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INVITED REVIEW

Probiotic dairy dessert from camel milk – A review

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Abstract: A staple food in many regions of the world, especially in the arid and semi-arid regions, is camel's milk. Health-promoting ingredients found in camel's milk include lactoferrin, zinc, lactoactive peptides, and mono- and polyunsaturated fatty acids. Some significant human ailments, such as TB, asthma, gastrointestinal disorders, and jaundice, may be treated with the use of these drugs. Compared to cow's milk, camel's milk has a more varied composition. In camels, nutrition, breed, age, and lactation stage have a greater impact on milk composition. The percentage of components in camel's milk varies greatly depending on the region and season. These whey proteins have distinctive qualities, such as physical, chemical, physiological, functional, and technical traits that are advantageous in the food application, in addition to their high nutritional value. Camel's milk proteins are hydrolyzed to create bioactive peptides, which have an impact on the body's primary organ systems and provide them physiological activities. The antidiabetic, antibacterial, antioxidant, and anti-cholesterol properties of camel milk are highlighted in this article.

Keywords: Autism, Anti-diabetic, Bioactive peptides, Camel milk

Introduction

Functional foods are good for the human body and thus they are in demand. This evaluation demonstrates the worth of camel milk, its derivatives, and milk-based products. A probiotic dessert is a perfect example of a functional meal with sensory attributes that the customer accepts, which may result in health-beneficial feedback (Valencia et al. 2016). The order Artiodactyl includes the family Camelidae, which includes camels. Numerous communities, notably those in the Middle East and Arabian Peninsula's arid regions, depend heavily on camels for their way of life (Kaskous, 2016; Sisay and Awoke, 2015). Camels could adapt to many environmental situations. They are used for transportation, recreation, and as sources of meat and milk, boosting the economy and ensuring that people have access to food (Suliman et al. 2019; Swelum et al. 2020). According to the latest Food and Agriculture Organization (FAO) figures, there are over 29 million camels in the globe, with about 95% of them being dromedary (one humped) camels (Sikkema et al. 2019). The amount of milk produced is influenced by a variety of variables, including breed, animal health, lactation stage, and living circumstances (Swelum et al. 2020). Even though camel udders are like cow udders in structure, camel milk output is smaller and more variable than cow milk yield. However, improved nutrition, water, and veterinary procedures may raise camel milk yield (Park & Haenlein, 2013). Milk is used every day by millions of people throughout the world because it has so many nutritional advantages, including helping young children's bones develop since milk is a strong source of calcium and vitamin D. It has been shown to be advantageous for older individuals, particularly for menopausal women where calcium shortage is a high-risk factor for osteoporosis development (El-Hatmi et al. 2015). For most individuals in underdeveloped nations, milk production not only provides nourishment but also a source of money and food security. The production of milk is a home activity in about 150 million families worldwide (FAO, 2012). Small-scale producers especially benefit from it because of the immediate financial flow.

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Human sustenance is provided by camel's milk. Additionally, it has medicinal advantages (Bai et al. 2015). The review focuses on the physicochemical properties of camel milk versus cow milk, health benefits, bioactive peptides derived from camel milk protein fractions, and camel milk value added products.

Comparison of physicochemical properties of camel milk with cow milk

Colour of camel milk is opaque white, has a typical milky odour and combination of both taste salty-sweet with a high acid content. These characteristics of camel milk are mostly dependent on the type of fodder or flora present in the grazing region (Singh et al. 2017) and also depends on the phase of lactation (El-aziz et al. 2022). The ratio of components in camel's milk is considerably influenced by season and region is shown in Table 1 (El-Hatmi et al. 2015). The taste may vary according on the camels' habitat. On the American continent, camel milk stands out for its sweet flavour and creamy texture. In the Middle East, camel milk has a taste akin to hazelnut (Galali and Al-Dmoor, 2019; Abbas, 2013). After coagulation, camel milk whey is white in colour, in contrast to the greenish hue of cow milk whey. The presence of casein and tiny fat globules, which scatter light, may be the reason of greenish colour of cow milk whey. Due to the presence of carotene, cow's milk has an opaque white colour with a yellowish tint whereas Camel milk's white colour results from a lower level of -carotene (El-aziz et al. 2022). Camel milk has an average density of 1.029 g/cm^3 (Singh et al. 2017). When compared to cow's milk and human milk, camel milk has the highest viscosity, which ranges between 1.3 and 1.44 mPa.s. The fat globules of camel milk are like small floccules, and this is the reason for the high viscosity of camel milk (El-aziz et al. 2022). Specific gravity of camel milk depends on the breed of the camel and ranges from 1.028 kg. L^{-1} and 1.033 kg. L^{-1}. These values are comparable to values for both cow milk and human milk, which are varies from 1.026 kg.L^{-1} and 1.034 kg.L^{-1} (Sakandar et al. 2018). The pH varies from 6.2 to 6.5, which is lower than that of cow's milk (6.5-6.7) (El-Hatmi et al. 2015). Camel milk has a pH between 6.5 and 6.75, similar to that of cow milk, (Sakandar et al. 2018) but lower than that of human milk, which has a pH between 6.75 and 7.42 with a mean of 7.09.(El-aziz et al. 2022). Fresh camel milk has a pH between 6.4 to 6.7, which is comparable to sheep milk but somewhat lower in bovine milk (Singh et al. 2017). While skim cow milk has a maximum buffering capacity at about pH 5.65, skim camel milk has a maximum buffering capacity of 4.95. This indicates that camel and cow milk have distinct compositions of components with buffering capacity (Sakandar et al. 2018; Sabahelkhier, 2012). Camel milk has an acidity that ranges from 0.14 to 0.15 percent, which is comparable to the 0.15% acidity of cow milk (El-aziz et al. 2022). Fresh camel milk has a titratable acidity that ranges from 0.13 to 0.16 percent lactic acid, which is slightly less than the average for cow milk of 0.17 percent and may vary by breed (Sakandar et al. 2018). The freezing point of camel milk varies from -0.57 °C to -0.61 °C (Singh *et al*. 2017). The freezing point of camel milk is lower than that of cow milk, which lies between -0.51 °C and -0.56 °C. Some physicochemical properties of camel milk are shown in Table 2. As the salt and lactose concentration of camel milk is higher in comparison to cow milk, this results in the lower melting point of camel milk (Sakandar et al. 2018). The calorific value of camel milk is 665 kcal/L, which is lower than the calorific value of cow milk, which is 701 kcal/L. The variation in calorific value may be due to differences in the concentration of fat, lactose, and protein contents. Camel milk has a steady lactose level that ranges between 3.5 and 4.5% (Devendra et al. 2016). In camel milk, the fat globule's average size is lower than that of globules seen in milk from cows, buffalo, and goats (Khalesi et al. 2017). Total milk solids in camel milk are comparatively low. The water content of camel milk lies between 87% and 90%. An inverse connection was observed between the total solids in camel milk and the camel's intake of water (Singh et al. 2017).

