18.33
7.	Income from dairy farming	Low (<6000)	16,180	9,836.13	15	12.50
		Medium (6000-25000)			87	72.50
		High (>25000)			18	15.00
8.	Land holding	Marginal (<1 ha)			40	33.33
		Small (1-2 ha)			45	37.50
		Semi-medium (2-4 ha)			25	20.84
		Medium (4-10 ha)			10	8.33
		Large (>10 ha)			0	0
9.	Economic motivation	Low	19.83	1.77	10	8.33
		Medium			90	75.00
		High			20	16.67
10.	Scientific orientation	Low	23.15	2.22	15	12.50
		Medium			93	77.50
		High			12	10.00

The findings are in accordance with the findings of Lawrence and Ganguly (2012) and Tekale et al. (2013) that most of the dairy respondents had medium level of innovativeness. It might be due to proper education and medium level of dairy experience.

Achievement motivation

It is visible from Table 2 that, majority of the dairy farm women (57.5%) had medium level of achievement motivation whereas 35 per cent had high level of achievement motivation and only 7.5 per cent had low level of achievement motivation. Women engaged in dairy farming were found to be in medium to high level of achievement motivation which characterized them as successful entrepreneurs.

The findings are similar with the findings of Lawrence and Ganguly (2012), Tekale et al. (2013) and Patel et al. (2014) who also reported that majority of the respondents had medium level of achievement motivation.

Decision making ability

It is apparent from the Table 2 that 70 per cent dairy farm women had medium level of decision making ability, followed by 15 per cent who had high level of decision making ability and 15 per cent had low level of decision making ability.

Similar findings were reported by Lawrence and Ganguly (2012), Rathod et al. (2012), and Patel et al. (2014) who concluded that

Table 2 Distribution of dairy farm women based on their components of entrepreneurial behaviour (n=120)

S. No.	Components	Mean	SD	Category	Frequency	Percentage
1.	Innovativeness	21.53	3.64	Low (<18)	19	15.83
				Medium (18-24)	56	46.67
				High (>24)	45	37.50
2.	Achievement motivation	3.68	1.34	Low (<2)	9	7.50
				Medium (2-4)	69	57.50
				High (>4)	42	35.00
3.	Decision making ability	14.36	1.26	Low (<13)	18	15.00
				Medium (13-15)	84	70.00
				High (>15)	18	15.00
4.	Risk orientation	7.43	1.66	Low (<6)	27	22.50
				Medium (6-8)	60	50.00
				High (>8)	33	27.50
5.	Coordinating ability	7.16	1.48	Low (<6)	20	16.67
				Medium (6-8)	81	67.50
				High (>8)	19	15.83
6.	Planning ability	2.09	0.58	Low (<1)	19	15.83
				Medium (1-2)	71	59.17
				High (>2)	30	25.00
7.	Cosmopoliteness	7.84	1.20	Low (<7)	15	12.50
				Medium (7-9)	99	82.50
				High (>9)	6	5.00
8.	Self-confidence	4.78	1.33	Low (<3)	8	6.67
				Medium (3-5)	68	56.67
				High (>5)	44	36.66
9.	Information seeking behaviour	3.13	1.40	Low (<2)	9	7.50
				Medium (2-4)	88	73.33
				High (>4)	23	19.67

majority of the dairy respondents had medium level of decision making ability.

Risk orientation

It is evident from the data in Table 2 that half of the dairy farm women (50%) had medium risk orientation, whereas 27.5 per cent dairy farm women had high risk orientation and 22.5 per cent had low risk orientation. Risk bearing capacity of an individual depends on its socio-economic, personal and psychological aspects. A woman having better income with medium level of experience has medium risk orientation.The findings are similar with the findings of Lawrence and Ganguly (2012), Patel et al. (2014) who reported that maximum numbers of dairy respondents had medium level of risk orientation.

Coordinating ability

The data in Table 2 indicates that majority of the dairy farm women (67.5%) had medium level of coordinating ability, followed by 16.67 percent who had low level of coordinating ability and 15.83 per cent had high level of coordination ability. The medium level of coordinating ability possessed by majority of dairy farm women exhibits their ability to manage dairy as a successful venture.Similar findings were reported by Patel et al. (2014) who found that most of the dairy respondents had medium level of coordinating ability.

Planning ability

The results from the Table 2 reveals that most of the dairy farm women (59.17%) had medium level of planning ability, whereas 25 per cent had high level of planning ability and 15.83 per cent had low level of planning ability.Similar findings were reported by Lawrence and Ganguly (2012), Tekale et al. (2013), and Raina et al. (2016) who concluded that majority of the dairy respondents had medium level of planning ability.

Cosmopoliteness

It is inferred from data in Table 2 that 82.5 per cent dairy farm women had medium level of cosmopoliteness, whereas 12.5 per cent had low level of cosmopoliteness and only five per cent had high level of cosmopoliteness. It is evident that more than 80 per cent dairy farm women had medium level of cosmopoliteness. This shows that dairy farm women are actively engaged in seeking information from sources other than their own social system.

The findings are in accordance with the findings of Lawrence and Ganguly (2012), Tekale et al. (2013) and Raina et al. (2016) who also concluded that majority of the respondents had medium level of cosmopoliteness.

Self-confidence

It is evident from the Table 2 that more than half of the dairy farm women (56.67%) had medium level of self-confidence, followed by 36.66 per cent who had high level of self-confidence and 6.67 per cent had low level of self-confidence. This might be due to

Table 3 Distribution of the dairy farm women based on their overall entrepreneurial behaviour (n=120)

S. No.	Category	Frequency	Percentage
1.	Low (<52)	21	17.50
2.	Medium (52-67.5)	75	62.50
3.	High (>67.5)	24	20.00
	Mean	59.82	
	SD	7.65	

Table 4 Relationship between Entrepreneurial behaviour of dairy farm women with their profile characteristics (n=120)

S. No.	Profile characteristics	Correlation coefficient	t value
1.	Age	0.096	0.95
2.	Education	0.247**	2.53
3.	Family size	0.448*	4.96
4.	Dairy experience	0.237**	2.41
5.	Herd size	0.368*	3.92
6.	Milk production	0.449*	4.97
7.	Monthly income	0.614*	7.70
8.	Land holding	0.404*	4.37
9.	Economic motivation	0.500*	5.72
10.	Scientific orientation	0.417*	4.54

*Significant at 0.01 level of probability
t_{tab} = 2.62 (at 1% level of significance)

**Significant at 0.05 level of probability
t_{tab} = 1.98 (at 5% level of significance)

reason that most of the dairy farm women had medium economic motivation and medium level of income. Good income and economic status boost self-confidence of dairy farm women.

Similar findings were reported by Tekale et al. (2013), Patel et al. (2014) and Raina et al. (2016) who concluded that most of the dairy respondents had medium to high level of self-confidence.

Information seeking behaviour

It is clear from the Table 2 that majority of the dairy farm women (73.33%) had medium level of information seeking behaviour, followed by 19.67 per cent who had high level of information seeking behaviour and only 7.5 per cent had low level of information seeking behaviour.

The results are in accordance with the results of Rathod et al. (2012), Patel et al. (2014), and Raina et al. (2016) who also concluded that majority of the dairy respondents had medium level of information seeking behaviour.

Overall entrepreneurial behaviour of dairy farm women

On the basis of total entrepreneurial scores obtained by dairy farm women, they were grouped into three categories viz. low, medium and high and their frequency and percentage distribution are given in Table 3.

It is evident from the Table 3 that 62.5 per cent dairy farm women had medium level of entrepreneurial behaviour, whereas 20 per cent dairy farm women had high level of entrepreneurial behaviour and 17.5 per cent dairy farm women had low level of entrepreneurial behaviour. Thus, it can be concluded that most of the dairy farm women had medium to high level of entrepreneurial behaviour. This might be due to high dairy experience, good schooling, medium milk production, economic motivation and scientific orientation.

