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INVITED REVIEW

Nutritional and nutraceutical properties of goat milk for human health: A review

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Abstract: Milk are considered as the best source of nutrients in well balanced ratio, and it also performs a number of beneficial biological activities in the human body that improves digestive system, metabolic activities to ingested nutrients, organ's growth maintenance, development and resistance against diseases. Goats are considered to be the oldest domestic animals, domesticated for meat and milk and are known as the poor man's animal. Goat milk is nature's closest thing to perfect food. The constituent and composition of goat milk is totally different from cow or buffalo milk. Goat milk is nutritionally more or less matches to human milk which is not very much similar to cow's milk. It also possesses lots of medicinal properties. Lactose intolerance patients will be benefited from goat milk because it has less lactose than human and cow milk. It contains proteins, vitamins, minerals, fatty acids, trace elements and enzymes that can be easily assimilated in our body. Smaller and uniform fat globules of goat milk make them easily digestible in the body. It contains higher selenium concentration which acts on regeneration of blood platelet suffering from dengue fever. Protein content in goat milk is lower than cow milk and is easily digestible when compared to cow or buffalo milk. Taurine is present in goat milk which has inhibitory effect on hypertension, cardiovascular disease and synthesizes essential amino acids in human body. Asthma, anemia, stomach ulcers, constipation, sleeplessness, and neurotic indigestion have all been shown to be treated with goat milk. The goal of this review is to look at the differences in goat milk composition from cow and human milk, as well as the nutraceutical benefits on human health.

Keywords: Cardiovascular diseases, Enzymes, Goat milk, Hypertension, Lactose intolerance, Nutraceuticals

Introduction

The global dairy goat industry is growing rapidly. Dairy goats are not only for wholesome and nutritious milk products but also to provide smallholders with sustainable livelihoods and assets. The world's goat population is believed to be at 1 billion, with the majority (94%) living in Africa and Asia's poorest countries (FAOSTAT 2019). As per the 20th Livestock census of India, the total livestock population in the country was 535.78 million in 2019, among which goats were 148.88 million, increased by 10.14% over the previous census 2012 (GOI, 2019). There are several reasons that goats (*Capra hircus*) can be kept under the category of important and useful animals. They have the good ability to adapt to nearly any environment, low feed value acceptance, and extreme weather conditions, plus their versatility and relatively high productivity. These are some reasons because goat is one of the oldest domesticated animals and hence is called as cow of the poor people (Monteiro et al. 2017). Major areas of goat milk application are domestic consumption, milk products, particularly cheeses and yoghurt, and goat milk is also beneficial to people who are allergens towards cow milk and other gastro-intestinal diseases. The composition of human milk is such that it is in the biological norm for infant food. A great deal of its chemical composition provides protection against inflammation, infection and provides nutrition, aids digestion and organ development, as well as protecting against food poisoning. However, time constraints, health conditions and urbanization may cause the early termination of breastfeeding. Even national guideline of India also recommended in the pandemic of COVID-19 that breastfeeding in this condition is very dangerous and it could not be possible during isolation (Chawla et al. 2020). Therefore, there is need to find alternative milk for infant those who cannot have sufficient breast feeding. A lot of researchers have recommended Goat milk as the best substitute of cow milk, buffalo milk and human milk. Goat milk has got an excellent medicinal and nutritional property. It has

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been established that the goat milk is best option for the infant suffering from allergy of cow milk and other problems related to gastro intestinal tract (Formiga de Sousa et al. 2015). Summarized health benefits of goat milk are shown in figure 1. This demand is also increasing as individuals become more aware of the issues with standard medical treatments for such illnesses. Because of its simple digestion and lower allergic qualities than bovine milk, goat milk has been considered a nutraceutical in recent decades (Clark et al. 2017). Goat milk are considered as one of the best alternative with great potential that can easily replace the indigenous dairy products to improve human health, especially people suffering from lactose intolerance. The recent trends in the eating habits of developed countries involve milk which has very high nutritional values. In developed countries milk are consumed either alone or with probiotic bacterial strains and some other physiologically active metabolites and made into new dairy functional products (Lima et al. 2018). The main properties of goat milk and its composition have been compared with other species milk. Other than providing nutrition, goat milk has antimicrobial, anti-allergic, antioxidant and other nutraceutical properties which are reviewed in this article.

There are many bioactive compounds that are useful in curing various chronic diseases and goat milk are rich source of these bioactive compounds. There are various peptides and oligosaccharides that are present in goat milk are useful in curing metabolic disorders, cardiovascular disease, and neurological degeneration and in improving the gastrointestinal health. Goat milk is also helpful in cancer treatment. The oligosaccharides that are present in goat milk have immunomodulatory properties which prevent the attack of harmful and deadly microorganisms and oligosaccharides also act as prebiotic (Peng et al. 2020). Due to these mentioned important and potential health benefits of goat milk, infants, older people and diseased persons are recommended for goat milk.

Nutritive value of milk

As per the census of 2017, goat population was estimated to be approximately 218 million, with an average milk production of 18.7 MT (FAO, 2019). The average composition and constituents of cow milk, goat milk and human milk are shown in Table 1. These compositions vary with respect to different type of breeds, feeding system, environmental condition, temperature, stage of lactation, disease of udder and health status of animal (Yadav et al. 2016). Due to so much health benefits, nutritional and compositional values of goat milk are used in treatment of variety of health related issues. The most common of which is food allergens of protein of cow milk which is the most common culprit. Mal-absorption syndromes, coronary by-pass, hyperlipo-proteinemia, chyluria (milky urine), childhood epilepsy, gastric ulcer, allergies, and gallstones have all been cured with goat milk (Park, 2017). It has various advantages not only nutritionally but technologically as well over cow milk, like smaller and uniform fat globule, which results in a smoother and finer texture in processed products, less quantities of alpha-1-casein, which results in softer gel type products, lower viscosity and higher water holding capacity (Gomes et al. 2013).

Composition of Goat milk

Over the last few decades, research has expanded our understanding on the basics and distinctive characteristics of goat milk composition and capabilities. Milk, which is mammal's initial food, provides almost all of the required energy and nutrition for the normal growth and functioning of neonate's and their development. Milk consumption is stopped in all mammals after weaning stage, except in human. The flavor of goat milk is little bit intense due to presence of fatty acid of short chain and is white in color due to carotene, as all the beta carotene are converted into Vitamin A. (Yakan et al. 2019; Ranadheera et al. 2019). The composition varies depending on numerous factors, including the animal, breed, nutrition, lactation age, stage, and habitat.

Fat

Fat or Fatty acid profile is one of the most important constituent in goat milk. It differs significantly from cow milk as shown in Table 2. Goat milk contains short and medium chain fatty acid (MCFA) and possesses around double amount (16%) of MCFA while cow milk contains lower fatty acid (8%) (Kumar et al. 2016), being much higher in caproic (C6:0), butyric (C4:0), capric (C10:0), caprylic (C8:0), palmitic (C16:0), lauric (C12:0), myristic (C14:0), and lower in longer chain fatty acids content like stearic (C18:0) and oleic acid (C18:1). It was named after goats because high amount of caproic acid (C6:10), caprylic acid (C8:0) and capric acid (C10:0) found in goat's milk. It has been demonstrated that the fat of goat milk are easily utilized more efficiently digested than the fat of cow milk because MCFA is more easily digestible of its low molecular weight, the action of digestive enzymes, and water solubility (Gallier et al. 2020).

Goat milk had found its wide use in clinical treatment like infant malnutrition, intestinal rectification, hyperlipo-proteinemia, cystic fibrosis, epilepsy, gallstones and coronary disease because of longer chain fatty acids and medium-chain triglycerides (MCTs) which have unique metabolic ability to provide direct energy without depositing in adipose tissues (Prosser, 2021; Mehra et al. 2021) and reduced the level of lipoprotein (LDL) while triglyceride (TRI) and high density lipoprotein (HDL) level remained normal, therefore goat milk fat is utilized more efficiency than cow milk fat. MCTs used for "milky urine" (chyluria) and chylothorax (lung conditions) (Triffoni-Melo et al. 2013). By altering the feed of goat, the fatty acid profile towards unsaturated fatty acid can also be altered for more beneficial purposes.

Goat milk is comprised of very smaller and finer fat particles which converts into very smaller and softer curd in the human stomach

which can easily be broken by the stomach enzymes and thus are easily digestible as compared with cow milk. The diameters of goat milk's fat globules are ranges from 0.73 to 8.58 micrometers (Sudharsan et al. 2020). The range of diameter in ewes is between 2.79 mm, while in goats the average diameter of milk fat globules is smaller than in cows and sheep, and is between 2.2 mm and 2.5–2.8 mm (Martini et al. 2016).

Protein

The Protein percentage of goat milk and cow milk are very much same and protein percentage are very much influenced by genetically differences of breeds, species, ration, influences of stage of lactation, mastitis and climate (Silanikove et al. 2016). The protein in goat's milk is broken down into its individual components. In which the high level of casein and the structure of micelles are the main ones. They contain a wide range of bioactive peptides along with minor protein and non-protein portion which include nucleosides, amino acids and nucleotides. Roy et al. (2020) shows that 96% parts of casein of goat milk are entirely hydrolyzed in vivo by trypsin as compared with 76 to 90 % casein of cow milk. These physical properties of protein present in goat milk make it health beneficial. Therefore, goat milk is more degraded significantly faster by human gastric and duodenal juices than cow milk. The protein of any milk plays vital role in the nutritional, body maintenance and processing techniques. The goat milk protein is composed of casein which is the major

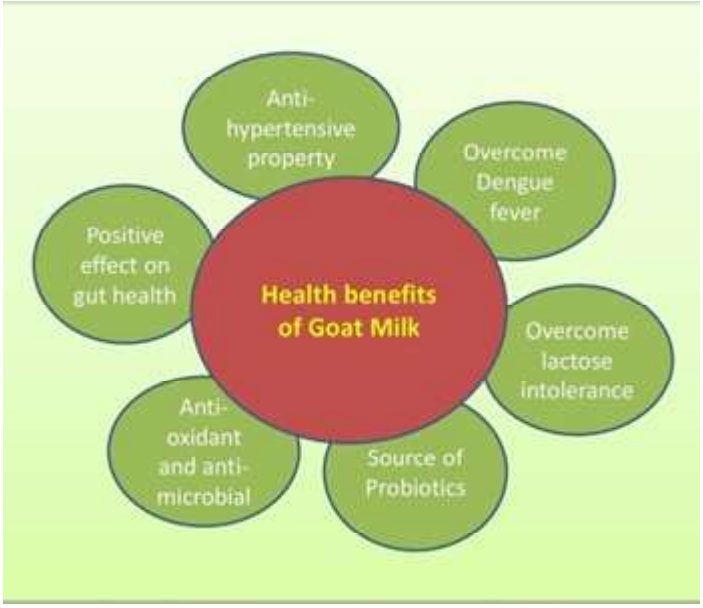


Fig 1. Health benefits of goat milk

Table 1 Basic constituents present in goat milk, cow milk and human milk (per 100 g)

Milk composition	Goat Milk	Cow Milk	Human Milk
Water	87.5	87.7	86.7
Total solid	12.2	12.3	12.3
Lactose	4.6	4.7	7.2
Fat	4.0-4.5	3.8	4.1
Protein	3.2	3.3	1.3
Ash	0.8	0.7	0.2
Energy (kcal)	70	69	68

(Source: Lima et al. 2018)

Table 2 Average Fatty acid and cholesterol contents of goat milk, cow milk and human milk

S. No.	Fatty acid	Goat Milk	Cow Milk	Human Milk
1.	(C4:0) Butyric acid	0.13	0.11	-
2.	(C6:0) Caproic acid	0.09	0.06	-
3.	(C8:0) Caprylic acid	0.10	0.04	-
4.	(C10:0) Capric acid	0.26	0.08	0.06
5.	(C12:0) Lauric acid	0.12	0.09	0.26
6.	(C14:0) Myristic acid	0.32	0.34	0.32
7.	(C16:0) Palmitic acid	0.91	0.88	0.92
8.	(C18:0) Stearic acid	0.44	0.40	0.29
9.	(C16:1) Palmitoleic acid	0.08	0.08	0.13
10.	(C18:1) Vaccenic acid	0.98	0.84	1.48
11.	(C18:2) Linoleic acid	0.11	0.08	0.37
12.	(C18:3) α-Linolenic acid	0.04	0.05	0.05
13.	Cholesterol (mg)	11	14	14

(Source: Turkmen et al. 2017)

part of proteins and the whey protein which is present in lesser amount (Table 3). Casein in goat milk contributes ~80% and whey protein contributes 20%. The occurrence of s1-casein in goat milk has been extensively examined in past recent years, after that it was also revealed that, this form of casein is available in six forms: A, B, C, E, F, and "null." α_{S1}-casein is the most abundant α_s-casein in cow milk. The "null" kind or lack of certain goat milk suggests that the primary (α_s-casein variant) in various goats is the α_{s2}-casein variant, which has variable digestion and cheese-making capabilities (Kumar et al. 2012). Soft cheeses are easier to manufacture because goat milk has a low s1casein content, which allows for speedier coagulation and softer curds. As part of the protein fraction, amino acids are also included. Goat milk, cow milk and human milk are very much similar in their amino acid profile with exception that goat milk contains lower amount of cysteine than human milk (Silanikove et al. 2010). The nutritional value of protein is decided by the essential amino acid content present in the milk. Goat milk is good source of essential amino acids i.e. tyrosine, valine, leucine, cysteine, lysine, threonine, Phenylalanine and non-essential amino acids are glutamic acid and Proline (Lima et al. 2018). Taurine is present in goat milk, which is representative of free amino acid. Taurine is useful for growth and development of the body (Zenebe et al. 2014). Another characteristic of goat milk's protein is its unique shape, size and structure and the amounts and types of micelles. The micellar size of goat milk protein is smaller (180 nm) in size than cow milk protein micellar size (260 nm).

Minerals

Mineral content in Goat milk is higher than cow and human milk as shown in Table 4. Mineral factors makeup approximately (4-6) percentages of the human frame weight and play an essential position in the growth, development, and management of various key activities. These minerals are also necessary for healthy bones and teeth, as well as maintaining the ionic balance of physiological fluids (Singh et al. 2019). The amount of minerals depends on the different factors like breed, age of the goat, lactation period, diet and individual health. Goat milk should be preferred over human and cow milk as it contains higher mineral concentration. Goat milk possesses 121 mg Phosphorus and 134 mg calcium per 100 g, which is higher as compared to human milk

Table 3 Protein profile (g L^{-1}) of milk from goat milk, cow milk and human milk

S. No.	Protein fractions	Goat Milk	Cow Milk	Human Milk
1.	Total casein	23.3-46.3	24.6-28	2.4-4.2
2.	Total whey protein	3.7-7.0	5.5-7.0	6.2-8.3
3.	Casein-to-whey protein ratio	78:22	82:18	2911;71-33:67
	Major caseins			
4.	α_{s1}-casein	0-13.0	8-10.7	0.77
5.	α_{s2}-casein	2.3-11.6	2.8-3.4	-
6.	β-casein	0-29.6	8.6-9.3	3.87
7.	k-Casein	2.8-13.4	2.3-3.3	0.14
	Major whey protein			
8.	β- Lactoglobulin	1.5-5.0	3.2-3.3	-
9.	α-Lactalbumin	0.7-2.3	1.2-1.3	1.9-3.4

(Source: Roy et al. 2020)

Table 4 Average minerals contents of goat milk, cow milk and human milk (per 100g)

S. No.	Minerals (mg)	Goat Milk	Cow Milk	Human Milk
1.	Potassium (K)	181	152	55
2.	Chloride (Cl)	150	100	60
3.	Calcium (Ca)	134	122	33
4.	Phosphorus (P)	121	119	43
5.	Sodium (Na)	41	58	15
6.	Sulphur (S)	28	32	14
7.	Magnesium (Mg)	16	12	04
8.	Selenium (Se)	1.33	0.96	1.52
9.	Zinc (Zn)	0.56	0.53	0.38
10.	Iron (Fe)	0.07	0.08	0.20
11.	Iodine (I)	0.022	0.021	0.007

(Source: Kumar et al. 2016)

and cow milk. Goat milk is higher in concentration of Calcium, Manganese, Phosphorus, Potassium, Magnesium, Chorine, Selenium, and less concentration of Sodium and Sulphur. As well as, In Comparison to human milk, goat milk possess higher concentration of zinc, Iodine, whereas, less concentration of Iron and also total peroxidase activity is greater than cow milk and human milk (Kumar et al. 2012) which is very important for human health, since iodine and thyroid hormones both play an important role in metabolic and physiological body functioning. Goat milk contain around 0.70 to 0.85% mineral which is higher than cow and human milk. Mineral concentration does not fluctuate but it varies according to different factors according to (Kumar et al. 2016). From the human nutrition point of view goat milk can be beneficial more than the cow milk or human milk and can be used as a complimentary diet (Lad et al. 2017). Goat milk contains higher percentage of zinc than human milk. Zinc is an important mineral for wound healing, maintenance of healthy skin and enhancing immunity.

Vitamins

Vitamins are organic substances found in milk that are biochemical, physiological, and metabolically active. Table 5 shows the vitamin content of goat milk, cow milk and human milk. Milk of goat possess significant amount of Vitamin A just like human milk. The reason behind this is that goats can convert β-carotene into Vitamin A in the milk. Due to this goat milk is whiter than cow milk and also goat milk contains high concentration of Vitamin C than cow milk, which is a good for boosting the immunity of the consumers Kumar et al. (2016). Apart of these entire goat milk is good source of Vitamin E, riboflavin, Vitamin D, Thiamin, Niacin (Lad et al. 2017; Getaneh et al. 2016). Goat milk lack Vitamin B_{12} as well folic acid, feeding human or infant diet with only goat milk can cause megaloblastic anemia (Barłowska et al. 2011). As goat milk contains sufficient amounts of thiamine, vitamins B, niacin, riboflavin, and pantothenic acid. If human infants are fed with goat milk only then they are fulfilled with all of these vitamins and minerals. The bioavailability of goat milk's vitamin is more than cow milk. Goat milk also contains precursor of Vitamin A which are readily bioavailable.

Goat milk products

The processed goat milk products are having good market approach these days. There are different goat milk products are available in the market like- frozen yoghurt, fortified or flavored milk, ice cream, butter milk, dried milk or condensed milk product, dried granulated milk, fruit yoghurt, hard cheese, soft cheese, blue cheese, dry whole milk, tvorog (strawberry, full cream, garlic and 'french-style''), butter oil, dahi, cream, butter, powder, cultured cream butter, soap, whey protein concentrate and ghee. Indian traditional products like- Channa, Paneer, Ghee, etc.

Some goat milk products for human consumption are-

1. Cheese- The origin of goat milk cheese was Mesopotamia. Goat cheese was made from raw and pasteurized goat milk. Ripening and maturation of cheese of goat milk is depend on different factors like- Incubation period, pressing and forming techniques species and amount of organism used as culture. Numbers of varieties of goat milk cheese are manufactured in all over the world. Manufacturing of cheese by raw goat milk is prohibited in many countries as microbial load are higher in raw milk. Storage condition and ageing time are the important factors that decide the flavor, texture and body of the cheese.

2. Fluid milk- Goat milk can be fortified with vitamins, minerals and several additives with different processing steps and packaging techniques. Fluid goat milk is packed in Glass, Plastic, or paper carton. Product shelf life depends on many factors like- type of packaging, processing etc.

3. Desserts- Goat milk ice cream is an alternative for consumers, especially children, because of its Anti-allergenic and nutritional properties. Goat milk ice and yoghurt are usually flavored. Mainly Chocolate, Vanilla is the most common flavors used.

Table 5 Average vitamin contents of goat milk, cow milk and human milk (per 100g)

S. No.	Vitamin	Goat Milk	Cow Milk	Human Milk
1.	Vit.-A Retinol (IU)	185	126	190
2.	Vit.-D Cholecalciferol (IU)	2.3	2.0	1.4
3.	Vit.-B_1 Thiamin (mg)	0.068	0.045	0.017
4.	Vit.-B_2 Riboflavin (mg)	0.21	0.16	0.02
5.	Vit.-B_3 Niacin (mg)	0.27	0.08	0.17
6.	Vit.-B_5 Pantothenic acid (mg)	0.31	0.32	0.20
7.	Vit.-B_6 Pyridoxine (mg)	0.046	0.042	0.011
8.	Vit.-B_8 Biotin (µg)	1.5	2.0	0.4
9.	Vit.-B_9 Folic acid (µg)	1.0	5.0	5.5
10.	Vit.-B_{12} Cobalamin (µg)	0.065	0.357	0.03
11.	Vit.-C Ascorbic acid (mg)	1.29	0.94	5.00

(Source: Lad et al. 2017)

Sweets are also produced from goat milk. Cajeta is a Mexican candy, manufactured by goat milk found in different manufactured by goat milk found in different flavors. Cookies are also made of goat milk. Ghee, whey products, fermented products, evaporated dried milk products, etc. is produced by goat milk (Ribeiro and Ribeiro, 2010).

Pal et al. (2017) has reported that goat milk and its product are very beneficial as nutritional or functional food and helps to maintain the health of consumers especially to those who are allergic to cow milk. It was mentioned that different products are available in market such as- Cheese, Butter, Butter milk, yoghurt, condensed milk, flavored milk, candies, sweets, etc.

Nutrient Functionality

Nutraceuticals or functional foods contain some active compounds which have beneficial effect on human body other than its nutritional benefits. Milk has the capacity to develop immune system in the newborn baby, limit the growth of the bacteria, and provides antioxidant and anti-inflammatory protection (Lepage and Van de Perre 2012). Another factor that could influence immunological and inflammatory processes is the power of milk and its product to supply nutrients to the human body that can alter the gut beneficial microbiota, an important regulator of immunity (Ceapa et al. 2013; Kau et al. 2011). Hsieh et al. (2015) recently published reviews on the benefits of several bioactive components present in milk and dairy products resulting in reducing the inflammation and functioning as coadjutants in traditional therapies.

Bioactive peptides

Now day's food scientists, researchers and even consumers are showing more interest towards functional foods and bioactive components present in them. Any functional food are just like any conventional food and it could be the part of diet as it has its basic nutritional values, in addition of that, functional food have many physiological benefits like reduce the chances of various chronic diseases and immune modulation.

As per records of the National Cancer Institute (USA), bioactive components are "one type of chemical food in small amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). Bioactive compounds have actions in the body that may promote good health. They are being studied in the prevention of cancer, heart disease, and other diseases". Bioactive compound is "a compound which has the capability and the ability to interact with one or more component(s) of the living tissue by presenting a wide range of probable effects". Bioactive compounds can be obtained naturally, terrestrially or aquatic, may be a plant source, animal source or other sources like micro organisms or can be even synthetic. The word "bioactive compound" is not related to the basic nutrient content of the food that are essential and important for metabolic activities of living organism such as primary metabolites (Claps et al. 2017).

Bioactive peptides basically are protein fragments that have beneficial and fruitful impact on physiological processes or circumstances and may influence human health in good way (Huang et al. 2013). Goat milk is considered as close as a perfect diet (Atanasova and Ivanova 2010). Protein of goat milk is composed of 80% caseins and 20% whey proteins. Maximum portion of goat milk protein are readily biologically available while the rest part of milk peptides could be digested on the action of proteolytic enzymes. When chymosin act on k-casein, during coagulation of milk during the cheese manufacturing a soluble C-terminal of caprine calmodulin (calcium-binding protein) is formed is an active component of caprine calmodulin (calcium-binding protein). These peptides are also key sources of bioactive peptides, anti-hypertensive and ACE-inhibitory peptides (Espejo-Carpioet al. 2013; Hernandez-Galan et al. 2017). Other small proteins with biological action found in goat milk areprotease peptone, lactoferrin, immune-globulins, transferring, prolactin, ferritin and folate-binding protein. Fermented dairy products like yoghurt and cheese already have variety of naturally produced bioactive peptides. The lactoferin value approximately 0.107 ± 19 mg / mL in goat milk is higher than cow milk (Wang et al. 2019). Many epidemiological studies had proved that consumption of milk and dairy products leads to lower incidence of hypertension. The anti-hypertensive effect in milk and milk products is due to its high mineral content (particularly potassium, calcium and magnesium) and certain particular protein and their hydrolysates (Mullins et al. 2021).

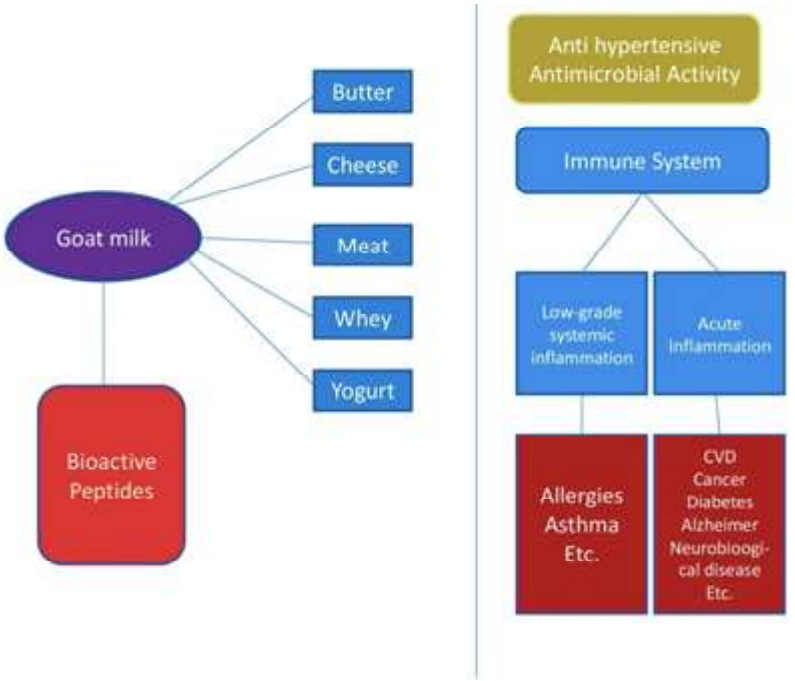
Quality and safety

For quality production, processing and successful business of goat milk and its processing for processed products, high-quality raw milk is required. Milk and its products must be free of harmful microorganisms, antibiotics, insecticides, and herbicide chemicals before they may be consumed. They should have a pleasant flavor with no unpleasant aftertaste and be free of rotting microorganisms. The quality of raw milk depends on various factors such as processing ways, health state of animal, environment, acceptability levels etc. Milk quality can be assessed using total bacteria count (TBC), the presence or absence of certain types of pathogenic bacteria, as well as somatic cell count (SCC) or the number of leukocytes in milk (Alhussien and Dang, 2018).

Benefits of goat milk

Goat milk is totally different from human milk and cow milk in nutritional values and its composition. Goat milk possesses many bio-active compounds that would be beneficial for the person suffering from several chronic diseases summarized in figure 2. Goat milk contains oligosaccharides, peptides, and lipids that

Fig 2. Primary health impacts of goat milk



can help with neurological degeneration, cardiovascular disease, and metabolic disorders, as well as intestinal health. Goat milk has probifidogen and probiotic effect because of its health benefits and Nutritional properties goat milk is preferred for older adults, infants (Lima et al. 2018). Bioactive lipids present in goat milk helps to keep immune system healthy. Goat milk possesses MCT i.e Medium Chain Triglycerides, which helps in energy production and improving the nutrition absorption in the body. It also helps in preventing the bacterial infections. Goat milk Oligosaccharides is beneficial for human health as it possess anti-infective and probiotic properties. Vitamins present in goat milk can be helpful in boosting the immunity of consumers as it possesses Anti-oxidant and Anti-viral properties. Minerals in goat milk helps in maintaining the physico-chemical properties of body.

Goat milk is beneficial for infants and adult because it possesses many medicinal properties like fermented goat milk are beneficial for the cardio vascular diseases as it contain anti-thrombotic, anti-oxidative and anti-atherogenic effects. Also it is useful for the treatment of Gastro-intestinal diseases, Cancer, Allergies, and Immunological properties (Zenebe et al. 2014). Kumar et al. (2016) has reported in his article that goat milk helps in curing dengue fever, brain development, Lactose intolerance etc. and also goat milk act as probiotic and it is a source of antioxidant. Peptides, oligosaccharides and non-digestible sugars present in goat milk act as prebiotic. Lack of selenium may cause lowering in blood platelets which is the main issue in dengue fever. The goat milk is prescribed to the patient for balancing the blood fluid level in the body of the patient. Salic acid found in colostrum and milk which plays an important role in the development of brain and also in improving immunity. Goat milk possesses less amount of lactose, so it helps to prevent to symptoms of lactose intolerance. Antioxidant peptides present in milk have ability to delay or prevent oxidative spoilage. Oligosaccharide present in goat milk has scavenging property against *E.coli*.

Goat milk is also used to cure Insomnia, Stomach ulcer, Asthma, Acidity, Constipation etc. It contains high amount of Non protein Nitrogen, Protein, phosphate which helps to make it more digestible. Goat milk possesses prolactin and Lactoferrin which helps for good functioning of body like production of milk and immunity. As it is good source of nitrogen and potassium, it helps to cure Cardio-vascular diseases and also be useful for maintaining the blood pressure as well (Lad et al. 2017). It can be useful to enhance bone mineralization because it helps to enhance the metabolic and digestive use of Calcium and Phosphorus. Arthritis can be treated by bio organic sodium present in goat milk. When compared with other milk, goat milk is preferred by consumers when it comes as medicinal point of view as it possesses many therapeutic values, less chances of allergy, easily digestible and act as buffer. So it can be preferred for infants also due to its easy digestible property and its compositions are similar to human milk (Lund et al. 2021; Risko and Csapo et al. 2019). Lactic acid bacteria can be used to ferment the goat milk protein or this protein could easily be hydrolyzed by enzymes to produce the antioxidant peptides. These antioxidant peptides have iron chelation activity, radicals scavenging activity and inhibition of auto oxidation of polyunsaturated fatty acids (Kumar et al. 2016). Goat milk is the vital source of bioactive peptides and its nutraceutical properties could be exploited to validate and promote goat milk production.

Nutraceuticals are the bioactive components of food that are first isolated, purified and then sold in form of medicine that gives beneficial effect against some chronic diseases and enhances the immune system as well. Now days the protein portion of goat milk is used to produce nutraceuticals. Isolated peptides are used in relaxing blood vessels in people suffering from high blood pressure. Lactotripeptide are inactive when present in milk but become active when isolated and are more effectively deal with high blood pressure. Hydrolyzed casein is found to be effective against high blood pressure. The casein and whey protein of Goat, are rich source of ACE inhibitory peptides as ACE inhibitory drugs have various side effects in the control of blood pressure. Some other protein components in isolated form were used in curing a specific metabolic syndrome characterized by obesity due to improper glucose metabolism. Protein isolated from goat milk also found its importance in curing congenital antithrombin. Beta casein has immune modulatory property which is already a peptide. Beta casein is found in higher amount in goat milk as compared to cow milk (Mwenze, 2015).

Albenzio et al. (2021) has reported that many children have allergy against cow milk like gastrointestinal allergy and chronic enteropathy which can be efficiently cured by goat milk. Goat milk therapy is beneficial for the pediatric diseases with good results. That's why Goat milk is considered more suitable for elders and infants. Goat milk contains appropriate amount of selenium, which helps in improving immune system. So, goat milk is helpful in boosting the immunity of the consumer and cure diseases. It was studied that goat milk is able to release active Nitric Oxide from blood cells which triggers the production of Cytokine. The Nitric Oxide release can cure infections by exposing Antibacterial activity and also can have Cardio-protective effects in consumers. Due to its protein composition, Curd formed by goat milk is softer which promotes the healthy digestion process. Goat milk doesn't cause irritation inside the gut because of its fat globules size which is much smaller than cow milk fat globules (Lad et al. 2017).

In dengue cases doctors recommend to drink goat milk as it increase the blood platelets. Studies are still going on to find out the factors which are responsible of increasing platelets in goat milk. Goat milk increases the formation of high density lipoprotein cholesterol which is good cholesterol and decrease the formation of low density lipoprotein cholesterol which is bad cholesterol. Thus, along with nutritional values goat milk have several medicinal values. Got milk could easily be digested by people suffering from lactose intolerance who cannot take cow milk.

Goat milk can be beneficial for bile salt formation, development of brain, growth of the body because goat milk contains higher concentration of taurine, an amino acid. Potassium in goat milk helps in maintaining the function of kidney, muscles and nerves. Chloride helps to maintain the Osmotic pressure, fluid balance, blood pH of the body and also balance the function of liver. Zinc helps to maintain the wound healing, Healthy skin and also it helps in adaptive immunity. Oligosaccharide in goat milk has anti-inflammatory property; it also helps to reduce clinical symptoms like bloody stools and diarrhea. Goat milk possesses high concentration of Conjugated Linoleic Acid (CLA), which act as anti-carcinogenic against mammary and colon cancer (Zenebe et al. 2014; Claps et al. 2014).

Due to higher percentage of oligosaccharides in goat milk powder it is widely used as prebiotic for intestinal microbiota. Goat milk increases the biliary secretion of cholesterol and decreases plasma cholesterol level in blood. Regular consumption of goat milk increases the bone mineral density and less fat deposition. The increase in the bone mineral density is due to higher plasma mineral density in goat milk which is due to higher percentage of essential minerals in the composition of goat milk. Taurine present in goat milk which is derived from sulphur-containing amino acids is very much important for the metabolic activities of neonates.

Conclusion

From the facts of this paper we could say that goat milk is one of the best alternative milk of cow milk and human milk. Goat milk is the major source of bioavailable fats, proteins, minerals, vitamins and with great suitability for infant and human. Several value-added products are available in the market that are made from goat milk due to their therapeutic importance, easy digestion, and buffering capacity, which are exceptional when compared to the milk from other animals. Compositional values of Goat milk are more or less similar or even better than cow milk and human milk. Goat milk possesses several nutraceutical and functional property which can be beneficial for the human health and also helpful in growth, development and maintenance of the body. Goat milk poses high nutritional values in addition of this it also poses high nutraceutical properties thus more suitable for infants, older people and convalescent people. In respect to food industry, goat milks various physiological functions and it has indicators of bioactive molecules thus very much important in designing functional foods. It also helps to cure many chronic diseases like Dengue, cardio-vascular diseases, immunological disorder, etc. All these above reasons are responsible why goat milk is preferred as an alternative of cow milk and human milk. In the developing countries where poverty and health sector are not so much advanced and malnutrition is of greatest concern, there, production and consumption of goat milk should be promoted due to its high nutritional, therapeutic, nutraceutical and physiological values.

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RESEARCH ARTICLE

Preparation of curcumin fortified buffalo *ghee*

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Abstract: Attempts were made in the present investigation to standardize the *ghee* manufacturing method for preparation of curcumin fortified *ghee*. Sensory evaluation and fat recovery revealed that *ghee* preparation using creamery butter method was more preferable than *desi* and direct cream method. Fermenting cream using NCDC 352 starter @ 2% inoculation rate yielded sensorially superior *ghee* in least fermentation time. Sensory attributes of *ghee* were affected upon addition of curcumin beyond 250 ppm; however, the samples containing upto 350 ppm of curcumin were acceptable to the sensory panelists. During cooking, it was observed that increasing the heating temperature improved the sensory attributes while increasing the heating duration decreased the same. The study will be useful for dairy industries looking for functionality enhancement of *ghee*.

Keywords: Curcumin, Fermentation and Sensory evaluation, *Ghee* preparation

Introduction

India is the largest milk producer in the world with an annual production of about 186,000 tonnes in 2018. About 46% of milk produced is consumed as liquid milk and 50-55% is converted into traditional Indian dairy products (TIDP), which are deep rooted from ancient traditions and have a strong cultural heritage (Nigam et al. 2016). *Ghee* is one of the most widely used traditional dairy products of India which essentially contains minimum 99.70% milk fat. Currently India represents both the world's largest producer and consumer of *ghee* with about 30-35% of the milk annually produced in India was converted into *ghee* (GAIN, 2014). The market for *ghee* in India is the second highest after fluid milk in both value and volume. It occupies a premier position among the milk products produced in India since time immemorial (Lodh et al. 2018). *Ghee* is an important source of fat in the diet of people of Indian subcontinent. Eighty percent of *ghee* produced is used for culinary purposes while the remaining 20% is used for confectionery, including small amounts consumed on auspicious occasions like religious ceremonies (Ganguli & Jain, 1972). *Ghee* is manufactured mostly from buffalo milk as it constitutes more than 55% of the total milk production in India and higher fat content (6-7%). *Ghee* production is an important activity of organized dairy sector as it obtained from surplus fat, collected during the standardization of liquid milk (Aneja et al. 2002).

Utilization of *ghee* in Indian diet without any adverse health effects has been reported for centuries. Apart from a rich source of fat, it is also a good source of essential fatty acids and fat soluble vitamins like A, D, E and K. Scientific findings indicated that *ghee* contains several therapeutic components like conjugated linoleic acids (CLA), sphingomyelin, butyric acid, myristic acid etc. CLA is considered as an important biologically-active compound of *ghee* due to its proven anticarcinogenic, antiallergic and anti-inflammatory properties (Smit et al. 2010). CLA also improve cardiovascular health by its antiatherogenic as well as antioxidant properties (Chinnadurai et al. 2008). Despite of several health implications, the milk fat including *ghee* has often been implicated in atherosclerosis, coronary heart disease because of its cholesterol content and composition of fatty acids. Oxidative deterioration of *ghee* is one of the major factors that limit the shelf life of *ghee* (Mehta et al. 2015). The onset of rancidity in *ghee* is mainly due to the oxidation of unsaturated fatty acids leading to development of peroxides and/or due to hydrolysis of glycerides resulting in increased levels of free fatty acids. Hydroperoxides and their subsequent break down products not only produce off flavour also accompanied by nutritional loss. Numerous scientific evidences correlated oxidized lipids with negative health implications (Pukalskas et al. 2005). Oxidized oils

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cause cancer, heart disease and early aging in consumers who consume oxidized oils and fats. The use of antioxidants is the most appropriate way to stabilize oils, prevent lipids oxidation and to protect oils from the damages caused by oxidized products such as free radicals (Yassari & Yasari, 2013).

Numerous herbs have been known to exhibit antioxidant potential in food lipids and retard lipid oxidation during storage (Parmar et al. 2013). Hence, it is no wonder that the use of herbs and spices as a resource of safer and natural antioxidants has drawn much attention as they not only impart taste, aroma and colour to food but also help to enhance healthful attributes because of their functional characteristics. One such beneficial phytochemical is curcumin (diferuloylmethane), a fat soluble bioactive, yellow pigment present in Indian spice turmeric (*Curcuma longa* L.), well known for its various functional attributes. Turmeric has long been used in Ayurveda for the treatment of different diseases as it possesses numerous functional attributes e.g., antiinflammatory, antioxidant, anticarcinogenic, antimutagenic, hypotensive, hypocholesteremic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, etc. (Hosseini et al. 2011). Curcumin, which makes up 2 to 5% of the spice, reported to activate the rate limiting step in cholesterol catabolism, that is, cholesterol 7-á-hydroxylase thereby stimulating the conversion of cholesterol to bile acid, an important pathway in the degradation of cholesterol (Babu & Srinivasan, 1997). Majithiya et al. (2004), reported the ability of curcumin inhibit to LDL oxidation and hypocholesterolemic effect in mice. Curcumin also lower lipid peroxidation by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase at higher levels (Arafa, 2005).

Although turmeric has been used in Ayurvedic medicine since ancient times in various medical applications, very limited information is available concerning the application of curcumin, the natural pigment found in turmeric as natural antioxidants in *ghee* for preventing auto-oxidation and prolonging its shelf life. As curcumin possesses a wide spectrum of biological functions, it is also expected that its incorporation will help to improve the functional attributes especially its antioxidant behaviour and hypocholesterolemic effect. Hence, the present project was undertaken with the aim to develop the portocol for preparation of curcumin fortified buffalo *ghee* preparation.

Materials and Methods

Raw materials

Buffalo milk, cream and white butter was procured from Experimental Dairy (ICAR-National Dairy Research Institute, Karnal, Haryana, India), was used for the manufacture of curcumin fortified *ghee* during this investigation. Curcumin (97% w/w) was procured from M/S Hi-Media Chemicals Pvt. Ltd., India. Starter cultures (NCDC 167 and 352) were procured from National Collection of Dairy Cultures of Dairy Microbiology Division, ICAR-NDRI, Karnal and the cultures were maintained at -10 °C before using the product preparation.

Preparation of curcumin fortified buffalo ghee

Ghee was prepared using cream or butter using the method prescribed by Aneja et al. (2002). For selection of satiable *ghee* preparation method, different methods *viz*., desi, direct cream and creamery butter methods were employed. For preparation of *ghee* from fermented cream, two cultures (NCDC 167 and 352) were used at 1 and 2 % level. After the pH was decreased to 4.50, it was churned and the white butter so obtained was used for *ghee* preparation. For curcumin fortification, curcumin was added at different levels into white butter, remaining protocol was the same as that for preparation of *ghee* from creamery butter method using fermented cream.

Sensory evaluation

Samples obtained during the course of investigation were analyzed by trained panellists from the scientific faculty of NDRI, Karnal using the scorecard prescribed by Bureau of Indian Standards (BIS, 1976). In brief, representative *ghee* sample was drawn from the bulk lot in a clean and dry glass bottle. This was followed by capping the bottles properly. The capped samples were presented to the panelists for sensory evaluation. The samples were also provided with 1% NaCl solution (luke warm) and a score card. During the sensory evaluation, first the samples were visually examined for color and suspended impurities (residue). This was followed by perception of odour by inhaling the smell concentrated at the headspace of the sample immediately after removing the lid. Body & texture was evaluated with the help of a spatula or glass rod. At last, a spoonful of *ghee* was taken in the mouth for taste and aroma evaluation. After evaluation, scores were awarded to the samples based upon the perception by the panelist.

pH

During fermentation, pH of the cream was measured using pH meter (Model No PHS 25 CW, SDFCL, Mumbai).

Fat recovery

Fat recovery in the product was determined using the following formulae:

$$Fat\ recovery = \frac{Amount\ of\ fat\ in\ ghee * 100}{Amount\ of\ fat\ in\ the\ raw\ material}$$

Statistical analysis

The results obtained during the course of investigation were tested statistically using SPSS software (Version 20, USA) and

data were expressed as means ± standard error (S.E.). Analysis of variance (ANOVA) test was employed to identify significant differences ($p < 0.05$) between data sets.

Results and Discussion

Selection of ghee preparation method for curcumin fortified buffalo ghee

Studies were conducted to find out the suitable method for preparation of curcumin fortified buffalo *ghee*. *Ghee* was prepared using direct cream method (DC), desi method (Desi) and creamery butter method (CBM) and the samples were subjected to sensory evaluation. The sensory scores given by the semi trained panelist for the *ghee* samples prepared by different methods are presented in Table. 1. It was observed that the highest flavor score was observed for the *ghee* prepared by CBM, which was significantly higher ($p<0.05$) from the *ghee* prepared using *desi* and DC ($p<0.05$) method. Texture score of the sample were highest ($p<0.05$) from *desi* method, followed by CBM and DC method. On the other hand, sensory scores were significantly not different ($p<0.05$) for color and appearance and freedom from suspended impurities attributes. Highest overall acceptability was observed for the sample prepared using CBM method, followed by desi method and the *ghee* prepared using DC method obtained significantly ($p>0.05$) lowest score among the three samples. Further, fat recovery from the *Desi*, DC and CBM methods were 83.50±0.28, 80.16±0.44 and 89.0±0.57 %, respectively. From this, it was observed that highest sensorial acceptability and fat recovery obtained with CBM method. Moreover, in large mechanized plants, CBM method is most popular for manufacturing *ghee*. Hence, it was selected for further studies.