Compared to cow milk, camel milk has significantly less heat stability. Cow milk coagulates at 130 °C and heat coagulation time is about 40 minutes at pH 6.7, while camel milk coagulation time is 2-3 minutes at 130 °C and pH 6.7 (Sakandar et al. 2018). As the temperature increases, the heat stability of camel milk decreases when compared to cow milk and cannot sterilized at natural pH because of the casein-micelle size as well as lack of β-lactoglobulin (β-LG) (Hinz et al. 2012) and lesser percentage of k-casein (k-CN) in camel milk (El-aziz et al. 2022). The main problem of the camel milk preserved by ultra-high temperature (UHT) is sedimentation of proteins which need the use of selected additive to attain physical stability. Sterilized camel milk shows the maximum heat stability in the pH ranges of 7.0–7.2 and the minimum heat stability in the pH range of 6.5–6.8. Camel milk can be pasteurised well by the VAT or HTST methods of pasteurisation with zero precipitation of proteins (Felfoul et al. 2015). The proteins of camel milk classified into three wide-ranging

Table 1: Variability of physicochemical parameters of camel milk among different regions

Parameters	Irrigated Plains	Sandy Desert	Coastal Mangroves
pH	6.50	6.49	6.48
Titratable acidity (%)	0.165	0.169	0.178
Specific gravity	1.0319	1.0285	1.0301
Viscosity (cps)	1.7767	1.6500	1.6856
Conductivity (mS/cm)	5.0389	4.6971	4.8622
Refractive index	1.3452	1.3448	1.3450

Source: (Baloch et al. 2018)

classes, viz. caseins, whey proteins, and milk fat globule membrane proteins (Hailu et al. 2016) . Total protein content of camel's milk fluctuates from 2.15 to 4.90% (Swelum et al. 2021). Casein composition in camel and cow's milk is comparable, however whey protein level varies. As a result, the ratio of casein to whey proteins in cow's milk is larger than in camel's milk (Park & Haenlein, 2013). The primary protein in camel's milk is casein, which accounts for 52–87% of the total proteins. Whey proteins make for 20–25% of the total proteins (Devendra et al. 2016). Compared to cow's milk, the whey proteins of camel milk were more heat stable. At a temperature of 80 °C for 30 min, the whey proteins of camel milk were 32–35% less denatured than the whey proteins of cow milk, which were 70–75% denatured. The denaturation temperature for sweet whey of camel milk was 73.8 °C and for acidic whey 60.50 °C, and 70.5°C for sweet whey of cow milk and 63.9°C for acidic whey of cow milk, indicating that whey proteins obtained from camel milk are more sensitivity towards acidity when compared to whey proteins obtained from cow milk (Felfoul et al. 2017).

Both camel and cow milk have similar casein contents, but they have different whey proteins content. Hence, casein to whey proteins ratio of cow milk is greater than that of camel milk. This affects the firmness of coagulum. Camel's milk forms softer gel than cow's milk. Camel's milk casein has four fractions (α_{s1}, α_{s2}, ß, and k-casein) (Jilo, 2016) and presents in the ratio of ás1 to ás2 to ß to k-casein- 22:9.5:65:3.5 respectively (Swelum et al. 2021). Composition of casein in camel and bovine milk is shown in Table 3. Along with having many soluble proteins, camel's whey protein contains native proteases such chymotrypsin A and cathepsin D. (Alhaider et al. 2013). As a result, camel's milk proteins may be bioactive on their own or act as building blocks for bioactive peptides. As β-casein and α-casein were discovered to be 28.6 kDa and 35 kDa, respectively, it has been claimed that camel's milk caseins had greater molecular weights than bovine caseins. While in cattle, β-casein is 24 kDa and alpha-casein is 22–25 kDa (Al Haj et al. 2018). Camel's milk contains more β-casein (65%) than α-casein (21%). Compared to cow milk, camel's milk has nearly the same β-casein and á-casein concentration i.e., 36 and 38%, respectively and higher percent of k -casein i.e., 13%, which is around 4 times lesser in camel's milk i.e., 3.47% (Devendra et al. 2016). The â-casein is more digestible in human body and has less allergic effect, as it is more susceptible to peptic hydrolysis in the gut. The presence of higher ß-casein makes camel's milk more beneficial for human health. (Swelum et al. 2021). Casein micelles in camel's milk range in size from 20 to 300 nm, whereas those in cow's milk range from 40 to 160 nm (Park & Haenlein, 2013). Overall, camel's milk has casein micelles with a greater average diameter and a higher mineral charge (Attia et al. 2001). The primary whey protein in camel's milk is called α-lactalbumin (α-LA). α-LA from camel milk is preferred over á-LA from cow's milk because it is more easily digested and has more antioxidant action (Park & Haenlein, 2013).

Camel milk lacks ß-lactoglobulin, which makes it less allergenic, but contains other whey proteins such lactoferrin and immunoglobulins (Devendra et al. 2016). The primary immunoglobulin in camel milk is immunoglobulin G (IgG), whose molecular weights are different from those of IgG from cattle, sheep, goats, and humans (Alavi et al. 2017). The primary component in bovine whey (50%) is ß-LG, which is absent in camel's whey (El-Agamy et al. 2009). Camel's whey proteins offer a unique source of proteins that may produce bioactive peptides that have the potential to improve health.

Camel's milk varies in its overall mineral concentration between 0.60 and 0.90 percent. The increased chloride content gained from the feed the animals eat can be used to explain why camel's milk has a salty flavour (Devendra et al. 2016). calcium, phosphorous, magnesium, sodium, and potassium content in camel milk are comparable to that in cow milk in terms of minerals (Kaskous, 2016). zinc, copper, iron, and magnese content make up most of the difference since camel's milk contains greater levels of these minerals. The prevention of iron-deficiency anaemia may be helped by camel's milk's higher iron content. Additionally, because lactoferrin requires low amounts of citrate to be effective, camel milk has a lower concentration of citrate than cow milk, which boosts lactoferrin's antibacterial action (Park & Haenlein, 2013).