The findings are in accordance with the findings of Lawrence and Ganguly (2012), Raina et al. (2016) and Sadashive et al. (2017) who also concluded that maximum numbers of dairy respondents possessed medium to high level of entrepreneurial behaviour.

Relationship between profile characteristics and Entrepreneurial behaviour of dairy farm women

Data related to relationship between profile characteristics and entrepreneurial behaviour of dairy farm women are presented in Table 4. It revealed that family size, herd size, milk production, monthly income from dairy farming, land holding, economic motivation and scientific orientation had positive and significant relationship at 0.01 level of probability, whereas education and dairy experience had positive and significant relationship with entrepreneurial behaviour at 0.05 level of probability but age had positive and non-significant relationship with entrepreneurial behaviour of dairy farm women.

It indicates that family size, herd size, milk production, monthly income from dairy farming, land holding, economic motivation and scientific orientation were highly correlated with entrepreneurial behaviour of dairy farm women. Moreover, age did not have much influence on entrepreneurial behaviour of dairy farm women. It might be due to the reason that maximum respondents had medium family size (5-8 members). Family members cooperates in doing domestic as well as dairy farming work, helps in making best decisions, taking actions and boost self-confidence of a dairy farm woman.

Land holding had exhibited positive and significant relationship with entrepreneurial behaviour of dairy farm women. It might be due to reason that the respondents could use her land for growing green fodder for the dairy animals and for practicing and adopting new improved technology related to dairy farming. Thus, it was concluded that larger the land holding, higher the entrepreneurial behaviour of dairy farm women.

Monthly income and economic motivation showed positive and significant relationship with entrepreneurial behaviour of dairy farm women. It might be due to reason that economic motivation is a psychological condition of an individual which drives the respondents to strive hard and achieve higher income. These findings are similar in accordance with the findings of Patel et al. (2014) and Raina et al. (2016).

Conclusions

Entrepreneurial behaviour of a dairy farm woman is depended upon innovativeness, achievement motivation, decision making ability, risk orientation, coordinating ability, planning ability, information seeking behaviour, cosmopoliteness and self-confidence. Thus, effective entrepreneurship development programmes should be initiated on these parameters so that women can empower themselves. The results of the study showed that less percentage of women had high milk production. Therefore, efforts by veterinary experts, extension agents should plan training programmes for enhancing milk productivity. The findings indicate that very few dairy farm women had large herd size. Thus, efforts should be made to educate women about subsidies and other government schemes which enable them to increase the herd size. It is also inferred from the study that economic motivation, scientific orientation, milk production and herd size were positively related with entrepreneurial behaviour. The policy makers and training institutes on entrepreneurship should put more emphasis for designing training on these aspects.

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RESEARCH ARTICLE

Farmers' perception towards dairy farm automation in north India

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Abstract: Automation in dairying is operationalized as "art and science of utilizing mechanical, electronic equipment and technologies for optimising the production & processing of milk and minimising the need of labour". The study was conducted in five states of northern India. The purpose of this study to assess the commercial dairy farmers perception towards the 'dairy farm automation technologies'. The findings of the study indicated that majority (72.00%) of the farmers considered ICAR, SAU, SVS personal as the most important source of information to update the knowledge on dairy automation/ mechanization technologies. The perception of 90 respondents from total of 5 states towards automation/ mechanization technologies was assessed using snowball sampling technique. The results revealed that majority of the farmers considered benefit cost ratio as the most important with mean value of 2.88 prior to purchase dairy automation/ mechanization technologies. It was also observed that commercial dairy farmers had high scientific orientation, high risk orientation,high level of economic motivation and possessed high innovativeness in their occupation. More than 54.44 percent respondents had high perceptual level, while only 10.00 per cent of the farmers were in the category of low perceptual level.

Keywords: Dairy automation, Farmers, mechanization, north India

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Introduction

Urbanization and globalization have driven the modernization (industrialization) of agriculture and the dairy industry worldwide. As people move from farms and rural villages to urban centers, agriculture adopts mechanization and automation to produce food products for urban consumers. Urbanization has driven international trade because of increased earnings of urban dwellers. Mechanization in dairy industry started a way back in the era of 1830 (Shahhosseini, 2013) however, several advances in dairy farm mechanization have taken place only recently due to increased number of heads per farm. Dairy farm modernization leads to implementation of sanitation and quality standards and adoption of standardized handling equipment such as stainless-steel containers, milking machines, bulk milk cooler, etc on the farm. Larger and more specialized dairy processing plants impose requirements on milk producers to increase quality and volume. Overall, modernization of dairy farms and specialization of processing reduces costs for dairy products for consumers in urban areas (Nicholson et al. 2011). The increasing demand was expect to push up intensification in smallholder livestock farming (Wright et al. 2012). Demand for dairy products and technologies will grow during the next 50 years for 2 reasons. First, increased per capita income worldwide will boost demand for dairy and other food products from animals and these products increasingly will provide essential nutrients in developing countries. A mechanized dairy farm and an effective waste disposal system are recommended for efficient dairy farming (Rahman, 2004). The progress in life cannot be achieved without adequate evaluation, assessment of knowledge. The farmers' perception about dairy farm automation technologies is of paramount issue. Tireless effort of scientist and research will amount to waste of time and resources when proper and long term adoption is not achieved at the farmers' end. The study therefore focus on farmer's perception on dairy farm automation. Perception of farmers toward dairy automation is very important for the adoption of the innovations/ technologies.

Materials and Methods

The present study was conducted in north India. North India consist of 7 states (ISCS), out of these 7 states, 5 states namely

Haryana, Punjab, Delhi, Rajasthan and Uttar Pradesh were selected for the study. From each state, one district purposively selected on the basis of highest milk production in the states. Due to unavailability of documented records of commercial dairy farmers, snowball sampling technique was used to reach those progressive commercial dairy farmers. From each district, 18 commercial dairy farmers were selected constituting a total of 90 respondents practising commercial dairy farming. The primary information on socio- personal, sources of information, communication, Pre-Purchase Considerations for dairy Automation Technologies, Scientific Orientation, Risk Orientation, Economic Motivation, Innovativeness and Perception towards Dairy Automation/ mechanization Technologies was collected through survey with the help of well-structured interview schedule. An exclusive scale developed by Adrian et al. (2005) was used to measure the perception of the respondents towards automation/ mechanization technologies. The responses for 18 statements were recorded on 5 point continuum (namely, strongly agree, agree, undecided, disagree and strongly disagree) . The total Perception score for individual respondent was calculated by summing up the score of each statement as perceived by the respondent and based on the total score obtained, perception was categorised into low, medium and high categories using CSQRT. On the basis of perception, the respondents were categorised into three groups *viz.,* low perceptual level, moderate perceptual level and highly perceptual level. Then frequency, percentage, weighted mean score and rank was calculated for each component and each statements.

Results and Discussion

Profile characteristics of the respondents gives an idea about the respondent's background, living condition, surroundings and belongings which consecutively will help in drawing suitable implication based on the results. Thus, profiling of the commercial dairy farmers from five states of North India was carried out to get a clear understanding about the respondents and their perspective on precision farming technologies.

General profile of the respondents

The socio- personal characteristics such as age, education, experience in dairy automation, occupation, operational land holding, annual income, herd size, herd composition, source of information, pre-purchase considerations for dairy automation technologies, scientific orientation, risk orientation, economic motivation and innovativeness of commercial dairy farmers were assessed.

Age

The age of respondents was selected as an independent variable as it influences the farmer to choose and adopt an innovative technology. It plays a vital role as it indicates the mental maturity of the individual and also affects the decision making capability of the person. An appraisal of the Table 1 revealed that majority (58.89%) of the farmers belonged to middle age group (36-50 years) followed by young age group (up to 35) and old age group (> 50 years) which accounts for 21.11 per cent and 20.00 per cent respectively. The mean value of age was found 43.19 years among the respondents. These results were in agreement with Kudari (2014), who reported that most of the precision farmers were middle-aged. This could be because dairy automation being an innovative high tech approach, logically farmers above 50 years of age may not have the leaning to adopt the technology. Young aged people though attracted towards opportunities to adopt the dairy automation/ mechanization technologies need more opportunities to avail the advantages of precision dairy farming.