Selection of type and level of culture to manufacture curcumin fortified buffalo ghee

Buffalo *ghee* was prepared form fresh cream butter (FC) and ripened cream butter (RC) and the sensory scores obtained for these samples are presented in Table. 2. It was observed that *ghee* prepared from RC butter obtained significantly ($p<0.01$) higher flavour, texture and overall acceptability score than the *ghee* prepared from FC butter, which could be attributed to the changes brought about by fermentation in the cream. Fermentation of cream produces diacetyl, which is a major contributor of flavour in sour cream and butter (Longo & Sanroman, 2006). Color and appearance score and free from suspended impurities score for the two *ghee* did not differed significantly ($p>0.05$) from each other. Based upon the obtained results, *ghee* preparation from RC butter was selected for further studies.

Starter culture plays an important role in fermentation of cream, therefore trials were conducted with different cultures for obtaining shortest possible time of fermentation with superior sensory quality of *ghee*. For this, initially two mesophilic *dahi* cultures namely NCDC 167 and NCDC 352 (1 and 2% w/w) were employed and their fermentation characteristics in terms of ability to reduce pH of standardized cream having 40% fat with time were studied. The pH reduction pattern of NCDC 167 and NCDC 352 are presented in Fig. 1. It could be observed that 2% (w/w) NCDC 352 took shortest time *i.e.*, 7.5 h to reduce the pH of cream to the end point *i.e.*, 4.5. Flavour development in butter or sour cream is controlled by pH, which control the amount of lactic acid production and also helps in the biosynthesis of diacetyl. At pH above 5.4, the activity of *acetolactate synthetase* is inhibited, consequently the production of acetolactate, precursor of diacetyl is very limited (Cogan & Hill, 1993).

Besides of the fermentation time, sensory quality of *ghee* is also affected by the starter culture used in its preparation. Joseph & Appachar (1980) prepared *ghee* using the cream fermented by different starters and reported that the product prepared using *S. diacetylactis* (DRC 1) and *S. lactis* (C 10) had higher flavour score, while no such effect was observed with *S. thermophilus* (HST) and *L. bulgaricus* (LBW) culture. Considering this, *ghee*

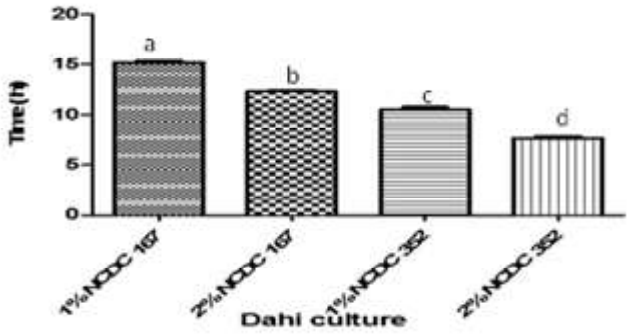

Fig. 1: pH reduction pattern of cream by different cultures
Values are means for the same parameter with different superscripts differ significantly ($p<0.05$

Table 1 Effect on sensory attributes of buffalo ghee prepared by different methods

Sensory attributes	Direct cream method	*Desi* method	Creamery butter method
Flavor	40.56[a]	43.49[b]	45.15[b]
Texture	20.19[a]	24.55[b]	22.74[c]
Color and appearance	7.14[a]	8.55[a]	8.51[a]
Freedom from suspended impurities	9.00[a]	9.00[a]	9.00[a]
Overall acceptability	76.89[a]	85.59[b]	85.40[b]

Values are mean (n=3); means for the same parameter with different superscripts differ significantly ($p<0.05$

prepared from the cream inoculated with 2% culture of both the cultures (NCDC 167 and 352, individually) was evaluated for sensory attributes and the results are presented in Table 3. It was observed that the sample prepared using NCDC 352 obtained higher flavour and overall acceptability scores than the sample prepared using NCDC 167. This could be due to differences in the metabolites produced during fermentation by these cultures. Based upon the obtained results, 2% (w/w) NCDC 352 (mesophilic *dahi* culture) was selected for further studies.

Effect of different levels of curcumin on sensory score of curcumin fortified buffalo ghee

Being the main functional ingredient in the curcumin fortified buffalo *ghee*, trials were conducted to select the appropriate range of curcumin to be fortified in the product. The required minimum level of curcumin for the desired health benefits (Lim et al. 2001), was selected as 160 ppm which had been proved to give anti-Alzheimeric effect. Six levels of curcumin *viz.*, 160, 200, 250, 300, 350 and 400 ppm were tried in the preparation of curcumin fortified buffalo *ghee* and the samples were evaluated for their sensory attributes. It could be observed from Table 4. that the flavour score was not affected ($p>0.05$) upto 250 ppm of curcumin addition into buffalo *ghee* beyond that the score was significantly decreased ($p<0.05$) with increasing level of curcumin content. The lowest sensory score was obtained in case of *ghee* sample added with 400 ppm of curcumin, which could be due to undesirable flavour of curcumin at such high level. Prasad et al. (2017) also reported that *burfi* prepared using higher level of turmeric had undesirable (bitter) flavor. Similar to flavor score, colour and appearance score was also significantly decreased ($p<0.05$) with increasing the level of curcumin addition, which could be due to the intense yellow colour imparted by curcumin (Lodh et al. 2018). Whereas, texture and free from suspended impurities scores were not significantly affected ($p>0.05$) by increasing level of curcumin addition. Overall acceptability score was also decreased significantly ($p<0.05$) with increasing level of curcumin, but the effect non-significant upto 250 ppm, beyond which the decrease in score became significant ($p<0.05$) with the increasing level of curcumin. Moreover, the product manufactured using 400 ppm of curcumin was of deep yellow in colour, devoid of *ghee* flavour as curcumin flavour was dominated over the natural *ghee* flavour in the product and hence was almost rejected by the sensory panelists. Further, it was also observed that although the flavour score decreased significantly beyond

Table 2 Effect on sensory attributes of buffalo ghee prepared from fresh cream butter and ripened cream butter

Sensory attributes	Fresh cream butter	Ripened cream butter
Flavor	42.15[a]	45.77[b]
Texture	23.41[a]	26.06[b]
Color and appearance	8.10[a]	8.44[a]
Freedom from suspended impurities	9.00[a]	9.00[a]
Overall acceptability	82.66[a]	89.27[b]

Values are mean (n=3); means for the same parameter with different superscripts differ significantly ($p<0.05$

Table 3 Effect on sensory scores of buffalo *ghee* prepared from mesophillic *dahi* culture

Sensory attributes	NCDC-167	NCDC-352
Flavor	41.18[a]	44.41[b]
Texture	26.02[a]	25.99[a]
Color and appearance	8.46[a]	8.75[a]
Freedom from suspended impurities	9.00[a]	9.00[a]
Overall acceptability	84.66[a]	88.15[b]

Values are mean (n=3); means for the same parameter with different superscripts differ significantly ($p<0.05$

Table 4 Effect of curcumin level on on sensory scores of curcumin fortified buffalo ghee

Sensory attributes	160	200	250	300	350	400
Flavor	45.44[a]	43.12[a]	43.43[a]	40.16[b]	36.75[c]	30.10[d]
Texture	25.44[a]	25.10[a]	26.43[a]	26.06[a]	26.77[a]	26.01[a]
Color and appearance	7.80[a]	7.04[a]	6.43[b]	6.04[c]	5.61[d]	4.50[e]
Freedom from suspended impurities	9.00[a]	9.00[a]	9.00[a]	9.00[a]	9.00[a]	9.00[a]
Overall acceptability	87.68[a]	84.26[a]	85.29[a]	81.26[b]	78.13[c]	69.61[d]

Values are mean (n=3); means for the same parameter with different superscripts differ significantly ($p<0.05$

Table 5 Effect on heating temperature on sensory scores of curcumin fortified buffalo *ghee*

Sensory attributes	110	115	120
Flavor	38.44[a]	42.05[b]	45.16[c]
Texture	21.00[a]	24.15[b]	26.72[c]
Color and appearance	8.55[a]	7.80[a]	7.07[b]
Freedom from suspended impurities	9.00[a]	9.00[a]	9.00[a]
Overall acceptability	76.99[a]	83.00[b]	87.95[c]

Values are mean (n=3); means with different superscripts differ significantly ($p<0.05$)

Table 6 Effect on heating duration on sensory scores of curcumin fortified buffalo *ghee*

Sensory attributes	16	20	24
Flavor	45.05[a]	40.16[b]	38.72[c]
Texture	22.66[a]	25.16[b]	24.23[c]
Color and appearance	8.55[a]	7.82[b]	7.43[c]
Freedom from suspended impurities	9.00[a]	9.00[a]	9.00[a]
Overall acceptability	85.26[a]	82.14[b]	79.38[c]

Values are mean (n=3); means with different superscripts differ significantly ($p<0.05$)

250 ppm of curcumin addition but the product was acceptable upto 350 ppm addition of curcumin hence this level (350 ppm) was selected for further studies.

Effect of heating temperature on sensory score of curcumin fortified buffalo ghee

Three different heating temperature *viz.*, 110°C, 115°C and 120°C for 20 minutes were studied for the preparation of curcumin fortified buffalo *ghee* and the samples so obtained were evaluated for sensory attributes (Table 5). It could be observed that the sensory score for all the attributes (except colour and appearance and free from suspended impurities) for curcumin fortified buffalo *ghee* increased significantly ($p<0.05$) with increasing the heating temperature. During heat clarification, higher temperature is needed to remove water bound to the SNF and to develop the characteristic flavour. Our results are in agreement with Ganguli & Jain (1972) who reported that *ghee* clarified at lower temperature i.e., below 110°C had curdy flavour, which might have decreased the score. In addition, higher heat treatment would have caused higher interaction between protein and lactose, which resulted into Maillard's reaction along with caramelization (Duhan et al. 2020). Wadodkar et al. (2002) reported that lactones and lactones are among the major classes of lactones that comprise the typical *ghee* flavour. Considering the results obtained from sensory evaluation, heating temperature of 120 °C was selected for further studies.

Effect of duration of heating on sensory scores of curcumin fortified buffalo ghee

After selecting the heating temperature (120 °C), heating duration was optimized by varying the heating duration to 16, 20 and 24 min during the preparation of curcumin fortified buffalo *ghee*. The samples so obtained were evaluated for sensory attributes and the results are presented in Table 6. It could be observed that average score of different sensory attributes i.e., flavour, texture and overall acceptability for curcumin fortified buffalo *ghee* decreased significantly ($p<0.05$) with an increase in the heating duration. Moreover, the product prepared by heating for 24 min had burnt flavour, which might be the reason for lower scores for this sample. Decrease in the sensory scores with increasing heating duration could be attributed to higher extent of changes taking place during heating *viz*., Maillard's reaction, caramelization, etc. which besides of making the product darker in color, also resulted into decreasing the flavor attribute of *ghee* (Ahmad et al. 2018).

Conclusions

Curcumin fortified buffalo *ghee* preparation methodology was optimized in this study. *Ghee* preparation from creamery butter method was found to be more preferable based upon sensory evaluation and fat recovery. *Ghee* prepared from fermented cream (using NCDC 352, @ 2% inoculation) was found to yield sensorially superior *ghee* at least fermentation time. Curcumin addition beyond 250 ppm resulted into significant ($p<0.05$) decrease in the sensory attributes of *ghee,* but the samples containing upto 350 ppm of curcumin were acceptable to the sensory panelists. Increasing heating temperature improved the sensory attributes while increasing the heating duration decreased the same.

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RESEARCH ARTICLE

Designed colostrum cake: Innovative process to prepare-traditional indigenous milk product

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Abstract: Colostrum is a traditional food having its health benefits to the human. The limited availability of natural colostrum, designed colostrum cake was prepared by blending egg-white, whey protein powder, Alphonso mango pulp, and other ingredients. In phase-I, six different mixes were prepared to optimize the level of egg-white and whey protein powder to be used for the preparation of the designed colostrum cake based on sensory evaluation. The treatment T_3 having the combination of cow milk, egg-white, whey protein powder, and skim milk powder 50%, 15%, 10%, and 25% respectively were found to have the highest sensory score. In phase-II, Mango pulp was used @ 05, 10, 15, and 20 per cent of the colostral mix. It was found that among all the treatments addition of 15% alphonso mango pulp (T_3) used with the most acceptable treatment T_3 found in phase-I was recorded highest overall acceptability score of 8.07. This is followed by treatment T_2 (7.84) while the lowest score was recorded of treatment T_4 (7.50). The addition of Mango pulp in the designed colostrum cake improved sensory quality and overall acceptability. The most acceptable designed colostrum of 15% mango pulp recorded on an average total solid, fat, protein, ash, and acidity as 40.62, 2.55, 14.22, 2.31, and 0.38 per cent respectively. The cost of production of designed colostrum cake at T_1, T_2, T_3, and T_4 was ₹42.80, 43.10, 42.66, and 43.66 per 100 gm. The designed colostrum cake was successfully prepared with a high protein and low-fat content which further provides the scope to the food industries.

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Introduction

In a human being, the intake of nutritional food is important for a good lifestyle and it depends on the intake of nutritional food to fulfill the requirements of the body. Much traditional food of India is considered as a "complete food" because it helps to promote growth and acts as an immune booster (Rajamanickam et al. 2016; Muehlhoff et al. 2013). One of such natural traditional functional foods is milk and its associated products because it is rich in antioxidative peptides, Vitamins, organic acids, calcium, probiotic bacteria with other biologically active peptides that are liberated from milk proteins by different hydrolytic methods (Bhat and Bhat 2011; Visioli et al. 2014; Guha et al. 2021). Thus, the importance of milk and processed dairy products for human health due to their nutritional value cannot be ignored. Nowadays humans are more conscious about their health, the consumption of dairy products is increasing at an annual growth rate of about 20% (Rasone et al. 2015). Moreover, in India, about half of milk is used as a fluid, whereas more than 50% is converted into dairy products such as khowa, milk cake, kalakand, yogurt, butter, ghee, milk powder, ice cream, paneer, and other value-added products (Visioli et al. 2014). The Indian traditional products have huge export per year due to the strong existence of the Indian Diaspora worldwide.

Throughout the country, for many years various methods are used to prepare Indian traditional food. "Shodhana therapy" is one of the traditional food preparation native methods which eliminate the toxic substance present in the food (Sarkar et al. 2015). Among all the dairy products colostrum cake is also a nutritious milk product. It is a yellowish viscous liquid secreted at a time of parturition, also called beesting, bisnings, or first milk (Buttar et al. 2017; Kandasamy 2016; Godhia and Patel 2013). It is differing in milk which is secreted later, by rich in immunoglobulins that confer passive immunity to the newborn (Zwierzchowski et al. 2016), macro-and micronutrients (El-Fattah et al. 2012), lactalbumin, and lactoprotein (Godhia et al. 2013), cytokines hence colostrum also known as *Elixir of life*. Historically, in Ayurveda bovine colostrum has been used for both medicinal as well as

spiritual purposes since cows were first domesticated in India. During the 18th century, western medicine took an interest in the study of the potential health benefits of colostrum on human health. As a result, it was prescribed for immunity building. Moreover, in the 20th century until the discovery of the antibiotic drug penicillin and other artificial antibiotics, colostrum was commonly used against bacterial infections. The colostrum cake is an indigenous dairy product prepared from colostral milk. It is prepared for many years by using a traditional method or recipe i.e., by steam cooking of the mixture of colostral milk admixed with normal milk, sugar, and condiments. Thus, a smooth texture cake is formed which is then cut into pieces of desirable sizes and served as a delicacy, either in a hot or cold state.

Materials and Methods

Material

The fresh cow milk and eggs were collected from the Instructional Dairy Farm of College of Agriculture, Dapoli under a hygienic condition and transported to the laboratory. Other ingredients like sugar, alphonso mango pulp, whey protein powder (WPP), skim milk powder (SMP) and stabilizer (Gelatin) were purchased from the local market at Dapoli. The experiment was carried out in two phases.

Estimation of optimum level of egg-white and whey protein powder

In the first phase, the optimum level of egg-white and whey protein powder for preparation of good quality designed colostrum cake was determined using six treatment combinations (Table 1). The mean values of three replication of each treatment were used for analysis. Three levels of egg-white (10, 15, and 20%) and two levels of whey protein powder (WPP) (10 and 15%) were used simultaneously in combination. For the preparation of the designed colostrum, mixed cane sugar was used @ 20 per cent and stabilizer (gelatin) @ 0.5 per cent for all treatments. The most optimum combination was determined based on sensory evaluation of product carried out by 9 points hedonic scale by a panel of not less than eight semi-trained judges. The designed colostrum cake was prepared as per the procedure given by (Dumbre, 1991) with slight modification. During the preparation, the milk was first pre-heated at 35-40 ºC followed by filtration. SMP was added at the rate of 25 per cent and filtrate by gentle mixing to which egg-white and WPP ingredients were added as per treatment in phase I (Table 1). A smooth uniform mixture was obtained, and then 20 per cent sugar and 0.5 per cent stabilizer were added sequentially into the mixture. The designed colostrum mix was then cooked for 25 to 30 min and used for sensory evaluation. The mean value of each treatment was taken for further analysis.

Estimation of optimum level of mango pulp

In phase- II to increase the nutritional value and flavor, enrichment of the designed colostrum cake was carried out by blending with alphonso mango pulp. The trial was conducted with four treatments and six replications. The most acceptable combination of egg-white and whey protein powder (WPP) found in phase-I based on the sensory evaluation was used as a base material for colostrum cake mix. Cane sugar and stabilizer were added in a fixed amount given in phase-I. All ingredients were mixed properly to form a uniform smooth colostrum cake mix. The mixture is then cooked on low flame for 25 to 30 min; the designed colostrum cake was obtained. The alphonso mango pulp was used to enrich the quality of colostrum cake, in proportion as given on a w/w basis (Table 1). The mean values of each replication of treatment were taken for further analysis.

Estimation of chemical properties

The ingredients used for the preparation of the designed colostrum cake such as cow milk, egg-white, and mango pulp and design colostrum cake were analyzed. The total solids content was determined by the gravimetric method as per IS: 1479 (Part II). The fat content was determined by using the standard Gerber method as described in IS: 1224 (Part I). The protein content was determined by estimating the per cent nitrogen by the micro kjeldhal method as recommended in IS:1479 (Part II). The per cent nitrogen was multiplied by 6.38 to find out the protein percentage in milk. Per cent ash content was determined by the method described in A.O.A.C. (1975) The acidity of milk expressed as per cent lactic acid was determined by the method described in IS:1479 (Part II). To minimize experimental error, at a time five samples were analyzed from every treatment during each replication.

Sensory evaluation

The samples of the designed colostrum cake of every palatability trial were subjected to sensory evaluation by a panel of around 8-10 semi-trained judges. Scoring was done using the nine-point hedonic scale method as described in IS:6273 (Part-II) for sensory evaluation. The designed colostrum cake was evaluated by the judges for sensory attributes like color, general appearance, body texture, and flavor.

Economics of product

The cost of artificial colostrum cake production under different treatments was worked out by using prevailing market rates of ingredients only.

Statistical Analysis

Randomized Block Design (RBD) was employed for statistical analysis. The data were tabulated and analyzed for significance (Snedecor and Cochran, 1967). Further, Standard Error (SE) and CD were calculated.

Results and Discussion

Estimation of optimum level of egg-white and whey protein powder

The benefit of preparation of designed colostrum cake over the natural colostrum cake which is prepared using natural colostrum cake is more. An important factor is the all-time availability of natural cow colostrum. Also, the nutritional composition of fresh colostrum varies with the feed, length of the dry period, days to parturition, age, etc. Hence this study was focused on the preparation of designed colostrum cake enriched with protein and mango pulp. However, no comparable data was available because no studies were published yet on the preparation of designed colostrum cake. Phase I was aimed at the determination of optimum level of egg-white and whey protein powder for the preparation of good quality designed colostrum cake. The data recorded for the variation in color and appearance of the designed colostrum cake was found to be significant. The perusal of Fig. 2a indicates that the designed colostrum cake mix prepared by using 50 per cent normal milk, 15 per cent egg-white, 10 per cent whey protein powder, and 25 per cent skim milk powder (T_3) scored the highest points followed by T_6. In the case of treatment T_3, a typical combination of ingredients might have resulted in providing desirable yellowish color to the product. It was observed that a 15 per cent level of whey protein powder gives denser color which is not appreciated by judges. Similarly, an increase in the level of egg-white at 20 per cent and reducing the level of whey protein powder at 10 per cent adversely affected the colour score. In the case of flavor, the variation was observed in designed colostrum cake was found to be significant. It is found that treatment T_3 secured the highest and treatment T_4 lowest score for flavor. However, addition of egg-white and whey protein powder at an identical proportion of 15 per cent was found least scored (Table 1). The data regarding the body and texture attribute of designed colostrum cake indicate variation in body and texture of designed colostrum cake was found to be significant. T_3 has scored a convincingly higher point over the rest of the treatments. It probably may be due to the fact that typical composition of T_3 treatments. Moreover, when came to the overall acceptability of the product which depended upon all the sensory attributes of products as treatment T_3 secured the highest score for all these sensory parameters. A higher level of egg-white was found very compact texture whereas in lower levels gives very soft and brittle texture to the cake. Thus, based on findings of phase I, it can be conclusively stated that treatment T_3 i.e., designed colostrum cake prepared by using cow milk, egg-white, whey protein powder, and skim milk powder in a proportion of 50%, 15%, 10%, and 25% obtained highest score in sensory evaluation and hence it was selected for further studies in phase II (Table 1).

Estimation of optimum level of mango pulp

Tactile properties are an integral and important component of overall sensory quality and consumer's acceptability of the product. For overall acceptability, the mean score obtained for color and general appearance, flavor, and body and texture were considered. Based on the results, we can affirmatively state that amongst the different levels of mango pulp, T_3 (15% mango pulp) treatment was most acceptable by the judges. The treatment differences are significant at a 1per cent level of significance. It was recorded the maximum score (8.07) followed by designed colostrum cake with 10 per cent mango pulp (7.83). The lowest score was obtained by the product with 20 per cent mango pulp (7.57). From the results, it was observed that the designed colostrum cake with 15 per cent mango pulp showed slight yellowish mango color with a clear and clean general appearance which was liked very much by the judges. Acceptance of any milk product by customers largely depends upon the colour and appearance of the product. The flavor is an important criterion for the acceptance of any food article. The data about the sensory score for flavor in respect of designed colostrum cake, the highest score was obtained by designed colostrum cake with 15 per cent mango pulp (8.10) followed by 20 per cent mango pulp (7.51). Each dairy product possesses its characteristic properties of mouthfeel, which are commonly defined in terms of body and texture. From the average figures for body and texture, it is observed that the higher score was obtained by the designed colostrum cake with 15 per cent mango pulp (8.04) followed by the artificial colostrum cake with 10 per cent mango pulp (7.76). The reasons for the low score in the remaining treatments may be due to the deep burnt aroma of pulp at a higher level whereas

Table 1 Treatment combinations used for determination of optimum level of egg-white, whey protein powder, and mango pulp for preparation of colostrum cake in phase-I and II.

	Phase-I					
Ingredients (%)	T_1	T_2	T_3	T_4	T_5	T_6
Cow milk	55	50	50	45	45	40
Egg-white	10	10	15	15	20	20
WPP	10	15	10	15	10	15
SMP	25	25	25	25	25	25
Total	100	100	100	100	100	100
			Phase-II			
Mango pulp	5	10	15	20	-	-

*Percent values represent the concentration used in phase-I

mild dull flavor at the lower level was not liked by judges. At a higher level of mango pulp, the score for the product was decreased simultaneously which may be due to sticky texture with a very soft body that ultimately affected the softness of the product.

Estimation of chemical properties

Many factors can influence the nutritional composition of cow milk, which included the lactation stage, animal genotype, influence of environmental factors, feed intake, season (Gustavsson et al. 2014; Welter et al. 2016). The analysis of chemical properties of cow milk used for designed colostrum cake preparation revealed that it was of good quality. It is clear from the figures of total solids (13.09%), fat (4.32%), protein (3.57%), ash (0.77%), carbohydrate (4.9%) and acidity (0.13%) (Table 2). It lay within the limits of legal standards for cow milk in Maharashtra state as prescribed by PFA rules (De, 2011).

Further, the analytical study of egg-white found that the average protein content was 10.50 per cent, fat 0.0 per cent, carbohydrates 0.70 per cent, total solids 26.2 per cent, and ash 0.75 per cent. Anonymous (2012) reported 88 per cent moisture, 10.60 per cent protein, 0.03 per cent fat, 0.90 per cent carbohydrates and 0.60 per cent minerals. Another study by Tolik et al. (2014) analyzed moisture 87.9 per cent, protein 10.6 per cent, fat 0.0 per cent, and minerals 0.6 per cent. Similarly, the chemical composition of mango pulp was also analyzed, total solids 29.82 per cent, fat 0.89 per cent, protein 0.92 per cent, ash 0.31 per cent, carbohydrate 15.1 per cent, and acidity 0.43 per cent. Similar results were also reported by Maldonado-Celis et al. (2019).

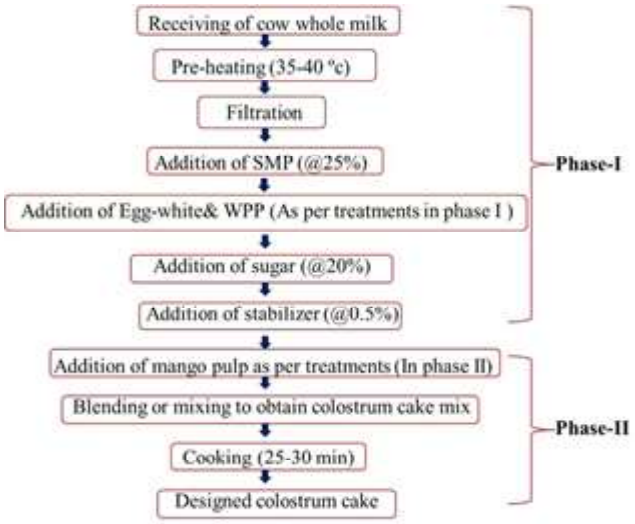

Fig.1 Flowchart for the preparation of designed colostrum cake

The proximate analysis of the designed colostrum cake prepared by using different levels of mango pulp was carried out for total solids, fat, protein, ash, and acidity. The total solids content of the designed colostrum cake shows a significant decrease with the increase in the level of mango pulp with values of 41.60, 41.05, 40.62, and 40.36 per cent at 5, 10, 15, and 20 per cent level of mango pulp, respectively. This was due to a very low total solids content of mango pulp as given in Table 3 (29.82 per cent). In the present investigation, the composition of designed colostrum cake was blended in such a way that it will give about 42 per cent total solids in the finished product. Literature on this type of research by using mango pulp or any other fruit pulp for quality

Table 2 Chemical composition of cow milk, egg-white, and mango pulp

Parameter (%)	T1	T2	T3	T4	SE±	CD
Total solids	41.60	41.05	40.62	40.36	0.133	0.399
Fat	2.71	2.63	2.55	2.48	0.0325	0.097
Protein	15.49	14.82	14.22	13.66	0.156	0.469
Ash	2.52	2.41	2.31	2.23	0.0318	0.0957
Acidity	0.34	0.35	0.38	0.40	0.011	0.033

Mean value treatment of four replications (*Significant at 1% level of significance)

Table 3 Physcio-chemical analysis of design colostrum cake in phase-II

Constituents (%)	Cow milk	Egg-white	Mango pulp
Total solids	13.09	26.2	29.82
Fat	4.32	Nil	0.89
Protein	3.57	10.5	0.92
Ash	0.77	0.75	0.31
Acidity	0.131	Nil	0.43
Carbohydrate	4.9	0.7	15.1

*Mean value treatment of four replications (*Significant at 1% level of significance)

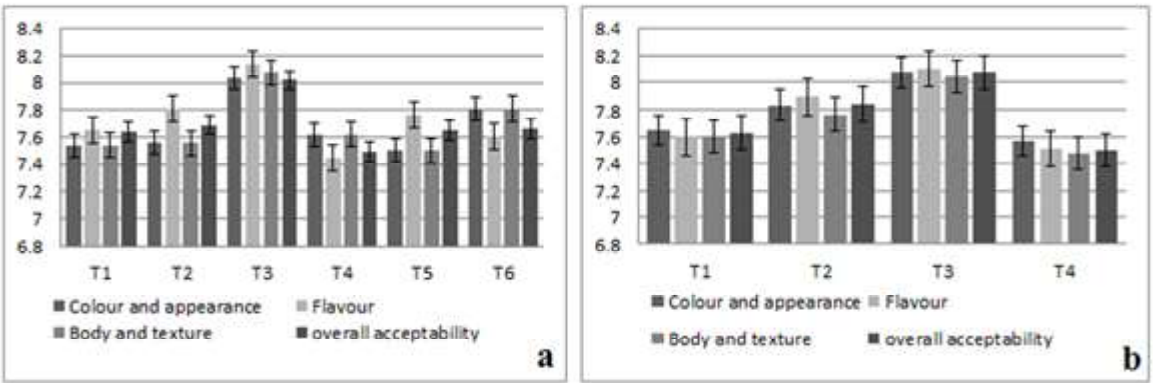

Fig. 2 Sensory evaluation of designed colostrum cake in Phase I (2a) and phase II (2b)

enhancement of designed colostrum cake is not undertaken. So no comparative data is available. The perusal of data revealed that the variation in the fat content of designed colostrum cake due to different levels of mango pulp was found to be significant. The fat content decreased significantly with the increase in the level of mango pulp. The average fat percentage of designed colostrum cake at 5, 10, 15, and 20 per cent level of mango pulp was 2.71, 2.63, 2.55, and 2.48 per cent, respectively. Since the natural colostrum has more caloric density, 2.18 g (Dande and Nande, 2013; Wu, 2013), reducing the level of fat content is currently a primary trend in food industries (Ma and Boye, 2013). However, the successful development of such low-fat content food products remains a challenge as fat is responsible for providing smooth texture and rich flavor to the product. It is very obvious because in the present investigation egg-white as well as whey protein powder was used for blending the designed colostrum cake mix. Both these constituents contain negligible fat and give a good texture to the cake.

The protein content of the designed colostrum cake was highly significant due to the addition of pulp. It decreased with the increase in the level of pulp which may be due to its very low protein content (0.92 %). The values are 15.49, 14.82, 14.22, and 13.66 per cent protein content at 5, 10, 15, and 20 per cent addition of mango pulp, respectively. The main attention was given to maintaining the protein percentage of the mix rather than the fat percentage. In the present investigation, it can be seen that at 15 per cent addition of mango pulp the protein content was 14.22 per cent. In cow, buffalo, and goat colostrum found 14.9 per cent, 4.3 per cent, and 3.4 per cent protein content, respectively (Ahmad et al. 2013). These results are more or less comparable with the results obtained in this study. Whereas, Dande and Nande (2020) reported a very low level of protein (7.98%) in bovine raw colostrum. The previous comparative study on humans, cow, buffalo, and goat colostrum revealed that cow colostrum contains more nutritional constituents than other species (Ahmad et al. 2013).

The perusal of data revealed that an increase in the level of mango pulp resulted in a significant decrease in the ash content of designed colostrum cake. This may be due to the very low ash content of mango pulp (0.31 %). The average ash content of designed colostrum cake at 5, 10, 15, and 20 per cent added pulp was 2.52, 2.41, 2.31, and 2.23 per cent, respectively. It was observed that the addition of mango pulp at the rate of 5,10, 15, and 20 per cent, resulted in a slight increase in acidity of designed colostrum cake i.e. 0.34, 0.35, 0.38, and 0.40, respectively. This increase in acidity was statistically significant. The increase in acidity may be due to the higher (0.43%) acidity of mango pulp. The Indian standard provides a guideline for sensory evaluation. A hedonic rating test is used to measure the degree of pleasurable or un-pleasurable experiences with foods.

Cost of production

While calculating the cost of production of the designed colostrum cake the cost of ingredients used was taken into account considering then prevailing market rates. It can be said that the cost of designed colostrum cake prepared by using different levels of mango pulp and added ingredients was different from treatment to treatment. The actual quantity of colostrum cake obtained was considered because the recovery of the designed colostrum cake was different. While calculating the cost of designed colostrum cake production of phase-I, the cost of material, based on added ingredients only was considered. The lowest phase-I cost of production was observed in the case of treatment T_1 (36.03) and highest of T_6 (54.05). However, the lowest phase-II cost of production was observed in the case of treatment T_3 (42.66) and the highest of T_4 (43.66).

Conclusion

From the results of the present investigation, it can be concluded that mango pulp could be successfully utilized for the manufacture of designed colostrum cake. The most acceptable quality designed colostrum cake can be prepared by using cow milk 50 per cent, egg-white 15 per cent, whey protein powder 10 per cent,

skim milk powder 25 per cent and addition of sugar 20 per cent of the whole mix and by using 15 per cent mango pulp. Further, the designed colostrum cake contains a more protein and less fat hence it was most acceptable by the consumers as a high protein food. Mango had a positive effect on the sensory attributes of designed colostrum cake on its acceptability and consumption. Besides peculiar flavor, it also adds nutritional importance to the product. The production cost of the most acceptable quality designed colostrum cake in phase I (T_3-15 % egg-white and 10 % whey protein powder) was 41.39 per 100 gm and the cost of production of most acceptable quality designed colostrum cake in phase II (T_3-15 % Mango pulp) was 42.66 per 100 gm.

Acknowledgments

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RESEARCH ARTICLE

Development of probiotic tomato *kulfi*

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Abstract: *Kulfi* is a traditional Indian frozen dessert with nutritional significance but, lacks therapeutic properties. *Kulfi* can be used as a vehicle for probiotics and prebiotics, with the added advantage of being appreciated by the people of all age groups. This study involves the production of *kulfi* by incorporating probiotic culture of *Lactobacillus acidophilus* La-5 (10^7 – 10^8 CFU/g) and tomato juice (prebiotic) (25% and 37.5%) to enhance its functional value and create a synbiotic system. The effect of tomato juice concentration was evaluated for physiochemical properties, culture's survival, and sensory characteristics. Data revealed a reduction in pH of *kulfi* variants with high tomato juice concentration. It was also found that, the probiotic tomato *kulfi* variants (25% and 37.5% variants) possessed high acidity (0.225% and 0.216%) and sucrose content (13.98% and 13.50%), respectively. The protein content was found to remain constant in all the *kulfi* variants, indicating no association between probiotic culture, tomato and *kulfi*. A decrease in fat content was observed with an increasing concentration of tomato juice. A reduction in viable counts was noted in 25% and 37.5% *kulfi* variants during one week of frozen storage. All *kulfi* variants received a fair score in the organoleptic evaluation, however, probiotic *kulfi* containing 25% tomato juice attained the highest score for all the parameters. Hence, proving that the incorporation of probiotic culture and tomato not only enhanced the nutritional properties of the *kulfi,* but also improved the sensory properties of the product.

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Introduction

Kulfi, *or Malai-ka-baraf*, a traditional Indian frozen dairy-based dessert which is an Indian analogue of ice cream, is the most savoured frozen dessert attributing to its palatability as well as low cost (Aneja et al. 2002). The chief difference between ice cream and *kulfi* is that the former is whipped with air or overrun, while the latter is not and hence, comprises no air (Kumar et al. 2017). Traditionally, *kulfi* is prepared using sweetened milk (containing 20-25% added sugar and concentrated to about half of its volume) and *malai*/cream, crushed nuts (almonds and pistachios) as well as flavouring ingredients (vanilla and/or rose essence. Subsequently, the prepared mix is poured and frozen in small conical shaped containers till consumption (Siva et al. 2019). At industrial level, a *kulfi* mix is composed of milk fat (10-16%), milk solids-not-fat (9-12%), sucrose (9-12%), corn syrup solids (4-6%), stabilizers/ emulsifiers (0-0.5%), total solids 36-45%, and water 55-64% (Kumar et al. 2017).

In the recent past, sociodemographic transition across the globe has resulted in major modifications in the dietary behaviours and health index of populations of both developed and developing countries. Reportedly, over the past few decades, global consumption of diets high in fat, especially saturated fat, low in unrefined carbohydrates, free sugars or salt/sodium has increased, in comparison to consumption of fruit, vegetables and dietary fibre (Agarwal et al. 2016; World Health Organization, 2015). This trend has resulted in the rising prevalence of various metabolic disorders, cancers, and other ailments. Considering these challeneges, the scientists are now aimed at developing functional foods that possess nutritive and therapeutic values (Putnik et al. 2018; Granato et al. 2020). Various studies conducted in past few years have emphasised on the significance of fruits, vegetables, whole grains, pre-and probiotics in mitigating several ailments. This has subsequently contributed to the burgeoning of the functional food market. A functional food may be defined as a food to which a component has been supplemented (macronutrient, micronutrient, phytochemicals or probiotics) or

eliminated by employing certain techniques or a food which has its bioavailability modified (Rahimian et al. 2019).

The formulation of probiotic-based food products is a significant field of research for the emerging functional food market. Probiotics (such as *Lactobacillus* and *Bifidobacterium* genera) may be defined as living microorganisms that when ingested in appropriate quantity, can survive in an acidic environment and in the presence of bile salts in the human body, and promote health benefits to the human body (Hill et al. 2014; Champagne et al. 2018). The potential health benefits probiotic consumption are not limited to immunomodulatory, anti-tumoral and hepato-protective action, and maintaining equilibrium of intestinal microflora (Maldonado et al. 2017).

In the past few years, various fruits and vegetables have been considered as a potential functional ingredient for the development of functional foods. Tomato is one such notable vegetable which belongs to the Solanaceae family and is cultivated widely across the world. It has gained immense popularity in various nations attributing to its year-round availability, pleasant taste, and potential nutraceutical effects. With respect to nutrient content, tomatoes are composed of 95% moisture, 3% carbohydrates, 1.2% protein, 1% total fat, minerals including sodium, calcium, potassium, magnesium, manganese, phosphorus, and zinc, and vitamins including vitamin A, B (thiamin, riboflavin, niacin, pantothenic acid and pyridoxine) and C (Melfi et al. 2018). Additionally, it is an excellent source of carotenoids like lycopene, β-carotene; phenolic compounds like phenolic acids and flavonoids; and glycoalkaloids like tomatine (Salehi et al. 2019). Lycopene, a compound which imparts the red colour to tomatoes aids in reducing risk of a range of chronic ailments including cardiovascular diseases, cancers, and skin erythema induced by ultraviolet light; (Larbat et al. 2014). Recent studies have recognized the significance of the whole tomato as a food with low energy-density and a range of health promoting properties and have attempted to investigate its applications as a functional ingredient for the development of a variety of functional foods (Theresa Ademosun et al. 2019). Tomato juice and papaya pulp have been reported to facilitate *Lactobacillus acidophilus* growth and thus, effectuating higher viable counts, shorter generation times and enhanced utilisation of sugar. This facilitation may be ascribed to ampler simple sugar (chiefly glucose and fructose) and minerals (magnesium and manganese) availability, which are recognised as growth promoters for *L. acidophilus* (Kailasapathy and Chin, 2000).

Taking into consideration the current consumer demand for foods with functional and nutraceutical properties, various studies have been conducted to reformulate *kulfi* by enriching it with a variety of functional ingredients including guava pulp and palm sugar (Sobana et al. 2021), amaranthus (Patel et al. 2020), flaxseed powder (Siva et al. 2019), and wood apple (Kumar et al. 2017). Till date probiotics have been integrated in a range of food matrices, of which the dairy products have been considered as a befitting vehicle to deliver probiotic. Since past few decades, the application of probiotics in cheeses, fermented milks, and now in ice-creams has been widely explored in the literature. However, *kulfi* is a relatively innovative matrix for the application of probiotics. Thus, based on the findings from the literature, this study has been undertaken to create a symbiotic system and to enhance the functional value of traditional *kulfi* by incorporating of probiotic cultures *Lactobacillus acidophilus* La-5 at the rate of 10^7 & 10^8 cfu/g and tomato at 25% and 37.5% concentration.

Materials and Methods

Materials

To develop probiotic tomato *kulfi*, full cream milk (Mother Dairy brand) was procured from Mother Dairy shop, Mandi House, New Delhi, and honey (Patanjali brand) was procured from Patanjali store, Vasundhara, Ghaziabad, U.P. Other ingredients like tomatoes, cardamoms, cardamom powder were purchased from local market. Freeze dried culture of *Lactobacillus acidophilus* strain LA-5 was procured from National Collection of Dairy Cultures- ICAR National Dairy Research Institute, Karnal, Haryana. Three samples of *kulfi* were prepared with ingredients enlisted in Table 1.

The steps involved in the preparation of *kulfi* variants have been represented in Figure 1. Fresh tomatoes were sorted, blanched, peeled and blended in a food mixer. The blended tomatoes pulp was strained, until a clear filtered juice was obtained. The process of preparing *kulfi* involved concentrating milk to about two folds; adding honey and cardamom powder to the concentrated milk; cooling the prepared mixture at room temperature; and finally, adding the prepared tomato juice to the mixture under sterilized conditions with constant stirring for 10 minutes. Probiotic strain was then added to the prepared mixture in the laminar flow chamber, following which the mixture was incubated for 1-2 hours at chilling temperature (5°C). Subsequently, the prepared *kulfi* mixture was poured into 80 ml conical mould which were frozen at -18 to -20°C for 10-12 hours. Figure 2 displays the developed *kulfi* variants.

Proximate analysis

The moisture, protein, and ash content were estimated by the procedure provided by (AOAC, 2000). pH and titratable acidity of all the *kulfi* samples were estimated by the procedure as given by (AOAC, 2000). The fat and sucrose content in the samples was determined by the Gerber fat test (AOAC, 2000) and Lane and Eynon method (James, 2013), respectively.