Vitamin and mineral salt concentrations in camel milk vary depending on the breed, diet, amount of water consumed, and stage of lactation. It also has a high vitamin C content—up to ten times that of cow milk and a high vitamin E content level (Benmeziane–Derradji, 2021). As a result, it may boost the antioxidant and antiradical properties and lengthen shelf lives (Izadi et al. 2019). The fundamental distinction between camel

Table 2: Physicochemical properties of camel milk and camel milk whey

Parameters	Camel Milk	Camel Milk Whey
Acidity (%)	0.21	0.3
Ash (%)	0.97	0.9
Fat (%)	3.4	0.4
pH	6.5	5.4
Protein (%)	3.3	1.5
Total solids (%)	9.9	3.3

Source: (Ahmed NNA, 2011)

Table 3: Composition of casein in camel and cow milk

Protein	Camel (g/l)	Bovine (g/l)
α_{s1}-casein	5.3	9.5
α_{s2}-casein	2.3	2.5
ß-casein	15.6	9.8
k-casein	0.8	3.3
Total casein	24.0	25.1

Sources: (Hailu et al. 2016 ; Swelum et al. 2021)

milk and other milks is that camel milk has a low lipid and saturated fatty acid content, which results in a low cholesterol level (Benmeziane–Derradji, 2021). However, it lacks folic acid, pantothenic acid, and thiamine, riboflavin, and retinol. Pyridoxine and Cyanocobalamin have about the same amounts in both camel and cow's milk (Devendra et al. 2016; Elhosseny et al. 2018). Compared to cow's milk, camel's milk contains more inhibitory structures, particularly lysozyme and lactoferrins, which are significantly more abundant. Camels' milk contains glycoprotein lactoferrin between 0.02 and 2.1 g/L. It possesses antibacterial, anti-inflammatory, immune-suppressing, and anticancer properties. Another milk antibacterial component called lysozyme is found in camel's milk at a concentration of around 150 g/L, which is greater than that of cow's milk (70 g/L) (Park & Haenlein, 2013). Fig. 1 represents the comparison between camel milk and cow milk

Nutritional benefits of camel milk

Camel's milk has high level of digestibility (Meena et al. 2014). Vitamin C, which is three to five times more than in cow's milk, is crucial for nutrition (Bai et al. 2015; Kamal & Karoui, 2017). Comparable to human milk, camel milk has a high amount of ß-casein; this casein is more easily digested and results in fewer new born gastrointestinal sensitivities. Camel's milk offers a variety of nutrients and medicinal qualities, including antibacterial, anticancer, antioxidant, anti-hypertensive, and anti-diabetic effects (Ayoub et al. 2018; Sharma and Singh, 2014; Bakr Shori, 2015). During storage or processing, native protease enzymes such as milk plasmin can hydrolyse proteins and liberate bioactive peptide fragments (Mohanty et al. 2016). In addition to enhancing the bioactive qualities of milk proteins, enzymatic hydrolysis is known to boost their functional properties (Jrad et al. 2014). Making bioactive hydrolysates from camel's milk proteins and examining their potential bioactivity in in vitro and in vivo settings are the main topics of recent study (Mudgil et al. 2018). The probiotic bacteria can manufacture these beneficial components from milk proteins during the fermentation process (Devendra et al. 2016). Due to its high content of anti-inflammatory proteins, polyunsaturated fatty acids, and vitamins that speed up carbohydrate metabolism, it has a positive impact on stomach and intestinal illnesses (Kaskous, 2016). Due to the presence of lactoferrin, lysozyme, lactoperoxidase, hydrogen peroxide, and immunoglobulins, camel's milk possesses antibacterial and antiviral characteristics. These substances can inhibit both gram-positive and gram-negative bacteria, such as *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Lactoferrin in camel milk suppressed the growth of *Salmonella typhimurium* by binding iron and preventing it from being used for its growth. Camel milk has more antimicrobial ingredients than cow milk does. However, milk's beneficial characteristics are fully rendered inactive after being exposed to 100°C for 30 minutes. Additionally, camel milk's whey proteins improve the anti-rotaviruses' capacity to treat non-bacterial gastroenteritis. Camel's milk has a