Education

The level of education moulds the farmer's response to improved technology since enlightened farmers have a higher motivation to adopt new technologies and to earn more profit. A close analysis of Table 1 revealed that 46.67 per cent of the farmers had higher secondary education followed by graduate and above (27.78%). It was observed that about 74.45 per cent of the farmers had higher secondary and above level of the education. As observed in the age category, majority were middle aged and young aged people were getting attracted towards dairy automation technologies and that is reflected in the educational level also. The findings of the study are in line with the study of Sudha (2008) who stated that most of the precision farmers were educated up to higher secondary level followed by college education of the respondents.

Experience in dairy automation / mechanization

Findings revealed that 45.56 per cent of the respondents had medium experience (5 to 8 years) in dairy automation technologies followed by high (>8 years) and low experience (<5 years) i.e. 32.22 and 22.22 per cent, respectively. The average experience of the farmers in dairy automation/ mechanization in the study area was 6.33 years as indicted in table 1. This could be due to the reason that the application of dairy automation technologies is still at its infancy stage and some discrete initiatives have been started towards the application of this technology in the study area. This result is in agreement with the findings of Sudha (2008) who reported that most of the precision farmers had medium level of experience in precision farming.

Occupation

Findings in Table 1 exposed that majority (72.22%) of the farmers were engaged in crop farming along with dairy, 18.89 per cent in dairy + crop farming + business activity, 6.67 per cent in dairy + crop farming + service, 2.22 per cent were involved in dairy farming whereas none of the farmers were engaged in crop farming exclusively as their occupation.

Operational land holding

Results presented in Table 1 revealed that 33.33 per cent of the farmers had medium land holdings followed by large, semi-medium, small and marginal land-holding i.e. 26.67, 16.67, 14.44 and 8.89 per cent, respectively. The average land-holding of farmers was 9.71 hectare. This finding is consistent with Kudari (2014) who reported that most of the precision farmers were having medium land holding followed by large land holding.

Annual Income

Annual income of the respondents was presented in Table 1 and it was found that majority (62.22%) of the farmers were in medium income category (5 to 8 lakhs) followed by high and low category of annual income comprising of 30.00 per cent and 7.78 per cent respectively. The study revealed that majority of the respondents were having annual income more than 5 lakhs which can be attributed to their adoption of dairy automation technologies and larger land holding size. The findings of the study are in accordance with Padma (2013) who reported that majority of the precision farmers had medium income.

Herd size

The results presented in Table 1 revealed that 41.11 per cent of the farmers were rearing medium herd size (16 to 43 animals), followed by 31.11 per cent who were rearing small (less than 16 animals) and 27.78 per cent of the user farmers possessed large herd size of dairy animals. The average herd size of user farmers in the study area was 28.22 animals per dairy farm.

Herd composition

The collected data was further analysed to see the category wise distribution of dairy animals (Table 1) in the study area. The findings revealed that the farmers had 57.25, 33.26 and 9.49 per cent crossbred cow, buffalo and indigenous cow as major composition of herd size in the study area, respectively. Respondents had shown higher preference towards crossbred cattle because of higher milk production and ease of operation of

Table 1 General (socio-personal) profile of the respondents

S. No.	Variable	Category	n=90	
			Frequency	Percentage
1	Age	Young (up to 35 years)	19	21.11
		Middle (36 to 50 years)	53	58.89
		Old (more than 50 years)	18	20.00
		Mean	43.19	
2	Education	Illiterate	0	0.00
		Primary	0	0.00
		Middle	6	6.67
		High school	17	18.88
		Higher secondary	42	46.67
		Graduate& above	25	27.78
3	Experience with dairy automation	Low experience (<5 years)	20	22.22
		Medium experience (5 to 8 years)	41	45.56
		High experience (>8 years)	29	32.22
		Mean	6.33	
4	Occupation	Dairy Farming	2	2.22
		Dairy + Crop farming	65	72.22
		Dairy + Crop farming+ Service	6	6.67
		Dairy + Crop farming+ Business	17	18.89
5	Annual income	Low (<5 lakhs)	7	7.78
		Medium (5 to 8 lakhs)	56	62.22
		High (>8 lakhs)	27	30.00
		Mean	8.79	
6	Herd size	Small (<16 animals)	28	31.11
		Medium (16to 43animals)	37	41.11
		Large (>43 animals)	25	27.78
		Mean	28.22	
7	Herd Composition	Indigenous cow	241	9.49
		Crossbreed cow	1454	57.25
		Buffalo	845	33.26
		Total	2540	100.00

semi-automatic milking machine. Meena et al. (2017) also reported that 4067 percent of the farmers in Haryana had crossbred animal in their herd composition.

Source of Information

It was clear from Table 2 that majority (72.00%) of the farmers considered ICAR, SAU, SVS personal as the most important source of information to update the knowledge on dairy automation/ mechanization technologies followed by State department of Agriculture / Dairy/ Animal Husbandry personal, KVK / ATMA personal, Govt. organizations published Magazine/ bulletins/folder, Input dealers, Mobile Apps/Media/ ICT, Rural retails Hubs and Output buyers. Majority (67.15%) of the farmers considered public sources as the major sources of information. This might be due to the contact of the farmers with the public personnel to avail the subsidy benefits on dairy automation technologies.

Pre-Purchase Considerations for dairy Automation Technologies

From the results given in Table 3, it was found that majority of the farmers considered benefit cost ratio as the most important with mean value of 2.88 prior to purchasing dairy automation/ mechanization technologies followed by total investment cost, compatibility with existing practices and systems, simplicity and ease of use, time involved using the technology, proven performance through independent research and availability of local support having mean values 2.74, 2.68, 2.63, 2.55, 2.37 and 2.19, respectively. The findings of the study are in line with Bewley (2010) who indicated that producers considered benefit cost ratio, total investment cost and simplicity and ease of use to be most

Table 2 Sources of Information for dairy automation / mechanization

Information sources	S. No.	Category	Frequency	Ranking
Public sources	1	KVK / ATMA personal and on webpage	38 (42.22)	IV
	2	ICAR, SAU, SVS personal and on webpage	65 (72.22)	I
	3	State department of Agriculture, Dairy, Animal Husbandry in personal and on webpage	46 (51.11)	II
	4	Govt. organizations published Magazine/ bulletins/folder, etc	35 (38.89)	III
Private sources	1	Private practitioners / Para vets	17 (18.89)	III
	2	Input dealers	29 (32.22)	I
	3	Output buyers	11 (12.22)	V
	4	Rural retails Hubs	12 (13.33)	IV
	5	Mobile Apps, Media, ICT	21 (23.33)	II
		Public sources	184 (67.15)	I
		Private sources	90 (32.85)	II

Table 3 Distribution of users according to their pre-purchase considerations for dairy automation technologies

Category	Mean	Percent	Ranks
Benefit to cost ratio	2.88	96.00	(i)
Total investment cost	2.74	91.34	(ii)
Simplicity and ease of use	2.63	87.67	(iv)
Proven performance through independent research	2.37	79.00	(vi)
Availability of local support	2.19	73.00	(vii)
Compatibility with existing practices and systems	2.68	89.34	(iii)
Time involved using the technology	2.55	85.00	(v)

important prior to adopt a technology. Dairy automation demands high installation and repair costs (Hamadani and Khan 2015).

Scientific Orientation

The scientific orientation of farmers towards possibility of adoption of dairy automation technologies was measured using structured interview schedule in which statements regarding the readiness to adopt dairy automation technologies were used. From the results given in Table 4, it was found that majority (58.89%) of the farmers had high scientific orientation followed by medium and low scientific orientation i.e. 30.00 and 11.11 per cent respectively. High scientific orientation could be the reason for adoption of dairy automation technologies by the farmers. The findings of the study are in line with Gabriel (2014) who reported that majority of the precision farmers had high level of scientific orientation.