Vitamin C analysis

The standard curve was estimated by pipetting standard ascorbic acid (1, 2, 2.5, 3, 4 and 5 ml) to dry cuvettes to which 2% HPO_3

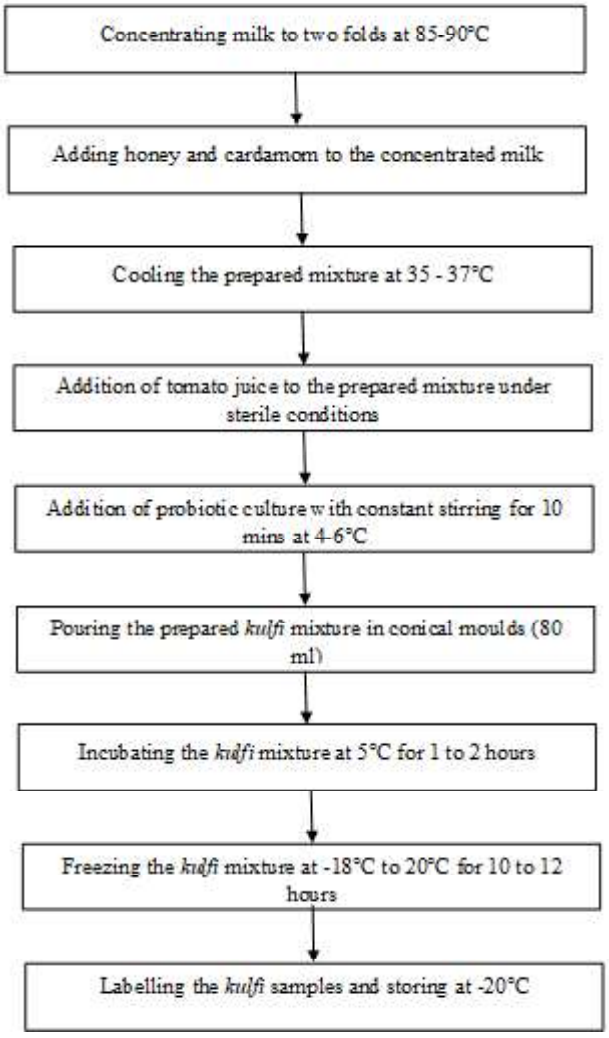


Fig 1. Process flow diagram for processing of probiotic tomato *kulfi*

was added to obtain the final volume 5ml. Following which, 10 ml dye was pipetted into the cuvettes. The readings were noted within 20 seconds at 100% transmission using blank 5 ml of 2% HPO3 solution and 10 ml of water. To estimate vitamin C content of samples 10g sample was blended with 3% HPO_3 to make 100 ml final volume was obtained. Subsequently, the blended mixture was filtered and centrifuged. 5 ml extract was taken in a dry cuvette to which 10 ml of 2, 6- dichlorophenol indophenol was added. The red colour was measured at 518 nm and absorbance was plotted against concentration (AOAC, 2000). The formula used to calculate the vitamin C content was:

$$\text{mg of vitamin C per 100g} = \frac{\text{Vitamin C content} \times \text{volume made up to} \times 100}{\text{g of solid taken for estimation} \times 1000 \times \text{weight of sample taken}}$$

Lycopene content analysis

The lycopene content of *kulfi* samples was evaluated by taking 5-10 g of sample and grinding it with acetone in a pestle and mortar repeatedly to obtain acetone extract. The acetone extract was transferred to a separating funnel containing 10 - 15 ml of petroleum ether. The obtained carotenoid pigment was transferred into petroleum ether by diluting the acetone with water. The lower phase thus obtained was transferred to another separating funnel, while the petroleum extract containing carotenoid pigment was transferred to an amber-coloured bottle. The acetone phase was discarded and to the petroleum ether extract, a small quantity of anhydrous Na_2SO_4 was added and transferred to 50 ml volumetric flask and the final volume was obtained using petroleum ether. Subsequently, it was measured at 503 nm in spectrophotometer using petroleum ether as blank (Ranveer et al. 2013).

Microbiological analysis

***Lactobacilli* Count**

Lactobacilli count was carried out as per the procedure described by the Indian standards (IS): 16068 (2013). Serial dilutions of the samples were prepared and 1 ml from selected dilutions was poured in duplicates plates and mixed with tempered de Man, Rogosa and Sharpe medium (5-7 ml) was poured. The plates were then incubated at 37 ± 2 ^{0}C for 72 hours. After incubation, plates were removed for counting the colonies. The *lactobacilli* count was expressed as CFU/g.

Total viable count (TPC)

Total viable count was carried out as per the method described by AOAC (1990) using pour plate method with plate count agar. From suitable dilutions, 1 ml was transferred into the plate following which the medium poured. The plates were incubated

Table 1 Percent ingredients for *kulfi* (weight %)

Kulfi variant	Code	Ingredients				
		Milk	Tomato juice	Honey	Cardamom	*Lactobacillus acidophilus*
Control (Plain *kulfi*)	C1	80		15	5	-
Control (Tomato *kulfi*)	C2	60	20	15	5	-
Probiotic tomato *kulfi* (25%)	P1	60	20	15	3.75	1.25
Probiotic tomato *kulfi* (37.5%)	P2	50	30	15	3.75	1.25

at 37ºC for 2 days. Colonies were expressed as CFU/g for solid samples.

***E. coli* count**

1 ml sample from 10^3 dilutions was added to sterilized petri dish by using sterilized pipettes. 10 ml eosin methylene blue agar medium was added in petri dish, mixed, and spread uniformly by rotating clockwise and anti-clockwise and allowed to set at room temperature for 10 to 15 minutes. Petri dishes were incubated at 44! for 22-24 hours. The colonies were counted by using colony counter and calculated (CFU/g) for milk (AOAC, 1995).

Yeast and mould count

1 ml sample from 10^3 dilutions was added to sterilized petri dish by using sterilized pipettes. 10 ml plate count agar medium was added to petri dish at a temperature of 45!. The medium and inoculums were mixed, spread uniformly by rotating clockwise and anticlockwise and allowed setting at room temperature for 10-15 minutes. The petri dishes were inoculated in duplicate. These dishes were then incubated at 10! for 7 days. After incubation period, colonies were counted by using colony counter and organisms were recorded as per ml of milk and per gram for milk products (AOAC 1997).

Sensory analysis

Sensory evaluation of *kulfi* samples was conducted on the day of preparation. The panellists consisted of eight untrained and two semi-trained members who were graduate students and staff members in the Department of Food Science and Nutrition, University of Delhi. The panellists were selected based on their interest and availability. Training sessions for the panellists were conducted prior to evaluation where the panellists were trained to be familiar with attributes and scaling procedures of *kulfi* samples. Sensory evaluation was conducted to for the sensory characteristics including - appearance, colour, odour, texture, taste, and overall acceptability. Sensory attributes were evaluated using a 9-point hedonic scale with 1 as the lowest score or extremely dislike and 9 as the highest score or extremely like. All samples were randomly coded and presented to the panellists. Water was provided to the panellists to cleanse their palates between samples. Lighting of the room was maintained throughout the analysis, which was conducted in the Food laboratory at University of Delhi.

Results and Discussion

Proximate composition and physicochemical properties

The results obtained for proximate composition and physicochemical properties of four *kulfi* variants are represented in graph 1. The moisture content of probiotic *kulfi* under different treatments was 73.53 % (25% variant) and 75.23 % (37.5% variant). The moisture content of C1 and C2, was in concordance with those of Kaur et al. (2021) and Kumar (2012). The protein content of the *kulfi* samples in the present study was consistent with the IS: 10501-1983, where the minimum protein content specified for plain and fruit *kulfi* is 3.5%. the results obtained for the protein content in present study were similar to those observed by Jayalalitha (2016) and Kumar (2012). The fat content in *kulfi* variants observed in the current investigation were as recommended by (BIS, 1970), where the minimum fat content specified for plain and fruit ice-cream is 10% and 8%, respectively; and was similar to that observed by Jayalalitha (2016) and Kumar (2012). It was found that the fat content reduced with increasing tomato juice concentration (Nalkar et al. 2018). A similar observation was observed in pH content of *kulfi* variants where the values were in concordance with those observed by several researchers (Sobana et al. 2020; Kumar et al. 2017; Kumar, 2012). In addition, the increasing tomato juice concentration resulted in increased titratable acidity. The acidity among *kulfi* variants of the present study was found to be similar to those reported by Siva et al. (2019). The sucrose content was observed to increase with the increase in the concentration of tomato juice. The levels of sucrose in four *kulfi* variants observed in the current study were in concordance with the standards by IS: 10501-1983, where the minimum sucrose content specified for plain and fruit *kulfi* is 13% and the results reported by Kumar (2012).

Vitamin C content

Graph 2 represents the vitamin C content in developed *kulfi* variants. The vitamin C content of probiotic *kulfi* variants under different treatments (25%, 37.5%) was 0.16 mg/100g and 0.28 mg/100g, respectively. The results indicated that there was an increasing trend in the vitamin C content with an increase in the concentration of tomato juice. The result obtained in the present study were similar (230 ± 6 µg/100 g fresh matter of tomato fruit pulp) to those reported by Gahler et al. (2003).

Lycopene content

The observations with respect to lycopene content of *kulfi* variants are graphically presented in Graph 3. The lycopene content of probiotic *kulfi* under different treatments (25% and 37.5%) was 0.0188 mg/g and 0.0326 mg/g, respectively. The results indicated that there was increasing trend in the moisture with an increase in the concentration of tomato juice. Similar results were also reported by Wawrzyniak (2005).

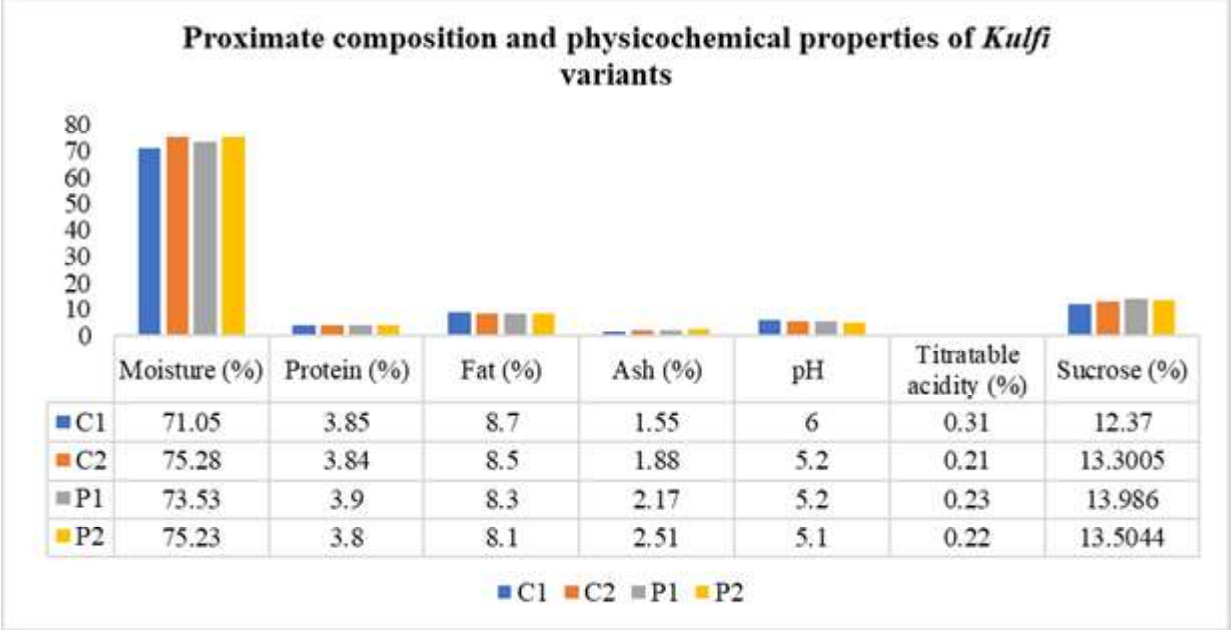
Microbiological analysis

Table 2 Viability of probiotic bacteria in *kulfi* variants

Kulfi variants	Viable count CFU/g	
	Day 0	Day 7
P1	1.5×10^8	3.71×10^7
P2	2.14×10^8	8.5×10^7

a. Control (C1) plain *kulfi* b. Control (C2) tomato *kulfi* c. Probiotic tomato *kulfi* – 25% (P1) d. Probiotic tomato *kulfi* – 37.5% (P2)

Fig 2. The developed *kulfi* variants (C1, C2, P1 and P2)



	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH	Titratable acidity (%)	Sucrose (%)
C1	71.05	3.85	8.7	1.55	6	0.31	12.37
C2	75.28	3.84	8.5	1.88	5.2	0.21	13.3005
P1	73.53	3.9	8.3	2.17	5.2	0.23	13.986
P2	75.23	3.8	8.1	2.51	5.1	0.22	13.5044

Fig 3. Proximate composition and physicochemical properties of *Kulfi* variants

Table 3 Sensory evaluation of *kulfi* variants

Kulfi variants	Colour	Body and texture	Flavour	Overall acceptability
C1	7.60 ±0.52	6.05±0.83	7.60±0.70	7.85±0.33
C2	7.3±0.33	7.7±0.67	6.7±0.99	6.6±0.69
P1	8.05±0.49	8.30±0.67	8.45±0.68	8.55±0.68
P2	7.10±0.99	7.30±1.05	6.40±1.31	6.60±0.82

The results obtained from microbiological analysis (total plate count, yeast and mould count and *E.coli* count) of *kulfi* variants exhibited that the concentration of probiotic culture had a significant effect on the microbial count in *kulfi* variants The results obtained were in concordance with those obtained by Sobana et al. (2021) who substituted the milk fat and sugar added in the conventional *kulfi* with guava fruit pulp fat as well as palm sugar. The coliform count per gram in different types of probiotic *kulfi* observed in the current study were as within the standards given by IS: 10501-1983, where the maximum count specified for plain and fruit *kulfi* is 100 CFU/g.

The results presented in Table 2 indicate the viability of *L. acidophilus* culture at -18 to -20 ! on 0^{th} and 7^{th} day as 1.5 x 10^{8}

Fig 5. Lycopene content (mg/g) of *kulfi* variants

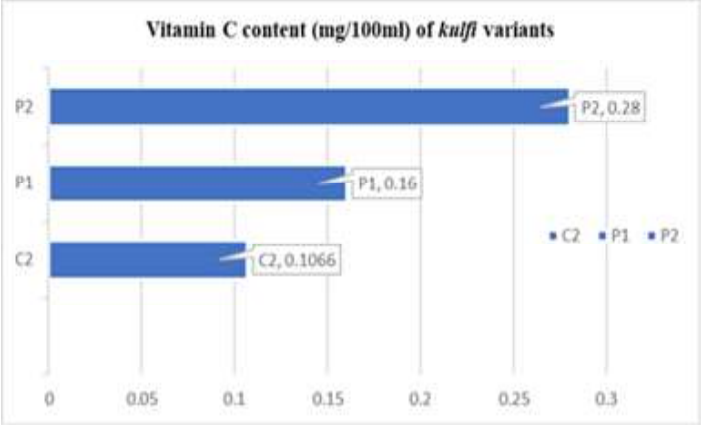


Fig 4. Vitamin C content (mg/100ml) of *kulfi* variants

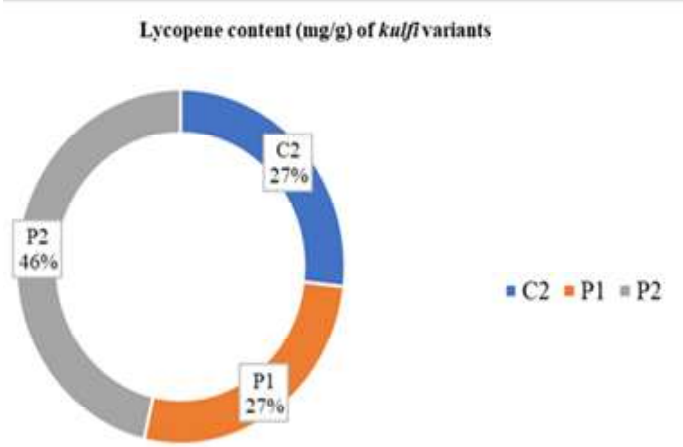
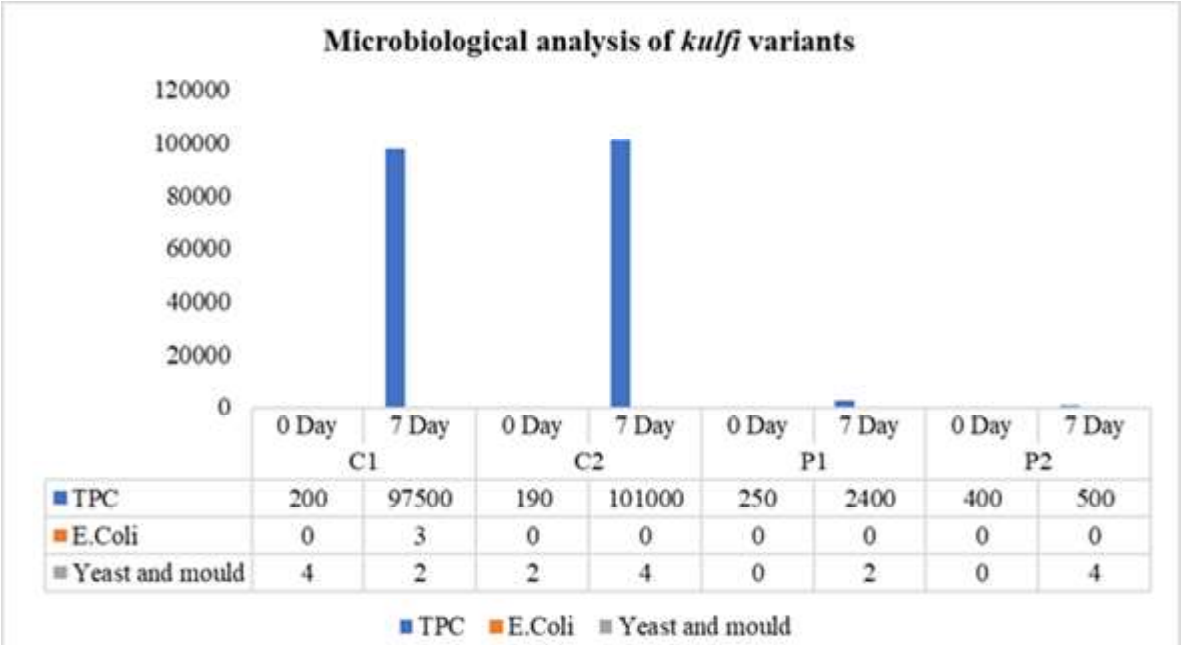


Fig 6. Microbiological analysis (TPC, *E.coli* count and yeast and mould count) of *kulfi* variant



	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day
	C1		C2		P1		P2	
TPC	200	97500	190	101000	250	2400	400	500
E.Coli	0	3	0	0	0	0	0	0
Yeast and mould	4	2	2	4	0	2	0	4

Fig 6. Microbiological analysis (TPC, *E.coli* count and yeast and mould count) of *kulfi* variant

CFU/g and 3.71 x 10^7 CFU/g for 25% variant and 2.14 x 10^8 CFU/g and 8.5 x10^7 CFU/g respectively. It is to be note that, even after a week of storage period, large number of viable cells of probiotic cultures were found. The decrease in viable count is attributed to thermal and osmotic shock that inevitably affects viability of organisms.

Sensory Analysis

Sensory evaluation of the develop *kulfi* samples conducted on 0^{th} day for colour, flavour, body and texture and overall acceptability by sensory panellists. The scores obtained for *kulfi* variants are graphically represented in graph 5, which exhibits that all the *kulfi* samples obtained a fair score in the organoleptic evaluation, however, P1 attained the highest score for all the parameters. Similar results were observed by Salem (2015) and Kumar (2012). It can be observed from table 2 that there was significant difference in the scores obtained for the colour and appearance attribute between the control and treated samples.

Conclusion

The current study was undertaken to explore the possibility of utilizing probiotic culture of *Lactobacillus acidophilus La-5* and tomato juice in the formulation of *kulfi* and hence, a value-added frozen dessert with synbiotic system was developed. The study findings indicated that the incorporation of probiotic culture and tomato juice resulted in no significant difference on

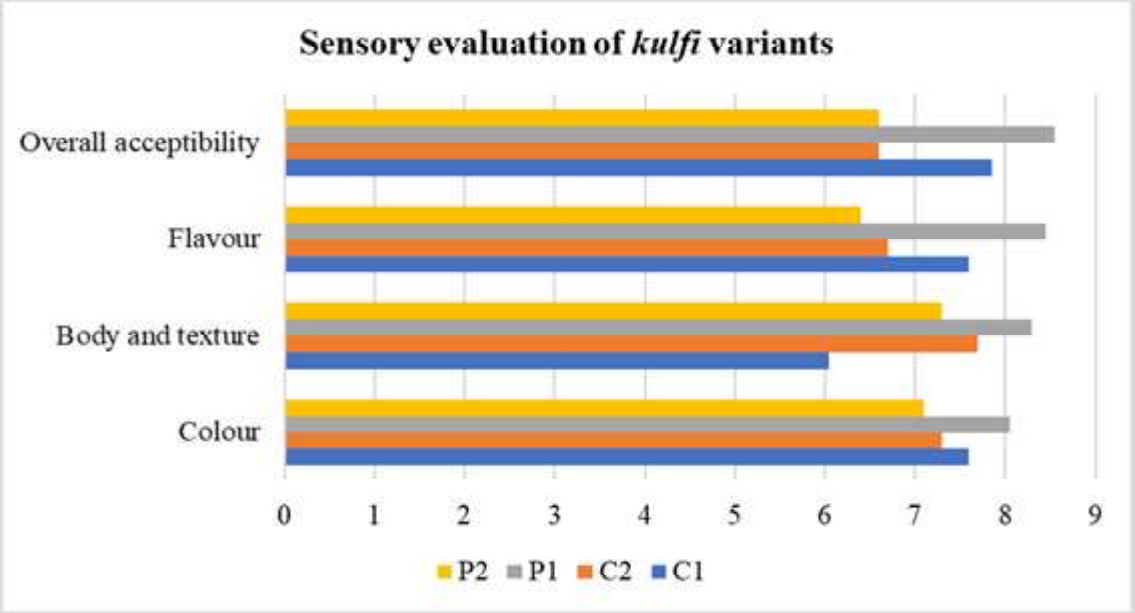


Fig 7. Sensory evaluation of *kulfi* variants

protein content of *kulfi* variants, significantly enhanced their sensory attributes. Incorporation of tomato juice at 25% level and 1.25% *L. acidophilus* in *kulfi* mix was found to be desirable for the production of organoleptically appealing *kulfi*. Based on the findings obtained from this study, it can be proposed that further research can be envisaged to investigate that effect of different levels of inoculation of *L. acidophilus* culture on physicochemical, microbiological, and organoleptic quality of *kulfi;* and its correlation with different concentrations of tomato juice to develop an organoleptically acceptable *kulfi* variant.

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RESEARCH ARTICLE

Storage stability of fluid bed dried solid state fermented (SSF) lactic cultures in milk

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Abstract: The present investigation was intended to study the effect of storage at ambient temperature on the fluid bed dried powder of Solid State Fermented (SSF) lactic cultures such as dahi, yoghurt and probiotic cultures and also made an attempt to check the performance in milk. The SSF cultures were prepared from various dhals and screened for the survival of aerobic spore farming bacteria, the raw black gram dhal showed lowest spore count of 1.52 $\log_{10}$cfu/g. Combination of hot air oven exposure of black gram dhal at 100^{0}C/1 h and sterilization at 121^{o}C/30 min completely destroyed the spores. On fermentation the maximum viable counts of dahi (8.41$\log_{10}$cfu/g), yoghurt (8.98$\log_{10}$cfu/g) and probiotic (9.47$\log_{10}$cfu/g) cultures were obtained on black gram dhal containing 1:0.8 ratio of moisture was supplemented with 1% level of skim milk powder, ash gourd, carrot and tomato juices as they provided growth promoting agents. The fermented SSF cultures were subjected for fluid bed drying for 1.5 h at ambient temperature (25^{o}C). The SSF culture viability upon fluid drying decreased from 8.65 to 8.33, 9.01 to 8.85 and 9.65 to 9.34$\log_{10}$cfu/g in case of dahi, yoghurt and probiotic cultures, with the moisture content of 9.4%. When these dried powders of SSF cultures of dahi, yoghurt and probiotic cultures were inoculated at 1,0.5 and 3% to heated milk, the cultures took 5:00, 3:30 h and 9:30 h to set the heat treated milk with acidity of 0.68, 0.71 and 0.59% lactic acid respectively. The dried SSF lactic cultures possessed viability of 6 $\log_{10}$cfu/g till 24th days of storage at ambient temperature (28±1^{o}C) and these cultures showed absence for the contaminants like coliforms, aerobic spores and yeast and molds. With respect to performance, the curdling time increased with the increased storage days at ambient temperatures due to the reduction in viable lactic counts.

Keywords: Black gram dhal, Heat treatments, Fluid bed drying, Solid state fermented lactic cultures, Supplements,

Introduction

Fermentation of milk preserves its nutrients for long time and fermentation is made possible by inoculating the heat treated milk with lactic acid bacteria. Fermented milk products have their own importance in the human diet by extending potential therapeutic benefits to the consumers. People have the concept of using fermented milk products as they are safe for consumption due to fermentation end products of the lactic cultures. The recent classification of fermented milk products was given by Tamime and Robinson (1998) that includes mesophilic (curd), thermophilic (yoghurt), therapeutic (acidophilus milk) and yeast-lactic (kefir) fermented milk products. Fermentation is the process of transformation of simple raw materials into a range of value added products by utilizing the phenomenon of growth of microorganisms or through their activities on various substrates. It is one of the oldest technology used for the food preservation and science of fermentation termed as Zymology. The term fermentation is derived from Latin the word “Fereve” means to ‘Boil’.

Based on the substrate, fermentation may be submerged fermentation (SmF) in liquid media or Solid State Fermentation (SSF) on solid substrates like nutritive (dhal, rice) or inert substrates like paddy husk etc (Kumar et al. 2003). Solid state fermentation technique has been widely used in preparation of fermented foods, enzymes, organic acids, polysaccharides, biomass of lactic acid bacteria, colours and flavours that involve the controlled growth of microorganisms on solid substrates in the absence of free moisture, so as to obtain large number of viable cells in concentrated form (Bhargav et al. 2008).

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Lactic acid bacteria (LAB) are a group of micro aerophilic, Gram positive, non-spore forming bacteria that ferment lactose to produce primarily lactic acid. LAB include a variety of industrially important genera such as *Lactococcus, Streptococcus, Leuconostoc, Pediococcus* and *Lactobacillus*. (Yantyati et al.2014). LAB are known commonly as starter bacteria. They are naturally present in raw food material such as milk, vegetables and also in the human gastro-intestinal tract (probiotics), playing an important role as starter cultures for fermentation in dairy and food industries. Starters are selected strains of microorganisms deliberately added to milk to attain desirable final product. Apart from fermentation of milk, certain starter bacteria also possess health promoting benefits and are called as probiotics (Krishna et al. 2011).

Probiotics like *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* are live microorganisms thought to be beneficial to the host organism when ingested through food. On the contrary, the term prebiotic generally refers to a food component like carrot juice, chicory root, tomato juice and many others that can be hydrolyzed only by selective microflora termed probiotics in colon of gastro-intestinal tract, activating them to manifest potentially beneficial effects on the host (Hussain et al.2016).

Edible substrates such as dhals are used as solid substrates to grow the cells to maximum level up to 10^{10} cells per gram due to more surface area for growth. In conventional liquid culture, the cell growth is limited maximum up to 10^8 cells per millilitre, due to acidic pool during the growth of cells in the presence of free moisture (Koyani and Rajput 2015). After growth of lactic cultures on solid substrates with proper supplementation, needs suitable preservation methods to reduce moisture without affecting much of the cell viability. Some of the practiced methods are air drying, freeze drying and fluid bed drying. Among the air drying, freeze drying and fluid bed drying, the best method seemed to be the fluid bed drier to dry SSF lactic cultures on supplemented black gram dhal with respect to higher viability and less moisture retainment compared to other two (Vandenberghe et al. 2000).

Hence, in this study attempt has been made to grow lactic cultures on suitable edible substrate with proper supplementation, followed by a fluid bed drying method to keep solid state fermented lactic cultures viable for longer time and storage of dried powder of solid state fermented (SSF) lactic cultures on viability at ambient temperature indicating that SSF culture requires low temperature storage to maintain viable cells to use as ideal inoculums for preparation of fermented milk products.

Materials and Methods

Lactic acid bacterial cultures: The dahi cultures consisting of *Lactococcus lactis* ssp.*lactis* MTCC1742 and *Lactococcus lactis* ssp.*lactis* bv.*diacetylactis* MTCC1745, yoghurt culture that included *Streptococcus thermophilus* MTCC1746 and *Lactobacillus delbrueckii* ssp.*bulgaricus* MTCC1749 and *Lactobacillus acidophilus* MTCC1753 as probiotic culture, which had been maintained in sterile Yeast glucose chalk litmus milk (YGCLM) in the department of Dairy Microbiology, Dairy science college, KVAFSU, Hebbal, Bengaluru-24 were used in this study.

Collection and screening of various dhals for aerobic spore counts

Dhal of 8 various types, commonly available in local market of Bengaluru such as raw Bengal gram dhal, raw and roasted Bengal gram dhal, Black gram dhal, Green gram dhal, Hyacinth dhal (avaraebele), Masoor dhal, Red gram dhal, Soybean dhal were purchased, cleaned to remove stones and unwanted plant materials and stored in a self-sealing polythene pouches. To determine the extent of spores present in dhal, they were subjected to aerobic spore counts by plating method as per Harrigan (1998).

Various sporicidal treatment given to black gram dhal to use as solid substrate

Various treatments like dry heat treatment such as dry frying for 5 min, exposure to microwave for 1min and exposure to 100°C for 1 h in hot air oven and wet heat treatments like hydration of dhal for 30 min, 12 h and 24 h, 0.01% and 0.05% treatment with hydrogen peroxide and tyndallization (steaming for 3 successive days) were given to dhal to reduce aerobic spore count and after treatment analysed the treated dhal for aerobic spore count as mentioned in Harrigan (1998).

Supplementation of treated black gram dhal for the growth of lactic cultures

Best sporicidal treatment to completely destroy the aerobic spore was selected and then supplemented with skim milk powder, ash guard juice, carrot juice and tomato juice.

Preparation of ash guard juice, carrot juice and tomato juice

Ash guard, carrot and tomato were obtained freshly from local market. The edible portions were obtained, washed with potable water, grated, steamed for 15 min and mashed in clean, dry, mixer. The obtained puree was filtered through muslin cloth. After filtration the juices of ash guard, carrot and tomato were collected separately in a sterile conical flask.

Final supplementation to black gram dhal

The aerobic spore free black gram dhal was supplemented with each of SMP, ash guard juice, carrot juice and tomato juice at 0.5, 1, 1.5 and 2% level. The moisture maintained was 1:0.8 level, including volume of water in juices.

Growth study of SSF cultures on supplemented black gram dhal

The maximum growth period required for good biomass of lactic culture on supplemented black gram dhal was determined at optimum growth temperature for 48 h. At every 6 h interval, aseptically drawn samples of SSF dahi, yoghurt and acidophilus cultures were subjected for viability determination.

Determining the viability of lactic culture grown on supplemented black gram dhal

SSF cultures of dahi, yoghurt and probiotic cultures were drawn at different growth periods, cultures were aseptically transferred to sterile pestle and mortar, triturated with sterile 99 ml of phosphate buffer separately, required dilutions were prepared and plated using yeast glucose agar for total lactic count. The plates were incubated at 30°C for dahi culture and while in case of yoghurt and probiotic cultures at 37°C. The viable lactic counts were expressed as $\log_{10}$cfu/g.

Drying of SSF lactic cultures using fluid bed drier

SSF fermented lactic cultures were transferred to the sterile fluid bed drier and dried at ambient temperature (25±1°C) for periods of 1, 1.5 and 2 h and the samples were aseptically drawn to determine the moisture and viable lactic counts.

Preparation of powder of dried SSF lactic cultures

The best dried SSF lactic cultures grown on supplemented black gram dhal, based on less retained moisture and higher viable count were made into powder using sterile dry mixer. Powdered SSF lactic cultures were transferred to labeled self-sealing polythene pouches.

Effect of storage of SSF lactic cultures on viability at ambient temperature (28±1°C) and their performance in sterile milk

In order to determine the viability, the dried powder of SSF lactic cultures were packed in self-sealing polythene pouches were stored at ambient temperature (28±1°C). Once in every 3 days of storage at ambient temperature, the viability of SSF cultures were determined using YGA and at the same time inoculated to heat treated milk and incubated. Curdling time and titratable acidity were determined for the set milk. The stored SSF cultures were also tested for the safety through enumeration of coliforms, aerobic spore and yeast and molds count.

Statistical Analysis

The data was analyzed using R software [R. version 3.1.3 (2015-3-09), copyright © 2015, R foundation] for statistical computing both one way and two way Completely Randomed Design (CRD) which is the most appropriate for the study. Data on the response variables were collected for three replications for each of these treatments. ANOVA tables were prepared to analysed the data and where the F value was significant the critical difference was calculated and used to identify where significant differences existed and was indicated in the table use superscripts.

Results and Discussion

The cultures produced should be available all the time, it should be easily transported, stored for long time without much loss of viability. So under commercial conditions it is important that the final culture should be viable to set the milk and use as an direct vat set culture even though stored at ambient temperature. In order to check the efficiency of SSF lactic cultures, they were made into powder and stored in self-sealing polythene pouches at ambient temperatures. After every 3 days at ambient temperatures, samples were drawn to observe their viability and performance in milk after inoculation.

The viable count of dahi SSF culture started decreasing from 8.55 to 6.05 $\log_{10}$ cfu/g at 24th days of storage. Up to 12th day of storage, the viable count reduction was around 0.70 and later

Table 1 Effect of storage of fluid bed dried powdered SSF dahi culture on viability at ambient temperature (28±1°C) and their performance in heated milk

Storage days	Viable counts in dried powder of SSF culture ($\log_{10}$ cfu/g)	Performance of dried powder of SSF culture in milk	
		Curdling time (h)	Titratable acidity (%LA)
0th	8.55^{a}	5:00	0.68
3rd	8.33^{b}	5:15	0.69
6th	8.22^{c}	5:30	0.71
9th	8.03^{d}	6:00	0.74
12th	7.81^{e}	6:30	0.78
15th	7.13^{f}	7:00	0.81
18th	6.69^{g}	7:30	0.85
21th	6.32^{h}	8:00	0.89
24th	6.05^{i}	8:30	0.94
CD($p \leq 0.05$)	0.16		

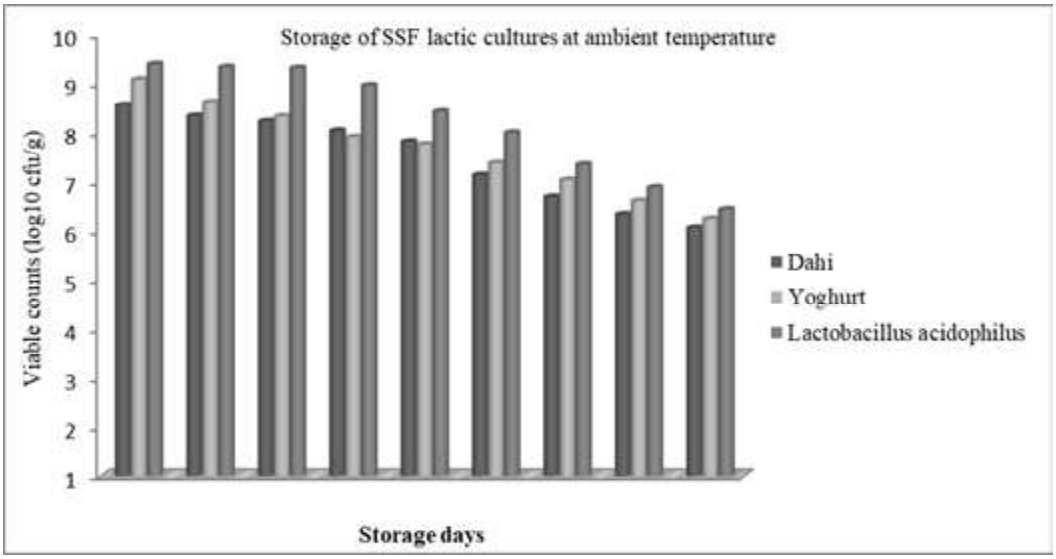

Fig. 1 Effect of storage of fluid bed dried powdered SSF lactic cultures on viability at ambient temperature

Table 2 Effect of storage of fluid bed dried powdered SSF yoghurt culture on viability at ambient temperature (28±1ºC) and their performance in heated milk

Storage days	Viable counts in dried powder of SSF Culture (log_{10} cfu/g)	Performance of dried powder of SSF culture in milk	
		Curdling time(h)	Titratable acidity (%LA)
0th	9.07[a]	3:30	0.71
3rd	8.61[b]	4:00	0.73
6th	8.32[c]	5:00	0.76
9th	7.90[d]	5:15	0.78
12th	7.75[de]	6:00	0.79
15th	7.37[f]	6:30	0.80
18th	7.03[g]	7:15	0.83
21th	6.61[h]	8:00	0.85
24th	6.23[i]	8:30	0.88
CD($p \leq 0.05$)	0.25		

storage showed reduction up to 2.50. Coliforms, aerobic spores and yeast and molds were absent in dried powder of SSF dahi cultures till the conclusion of storage period at ambient temperature. The same culture when inoculated at 1% level to heated milk, curdling time ranged from 5:00 to 8:30 h at incubation of 30ºC with acidity of 0.68 to 0.94% LA. Thus more the viable count in inoculums, shorter the curdling time. Significance difference was noticed in the viable count of dried powder of SSF dahi culture among storage days from 18th days onwards. At 24th day of storage the viable count was 6.05 log_{10} as shown in Table 1, a minimum inoculum for early setting of dahi and hence the storage study was concluded.

The absence of coliforms, aerobic spores and yeast and molds in dried powder of SSF dahi cultures till the conclusion of storage period at ambient temperatures indicate the hygienic procedures adopted during propagation, inoculation and preparation of fermented milk products using SSF lactic cultures.

A similar study by Deepa (2011) showed reduction of 2 log in the viability of SSF mixed dahi culture on supplemented black gram dhal stored at ambient temperature (24ºC), while at 60th days the reduction was 4.50 log. But when SSF culture was stored at refrigeration temperature (7ºC), the reduction in viable count was slower accounting for 2 log at 49th days of storage while at 60th day of storage, the reduction was 3.50 log.

SSF yoghurt culture having 9.07 log count reduced by 1 log at the end of 9th day of storage and further by 2 log reduction on 18th day and latter it was 2.8 log at the end of 24th day. The SSF yoghurt culture when inoculated at 0.5% level to heated milk, curdling time of 3:30 extended to 8:30 h at incubation of 42ºC which may be attributed to lower viable counts in the latter stages of storage due to faster moisture removal from SSF cultures (Table 2).

The viable counts of yoghurt SSF culture reduced by 1 log on 20th day of storage (from 9.07 to 8.03 log) and later reached 6.94

Table 3 Effect of storage of fluid bed dried powdered SSF probiotic culture viability at ambient temperature (28±1ºC) and their performance in heated milk

Storage days	Viable counts in dried powder of SSF culture log_{10}cfu/g	Performance of dried powder of SSF culture in milk	
		Curdling time (h)	Titratable acidity (%LA)
0th	9.38[a]	9:30	0.59
3rd	9.32[a]	10:00	0.62
6th	9.30[a]	10:15	0.64
9th	8.95[b]	10:30	0.65
12th	8.42[c]	11:00	0.67
15th	7.99[d]	11:30	0.69
18th	7.34[e]	12:15	0.73
21th	6.88[f]	13:00	0.75
24th	6.42[g]	13:45	0.81
CD($p \leq 0.05$)		0.12	

log on 35th day and finally to 6.85 log at the end of 40 days of storage at 7 ºC. Short set yogurt prepared using stored SSF yoghurt culture took 5 h extra from 1st to 40th day of storage at 7ºC.

SSF yoghurt culture stored at ambient temperatures did not have coliforms, aerobic spores and yeast and mold still the end of storage indicating hygienic methods adopted at each step of processing and production.

Contradicting the present study, Vanisri (1995) experienced more curdling time when freeze dried SSF yogurt culture was used as inoculums. She found that freeze dried SSF yoghurt culture on black gram when stored at 30ºC showed reduction of 3.00 log viable count at the end of 42th day of storage. When the viable count dropped to 6.00 log at the end of 42 day of storage at 42ºC took 24 h to curdle with 0.50% acidity against 18 h of curdling when fresh SSF culture was inoculated at 1% level (9.39 log based cfu/g).

When the SSF culture was stored at 7ºC viable count reduced to 2 log which curdle at 24 h with acidity of 0.9% LA when inoculated at 1% level into sterile skim milk (Vanisri, 1995).

Ramachandra (2008) reported that the dried SSF yoghurt culture on paddy husk was stored in heat sealed polythene bags at room temperature (30ºC). Samples drawn at intervals were examined for the viable count and activity. The initial viable count of 8.70log10 cfu/g gradually decreased to 7.60 log10 cfu/g after 30 days of storage. The activity of the culture in terms of curdling was 18 h with acidity of 0.50%LA

Prabha et al (2010) studied that the dried SSF yoghurt culture on paddy husk was stored in heat sealed polythene bags at room temperature (30ºC). Samples drawn at intervals were examined for the viable count and activity. The initial viable count of 8.70log_{10} cfu/g gradually decreased to 7.60 log_{10} cfu/g after 30 days of storage. The activity of the culture in terms of curdling was 18 h with acidity of 0.50%LA.

The viable count of SSF probiotic culture decreased from 9.38 to 6.42 log_{10} cfu/g at 24th day of storage. Up to 12th day of storage, the viable count reduction was around 1 log and further, storage, showed reduction up to 3 log Colifrom count, aerobic spore count and yeast and mold counts were absent throughout the ambient storage temperature in dried powder of SSF probiotic culture. The stored SSF culture when inoculated at 3% level to sterile milk, curdling time was ranged from 9:30 to 13:45 h at incubation of 37ºC with acidity of 0.59 to 0.81% LA from 1st to 24th day of storage. Significance difference was noticed in the viable count of dried powder of SSF probiotic culture among storage days from 18th day onwards. At 24th day of storage the viable count was 6.42 log, a minimum inoculum for early setting as well as needed probiotic count for the extension of therapeutic benefit to consumers and hence the storage study was concluded at 24th day itself.

No reports were available for fluid bed dried cultures. But reports were available for the freeze and air dried SSF lactic cultures. Vanisri (1995) observed decrease in viable count of freeze dried SSF acidophilus culture on black gram dhal stored at room temperature by 2.50 log at the end of 42th day of storage may be responsible for longer curdling time of 24 h with initial viable count of 7.00 and acidity of 0.44 %LA.

In a similar study, Ramachandra et al. (2009) dried SSF culture of *Lactobacillus acidophilus* 111 on paddy husk and stored in heat sealed polyethylene bags at room temperature (30ºC). The initial viable count of 8.81 log_{10} cfu/g gradually decreased to 6.14 log_{10} cfu/g after 30 days of storage. The activity of the culture in terms of curdling was 34 h against 26 h of fresh SSF culture.

The air dried fermented black gram dhal containing viable count of 7.00 of *Bifidobacterium longum* PF1 and incorporation into ceralac weaning food retained appreciable number of viable counts accounting for 6.70 log even after the storage period of 6.00 months at ambient temperature of 30ºC (Prabha et al. 2010).

Conclusion

In order to study the viability of stored fluid bed dried powder of SSF lactic culture such as dahi, yoghurt and probiotic culture at ambient temperature. The biomass production of SSF lactic cultures on supplemented black gram dhal was aseptically dried using fluid bed and storage was carried out till the end of 24th days of storage at ambient temperature (28±1ºC). Minimum viability of dahi, yoghurt and probiotic cultures had 6.05, 6.23 and 6.42 $\log_{10}$ cfu/g Viable counts respectively at ambient temperature on the 24th day of storage. And none of the stored samples showed the presence of coliforms, aerobic spores and yeast and molds.

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RESEARCH ARTICLE

Design and development of solid state cooling module for rawmilk cooling

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Abstract: The global increasing demand for refrigeration in field of cooling and storage led to usage of more electricity and release of ozone depleting gases over the world which is a contributing factor of global warming. Thermoelectric cooling is a novel technique for producing cooling and heating effect. Hence, a solid state cooling module for cooling of raw milk was designed and developed. The milk cooling chamber was designed with the dimensions of 260 mm in diameter, 140 mm in height and 2 mm in thickness. The volume of the milk and water cooling chamber was 5 and 4 L, respectively. Three numbers of TEC1-12715 Peltier module, with the dimensions of 40 mm in length, 3.6 mm in thickness and 40 mm in width, cold side fins (36 fins), hot side fins (100 fins) and exhaust fans (3 Nos.) were used in the cooling module. The 12 V, 10A DC power supply for three module and exhaust fans was drawn from SMPS in parallel connection. The thermoelectric cooling module was able to cool raw milk from 37 to 9 °C. The highest cooling load obtained was 229.74 kJ at 60 min. The minimum and maximum power consumption recorded was 0.375 kWh and 1.125 kWh, respectively. The COP of the module was ranged from 0.036 to 0.259.