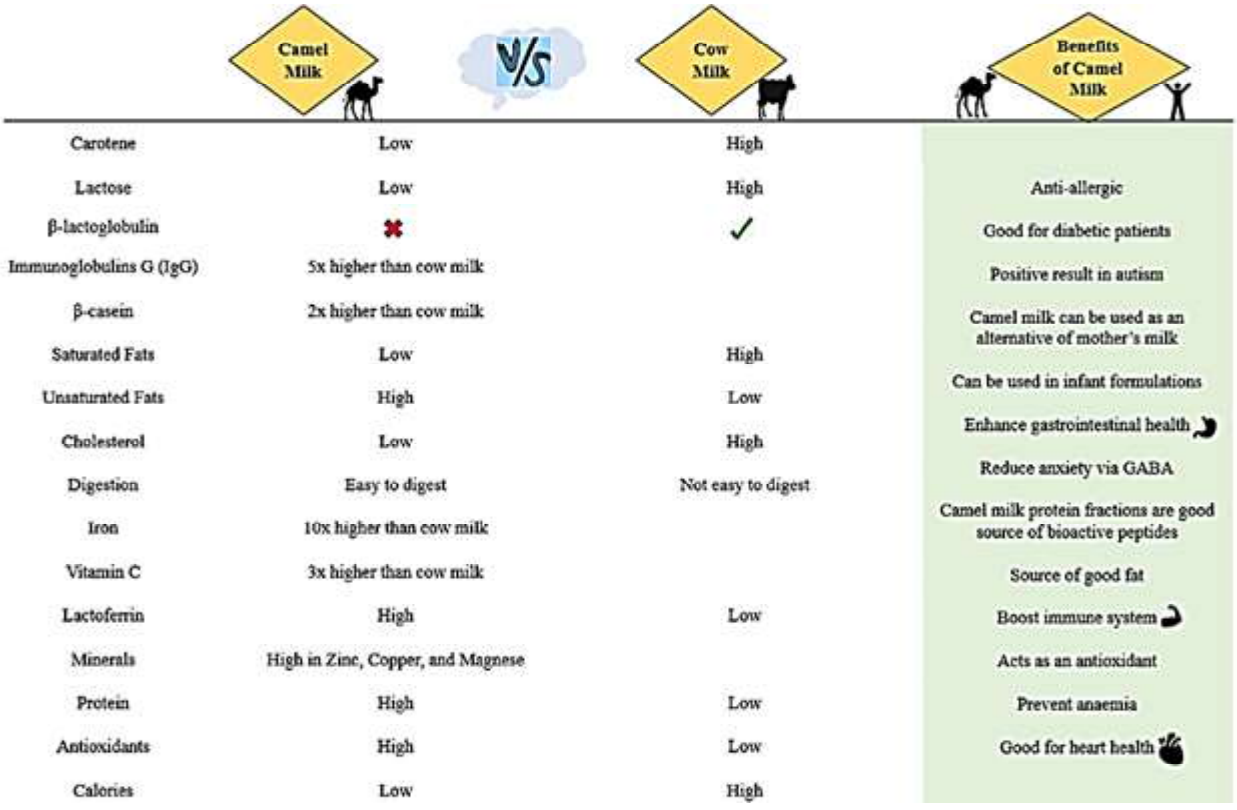

Fig 1. Comparison between camel and cow milk and health benefits of camel milk

therapeutic impact on drug-resistant tuberculosis (TB) due to the number of antibacterial components in it. (Devendra et al. 2016). Hepatitis C viruses can be inhibited and their multiplication in cells prevented by the lactoferrin and IgG found in camel's milk. When human IgG cannot detect the presence of the virus, the Camel milk's IgG can recognize hepatitis C viral peptides. Saltanat et al. (2009) reported that consumption of camel milk for 1 year can help treat hepatitis B because it controls the expression of type 1 helper T cell/ type 2 helper T cell (Th1/Th2)-type cytokines and balances the Th1/Th2 cytokine network, thus strengthening cellular immunity, preventing virus deoxyribonucleic acid (DNA) replication, and enhancing the recovery of chronic hepatitis B patients.

Milk proteins, like many other dietary proteins, have angiotensin converting enzyme (ACE)-inhibitory peptides as part of their main structure. The fermented camel milk also contains ACE-inhibitory peptides (Moslehishad et al. 2013). Proteins are broken down into peptides and amino acids by probiotic bacteria used in fermentation. The bioactive peptides in camel milk that has undergone fermentation may help to reduce cholesterol. From camel's milk, certain probiotic strains of *Lactobacillus, Bifidobacterium, Enterococcus,* and *Streptococcus* were identified and employed in the dairy business (Shori & Baba, 2014). Orotic acid, which is present in camel's milk, is known to lower cholesterol levels in people (Devendra et al. 2016). El Hatmi et al. (2017) compared the physicochemical and sensorial characteristics of fermented camel (dromedary) milk (FDM) and fermented cow milk (FCM) by fermenting milk with *Enterococcus faecium* (W1), *Streptococcus macedonicus* (W2), and by the combination of W1 and W2. More viscous and acidic fermented milks were produced by the combination of W1 and W2 than by a single strain. The advantageous fatty acids were unaffected by the fermentation process. Both FDM and FCM milks included 19 aroma components, the majority of which were ethanol, acetoin, and diacetyl. When fermented by the W1 strain, FDM had the highest radical scavenging activity. In contrast to peptide fractions produced by imitating gastro-intestinal digestion, El-Hatmi et al. (2016) found that peptide fractions made following fermentation with Streptococcus thermophilus exhibit a higher level of free radical scavenging activity.

Due to the tiny size of the immunoglobulins and the presence of insulin and compounds like insulin e.g., half cysteine, camel's milk can be utilized to treat both type 1 and type 2 diabetes (Devendra et al. 2016; Malik et al. 2012). Limon et al. (2014) reported that the camel and goat milks can activate GABA (gamma- aminobutyric acid) receptors and contain much more bio accessible GABA than cow and human milks. There are 52 units of insulin per litre in camel milk, which is a high concentration (Ayoub et al. 2018). Additionally, these substances have an impact on the liver and pancreas, which improves insulin production, lowers the amount of insulin needed to control blood sugar level, lessens insulin resistance, and enhances lipid profiles (Kaskous, 2016; Ayoub et al. 2018).

Decreased allergenicity, particularly in kids with cow's milk allergies, is another potential health advantage of camel's milk. This allergy is brought on by the presence of ß-lactoglobulin as well as the high concentration of á-casein and low content of hypoallergenic ß-casein. The immunoglobulins in camel milk resemble those in human milk hence, it is safe for infants to drink (Devendra et al. 2016; Izadi et al. 2019). Also, camel's milk is safe to drink for those who are lactose intolerant. Compared to cow's milk, which is high in D-lactate, camel's milk has more L-lactate. L-lactate reduces the allergenicity of milk. Because camel milk immunoglobulins do not react with the immunoglobulin E (IgE) of children who are allergic to cow's milk, they reduce allergic symptoms (Kaskous, 2016). Furthermore, those who have autism may benefit from drinking camel's milk. ß-Casomorphin, a potent opioid peptide that is formed by the incomplete metabolization of casein protein in the intestine, causes diarrhea and alter appetite. This opioid peptide may also cause brain damage in youngsters. Cow's milk has a high concentration of ß-casein and ß-lactoglobulin, which increases the likelihood that opioid peptides will develop (Devendra et al. 2016; Kaskous, 2016). Al-Ayadhi and Elamin (2013) examined the effects of the consumption of 500 mL of camel milk by autistic children for a period of two weeks. The results showed that by changing the levels of antioxidant enzymes and nonenzymatic antioxidant compounds, camel milk may have a significant impact on reducing oxidative stress and improving autistic condition.

Additionally, the protective proteins lactoferrin, lysozyme, and immunoglobulins in camel's milk may help with brain development (Devendra et al. 2016). Another camel's milk advantage is the treatment of breast, lung, liver, and blood cancer (Kaskous, 2016). It prevents the growth of human hepatoma (HepG2) and human breast (MCF7) cells as well as the activation of cell-line-specific death receptors and oxidative stress-related processes (Korashy et al. 2012). The drinking of camel's milk contributes to the development of a larger abundance of Allobaculum, Akkermansia, and Bifidobacterium, which enhances the gut microbiota. According to a study by Wang et al. (2018), camel's milk may increase the amount of Allobaculum, which may have a good impact on the organism's physiological function. Short-chain fatty acids produced by this species help to reduce inflammation, prevent obesity, and promote colon health. A mucin-degrading probiotic known as Akkermansia is well known for its advantages against obesity, metabolic diseases, diabetes, and inflammation. Abdel-Salam et al. 2016 study the impact of a diet including Camel whey protein on wound healing in malnourished mice. They discovered that the ability of malnourished mice supplemented with camel whey protein to heal wounds improved due to a reduction in oxygen free radicals and an increase in glutathione levels (an antioxidant). Ayyash et al. (2018) in his

study showed the health-encouraging benefits of water-soluble extract (WSE) of fermented camel milk. They found that WSE of fermented camel milk has ACE-inhibition antiproliferative and antioxidant activities.