Risk orientation

The findings in Table 5 revealed that majority (51.11%) of the farmers had high risk orientation followed by medium and low risk orientation i.e. 36.67 and 12.22 per cent respectively. A successful farmer always takes calculated risk and plays with nature in order to get higher profits. Table 5 clearly indicated that 92.22 per cent of the farmers had annual income more than 5 lakhs which itself indicated high risk taking nature of the farmers. High risk orientation might be the probable reason for adoption of dairy automation technologies by the farmers. The findings of the study are consistent with Gabriel (2014)who stated that majority of the precision farmers had high level of risk orientation.

Economic Motivation

A cursory look at the Table 6 revealed that majority of the farmers (87.78%) have medium to high level of economic motivation, this might be due to the high profit orientation of the farmers and their desire to stabilize and improve further economically by adopting and considering the adoption of more dairy automation technologies. The results of the study are in contrary with Padma (2013) who indicated that majority of the precision farmers have medium to high level of economic motivation.

Innovativeness

Innovativeness is a cognitive aspect of change, which affects the readiness of an individual to accept new technology. From the results given in Table 7, about 50.00 per cent of the farmers possessed high innovativeness followed by medium (35.56%) and low (14.44%) innovativeness. Majority of the farmers possessed medium to large size land holdings and hence they would like to obtain higher returns by adopting the innovations.The findings of the study are consistent with Floralavanya (2007) who reported that higher number of the precision farmers had high level of innovativeness.

Table 4 Distribution of respondents according to their scientific orientation

Category	Frequency	Percentage
Low (<13)	10	11.11
Medium (13to 26)	27	30.00
High (>26)	53	58.89

Table 5 Distribution of respondents according to their risk orientation

Category	Frequency	Percentage
Low (<13)	11	12.22
Medium (13to 26)	33	36.67
High (>26)	46	51.11

Table 6: Distribution of respondents according to their economic motivation

Category	Frequency	Percentage
Low (<13)	11	12.22
Medium (13to 26)	28	31.11
High (>26)	51	56.67

Table 7 Distribution of respondents according to their innovativenes

Category	Frequency	Percentage
Low (<13)	13	14.44
Medium (13to 26)	32	35.56
High (>26)	45	50.00

Perception of Farmers towards Dairy Automation/ mechanization Technologies

Perception of Farmers towards Dairy Automation/ mechanization Technologies were measured with the scale developed by Adrian et al. (2005). The response of the respondents for 18 statements

Table 8 Distribution of user farmers on the basis of perception scale values

Sl. No.	Statements	SA	A	UD	DA	SDA
1	Dairy automation/ mechanization technologies are useful for me	52 (57.78)	29 (32.22)	0 (0.00)	6 (6.67)	3 (3.33)
2	Dairy automation/ mechanization technologies improve the resource use efficiency	48 (51.11)	36 (40.00)	0 (0.00)	6 (6.67)	2 (2.22)
3	Dairy automation/ mechanization tools have relative advantage as compared to traditional practices	55 (61.11)	35 (38.89)	0 (0.00)	7 (7.78)	2 (2.22)
4	Dairy automation/ mechanization technologies provides information for decision making	29 (32.22)	41 (45.56)	0 (0.00)	14 (15.56)	6 (6.67)
5	Dairy automation/ mechanization technologies are easy to use	35 (38.89)	42 (46.67)	0 (0.00)	10 (11.11)	3 (3.33)
6	I am able to remember how to perform task using Dairy automation/ mechanization technologies	37 (41.11)	43 (47.78)	0 (0.00)	10 (11.11)	0 (0.00)
7	Dairy automation/ mechanization technologies are clear and understandable	28 (31.11)	42 (46.67)	0 (0.00)	15 (16.67)	5 (5.56)
8	Dairy automation/ mechanization technologies can increase the yields	58 (64.44)	26 (28.89)	0 (0.00)	6 (6.67)	0 (0.00)
9	Dairy automation/ mechanization technologies increases the cost of production	11 (12.22)	28 (31.11)	0 (0.00)	37 (41.11)	14 (15.56)
10	Dairy automation/ mechanization technologies reduces environmental hazards caused by blanket use of resources	38 (42.22)	46 (48.88)	0 (0.00)	6 (6.67)	2 (2.22)
11	Dairy automation/ mechanization technologies will be more profitable in future	28 (31.11)	53 (58.89)	9 (10.00)	0 (0.00)	0 (0.00)
12	Dairy automation/ mechanization technologies improves animal health	34 (37.78)	43 (47.78)	0 (0.00)	11 (12.22)	2 (2.22)
13	Sustainable dairy / Agriculture can be achieved through Dairy automation/ mechanization	25 (27.78)	33 (36.67)	4 (4.44)	15 (16.67)	13 (14.44)
14	Dairy automation/ mechanization technologies requires sophisticated equipment's, which requires technical support from the R &D	5 (5.56)	13 (14.44)	0 (0.00)	43 (47.78)	29 (32.22)
15	Dairy automation/ mechanization technologies are more suited to small and marginal farmers as compared to large or commercial farmers	9 (10.00)	16 (17.78)	5 (5.56)	34 (37.77)	26 (28.89)
16	Dairy automation/ mechanization technologies demands more labour	6 (6.67)	13 (14.44)	2 (2.22)	41 (45.56)	28 (31.11)
17	Dairy automation/ mechanization technologies helps in underground and surface water conservation	28.00 (31.11)	44 (48.89)	2 (2.22)	11 (12.22)	5 (5.56)
18	Adoption of Dairy automation/ mechanization technologies empowered me as a farm manager	25 (27.78)	46.00 (51.11)	3 (3.33)	10 (11.11)	6 (6.67)

Table 9 Distribution of respondents on the basis of their overall perception level

Category	Frequency	Percentage
Low perceptual level (<47.57)	9	10.00
Moderate perceptual level (47.57 to 70.25)	32	35.56
High perceptual level (>70.25)	49	54.44

were recorded on 5 point continuum. (Strong Agree, Agree, Undecided, Disagree and Strongly Disagree. The total Perception score for individual respondent was calculated by summing up the score of each statement as perceived by the respondent. Based on the total score obtained, perception was categorised into low, medium and high categories using CSQRT.

A critical look on Table 8 shows that the majority of the respondents had positive perception about the 'Dairy Automation/ mechanizationtechnologies'. Farmers strongly agreed with the relative advantage aspect of dairy automation/ mechanizationtechnologies as compared to the conventional practices with mean value of 4.79. Increase in yield was perceived strongly by 64.44 per cent of the farmers with mean value of 4.51. This could be due to the high yield achieved by the farmers after adoption of dairy automation/ mechanizationtechnologies. Majority (51.11%) of the farmers strongly favoured the improvement in resource use efficiency with dairy automation/ mechanizationwith mean value of 4.42 as farmers might have experienced optimization of resource use and increase in efficiency with the help of technologies. Usefulness of dairy automation/ mechanizationtechnologies was perceived strongly by 57.78 per cent of the respondents having mean value of 4.34.

About 48.88 per cent of the respondents were in agreement with the statement that dairy automation technology leads to reduction in the environmental hazards caused due to blanket use of resources with mean value of 4.31. Profitability of dairy automation technologies in the future was perceived by 58.89 per cent of the respondents with mean score of 4.21, whereas ability to remember how to perform task using dairy automation technologies was perceived by 47.78 per cent of the user farmers with mean score of 4.19. 46.67 per cent of the user farmers were in agreement with respect to ease of usage of dairy automation technologies. This might be due to their high confidence because of adoption of dairy automation technologies. Improvement in the animal health was perceived by the 47.78 per cent of the respondents. Water conservation due to dairy automation technologies was perceived by 48.89 per cent of the farmers with mean score of 3.88. Empowerment of farmers as a farm manager was favoured by majority (51.11%) of the farmers, whereas it was found that 46.67 per cent of the farmers agreed with respect to clarity and understand ability aspect of the dairy automation technologies with mean score of 3.81. Relative information provided by the dairy automation technologies for making decisions was agreed by 45.56 per cent of the farmers with mean score of 4.79. From this we can conclude that there is a trend of positive response towards dairy automation/ mechanization technologies, and more number of farmers was in agreement with the perception statements on dairy automation/ mechanization.