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Introduction

Spoilage of milk and milk products results from growth of fermentative bacteria when storage temperatures are sufficiently high. The most common micro-organisms for spoilage of milk and milk products are gram-negative (rod-shaped bacteria, *Pseudomonas spp., Coliforms*), gram-positive spore-forming bacteria (*Bacillus spp., Clostridium spp*.), lactic acid producing bacteria (*Streptococcus spp.),* yeasts and moulds (Giffel and Wells Bennik, 2010). Insulation and refrigeration of milk tankers may be necessary under some climatic conditions. Praveen and Pranay, 2018; Soylemez et al. 2018reported that the present refrigeration system produces cooling effect by using refrigerants like Freon, Ammonia, *etc*. Using these refrigerants one can get maximum output but one of the major disadvantages is emission of harmful gases like CFCs and HCFCs which leads to global warming and also these systems are not reliable and economical where compact design and noiseless operation is required. To address these problems, there is a need for energy efficient, green and eco-friendly technologies. In this regard, solid state cooling is one among them. Thermoelectric cooling or solid state cooling is an alternative for the conventional cooling systems. Thermoelectric cooling (TEC) involves thermal and electrical energy. Thermoelectric devices are solid state devices, meaning that doesn't have any moving parts and have a lot of applications in different fields like food preservation, storage applications, electronic cooling, military field, automobiles cooling, storage of pharmaceutical products, refrigeration and air-conditioning field (Sujithet al. 2016).

A basic solid state module consists of semiconductor elements (p-type and n-type) that work as two dissimilar conductors arranged in specific order. The layer of elements is soldered between two ceramics plates, they are placed electrically in series structure and thermally in parallel structure. The temperature will decrease at that junction, when the direct current passes through one pair or multiple pairs of elements from 'n' to 'p' resulting in the absorption of heat from the surrounding(Gokcek and Sahin, 2017*;* Dongare et al. 2018). Solid state refrigeration system works

on the thermoelectric effects such Seebeck effect, Thomson effect and Peltier effect. COP of compression refrigerators decreases with the decrease in capacity. Therefore, when it is necessary to design a low capacity refrigerator, solid state refrigeration system is preferable.Hence, solid state refrigeration system is good option for food preservation and cooling of liquid products (Beretta et al. 2019). Keeping the view, the importance of raw milk cooling in small capacity production catchments and the need of eco-friendly refrigeration systems, the solid state cooling module was designed and developed.

Materials and Methods

The design was progressed thorough series of steps. These steps were identification of the problem, analyse the problem, decide upon a design selection and implement design. The steps in design and development of solid state cooling module were selection of fixed parameters such as material work piece, temperature difference, estimation of quantity of water and milk, estimation of cooling load, selection of number of modules and their parameters, fabrication work of cooling module and assembling of all components. The brief description of design procedure is explained below.

Selection of material

The AISI-316 SS material was selected for the fabrication of the cooling module

AISI-316 SS is Austenitic Grade Stainless steel has good wieldable and forming properties. It is resistant to both acid and alkali and has good corrosion-resistant properties. The SS-316 sheet of 660×460×2 mm and two SS vessels of 260 dia×140 height×2 mm wall thickness mm and 290 dia×150 height×2 mm wall thickens were used for the fabrication of the cooling chambers.The insulating materials were used to protect the inside cooling space from the hotter atmosphere. The cooling module was insulated with 20 mm thick puff material, which has thermal conductivity 0.02 W/mK. The water cooling chamber has capacity of 4 litre and milk cooling chamber has 5 litre. The overall dimensionsof developed solid state cooling module is mentioned in Table 1.

Design of thermoelectric components

Cooling load calculations

The important factor to be accurately calculated for a TEC is the amount of heat to be removed or absorbed by the cold side of the TEC. In this study cooling load was calculated by knowing the mass of water, specific heat of water and temperature difference. The mathematical equation for calculation of cooling load (Balakrishanet al. 2016) is given in Equation (1).

$$\text{Cooling load, } Q_c = \text{Refrigeration Effect} = \frac{m \times C_p \times \Delta T}{t} \quad \ldots(1)$$

where,

Amount of heat removed or the cooling load, kW

M = Mass of water, kg

C_p = Specific heat of water, kJ/kg K

ΔT = Temperature difference, K

t = Time in seconds

After calculation of cooling load, the number of Peltier modules was calculated(Totalaet al. 2014).

Fixed Parameters: 1) Mass of water = 4 kg

2) Cp for water = 4.18 kJ/kgK

3) Temperature difference = 21 °C

4) Time = 30 min=1800 seconds

Cooling load = $\frac{4 \times 4.18 \times 21}{1800}$ 0.1950 kW

Number of modules = $\frac{\text{Refrigeration effect (W)}}{\text{Capacity of one Peltier module (W)}}$ =2.13

(The capacity of Peltier module (TECH1-12715)is 90W)

Efficiency of thermoelectric refrigeration is around 0.2 to 0.4 and due to surrounding heat losses, three Peltier modules for design of solid state cooling system was selected considering the safety factor.

Table 1 Dimensions of cooling module

S. No.	Parameter		Value
1	Outer dimension (mm)	Diameter	260 mm
		Height	140 mm
		Thickness	2 mm
2	Inner dimension (mm)	Diameter	290 mm
		Height	150 mm
		Thickness	2 mm
3	Volume of cooling chamber for water		4 L
4	Volume of cooling chamber for milk		5 L
5	Total height of cooling module		460 mm

Selection and operating parameters of a thermoelectric module

Once it was decided that thermoelectric cooler is to be considered for cooling system, the next step was to select the thermoelectric module that can satisfy a particular set of requirements. Modules are available in great variety of sizes, shapes, operating currents, operating voltages and ranges of heat pumping capacity. The minimum specifications for finding an appropriate TEC by the designer must be based on the following parameters (Totala et al. 2014).

a. Cold side temperature (T_c)

b. Hot side temperature (T_h)

c. Operating temperature difference, which is the temperature difference between T_h and T_c.

d. Operating current (I) and operating voltage (V) of the TEC

e. Amount of heat to be absorbed at the TEC's cold surface. This can also be termed as heat load.

a. Cold side temperature

In this study the water was first cooled by indirect contact with the cold surface of TEC. The required cooling temperature was considered as the temperature of the cold side of TEC (Tc). The cold side temperature was varied with the number of modules. In this study, it was necessary to cool the milk below 10 °C, based on this, the outlet temperature the thermoelectric module (TECH1-12715) was selected.

b. Hot side temperature

The hot side temperature (Th) was mainly based on two factors. First parameter was the temperature of the ambient air to which the heat was rejected. Second factor was the efficiency of the heat sink which is between the hot side of TEC and the outside environment (Rakesh, et al. 2016).

c. Temperature difference

The two temperatures & and difference between them (ΔT) is a very important factor. "T is to be accurately determined if the cooling system is expected to be operating as desired. The ΔT was calculated by using the following equation (Totalaet al. 2014).

$$\Delta T = T_h - T_c \quad ...(2)$$

where,

T_h = Initial temperature of water, °C

T_c = Finial temperature of water, °C

Actual ΔT is not same as the system ΔT. Actual ΔT is the difference between the hot and cold side of the TEC. On the other hand, system "T is the temperature difference between the ambient and the system.

d. Operating current and voltage

Operating current and voltage are the two important parameters that must be considered for a successful TE system. Current and voltage of a TEC was determined by the charts provided by the manufacturer. TEC's power is the product of required voltage and current (Totala et al. 2014).

$$P = I \times V \quad ...(3)$$

where,

I = Maximum current Peltier module can withstand, A

V = Maximum voltage Peltier module can withstand, V

Peltier modules

A standard module consists of any number of thermocouples connected in series and sandwiched between two ceramic plates (Fig. 1). By applying a current to the module one ceramic plate is heated while the other is cooled. The direction of the current determines which plate is cooled (Jaziri et al. 2019). The number and size of the thermocouples as well as the materials used in the manufacturing determine the cooling capacity. Cooling capacity varies from fractions of Watts up to many hundreds. Different types of TEC modules are single stage, two stage, three stage, four stage, centre hole modules etc. In this study, the TEC1-12715 Peltier module with dimensions of 40×40x3.6 mm was selected based on the cooling load. The detailed specification of Peltier module is given in Table 2. Peltier module was made up of thin ceramic wafers with P and N type semiconductors connected in series between them. The thermoelectric couples are thermally in parallel while electrically in series.

Extended fins with exhaust fan (heat sink)

The extended fins were used to increase the rate of heat transfer, where the hot side heat sink was used to dissipate the heat and cold side extended fins were used increase the rate of heat transfer between surface of the module and cooling fluid. Heat sink consists of two parts; one is a 12 V DC fan and aluminium finned surface. Aluminium (290 W/m K) fins were selected for the study due to its specific weight, high thermal conductivity and low price comparedto other materials. Fig. 2 shows the extended fin with exhaust fan. The hot side heat sink of 90×90×40 mm and cold side heat sink of 70×62×40 mm.

Switch mode power supply (SMPS)

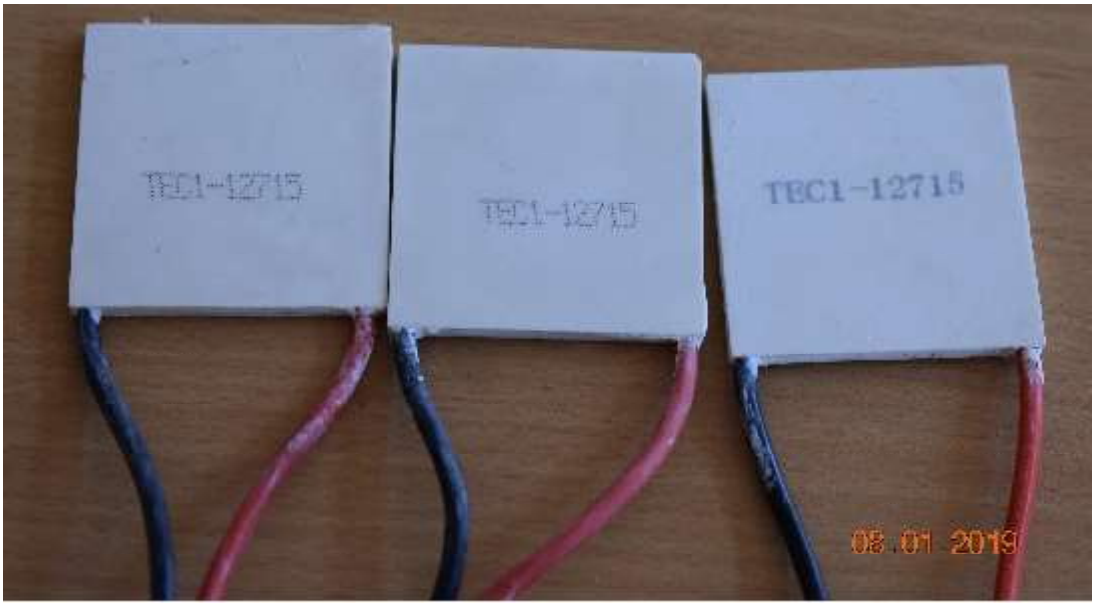

Fig. 1: Peltier modules TEC1-12715

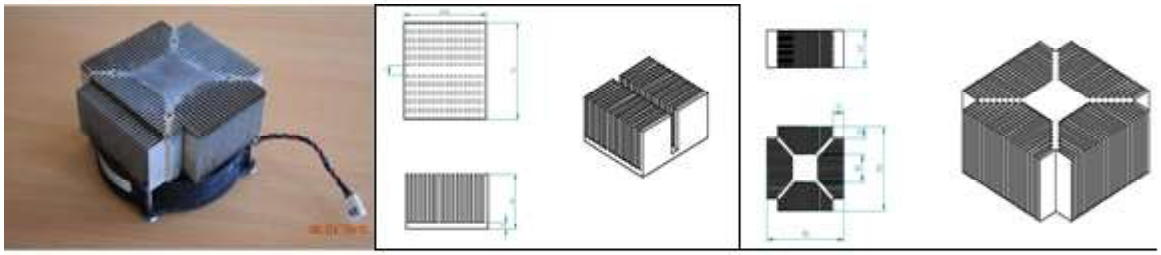

Fig. 2 Extended fin with exhaust fan

Fig. 3 Switch mode power supply unit (SMPS)

Table 2 Specifications of thermoelectric module

	Model : TEC1-12715	
Sl. No.	Parameters	Range
1	Voltage	15 Volts
2	Current	15 Amps
3	Dimension	40×3.6×40 mm
4	Q_{max}	230 Watts
5	T_c and T_h	-10 to -20 °C and 138 °C
6	$ŠT_{max}$	68 °C
7	Resistance	0.80 Ohm
8	Weight	50 g

The switch mode power supply is a device which converts alternate current (AC) to direct current supply (DC). The solid state cooling module was a low-impendence semiconductor device that presents a resistive load to its power source and these solid state modules are sensitive to voltage variations and requires a regulated DC voltage supply, usually referred to as a 'switch-mode power supply' (SMPS).Fig. 3 shows the switch mode power supply unit (SMPS). Since the heat sink fans and Peltier modules uses the DC power to work but the power available is in AC. Hence, converter is required for the fans and modules to work (Rokde et al. 2017). The SMPS, with intake of 110 or 220 V/60Hz AC and capable of converting to 12 V, 40 A DC power supply was selected.

Stainless steel material

Cooling cabinet had to be fabricated with a material, which would easily conduct heat, it should be strong enough to bear the pressure of milk that will be filled inside it and it should be easier to handle and more importantly (Awasthi and Mali, 2012), it should have a flat surface for the modules to be mounted on. To

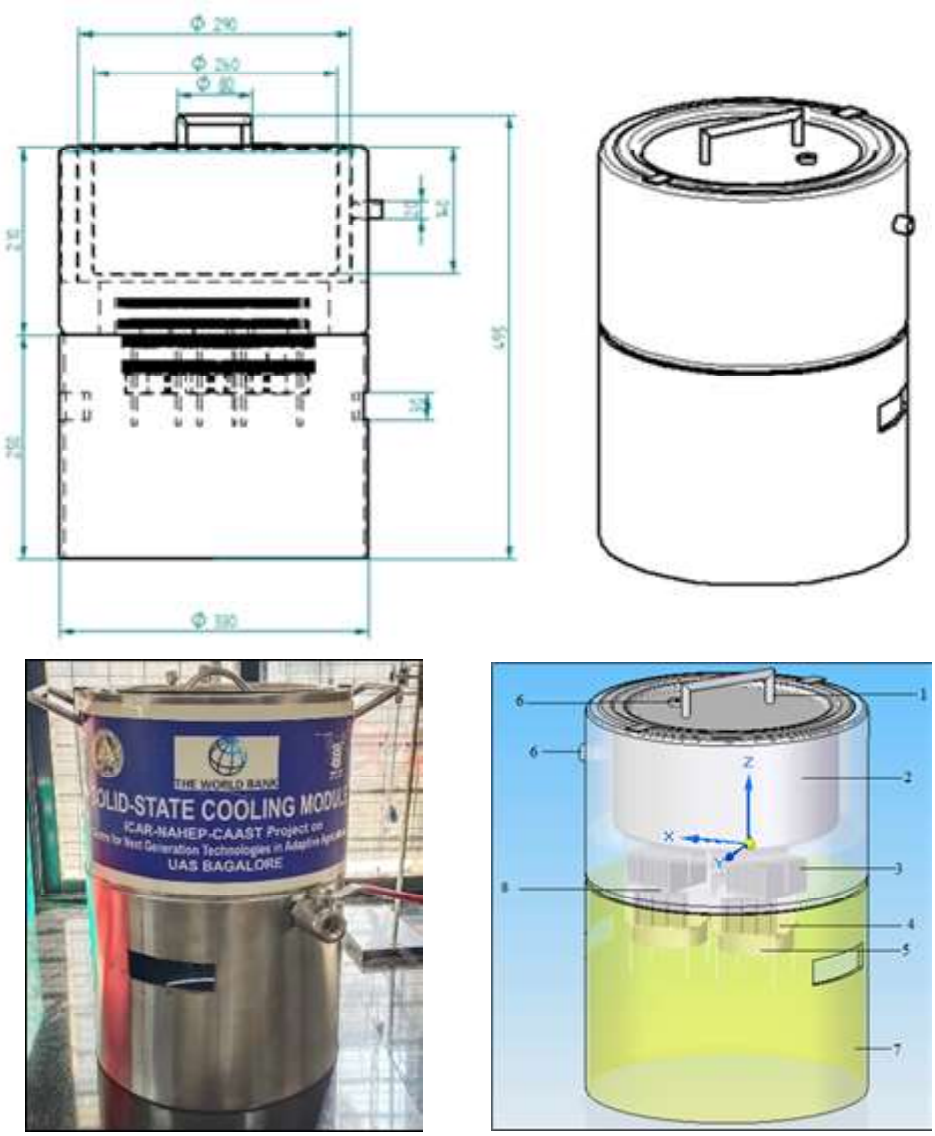

Fig. 4 Side view and 3D isometric view of developed solid state cooling module

Part description: 1 Lid; 2 Cooling chamber; 3 Cold side heat sink; 4 Hot side heat sink; 5 Exhaust fan; 6 Milk and water temperature sensor ports; 7 Supporting stand; 8 Peltier module

Table 3 Specification of heat sink, cold sink and exhaust fan

S.No.	Parameters	Hot side heat sink	Cold side heat sink	Exhaust fan
1	Material of construction	Aluminium	Aluminium	Plastic
2	Length	90 mm	70mm	90 mm
3	Width /diameter	90 mm	62 mm	42 mm
4	Height	40 mm	40 mm	25 mm
5	Total number of fins/wings	100	36	7

achieve these requirements, stainless steel AISI-316 stainless steel material was used with thermal conductivity of 52 W/mK.

Insulating material

Thermal insulation was used to prevent heat losses from all hot surfaces to conserve energy. The cooling module was insulated with 20 mm thick puff material, which has thermal conductivity of 0.02 W/mK.

Temperature sensors

In the present study two temperature sensors were used to record the temperature of water and milk. The K-type thermocouple (-200 °C to +1350 °C) was used to record the temperature of water.

Fabrication work

As per fabrication drawings, visible marking was made on selected SS-316 sheet to develop geometry of intended equipment's by

using metal markers. SS sheets were cut exactly on marks by using manual metal cuter of with 100% accuracy. A sheet rolling machine was used to form cylinders. All welded joints were finished by Bosch Die and angular grinder using 60 grit grinding wheels. All corner joints were ground to minimum 2 mm radius of curvature as per the sanitary standards. The non-ferrous free grinding and polishing wheels were used for grinding and polishing. Pickling was carried using a pickling paste (combination of acids) to remove rust and metal scale formed during high temperature operation like welding, grinding, heat treatment *etc.*

Experimental setup

The solid state cooling module consisted of jacketed type cooling chamber, three Peltier modules, extended fins, CPU exhaust fans and Switch Mode Power Supply (SMPS) with

12 V, 40 A power supply. The solid state cooling module works on the principle of Peltier effect. The Peltier or TEC modules were sandwiched between the two extended fins. Heat sink was attached to the hot side of the module to transfer heat by air to the surrounding. The cold side of module with the extended fins is called cooling unit whereas the cold side of the module is fixed to the bottom of the jacketed type cooling chamber. The cooling chamber consists of an inlet and outlet channels for water where inlet and outlet for the milk were provided at top of the cooling module which was open and close cap system. The cylinder shaped cooling unit was surrounded by 20 mm thick puff type insulation material to reduce the rate of heat transfer. The solid state cooling module was operated by direct current (DC) and power is directly transmitted to the modules and heat sink fans through SMPS. When DC power is transmitted to the modules, one side of modules was heated and the other side was cooled. The heat from hot side of the module was exhausted by air and cold side of module was used to provide cooling module to the water. Fig.4 shows side view and 3D isometric view of developed solid state cooling module.

Three Peltier modules were used and were sandwiched between the cold and hot side heat sinks. The power supply for Peltier modules and exhaust fan were drawn from 12V, 40A SMPS. The Peltier modules and exhaust fans were connected to the SMPS. For performance evaluation of the cooling module, 4L of water was fed into the water cooling chamber and temperature was recorded with respect to time. The experiment was repeated with increasing in number of modules. The milk and cooling fluid temperature was continuously recorded by temperature sensors.

Results and Discussion

The performance of the solid state cooling module was evaluated in terms of cooling load, coefficient of performance and power consumption. The experiments were conducted with selected number of modules (1, 2 and 3 numbers) and time (60, 120 and 180 min). For each experiment 4 L of water at ambient temperature (30 °C) were taken and experiments were replicated thrice. The cooling fluid was filled into the water cooling chamber and it was kept for cooling (3h). For performance evaluation of the cooling module, 5 L of raw milk was fed into the cooling cabinet (milk). The milk and cooling fluid temperature was continuously recorded by temperature sensors.

The minimum temperature of 9 °C was recorded at 180 min, while maximum temperature of 19 °C was obtained at 60 min. The performance of the cooling module was evaluated by the temperature achieved with water as cooling media. Among all the modules, the temperature varied from 30 to 9 °C. The lowest temperature achieved was 9 °C at 180 min with three module combination. Fig. 5 shows the effect of number of modules and time on temperature of water. The temperature of water was decreased with increase in time due to removal of heat from water. The temperature of water decreased with increase in number of modules due to increase in rate of heat transfer.

The experimental results of cooling load of the unit decreases with increase in time and number of modules as shown in Fig. 6. Among all the modules, the cooling load varied from 229.74 to 69.57 kJ. The lowest cooling load of 69.570 kJ was obtained at 180 min with one module. The highest cooling load was 229.74 kJ obtained at 60 min with three modules.It was observed from the results that the cooling load was decreased with increasing in time and number of modules and it was due to decrease in temperature difference with increase in time.

The power consumption of the unit increased with increase in time and number of modules as shown in Fig. 7. The 12 V, 10 A power supply was required for one module to operate.Due to increase in number of modules and time, the power consumption was increased. The minimum power consumption of 0.375 kWh was recorded at 60 min, while maximum power consumption of 1.125 kWh was recordedat 180 min.

The COP of the cooling module was decreased with increase in time and number of modules as shown in Fig. 8 (Huanget al. 2000). Among all the modules, the COP varied from 0.036 to 0.259. The lowest COP of developed cooling module was 0.036 at 180 min with three modules combination. The highest COP of 0.259 was seen at 60 min with one module.

Performance evaluation of developed solid state cooling module with raw milk

The three module combination was optimised and the optimised combination was used for the performance evaluation of solid state cooling module with raw milk. The experiment was carried out with 5 litres of raw milk at 37 °C as initial temperature. The experimental results showed that the milk temperature was decreased with time.

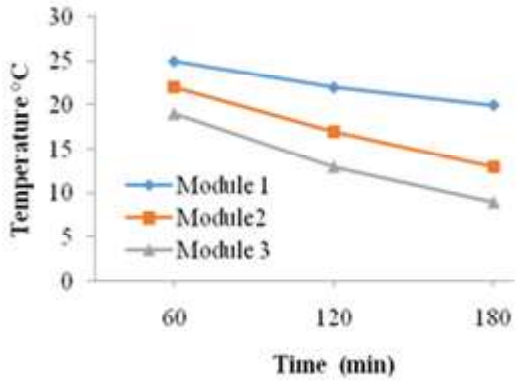

Fig. 5 Effect of number of modules and time on temperature of water

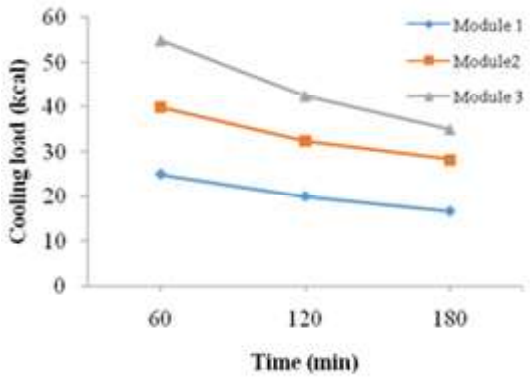

Fig. 6 Effect of number of modules and time on cooling load

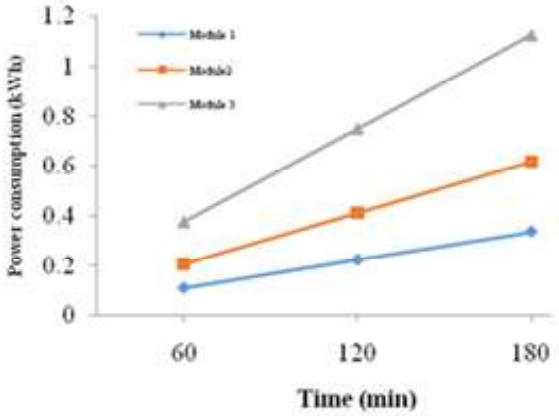

Fig. 7 Effect of number of modules and time on power consumption

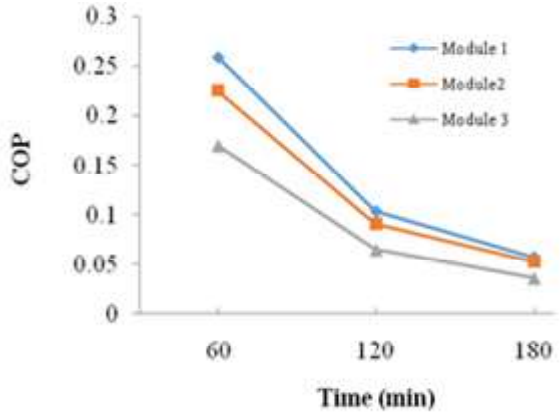

Fig. 8 Effect of number of modules and time on COP

The experimental results showed that the raw milk temperature was decreased with increase in time, at 2 min 30s, the temperature of milk and water was reached to 17 °C. The temperature of water and milk reached to equilibrium state in 1 min 50s as shown in Fig. 9. The temperature was maintained at 17 °C for next 1 h and the mean temperature of milk for next three hours was 19 °C. The decrease in temperature of milk with increase in water temperature and time was due to heat transfer between the water and milk.

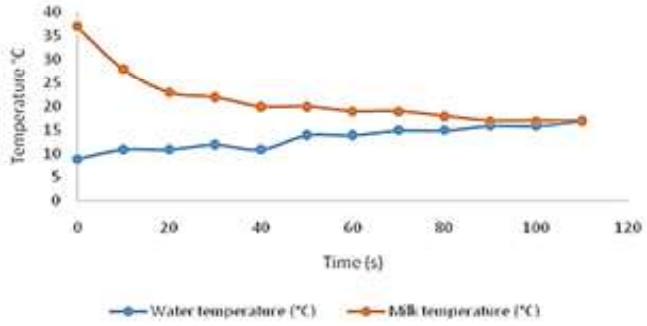

Fig.9 Effect of time on temperature of milk and water

Conclusions

A solid state cooling module for cooling of raw milk was designed and developed. Three thermoelectric modules were used for achieving the cooling. It is shown from testing results that, the cooling system is capable of cooling raw milk. Thermoelectric cooling was able to cool a raw milk temperature from 37 to 9 °C. The minimum temperature of water in solid state cooling module was found to be 9 °C in 3 h with three modules. The temperature of

9 °C in 180 min with three module combinations was the best result obtained. The temperature of milk was reached to equilibrium stage at 17 °C in 1 min 50 s. The mean temperature of 19 °C was maintained for next four hours. Overall dimension of solid state cooling module was 460 mm height, 330 mm diameter and 20 mm thickness. Performance of solid state cooling module can be increased by using new generation heat transfer fluids like nano fluids, glycol *etc.* for the better results.

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RESEARCH ARTICLE

Heavy metal contamination of milk and milk Products produced in Boumerdes (Algeria)

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Abstract: Milk and its derivatives are major source of micronutrients however an overdose of these can be harmful. This study was aimed to measure heavy metals concentration and their derivatives from the Factory LFB (Boudouaou Cheese Dairy) in Boumerdes, Algeria. The concentrations were measured using a graphite furnace spectrophotometer, the all data processing was carried with XL-STAT 2014 software. The mean values (Cu, Zn, Fe, Mg, Cd, Cr and Pb) lie respectively between: (0.019-0.45), (0.29-7.24), (0.21- 0.56), (1.09-120.77), (0.012-0.41), (0.006-0.027), (0.086-0.72mg/L) Higher levels of Cd, Fe, Cr and Pb were observed in pasteurized milk and cheese, and higher levels of Zn and Cu in cheese than in other samples, all exceeding World Health Organization (WHO) limits. The highest non-carcinogenic risk quotient (NCR) values were attributed to Pb and Cd in raw milk, pasteurized milk and cheese, which were above the values recommended by the Health Protection Agency United States Environment (USEPA) as for the carcinogenic risk factor (CR), despite the low value found, consumption of milk and cheese over a long period of time does not exclude the risk of developing cancer.

Keywords: AAS, Carcinogenic factor, Heavy metals, Milk and derivatives, Risk quotient

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Introduction

In recent years, food industry has undergone an important evolution in consumers favour, through the search for quality products adapted to their basic health and safety needs (Bréger, 2018) Today, in order to conquer international markets, the dairy industry must control the qualitative evolution of its products, label them and thus gain the confidence of its customers (Charlebois and Haratifar, 2015). Of all food, milk is practically complete food, it is the closest to an ideal food, it can cover all the body's needs during the first stage of life, it contains mainly all the elements necessary for the growth and body development. This richness and diversity of constituents makes milk one of the basic elements of a balanced diet (Kim et al. 2003; Ellen et al. 2013). Depending on the different treatments, drinking milks currently available on the Algerian market (raw and pasteurized milk) may be subject to a number of alterations and contaminations (e.g. heavy metals) responsible for food poisoning and may cause various diseases (Amiot et al. 2002). Heavy metals are chemical contaminants that can be found in consumer foods and also as residues in milk (AFSCA, 2018). Contamination of milk with heavy metals presents chemical hazards that may be present during the production, processing and packaging of milk (Lukáčcvá et al. 2012; Zain et al. 2016).

Most of the heavy metals present in milk are essential cofactors for many enzymes and in many physiological functions in humans where deficiencies can lead to disturbances and pathologies (Tripathi et al. 1990; Quin et al. 2009; Daşbaşi et al. 2016). However, at high concentrations, these metals may also pose a risk of toxicity (Amir et al. 2014). On the other hand, the International Agency for Research on Cancer (IARC, 2016) classifies arsenic (As), lead (Pb) and chromium (Cr) as carcinogenic metals. Therefore, consumers must be attentive to the health qualities of milk (Marta et al. 2018). Thus, during milking, collection and the various stages of milk and its derivatives processing may appear changes in physico-chemical parameters and contamination that may constitute health risks for consumers (Fischer et al. 2011). Despite the evolution of technological processes that ensure a certain hygienic guarantee of milk, the consumer remains very attached to dairy products (Eduardo, 2016). For decades, all milk marketed has been subject

to official quality control and all has been subject to official quality control. This control is the subject of particular attention and requirements for the marketing of this product have been determined for the evaluation of its nutritional and hygienic quality (Michael, 2018). Keeping these views in mind, the present study was conducted to asses the existence of heavy metals in Algerian milk and its derivatives.

Materials and Methods

Biological material

The samples used in this study were taken from the dairy and cheese factory LFB, Boumerdes, Algeria **(Fig 1)** to investigate the effect of environmental pollution on the heavy metal content of raw milk and consisted of:

- The raw materials used at the LFB, which were mainly: Milk powder (0 and 26% fat), raw milk, stored in well-ventilated sheds.
- The finished products: milk in bags, whey and cheese, stored in cold rooms.

The samples to be analyzed are shown in Table 1.

Reagents

All reagents: Nitric acid (65%), Hydrogen peroxide (32%), Standards for all heavy metals (1000 ppm) Copper, Zinc Iron, Magnesium, Cadmium, Lead, Chromium were supplied by Sigma-Aldrich, St. Louis Missouri, USA. (Sigma). All sample preparations and measurements were performed with ultrapure water (CJ-002-ULTRAPURE-DI-1000 purifier) having a conductance of 0.055 µS/Cm and a resistivity of 18.2 MΩ.Cm.

Equipment and apparatus

Analytical balance (Mettler Toledo) with a precision of ± 0.001 g, volumetric flasks (25, 50, 100 mL), measuring cylinders, pipettes, micro-pipettes, muffle furnace, Centrifuge, pH meter, hot plate, desiccator, porcelain crucible, Nylon syringe filter 0.45µm, atomic absorption spectrophotometer (Agilent) type 240 FS (Fast Sequential), graphite furnace atomization unit 240 ZAA which allow a high sensitive analysis of inorganic elements by electro-thermal atomic absorption spectrometry (the furnace method) and hydrid vapour atomic absorption spectrometry was used.

Samples preparation

For the unit water, 100 mL is added to a 250 mL beaker. 5 mL of HNO_3 (65% nitric acid) and 2 mL of H_2O_2 (32% hydrogen peroxide) are added. This is allowed to stand until the sample is dissolved and then heated on a hot plate to concentrate the mixture to about 15 mL (ISO 5961, 1994).

To raw milk and whey, the samples (500 mL) were acidified at room temperature for 48 hours until the pH reached 4.6, then centrifuged at 1000 rpm for 10 min. The milk supernatant was collected in a 250 mL beaker, 5 mL of 65% HNO_3 was added. The contents were heated on a hot plate at 90°C for 10 min until complete evaporation (Moradi et al. 2012).

For milk powder, 1 g of milk was placed in a 100 mL beaker, then 5 mL of $HN0_3$ (16 N) and 5 mL of 30% H_20_2 were added. The mixture was left to allow the reaction to proceed, then the beaker was placed on a hot plate at 60 °C for 30 min. The beaker was removed and allowed to cool for 5 minutes, after which 10 ml of

Table 1 Raw and final materials taken from the LFB dairy

Raw material	Finished product
Milk powder (0 % fat)	-
Milk powder (26 % fat)	Pasteurized milk (semi-skimmed)
Water	Water
Raw milk	Cheese, Whey

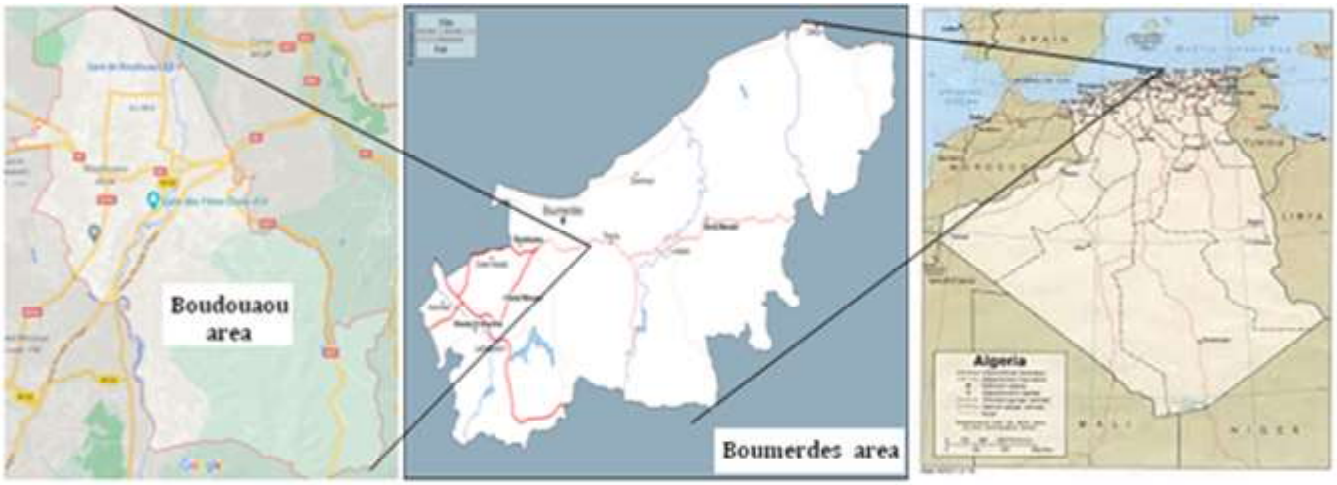

Fig.1 Study area location

HNO_3 and 5 ml of H_20_2 were added. The beaker is then placed on a hot plate, gradually increasing the temperature. The addition of HNO_3-H_2O_2 was continued until the sample became clear (Agemian et al. 1980).

For cheese, 5 g of sample was placed in a muffle furnace for 5 hours at 500°C. The ash was collected in a 100 mL beaker and 10 mL of 20% HNO_3 was added. The beaker was placed on a hot plate. After evaporation, 50 mL of pure HNO_3 was added (AOAC, 2002). All solutions were filtered through Whatman filters (0.45µm) into a 100 mL volumetric flask and made up to the mark with ultrapure water.

The following equation used to convert mg/l to mg/kg concentration is: (Uwah et al. 2012).

$$[C]\left(\frac{mg}{Kg}\right) = \frac{[C]\left(\frac{mg}{L}\right) * V}{P_e}$$

Where:

[C] is the concentration.
V is Final volume of the solution in mL.
P_e is Test sample in g.

Human Health Risk Assessment

In this study, a number of standard measures, namely, the estimated daily intake (EDI) for each metal in milk and its derivatives samples, non-carcinogenic risk quotient (NCR) and carcinogenic risk (CR), were determined to assess the health risks from milk consumption (Muhib et al. 2016).

Estimated Daily Intake (EDI)

$$EDI\left(\frac{mg}{l}\right) = \frac{C_m * C_i}{70}$$

Where:

Cm is the metal concentrations in milk (mg/g).

Ci is the daily milk consumption rate for Algeria (Belete et al. 2014).

and 70 kg is average body weight of an adult resident.

The non-carcinogenic risk quotient

The non-carcinogenic risk quotient is given by the following formula

$$NCR = \frac{EDI}{R_fD} * 10^{-3}$$

Where:

EDI is the estimated Daily Dose of Metal (mg/day).

R_fD is the oral reference dose (mg/kg/day).

In this case, R_fD is 0.001 for Cd, 0.004 for Pb, 0.003 for Cr, 0.7 for Fe, 0.04 for Cu, and 0.3 for Zn. QR e" 1 indicates potential health risk.

The carcinogenic risk factor

(lifetime cancer risk) is estimated as follows

$$CR = \frac{(EF_r * ED * EDI * CSF_0) * 10^{-3}}{AT}$$

Where:

EF ris the frequency of exposure (350 days/year).
ED is the duration of exposure (30 years).
AT is the mean time to carcinogen (365days/year×65 years depending on life expectancy in Algeria).

And CSFo refers to oral carcinogenicity slope factor of the integrated information on risks System database, i.e. 8.5 x 10^{-3} (mg/kg/day for Pb) (Akele et al. 2017).

Statistical analysis

The statistical analysis of the various data was carried out using the software XLSTAT 14. A single criterion analysis of variance (ANOVA 1) was performed and followed by the Tukey test. The significance level was evaluated at the 5% ($P<0.05$) threshold. A principal component analysis (PCA) was carried out in order to highlight a group structure according to physico-chemical parameters.

Results and Discussion

Heavy metals determination in milk and its derivatives

Milk is a valuable source of essential minerals and micronutriments but some toxic elements may be accidently added during handling, processing, and milk remixing. The mean concentrations of various essential and toxic heavy metals are presented in table 2

Table 2

indicating mean values±SD and differences (ANOVA/Tukey test) among the various milk categories. Mean values of the mineral

elements were closest to the permissible values as determined by international standards (FSA, 1999; CE, 2001). The differences in mean values (Cu, Zn, Fe, Mg, Cd, Cr and Pb) of the seven food matrices were observed to be significant, ($P<0.05$), whereas there were significant ($P<0.05$ ANOVA/Tukey) differences in case of manganese. The mean differences of cadmium were significant ($P<0.05$), in the dried and liquid milk groups.

It was noted that all values of Copper were below the permissible standard except for 0% milk and cheese which was about 0.052 mg/l for milk (Renner et al. 1989) and 2 mg/l for water (UE, 1998). The results obtained in this study showed a value of 0.148 mg/l for cheese, a value which was below that obtained by Öztürk et al. (2012), from which they found a mean copper value of 0.5036 mg/l. On the other hand, our found value for raw milk is in the range determined by Rubina et al. (2016), which is between 0.001 and 0.098 mg/l.

Zinc is a trace element necessary as a cofactor for the enzymes present in milk; it is also associated with lactoferrin. Zn deficiency leads to a multitude of health problems such as poor growth, late puberty and infertility (Prasad, 1983). In the light of the results obtained in this study we note that our zinc concentration in cheese was lower than the one found by Gogoasă et al. (2006), where they found values between 17.39 and 23.17 mg/l. On the other hand, our zinc concentration in raw milk was around 3.18 mg/l. It remained relatively lower than the norm and the same for the one found by Rubina et al. (2016), who determined the zinc concentration in raw milk and found a value between 2.086 and 5.498 mg/l.

Iron is a necessary trace element, the found values during this work are lower than those found by Salah et al. (2013), they found an iron concentration value equal to 20.41 mg/l for milk powder, it is the same for El-Bassiony et al. (2016), they found an Iron value equal to 1.44 and 0.73 mg/l for water and raw milk respectively, the same for Ghosia et al. (2014) so they found 2.42 mg/l of Iron in powdered milk. In addition, our values are similar to those obtained by Rubina et al. (2016) who reported that values of different milk samples ranged between 0.001 and 3.260 mg/l. The excess of iron in milk and its derivatives does not reflect its concentration in the dairy animals blood. It is probably the iron intake per diet that favours its content in milk. High iron concentrations may also be likely to occur due to the equipment used during milk collection and processing (Coni et al. 1995; Qin et al. 2009).

Magnesium is an essential mineral to the proper human body functioning. It is involved in more than 300 metabolic reactions in the body (Pérez-Carrera et al. 2016). Our mean concentration is 2.32 mg/l for raw milk is higher than that determined by Renata et al. (2013), who worked on cow's milk from two breeds, Simmental and Holstein-Friesian, found a mean of 0.131 and 0.119 mg/l for the two breeds respectively.

Cadmium has a very significant harmful effect on human health it can cause many disorders (Benecke et al. 2004; Il'yasova et al. 2005). Cd values are between 0.012 and 0.041 mg/l for raw milk and 26% milk powder respectively, these values exceed the tolerated standard of 0.01 and 0.005 mg/l for milk and water respectively according to the UE, 1998 codex; the same applies to the results found by El-Bassiony et al. (2016), which were between 0.03 and 0.05 mg/l for water and raw milk respectively. Finally comparing our results with those of Abdalla et al. (2013) they are much higher than their value (0.0006 mg/l) for raw milk. In general Milk and dairy products contain a low concentration of Cd except when dairy animals consume contaminated food and water (Cabrera et al. 1995; Okada et al. 1997). In addition, contamination during storage, marketing and container leaching can be considered a source of Cd in milk and other dairy products.