Camel milk proteins*:* The concentration of amino acids in camel milk are high except for lysine, glycine, threonine, and valine (Shamsia 2009). Recently, increasing attention has been paid to the components of MFGM (Milk Fat Globule Membrane), especially to their protein components (Yang et al. 2015). MFGM proteins, which account for 1–4% of the total milk protein, depending on the breed of the animal. The proteins of camel MFGM are mainly involved in protein processing, bio-synthesis of fat, and actin cytoskeleton organization (Sabha et al. 202). The main MFGM proteins are fatty acid synthase, xanthine oxidase, butyrophilin, lactadherin, and adipophilin (Bakry et al. 2021; Saadaoui et al. 2014).

Camel milk fat*:* Camel milk ranges in fat content from 1.2 to 4.5% (Devendra et al. 2016). However, camel's milk has a fat level that may reach up to 6.4%, according to (Park & Haenlein 2013), and its fatty acid composition is characterized by the presence of unsaturated and long-chain fatty acids like linoleic acid in greater concentrations. Short chain fatty acids in camel milk are low. The amount of lipids in human serum is decreased as a result of this. Ninety-two to ninety-nine percent of the fatty acids are long-chain, and between 35 and 50 percent are unsaturated (Izadi et al. 2019). Because of these structural variations, the camel's milk fat has a "waxy feel

Camel milk water content: The most important factor in camel milk is water content. The total solid content is similar to that of human milk. Unlike other animals the water content of camel milk increases during dehydration. When water is easily available the water content of the milk is 86 percent, but when water is restricted the water content of milk rises to 91 percent (Rahim et al. 2020). This is useful as water source for dehydrated calf and the humans in area where water is scarce. The reasons for increment of water content of milk of dehydrated camel are antidiuretic hormone **(**ADH) secretion is elevated in the dehydrated camel, a decrease in the fat content and type of forage eaten by the animal (Ahmed, 2015).

Camel milk carbohydrate: The major carbohydrate fraction in camel milk is lactose sugar with range between 3.3 to 5.80 percent. The nature of vegetation eaten by the camels in desert areas could be a significant factor for extensive variation in lactose contents. Camels generally like to consume halophilic plants like Celosia, Acacia and Atriplex to fulfil their physiological necessities of salts intake. However, in some dromedary varieties of the camel lactose contents found to be changed slightly over a period. Lactose can be readily digested by human lactase with no signs of lactose intolerance. Additionally, it contains a modest number of various oligosaccharides that defend new born against viruses, encourage the growth of Bifidobacterium environments, and aid in nervous system development (Park & Haenlein, 2013).

Bioactive peptides

Potential amino acids (bioactive peptides) are encoded in milk proteins as inactive sequences that are liberated from milk proteins either naturally during digestion, through proteolysis using enzymes, or by fermentation. Bioactive peptides found in camel milk have a wide range of potential uses, including anti-bacterial (gram positive and gram negative), anti-hypertensive, ace inhibition, anti-inflammatory, mineral binding, cytotoxicity, anti-oxidant, and immunomodulatory effects (El-Salam and El-Shibiny, 2012; Zibaee et al. 2015; Soleymanzadeh et al. 2016). As a result, interest in bioactive peptides derived from camel milk proteins is increasing nowadays. Three methods are used to generate peptide fractions from camel milk proteins (i) fermentation with different proteolytic bacterial strains (ii) enzyme hydrolysis using purified proteases and (iii) a combination of pepsin and pancreatin to mimic gastrointestinal digestion (Mati et al. 2017). Ibrahim et al. (2018) isolated bioactive antioxidant peptides from camel milk's protein fractions. Their findings suggest that the casein and whey protein fractions of camel milk both contain bioactive peptides with significant radical-scavenging activities. This opens a fascinating possibility for their potential use as nutraceuticals or therapeutic peptides for the prevention and treatment of diseases linked to oxidative stress. Fourteen peptides with masses ranging from 913.12 to 2,951.68 m/z were derived from the casein hydrolysate. From casein hydrolysate, most of the peptides were generated by ß-caseins in comparison to $á_{s1}$, $á_{s2}$ and ê-casein. There were eight peptides derived from the whey hydrolysate, with masses ranging from 1,168.52 to 1,861.14 m/z. In the whey hydrolysate few peptides were derived from lactophorin and cysteine-rich proteins, but most of the peptides were derived from lactoferrin. Kumar et al. (2016) Compared to alcalase and papain, á-chymotrypsin hydrolyzed camel milk whole casein displayed higher antioxidant activity. According to Mudgil et al. (2019) study, camel milk proteins are an intriguing source of antihypertensive and anti-inflammatory peptides. Also, it was revealed that the type of enzymes and the timing of the hydrolysis had a significant impact on the bioactivities of the peptides generated during enzymatic hydrolysis, which released powerful antihypertensive and anti-inflammatory peptides. Alcalase and papain enzyme hydrolysis of camel milk proteins for 6 hours increased ACE inhibitory action, while 9-hour enzyme hydrolysis resulted in a decrease in this activity, demonstrating the significance of controlled hydrolysis. Al-Shamsi et al. (2018) verified that the performance of camel milk protein hydrolysates (CMPH) was impacted by the type of enzymes utilised in their production. Alcalase and bromelain both had lower hydrolysis efficiency than papain. The foaming capacity and protein solubility were increased by all the enzymes, with papain having the most activity. After papain hydrolysis, antioxidant activities were also elevated. Nongonierma et al. (2018) identified nine