In contrast, 31.11 per cent of the farmers strongly disagreed with more labour required for dairy automation/ mechanization technologies with mean score of 3.80. Requirement of sophisticated equipment's for dairy automation/ mechanization technologies was strongly disagreed by 32.22 per cent of the farmers. Suitability of dairy automation/ mechanization technologies more towards small and marginal farmers was strongly disagreed by 28.89 per cent of the respondents. Also, 41.11 per cent of the farmers disagreed with the increase in production cost due to adoption of dairy automation/ mechanization technologies. The increase in cost of production due to adoption of dairy automation machineries, technical sophistication in their use, relative advantage of technology for small farmers and increased demand for labour after use of the machineries were those statements which majority of farmers disagreed because they were negative statements impeding the adoption of automation technologies to farmers. The reason behind this observation was that overall statements of the scale revealed the positive perception of commercial dairy farmers towards adoption of dairy automation/ mechanization technologies.

Overall Perception of respondents towards dairy automation/ mechanization Technologies

Table 9 revealed that the maximum number of the farmers (54.44%) was having a high perceptual level, while only 10.00 per cent of the farmers were in the category of low perceptual level. However, 35.56 per cent of the farmers were found in the category of having moderate perceptual level. The results indicated that 81 farmers were having moderate to high perceptual level towards dairy automation/ mechanization technologies. From this we can conclude that there is a trend of positive response and concern for dairy automation/ mechanization among farmers and a significantly 90.00 percent of respondents were having a moderate to high perceptual level towards dairy automation/ mechanization technologies.The results of the study are consistent with the findings of Gabriel (2014) who reported that more number of the precision farmers had high perceptual level.

Conclusions

Socio economic profile of respondents showed that economic progressiveness is an important requirement needed by commercial dairy farm. Majority (72.00%) of the farmers

considered ICAR, SAU, SVS personal as the most important source of information to update the knowledge on dairy automation/ mechanization technologies. It was found that majority of the farmers considered benefit cost ratio as the most important with mean value of 2.88 prior to purchasing dairy automation/ mechanization technologies. It was also observed that commercial dairy farmers had high scientific orientation, high risk orientation, high level of economic motivation and possessed high innovativeness in their occupation. More than 54.44 percent respondents had high perceptual level, while only 10.00 per cent of the farmers were in the category of low perceptual level. However, 35.56 per cent of the farmers were found in the category of having moderate perceptual level. The results indicated that 81 farmers were having moderate to high perceptual level towards dairy automation/ mechanization technologies. From this we can conclude that there is a trend of positive response and concern for dairy automation/ mechanization among farmers and a significantly 90.00 per cent of respondents were having a moderate to high perceptual level towards dairy automation/ mechanization technologies.

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SHORT COMMUNICATION

A research on the pollution parameters of buttermilk

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Abstract Buttermilk and butter-washing water obtained during butter production in some production facilities in Turkey are released directly into the sewer system without being evaluated or tested for their components. These byproducts can cause environmental pollution from their nutrient composition and concentrations. Buttermilk is an industrial waste that has a high organic load because it contains all the components of milk solids. The aim of this study was to determine the waste composition and chemical oxygen demand (COD) of buttermilk, one of the main pollutants from the dairy industry because of its levels of organic matter. Nine buttermilk samples obtained from three different butter-production plants in Burdur and Isparta, Turkey, were sent to the laboratory under cold chain (+ 4°C). The average values of fat, protein, lactose, solids, and ash of the buttermilk samples were 1.38 ± 0.37, 0.77 ± 0.123, 5.12 ± 0.11, 7.92 ± 0.36, and 0.676 ± 0.027 mg/L, respectively, at pH 4.70 ± 0.05. COD was 94866.67 ± 10091.82 mg/L.

Keywords: Butter, Buttermilk, COD, Dairy processing waste, Waste

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The main pollutants from the dairy industry are yogurt whey, whey, butter washing water, brine solutions, and cleaning waters formed in the production of milk, buttermilk, and strained yogurt. In terms of environmental pollution, the composition and concentration of the wastewater, rather than the amount of water used, is important (Kirdar et al. 2017).

The production of butter, which has a very high nutritional value and a higher protein content than other fats, is very common in Turkey and has been reported to date back to the Sumerians in 3000 BC. According to the calculations made for milk and other dairy products, butter consumption in Turkey is 1.52 kg/capita, and 295000 tons cream/year is processed into butter, creating 177000 tons buttermilk. The nutritional composition of buttermilk is similar to that of skim milk and is very rich. For every 100 kg butter produced, 166 kg buttermilk is produced as a byproduct and released into the sewer system with no evaluation or testing of its components (Kirdar, 2016). When the buttermilk is released into the receiving environment without any testing and/or refinement, it causes extreme water pollution from their nutrient composition and concentration and economic losses (Tan et al., 2003; Anonymous 2016).

The aim of this study was to determine the waste composition, concentration, and COD of buttermilk from the dairy industry.

Nine buttermilk samples from three different butter production plants in Burdur and Isparta, Turkey, were sent to the laboratory under cold chain (+ 4°C).

The samples were prepared according to the methods described by the Association of Official Analytical Chemists (AOAC, 1990). In the analysis of COD, Lange LCK 1014 kit and method were used in Hach-DR 5000 spectrophotometer.

The average values of the components of the buttermilk samples are given in Table 1. The mean fat, protein, lactose, total solid, and ash values in the buttermilk samples were 1.38 ± 0.37, 0.77 ± 0.123, 5.12 ± 0.11, 7.92 ± 0.36, and 0.676 ± 0.027 mg/L, respectively, and the mean pH was 4.70 ± 0.05.

Table 1 Properties of buttermilk

Properties	Mean Values
Fat(%)	1.357±0.367
Protein (%)	0.77±0.123
Lactose (%)	5.117±0.114
Total Solid(%)	7.92±0.362
Ash(%)	0.676±0.027
pH	4.70±0.052
COD(mg/L)	94 866.666±10 091.818

Buttermilk is an industrial waste with a high organic load of all the components found in milk solids. This byproduct from the production of butter is directly released into the sewer system and is never evaluated or tested for its components. COD is the amount of oxygen required to chemically oxidize the oxidizable substances in water and is one of the most important parameters by which to determine the degree of pollution from domestic and, especially, industrial wastewaters. Because the amount of compounds that can be chemically oxidized is higher than can be biologically oxidized, COD is higher than the biological oxygen content (BOD) (Peker 2007).

COD was determined to be 94866.67 ± 10091.82 mg/L in the buttermilk samples; however, these results were lower than those reported by Kessler (1981) and Odlum (1990).

In butter production, ~1.06–1.45 m^3/ton wastewater is produced. The pollutant load is 1.96–2.60 kg BOD/1000 kg milk (Atamer, 2005). Zeytinoglu (1993) has reported that the highest BOD value (40000 mg/L) in factories in Bursa, Turkey, was determined in milk and dairy plants. Toprak et al. (2013), in their study on waste composition and concentration and pollution parameters caused by yogurt whey, one of the main pollutants in the dairy industry resulting from organic substances, have determined that the COD value of yoghurt whey samples were 38223 mg/L.

The COD values of buttermilk wastewaters were reported to be 18400-70000 mg/L by Altunýsýk et al. (2002), 2300-6500mg/L by Ozturk et al. (1993), 70000 mg/L by Mawson (1994), 2148-5134 mg/L by Tanik et al. (2002), 60000 mg/L by Peker (2007), 61250 mg/L by Kavacik et al. (2007), 56000 mg/L by Gurtekin (2011), and 60000–80000 mg/L by Yazar et al. (2011). The results of the present study were higher than those from these studies.