Chromium is a trace element whose main function is major for the proper functioning of the body: it regulates the secretion of pancreatic insulin in order to maintain a constant blood sugar level (Pallavi et al. 2020). However, its chronic toxicity is characterized by renal and hepatic lesions as well as nervous tissue disorders (Azeh et al. 2019). The mean concentrations of Cr determined vary between 0.024 and 0.027 mg/l for milk powder and pasteurized milk, then they are higher than the values reported by Ghosia et al. (2014), which found values of 0.0014 and 0.0001 mg/l for both samples mentioned above, and similar to the value reported for Rubina et al. (2016) where they found a value between

Table 2 Heavy metal concentration detected in milk and its derivatives

	Cu	Zn	Fe	Mg	Cd	Cr	Pb
Water	$0.447\pm0.016^{(e)}$	$0.377\pm0.053^{(a)}$	$0.386\pm0.007^{(c)}$	$1.089\pm0.016^{(a)}$	$0.006\pm0.0015^{(a)}$	$0.0062\pm0.0008^{(a)}$	$0.184\pm0.008^{(a,b)}$
0% milk	$0.089\pm0.007^{(c)}$	$0.471\pm0.038^{(a)}$	$0.561\pm0.01^{(f)}$	$9.223\pm0.203^{(f)}$	$0.022\pm0.0013^{(c,d)}$	$0.024\pm0.003^{(ec)}$	$0.072\pm0.028^{(a)}$
26% milk	$0.033\pm0.004^{(a,b)}$	$0.534\pm0.025^{(a)}$	$0.254\pm0.009^{(a)}$	$7.184\pm0.094^{(a)}$	$0.041\pm0.0012^{(e)}$	$0.024\pm0.0016^{(b,c)}$	$0.086\pm0.038^{(a)}$
Raw milk	$0.020\pm0.006^{(a)}$	$3.18\pm0.242^{(c)}$	$0.219\pm0.015^{(a)}$	$2.322\pm0.027^{(a)}$	$0.012\pm0.0013^{(a,b)}$	$0.015\pm0.001^{(a,b)}$	$0.251\pm0.0768^{(b)}$
Pasteurized milk	$0.042\pm0.006^{(b)}$	$2.69\pm0.24^{(b)}$	$0.512\pm0.011^{(e)}$	$120.77\pm1.248^{(e)}$	$0.014\pm0.0047^{(b)}$	$0.028\pm0.0013^{(c)}$	$0.256\pm0.036^{(b)}$
Cheese	$0.148\pm0.007^{(d)}$	$7.243\pm0.225^{(d)}$	$0.438\pm0.019^{(d)}$	$65.443\pm0.684^{(d)}$	$0.016\pm0.0034^{(b,c)}$	$0.008\pm0.006^{(a)}$	$0.228\pm0.047^{(b)}$
Whey	$0.022\pm0.005^{(a,b)}$	$0.292\pm0.023^{(a)}$	$0.343\pm0.014^{(b)}$	$55.326\pm0.565^{(b)}$	$0.024\pm0.0004^{(d)}$	$0.013\pm0.0043^{(a)}$	$0.14\pm0.037^{(c)}$
Water standard $^{(*)}$	2	5	0.2	50	0.005	0.05	0.01
Milk standard $^{(**)}$	0.052	4.2	0.21	120	0.01	0.017	0.02

(*)UE, 1998; (******) Renner et al. 1989.

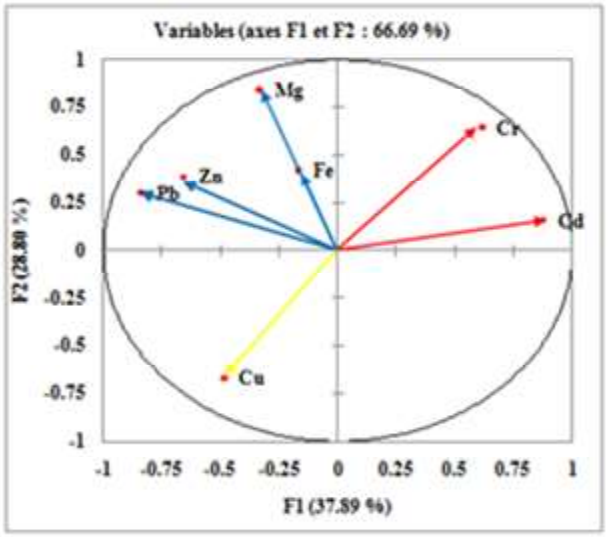

Fig.2 Graphical representation of the analysis of heavy metals in milk and its derivatives (distribution of inertia between the F1 and F2 axes)

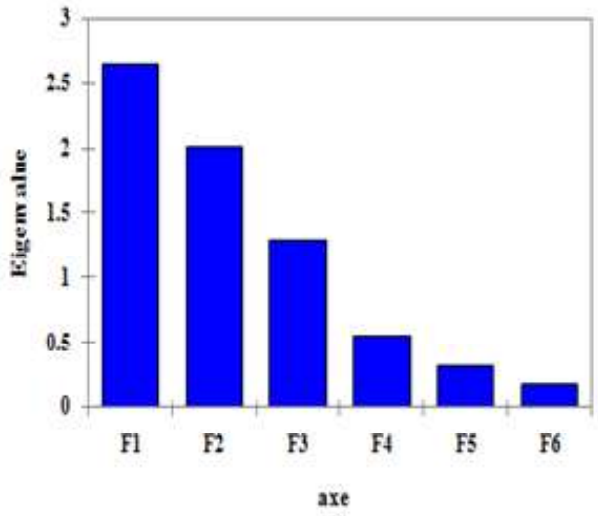

Fig.3 Eigenvalues of the two components F1 and F2

Table 3 Distribution of heavy metal inertia between the F1 and F2 axes in milk and these derivatives

	F1	F2
Eigenvalue	2.652	2.016
Variability (%)	37.889	28.802
Cumulated (%)	37.889	66.691

0.001 and 0.232 mg/l. However, our results are inferior to those found by Younus M et al. (2013) for values of 3.750 and 8.400 mg/l.

All milks contained lead residues in excess of the levels predicted in the Codex Standard, (1995). According to Swarup et al. (1997) and Oskarsson et al. (1998) the main sources of milk lead contamination are industrial origin, including the handling of paint, gasoline or batteries. In addition, in their technical report published in 1972, FAO and WHO indicated that the possible presence of lead residues in milk comes from three possible sources of contamination: air, food, and water (FAO/WHO, 1972). The Pb concentrations found in this work exceed the tolerated standard according to the IRAC Codex standard, (1992), which is 0.02 mg/l for milk and its derivatives, and according to the UE standard, (1998), which is 0.01 mg/l for water. Given the lead concentration values obtained in this work for powdered and pasteurized milk, its values are significantly higher than those found by Ghosia et al. (2014), whose Pb concentration they determined in similar samples and found 0.017 and 0.0006 mg/l respectively. As regards the concentration of lead in the 0% powdered milk sample, is similar to that obtained by Salah et al. (2013), whose value obtained is 0.791 mg/l.

From the results, it is evident that the samples of milk and its derivatives examined were contaminated with lead. Higher values in powdered milk were raised as a consequence of contamination during handling, exposure and processing (Onianwa et al. 1996). These contaminations constitute a danger for the consumer because according to Vidal, (2008), the accumulation of lead residues in the body through the milk consumption could cause several diseases, including autism and neurodegenerative diseases.

Analysis and typological structure of the metallic contamination of milk and its derivatives

Principal component analysis (PCA) allowed us to classify and process the information on the heavy metals studied by establishing correlations between the all variables. This PCA is carried out on a data matrix made up of 7 food products (water, milk and its derivatives) during which the seven variables (Cu, Zn, Fe, Mg, Pb, Cr and Cd) were measured. The eigenvalues of the two components F1 and F2 and their contribution to the total inertia are shown in Table 3 and Figure 3.

The two axes taken into consideration to describe the correlations between variables related to spatial structures, hold alone 66.69% of the total information with respectively 37.89% for axis 1 and 28.80% for axis 2. Examination of the correlation matrix between variables (Table 3) reveals the presence of a significant correlation between Cu/Pb (r=0.069), Zn/Fe (r=0.083), Zn/Mg (R=0.401), Fe/Cu (r=0.160), Fe/Mg (r=0.468), Fe/Cr (r=0.272), Mg/Cr (r=0.278), Mg/Pb (r=0.492) and between Cd/Cr (r=0.5), and a moderately significant correlation between Zn/Pb (r=0.646).

In the factorial plan “F1 x F2” (Figure 2 and Table 4),

The first component (Axis F1) partially described the contamination of milk and its derivatives with Chromium (0.615) and with Cadmium (0.875) With an inertia of 37.88 %. The second component (Axis F2) described the enrichment of milk and its

Table 4 Correlation matrix (Pearson (n)) between heavy metal variables in milk and its derivatives

Variables	Cu	Zn	Fe	Mg	Cd	Cr	Pb
Cu	1	-0.088	0.160	-0.295	-0.562	-0.623	0.069
Zn	-0.088	1	0.083	0.401	-0.297	-0.291	0.646
Fe	0.160	0.083	1	0.468	-0.254	0.272	-0.077
Mg	-0.295	0.401	0.468	1	-0.132	0.278	0.492
Cd	-0.562	-0.297	-0.254	-0.132	1	0.500	-0.696
Cr	-0.623	-0.291	0.272	0.278	0.500	1	-0.268
Pb	0.069	0.646	-0.077	0.492	-0.696	-0.268	1

Table 5 Variable codes and their coordinates

Variables	Codes	F1	F2
Copper	Cu	-0.485	-0.672
Zinc	Zn	-0.657	0.385
Iron	Fe	-0.169	0.423
Magnesium	Mg	-0.336	0.842
Cadmium	Cd	0.875	0.156
Chromium	Cr	0.615	0.643
Lead	Pb	-0.837	0.299

derivatives with Zinc, Iron, Magnesium, Cadmium, Chromium and Lead. The variable codes and their coordinates are shown in Table 5.

From left to right, the F1 axis thus translates an increasing gradient of milk and its derivatives into Cd and Cr. From bottom to top, the F2 axis therefore reflects an increasing gradient of contamination of milk and its derivatives by other metals (Figure 2). The typological structure shown by plan F1xF2 shows the individualization of three areas according to their degree of contamination (Figure 4):

An area formed by individuals (Milk 0 and 26% and whey) where there is a medium pollution in Cd, Cr. An area formed by individuals (Pasteurized milk and cheese) characterized by pollution in Pb, Fe, Zn and Mg. Finally, an area formed by individuals (raw milk and water) where there is a low pollution in Cu.

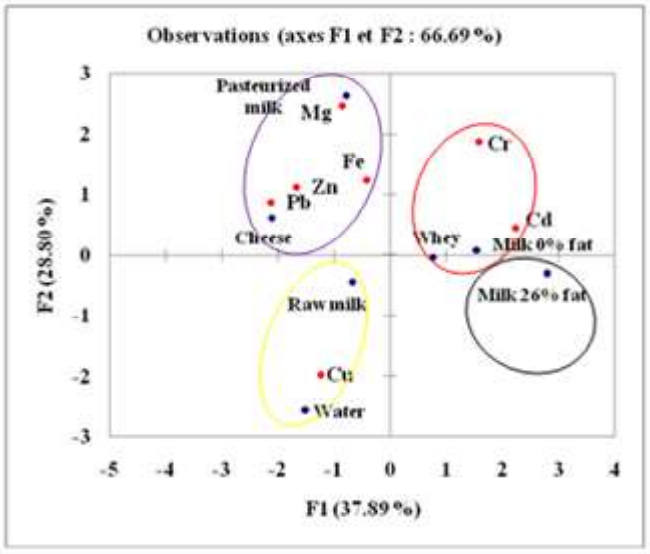

Fig.4 Milk and its derivatives factor map

Human Health Risk Assessment

The Estimated Daily Intake (EDI) values for consumers, the Non-Carcinogenic Risk Quotient (NRQ), and the Carcinogenic Risk to Human Health (CR) of heavy metals due to the consumption of raw milk, pasteurized milk and cheese in Algeria are shown in Table 6.

The daily consumption of raw milk, pasteurized milk and cheese in Algeria is low, estimated at 0.5; 0.6 L/day; and 6850 mg/day respectively. Non-carcinogenic risk quotients (NCR) were developed to estimate the potential health risks associated with long-term exposure to heavy metals. An NCR <1 indicates that the exposed population can be assumed to be safe, whereas a potential health risk exists and intervention measures are mandatory if NCR e" 1(Akele et al. 2017).

In this study, the non-cancer risk quotient (NCR) value for each metal was less than 1 (Table 6) for all samples (pasteurized milk, raw milk). They are also lower than those found by Akele et al. (2017). On the other hand, the NCR value in cheese clearly exceeds the value 1, so the risk certainly exists and safety measures must be put in place to protect the consumer.

The first value of NCR was below the alert threshold for the first two samples (raw milk and pasteurized milk). The potential health risks attributable to trace elements via milk consumption should not be underestimated in the long term. With regard to the carcinogenic risk factor (CR) the values found in the cheese fraction exceed the threshold tolerated by United States Environmental Protection Agency (USEPA) which is 10^{-6} (USEPA, 2013).

Table 6 EDI, NCR and CR heavy metal values

Metal	Raw milk			Pasteurized milk			Cheese		
	EDI	NCR	CR	EDI	NCR	CR	EDI	NCR	CR
Cu	1.412E-4 ± 3.942E-5	3.53E-06 ± 9.855E-07	-	3.633E-4± 5.272E-5	9.08E-6 ± 1.318E-6	-	14.490 ± 0.6604	0.362 ± 0.016	-
Zn	2.271E-2 ± 1.73E-3	7.57E-5 ± 5.781E-6	-	2.305E-2 ± 2.062E-3	7.69E-5 ± 6.87E-6	-	708.74 ± 22.0607	2.362 ± 0.073	-
Fe	1.566E-3 ± 1.109E-4	2.24E-6 ± 1.584E-7	-	4.386E-3 ± 9.474E-5	6.27E-6 ± 1.353E- 7	-	42.837 ± 1.8687	0.0611 ± 2.668E-3	-
Mg	1.658E-2 ± 1.917E-4	1.96E± 00	-	1.035 ± 1.069E-2	2 .535E-3± 0.00	-	6.40E+3 ± 66.918	30,582± 0.00	-
Cd	8.64E-5 ± 9.285E-6	8.64E-5 ± 9.285E-6	1.99E-8± 2.143E-9	1.237E-4± 4.09E-05	1.24E-4 ± 4.09E-5	2.85E-8± 9.438E-9	1.55 ± 0.3422	1.552 ± 0.342	5.475 ± 0.00
Cr	1.07E-4 ± 7.145E-6	3.57E-5 ± 2.381E-6	-	2.376E-4 ± 1.081E-5	7.92E-5 ± 3.603E-6	-	0.795 ± 0.5812	0.265 ± 0.193	-
Pb	1.795E-3 ± 5.487E-4	4.49E-4 ± 1.371E-4	7.04E-8± 2.153E-8	2.1997E-3± 3.1164E-4	5.50E-4 ± 7.791E-5	8.63E-8 ± 1.222E-8	224 ± 4.683	5.591 ± 1.170	0.93 ± 0.00

Conclusions

This study provides very important information on the levels of heavy metals (lead, chromium and cadmium) and trace elements (zinc, copper, iron and magnesium) in milk and its derivatives. Zinc and copper were the metals detected in the highest amounts in cheese, while cadmium, lead and zinc were present in higher amount in all samples. Significantly higher levels of iron were observed in the unit water. The results of the study clearly indicate that the consumption of milk and milk products corresponds to a significant proportion of the tolerable daily intake of heavy metals. The consumption of milk and milk products in this area is practically at risk, and the bioaccumulation of lead and cadmium in the milk chain and the intake of other foodstuffs should also be of concern. Particular attention should be paid to heavy metals, as once they are present in concentrations above the acceptable daily intake, they must be reduced to acceptable levels during processing.

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RESEARCH ARTICLE

Influence of WPC and coagulation temperature on the physico-chemical and sensory quality of goat milk *chhana*

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Abstract: This study was carried out to select a suitable level of WPC in milk and coagulation temperature for manufacture of *chhana* prepared from Surati goat milk. WPC was evaluated at three levels of addition, viz. 0.25, 0.50 and 0.75 % w/w of milk and three coagulation temperatures were evaluated viz. 75, 80 ºC and 85 ºC. A significant reduction in moisture content as well as increase in fat and protein content of *chhana* was observed with increase in the level of WPC and coagulation temperature. Their interaction also showed similar effect. *Chhana* prepared using a coagulation temperature of 85ºC had the highest pH (i.e. 5.69) and acidity (0.51% lactic acid) which was significantly higher compared to *chhana* samples prepared using lower coagulation temperatures viz. 75 and 80ºC. *Chhana* prepared from milk incorporated with 0.5% WPC and coagulated at 80ºC was preferred the most with respect to sensory attributes.

Keywords: *Chhana*; Coagulation temperature; Goat milk; Quality; Whey protein concentrates; Soft cheese

Introduction

Chhana, a direct acidified traditional Indian soft cheese, is used as a base material for the preparation of several sweets such as *sandesh*, *rasogolla*, *cham cham*, *kheermohan* etc. Since the quality of sweets largely depends on the quality of *chhana*, it is highly essential to produce excellent quality *chhana*.

The popularity of goat milk is increasing due to its superior digestibility and absorption of micronutrients. The superior digestibility of goat milk is because of the nature of the proteins and the fat molecules (Park et al. 2007). Goat milk does not contain the protein agglutinin that promotes clustering of fat globules. The absence of cluster facilitates rapid digestion and absorption. Goat milk contains high levels of caprylic and capric acids, which are known for their antimicrobial quality. These fatty acids are used in dietary supplements to inhibit the growth of *Candida albicans* and other yeast species. Goat milk helps to increasing the pH of the blood stream. Lower blood and intestinal pH levels have been associated with fatigue, headaches, muscle aches and blood sugar imbalances (Mwenze, 2015).

Chhana made from goat and cow milk had significantly ($P<0.05$) higher moisture contents than buffalo milk *chhana*. Devangare et al. (1995) obtained acceptable quality of *chhana* from goat milk by partial replacement of cow milk (26 to 50%). *Chhana* was prepared from Jamunapari and Barbari goat milk using different level of coagulant concentration and coagulation temperature. Jamunapari milk *chhana* was rated better than Barbari milk *chhana* for colour and appearance as well as texture while flavour was almost at par. Lactic acid produced a granular product while other coagulants gave a smooth-textured *chhana* (Sharma et al. 1995). Coagulating milk at higher temperature, i.e. above 80°C, yielded lower moisture content and firm body (Sharma et al. 1998). Whey protein concentrate (WPC) has been used in many food applications as a functional ingredient due to its superior nutritional quality and excellent functional properties. Incorporation of WPC resulted in higher yield and better organoleptic properties of cheese (Korhonen et al. 1998; Vidigal et al. 2012). WPC can serve as a texture enhancer also in soft cheeses (Herceg et al. 2007)

Chhana samples prepared from goat milk possess very loose and sticky body which is difficult to handle. Hence, this investigation was carried out to formulate an acceptable quality *chhana* from goat milk using WPC as a functional ingredient and standardize

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certain processing parameters to manufacture acceptable quality goat milk *chhana*.

Materials and Methods

Fresh Surati goat milk was obtained from livestock research station of the university. The fat and SNF content of the milk was varied from 3.5 to 4.2% and 8.5 to 8.7% respectively. The goat milk was pasteurized at 75°C/15 s and stored at 4±2°C until used. WPC-80 was obtained from Charotar Casein Company, Malharpura, Nadiad, India containing 80.3% protein. Citric acid, supplied by Loba-Chemical Pvt. Ltd., Mumbai was used as a coagulant.

Method for manufacture of goat milk *chhana*

Calculated quantity of WPC at different rates, viz. 0.25, 0.50 and 0.75% w/w of goat milk, were blended with milk at about 40°C (about 50 times of its weight) and allowed to hydrate for 30 min before mixing in milk. Goat milk was pre-heated to 90°C/5 min with continuous agitation followed by cooling to coagulation temperature (viz. 75, 80 or 85°C). Coagulation was carried out by adding freshly prepared 1.0% citric acid solution until clear whey was obtained with constant stirring. Whey was immediately drained after coagulation. The coagulated mass was kept hanging in a muslin cloth for 20 min. The *chhana* so obtained were packed in polyethylene pouches and stored in refrigerator (5±1°C).

The fat, SNF and moisture content of goat milk and goat milk cream; fat, protein and ash content of goat milk *chhana*; titratable acidity and pH of goat milk and *chhana* were estimated as per the procedure described by FSSA (2015).

Sensory evaluation

Chhana samples were tempered to 25±2°C before judging. Sensory analysis of samples was performed in isolated booths illuminated with incandescent light maintained at 23±2°C. *Chhana* cubes (25 g) were served in petri dishes. The plates were labelled with randomized three-digit codes. The order in which samples were presented was randomized across the subjects. Subjects assessed a maximum of 5 samples in one session. The sensory panel (n=10) was composed of staff members working in the institution. The selection criterion was that the subject has to be regular consumer of the product and consistent in behaviour during sensory evaluation sessions. Lukewarm water was used as a rinsing agent. The sensory attributes of the *chhana* were evaluated using the 9-point hedonic scale scorecard.

Statistical analysis

The mean values generated from the analysis of duplicate samples of *chhana* were subjected to statistical analysis using completely randomized design (Steel and Torrie, 1980).

Results and Discussion

Effect of level of fat in goat milk on quality of *chhana*

Preliminary trials revealed that *chhana* prepared from goat milk containing less than 3.0% fat was unacceptable due to hard body, coarse and rubbery texture as well as flat and unacceptable flavour. Hence, it was not suitable for further use. Fat content above 4.0% yielded *chhana* with very soft, pasty and sticky body which caused difficulty in kneading and ball formation. This could be because goat milk derives many of its most distinctive properties from its lipid fraction. Average size of fat globules is 3.5 µm in goat milk (Park and Guo, 2006). This effect could also be attributed to the fact that goat milk has slightly lower casein content than cow milk, with a very low proportion or absence of αs_1-casein, and a higher degree of casein micelle dispersion (Remeuf and Lenoir, 1986; Vegarud et al. 1999). Moorthy and Rao (1982) prepared goat milk *chhana* from goat milk standardized to 3, 4 and 5% fat and reported that better quality *rasogolla* was obtained from *chhana* made from 4% fat. Bhargava et al. (1992) also postulated that goat milk with 3 to 4% fat could successfully prepare *chhana* suitable for *rasogolla* making. Hence, in current investigation, a level of 3.5% fat was selected and used for manufacture of goat milk *chhana*.

Effect of WPC levels and coagulation temperature on yield and recovery of total solids

Yield

The average yield of goat milk *chhana* varied from 12.23% to 17.70%. The yield of *chhana* decreased with increase in level of WPC and coagulation temperature. *Chhana* coagulated at 75°C without addition of WPC gave maximum yield whereas the lowest yield was observed in *chhana* coagulated at 85°C in which WPC was incorporated @ 0.75% w/w of milk. *Chhana* made from goat milk without addition of WPC had significantly ($P<0.05$) higher yield than all the other levels of WPC, whereas *chhana* prepared using the highest level of WPC had significantly ($P<0.05$) lower yield than all the other levels. The yield of *chhana* decreased significantly ($P<0.05$) with increase in coagulation temperature (Table 1).

The yield of *chhana* was slightly higher than that reported by Bhargava et al. (1992) who prepared *chhana* from goat milk containing 3 and 4 % milk fat which could be ascribed to the use of WPC as well as differences in breed of goat, SNF content of milk, method of manufacture and coagulant used. Sharma et al. (1998) also reported reduction in the yield of *chhana* with an increase in coagulation temperature.

Total solids recovery

The rate of addition of WPC as well as coagulation temperature significantly ($P<0.05$) increased the total solids (TS) recovery in *chhana.* The highest TS recovery was observed at coagulation temperature 85°C and WPC addition at 0.75% w/w of milk (Table

1). Several researchers obtained higher TS recovery with increase in cogulation temperature (Sharma et al. 1998; Agnihotri and Pal, 1996).

Effect of WPC levels and coagulation temperature on compositional attributes of goat milk *chhana*

Moisture

The moisture content of *chhana* significantly (P<0.05) decreased with increase in rate of addition of WPC in milk and coagulation temperature. Their interaction also had significant (P<0.05) impact as well (Table 2). Adding WPC to milk leads to incorporation of WPC in the pores of casein network of cheese matrix along with fat globules. This results in a higher protein/fat ratio which gives way to the compact and continuous relatively large area of protein matrix (Lobato-Calleros et al. 2007; Hanafy et al. 2016). The reduction in moisture content in *chhana* could partly be attributed to this phenomenon.

The addition of WPC to milk increased total solids content in buffalo milk soft cheeses (Tashakori et al. 2013). Moisture content gradually decreased (PÂ0.05), while TS, protein, fat, ash and lactose significantly increased by adding WPC to milk in non-fat fresh soft cheeses (Hanafy et al. 2016).

Increase in coagulation temperature from 70 to 90°C decreased moisture retention in goat milk *chhana* from 57.20 to 55.20% (Sharma et al. 1998). Goat milk paneer had lower moisture content when it was coagulated at higher temperatures (Agnihotri and Pal, 1996). WPC – treated rennet and acid cheeses had lower moisture content than control (Risch, 1992).

Protein

Protein content of *chhana* significantly (P<0.05) increased with increase in level of WPC and coagulation temperature. Their interaction also had significant (P<0.05) impact on protein content. A reduction in moisture content explains increase in the content of other constituents. WPC will also directly contribute to protein content of *chhana*. Similar trend was demonstrated by Sakr and Mehanna (2011) and Ismail (2012) in WPC-treated Ras cheese. Goat milk paneer had higher protein content when it was coagulated at higher temperature (Agnihotri and Pal, 1996).

Fat

Fat content of *chhana* significantly (P<0.05) increased with increase in level of WPC and coagulation temperature. Their interaction also had significant (P<0.05) impact on fat content. The lower fat content in experimental samples made using a coagulation temperature of 75°C could be attributed to the higher fat losses in whey. Higher coagulation temperature leads to low moisture retention in *chhana* resulting in higher total solids and fat content (Sharma et al. 1998). The values of fat obtained in the present study are comparable with the results obtained by Agnihotri and Pal (1996) who reported an average fat content of 24.5 to 29.5% fat in Barbari goat milk paneer prepared from milk containing fat ranging from 3.4 to 5.6%. Adding of WPC to milk significantly (P<0.05) increased fat content of rennet and acid cheeses (El-Sheikh et al. 2001; Yazici and Akgun, 2004). An opposite trend of results was observed by Lobato-calleros et al. (2007) when WPC was added in cheeses.

Table 1 Influence of WPC levels and coagulation temperature on yield and TS recovery of *chhana*

Level of addition of WPC (% w/w of milk)	Yield (%) Coagulation Temperature (°C)			Average for WPC
	75°C	80°C	85°C	
0	17.70±0.46^{p}	17.00±0.56^{p}	15.30±0.69^{p}	16.60^{a}
0.25	16.40±0.59^{p}	16.00±0.67^{p}	14.70±0.71^{q}	15.70^{a}
0.50	15.90±0.60^{p}	15.47±0.56^{p}	13.26±0.75^{q}	14.88^{b}
0.75	14.87±0.46^{q}	13.53±0.78^{q}	12.23±0.70^{r}	13.54^{c}
Average for coagulation temperature	16.21^{x}	15.50^{x}	13.87^{y}	
		CD (0.05) W=1.26; T=1.30; W x T=2.43		
		Total solids recovery (%)		
0	46.29±0.82^{p}	51.66±0.69^{q}	52.85±0.87^{q}	50.27^{a}
0.25	47.02±0.68^{p}	52.60±0.96^{q}	54.32±0.72^{r}	51.31^{a}
0.50	47.24±0.90^{p}	53.07±0.89^{q}	55.13±0.93^{r}	51.82^{b}
0.75	47.70±0.97^{p}	53.67±0.97^{q}	55.56±0.78^{r}	52.31^{b}
Average for coagulation temperature	47.06^{x}	52.75^{y}	54.46^{z}	
		CD (0.05) W=1.22; T=1.26; W x T=2.45		

Each observation is mean ±SD, n=4; W = level of WPC; T=coagulation temperature Each observation is mean±SD, n=4; a-d Values with same superscript in each column do not differ significantly (p<0.05); x-z Values with same superscript in each row do not differ significantly (p<0.05); p-r Values with same superscript in each row and column do not differ significantly.

Fat on Dry Matter (FDM)

Incorporation of WPC in goat milk had significantly (P<0.05) reduced FDM content in goat milk *chhana*. The FDM content of goat milk *chhana* coagulated at 80°C was significantly (P<0.05) higher than *chhana* made using coagulation temperatures of 75 and 85°C. The interaction effect of WPC and coagulation temperature had significant (P<0.05) effect on the FDM content of goat milk *chhana*. FDM in *chhana* is affected by various parameter like fat, TS, fat recovery, TS recovery moisture content of paneer as well as ratio of fat to casein content in milk. The fat and MSNF content of Surati goat milk used for *chhana* making was 3.5% and 8.5% respectively. The lower FDM content in goat milk *chhana* observed in this study could be attributed to the lower fat content in proportion of MSNF of milk used in this study.

Ash

Ash content of *chhana* significantly (P<0.05) increased with increase in level of WPC and coagulation temperature. Their interaction also had significant (P<0.05) impact on ash content.

Table 2 Influence of WPC levels and coagulation temperature on compositional attributes of *chhana*

Level of addition of WPC (%w/w of milk)	Coagulation temperature °C			Average for WPC
	75 °C	80 °C	85 °C	
		Moisture (%)		
0	66.20±2.38^{P}	61.11±2.50^{P}	55.87±1.97^{Q}	61.06^{a}
0.25	62.11±273^{P}	57.07±2.55^{Q}	51.84±1.82^{R}	57.01^{b}
0.50	59.85±2.62^{Q}	54.37±2.62^{Q}	44.76±2.14^{S}	53.00^{c}
0.75	55.52±1.90^{Q}	46.26±1.64^{R}	38.40±1.70^{T}	46.77^{d}
Average for coagulation temperature	60.92^{x}	54.70^{y}	47.71^{z}	
		CD(0.05) W = 2.89; T = 3.03; WxT = 5.90		
		Protein (%)		
0	16.94±0.86^{P}	17.75±0.78^{P}	19.75±0.99^{P}	18.15^{a}
0.25	19.17±0.95^{P}	19.84±0.81^{Q}	21.66±0.92^{Q}	20.22^{b}
0.50	20.69±0.83^{Q}	21.50±0.84^{Q}	25.16±1.08^{R}	22.45^{b}
0.75	23.20±0.82^{R}	25.75±0.86^{R}	28.58±1.25^{S}	25.85^{c}
Average for coagulation temperature	20.00^{x}	21.21^{x}	23.79^{y}	
		CD(0.05) W = 1.40; T = 1.46; WxT = 2.84		
		Fat (%)		
0	14.92±0.75^{P}	19.37±1.19^{Q}	21.54±1.21^{Q}	18.61^{a}
0.25	16.51±0.90^{P}	21.23±0.92^{Q}	23.12±1.24^{R}	20.28^{a}
0.50	17.50±0.94^{P}	21.92±0.94^{Q}	25.62±1.18^{R}	21.68^{b}
0.75	19.50±0.86^{Q}	25.04±1.23^{R}	27.79±1.31^{S}	24.11^{c}
Average for coagulation temperature	17.11^{x}	21.89^{y}	24.52^{z}	
		CD(0.05) W = 1.67; T = 1.73; WxT = 3.35		
		FDM (%)		
0	44.16±1.37^{P}	49.82±1.15^{Q}	48.82±1.13^{Q}	47.60^{a}
0.25	43.59±0.84^{P}	49.45±1.13^{Q}	48.06±1.09^{Q}	47.03^{a}
0.50	43.49±0.90^{P}	48.03±1.15^{Q}	46.38±1.10^{P}	45.97^{b}
0.75	43.84±1.09^{P}	46.61±1.12^{P}	45.12±1.12^{P}	45.19^{b}
Average for coagulation temperature	43.77^{x}	48.47^{y}	47.09^{y}	
		CD(0.05) W = 1.11; T = 2.14; WxT = 3.26		
		Ash (%)		
0	0.92±0.12^{P}	1.10±0.13^{P}	1.25±0.14^{Q}	1.09^{a}
0.25	0.88±0.10^{P}	1.14±0.15^{P}	1.32±0.13^{Q}	1.11^{a}
0.50	0.78±0.10^{P}	1.19±0.11^{P}	1.42±0.14^{Q}	1.13^{a}
0.75	0.89±0.15^{P}	1.37±0.12^{Q}	1.57±0.15^{Q}	1.28^{b}
Average for coagulation temperature	0.87^{x}	1.20^{x}	1.39^{y}	
		CD(0.05) W = 0.12; T = 0.35; WxT = 0.45		

Each observation is mean ±SD, n=4; W = level of WPC; T=coagulation temperature Each observation is mean±SD, n=4; a-d Values with same superscript in each column do not differ significantly (p<0.05); x-z Values with same superscript in each row do not differ significantly (p<0.05); P-T Values with same superscript in each row and column do not differ significantly.

The values of ash obtained in the present study are comparable with the results obtained by Agnihotri and Pal (1996), who reported an average ash content of 1.53 to 2.23%. Hanafy et al. (2016) made non-fat fresh cheese from skim milk powder with incorporation of WPC (34) and reported that addition of WPC in cheese milk increased ash content 0.60 to 1.10 per cent in non-fat fresh cheese compared to cheeses prepared without addition of WPC in milk. Hinrichs (2001) reported that addition of WPC in cheese increase the ash content of cheese. Sharma et al. (1998) made *chhana* from Jamunapari and Barbari goat milk coagulated at 70 ºC, 80 ºC and 90 ºC and reported that *chhana* coagulated at higher temperature had higher ash content.

Effect of WPC levels and coagulation temperature on pH and acidity of goat milk chhana

pH

The average pH values of *chhana* samples prepared without addition of WPC was 5.70 which was significantly (P<0.05) higher than the average values for samples incorporated with WPC. Experimental samples of *chhana* made at coagulation temperature of 85°C had the highest pH i.e. 5.69 which was significantly (P<0.05) higher than *chhana* samples prepared using lower coagulation temperatures viz. 75 and 80 ºC. Interaction effect between level of WPC in milk and coagulation temperature on pH was found to be non-significant (Table 3). Agnihotri and Pal (1996) also reported that pH of paneer made from goat milk ranged from 5.69 to 6.13. This effect could be attributed to the fact that goat milk is slightly alkaline since the pH is higher than bovine milk due to the higher buffering capacity of goat milk proteins (Park, 1994).

Hanafy et al. (2016) reported that the addition of WPC in non-fat fresh cheese had a significant effect on the pH of soft non-fat fresh cheese and when the level of WPC increases in cheese, there was a significant reduction in pH was noticed. Tashakori et al. (2013) observed that Feta cheese with WPC had lower pH values than control. Elbarbary and Saad (2019) reported that the addition of camel milk WPC in buffalo milk cheese leads to decrease pH in cheese. Similar observations were recorded in the current experiment. Thus, the results obtained in this part of the study corroborates with those reported in the literature.

Acidity

Experimental *chhana* samples prepared without addition of WPC had an average acidity of 0.44 % LA which was significantly (P<0.05) higher than samples prepared from milk incorporated with WPC. *Chhana* made at 85 °C coagulation temperature had an average acidity of 0.51 % LA which was significantly (P<0.05) higher than *chhana* prepared using lower coagulation temperature i.e. 75 and 80 °C. The interaction effect of WPC and coagulation temperature on the acidity of *chhana* was found to be non-significant (Table 3).

Hanafy et al. (2016) reported that the addition of WPC in non-fat fresh cheese had significant effect on the acidity of cheese. When the level of WPC increased in cheese there was a significant increase in acidity of cheeses. Tashakori et al. (2013) also observed

Table 3 Effect of WPC levels and coagulation temperature on pH and acidity of goat milk *chhana*

Level of WPC (% w/w of milk)	Coagulation Temperature (^{0}C)			Average for WPC
	75°C	80°C	85°C	
		pH		
0	5.66±0.09	5.72±0.14	5.75±0.09	5.70^a
0.25	5.60±0.11	5.64±0.12	5.68±0.07	5.64^a
0.50	5.54±0.08	5.60±0.11	5.64±0.07	5.59^b
0.75	5.30±0.07	5.53±0.12	5.60±0.08	5.48^c
Average for coagulation temperature		5.52^x	5.60^x	5.69^y
		CD (0.05) W=0.09; T=0.11; W x T = NS		
		Acidity (% LA)		
0	0.43±0.07	0.43±0.08	0.47±0.09	0.44^a
0.25	0.46±0.09	0.48±0.09	0.49±0.09	0.48^a
0.50	0.49±0.08	0.50±0.09	0.51±0.08	0.50^a
0.75	0.51±0.07	0.52±0.07	0.55±0.09	0.53^b
Average for coagulation temperature		0.47^x	0.48^x	0.51^y
		CD (0.05) W=0.08; T=0.03; W x T = NS		

Each observation is mean ±SD, n=4; W = level of WPC; T=coagulation temperature Each observation is mean±SD, n=4; a-d Values with same superscript in each column do not differ significantly (p<0.05); x-z Values with same superscript in each row do not differ significantly (p<).

that Feta cheese with WPC had lower pH values and higher acidity than control. Elbarbary and Saad (2019) reported that the addition of camel milk WPC in buffalo milk cheese leads to increased acidity in cheese. Similar results were observed in the current experiment. Thus, the results obtained in this part of the study corroborates with those reported in the literature.

Effect of WPC levels and coagulation temperature on organoleptic characteristics of goat milk *chhana*

The organoleptic characteristics of *chhana* samples have been presented in Table 4.

Colour and appearance

Incorporation of WPC and coagulation temperature and their interaction had significant (P<0.05) impact on colour and appearance of the product. Maximum colour and appearance score was obtained when WPC was added at the rate of 0.5% and coagulation temperature was 80°C. The colour was dull at lower coagulation temperature while there was dry appearance at higher coagulation temperature. A very moist appearance was observed at lower level of addition of WPC while the product was very dull and unattractive when WPC was added at higher level. Sharma et al. (1995) obtained similar results, who prepared *chhana* from Jamunapari and Barbari goat milk using different coagulation temperatures. They reported that coagulation temperatures 70°C and 80°C yielded *chhana* with better appearance compared to 90°C. Jailkhani and De (1980) also observed that that 80°C coagulation temperature gave the highest score for appearance and colour as compared with 75°C and 85°C. Sensory quality of caprine milk cheeses can be highly influenced by the types of feeds fed to the lactating animals. Off-flavour in goat milk can be attributed to the feeds, weeds, forages, chemicals, building materials, colostrum, estrus, mastitic milk, filth, utensils and

Table 4 Influence of WPC levels and coagulation temperature on sensory scores of *chhana*

Level of addition of WPC (%w/w of milk)	Coagulation Temperature °C			Average for WPC
	75 °C	80 °C	85 °C	
		Colour and Appearance score		
0	7.05±0.33^{p}	7.26±0.34^{p}	7.13±0.24^{p}	7.14^{a}
0.25	7.40±0.27^{p}	7.95±0.24^{q}	7.85±0.34^{q}	7.73^{b}
0.50	7.79±0.28^{q}	8.38±0.35^{r}	8.05±0.33^{q}	8.07^{c}
0.75	7.65±0.34^{q}	8.11±0.26^{q}	7.65±0.41^{q}	7.80^{b}
Average for coagulation temperature	7.47^{x}	7.92^{y}	7.67^{x}	
		CD(0.05) W = 0.26; T = 0.27; WxT = 0.51		
		Flavour score		
0	6.50±0.34^{p}	6.83±0.19^{p}	6.85±0.20^{p}	6.73^{a}
0.25	6.62±0.23^{p}	6.90±0.24^{p}	6.90±0.23^{p}	6.80^{a}
0.50	6.93±0.19^{p}	7.60±0.21^{q}	7.30±0.21^{q}	7.28^{c}
0.75	6.92±0.22^{p}	7.17±0.21^{q}	6.96±0.24^{p}	7.01^{b}
Average for coagulation temperature	6.74^{x}	7.13^{y}	7.00^{x}	
		CD(0.05) W = 0.21; T = 0.29; WxT = 0.49		
		Body and Texture score		
0	5.85±0.33^{p}	6.10±0.37^{p}	6.04±0.33^{p}	5.99^{a}
0.25	6.43±0.46^{p}	6.74±0.43^{q}	6.73±0.32^{q}	6.63^{b}
0.50	7.20±0.39^{q}	7.85±0.44^{r}	7.34±0.34^{q}	7.46^{c}
0.75	6.96±0.34^{q}	7.05±0.33^{q}	6.94±0.24^{q}	6.98^{b}
Average for coagulation temperature	6.61^{x}	6.93^{y}	6.76^{x}	
		CD(0.05) W = 0.37; T = 0.22; WxT = 0.64		
		Overall Acceptability score		
0	6.04 ±0.29^{p}	6.13 ±0.22^{p}	6.35 ±0.23^{p}	6.17^{a}
0.25	6.65 ±0.24^{q}	7.02 ±0.19^{r}	6.55 ±0.24^{q}	6.74^{b}
0.50	6.93 ±0.33^{q}	7.67 ±0.16^{s}	7.20 ± 0.29^{r}	7.27^{c}
0.75	6.73 ±0.24^{q}	7.16 ±0.18^{r}	6.74 ±0.17^{r}	6.87^{b}
Average for coagulation temperature	6.66^{x}	7.00^{y}	6.7^{x}	
		CD(0.05) W = 0.23; T = 0.25; WxT = 0.46		

Each observation is mean ±SD, n=4; W = level of WPC; T=coagulation temperature Each observation is mean±SD, n=4; a-d Values with same superscript in each column do not differ significantly (p<0.05); x-z Values with same superscript in each row do not differ significantly (p<0.05); p-s Values with same superscript in each row and column do not differ significantly.

strainer, unclean milking equipment, slow cooling, odors from bucks, barn and milk room. Feeding odorous feeds at least two hours before feeding is not recommended (Moatsou and Park, 2017).

Flavour

Incorporation of WPC and coagulation temperature and their interaction had significant (P<0.05) effect on the flavour of *chhana*. Maximum flavour score was obtained when WPC was added at the rate of 0.5%, and coagulation temperature was 80°C. A flat flavour was noted at lower coagulation temperature while there was typical goat-milk flavour at higher coagulation temperature. Sharma et al. (1995) prepared *chhana* from Jamunapari and Barbari goat milk using different coagulation temperatures. A slight better but non-significant (P<0.05) flavour score was obtained at 80°C coagulation temperature compared to 75°C. However, there was a significant (P<0.05) reduction in flavour score when *chhana* was coagulated at 85°C. Similar results were obtained in this study. Jailkhani and De (1980) also found 80°C temperature as the most effective for giving the highest scores for flavour which agrees with the present study. Interaction between level of WPC and coagulation temperature was found to have had significant (P<0.05) effect on the flavour scores of *chhana*. The decrease in flavour scores of *chhana* can be attributed to sharp goaty flavour, which gave an unpleasant aftertaste and was found to be unacceptable by panelists. Goat milk has a higher proportion of fatty acids, including capric, caprylic and caproic acids. The composition of these short chain fatty acids gives goat milk and its cheeses for their unique tangy flavour (Kosikowoski and Mistry, 1999). Goat milk with little or no αs_1-casein has a lower fat content and higher lipolysis levels, showing a more intense goaty flavour (Moatsou and Park, 2017). A progressive improvement in the flavour scores of samples was observed wherein WPC was incorporated up to 0.5%; after that, there was a slight reduction in flavour scores (i.e. from 7.28 to 7.01) with the increase in rate of addition of WPC. The improvement in flavour scores could be attributed to the masking effect of WPC on the goaty flavour of *chhana*. No reports are available for effect of WPC and coagulation temperature on the flavour scores of goat milk *chhana*. The results obtained in this part of the study agree with that reported by Elbarbary and Saad (2019) who stated that camel milk WPC improved the flavour of the buffalo milk soft cheese. Hanafy et al. (2016) also demonstrated that the flavour score after the addition of WPC was better than the control samples in non-fat fresh soft cheese. Sakr and Mehanna (2011) also found that adding WPC significantly improved the flavour of low-fat Ras cheese. In addition, it also enhanced the organoleptic properties of cheese. On the other hand, Pinto et al. (2007) replaced cheddar cheese solids with WPC solids at different levels 1.5, 3.0 and 4.5% in processed cheese spread and reported that when rate of WPC increased in processed cheese spread, there is significant reduction in flavour score this was because addition of WPC at higher levels imparted a milder flavour to the product. Thus, the results obtained in this part of the study are in agreement with those reported in the literature.