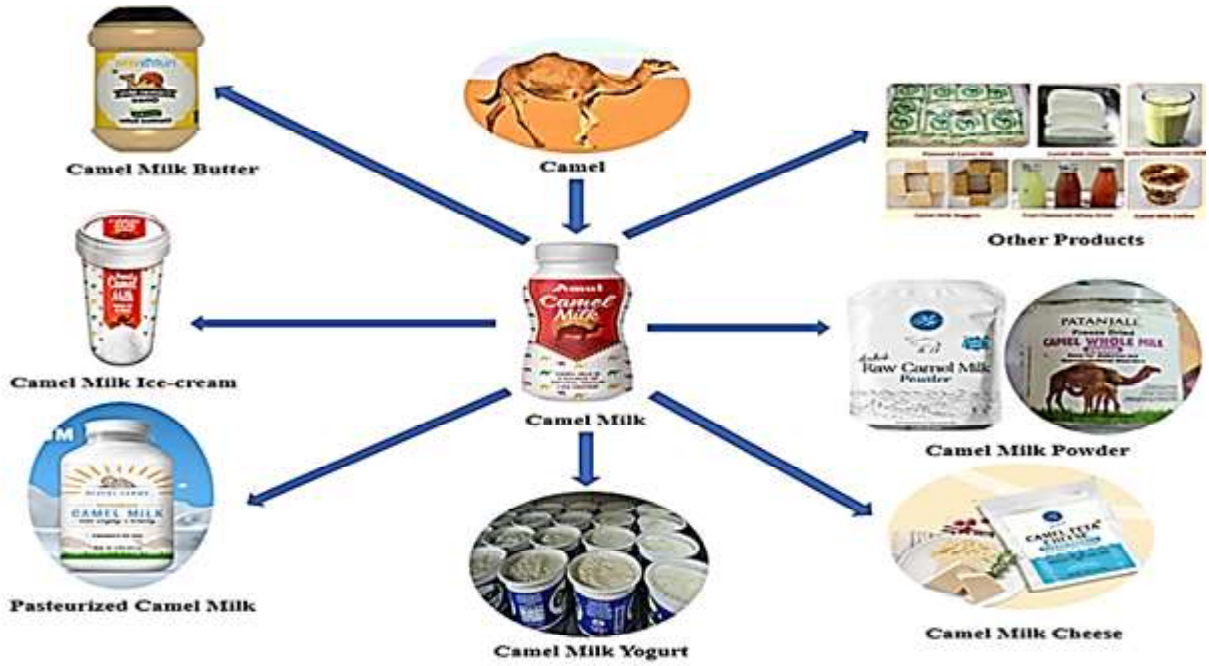

Fig. 2 Value-added camel milk products

unique camel milk peptides including FLQY, SPVVPF, ILDKEGIDY, LQALHQGQIV, LLQLEAIR, LPVP, ILELA, MPVQA and FQLGASPY with high dipeptidyl peptidase (DPP-IV) inhibitory capability. The most potent anti-diabetic peptides were MPVQA and LPVP. Nongonierma et al. (2019) extracted Val Pro Val (VPV) bioactive peptide from camel whey protein hydrolysate that was reported to be the second most powerful DPP-IV inhibitor known to date after Ille Pro Ille (IPI) (Diprotin A), a commercial DPP-IV inhibitory. Mudgil et al. (2021) found that when camel milk caseins were hydrolyzed with enzymes alcalase and pronase E for 3 and 6 hours, followed by mimic gastro-intestinal digestion, it resulted in the generation of potential anti-diabetic peptides that had a strong inhibitory effect against enzymatic markers that are involved in diabetes like DPP-IV, α-amylase, and α-glucosidase. Their findings indicated that the peptide FLWPEYGAL was the most effective inhibitor of á-amylase, LPTGWLM, GPAHCLL, and MFE peptides were the most effective against α-glucosidase; and the peptides HLPGRG, QNVLPLH and PLMLP were most effective against DPP-IV. Kamal et al. (2018) investigated the in-vitro anti-liver cancer potential of camel milk whey protein hydrolysates, which were produced by hydrolysing the protein using pepsin, chymotrypsin, and trypsin at varying times. Just 4.5–6.5% of the viable hepatoma G2 (HepG2) cells remained after the treatment with chymotryptic hydrolysates that are generated after 3 hours of hydrolysis, which had the highest anti-proliferative effect. Tryptic hydrolysates generated after 3 hours of hydrolysis and peptic hydrolysates generated after 3 and 6 hours of hydrolysis have both shown a notable antiproliferative effect by reducing the viability of cancer cells.

Value added camel milk products

Large-scale camel milk production and processing facilities have recently been built in several nations as a result of the growing demand for camel milk, particularly from non-camel-producing communities (Seifu 2023). There is scope for manufacturing a variety of processed products from camel milk because, in terms of nutritional value, camel milk is superior to cow or buffalo milk and relatively similar to human milk. It has high quantities of a variety of bioactive substances that are vital for maintaining human health. Compared to bovine milk, the number of food products made from camel milk is still quite small, despite its significant nutritional and health benefits. To maintain the nutritional content of camel milk while obtaining desired qualities in the finished products, a thorough understanding of the composition, bioactive components, and thermal stability of camel milk is crucial (Ho et al. 2022). Value added products from camel milk is shown in Fig. 2

Pasteurized camel milk

For camel milk, different countries use different pasteurization time-temperature combinations, like 63°C for 30 min., 72°C for 15 sec., and 80°C for 20 sec. Countries that produce camel milk do not have any specific regulations or standards for camel milk. As a result, camel milk is pasteurized in accordance with the same criteria as milk from cows. The best temperature to improve milk stability is thought to be 80°C for 20 seconds during pasteurization of camel milk. Camel milk undergoes separation issues at temperatures exceeding 80°C (Seifu 2023). Different time-temperature combinations for the pasteurization of camel milk

were reported in different scientific literatures, such as Rahman et al. (2012), reported 60°C for 30 minutes; Alhaj et al. (2013), reported 75°C for 15 seconds; and Oselu et al. (2022), reported 90°C for 30 minutes. Thus, in various studies, several time-temperature combinations varying from 50 to 95 °C were evaluated for the inactivation of microorganisms (Muthukumaran et al. 2022). Due to protein sedimentation, ultra-high temperature pasteurization (UHT) of camel milk was unsuitable. Only mild UHT processing, i.e., 150°C for 2 seconds followed by refrigeration, was needed to extend the shelf life of camel milk up to 5 weeks (Seifu 2023). To find an effective pasteurization indicator, Lorenzen et al. (2011) examined the alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactoperoxidase (LPO), lipase (LIP), and leucine aryl amidase (LAP) activities in raw and pasteurized camel milk. They concluded that in pasteurized camel milk, GGT, ALP, and LIP were still present, but lactoperoxidase (LPO) might have been a better indicator of pasteurization.

Camel milk powder

Food and dairy industries may have a wonderful opportunity to expand their product lines by using camel milk powder to promote new milk and milk products. Camel milk powder are relatively recent consumable product in international dairy market, credit goes to the emergence of powder milk manufacture, Drying is the best method to store this perishable commodity for subsequent use. Furthermore, because camel milk is typically produced far from the consumption basin, the only way to transport a significant quantity of it is to remove the water that makes up 88–90% of its weight. This approach also preserves liquid milk's nutritional value, which is a bonus. Camel milk powder is currently produced using two basic drying techniques: freeze-drying and spray-drying (Chhasatiya and Tagalpallewar 2022). Deshwal et al. (2020) prepared spray-dried whole camel milk powder (SDW), spray-dried skimmed camel milk powder (SDS), and freeze-dried whole camel milk powder to study the impact of spray and freeze drying on the physico-chemical and functional qualities of camel milk. The authors' findings stated that the SDS showed higher moisture content, followed by the SDW, and then the FDW. Powders' calcium and iron contents significantly decreased as a result of spray dying. SDW exhibits strong flowability. The best wettability and solubility were found in FDW. SDS showed the best foaming ability and stability due to the lack of fat and absence of LG and higher LA in camel milk. Ibrahim and Khalifa (2015) evaluated the effect of lyophilization process on the nutritional value of camel milk. The result showed that the protein (casein and whey), amino-acids ash fat, and lactose content were enhanced after lyophilization. Vitamins and minerals content also increases except for vitamin c and calcium, potassium, and phosphorus.