Conclusion

As is the case in whey, the organic load content of buttermilk can be reduced by recovering some nutritional elements using technological methods. The products obtained through recovery can be used as supplements in food production and could reduce the economic losses caused from dumping the waste products into the sewer system. It is possible to suggest alternative products by changing the direction of the studies to be done on this subject. Studies on the use and evaluation of industrially produced buttermilk are needed.

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SHORT COMMUNICATION

A study on change of fatty acids profile in ghee adulterated with palm oil

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Abstract: Ghee, is clarified butter or anhydrous milk fat, has an integral relation with Indian culinary culture. Due to the importance and seasonal decline in the production; unscrupulous traders tend to add cheap vegetable oils like palm oil in ghee. The composition of milk fat and oil vary in terms of fatty acids. Milk contains mainly short and medium chain fatty acids whereas vegetable oil contains long chain fatty acids. Due to different fatty acids composition between milk fat and palm oil, we tried to exploit this fatty acids difference to identify the adulteration of palm oil in ghee, using Gas chromatography. Study suggested that due to palm oil adulteration in ghee, the concentration of short chain fatty acids get decreased and long chain saturated and unsaturated fatty acids showed an increase. In addition to the above, change was also observed in the ratio of the total of saturated fatty acids to unsaturated fatty acids. Fatty acids analysis could be very effective for the detection of palm oil adulteration in ghee to the tune of 5 %. Our current study suggested that fatty acids study was able to detect foreign fat like palm oil in milk fat but this study is not suitable to confirm the type of foreign fat in milk fat or ghee.

Keywords: Adulteration, Butyric acid, Ghee, Gas liquid Chromatography, Linoleic acid, Palm oil

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India is the highest milk producing country in the world. In India after liquid milk (~ 46 %), ghee (~ 28 %) is the second largest dairy product that is consumed throughout the country (GAIN, 2014). A very complex situation arises during the lean season of the year i.e., in the summer months (May to August) when the supply of milk and ghee drastically reduces. In this situation, unscrupulous traders use to add cheaper fats such as vegetable oils (palm oil, cottonseed oil etc.) in ghee, for gaining more money. Traditionally such type of adulteration is checked by analysing different fat constants of milk fat like- Reichert–Meissl value (RM value), Polenske value and Butyro Refractometer (BR) reading. Recently, dry fractionation technique coupled with fat constants analysis, was proved more suitable than simple fat constants analysis, to detect adulteration in ghee (Kumar et al. 2017); however, these technique are time consuming.

Recent trend being followed by the unscrupulous traders adulterate ghee in such a way that cannot be detected by simple fat constant analysis based approaches. Very recently, Pathania et al. (2020) applied a new approach of zooming in and superimposing of selected peaks in the chromatograms of triglycerides (using Gas chromatography), to detect refined palm oil along with other manipulated fats. Detection of palm oil adulteration in ghee is always a challenge for the scientific community; as there are very few studies available for the detection of oil in ghee (Ramani et al. 2019). Bector and Sharma (2002) were able to detect palm oil adulteration in ghee using a colorimetric test ; however these test was unable to detect BHA added ghee and ghee adulterated with palm oil. Researchers developed methods using fourier transform - near infrared (FT-NIR) and fourier transform - middle infrared (FT-MIR) spectroscopy to identify palm oil adulteration in ghee (Mehta et al. 2018; Aparnathi et al. 2019). However, FT-IR based analytical approaches were not suitable to detect palm oil in ghee lower than 10% level.(Aparnathi et al. 2019)

Fatty acid composition of milk fat is greatly affected by season, breed, species, parity, stage of lactation etc. (Kumar et al. 2015). Bharwade et al. (2017) reported that short and medium chain fatty acids were predominated in ghee. Butyric acid is a unique fatty acid in milk fat; however, change in butyric acid indicated the presence of adulteration in ghee (Mehta, 2013). Upadhyay et

al. (2018) reported that for both pure cow and pure buffalo ghee, the major fatty acids were myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$). Orsavova et al. (2015) reported that major fatty acids in palm oil were palmitic acid, oleic acid, linoleic acid and stearic acid; however, those researchers reported the absence of short chain fatty acids. Rebechi et al. (2015) reported that fatty acids ratio (C10:0/C8:0, C12:0/C10:0, C14:0/C12:0 and C14:0/C18:) could not able to detect body fat adulteration in milk fat. In a different study Kumar et al. (2015) successfully used selected fatty acid ratios (C14:0/C18:1 and C14:0/C18:2) as a marker for the detection of soybean oil adulteration in ghee.

Milk fat is unique and distinct from other fats. It is the only fat that contains short chain fatty acids, while vegetable oils are rich in linoleic acid (Kumar et al. 2015). Therefore, by keeping all these aspects of the fatty acid composition of different oils/fats, an attempt was made to detect adulteration in ghee with palm oil by fatty acid analysis using Gas chromatography technique.

Mix milk were collected from a local dairy farmers of Amreli district, Gujarat. The ghee was prepared by creamery butter method (De, 2011). The final temperature of clarification was 110°C. The clarified fat was filtered using coarse filter paper to obtain ghee and stored in airtight glass bottles at room temperature.

Palm oil was procured from a local market of Amreli city. For the preparation of adulterated ghee samples, pure ghee samples were heated to 60-70°C for 10 min before adding and mixing of palm oil. The adulterated ghee samples were prepared by the addition of palm oil in four different proportions (5, 10, 15 and 20 % w/w) into pure ghee.

Methylation of the above samples for making esters was done by the sealed tube method of DeMan (1964) as modified by Luddy et al. (1968). An incubation temperature of 75°C was used instead of 60°C.

Ester tubes were broken at the time of analysis and the sample was injected into the Gas chromatography column without any further treatment. Fatty acid profile of the above said samples in methylated form were analyzed in the capillary column (length 30 m, internal diameter 0.25 mm) using Gas Chromatography (Thermo Fisher Scientific – Trace 1110) equipped with a flame ionization detector (240°C). The other conditions like Total Flow: 79 ml/min (N_2), Column Flow: 1.49 ml/min, Pressure: 13.4 Kpa and Purge flow: 3 ml/min were set for further analysis.

The reference standards (Loba Chemical) of different fatty acids methyl esters were used to standadize a 37 minutes Gas chromatography programme. The sample was injected at an initial temperature of 70°C maintained for 3 min and then raised after the emergence of butyric acid peak to 150°C by holding for 3 min till the emergence of myristic acid ($C_{14:0}$) peak with a ramp rate of 5°C/min, then again raised up to 200°C with same ramp rate till the emergence of oleic acid ($C_{18:1}$) and then maintained at 200°C for 5 min until emergence of the peak of linolenic acid on chromatograph. By using the above specifications, chromatographs of different fatty acids profile were obtained and the percentage of each fatty acid was calculated and

Table 1 Fatty acid profile (percentage of weight) of pure ghee and palm oil and adulterated ghee