Table 5 Composition of goat milk *chhana*

Constituents	
Moisture, %	54.37
Protein, %	21.50
Fat, %	21.92
FDM %	48.03
Ash, %	1.19
Acidity (% lactic acid)	0.50
pH	5.60

Body and texture

Addition of WPC at different rates had significant (P<0.05) effect on body and texture score of goat milk *chhana*. Addition of WPC in *chhana* improved the body and texture score of *chhana* up to certain level. Experimental *chhana* samples made from addition of 0.50% WPC in milk had significantly (P<0.05) higher sensory score compared to *chhana* samples made from 0.0, 0.25, and 0.75% WPC. Similarly, coagulation temperature had significant (P<0.05) effect on body and texture score of goat milk *chhana*. *Chhana* coagulated at 80°C had significantly (P<0.05) higher body and texture score (i.e. 6.93) compared to *chhana* coagulated at 75°C and 85ºC. The interaction effect between level of WPC and coagulation temperature was found to significantly (P<0.05) influence the body and texture score of goat milk *chhana*.

In this study we found that there was a progressive improvement the body and texture scores of samples in which WPC was incorporated up to 0.5% rate of addition and coagulation temperatures of 80°C, thereafter at higher coagulation temperature and higher rate of addition WPC there as a significant reduction in body and texture scores of *chhana*. *Chhana* coagulated at 80°C containing 0.5% WPC had optimum softness with a smooth texture with desired chewiness. Similar observations were reported by Sharma et al. (1995). These authors showed that 80°C temperature was most suitable in giving the desired smooth texture in *chhana* made from Jamunapari and Barbari goat milk. Bhargava et al. (1992) also noted that the 80°C temperature was most suitable in giving the desired smooth texture in goat milk *chhana*.

No reports are available for effect of WPC and coagulation temperature on the flavour scores of goat milk *chhana*. However, the results obtained in this part of the study agree with Elbarbary and Saad (2019) who reported that the addition of camel milk improved body and texture score of buffalo milk soft cheese compared to product prepared without WPC. This improvement increased with an increase in the concentration of WPC. Hanafy et al. (2016) also reported a progressive improvement in body and texture scores of non-fat fresh cheeses with addition WPC-

34 @ 2, 4 and 6% in buffalo milk. Lobatto-Calleros et al. (2007) also found that WPC caused significant modification on the body and texture of partial and full skimmed soft cheese. Tashakori et al. (2013) found that cheese prepared by the addition of 20% WPC improved the body, texture, and consistency of spread cheese. However, higher rate of addition of WPC caused a significant reduction of body and texture and total scores in rennet and acid cheese samples. El-Sheikh et al. (2001) found that the addition of particulate whey protein concentrate improved the body and texture quality of low-fat Domiati cheese. Sakr and Mehanna (2011) found that adding WPC significantly improved the body and texture and flavour of low-fat Ras cheese. Ibrahem and Zubeir (2010) found that cheese made from concentrated milk containing 20% TS with replaced 20% WPC had an acceptable firmness and smooth texture and the consistency of cheese spread. Thus, the results obtained in this study are in agreement with those reported in the literature.

Overall acceptability

The critical difference calculated Table 4 indicated that the addition of WPC at different rate had significant ($P<0.05$) effect on overall acceptability scores of goat milk *chhana*. WPC was found to improve the overall acceptability scores up to 0.5% level of addition in milk, after that, higher rates of addition of WPC resulted in a significant decrease in overall acceptability scores of *chhana*. *Chhana* made from different level of coagulation temperature had significant ($P<0.05$) effect on the overall acceptability scores of *chhana*. Interaction effect between level of WPC and coagulation temperature was found to have significant ($P<0.05$) effect on the overall acceptability scores of *chhana*. Goat milk *chhana* made from the combination of 0.50% WPC and 80°C coagulation temperature had a significantly ($P<0.05$) higher overall acceptability score 7.67 compared to other treatment combinations. Hence, it was preferred the most concerning all the attributes studied from amongst all the experimental samples. The composition of goat milk *chhana* is presented in Table 5.

Conclusions

It can be concluded from the current investigation that *chhana* can be successfully prepared using goat milk which is suitable for further product preparation. Acceptable quality *chhana* can be prepared by coagulating milk at 80°C using 1% citric acid and with addition of whey protein concentrate at the rate of 0.5% w/w of the quantity of milk. The final composition of goat milk *chhana* is presented in Table 5. The final product was also acceptable from sensory perspective.

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RESEARCH ARTICLE

Physico-chemical and microbiological changes occurring in Kuru Kaymak (a traditional dairy product in Turkey) during storage

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Abstract: The aim of this study was to determine the changes in some physico-chemical and microbiological properties of Kuru Kaymak (KK) (Dry Clotted Cream) during 30 days of storage at 4°C. Kuru Kaymak samples were produced in a local family business on a small scale using the traditional method. The dry matter, fat, protein, lactose and ash content, and the water activity value of KK samples on the 30th day were 97.4%, 64.2%, 23.5%, 4.40%, 1.69% and 0.59, respectively. Unlike other high-fat dairy products, Kuru Kaymak samples were found to contain a fairly high amount of protein (23.5%). The free fatty acid value showed an enormous increase of 266.7% from 0 to 30 days. Yeast and mould counts of KK samples were below the countable level (<2 log cfu/g) throughout storage. The presence of coliform bacteria in KK samples showed that there was contamination due to non-compliance with hygienic rules in production. Considering the properties examined, it was concluded that KK could be stored for more than 30 days at 4°C without any noteworthy deterioration.

Keywords:Kuru Kaymak, Physico-chemical and microbiological properties, Storage, Traditional foods

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Introduction

Food, which has discrete characteristics that clearly distinguish it from other similar products, is called traditional food. Traditional foods have been consumed and produced locally and regionally for a long time, and are prepared using special ingredients and methods that are passed on to succeeding generations (Weichselbaum et al. 2009). Therefore, traditional foods can be regarded as a reflection of the culture, history and lifestyles of societies (Trichopoulou et al. 2007). Unfortunately, throughout world, some traditional foods are at risk of disappearing due to altered lifestyles, economic reasons, primitive production techniques and failure to satisfy consumers' concerns about food safety and nutrition. Therefore, it is important to study and document traditional foods to protect them from the threat of extinction (Trichopoulou et al. 2007, Weichselbaum et al. 2009).

Anatolia is very rich in traditional foods because it has thousands of years of history and also hosted many different cultures. Among these traditional foods, especially dairy products such as different cheese, yoghurt and kaymak have an important place. Kaymak or kajmak, which is a fat-rich creamy dairy product, is produced in certain parts of Anatolia by different techniques and names (such as Afyon Kaymagi, Lüle Kaymagi, Ispir Kaymagi and Kuru Kaymak), and is mostly consumed for breakfast (Cakmakci and Hayaloglu 2011, Atamer et al. 2016,). It should contain at least 60 g/100 g milk fat according to Turkish Food Codex (TFC 2003). In general, Kaymak has a slightly acidic flavour and a spreadable structure (Cakmakci and Hayaloglu 2011). It is not similar to other well-known milk fat based dairy products such as butter, samin and ghee in terms of both composition and shelf life (Akalýn et al. 2006). While its shelf life is only 1-2 weeks, the shelf life of the other products mentioned can be as long as 12 months. It is reported that Kaymak is also manufactured in the Balkans, Middle East, Iran, Afghanistan, Central Asia, and India (Cakmakci and Hayaloglu 2011).

Kuru Kaymak (KK) (Dry Clotted Cream) is a popular traditional dairy product in Nevsehir, Sivas, Erzurum and Erzincan province in Turkey. It is quite different from other Kaymak types in that it includes foaming and drying step in its production to form a crispy texture and to extend shelf life. Therefore, it has different

structural and physico-chemical properties. Unlike other high-fat dairy products, it also contains uniquely high levels of protein (Atamer et al. 2016). Very limited studies are available on KK. Atamer et al. (2016) reported that the protein content of KK samples ranged from 19.27 to 20.84%. Some chemical, physico-chemical and microbiological properties of 10 KK samples were determined by Yildirim et al. (2019) as 6.19-6.83 pH, 0.46-1.21% titratable acidity (as lactic acid), 67.30% dry matter, 46.39% fat, 2.70% ash and 3.87 log cfu/g coliform group bacteria. The gross chemical composition and microbiological quality of Ispir Kaymagi, a type of KK, were investigated by Cakmakci and Hayaloglu (2011). Mean dry matter, fat, protein and ash contents of samples were 86.1%, 54.4%, 17.8% and 2.34%, respectively. The microbiological counts were 4.02, 3.06, and 0.33 log cfu/g for aerobic plate count, yeast and moulds, and coliform, respectively.

Products with high lipid contents, like KK and butter, have a tendency to chemical or biochemical degradation. Although very limited, there are some research articles in the literature on the composition and production techniques of KK. However, to the best of the researchers' knowledge, no research on examining the changes occurring during the storage of KK is available in the published literature. For this reason, in the present study, changes in some physico-chemical and microbiological characteristics of KK were investigated during 30 days of storage.

Materials and Methods

Production of Kuru Kaymak

Kuru Kaymak samples were produced on a small scale in a local family business using the traditional method. First, after filtering through cheesecloth, 10 kg of cow's milk was boiled for 20 minutes (Fig. 1A). The hot milk was then slowly poured into a shallow tray from a height of about 60-100 cm to produce foam (Fig. 1B). The purpose of the foaming process is to make the texture of KK crisp like wafer after drying. The milk in the shallow tray was kept at 50-60°C for 2-4 h to accelerate the movement of the fat globules onto the milk surface (Fig. 1C). This step is very critical for the fat globules to form a thick cream layer on the milk surface. The resulting cream layer was then carefully separated from the wall of the tray with a knife (Fig. 1D). After that, the cream layer was removed in a semicircle shape with a thin wooden rolling pin (Fig. 1E) and allowed to dry in the shade on a wooden colander for 2 to 4 days. Approximately 350 g of KK was produced from 10 kg of cow's milk. Kuru Kaymak samples were transferred to the laboratory at 4°C in sterile plastic bags on the day of production, and cut into small pieces and stored in sterile plastic bags in a waterproof manner at 4±0.5°C for 30 days. While the dry matter, fat, protein, lactose, ash and water activity analysis of the samples were performed on the 0^{th} and 30^{th} days of storage; pH, titratable acidity, peroxide, free fatty acid and microbiological analysis of the samples were carried out on the 0^{th}, 10^{th}, 20^{th} and 30^{th} days of storage.

Proximate composition

The dry matter content of KK samples was determined by the oven-drying method at 102±2°C (IDF 1977). Van Gulik method was used to analyse the fat content (Ardö and Polychroniadou 1999). The ash content of samples was determined by heating in a furnace at 550±10°C (Ardö and Polychroniadou 1999). The micro-Kjeldahl method was used to analyse the nitrogen content of the samples (IDF 1993). The value of 6.38 was used as protein conversion factor. For pH measurement, 10 g of sample was macerated with 6 mL of distilled water and the pH of the resultant slurry was directly measured using a digital pH meter (pHenomenal pH 1000L meter, VWR International Ltd., Lutterworth, UK) (BSI 1976). Titratable acidity (as lactic acid, % w/w) was measured by titrating 5-10 g of sample with 0.1 N sodium hydroxide solution in the presence of alcoholic phenolphthalein solution (1%, w/v) as a turning point indicator (Atamer and Kaptan 1982). The total carbohydrate content of the samples (as lactose) was determined with Anthrone reagent (Joseph and Roe 1955). The analysis of free fatty acid was carried out by applying the method of the International Organization for Standardization (ISO 2004) as the number of grams of oleic acid per 100 g of fat. The peroxide value of the samples was determined by using the methodology proposed by the Official Methods of Analysis of the AOAC (No 965.33) (AOAC 1997). Water activity measurements were performed with a water activity meter (Novasina Thermoconstanter, model TH200, Axair Ltd., Pfaffikon, Switzerland).

Microbiological analysis

Samples of Kuru Kaymak (10 g) were aseptically homogenized in 90 mL of 1% (w/v) peptone water by using a stomacher (BagMixer 400 P, Interscience, Saint-Nom-la-Bretèche, France) and diluted by serially. For counting of total mesophilic aerobic bacteria, 0.1 mL of inoculum was added to the surface of Plate Count Agar (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h (Maturin and Peeler 2001); for counting of yeast and moulds, 0.1 mL of inoculum was added to the surface of Potato Dextrose Agar (Merck, Darmstadt, Germany) and incubated at 25°C for 5 days (Harrigan 1998). Total coliforms were enumerated in Lauryl Sulphate Tryptose Broth (Merck, Darmstadt, Germany) at 32°C for 48 h by the most probable number method (Feng et al. 2017).

Statistical Analysis

Kuru Kaymak production and all analysis were repeated three times. Statistical analysis of the results was performed using the SPSS 22.0 (SPSS Inc., Chicago, USA). Data obtained from physico-chemical and microbiological tests were evaluated by analysis of variance (ANOVA). Differences between the mean values for storage days were determined using the Duncan test and were considered significant when $p < 0.05$.

Results and Discussion

Physico-chemical properties

Physico-chemical properties of KK are given in Table 1. Since KK samples were packaged in a waterproof manner, the dry matter, fat, protein, lactose and ash contents, as well as the water activity (a_w) values of the samples were determined only on the 0th and 30th day of storage.

The dry matter, fat, protein, lactose, ash and a_w values of samples on the 30th day of storage were 97.4%, 64.2%, 23.5%, 4.40%, 1.69% and 0.59, respectively. There were no statistically significant differences between the samples in terms of these features on the 0th and 30th days of storage ($p > 0.05$). This result was expected due to the waterproof packaging of the samples. The fat content of KK (64.2%) was higher than the legal minimum 60% (w/w) fat limit specified in the Turkish Food Codex (TFC 2003). Unlike other high-fat dairy products, Kuru Kaymak samples were found to contain a fairly high amount of protein (23.5%). This is probably due to the fact that the foam formed during production is primarily protein-based (Atamer et al. 2016). Since not much work has been done previously on KK, it was not possible to compare all the data obtained in the present study with the results of other researchers. Dry matter (97.4%) and fat (64.2%) content of KK samples were significantly higher than dry matter (67.30%) and fat (46.39%) content reported by Yildirim et al. (2019). The ash content (1.69%) was lower than 2.70% found by Yildirim et al. (2019), and 2.34% reported by Cakmakci and Hayaloglu (2011). The protein content of KK (23.5%) was higher than the value reported by Atamer et al. (2016) (20.84%) and by Cakmakci and Hayaloglu (2011) (17.8%). The presence of 4.40% lactose in the samples on the 30th day of storage indicates that there are enough energy sources to support microbial growth.

As can be seen from Fig. 2, the pH values of KK samples varied between 6.47 and 6.70. The highest pH value was observed on the 0th day of storage with 6.70. Although pH values showed a steady decrease during storage, there was no statistically significant difference among storage days ($p > 0.05$). The pH values of KK samples were similar to values determined by Yildirim et al. (2019) and Cakmakci and Hayaloglu (2011). It is clear that the pH value of KK samples is quite suitable for the growth of microorganisms.

The titratable acidity (as lactic acid%, w/w) of KK samples varied between 0.28% and 0.36% during storage (Fig. 3). The lowest titratable acidity value was observed on the 0th day of storage with 0.28%, and the highest was observed on the 20th and 30th days of storage with 0.36%. Similar to the pH values, there was no statistically significant difference between the titratable acidities of the samples throughout storage ($p > 0.05$). This result showed that there was no substantial microbial activity or chemical reactions to increase titratable acidity during 30 days storage at 4°C. The titratable acidity values (0.28-0.36%) determined for KK samples during storage were lower than the values (0.46-1.21%) reported by Yýldýrým et al. (2019), but higher than the values (0.09-0.25%) reported by Cakmakci and Hayaloglu (2011).

High-fat foods tend to have chemical degradation characterized by oxidative rancidity and hydrolytic rancidity (rancid off-flavors). Lipid oxidation is one of the most important factors that determine the shelf life of high-fat foods. At the initial stage of oxidation, peroxides are formed. Peroxides subsequently decompose to form lower molecular weight compounds such as, aldehydes, ketones, alcohols and free fatty acid, leading to oxidative rancidity. Since the peroxide value is a chief indicator for lipid oxidation, it is

Table 1 Changes in some physico-chemical properties of Kuru Kaymak samples during storage

Properties	Storage Period (Day)	
	0	30
Dry matter (%, w/w)	97.4±0.28*	97.4±0.54
Fat (%, w/w)	64.9±0.17	64.2±0.49
Protein (%, w/w)	23.0±0.14	23.5±0.14
Lactose (%, w/w)	4.43±0.04	4.40±0.02
Ash (%, w/w)	1.67±0.02	1.69±0.02
Water activity (aw)	0.60±0.03	0.59±0.01

All the data are expressed as mean ± SD and are the mean of three replicates (n).
*According to the Duncan test, the means of all parameters showed an insignificant difference between days 0 and 30 ($p > 0.05$).

Table 2 Changes in some microbiological characteristics of Kuru Kaymak samples during storage

Microorganisms (log cfu/g)	Storage Period (Day)			
	0	10	20	30
Total mesophilic aerobic bacteria	5.67±0.04[a]	5.57±0.02[a]	5.14±0.04[b]	5.03±0.03[b]
Yeast and moulds	< 2	< 2	< 2	< 2
Total coliforms	1.66±0.01[a]	1.18±0.01[b]	1.18±0.01[b]	1.18±0.01[b]

All the data are expressed as mean ± SD and are the mean of three replicates (n).
[a,b] Means followed by different letters within the same line represent significant differences ($p < 0.05$) according to the Duncan test.

Fig. 1 Production stages of Kuru Kaymak

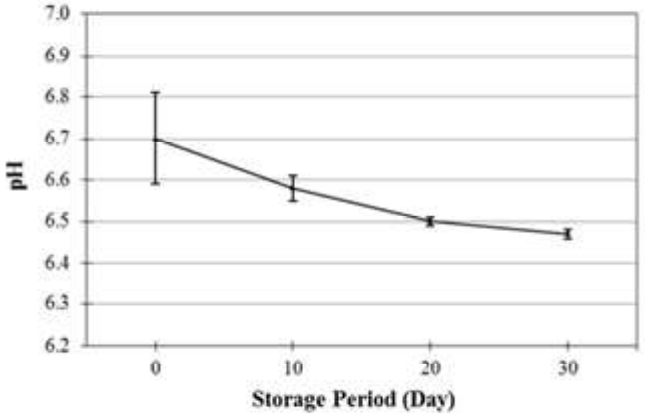

Fig. 2 Changes in pH values of Kuru Kaymak samples during storage. All the data are expressed as mean ± SD and are the mean of three replicates (n). Error bars represent standard deviations of values

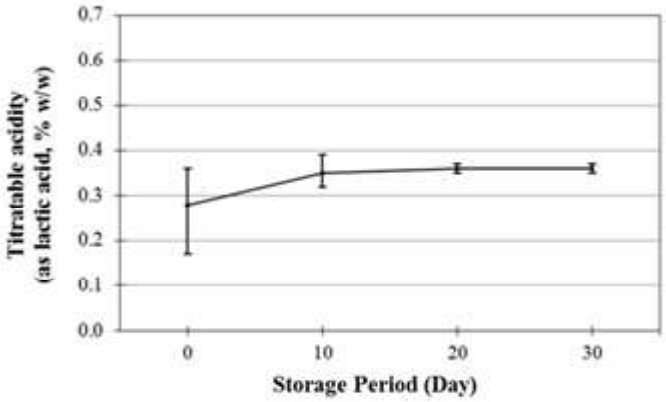

Fig. 3 Changes in titratable acidity (as lactic acid, % w/w) of Kuru Kaymak samples during storage. All the data are expressed as mean ± SD and are the mean of three replicates (n). Error bars represent standard deviations of values

widely used to monitor lipid oxidation (Ozkan et al. 2007). The products of peroxides, hydroperoxides and other compounds contribute to the papery, cardboard, or tallow off-flavors in high-fat dairy foods. The peroxide values of KK samples varied between 1.40 and 2.32 meq O_2 Kg^{-1} fat during 30 days of storage (Fig. 4). In parallel with the increase in storage time, peroxide value also showed a statistically significant increase ($p < 0.05$). Although the highest peroxide value was observed on the 30th day, this value did not differ significantly from the 20th day value ($p > 0.05$). The peroxide value showed an increase of 165.7% in the comparison between 0 and 30 days. The peroxide value (2.32 meq O_2 Kg^{-1} fat) reached on the 30th day of storage was slightly higher than those values reported by Erkaya et al. (2015) and Méndez-Cid et al. (2017), but lower than those values reported by Gosewade et al. (2017).

The free fatty acid value is a measure of the hydrolytic rancidity and is therefore an important quality criterion that determines the shelf life of high-fat dairy foods. The hydrolytic rancidity has been characterized as a bitter, sharp, astringent and soapy off-flavour. The free fatty acid values (as oleic acid in fat, % w/w) of KK samples varied from 0.15% to 0.40% during 30 days of storage (Figure 5). It was observed that free fatty acid values increased significantly with increasing storage time ($p < 0.05$). The highest free fatty acid value (0.40%) was determined on the 30th day of storage and this value was significantly different from values on other storage days ($p < 0.05$). The 0.40% free fatty acid level detected on the 30th day of storage, which is equal to the acid degree value (ADV) of 1.42, showed that the KK samples were in the extremely hydrolysed class (ADV > 1.40) according to the scale specified by Bradley et al. (1992). The free fatty acid value showed an increase of 266.7% in the comparison between 0 and

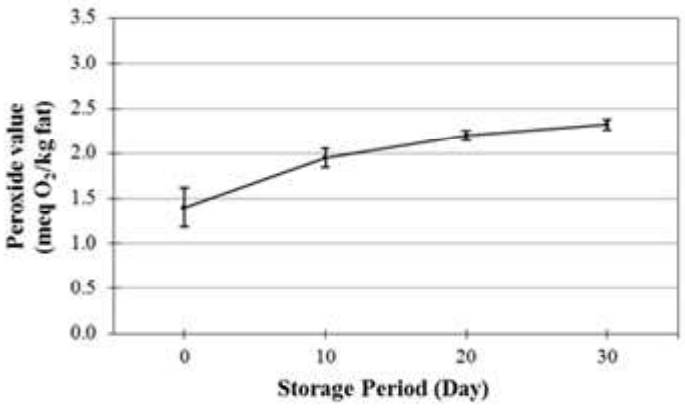

Fig. 4 Changes in peroxide value (meq O_2 Kg^{-1} fat) of Kuru Kaymak samples during storage. All the data are expressed as mean ± SD and are the mean of three replicates (n). Error bars represent standard deviations of values

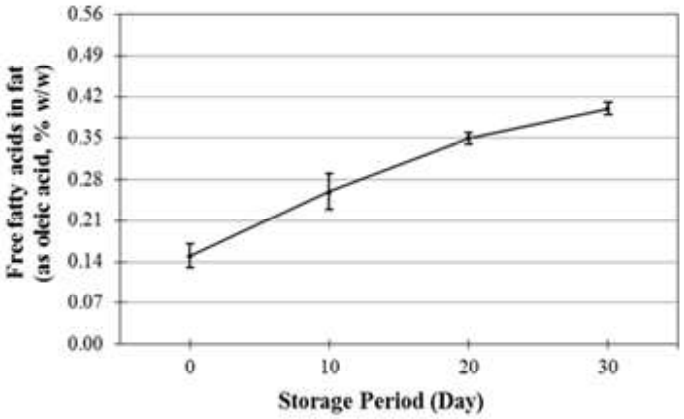

Fig. 5 Changes in free fatty acids in fat (as oleic acid, %w/w) content of Kuru Kaymak samples during storage. All the data are expressed as mean ± SD and are the mean of three replicates (n). Error bars represent standard deviations of values

30 days. Since the milk-borne lipase (indigenous milk lipase) enzymes were inactivated by the heat treatment applied during production, the most likely reason for the increase of free fatty acid values from 0 to 30 days of storage was lipolysis caused by the activity of heat resistant microbial lipase enzymes or by some specific chemical reactions. The free fatty acid value (0.40% for 30 days) was significantly lower than those reported for butter (Senel et al. 2011, Simsek 2011), but similar to those reported for kaymak (Akalin et al. 2006), for butter (Atamer and Kaptan 1982), and for fresh cow ghee (0.28-0.59%) (Gosewade et al. 2017). Similar to the results reported by Senel et al. (2011) and Simsek (2011), it was found that the free fatty acid values of KK samples increased constantly during 30 days of storage (Fig.5). Although both peroxide and free fatty acid values showed a high increase during the 30-day storage, this increase did not appear to cause any significant changes in the sensory properties of KK samples (data not shown).

Microbiological properties

The microbiological characteristics of KK samples are summarised in Table 2. Analysis of total mesophilic aerobic bacteria, yeast and moulds, and total coliform were performed to evaluate the hygienic-sanitary aspect of KK samples and their potential to support microbial growth.

Total mesophilic aerobic counts ranged from 5.03 to 5.67 log cfu/g (Table 2). The fact that the total mesophilic aerobic bacteria count in KK samples showed a high value immediately after production indicated that there was a substantial post-production contamination. The total mesophilic aerobic counts showed a statistically significant decrease during storage ($p < 0.05$) and its lowest level was observed on the 30th day of storage, indicating that KK was not supporting microbial growth. It has been observed that KK has a potential to support microbial activity on the one hand with its appropriate pH value and lactose content; on the other hand it limits microbial development due to its low water activity value.

Yeasts and moulds often cause surface discoloration and off-flavour in foods. The yeast and mould counts of KK samples were below the countable level (< 2 log cfu/g) throughout storage (Table 2). The probable reason for this result was that existing yeasts and moulds did not have a chance to grow due to low water activity. The yeast and mould count (< 2 log cfu/g) was lower than 3.06 log cfu/g and 3.88-7.53 log cfu/g reported by Cakmakci and Hayaloglu (2011) and Akalin et al. (2006), respectively.

Total coliform counts ranged from 1.18-1.66 log cfu/g during storage (Table 2). These values were lower than 3.87 log cfu/g determined by Yildirim et al. (2019), but higher than 0.33 log cfu/g found by Cakmakci and Hayaloglu (2011). The highest total coliform count was obtained at the beginning of the storage (day 0) ($p < 0.05$), then it decreased on the 10th day of storage and remained constant on the following days (20 and 30 days). Although both the pH value and lactose content of KK are suitable for the growth of coliform bacteria, the low water activity value probably restricted the growth of this group of bacteria. Therefore, it was concluded that KK is not a suitable medium for the development of coliform bacteria. The presence of coliform bacteria in KK samples showed that there was contamination due to non-compliance with hygienic rules in production.

Conclusions

This is the first report to examine the physico-chemical and microbiological properties of KK for 30 days of storage. Since KK was packaged in a waterproof manner, the dry matter, fat, protein, lactose and ash content and the water activity values of

the samples did not vary significantly between 0 and 30 days of storage. Considering the properties examined, it was concluded that KK could be stored for more than 30 days at 4°C without any noteworthy deterioration. Even though KK is not suitable for microbial growth, it is susceptible to oxidation and hydrolysis reactions, so precautions such as antioxidant supplements should be taken for a shelf life of more than 30 days. Microbiological results showed that KK was produced under very poor hygienic conditions; therefore, its manufacturing conditions should be improved using modern techniques and equipment while maintaining basic production steps.

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RESEARCH ARTICLE

Role of non-genetic factors on semen production performance in Murrah buffalo bulls

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Abstract: The present study aims to determine the effect of non-genetic factors on semen production traits of Murrah buffalo bulls maintained in an organized semen station. The data collected from 75 Murrah bulls maintained during the period 1997 to 2016 were used to analyse the non-genetic effects using least-squares analysis of variance under General Linear Model (GLM). The overall least-squares means for age at first semen collection (AFSC), age at first semen freezing (AFSF), age at last semen collection (ALSC), age at last semen freezing (ALSF), semen production period (SPP), frozen semen production period (FSPP), age at disposal (AD), number of ejaculates per bull per year, lifetime ejaculates per bull, frozen semen doses per bull per year and lifetime production of frozen semen doses per bull were 1149.71 days, 1160.67 days, 2745.32 days, 2728.73 days, 1313.97 days, 1292.23 days, 2819.09 days, 63.57, 637.88, 5738.60 doses and 69778.32 doses, respectively. Poor semen quality, old age, and poor libido were the predominant causes of disposal of bulls with 42.11%, 22.81%, and 12.28%, respectively. The period of birth of the bulls had a highly significant ($p<0.01$) effect on AFSC and AFSF and a significant ($p<0.05$) impact on AD. While the season of birth was not significant for any of the parameters studied. Period I (1997-99) recorded the highest values for AFSC, AFSF, and AD, whereas bulls born in period V (2009-10) exhibited lower AFSC and AFSF. These findings suggest that early selection and training of bulls under preferable managemental conditions will increase the semen production period and frozen semen doses.

Keywords: Disposal pattern, Non-genetic factors, Semen production performance, Murrah buffalo bulls

Introduction

Buffalo (*Bubalus bubalis*) plays an essential role in India's livestock genetic resources in terms of milk, meat, and draught power with a socio-economic impact on its rural population. India reigns the world with the largest livestock population of 192.49 million cattle and 109.85 million buffaloes (20th Livestock Census, 2019). Similarly, India ranks first in the world milk production with 187.75 million tonnes in 2018-19 of which a major share of 49% is contributed by indigenous and non-descript buffaloes (Basic Animal Husbandry Statistics, 2019). Murrah buffalo is one of the best Indian buffalo breed famous for milk production globally and recommended breed of choice for genetic up-gradation of non-descript buffaloes and crossbreeding with local breeds in the country. The frozen semen from Murrah bulls is extensively used for this purpose to augment milk production.

Artificial Insemination (A.I.) with frozen semen was proven to be the best technique worldwide for genetic improvement through the dispersion of superior germplasm. For production and distribution of quality frozen semen, it is very important that the bulls used in the A.I. programme satisfy quality standards, and the semen is collected and processed in accordance with the recommendations of Minimum Standards for Production (MSP) of Bovine Frozen Semen (2012), Department of Animal Husbandry and Dairying, Government of India. Identification of superior sires of high genetic merit is of utmost importance as genetic gain associated with the production performance comes mainly from sires because of their high reproductive differential and higher intensity of selection.

In order to achieve the production potential, genetic selection is the most scientific method adopted to make use of elite male germplasm for good quality frozen semen production. However, the number of superior bulls available to contribute for appreciable production in buffalo productivity is minimal. The

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technology of deep freezing of semen has remarkably increased spermatozoa's preservability and storage for artificial Insemination. This led to the development of methods for predicting semen production potential and identifying bulls with high sperm output characteristics at an early age, which can be used in breeding programmes to improve animal productivity. The information on the expected frozen semen dose producing ability per bull per year will help the semen station plan a suitable strategy to enhance the production to meet the growing demand for frozen semen doses.

Hence, the present study would provide valuable information on the frozen semen production potential of Murrah buffalo bulls reared under tropical semi-arid climatic region of the country. This information would be helpful to determine the breeding soundness of bulls and assess the production performance of the semen stations.

Materials and Methods

Data on semen production performance and disposal pattern of breeding bulls were collected from various records such as purchase record, daily performance record and auction record for 75 Murrah bulls maintained during the period 1997 to 2017 in the frozen semen production unit, Exotic Cattle Breeding Farm, Eachenkottai, Thanjavur, Tamil Nadu, India. The semen station was established in 1977 for semen production from imported Jersey and Holstein Friesian bulls. Later became an exclusive semen station for frozen semen production from genetically superior Murrah buffalo bulls. The farm is located in the tropical semi-arid climatic zone with an average rainfall of 914.31 mm. The latitude and longitude position of the semen station is 10.45'N and 79.29'E with an altitude of 50 meters above the mean sea level. The mean environmental temperature and relative humidity range from 22° to 39°C and 72 to 90%, respectively.

Age at first semen collection, age at first semen freezing, age at last semen collection, age at last semen freezing, semen production period, frozen semen production period, age at disposal, number of lifetime ejaculates per bull, number of ejaculates per bull per year, lifetime production of frozen semen doses per bull and frozen semen doses per bull per year were the parameters estimated to study the effect of non-genetic factors such as period of birth and season of birth. The birth year of the bulls was classified into five periods as, Period I (1997-1999), II (2000-2002), III (2003-2005), IV (2006-2008), and V (2009-2010) with an interval of three years except fifth period which is of two years. The season of birth was classified into four different seasons as, winter (December, January, and February), summer (March, April, and May), southwest monsoon (June, July, and August), and north-east monsoon (September, October, and November), respectively.

Statistical analysis

Single trait univariate analyses were performed under General Linear Model to study the effect of period of birth and season of birth on different semen production performance traits (Harvey, 1996). The difference between the least-squares means for sub-classes under a particular effect was tested by Duncan's Multiple Range Test.

The statistical model used for analysis:

$$Y_{ijk} = \mu + P_i + S_j + e_{ijk}$$

Where,

Y_{ijk} = k^{th} individual observation belonging to i^{th} period of birth and j^{th} season of birth

μ= overall mean

P_i = effect of i^{th} period of birth (i = 1 to 5)

S_j= effect of j^{th} season of birth (j = 1 to 4)

e_{ijk}= residual random error, NID (0 and σ^2_e)

The breeding bulls maintained in the frozen semen station were disposed off as per the guidelines of Minimum Standards for Production of Bovine Frozen Semen (2012). The major criteria considered for culling bulls comprises disease infection (Johne's disease, tuberculosis, brucellosis), chronic lameness, poor libido, poor semen quality (<40% initial motility and watery appearance of semen), poor freezability (<35% post-thaw motility), defective spermatozoa (>20%) and completion of semen production period, *i.e.,* eight years or three lakh semen doses. Hence, the total number of bulls culled in each category was calculated and expressed in percentage as per the statistical procedure of Snedecor and Cocharan (1989). The data analyses were performed using SPSS (Statistical Package for Social Sciences) software version 20.

Results and Discussion

The overall least-squares means for age at first semen collection (AFSC), age at first semen freezing (AFSF), age at last semen collection (ALSC), age at last semen freezing (ALSF), semen production period (SPP), frozen semen production period (FSPP) and age at disposal (AD) were 1149.71 days, 1160.67 days, 2745.32 days, 2728.73 days, 1313.97 days, 1292.23 days and 2819.09 days, respectively (Table 1). The least-squares analysis of variance for factors affecting semen production and performance traits is presented in Table 3.

The non-genetic factor, period of birth, had a highly significant ($p<0.01$) effect on AFSC and AFSF, besides significantly ($p<0.05$) influenced age at disposal. However, it did not affect any of the

Table 1 Least-squares means (± S.E.) for AFSC, AFSF, ALSC, ALSF, SPP, FSPP, and AD

Effect	Age at first semen collection (days)	Age at first semen freezing (days)	Age at last semen collection (days)	Age at last frozen semen collection (days)	Semen production period (days)	Frozen semen production period (days)	Age at disposal (days)
Overall	1149.71 ± 41.88(75)	1160.67 ± 42.51 (75)	2745.32 ± 289.83(55)	2728.73 ± 292.64(55)	1313.97 ± 295.64(55)	1292.23 ± 299.01(55)	2819.09 ± 284.73(55)
Period of birth	**	**	NS	NS	NS	NS	*
I (1997-99)	1393.48^{a} ± 97.96(5)	1393.83^{a} ± 99.43 (5)	4166.58 ± 497.17(5)	4157.19 ± 500.91(5)	1541.96 ± 507.14(5)	1537.61 ± 513.06(5)	4191.66^{a} ± 488.42(5)
II (2000-02)	1384.31^{a} ± 148.59(2)	1390.77^{a} ± 150.82(2)	2802.03 ± 742.28(2)	2799.86 ± 747.87(2)	1097.02 ± 757.17(2)	1087.99 ± 766.00(2)	2881.73^{b} ± 729.23(2)
III (2003-05)	993.72^{b} ± 32.82(46)	1009.29^{b} ± 33.31(46)	3118.74 ± 192.85(40)	3112.46 ± 194.30(40)	2118.60 ± 196.71(40)	2101.82 ± 199.01(40)	3174.99^{a} ± 189.45(40)
IV (2006-08)	1081.72^{b} ± 59.23(13)	1106.89^{b} ± 60.11(13)	2159.07 ± 391.48(7)	2125.49 ± 394.43(7)	1149.86 ± 399.34(7)	1096.07 ± 403.99(7)	2236.54^{b} ± 384.60(7)
V (2009-10)	895.32^{b} ± 71.99(9)	899.56^{b} ± 73.08(9)	1480.17 (1)	1448.64 (1)	662.42 (1)	637.66 (1)	1610.51^{c} (1)
Season of birth	NS	NS	NS	NS	NS	NS	NS
Winter (Dec. – Feb.)	1162.45 ± 73.42(13)	1170.87 ± 74.52(13)	2698.55 ± 413.32(12)	2677.09 ± 426.51(12)	1306.43 ± 431.82(12)	1284.89 ± 436.85(12)	2770.05 ± 415.88 (12)
Summer (Mar. – May)	1133.67 ± 71.47(10)	1132.76 ± 72.54(10)	3190.19 ± 517.98(5)	3176.57 ± 521.88(5)	1736.78 ± 528.38(5)	1730.08 ± 534.54(5)	3263.33 ± 508.87 (5)
Southwest monsoon (Jun. – Aug.)	1179.58 ± 54.75(25)	1200.01 ± 55.57(25)	2800.15 ± 342.76(17)	2784.09 ± 345.34(17)	1295.55 ± 349.63(17)	1267.57 ± 353.71(17)	2864.58 ± 336.73 (17)
Northeast monsoon (Sep. – Nov.)	1123.14 ± 49.53(27)	1139.05 ± 50.27(27)	2292.39 ± 344.73(21)	2277.16 ± 347.16(21)	917.13 ± 351.64(21)	886.39 ± 355.74(21)	2378.38 ± 338.66 (21)

** - Highly significant ($p<0.01$); * - Significant ($p<0.05$); NS – Non-significant; Means with at least one common superscript within classes do not differ significantly ($p>0.05$); Figures in parentheses indicate number of bulls.

other semen production parameters studied. Bulls in the period I recorded higher AFSC (1393.48 days), AFSF (1393.83 days), and age at disposal (4191.66 days), whereas a shorter period for these traits was observed, in period V (895.32, 899.56, and 1610.51 days, respectively). Similarly, the mean values for ALSC and ALSF were higher in period I (4166.58 and 4157.19 days) compared to that of other periods. The semen production and frozen semen production period were longest for the bulls in period III (2118.60 and 2101.82 days), and the shortest was noticed in period V, but only one bull had completed the production period, and the rest of the bulls are still in production phases. The season of birth did not influence any of the reproduction traits significantly. Interestingly higher mean value for most of the traits was observed in the summer season, and the lowest was spotted in the northeast monsoon season. Contrarily, the longest period for AFSC and AFSF was observed in the southwest monsoon.

On perusal of literature, it was noted that the information on semen production traits in Murrah buffalo bulls is very limited. Mukhopadhyay et al. (2010) while studying subfertility problems in breeding bulls, recorded an overall least square mean of 1,105.55 days, 1,205.57 days, 725.15 days, 559.03 days, and 1918.36 days for AFSC, AFSF, SPP, FSPP, and AD from 64 Murrah bulls. Similarly, Khatun et al. (2013) reported an overall least square means for AFSC, AFSF, SPP, FSPP, ALSC, ALSF, and AD as 1,113.7 days, 1198.1 days, 1043.2 days, 226.9 days, 2229.0, 2236.8 days, and 2458.8days, respectively. Shivahre et al. (2017) had the least-squares means of 1073.5 days, 1196.05 days, 644.28 days, 712.33 days, 1570.33 days, 1600.26 days, and 1668.25 days for AFSC, AFSF, SPP, FSPP, ALSC, ALSF, and AD. The overall least squares means for the semen production traits observed in the present study are relatively higher than those of previous studies. With respect to the effect of non-genetic factors, the results obtained in this study is in agreement with the findings of Mukhopadhyay et al. (2010) and Khatun et al. (2013), but contrary to Shivahre et al. (2017), who reported that ALSC was found to be significantly influenced by the period of birth.

Compared with the earlier reports, higher age at first semen collection was noticed in the present study, which could be reduced through genetic improvement and preparing the bulls for semen collection at an early age by providing adequate training with proper maintenance and management practices. Though AFSC was higher, the interval between AFSC and AFSF was comparatively lesser than earlier findings. This might be due to the selection of genetically superior bulls capable of producing quality frozen semen at an early age, appropriate training, good rearing environmental conditions, proper feeding practices, and better semen collection ambience. Basically, the bulls used for semen production are selected from the natural home tract of Murrah buffalo based on their parent's production performance, scrotal circumference, and body weight of the bulls. Semen production is a complex physiological process which is controlled by internal factors within the bull and external environmental

Table 2 Least-squares means (± S.E.) for annual and lifetime ejaculates and frozen semen doses in Murrah bulls

Effect	Number of life time ejaculates per bull	Number of ejaculates per bull per year	Lifetime production of frozen semen doses per bull	Frozen semen doses per bull per year
Overall	637.88 ± 100.01(75)	63.57 ± 11.19(55)	69778.32 ± 14146.99(75)	5738.60 ± 1686.71(55)
Period of birth	NS	NS	NS	NS
I (1997-99)	614.20 ± 233.92(5)	58.03 ± 19.24(5)	68903.56 ± 33088.22(5)	7480.70 ± 2898.69(5)
II (2000-02)	479.89 ± 354.82(2)	47.44 ± 28.68(2)	53522.91 ± 50189.83(2)	6260.99 ± 4320.99(2)
III (2003-05)	968.93 ± 78.38(46)	97.79 ± 7.49(39)	99932.21 ± 11086.32(46)	10008.29 ± 128.91(39)
IV (2006-08)	618.93 ± 141.42(13)	62.21 ± 15.12(7)	61540.33 ± 20004.70(13)	3713.36 ± 2278.50(7)
V (2009-10)	507.47 ± 171.93(9)	52.38 ± 40.59(2)	64992.59 ± 24319.18(9)	1229.65 ± 716.27(2)
Season of birth	NS	NS	NS	NS
Winter (Dec. – Feb.)	587.88 ± 175.32(13)	63.83 ± 16.37(12)	58124.72 ± 4799.5(13)	5093.22 ± 2466.61(12)
Summer (Mar. – May)	845.31 ± 170.66(10)	74.88 ± 20.01(6)	96945.56 ± 24139.67(10)	7441.34 ± 3014.88(6)
Southwest monsoon (Jun. – Aug.)	664.66 ± 130.74(25)	63.19 ± 13.25(17)	79942.73 ± 18492.75(25)	6425.95 ± 1996.92(17)
Northeast monsoon(Sep. – Nov.)	453.69 ± 18.28(27)	52.38 ± 13.41(20)	44100.27 ± 16729.97(27)	3993.89 ± 2019.80(20)

NS – Non-significant; Figures in parentheses indicate the number of bulls.

and management factors. Well-trained timid bulls would donate semen for longer periods than aggressive bulls. The semen collector, bull attendants, semen collection yard, exercise before semen collection, etc., affect semen production and collection. The probable reasons for the longer semen and frozen semen production period in this study could be due to better bull management in the semen station in the recent years and increased demand for buffalo frozen semen. The higher value for ALSC, ALSF, and age at disposal may be due to the prolonged period of frozen semen production and better reproductive status of the bulls. As per the MSP, bulls can be used for semen collection for a productive period of eight years or three lakh frozen semen doses, whichever is achieved earlier.