Camel milk cheese

The process of manufacturing camel cheese is novel. The difficulties in clotting observed in the preparation of camel milk cheese are due to the different casein proportions between cow and camel milk, particularly the lower amount of k--casein (3–4% of casein, compared to 13–15% in cow milk). Furthermore, camel milk's casein micelles cannot coagulate well with the bovine chymosin used in the dairy industry, resulting in a weak curd. So, the first difficulty confronted by researchers and dairy technologists is obtaining a hard coagulum (Konuspayeva and Faye 2021). Numerous researchers have tried to make cheese from camel milk using a variety of processing techniques, including starter cultures, heat treatments, the addition of calcium chloride ($CaCl_2$), rennet, and salting. Al-zoreky and Almathen (2021) prepared soft cheese from camel milk using recombinant camel chymosin. Recombinant camel chymosin (50 IMCU/kg) was used to curdle pasteurized camel milk. The result showed that thermophilic starter cultures coagulate camel milk faster. Konuspayeva et al. (2017), in their study, compared camel cheese with bovine cheese and prepared both dry and brine-salted soft camel milk cheese. They concluded that camel milk with good starter cultures and raising the protein content of camel milk maximized cheese yield and utilized thermophilic cultures to hasten the acidification of camel milk. They also reported that the recombinant camel chymosin was able to coagulate camel milk faster. El Zubeir and Jabreel (2008) prepared fresh using camifloc (a product that comprises calcium phosphate and vegetable rennet to curdle camel milk cheese.) and calcium chloride + Camifloc. They concluded that the yield of calcium chloride + Camifloc cheese was found to be higher than the camifloc cheese.

Camel milk ice-cream

Presently, the *United Arab Emirates* (UAE), Kazakhstan, and Morocco are countries where camel milk ice cream is manufactured commercially. 'Orom', a soured cream popular in Mongolia, is made from Bactrian camel milk. A good-quality, sensory-acceptable ice cream can also be produced by combining camel and cow milk. In the same way that bovine milk is processed, camel milk can also be processed to make ice cream, albeit the finish product may have distinct qualities and storage properties. However, camel milk ice-creams typically have a lower viscosity, a lower dry matter content, and a lower melting point than cow milk ice-creams when made using the same formulations. This is explained by the difference in total solids between camel milk (10.02%) and cow milk (12.30%) (Seifu 2023). Elkot et al. (2022) prepared a synbiotic ice cream using camel milk and black rice powder. They concluded that the incorporation of black rice powder improved the physicochemical and rheological characteristics of ice cream samples and had a substantial protective impact on the longevity of probiotic bacteria *Lactobacillus acidophilus LA-5*. Hajian et al. (2022) created low fat camel milk ice cream by incorporating chymotrypsin-generated camel milk casein antioxidant hydrolysates. The incorporation of antioxidant hydrolysates

improves free radical scavenging activity. The viscosity and consistency of the ice creams were also increased by the addition of casein hydrolysates because of their water-holding capacity.

Camel milk yogurt

Several strains of traditional lactic acid bacteria, including *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacteria*, have been tested. Unfortunately, the production of camel milk yoghurt has a textural issue, with the final product tasting sticky and unappealing (Konuspayeva and Faye 2021). The lack of ß-LG and lower level of ê-CN, as well as the high whey to casein ratio, are compositional factors that contribute to the weak texture and thin consistency of camel milk yoghurt (Seifu 2023). Trials with the addition of gelatine, alginate, or calcium were made to achieve a better texture. Ferments that produce exopolysaccharides were also utilized. Additionally, the introduction of a high-pressure treatment could improve the texture. Some authors have made sporadic attempts to enhance the production of camel milk yoghurt by combining it with milk from other species and by adding 0.75% biosynthesized xanthan, but it has given mixed results in terms of organoleptic qualities. Even with the addition of artificial or natural flavors, the finished product resembles 'drinking yogurt' without the expected taste. Shahein et al. (2022) reported that the addition of date syrup at the rate of 8% to fermented camel milk improve the taste. Frozen yoghurt has been seen as an alternative by several researchers as a product that falls between yoghurt and ice cream (Konuspayeva and Faye 2021). Galeboe et al. (2018) prepared yoghurt by the incorporation of 1.2% gelatine, 1.5 ml/L of $Cacl_2$, 40 ml/L of maple strawberry syrup, 5% bovine skim milk powder and 6% culture in camel milk and incubate for 18 hours at 42^0C. The findings demonstrated that the physical, chemical, and microbiological qualities of cow milk and camel milk yogurt were equivalent. However, the sensorial properties of camel milk yoghurt were not as well liked as those of cow milk yoghurt. Ibrahem et al. (2016) prepared yogurt by mixing camel milk with sheep milk in order to improve the processing aspects of camel milk and compared two different starter cultures used for fermentation. In comparison to yoghurt made from pure camel milk, yoghurt made from camel-sheep milk mixtures had higher fat, total solids, and protein contents. The quality and acceptability of camel milk yoghurt were enhanced by the addition of sheep milk.

Camel milk butter, ghee and sweet

Butter manufacturing from camel milk is exceedingly challenging, and the procedure for making butter from cow milk cannot be used to camel milk due to variations in the physical and chemical nature of their fats and proteins, even though their fat contents are quite comparable to those of bovine milk. Thus, several authors asserted that camel milk cannot be used to make butter. Camels' milk has little creaming ability due to the presence of tiny fat globules, the strong bonds between fat and proteins, and the absence of agglutinin, a protein that encourages the clustering of fat globules. Moreover, churning camel milk cream requires higher temperatures than those typically employed for bovine milk due to the high melting point of camel milk fat. Camel milk fat melts at a high temperature because of the large proportion of long-chain fatty acids in the fatty acid profile and the thicker globular membrane. Due to the probiotic properties of the microflora employed in traditional camel milk butter manufacturing, it is used for therapeutic purposes (Ho et al. 2022). Ghee (clarified butter), a well-known product in India, has also been made using camel milk; however, the yield of ghee was low when compared to buffalo or cow milk. Also, the finished product was more susceptible to rancidity. There is no information on making sweets from camel milk. Yet, conventional goods are accessible. For instance, a caramel known as 'Balkailmak' from Kazakhstan is produced following a lengthy heating process lasting almost 10 hours at boiling temperature. (Konuspayeva and Faye 2021).