Fatty acids	Type of fat/oil**		Level of Adulteration **			
	Pure ghee	Palm oil	5% PO	10% PO	15% PO	20% PO
$C_{4:0}$	2.43 ± 0.22	0.00	1.52 ± 0.008	1.28 ± 0.002	1.03 ± 0.01	1.01 ± 0.006
$C_{6:0}$	0.73 ± 0.001	0.00	0.64 ± 0.017	0.41 ± 0.004	0.39 ± 0.001	0.36 ± 0.001
$C_{8:0}$	0.62 ± 0.007	0.00	0.61 ± 0.003	0.45 ± 0.0006	0.43 ± 0.003	0.39 ± 0.01
$C_{10:0}$	1.59 ± 0.05	0.00	1.39± 0.001	1.29 ± 0.001	1.16 ± 0.001	1.01 ± 0.004
$C_{12:0}$	1.95 ± 0.01	0.00	1.37 ± 0.014	1.12 ± 0.0007	1.10 ± 0.005	1.08 ± 0.01
$C_{14:0}$	9.67 ± 0.03	0.96 ± 0.005	8.77 ± 0.024	8.37 ± 0.002	7.98 ± 0.01	7.47 ± 0.15
$C_{16:0}$	29.9 ± 1.64	39.68 ± 0.015	30.42 ±0.044	31.36 ± 0.02	32.04 ± 0.21	32.12 ± 0.81
$C_{18:0}$	18.24 ± 0.31	4.01 ± 0.017	17.14 ± 0.78	14.43 ± 0.19	12.34 ± 2.25	11.89 ± 7.76
$C_{18:1}$	30.94 ± 0.53	43.55 ± 0.04	34.17± 0.84	35.39 ± 0.02	36.29 ± 0.27	36.51 ± 1.13
$C_{18:2}$	1.91 ± 0.037	11.18 ± 0.007	2.22± 0.027	4.18 ± 0.16	5.68 ± 1.59	6.02 ± 6.01
$C_{18:3}$	1.57 ± 0.019	0.246 ± 0.001	1.36± 0.011	1.22 ±0.003	1.15 ± 0.02	1.12 ± 0.06
$C_{20:0}$	0.46 ±0.009	0.37 ± 0.001	0.43 ± 0.011	0.41 ± 0.014	0.40 ± 0.05	0.39 ± 0.11
Total saturated fatty acids	65.59±0.25	45.02±0.009	62.29±0.1	59.12±0.026	56.87±0.282	55.76±0.984
Total unsaturated fatty acids	34.42±0.195	54.976±0.016	37.75±0.292	40.79±0.061	43.12±0.626	43.65±2.4
Total saturated fatty acids/ Total unsaturated fatty acids	1.90	0.81	1.65	1.44	1.31	1.27
Sum of $C_{4:0}$ to $C_{16:0}$ fatty acids	16.99±0.05	0.96±0.005	14.30±0.01	12.92±0.001	12.09±0.005	11.32±0.03
Sum of $C_{18:0}$ to $C_{20:0}$ fatty acids	83.02±0.42	99.03±0.01	85.74±0.28	86.99±0.06	87.90±0.73	88.09±2.64

**Data represent the mean ± SE of six determinations

compared. The identical conditions as mentioned above and the retention time of these fatty acids were used to identify the fatty acids in the ghee (pure and adulterated) samples.

Pure ghee and palm oil revealed a wide difference in their fatty acid composition. Fatty acids composition of both palm oil and pure ghee is given in table 1. There were wide differences between the fatty acids composition of milk fat (ghee) and palm oil. It was observed from table 1 that in pure ghee short chain fatty acids like $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$; 2.43, 0.73, 0.62, 1.59 and 1.95 %, respectively; however, in case of palm oil no short chain fatty acids were determined. However, even myristic acid ($C_{14:0}$) was also found to be very nominal in the palm oil (0.96%) as compared to pure ghee (9.69%). The four main fatty acids in palm oil were identified as palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids at 39.68, 4.01, 43.55 and 11.18 %, respectively. Earlier Rebechi et al. (2015) reported that every oil or fat has different unique signature fatty acids make up; hence, ghee or pure milk fat content mostly short or medium chain fatty acids. However, among unsaturated fatty acids oleic acids were predominated in pure milk fat or ghee.

Comparing the palm oil to ghee, it was observed in table 1 that $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ were higher in case of palm oil than ghee. Though the $C_{18:3}$ and $C_{20:0}$ were 1.57 % and 0.46 %, those were higher in ghee as compared to palm oil. It was also observed in table 1 that mono-unsaturated fatty acids (oleic) in palm oil were higher as compare to ghee. However, total saturated fatty acids were higher in ghee as compared to palm oil. Our results were in line with the earlier observation of Kumar et al. (2015) who reported that short chain fatty acids were predominated in ghee, however, there were no short chain fatty acids identified in vegetable oil. Mehta (2013) analyzed different market samples of ghee and concluded that saturated fatty acids were predominated than unsaturated fatty acids in ghee. Same type of observation was earlier recorded by Upadhyay et al. (2018).

Earlier workers also suggested that unsaturated fatty acids were predominated in vegetable oil (Kumar et al. 2015; Upadhyay et al. 2018). Both ghee and vegetable oil contained very high amount of oleic acid; although the concentration of oleic acid was higher in the case of palm oil as compared to pure ghee. In a different study Upadhyay et al. (2018) reported that total unsaturated fatty acids were higher both in depot fat and vegetable oil.

The effect of palm oil adulteration was also significant on some selected fatty acids like butyric acid ($C_{4:0}$) that was found to be decreased when adulterated with palm oil (Table 1). However, oleic and linoleic acids were increased during adulteration of palm oil and this significance difference even visible after 5 % level of palm oil adulteration in pure ghee (Table 1).

From table 1, it was also revealed that during palm oil adulteration in ghee; the sum of saturated fatty acids decreased; however, sum of un-saturated fatty acids increased quantitatively. It was also observed that change of the fatty acids in ghee depends on the percentage of added palm oil in ghee. It was recorded from table 1 that total saturated fatty acid in pure ghee, pure ghee + 5 % palm oil, pure ghee + 10 % palm oil, pure ghee + 15 % palm oil and pure ghee + 20 % palm oil were 62.29, 59.12, 56.87 and 55.76 %, respectively; however, the total un-saturated fatty acids were 34.42, 37.75, 40.79, 43.12 and 43.65, respectively. This effect was clearly identified even in 5 % level of palm oil adulteration in ghee. Recently, FSSAI has implemented to detect vegetable oil adulteration in ghee using HPLC based method but that said method unable to detect palm oil in ghee (Ramani et al. 2019). However, our study suggested that palm oil could be efficiently detected by fatty acid analysis; especially the ratio of the sum of saturated fatty acids to sum of unsaturated fatty acids could be effectively use as a potent markers to detect palm oil as an adulterant in ghee. Rebechi et al. (2015) and Kumar et al. (2015) reported that during adulteration of both vegetable oil and depot fat in milk fat or ghee; fatty acids used to be changed and this change of fatty acid even visible very low level of foreign fat adulteration. So, from the current study it could be concluded that fatty acids study able to detect foreign fat in milk fat but this study is not fruitful to confirm type of foreign fat in milk fat.

Conclusions

The present study revealed that the palm oil could be easily detected at 5 % level in ghee/milk fat when butyric, oleic and linoleic acids were used as a marker. On the other side, ratio of sum of the total of saturated to unsaturated were found useful marker for detecting the different levels of adulteration in ghee. It can be concluded that fatty acids analysis could be very effective for the detection of palm oil adulteration in ghee to the tune of 5 %; but change of fatty acid could even observed during other types of foreign fat adulteration in milk fat or ghee.

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SHORT COMMUNICATION

Optimization of mango herbal Quarg type cheese with mango pulp and spices

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Abstract: The present study was carried out to optimize the levels of mango pulp and spices in quarg type cheese to prepare mango herbal Quarg type cheese. The sensory evaluation was carried out in respect of flavour, body and texture, colour and appearance and overall acceptability. The different level of mango pulp, cardamom and clove were studied in preliminary trials and five treatments were taken for experimental studies. Level selected for mango pulp was 27 per cent and levels of cardamom (0.6% and 0.7%) and clove (0.4% and 0.5%) were finalized for further study. The treatment T_2 (27 per cent mango pulp + 0.6 per cent cardamom + 0.4 per cent clove) was rated superior among all experimental treatments.

Keywords: Cardamom, Clove, Quarg type cheese, Herds, Mango pulp, Spices

Milk is a major diet component worldwide, and it is considered natural complete food providing fat, protein, carbohydrate, vitamin and minerals particularly calcium. It is considered as a vital component for good nutrition (Bidarkar et al. 2015). Milk is consumed in the form of fluid and also converted in to various milk products. The most common dairy products are categorized as heat desiccated, frozen dairy product, heat and acid coagulated and fermented dairy product. Fermentation is the oldest and most economical method in food preservation. It has been well documented that fermentation enhances mineral bioavailability and digestibility of proteins and carbohydrates as well as improves organoleptical qualities of the product. The most common fermented milk product in the world is cheese.