Annual and lifetime production performance

The overall least-squares means for the number of ejaculates per bull per year, lifetime ejaculates per bull, frozen semen doses per bull per year, and lifetime production of frozen semen doses per bull were 63.57, 637.88, 5738.60 doses, and 69778.32 doses, respectively (Table 2).

The non-genetic factors such as period of birth and season of birth did not significantly influence any of these traits. However, bulls born in period III (2003-2005) showed a higher value for the number of ejaculates and frozen semen doses than other periods. This increased value in period III may be due to the maintenance of maximum number of superior quality semen donating bulls for frozen semen production than the subsequent periods where the number of bulls reduced drastically. Among the seasons, surprisingly, a greater number of ejaculates and frozen semen doses were produced in the summer season (74.88, 845.31, 7441.34, and 96945.56 doses, respectively), followed by southwest monsoon (63.19, 664.66, 6425.95, and 79942.73 doses, respectively). There is a belief that semen production would be reduced in the summer season due to heat stress. The proper implementation of summer management practices in the semen station could probably deplete the environmental interactions and reduce the physical and physiological stress on the bulls. This fact has been proven in the effect of season on semen production traits. The lifetime frozen semen doses per bull ranged from 53522 (2000-2002) to 99932 doses (2003-2005) and 44100 (northeast monsoon) to 96945 doses (summer) between the period and seasons.

On perusal, the literatures on semen production performance in Murrah bulls is very scanty, Bhakat et al. (2011) recorded a mean of 53.27 ejaculates per bull per year, which is lower than that of the present result, howbeit a higher frozen semen doses per year per bull (6,879.49 doses) was observed. On the other hand, Roy (2006) reported 5,147.48 doses of frozen semen per bull per year from 32 Murrah bulls, which is lower than the present study. There is a dearth of literature in Murrah buffaloes to compare the outcomes on lifetime production of ejaculates and frozen semen doses. Hence, probably this is the first of its kind wherein the lifetime semen production performance is assessed. The evaluation of these semen production parameters helps determine the breeding efficiency of the bulls, which aids in identifying and culling bulls with poor performance. It would also provide valuable information in framing a suitable strategy for the semen station to improve the quality of semen and increase the number of doses as per market demand.

Table 3 Least-squares analysis of variance for factors affecting semen production and performance traits

Effect	df	AFSC	AFSF	SPP	FSPP	AD	Life-time ejaculates /bull	Number of ejaculates /bull/year	Lifetime production of frozen semen doses/bull	Frozen semen doses/ bull/year
					Mean sum of squares					
Period of birth	4	735803.93**	722696.18**	2073983.91^{NS}	2147143.96^{NS}	2972293.18*	645016.97^{NS}	3918.32^{NS}	5413089833.19^{NS}	70967294.59^{NS}
Season of birth	3	14202.21^{NS}	17082.98^{NS}	979278.29^{NS}	1027242.91^{NS}	1226620.81^{NS}	377116.71^{NS}	750.49^{NS}	8284870883.06^{NS}	22846402.05^{NS}
Error	67	46775.63	48526.75	1086014.61	1111493.33	1007330.18	243410.69	1557.06	4870182726.57	35349163.48

** - Highly significant ($p<0.01$); * - Significant ($p<0.05$); NS – Non-significant

Table 4 Culling and disposal pattern in Murrah bulls

Reasons recorded for disposal	No. of bulls	Percentage
Poor semen quality (Watery semen, initial motility < 70% and sperm abnormalities)	24	42.11
Poor freezability (Post-thaw motility <50%)	4	7.02
Poor libido (No sexual thirst, inability to mount, and poor protrusion of penis if mounted)	7	12.28
Old age (More than eight years of productive life or three lakh doses of frozen semen doses production)	13	22.81
Disease and infection (Chronic laminitis, joint ill and specific diseases)	6	10.52
Aggressive behaviour	3	5.26
Total number of bulls	57	100

Disposal pattern of Murrah bulls

The disposal pattern of Murrah bulls maintained in the semen station is furnished in Table 4. Poor semen quality was estimated to be the single major cause of disposal with 42.11%. The second major disposal cause was retired breeders (22.81%), followed by poor libido, disease, and infection, poor freezability, and aggressive behavior (12.28%, 10.52%, 7.02%, and 5.26%), respectively. The findings of the present study are in agreement with earlier reports of Suryaprakasam and Rao (1993), Mukhopadhyay et al. (2010), and Shivahre et al. (2014), who had stated that reproductive inefficiency such as poor semen quality and libido are the important causes of disposal, however, it is contrary to Khatun et al. (2013) who revealed poor semen freezability as the most frequent cause of disposal in Murrah bulls. Rao et al. (1995) reported that diseases and infections were the major causes of disposal of breeding bulls (43.75%), followed by poor semen quality (19.79%), old age (13.54%), poor libido (13.50%), and infirmity (9.38%).

Conclusions

The non-genetic factor, period of birth, had significantly influenced the traits such as age at first semen collection, age at first semen freezing, and age at disposal, but the season of birth did not affect any of the traits studied. Early selection of genetically superior breeding bulls and training at an early age for semen collection under unified management and feeding practices will help in producing better quality semen and more frozen semen doses. The estimation of each bull's production performance will help the semen station to assess the productivity and improve the production based on the demand.

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RESEARCH ARTICLE

Dairy infrastructure development index: measuring regional inequalities across districts of Gujarat

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Abstract: Infrastructure development is critical to sustain and attain optimum output from the rapidly growing Indian dairy sector. The existing studies indicate the need to improve the dairy infrastructure; however, the need varies across regions. This study analyses the disparity in the dairy infrastructure development of one of the highly dairy progressive State – Gujarat - using a Principal Component Approach (PCA). The results indicate that there exists nearly a 54% disparity in the dairy infrastructure facility among the districts of Gujarat. The index value varies from 0.04 for Dang to 0.66 for Banaskantha districts of Gujarat. The findings pointed towards a higher association of existence of farmer collectives and infrastructure development in the state. Development indicators to be prioritized for improving the level of dairy infrastructure in the low performing districts are also highlighted. The study suggests that the existing inequalities can be reduced by supporting and strengthening the already existing institutions like milk cooperatives and farmer producer companies or by incentivizing farmer groups to create one.

Keywords: Dairy Infrastructure, PCA, Dairy cooperatives, farmer producing company, Gujarat

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Introduction

Infrastructure plays an important role in development of any sector. Though Indian dairy sector has seen a structural change since white revolution, the development of dairy and veterinary infrastructure is still poor (Kumar et al. 2013). With existing infrastructure, only about one fifth of the adult female bovines are artificially inseminated and there is only one veterinary institute available for 5800 animals. Further, only around 0.03 hectares of land per animal is presently under fodder cultivation (Kumar et al. 2013). Therefore, there is a need to improve the dairy and veterinary infrastructure to realize more sustainable growth and development of the dairy sector.

Gujarat is the fifth largest milk producer in the country. White revolution which brought about the structural change in dairying originated in Gujarat. It has one of the highly developed cooperative chains in the country (Birthal et al. 2017, Kumar et al. 2019). Despite this, there exist regional disparities in availability of veterinary facilities, dairying practices, dairy infrastructure, irrigation facilities and technology adoption among others (Mahida et al. 2018). There has been a lot of studies to account for the regional disparities in agricultural and dairy development (Narain et al. 2007, Ayyoob et al. 2013, Kale et al. 2016 and Mahida et al. 2018). But there are no studies specific to the dairy infrastructure development in Gujarat. Hence, this study attempts to fill the above research gap.

We use a range of dairy infrastructure development indicators extracted from the 'Bulletin of Animal Husbandry and Dairying Statistics (2016-17 to 2018-19)' published by the Department of Animal Husbandry, Gujarat. These indicators were then used to develop a dairy infrastructure development index (DIDI) using the principal component approach. The results of this study will be useful for the dairy development board, policymakers and researchers. The low performing districts can be identified, tracked and specific measures can be taken to improve the performance of these districts.

Infrastructure development through public and private investment is one of the major policy discussions in India. There is no evidence to the best of our knowledge on the level of dairy

infrastructure development in Gujarat. Therefore, the purpose of this study is to calculate an index value which measures the dairy infrastructure development in Gujarat, rank and identify the poor performing districts.

Materials and Methods

To measure the dairy infrastructure development in Gujarat, 26 districts (before bifurcation) are taken as the unit of study as there is a growing consensus about the need for micro level planning. Data on selected dairy infrastructure indicators were collected for the triennium ending 2018-19. The descriptions of all the variables used are presented in Table 1.

All the indicator variables were first normalized using the formula,

$$X_{normal} = \frac{(X_{minimum} - X_{observed})}{(X_{maximum} - X_{minimum})} \dots (1)$$

Further, the indicators were tested for their significance. Principal component analysis (PCA) was used to separate out the significant indicators from the non- significant indicators. PCA was employed on the normalized data with varimax method for the rotation of the factors using SPSS. The amount of variance present in all indicators was measured and are represented by the communalities obtained through the PCA. The thumb rule of communality used by (Ponnusamy et al. 2016), indicating that the communality value above 0.6 as a sufficient condition for an indicator to be considered for PCA. None of the indicators were dropped from the factor analysis model as all had communality value of more than 0.6. Thus, all the indicators were used to assign weights in the next step.

PCA based approach was used to assign weights to the indicators for constructing the Dairy Infrastructure Development Index (DIDI) using the formula in equation 2:

$$DIDI_{District} = \frac{\sum_{i=1} X W_i}{\sum_i W_i} \dots (2)$$

where, $W_i = \sum |L_{ij}| E_j$ and

$DIDI_{District}$ – Dairy infrastructure development index of each district

X_i - normalized value of i^{th} indicator

W_i - weight of i^{th} indicator

E_j - Eigen value of the j^{th} factor

L_{ij} - loading value of the i^{th} district on j^{th} factor

i = 1,2,3,…10 indicators

Table 1 Description of variables used in the index

Variable	Description	Source
VI	Veterinary Institutes (Nos.) – includes polyclinics, high-tech veterinary polyclinics, Veterinary Dispensaries/Branch Veterinary Dispensaries, First Aid Veterinary Centers, Mobile Veterinary Dispensaries under Department of Animal Husbandry, Mobile Veterinary Dispensaries (Per 10 Villages)	Bulletin of Animal Husbandry and Dairying Statistics, Directorate of Animal Husbandry, Gujarat (2016-17, 2017-18 and 2018-19) (GoG, various years).
VD	Vaccination done (in lakhs) – against R.P., H.S., B.Q., Anthrax, FMD, ARV, ET, Sheep Pox, Fowl Pox, Ranikhet (F1-Strain and R2B-Strain), Mareks and others	
ICDP	Number of centers under Intensive Cattle Development Programme (Nos.)	
AI	Artificial Insemination work done per Artificial Insemination Centre (Nos.)	
GS	Number of Gaushalas and Panjrapoles (Nos.)	
CC	Installed Capacity of Chilling Centers (1000 Litres/Day) per center	
AFF	Area of fodder production farms (hectares)	
AS	Number of Animals/Units subsidized under Individual Beneficiary Schemes/Programmes (Nos.)	
DCS	Number of functioning milk cooperative societies (Nos.)	
MPPD	Milk procured from cooperative societies (Lakh Kg per day)	

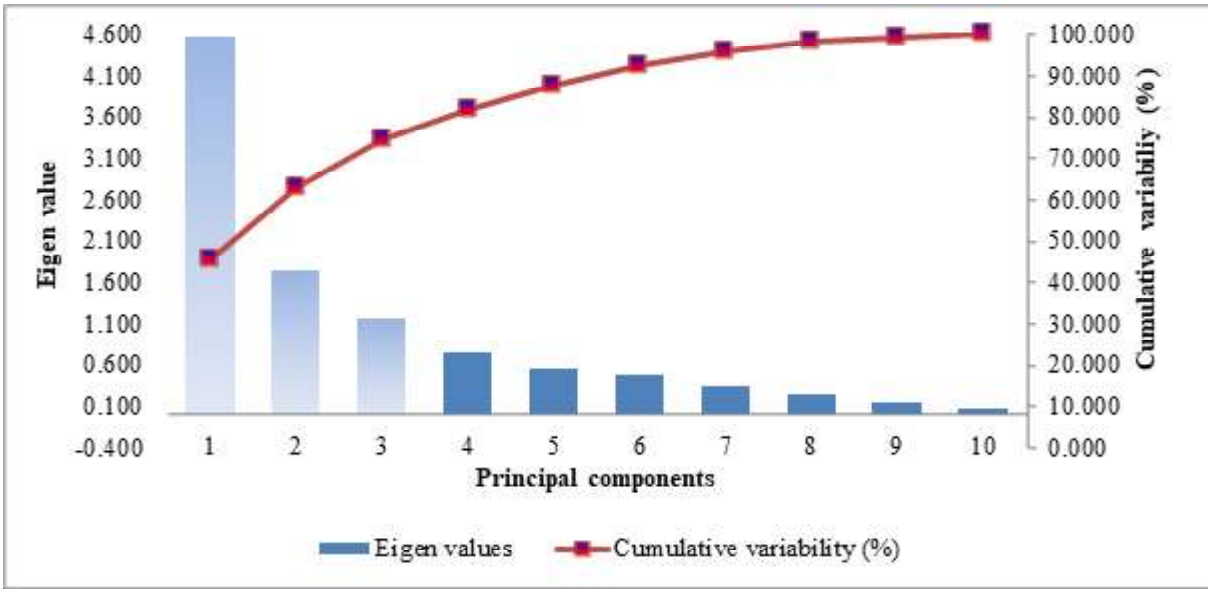

Fig. 1 Scree plot representing Eigen values and cumulative variability

Table 2 Factor loading values of major principal component

	Principal Component		
	1	2	3
VI	0.10	0.13	0.23
VD	0.28	-0.14	0.02
ICDP	-0.12	0.51	-0.04
AI	0.17	0.07	-0.27
GS	-0.14	0.24	0.42
CC	-0.11	0.49	-0.12
AFF	0.09	-0.25	0.55
AS	0.24	-0.04	0.05
DCS	0.21	0.03	-0.05
MPPD	0.29	-0.18	0.04

j = 1-3 Principal Components (PCs)

Based on the calculated composite index score, all the districts were categorized into three categories of dairy infrastructure development using,

$High = DIDI_{District} > Mean + 0.5\ SD$

$Moderate = Mean - 0.5\ SD < DIDI_{District} < Mean + 0.5\ SD$

$Low = DIDI_{District} < Mean - 0.5\ SD$

The PCA result extracted three PCs with eigen value greater than one accounting for around 75% of the variability (Figure 1). Eigen values and factor loading values represent the weights assigned to compute the index. The rotated factor loading values are given in Table 2.

To check the suitability of the data (in terms of sample adequacy) for the application of PCA, KMO (Kaiser-Meyer-Olkin) and Barlett's test was done. This sample adequacy measure of KMO indicates the amount of variance in the variables that might be common variance. According to Hair et al. (2006), a KMO value of more than 0.6 indicates that sample is adequate; this is being satisfied in our case. The Barlett's test of sphericity tests the proposition that the correlation matrix is an identity matrix or the variables are unrelated. Smaller (less than 0.05) the value of level of significance, more suitable the data for factor analysis. This condition was also satisfied in our case (Table 3).

Results and Discussion

The principal component analysis produced three principal components having eigen value greater than one as shown in the scree plot (Figure 1). The first PC captured the highest amount of variability around 46%, while the 2nd and 3rd PC captured around 17%, 12% variability, respectively. All together the four PCs accounted nearly 75% of variability in the transformed data as shown in secondary axis of the scree plot. The factor loading values obtained through vari-max rotation are presented in the Table 2. The factor loadings and eigen values together represent the weights assigned to each indicator for the index development.

The Dairy Infrastructure Development Index developed for 26 districts of Gujarat is presented in the Figure 2. The index values indicates that the Banaskantha, Sabarkantha and Vadodara were the top performing districts falling in the high dairy infrastructure development group with index value 0.663, 0.657 and 0.488, respectively. On other hand, Dang, Porbandar and Narmada are the districts having least dairy infrastructure development with index value 0.004, 0.082 and 0.100, respectively. On an average, Gujarat has a moderate level of dairy infrastructure facility with average index value 0.297.

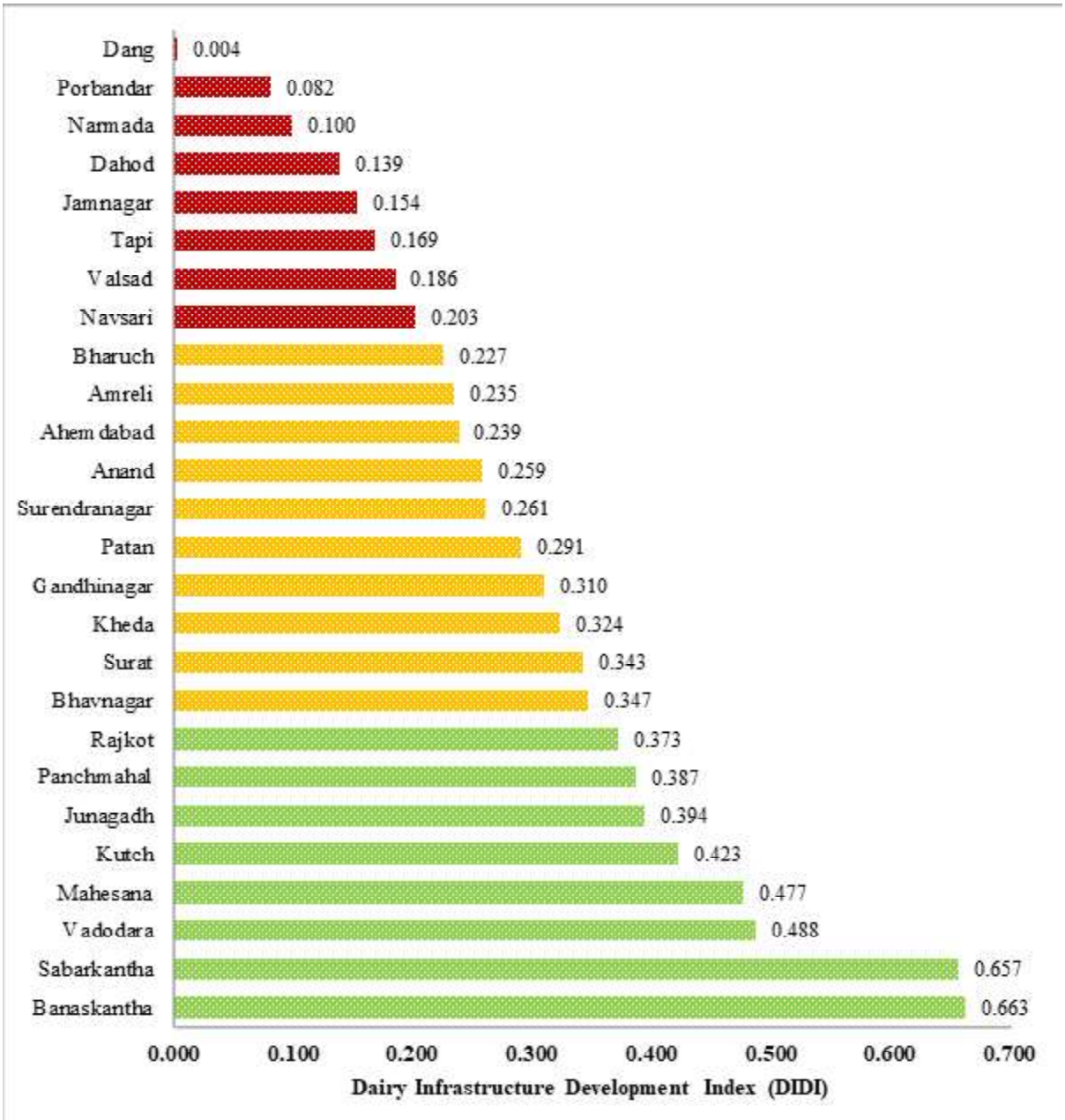

Fig. 2 Dairy Infrastructure Development index values and categorization of districts

Table 3 KMO and Bartlett's Test estimates

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.62
Bartlett's Test of Sphericity	Approx. Chi-Square	138.78
	Degree of freedom	45
	Significance level	0.00

It has been noted from weights that procurement capacity, vaccination facility, number of gaushalas, area under fodder farms, number of ICDP centres and chilling capacity per centre were contributing highest to the weights in developing index. It implies that improvement on these aspects can help to the districts in least DIDI category to cover up the gap in the level of dairy infrastructure development.

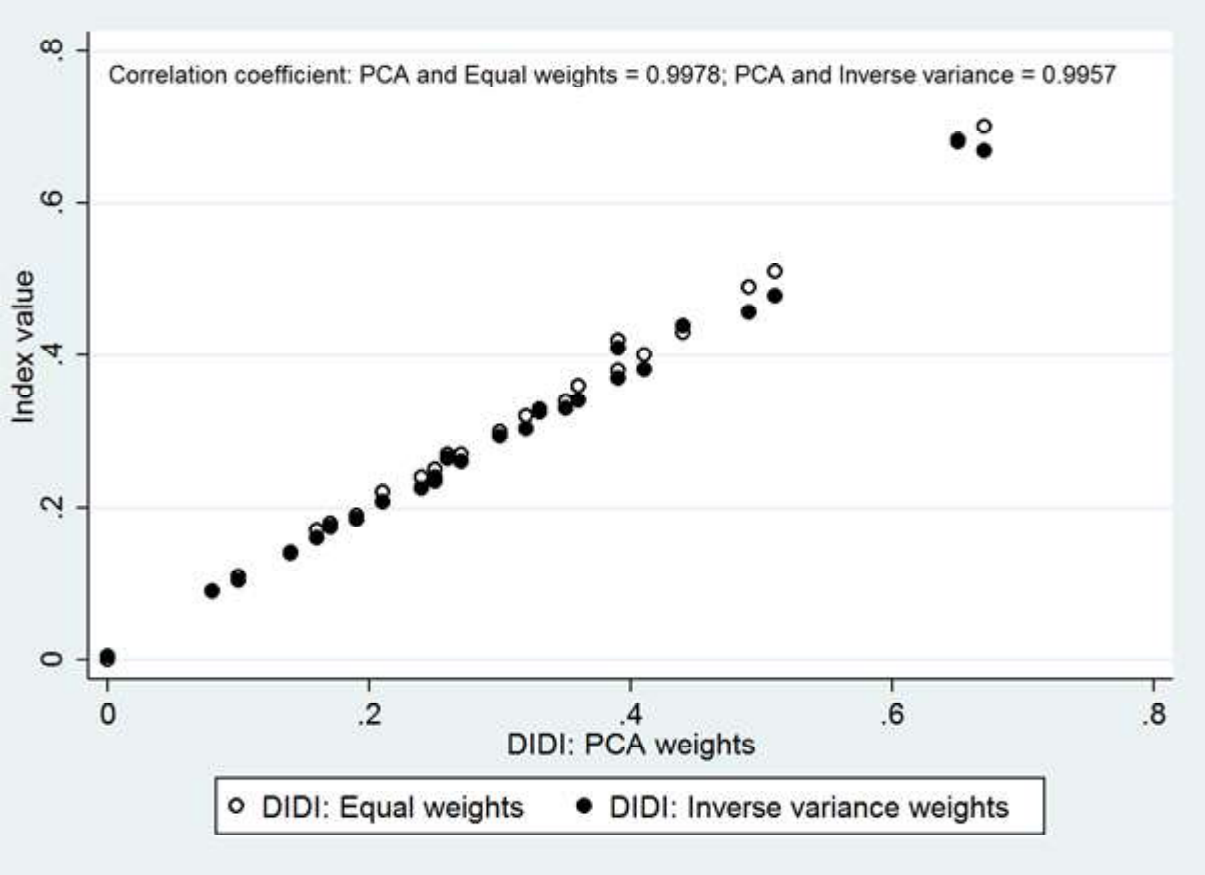

Fig. 3 Robustness of DIDI to different weighting methods

Categorization of districts based on the index value

Using Mean ± SD as a yard stick, all the districts were grouped into three categories based on the index value (Fig 2). Banaskantha, Sabarkantha, Vadodara, Mahesana, Kutch, Junagadh, Panchmahal, Rajkot were grouped into high dairy infrastructure development (DID) category. These eight districts cover around 50% of the state animal population, including nearly 48% of cows and 51% of the buffaloes. Bhavnagar, Surat, Kheda, Gandhinagar, Patan, Surendranagar, Anand, Ahmedabad, Amreli, and Bharuch were the districts with the moderate degree of DID covering around 34% of the dairy animal population comprising of 31% cow and 36% Buffaloes of the state. The districts with least DIDI include Navsari, Valsad, Tapi, Jamanagar, Dahod, Narmada, Porbandar and Dang. These eight districts cover 16% of the total dairy animal population containing 21% of cows and 12% of buffaloes. The degree of the variability in the level of dairy infrastructure development measured through coefficient of variation in the indices suggests that there exist nearly 54% disparity in the dairy infrastructure facility among the districts. The districts with very good infrastructure are also the districts with the strong cooperatives structure having substantial share in the local milk market. The cooperatives play critical role in supplying various veterinary services like artificial insemination, vaccination and other animal healthcare services at village level through the primary cooperative societies. The cooperative milk unions of top performing districts are more developed and are highly recognized for their role in the rural development through dairy development. The milk unions of these districts are well known as Sabar dairy (Sabarkantha), Banas dairy (Banaskantha), Dudhsagar dairy (Mahesana), AMUL (Kheda and Anand), Baroda dairy (Vadodara). The districts of Saurashtra region such as Rajkot, Junagadh, Bhavanagar, Amreli, and Kutch where along with cooperatives, Maahi, a milk producer company is another equally prevalent marketing channel contributing to the dairy infrastructure development.

To the contrary of our expectations, Anand and Kheda districts fell into moderate DID category. These districts were expected to be in the high DID category. This might be due to zero chilling capacity per centre in Anand, a smaller number of ICDP centres and few Gaushalas in both Anand and Kheda which are weighted more in index calculation. Since the largest dairy – AMUL – is present in the Anand district which manages both Anand and Kheda district, it reduces the requirement of preserving milk through chilling as the milk collected by DCSs directly go to the processing plants of AMUL dairy. Similarly, the presence of

AMUL also reduces the need of ICDP centres in the district. To our surprise, the Panchmahal district having very low dairy development (Mahida et al. 2018) possess high degree of infrastructure development, specifically in the aspects of availability of veterinary institutions and vaccination facilities. Our findings, however, to some extent are in consonance with Mahida et al. (2018) measuring regional disparity in dairy development of Gujarat as the districts namely Dang, Narmada, Valsad, Tapi and Dahod which are dominated by tribal population have poor level of infrastructure development. Our index values are robust to different weighting methods like equal weights to all the indicators and inverse variance-based weights (Fig. 3).

Conclusion

The disparity in the dairy infrastructure development among the regions is an obvious reason for the inequality in the dairy development. It prevents the producers to reap the optimum production from the dairy farming. The study found 54% disparity in dairy infrastructure development across various districts of Gujarat. Elimination of the inequalities in the infrastructure development is a long term and resource demanding process but considering the underlying causes can help us to reduce it by great extent through joint efforts by farmers, cooperative unions, government policies and other dairy related stakeholders.

The study finds that districts with well-established cooperatives like Banaskantha, Sabarkantha, Mahesana and Kheda and farmer producer companies (Maahi) like Rajkot, Bhavnagar, Kutch and Junagadh had better dairy infrastructure development. Also, underdeveloped districts with higher tribal population like Narmada, Tapi, Valsad and Dang had lower infrastructure development. The creation of dairy infrastructure, animal population and strong existence of farmers collectives (dairy cooperative or producer company) in a district seems to move in the same direction. Gujarat state "the heartland" of dairy cooperatives, therefore has pronounced opportunities to reduce the dairy infrastructure inequalities by forming policies to strengthen the already existing institutions such as dairy cooperatives and farmer producer companies or incentivizing farmer group to create one.

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RESEARCH ARTICLE

Economic analyses of milk production among members and non-members of farmer collectives in Saurashtra

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Abstract: Primary data collected for 2019-'20 from 416 members and non-members of dairy farmer collectives (where farmers come together to market their milk collectively – this study considers a co-operative union and a milk producer company – both prominent forms of farmer collectives) in Saurashtra region of Gujarat are used to work-out the cost and returns in milk production from local cow and buffalo, and across dairy farmer categories. The analyses reveal that farmer collective organizations like co-operatives and producer companies are profitable in case of both local cow and buffalo milk production, and also for the small (1-3) and medium (3-6) herd-size category (in Standard Animal Units or SAU) of farmers. Both the overall average feed cost and Total Variable cost (TVC) per animal per day incurred by members is 12 per cent and 11 per cent less than that of non-members, respectively. Members of the co-operative receive annual bonus and members of the producer company receive annual dividends and cash incentives; with this additional benefit received by the members, their total return per litre is 25 per cent more than that of non-members. The overall average annual net returns from dairy per household for members are higher at Rs. 11,641/- compared to the returns of non-members at Rs. 3955/-. Expanding the reach of farmer collective organizations would benefit more dairy farmers by making dairy a more profitable enterprise for them.

Keywords: Dairy farmer collectives, Milk production, Members and non-members

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Introduction

India has been the world's largest milk producer for more than two decades now. The feat has been possible through the pioneering Operation Flood programme initiated during 1970s. Some of the state milk federations have been functioning very well (Gujarat Cooperative Milk Marketing Federation (GCMMF), popularly known by the AMUL brand, and Karnataka Milk Federation (KMF), the Nandini brand). But with the advent of Milk Producer Companies (MPCs) in the country, organised dairy is getting yet another boost which is expected to ultimately benefit the member dairy farmers. There are over 200 MPCs in the country, 81 per cent of which are less than 5 years old (Neti et al. 2019; Thakur, 2020). Farmer collective organizations like milk co-operatives and producer companies provide input- and output-support services in the form of feed and fodder, veterinary and AI services, and remunerative milk price, etc., to their members. Payments on the basis of quality of milk (milk solid not fat or SNF, and milk fat) contributed by the member enhance transparency and strengthen trust in the organization. Most co-operatives also give annual bonus in cash or kind, while producer companies give dividends and/or incentives. Keeping in mind that the majority of milk producers in the country are small and marginal farmers with a small herd, it is imperative to investigate how these farmers benefit from membership of farmer collectives.

Gujarat is one of the largest milk producer states in India. The state has over 30 lakh milk producers who produced 14.49 million metric tonnes of milk in 2019 (Bulletin of Animal Husbandry and Dairying Statistics, 2017-18, Directorate of Animal Husbandry, Gujarat state). NDDB's Dairy Services (NDS) helped initiate a new venture, Maahi Milk Producer Company Ltd. (MMPCL) in the Saurashtra region of Gujarat in 2012. The company was incorporated under the provisions of Part-IXA of the Companies

Act 1956 and it began its commercial operations in March 2013 with milk procurement operations spread over seven districts of Saurashtra and Kutchh region covering 1,912 villages and 2,142 Milk Pooling Points (MPPs) at the village level. MPPs are similar to milk co-operative societies that test and collect milk from members in villages through the co-operative model. Maahi and four other MPCs (in other states) that NDS promoted have been able to achieve scale in terms of membership and business turnover, and have built-up internally-generated equity capital (Shah, 2016). Another strong farmer collective already operational in the region is the Rajkot District Co-operative Milk Producers Union Ltd., which is a member union of GCMMF. This paper presents a comparative analysis of the economics of milk production of members and non-members of the two farmer collective organizations in the area. We first compare the educational status and farm and household characteristics of members and non-members, and with the backdrop of input- and output-support services provided by the farmer collectives to their members, we proceed to present the cost and returns from local cow and buffalo milk production for members and non-members in the region. Cost and returns in milk production are also presented for members and non-members across categories based on SAU, followed by the overall average annual net returns from dairy earned by members and non-members by SAU categories.

Materials and Methods

The profiles of the two organizations are presented in Table 1.

Both the farmer collective organizations provide input- and output-support services to their members. Local cows are preferred over cross-bred in Saurashtra for their religious and sentimental value. The dominant indigenous cow breeds in the region are *Gir* and *Kankrej* and buffalo breeds are *Mahesani*, *Jaffarabadi*, and *Banni*.

To get the most representative sample from the population of dairy farming households in Saurashtra, the region was purposively stratified into four areas:

Area 1: where Maahi MPC is operational (Morbi and Botad randomly selected; 59 member households, 60 non-member households selected through stratified random sampling)

Area 2: where only co-operative union is active (Rajkot purposively selected for the presence of Rajkot Union; 67 member households, 74 non-member households selected through stratified random sampling)

Area 3: where both MPC and co-operative are active (Surendranagar and Bhavnagar randomly selected; 60 member households of Co-op, 60 member households of MPC selected through stratified random sampling)

Table 1 Profile of Maahi MPC and Rajkot union – the two major farmer collective organizations in Saurashtra (2020-'21)

Particulars	Maahi	Rajkot Union
Year of incorporation	2012	1961
Area of operation	Junagarh, Gir Somnath, Amreli, Botad, Bhavnagar, Surendranagar, Morbi, Jamnagar, Dev Bhumi Dwarka, Kutch, Porbandar	Rajkot, and parts of Junagarh, Amreli, Porbander, Jamnagar
Average daily milk procurement (LKgPD)	6.57	4.53
Products	Milk, buttermilk, SMP, flavoured milk, *ghee, kadhichaas, paneer, curd*	Milk, ghee, butter, buttermilk, flavoured milk, *peda*
Number of members	93500	65000
Number of DCS/MPP	2359	834
Board strength	15	19
Share capital (Rs. In crores)	35.43	25.37
Input support services	Mineral mixture, protein feed, vet. services, extension activities for promotion of Ration Balancing Programme and Fodder Development Programme	Animal feed and concentrates, vet. Services, trainings
Output support services	Payments through NEFT/bank transfer, Annual incentives in cash or kind, dividend	Online or cash payments; Annual bonus

Source: Compiled by author

Area 4: pockets of villages with no farmer collective organizations (Khakhada bela, Rojiya, Naranaka, Borda; 36 dairy households selected randomly)

In this way, data pertaining to socio-economic and farm characteristics and cost and returns in milk production from local cow and buffalo (both in the lean and flush seasons) were collected from 416 dairy farming households in the months of January-February 2020. The sample constituted 246 member households (119 members of Maahi MPC and 127 members of Rajkot Union), and 170 non-member households – all sampled through stratified random sampling. Care was taken to select non-member households that were similar to the member households with respect to observable characteristics.

A note on Standard Animal Units (SAU): Fixed assets such as cattle shed, dairy equipment and machinery, etc., are utilised for all dairy animals in the herd. The cost incurred on these items is joint costs, which must be apportioned to the animals on the basis of the extent of utilisation of fixed assets and labour by different categories of animals. To arrive at the total herd size of a household, it is not possible to simply take a sum of all animals the household possesses, as the animals differ in age and sex. Further, some animals may be in milk, while some may be dry or pregnant.

Therefore, all these different categories of animals are first converted into homogenous animal units with the help of certain conversion coefficients known as Standard Animal Units (SAUs) given by Sirohi et al. (2015). The total SAUs are then calculated for a household by taking a sum of its SAU of milch animals, heifers, adult male, and calf. Since Gujarat state falls in the West, the western region's conversion coefficients are used to arrive at the total herd size (in SAU) of a household.

Indigenous cow (or local cow) and buffalo are dominant in the study area for their religious and sentimental value. The SAUs for all 416 households in sample are calculated separately for local cow and buffalo before arriving at the total herd size. The households are then categorised into Small (1-3 SAUs), Medium (3-6 SAUs), and Large (>6 SAUs) herd-size categories on the bases of cumulative square root frequency method. Table 2 presents the distribution of member and non-member households across these categories.

Member households constitute a majority of medium farmers (51 per cent), followed by small (33 per cent) and large farmers (16 per cent), respectively. The non-members are mainly small farmers (48 per cent), followed by medium (42 per cent) and large farmers (9 per cent), respectively. Medium farmers dominate overall in the sample with about 47 per cent of total dairy households considered for the study.

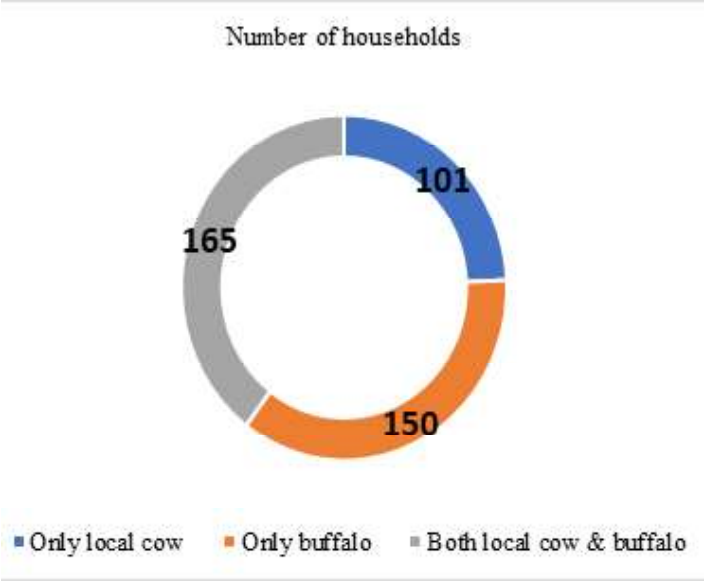

Fig. 1 Type of bovines owned by households in sample

Out of the total 416 households in sample, 165 households rear both local cows and buffaloes, while 101 households rear only local cows and 150 households rear only buffaloes. The type of cattle owned by dairy households in sample is presented in Figure 1.

On an average, the members own more cattle compared to the non-members (4.43 as opposed to 3.7 of the non-members). The average number of local cows and buffaloes with member households is 2.09 and 2.34 SAU, respectively. The corresponding numbers among non-member households are 1.6 and 2.1 SAUs, respectively (Table 3).

Cost and returns in milk production

The cost of milk production per animal per household is calculated by considering both fixed and variable costs. Fixed cost includes the cost of durable assets like farm inventory and equipment and cattle inventory. Capital Recovery Cost (CRC) method is used to calculate depreciation. CRC is the annual payment that will repay the cost of fixed input over the useful life of the input and provide an economic rate of return on investment. When this method is

Table 2 Distribution of member and non-member households across categories based on SAU

Member/Non-member	Small (1-3 SAU)	Medium (3-6 SAU)	Large (>6 SAU)	Total
Members FC	81(32.9)	125(50.8)	40(16.3)	246(100.0)
Non-members FC	82(48.2)	72(42.4)	16(9.4)	170(100.0)
Total	163(39.2)	197(47.4)	56(13.5)	416(100.0)

Note: FC is Farmer Collectives and SAU is Standard Animal Units; figures in parentheses are percentage to row totals

used, interest on fixed capital does not need to be calculated separately. CRC for farm inventory and equipment is calculated by the following formula:

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

Where Z = initial value of the capital asset;

r = rate of interest (taken as 12%); and

n = useful life of the asset in years

While calculating CRC for milch animals, useful life of local cow or buffalo is assumed to be 12 years. Total CRC is calculated and then apportioned to the individual animals using SAUs in the following manner:

$$Total\ CRC\ per\ day\ for\ LC = [Total\ CRC\ per\ day \times \left(\frac{Total\ SAU\ LC}{Total\ SAU\ of\ the\ HH}\right) + CRC\ per\ day\ LC];$$

$$Total\ CRC\ per\ day\ for\ Buffalo = [Total\ CRC\ per\ day \times \left(\frac{Total\ SAU\ buffalo}{Total\ SAU\ of\ the\ HH}\right) + CRC\ per\ day\ buffalo];$$

Where LC = Local cow and HH = Household

Variable cost includes feed and fodder cost, labour cost, veterinary cost, and miscellaneous cost. Feed and fodder cost comprises of all expenses incurred in feeding the animal with green fodder, dry fodder and concentrates. For purchased feed and fodder, the cost is calculated as product of quantity fed to the animal and purchase price of the respective feed. For home-grown feed and fodder, farm harvest prices are considered. In case of cultivated green fodder for which farm harvest prices were not available, the imputed value of crop is taken as the prevailing price of standing crop in the region. In case the animal was fed with collected grass and tree leaves from common property resources, its imputed value is their expected sale price and is considered in cost estimation. For home-made concentrate, the weighted average of prices of all the ingredients is taken to ascertain the price of concentrate.

Both family labour and hired labour are considered in labour cost. One man day is taken equal to 8 hours for estimation of labour cost and total time spent on different dairy activities is calculated and converted into man days. The cost of grazing of animals is also considered in the estimation.

Veterinary cost includes cost of natural service, artificial insemination (AI), medicines, vaccination of animal, charges of visiting veterinary doctor and other related expenses.

Table 3 Average number of Local Cow and Buffalo (SAU) owned by sample households

Member/Non-member	Local Cow	Buffalo	Total
Members FC	2.09	2.34	4.43
Non-members FC	1.60	2.10	3.70
Total	1.88	2.24	4.12

Miscellaneous expenditure consists of expenses incurred on repairs and fuel, electricity and water charges and other incidental charges.

Further, the following calculations are used to arrive at the final net returns per litre from local cow and buffalo and overall dairy:

Gross Cost = Total fixed cost + Total variable cost

Net cost = Gross cost – Value of dung

Gross returns = Milk yield per animal per day × Price per litre of milk

Net returns = Gross returns – Net cost

Cost per litre = Net cost / Average milk production per animal per day

Return per litre = Net returns / Average milk production per animal per day

While calculating return per litre for the members, annual bonus given by co-operatives and annual incentive given by the MPC is also considered. The co-operatives give bonus equal to 12 per cent of the total value of milk contributed by a member in a year.

Bonus per animal per day = 0.12 × (average milk production per animal per day × price of milk per litre)

Net return of co-operative member including bonus = Net return + Bonus per animal per day

Return per litre with bonus = Net return of co-operative member including bonus / Average milk production per animal per day

Total return per litre for co-operative member = Return per litre with bonus + Return per litre

Maahi MPC gives both annual incentives and dividend to its members. The incentive is equal to 1.15 multiplied by the quantity of milk contributed by a member in a year.

Incentive per animal per day = 1.15 × average milk production per animal per day

Net return of MPC member including incentive = Net return + Incentive per animal per day

Return per litre with incentive = Net return of MPC member including incentive / Average milk production per animal per day

Total return per litre for MPC member = Return per litre with incentive + Return per litre

Since the information on percentage of shareholding of each member was lacking, dividend could not be included in the calculation of returns from milk production in case of MPC members.

Results and Discussion

Table 4 presents the education status of sample household heads by their social group. About 7 per cent of members of farmer collectives belong to the scheduled group, in contrast to about 16 per cent of non-member households that belong to the scheduled group. On an average, it is the non-members (43 per cent) who are more educated than the members (37 per cent).

Farm and household characteristics of members and non-members are presented in Table 5. In variables like age and household size, both the groups are comparable. The member households are slightly more experienced in dairy (23 years) compared to the non-member households (19 years). The reported average annual income of the non-members is slightly more at Rs. 1.35 lakh compared to Rs. 1.14 lakh of the members. In terms of land-size measured in hectares, the members own more land (4 ha.) compared to that of non-members (3 ha.). There is an average price difference of about Rs. 5/- per litre of milk sold by members and non-members, with the non-members gaining an immediate price benefit.

Both the farmer collectives, i.e., Rajkot Union and Maahi MPC provide certain input- and output-support services to their members. The members' responses vis-à-vis each of the service is presented in Table 6. A majority of the members (72 per cent) receive online payments for the milk, while some (28 per cent) continue to receive cash payments. None of the households report collection of milk from their doorstep, which is why the service is not a part of the table. Among input-support services, 8 per cent of member households receive improved fodder variety seeds, 51 per cent receive concentrate, and 21 per cent receive mineral mixture, while 11 per cent receive salt and other supplements for their animals from the respective farmer

Table 4 Educational status of sample household heads by social group

Mem/Non-mem	Social Group	Below primary/illiterate	Primary or above (%)
Members FC	SC (6.5)	81.3	18.8
	ST (0.4)	100.0	0.0
	OBC (62.6)	63.0	36.8
	Others (30.5)	58.6	41.3
	Total (100.0)	63.0	37.0
Non-members FC	SC (14.7)	64.0	36.0
	ST (1.2)	100.0	0.0
	OBC (53.5)	53.9	46.2
	Others (30.6)	57.7	42.2
	Total (100.0)	57.1	42.9

Note: Figures in parentheses are percentages of the respective group

Table 5 Farm and household characteristics of members and non-members of farmer collectives

		Members FC		Non-members FC	
Sr. No.	Variable	Mean	SD	Mean	SD
1	Age (yrs)	44.4	9.8	42.5	8.2
2	HH size (number)	6.2	2.7	6.3	2.6
3	Yrs of dairy exp.	22.5	9.2	19.4	9.5
4	Annual income (Rs.)	114529	110272	135455	102722
5	Dist frm mkt (Kms)	5.8	3.3	6.6	3.2
6	Dist frm road (Kms)	0.4	0.5	0.6	0.1
7	Dist frm dairy (Kms)	0.5	0.5	0.6	0.1
8	Milk yield (Ltr/day)	16.5	10.8	17.6	14.7
9	Land-size (ha)	3.7	4.3	2.9	2.2
10	SAU	4.4	3.0	3.7	2.4
11	Milk price per litre (Rs.)	38.6	5.6	43.2	4.9
12	Value of farm equipment (Rs.)	110331.1	73684.0	100245.1	78825.3

collective organization. About 70 per cent of member households also receive veterinary services, 73 per cent receive medicines and 59 per cent receive AI services for their animals.

The cost and returns in milk production from local cow and buffalo for both members and non-members of farmer collectives in Saurashtra are presented in Table 7. The average feed and fodder cost per animal per day is higher for the non-members (Rs. 145/-) compared to that of members (Rs. 127), which is plausible, as the farmer collectives provide animal feed to their members at remunerative prices. The cost is Rs. 111/- and Rs. 143/- for the members in case of local cow and buffalo, respectively. The corresponding figures for the non-members are Rs. 132/- and Rs. 157/-, respectively.

The Total Variable Cost (TVC) incurred in case of local cow by members is Rs. 150/- per animal per day, while it is Rs. 177/- per animal per day for the non-members. In case of buffalo, the TVC is Rs. 191/- for the members as opposed to Rs. 209/- for the non-

Table 6 Input- & output-support services provided by farmer collectives to their members (no. of households)

Sr. No.	Service	Response of members	
1	Mode of payment	Online	Cash
		178(72.4)	68(27.6)
2	Improved fodder variety seeds	Yes	No
		19(7.7)	227(92.3)
3	Concentrate	126(51.2)	120(48.8)
4	Mineral mixture	51(20.7)	195(79.3)
5	Salt & other supple.	28(11.4)	218(88.6)
6	Vet. Services	172(69.9)	74(30.1)
7	Medicines	179(72.8)	67(27.2)
8	AI	146(59.3)	100(40.7)

Note: Figures in parentheses are percentage of households that gave the respective response

Table 7 Cost and returns from local cow and buffalo milk production for members and non-members of farmer collectives in Saurashtra, 2019-20 (Rs. per animal per day)

S. No.	Particulars	LC			Buffalo			Overall		
		Mem	Non-Mem	Overall	Mem	Non-Mem	Overall	Mem	Non-Mem	Overall
1	Capital recovery cost of fixed assets (TFC)	22.65	24.91	23.78	26.62	28.41	27.51	24.63	26.66	25.64
2	Green Fodder	40.42	44.27	42.34	49.64	51.34	50.49	45.03	47.81	46.42
3	Dry Fodder	14.38	18.66	16.52	26.32	28.53	27.43	20.35	23.59	21.97
4	Concentrate	55.99	69.47	62.73	66.80	76.95	71.87	61.39	73.21	67.30
5	Feed & fodder cost (2+3+4)	110.79	132.40	121.59	142.76	156.82	149.79	126.77	144.61	135.69
6	Labour cost	32.18	35.04	33.61	41.77	45.10	43.44	36.98	40.07	38.52
7	Veterinary cost	2.21	2.67	2.44	2.53	2.47	2.50	2.37	2.57	2.47
8	Miscellaneous cost	5.14	6.56	5.85	4.12	4.96	4.54	4.63	5.76	5.19
9	Total variable cost (TVC = 5+6+7+8)	150.31	176.67	163.49	191.17	209.35	200.26	170.74	193.01	181.87
10	Gross cost (TFC+TVC) (A)	172.95	201.58	187.26	217.78	237.76	227.77	195.37	219.67	207.52
11	Value of dung (B)	13.79	15.07	14.43	14.69	14.43	14.56	14.24	14.75	14.49
12	Net cost (C= A-B)	159.17	186.51	172.84	203.09	223.33	213.21	181.13	204.92	193.02
13	Price of milk	35.27	40.43	37.85	41.97	46.05	44.01	38.62	43.24	40.93
14	Average milk production/animal/day (E) (in Litres)	4.62	5.22	4.92	5.79	7.06	6.42	5.20	6.14	5.67
15	Gross return (D)	162.87	210.07	186.47	242.97	322.39	282.68	202.92	266.23	234.58
16	Net return (D-C)	3.70	23.56	13.63	39.88	99.06	69.47	21.79	61.31	41.55
17	Cost/litre (C/E)	34.48	36.14	35.31	35.29	33.60	34.44	34.89	34.87	34.88
18	Return / litre	0.78	4.29	2.54	6.68	12.45	9.56	3.73	8.37	6.05
19	Net Return including bonus/incentive (animal/day)	16.58	NA	NA	58.84	NA	NA	37.71	NA	NA
20	Total Return / litre	4.31	4.29	4.30	16.44	12.45	14.45	10.38	8.37	9.37

members. Overall, there is a TVC difference of over Rs. 20/- between members and the non-members of farmer collectives. The average milk production per animal per day is slightly higher in case of non-members (6 litres) as compared to the members (5 litres). The lower per litre price of milk realised by the members is compensated for by the annual bonus or incentive paid by the farmer collectives. In case of local cow, the return per litre for members and non-members is comparable at Rs. 4.31/- and Rs. 4.29/-, respectively. But in case of buffalo, the return per litre is higher by an amount of Rs. 4/- for the members. The overall returns from dairy are higher for the members (Rs. 10.38/-) compared to the non-members (Rs. 8.37/-).

The same analysis carried out across herd-size categories of dairy farmers is presented in Table 8. The analysis reveals that the feed and fodder cost per animal per day for the small farmers who are members is Rs. 114/- as opposed to Rs. 131/- for the non-members of that category. For medium farmers, the same figure is Rs. 138/- and Rs. 165/- for members and non-members, respectively. The TVC per animal per day incurred by small member farmers is Rs. 173/- compared to Rs. 190/- incurred by the non-members of same category. For medium farmers, the corresponding figure is Rs. 178/- and Rs. 204/- for members and non-members, respectively. The lower feed costs and TVC incurred by the large farmers who are members may be due to economies of scale (larger herd, lower per animal cost and remunerative feed prices). Here again, the immediate price benefit received by the non-members is overcome by the annual bonus or incentive paid by farmer collectives to their members. Both small and medium herd size farmers gain from collectivization, with a return per litre of Rs. 8/- and Rs. 14/-, respectively, in contrast to return per litre of Rs. 7/- and Rs. 9/- for the non-members of respective categories. Similar results have been reported by many researchers who have analysed economics of milk production of members of co-operatives versus that of non-members (Tanwar et al. 2012; Pandian and Selvakumar, 2013; Vishnoi, 2014; Priscilla, 2017; Chand et al. 2017; Athare et al. 2019; Kumari et al. 2020). The returns are comparable for large farmers with Rs. 8.9/- for the members and Rs. 9.2/- for the non-members.

Table 9 presents the overall average annual net returns from dairy earned by both members and non-members of farmer collectives in different herd-size categories. Small member farmers (with 1-3 SAU) earn Rs. 9,472/- annually from dairy, whereas the non-members of the same category earn annual returns of Rs. 2,935/- from the enterprise. The medium member farmers (with 3-6 SAU) also benefit from collectivization, as they reap higher returns at Rs. 13,843/- compared to the returns of Rs. 4,212/-

Table 8 Cost and returns in milk production in categories based on SAU for members and non-members of farmer collectives in Saurashtra, 2019-20 (Rs. per animal per day)

S. No	Particulars	Small (1-3)		Medium (3-6)		Large (>6)	
		Mem	Non-mem	Mem	Non-mem	Mem	Non-mem
1	Capital recovery cost of fixed assets (TFC)	30.87	29.67	25.61	27.61	20.89	20.39
2	Green fodder	39.82	43.78	50.51	54.77	40.96	45.24
3	Dry fodder	17.04	19.87	21.82	28.15	21.25	21.03
4	Concentrate	57.54	67.70	65.72	82.50	55.73	70.82
5	Feed & fodder cost (2+3+4)	114.40	131.35	138.06	165.42	117.93	137.08
6	Labour cost	53.11	51.67	31.80	30.68	21.95	21.82
7	Veterinary cost	2.16	1.92	2.55	2.33	2.24	3.32
8	Miscellaneous cost	3.53	6.04	5.44	5.94	4.26	4.19
9	Total variable cost (TVC = 5+6+7+8)	173.19	190.99	177.86	204.37	146.38	166.41
10	Gross cost (TFC+TVC) (A)	204.06	220.65	203.46	231.98	167.27	186.80
11	Value of dung (B)	15.01	14.17	14.04	15.73	13.51	13.01
12	Net cost (C= A-B)	189.05	206.48	189.42	216.25	153.77	173.79
13	Price of milk	39.58	43.45	38.07	42.19	37.51	41.58
14	Average milk production/animal/day (E) (in Litres)	5.12	5.93	5.86	6.55	4.50	5.49
15	Gross return (D)	205.43	259.76	225.13	277.94	169.63	230.43
16	Net return (D-C)	16.37	53.27	35.70	61.69	15.86	56.64
17	Cost/litre (C/E)	37.12	36.63	32.39	33.19	34.46	32.30
18	Return / litre	2.46	6.82	5.68	9.00	3.05	9.28
19	Net Return including bonus/incentive (animal/day)	31.75	NA	53.41	NA	29.70	NA
20	Total Return / litre	7.87	6.82	14.30	9.00	8.92	9.28

Table 9 Overall average annual net returns from dairy (Rs.) earned by members and non-members of farmer collectives by SAU categories

SAU Category	*Members FC	Non-members FC	Overall
Small (1-3 SAU)	9472(7733)	2935(5538)	6203(6635)
Medium (3-6 SAU)	13843(11485)	4212(5743)	9028(8614)
Large (>6 SAU)	9007(9245)	7801(5223)	8404(7234)
Overall	11641(10238)	3955(5723)	7798(7980)

Note: Figures in parentheses are standard deviations
*The values may be underestimated as dividend paid to members by Maahi MPC is not included

earned by non-members of the same category. The large member farmers (with >6 SAU) also enjoy higher annual returns of Rs. 9,007/- compared to that of large non-member farmers (Rs. 7,801/-). Overall, the average net annual returns from dairy for the members is Rs. 11,641/-, compared to Rs. 3,955/- for the non-members.

Conclusions

Farmer collective organizations are the backbone of organised dairy in India. A large proportion of total milk produced in the country comes from small and marginal farmers possessing a small herd of cattle. With benefits like feed and fodder at remunerative prices, AI services, milk quality-based assurance of price, and annual bonus or incentives that farmer collectives provide to their members, maximising their reach would be profitable to the dairy farmers. Dairy is profitable in case of both local cow and buffalo in Saurashtra region of Gujarat, where farmers are reaping higher returns from buffalo milk production. Collectivization is especially beneficial to the small and medium farmers with 1-3 or 3-6 SAUs of cattle. Both the overall average feed cost and Total Variable cost (TVC) incurred by the members is 12 per cent less than that of non-members. With the benefit of annual bonus or incentive received by members of farmer collectives, their total return per litre is 25 per cent more than that of non-members. The overall average annual net returns from dairy are also higher for the members at Rs. 11,641/- compared to the returns of non-members at Rs. 3955/. Both dairy co-operative as well as milk producer company model could be promoted in the area, so that more dairy farmers can enjoy better returns from the dairy enterprise.

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SHORT COMMUNICATION

Development of technology for manufacture of functional black rice (*Oryza sativa L.*) ice cream

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Abstract: Black rice (*Oryza sativa L.*) contain bioactive compounds i.e. anthocyanins and flavonoids which have potential to fortify ice cream as a functional food. The main objective of this study was to develop sugar free and antioxidant rich ice cream by addition of black rice flour. Based on the preliminary trials, black rice flour (BRF) was incorporated at 8, 10, 12 and 14 per cent during homogenization. Addition of more than 14 per cent black rice flour in ice-cream imparts increased hardness and viscosity. Based on sensory evaluation, ice cream prepared with 12 per cent black rice flour was accepted as optimized with highest values of acceptance for colour, flavor, texture and overall acceptability. The physico- chemical composition of optimized ice cream observed were 10.52 ± 0.02 % fat, 3.90 ± 0.12 % protein, 36.70 ± 0.02 % total solids, 72.56 ± 0.49 % overrun, 68.43 ± 4.21 (mg/GAE) total phenolic content and 40.21 ± 2.23 % DPPH.

Keywords: Anthocyanin, Black rice, Functional, Ice cream, Total phenolic content

Black rice belongs to *Oryza sativa L*; same species as white rice and red rice. This is a pigmented variety of rice and the colour of it is due to the presence of anthocyanins. Approximately, 26.3 % anthocyanins are found in black rice and cyanidin-3-O-glucoside and peonidin-3-O-glucoside are the main effective constituents accounting for about 90 % (Chang et al. 2010). Anthocyanin is one of the main antioxidant compounds that protect against the reactive oxygen species (ROS) which causes cellular damage in plants, animals and humans (Yamuangmorn and Prom-u-Thai, 2021). Chusak et al. (2020) reported that the consumption of anthocyanin improved the antioxidant status of the plasma. It contains 23 secondary metabolites, comprising anthocyanins, flavones, flavonoids glycosides (Quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside and myricetin-7-O-Glucoside), carotenoids, vitamin E (tocopherols and tocotrienols) and y-oryzanols which have been qualitatively and quantitatively characterized in the dehulled seeds of Japanese black–purple rice which provides health benefits and ensure the use of black rice as functional food. (Irakli, 2012; Sriseadka et al.2012). It also contains highest amount of proteins and dietary fibres of all rice varieties (Gani et al. 2012). Black rice is free of gluten, free of cholesterol, low in sugar, salt and fat. Black rice contains essential amino acids like lysine, tryptophan, functional lipids, dietary fibre, vitamins such as vitamin B1, vitamin B2, vitamin E, folic acid and phenolic compounds (γ-oryzanols, tocopherols, tocotrienols). Prasad et al. (2019) reported that the health properties of black rice are scientifically proven and the use of black rice in nutritional therapy is supported by the empirical data in respect of its physiological and pharmacological activity. One-fourth cup uncooked black rice contains approximately (in daily recommended values) 160 kcal energy, 1.5 g of fat, 34 g of carbohydrate, 2 g of fiber, 7.5 g of protein, no saturated fat and cholesterol (Kushwaha, 2016).

Ice -cream is a suitable dairy product that can be varied with different functional ingredients. Peoples of all age groups are very fond of ice cream and the number of studies about its functionalization has increased. Addition of black rice powder in ice cream can increase the health benefits and can serve as a functional food especially for diabetic patients as it lowers the starch digestion rates and maintains the blood glucose levels. Increased demand and liking of the consumers for nutritious products has drawn the attention of ice cream manufactures to find out some innovative options in nutritious and functional ingredients (Waterhouse et al. 2013). Addition of black rice flour in ice cream could improve the nutritional, functional and sensory properties. So, the major objective of this study was to optimize functional ice cream with black rice flour in different variations.

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Black rice (Chahao) was procured from market of Shalimar Bagh, New Delhi. Standardized milk, butter, cream (Amul) and stevia, were procured from local market of Varanasi. Rice was cleaned and milled to fine flour using mixer grinder and then the powder was transferred to low density polyethylene (LDPE) packages of size 12 cm x 9 cm, sealed and vacuum packed for further study.

The prepared black rice flour and white rice flour was analyzed for the physico-chemical composition and antioxidant activity. Black rice flour was extracted by method of Sutharut and Sudarat (2012). Total phenolic content were determined using the Folin-Ciocalteau reagent and Gallic acid as a standard (Slinkard and Singleton, 1997). The free radical scavenging activity was determined by using DPPH assay with modified method of (Brand-Williams et al.1995). A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%) was added to 0.1 mL of methanolic rice flour sample extract and shaken. The samples were kept in the dark room for 30 minutes after which absorbance was recorded at 575nm. The results were expressed as mg gallic acid/100g sample.

The flowchart for preparation of black rice flour (BRF) incorporated ice cream is given in (Figure 1). Table butter was used for roasting of black rice flour. The proportions used were decided based on the preliminary trials. Black rice flour (12.0g), standardized milk with 4.5 % milk fat (79 ml), cream with 25% milk fat (8.0g), butter with 80% fat (0.5g), stevia and sodium alginate (0.2g each) were used for preparation of 100g of ice cream. Different combinations were tried by varying the amount of black rice flour. The product was developed using different combinations of rice flour was organoleptically evaluated by semi-trained panel of 15 judges from Department of Dairy Science and Food Technology, Banaras Hindu University.

Fat, protein, acidity, total solids and pH were analyzed in control and black rice ice cream by following the AOAC (2000) methods. Overrun in control as well as in optimized black rice ice cream was analysed as per (Marshall et al. 2003) and viscosity (Brookfield Viscometer, Model DV-II +Pro, Brookfield Engineering Laboratories, USA). Texture profile analysis of ice cream samples was performed by using the texture profile analyser TA.XT plus, Exponent Lite (Stable Micro Systems, Surrey, UK) using probe P-32 up-to 80 mm distance at 2.0 mm/s test period. Total phenolic content in ice cream were determined using the Folin-Ciocalteau reagent and Gallic acid as a standard (Slinkard and Singleton, 1997). The free radical scavenging activity was determined by using DPPH assay with modified method of (Brand-Williams et al. 1995).

The data was analysed with the help of various statistical tools such as mean and standard error. To test the significant difference between the control and experimental samples, ANOVA, two tail t - test was applied using NCSS 19 software and MS Excel.

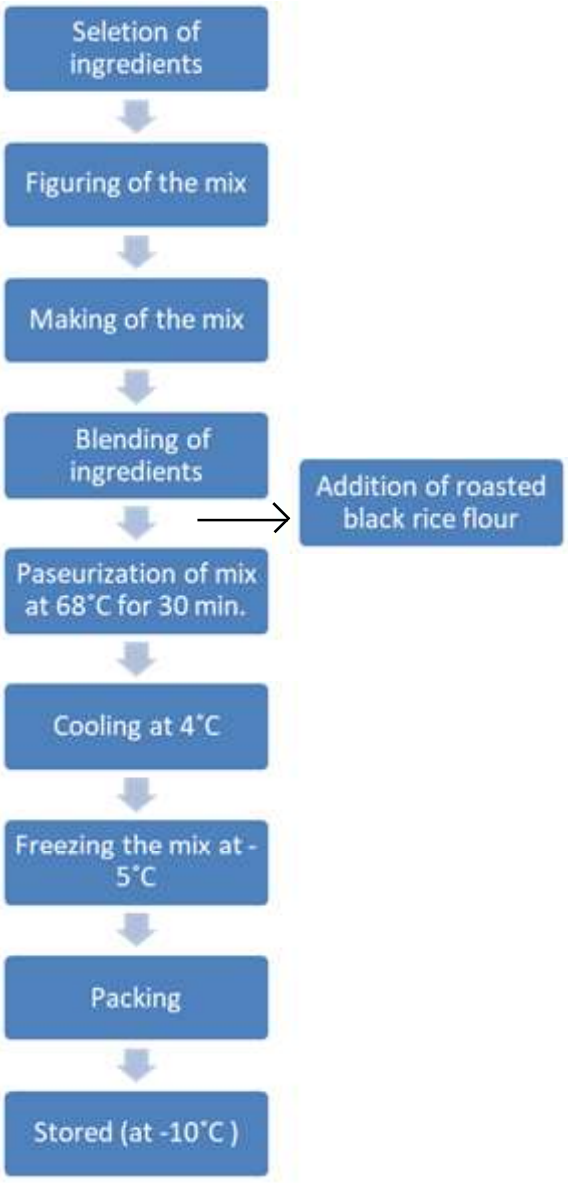

Fig. 1 Flow diagram for preparation of Black Rice Ice cream

Physico- chemical analysis of black rice revealed that it has good amount of protein % (9.00± 0.12), fat % (1.00 ± 1.20) and crude fibre % (0.51± 1.00). These results were in agreement with (Kumar et al. 2020). The study conducted by Thomas et al. (2013) reported the fat content of black rice was 0.70 % which is less than the present study. The difference in fat content may be due to the cultivation, regional difference or laboratory errors (Kang et al. 2011). Iron content black rice was 3.50 ±0.01mg/100gm. Total phenolic content of the black and white rice extracts were found to be 75.59 ±7.28 and 51.94 ± 1.97 mg catechin equivalent per gram, respectively. The results indicate that black rice extract contains higher total phenols as compare to the white rice extracts. The phenolic constituents of rice are mainly distributed in rice pericarp (Paiva et al. 2014). The main form of phenolic

Table 1 Physico-chemical composition, antioxidant properties and texture profile analysis of black rice ice-cream

Composition (%)	Control (B)	Optimised (B3)(Black rice flour ice cream)
Fat	10.50±0.20	10.52±0.02
Protein	2.50±0.02	3.90±0.12
Total solids	30.40±0.05	36.70±0.02
pH	6.32±0.02	5.65 ± 0.25
Acidity (%)	0.20±0.15	0.28 ± 0.51
Overrun (%)	90.31±1.32	72.56 ± 0.49
Viscosity (CP at 4°C)	293.0 ± 45.23	310.10 ± 65.23
Total phenolic content(mg/GAE)	5.05±0.01	68.43±4.21
DPPH (%)	NIL	40.21±2.23
Cohesiveness	0.05 ± 0.03[a]	0.09 ± 0.02[b]
Springiness	0.80 ± 0.04[a]	0.88 ± 0.05[a]
Gumminess	31.76 ± 0.02 [a]	46.30 ± 0.03[b]

Values are expressed as Mean± Standard deviation (n=3)

[a-b] Mean significant at (P<0.05) level of significance

Table 2 Cost estimation of black rice ice cream

Ingredients	Amount/ 100g cup	Cost (Rs.)
Black rice flour	12.0 g	3.60
Milk	79 ml	3.79
Cream	8.0g	8.0
Stevia	0.20 g	0.26
Table butter	0.50g	0.50
Sodium alginate	0.20g	0.15
Packaging cost	1 cup+ aluminium foil	1.0
	Total	17.30
Processing cost	Per cup	1.0
	Total	18.30
Marketing and distribution expenses @25% of product	Per cup	4.57
Total		22.87
Total cost (per 100g cup)		22.87 ≅23 Rs.

compounds present in rice is bound form (Saikia et al. 2012). Based on DPPH assay the antioxidant capacity of the extracts were found to be 56.23 ± 3.56 and 11.67 ± 1.18 % for black and white rice extract, respectively. These results were comparable with the observations given by (Sompong et al. 2011) that DPPH value ranged from 59.02 to 75.52 % for black rice varieties. The lowest value of antioxidant activity was observed in white rice. It may be due to reduction in polyphenol content as the concentration of the total soluble phenolic contents was related to lower antioxidant activity (Walter et al. 2013).

Based on sensory evaluation, black rice ice cream B3 (12% BRF) was most liked by the panelists followed by B2, B1 and B4. The flavour scores of control and B3 were at par (P>0.05) with each other. So, the BRF can effectively used as a fat replacer up to 40.05 % in ice cream. Body and texture score was also similar for control and B3. Combination B3 was selected for further study. There was decrease in pH and increase in acidity as compare to control sample. The acidity of the optimized black rice ice cream containing 12% black rice is more than that of the control i.e. 0.28 ± 0.03 and 0.20 ± 0.05 per cent, respectively. The lower overrun in the optimized ice cream may be due to relatively higher viscosity associated with such samples. According to Marshall and Arbuckle (1996) Ice cream mixes with high viscosities are known to have limited whipping ability. Viscosity of optimized ice cream was 310.10 ± 65.23. Addition of rice flour increased viscosity of ice cream mix (Cody et al. 2007). Similarly, Cottrell et al. (1980) also reported that polysaccharides such as starch increased the mix viscosity and restricted ice crystal growth during storage of ice cream. Table1 shows that the total solids values of ice cream were 36.70 ± 0.02 which is higher than the control 30.40± 0.05. This was due to the addition of black rice flour. Cody et al. (2007) also reported an increase in total solids with addition of rice flour in ice cream. Fat and protein content of optimized ice cream were 10.52±0.02 and 3.90 ±0.12, respectively. Incorporation of black rice flour also tended to increase the fat and protein content of

ice cream. Cohesiveness of optimized black rice ice cream was 0.08 ± 0.02 that is more than the control which represents an improvement in the sustainability of ice cream as it is compressed in the mouth before it breaks. Similar observations were reported by (Radocaja et al. 2011).

The estimated cost of black rice ice cream was found to be Rs. 22.87 per 100g/ cup which is quite comparable to the commercial ice cream available in the market which generally costs Rs. 20.0 or 30.0 per 100g of a cup (Table 2). Hence, from an economic point of view, the product was quite feasible.

Conclusions

Incorporation of black rice flour in ice cream will help to improve the nutritional as well as functional properties of ice cream. The high antioxidant activity of black rice makes it more suitable in dairy products. Being a rich source of anthocyanins, iron, antioxidants and overall nutritional profile made it as functional and novel ingredient in dairy as well as food products.

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SHORT COMMUNICATION

Dry cow therapy with ceftiofur to prevent postpartum mastitis in Indian cattle

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Abstract: Veterinary clinicians and academicians of India have limited locally generated data on the efficacy of the antimicrobial Ceftiofur hydrochloride in dairy cattle. The present study was undertaken to study the efficacy of Ceftiofur in dry cow therapy of cross bred Indian dairy cattle. Animals in the treatment group (N=254) received 500 mg of ceftiofur into all quarters on the day of drying while the animals in the control group (N=127) were left untreated. It was found that 98.03%, and 96.06% of treatment, and control group animals, respectively were exhibiting intramammary infections on the day of drying. Efficacy of Ceftiofur in terms of cure rates was 70.6% by day 28 postpartum with control group showing a spontaneous cure rate of 16.39%. The proportions of new intramammary infections by day 28 postpartum were higher in control group (73.77%) in contrast to treatment group (9.23%). Our study suggests an important role of ceftiofur as an effective antimicrobial agent in dry cow therapy.

Keywords: Mastitis, Dry cow therapy, Ceftiofur hydrochloride.

Dry cow therapy immediately after the last milking of lactation has traditionally been the most popular means of intramammary antimicrobial therapy and has become the most effective and widely used mastitis control method for dry cows (Eberhart, 1986). It plays a key role in eliminating existing infections in a herd and in preventing new intramammary infections (IMIs). The use of effective dry-cow therapy products results in 40 to 60 percent elimination of existing infections (Browning et al. 1990; Erskine et al. 1994; Osteras et al. 1999). For effective intramammary treatment, drugs should distribute throughout the udder and be rapidly absorbed into the general blood circulation (Du Preez J.H. 2007). However, in India, the treatment is predominantly done using parenteral antibiotics. Cephalosporins are an important class of antimicrobial agents that are in use today for both humans and animals. A few first and second-generation cephalosporins are approved worldwide strictly for treatment of mastitis infections in dairy cattle. A third-generation cephalosporin, Ceftiofur has been developed strictly for veterinary use. Ceftiofur has worldwide approvals for respiratory disease in swine, ruminants (cattle, sheep and goats) and horses, and has also been approved for foot rot and metritis infections in cattle. Spectramast DC (Zoetis, Mumbai) sterile suspension is a specially formulated intramammary infusion for dry cow therapy whose active ingredient is Ceftiofur hydrochloride. Most of the research for Spectramast DC was done in cows across the world and the data is plenty. However, Indian veterinarians and academicians have limited locally generated data on the efficacy of Spectramast DC in dairy cattle. Under these circumstances, the current study was undertaken to evaluate the efficacy of Spectramast DC in dairy cattle. The data generated from this study would help to accumulate data on efficacy of spectramast DC for dry cow therapy in Indian cattle and may be applied in other countries in future.

The animal farms in and around Hyderabad, India were visited weekly for a year in 2016 and cross bred cows were enrolled in the study on the day of drying off. Milk samples were collected and subjected for bacteriological examination for differential identification of mastitis pathogens such as *Staphylococcus sp., E. coli, Pseudomonas sp.,* and *Streptococcus sp.* by routine cultural and biochemical methods (Cruickshank et al. 1975; Bailey and Scott's., 2007). Cows that received dry cow therapy (N=254) *i.e.* 500 mg of Ceftiofur (Spectramast DC) into each quarter on the day of drying off were included in treatment group; cows that did not received any antimicrobial therapy on the day dry off served as control group (N=127). On day 5 and day 28 post calving of the enrolled cows, which were approximately 2 months

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days post dry cow therapy, milk samples were collected for bacteriological examination. Isolation of mastitis microorganisms from milk samples was taken to be indicative of IMI.

At the time of drying, before the experimental procedure, 98.03% of treatment group animals, and 96.06% of control group animals were exhibiting IMIs. The most prevalent pathogens at drying off were *Staphylococcus sp.* (66.25%) followed by *E. coli* (28.75%), *Pseudomonas sp.* (4.5%) and *Streptococcus sp.* (0.5%). Following treatment with Spectramast DC, cure rates of existing IMIs postpartum was 56.6% and 70.6% by day 5 and day 28 postpartum. The animals in the control group showed a spontaneous cure rate of 7.36% by day 5 and 16.39% day 28 post calving. In addition, following Spectramast DC therapy, the percentage of animals with new IMIs was low in the treatment group as compared to the control group. The percentage of animals that had developed new IMIs in control group was 64.75 and 73.77%, whereas in treatment group it was 4.01% and 9.23% by day 5 and day 28 post-partum, respectively. Only *E. coli* was found as new IMI agent in treatment group.

The primary goal of dry period, from an udder health perspective is to eliminate existing infections in the udder preparing for the next infection free lactation. The use of dry cow therapy is known to be associated with fewer cases of clinical mastitis post-partum (Smith et al. 1995; Erskine, 1998; Hamann et al. 1997; Williamson et al. 1995; Nickerson et al. 1999; Rainard 1988). The present study recorded the efficacy of ceftiofur antibiotic as dry cow therapy in eliminating existing infections, preventing new IMIs post-partum, and reducing incidence of clinical mastitis postpartum; results showed that ceftoifur was very effective in the above tested parameters. Very few studies were reported from India demonstrating the efficacy of dry cow therapy (Tiwari et al. 2018).

Conclusion

Findings from this study should add to the knowledge available on antimicrobials that are used in mastitis prevention and treatment. These findings suggest an important role of ceftiofur as an effective antimicrobial agent that can be used in curtailing either subclinical or clinical mastitis.

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SHORT COMMUNICATION

Dynamics of livestock population in Himachal Pradesh

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Abstract: A study was conducted to analyse the trends in livestock population in Himachal Pradesh in last two decades (1997-2019). Minor livestock species like Yak, Pig, Horse, Mule, Donkey and Camel population was excluded from the study as their all combined population accounted for only 1% of the total livestock population in the state in the 20th livestock census. The percent growth rates were calculated for all inter census period from 1997 to 2019. The study concluded that total livestock population in Himachal Pradesh has shown negative trend over the years 1997-2019. Except for the 190.2 % growth of crossbred/ exotic cattle population in the state, all other including Indigenous cattle, buffalo, sheep and goat population has shown negative growth in the study period i.e. 1997-2019. The recent census has recorded negative growth rate for all livestock species except crossbred/ exotic cattle. This may be due to reason that crossbred/ exotic cattle are constantly replacing indigenous cattle, owing to grading up with exotic breeds (Jersey/Holstein Friesian). The farmers prefer rearing of crossbred/ exotic cattle, as their milk yield per animal is higher than indigenous animals.

Keywords: Census, Himachal Pradesh, Livestock, Population

India has the largest livestock population and highest milk production across the world. The 20th livestock census, conducted in 2019, revealed the total livestock population of 536.7 million In Himachal Pradesh, more than 90 percent of the households rear one or other species of livestock. Various researchers such as Kumar and Pandey (1999), Kumar and Sharma (2004), Meganathan et al. (2004), Birthal and Taneja (2006), Kumar and Singh (2008), Baba et al. (2011), Patoo et al. (2011), Prabu et al. (2012) and Kale et al. (2016) have analysed the trends in livestock population growth in India and some states over the period since Independence. However, limited information is available on the growth trends of livestock in Himachal Pradesh. In this study, the growth trend of cattle and buffalo population in the state over two decades (1997 to 2019) has been analysed. The 16th, 17th, 18th, 19th and 20th livestock census reports were accessed from official webpages of the Department of Animal Husbandry and Dairying and information was analysed using various statistical tools.

The growth pattern of cattle (crossbred/exotic and indigenous), buffalo, sheep and goat was projected from the available data. Minor livestock species like Yak, Pig, Horse, Mule, Donkey and Camel were excluded from the study as their all combined population accounted for only 1% of the total livestock population in the state as per the 20th livestock census. The percent change in population was calculated using formula:

$$PR = \frac{(V_{Present} - V_{Past})}{V_{Past}} x100$$

Where: PR = Percent Rate: $V_{Present}$ = Present or Future Value; V_{Past} = Past or Present Value

India is having largest livestock population (536.76 Million) in the world, with 95.78% population found in rural areas and rest 4.22% in the urban India. According to 20th Livestock census, the total livestock population in Himachal Pradesh is 4.41 million (Table 1) which constituted 0.82% of the total Indian livestock population. About 99.1% of the livestock population in Himachal Pradesh is reared in rural areas while around 0.9% by urban population. This is due to the fact that majority of the population in the state resides in the rural areas and livestock rearing is practised as a mean of sustenance.

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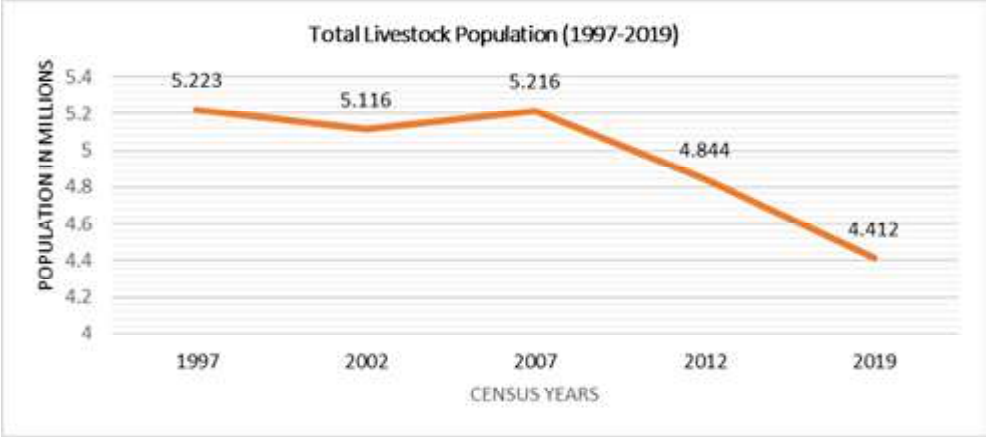

Fig. 1 Total Livestock Population trends (1997-2019)

Table 1 Himachal Pradesh Livestock Data as a component of All India (2019 census)

Livestock population	HP	All India	% share
Rural	4376116	514110739	0.851
Urban	36730	22650604	0.162
Total	4412846	536761343	0.822

Table 2 Total Milk Production in Himachal Pradesh and India (1997-2019)

Total Milk Production (Million Tonnes)	1997	2002	2007	2012	2019	% growth 1997-2019
HP	0.714	0.75	0.933	1.12	1.46	104.48
Growth %	0.00	5.04	24.4	20.04	30.35	
India	69.1	84.4	102.5	127.9	187.7	171.64
Growth %	0.00	22.14	21.45	24.78	46.76	
State's share in country's milk production (%)	1.033	0.889	0.910	0.876	0.778	

From table 2, India's total milk production in 2019 was recorded as 187.7 million tonnes and that of Himachal Pradesh as 1.46 MT, comprising 0.775% of the total national production. India's total milk production in 1997 was 69.1 MT with 0.71 MT from Himachal Pradesh. From 1997-2019, country's milk production saw an increase of 171.64% and that of Himachal Pradesh 104.48%. The percent growth in milk production in India for the census 17th, 18th ,19th and 20th over previous census was 22.14, 21.45, 24.78 and 46.76 respectively while the corresponding number for the state of Himachal Pradesh are 5.04, 24.4, 20.04 and 30.35 respectively. The numbers indicate that milk production in state increased at lesser rate than the milk production in the whole country. The share of the state in country's milk production has fallen from 1.03% in 1997 to 0.77% in year 2019. This is due to comparatively lower growth rate of the milk production in the state than the whole country. Himachal Pradesh has been categorised as least dairy progressive state, because of comparatively lesser focus on dairy development as compared to other dairy progressive states. Also, the state has lesser milk yield per animal in comparison to national average.

The total livestock population in Himachal Pradesh was recorded as 5.22 Million in 1997, which has fallen to 4.41 Million in 2019 signifying negative growth rate of 15.53% in the specified period. This decline has been recorded in every inter census period except for 2002-2007, in which the total livestock population in Himachal Pradesh showed a minimal positive growth rate of 1.95%. The decreasing livestock population in state is a matter of concern for policy makers as it signifies that farmers in the state are abandoning the livestock rearing due to various reasons like fragmentation of land holdings, nuclear families, preference towards tertiary jobs, scarcity of feed and fodder etc.

The population of crossbred/ exotic cattle in the state has shown a growth of 190.2% during the period 1997-2019. The number of crossbred/ exotic cattle in the state in 1997 was 0.368 Million while in 2019, the number has gone up to 1.06 Million. Only crossbred/ exotic cattle population has shown positive growth over the specified period. The wide adoption of Jersey, Holstein Friesian pure breeds and their crossbreds with native Indian breeds achieved through artificial insemination is the major reason behind growth of crossbred/ exotic cattle population in the state.

Table 3 Himachal Pradesh –Livestock Population in last 2 decades (in Millions)

Livestock Species	1997	2002	2007	2012	2019	% growth 1997-2019
Crossbred/Exotic Cattle	0.368	0.677	0.792	0.983	1.068	190.22
% Growth	0.00	83.97	16.99	24.12	8.65	
Indigenous Cattle	1.805	1.559	1.476	1.165	0.759	-57.95
% Growth	0.00	-13.63	-5.32	-21.07	-34.85	
Buffaloes	0.748	0.774	0.761	0.716	0.646	-13.64
% Growth	0.00	3.48	-1.68	-5.91	-9.78	
Sheep	1.08	0.926	0.901	0.804	0.791	-26.76
% Growth	0.00	-14.26	-2.70	-10.77	-1.62	
Goats	1.168	1.125	1.24	1.119	1.108	-5.14
% Growth	0.00	-3.68	10.22	-9.76	-0.98	
Total Livestock Population	5.223	5.116	5.216	4.844	4.412	-15.53
% Growth	0.00	-2.05	1.95	-7.13	-8.92	

The inter census growth rate is on the decline as this population had 8.6% growth rate between 2012-2019 as compared to 83.9% growth rate between 1997-2002.

The population of indigenous cattle breeds in Himachal Pradesh has fallen from 1.8 Million in 1997 to 0.7 Million in 2019, with negative growth rate of 57.9%. Their population has shown negative growth in each of last four inter census period. In a similar study, Tulachan (2001), reported negative growth of 1.06% in cattle population in Himachal Pradesh in the period 1982-1992. The wide adoption of exotic pure breeds and their crossbreds has led to this decline in population of indigenous cattle population in the state. In recent time Government of Himachal Pradesh has started various development schemes like Prakritik Kheti Khushhal Kissan, launched in 2018-19, which promotes rearing of Indigenous cattle breeds among farmers of the state.

The buffalo population in the state has declined from 0.74 million in 1997 to 0.62 million in 2019 with negative growth rate of 13.6%. Except for positive growth rate of 3.48% during 1997-2002, the buffalo population has shown negative growth in each of the inter census periods i.e. 2002-2007, 2007-2012 and 2012-2019.. The possible reasons for decline may be the fall in male buffalo population and preference for crossbred cattle over buffaloes. However, finding the concrete reasons for this decline is a matter of exploration in near future.

Sheep and goat population in Himachal Pradesh is majorly owned by the nomadic (Gaddi) people areas accounting for 60% of the total goat and sheep population of the state. The sheep population in the state stood at 1.08 Million in 1997 and declined to 0.79 Million in 2019. This amounts to a downfall of 26.79% over two decades. In each of the census conducted in the stipulated period (1997-2019) the sheep population has shown negative growth rate. One of the reason attributed to this trend is non adoption of sheep farming by the newer generations.

The goat population of the state has been recorded 1.108 Million in 2019 with a negative growth of 5.14% from the population of 1.168 Million recorded in 1997. This negative growth trend has been seen in every inter census period except for 2002-2007. State government is encouraging scientific goat farming of Beetal, Sirohi and Jamnapari breeds among farmers and a subsidy based development programme has already been operationalised in the state. The majority reasons may include reduction in pasture lands and forest grazing rights, disinterest of new generation in traditional nomadism, disease outbreaks, calamities and rustling during migration (Dogra et al. 2018).

Conclusions

The findings of this study revealed that total livestock population in Himachal Pradesh has declined significantly during the study period i.e. 1997-2019. According to 20th livestock census, Cattle constitute the majority of total livestock population followed by goat, sheep, buffalo and other livestock species. The production of milk in the state has increased, recording a rate of growth of 104.48% in the study period. The corresponding growth rate for nation stood at 171.64% in the period. In this period, all the livestock species have shown negative growth rate except for crossbred/exotic cattle. As livestock constitutes important component of the agriculture sector, immediate interventions in the form of capacity building in livestock management, improved feed and fodder development, veterinary services, improved credit facilities, push to farmer producer organisations etc. must be made to improve the livestock population in the state. The milk marketing infrastructure in the state happens to be weakest in the country, which has rendered dairy farming as non-commercial activity in the state. Suitable corrective actions need to be taken to boost the livestock development of the state.

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