The major cheese production has centred in western countries (Singh, 2011). At present cheese is highly diversified dairy product in world. There are more than 2000 varieties of cheese, although many have little difference. The manufacturing process and curing of cheese from immoral time, through centuries have resulted in the production of cheese with range in flavour from extremely mild to very sharp and in texture from semi solid to almost stone hard. The most popular variety of cheese are cheddar, mozzarella, feta, cottage and Quarg cheese. Quarg - the proper German name is speisequark, is a natural, unripened, fresh cheese produced on a large scale in Germany and is very popular there. It is essentially a milk protein paste, manufactured by acid coagulation of milk by proper bacterial cultures (e.g. *Steptococcus cremoris* and *Leuconostoc citrovorum*) with a small rennet addition for better separation of the protein coagulum from the whey and thus better yields.

It is common practice of using fruits in preparation of various dairy products like ice-cream, yoghurt and Shrikhand. Fruits are rich source of various important phytonutrients namely, vitamins, minerals, antioxidants and dietary fibers (Kanawjia et al. 2011). Mango (*Mangifera indica L.*) is commercially the most important fruit crop of India, accounting for > 54% of the total mango produced worldwide. Over 30 different varieties of mango are grown, the most important one is alphonso, which is rated best in the world. It is known for its strong aroma, intense peel coloration, delicious taste, and high nutritive value (due to its high content of vitamin C, â-carotene and minerals). It is also known as “Pride of Konkan Region”. It is recognized as the best variety for direct consumption as well as for processing purpose.

The flavour and palatability of most of the food is increased by the spices. The spices are used in comparatively small quantities in the food products. Most of the Indian spices have anti-inflammatory, antioxidant, antibacterial, anticancer, anti-

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carcinogenic properties. cardamom and clove are most common spices traditionally used in Indian culinary dishes.

A trend has been observed amongst customers towards minimally processed, highly nutritious, health promoting and flavour enriched food. Keeping the above fact in view with an aim to develop "Indian version of quarg type cheese", the present investigation was undertaken to prepare mango herbal quarg type cheese.

Milk sample of cow milk was obtained from Research-Cum Development Project on cattle, located at central campus, M.P.K.V., Rahuri. Freeze dried culture LF-40 was procured from Division of Dairy Microbiology, N.D.R.I., Karnal. Microbial "Meito" rennet was purchased from Mumbai. Jain's Farm fresh Alphanso variety mango pulp was procured from Ahmednagar. Cardamom powder, Clove powder was purchased from local market.

The quarg type cheese was prepared by using technology developed at NDRI for manufacturing of quarg cheese, prescribed by Gahane (2008) with some minor modifications.

The milk was standardized at 4 per cent fat level using Pearson's square method. Standardized milk was heated to 85 ºC for 15 min and mixed thoroughly and cooled to 30 ºC. The milk was inoculated by adding 1 per cent starter culture and incubated at temperature 30 ^{0}C. Two and half hrs after the addition of starter culture, microbial rennet @ 250 mg/100 kg milk was added and mixed thoroughly. The content was left undisturbed for curd setting in incubator at 30^{0}C, which took around 10-12 hrs. starting from culturing. The coagulum was then cut using knives and it was again left undisturbed for about 10-15 minutes. The curd was heated slowly and gradually increasing temperature to 55-60^{0}C @ 1^{0}C per minute and curd hold for 10 minutes at 60 ^{0}C. Cooked curd was then cooled to room temperature and filled in muslin cloth hanging for 3 to 4 hrs. The obtained quarg type cheese was mixed with Alphanso mango pulp and spices and homogenized by mixing thoroughly. The quarg type cheese prepared by using cow milk was packed in sterilized PVC containers and stored in refrigerator at 4 ± 1 ^{0}C.

Pre-experimental trials were conducted to decide the levels of mango pulp, spices (cardamom, clove) to be used in final experimental trials. On the basis of the results of sensory evaluation of pre-experimental trials, below mentioned levels of mango pulp and spices (cardamom and clove) were finalized for inclusion in the final experimental trials. T_0 (control), T_1 - Mango pulp 27%, T_2 - Mango pulp 27% + Cardamom powder 0.6% + Clove powder 0.4%, T_3 - Mango pulp 27% + Cardamom powder 0.6% + Clove powder 0.5%, T_4 - Mango pulp 27% + Cardamom powder 0.7% +Clove powder 0.4%, T_5 - Mango pulp 27% + Cardamom powder 0.7% + Clove powder 0.5%.

The quarg type cheese was evaluated sensorily by semi-trained panel of 5 judges from department of Animal Husbandry and Dairy Science, Post Graduate Institute, MPKV, Rahuri using quarg cheese score card, total score 100 (Kadiya et al. 2014). Out of 100, the maximum marks 50 were allocate for flavour, while 35 and 15 marks were allocated for body and texture and colour and appearance respectively. The obtained results were analysed by Completely Randomized Design (CRD) method (Snedecor and Cochran, 1994).

The sensory score for the flavour ranged from 47.00 to 47.85. The highest score was obtained at T_2 (47.85) followed by T_3 (47.67), T_1 (47.47), T_0 ((47.38), T_4 (47.11) and T_5 (47.00) table 1. Lowest score was observed for T_5 (47.00). T_2 was observed superior over other treatments, this might be due to the rate of added spices with mango pulp which resulted into a balanced flavour of both the spices i.e. cardamom and clove with flavour of mango. The decreasing score obtained might be due to increase in level of spices which may increase in little pungency of the product and dominating the mango flavour with hot pungent taste of spices and also decreasing cheesy flavour resulting into less acceptability by the judges.

The sensory score for body and texture obtained was ranged from 32.13 to 32.83. The highest score 32.83 was obtained by (T_1) i.e. 27% mango pulp followed by T_2 (32.73), T_3 (32.71), T_4 (32.69) and T_5 (32.66). The addition of mango pulp resulted into more lighter, smoother creamy texture of the product and also improved

Table1 Sensory evaluation of mango herbal quarg type cheese

Treatment	Flavour	Body and Texture	Colour and appearance	Overall acceptability
T_0	47.38^{cd}	32.13^{c}	13.77^{c}	93.29^{e}
T_1	47.47^{bc}	32.83^{a}	13.94^{b}	94.25^{bc}
T_2	47.85^{a}	32.73^{ab}	14.21^{a}	94.79^{a}
T_3	47.67^{ab}	32.71^{ab}	14.16^{a}	94.54^{ab}
T_4	47.11^{de}	32.69^{b}	14.09^{ab}	93.89^{cd}
T_5	47.00^{e}	32.66^{b}	14.06^{ab}	93.72^{de}
S.E.±	0.0924	0.0463	0.0550	0.1727
C.D.@5%	0.2878	0.1443	0.1714	0.5377

Values with different superscript differ significantly (P<0.05)

spreadability of product. Lowest score was received by T_0 (32.13), which may be due to high total solid results into less spreadability.

The score for the colour and appearance had ranged from 13.77 to 14.21. The treatment T_2 got maximum score (14.21), while lowest score obtained by T_0 (13.77). The colour of (T_0) was milky white. Addition of mango pulp changed the white colour to golden yellow colour in (T_1 to T_5) which resulted an enhanced acceptability of the product by the judges.

The treatment T_2 was rated superior among experimental treatments which was obtained 94.79 score for its overall acceptability.

Conclusion

It may be concluded that mango pulp and spices (cardamom and clove) could be used in combination for preparation of mango herbal quarg type cheese. The combination of (T_2) Mango pulp 27% + Cardamom powder 0.6% + Clove powder 0.4% is the best for overall acceptance.

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