Foaming agent

The quality of many dairy products' top foam layer, such as cappuccino coffee, impacts the overall product quality and market acceptance. Foam is typically made from cow's milk in coffee shops, which may not be safe for people with dairy allergies due to the proteins in cow's milk that cause allergies. Since, camel milk does not contain the allergen ß-lactoglobulin, it becomes a viable substitute for making foam. Under certain temperature and pH circumstances, the proteins in camel milk have foaming capabilities similar to those of cow milk. At pH 7 the foaming ability of camel milk sweet whey protein is slightly lower than cow milk sweet whey proteins. Camel acid whey proteins showed significantly higher foaming ability and stability after thermal treatment at 70 and 90 ^{o}C than their bovine counterparts. As α-lactalbumin makes up a larger fraction (>70%) of acid whey proteins therefore these proteins have good foaming properties. In purest form, ß -casein has greater foaming capabilities than α-lactalbumin. Because camel milk proteins denaturate and aggregate when heated at 70-100^{o}C for 30 minutes, surface hydrophobicity increases, electronegative charge decreases, and interfacial tension decreases, significantly improving camel milk's foaming capabilities (Ho et al. 2022). In a recent study, Lajnaf et al. (2022) examined the characteristics of camel and bovine milk's derived proteins, such as sodium caseinates, sweet whey, ß-casein, α -lactalbumin, and ß -lactoglobulin. The result of this study showed that camel milk proteins, particularly sodium caseinates and ß -casein, had the highest foaming ability, whereas bovine proteins had the highest foam stability, with greater foaming stability values for bovine ß -casein.

Packaging of camel milk dessert

Food packaging is one of the stages of food production that enables foods to reach consumers safely. By selecting the appropriate packaging material and technologies for different food products shelf life of food is increased and food quality and freshness can be preserved (Khalil et al. 2021)**.** Packaging of dairy products develops continuously along with advances in material technologies, which are in turn a response to demands of consumers. Novel dairy packaging systems include new packaging technologies such as the modified atmosphere packaging (MAP) that is widely used nowadays. Forms of active packaging relevant to dairy foods include oxygen scavenging, carbon dioxide absorbers, moisture and/or flavour/odour taints absorbers; releasing compounds (carbon dioxide, ethanol, antioxidants and/or other preservatives); maintaining temperature control and/or compensating temperature changes and antimicrobial packaging (Šèetar et al. 2019). When choosing packaging material for dairy products, various important factors need to be considered such as toxicity, compatibility with the product, impact resistance, maintenance of sanitation, odour, and light protection, chemically inactivity, shape and weight requirements, marketing appeal, printability, and cost (Karaman et al. 2015). The nature and the characteristics of the dairy product to be packaged define the selection of the appropriate packaging material and method. For example, if the product is susceptible to oxidation (such as butter) a selected material needs to have high barrier properties toward oxygen in order to enable the declared shelf life. Similarly, if the dairy product needs to be thermally treated after it has been packaged, the chosen material must be heat tolerant (Šèetar et al. 2019).

Shelf -life of camel milk

Raw camel milk has shelf life of (8 – 9) h at 37 °C and more than a week at (4 – 6) °C. Whereas shelf life of pasteurized milk is 22 days, when heated at 65°C for 20 minutes and kept at 7°C. The fresh milk can also be stored for one year in frozen condition. Camel milk is produced in areas where there is lack of milk cooling facilities coupled with high ambient temperature that exacerbates milk spoilage before it reaches the ultimate market and consumers. To overcome this problem lactoperoxidase system (LPS) is one the methods to preserve freshness of milk until it is marketed or reaches where there are milk cooling facilities (Amenu et al. 2017). In a study, lactoperoxidase system in fresh camel milk was activated within half an hour of the milking using various levels of thiocyanate and hydrogen peroxide (10–70:10–70 ppm ratios) and efficacy was evaluated. The best lowest activation level 20:20 is found to be effective in preserving raw camel milk up to 18–20 h at 37 °C. The enzyme activity in raw camel milk is high and the respective value in pasteurized milk is below the detection limit. In another study, acidity and pH of the pure fresh camel milk and milk diluted with water (1:1) stored at room temperature were 0.12 ± 0.03, 6.42 ± 0.18 and 0.09 ± 0.02, 6.65 ± 0.22, respectively. Other parameters, which include clot on boiling, alcohol, and alizarin alcohol tests, were observed negative in fresh camel milk. The study indicated that pure and milk diluted with water (1:1) can be stored for 8 and 10 h, respectively, at room temperature (Singh et al. 2017)

Camel milk- a need of future

The demand for milk and other dairy products is rising more quickly due to the increasing population, which is expected to reach 7.60 to 8.60 billion by 2030, 9.80 billion by 2050 and 11.20 billion by the year 2100 (Rehman et al. 2023). In addition, more than 6 billion people consume milk and milk products globally (Muthukumaran et al. 2022), with the majority of these consumers living in developing countries. Global milk production may also be constrained by unforeseen environmental factors, such as climate change and an increase in the likelihood of droughts, floods, and disease threats, all of which have a negative influence on the dairy business in various ways. Finding alternatives that are sustainable and climate-resilient is therefore urgently needed. Camel milk and its derived products have the potential to offer a more sustainable source of high-quality alternative of bovine milk (AL-Moosawi et al. 2023).

Conclusion

Camel milk is rich in nutrition and a nutraceutical dietary source. In addition, it lacks allergic â-lactoglobulin and contains high levels of â-casein, making camel milk suitable to be used as a daily drink for humans who are allergic to bovine milk. Camel milk has a high vitamin C content, a high iron content, and a lower fat content than cow's milk. Camel milk and its products have potential antimicrobial activities that can be attributed to the chemical composition of milk and the wide variety of valuable microorganisms present in them. Camel milk also has antidiabetic properties that are mostly due to the presence of bioactive peptides and insulin-like proteins. Overall, the medicinal benefits of camel milk are generating a lot of interest, and more thorough research is required to establish definite evidence of these benefits. However, the technologies used to transform camel milk into value-added products like pasteurized and sterilized milk, cheese, yoghurt, butter, powder, sweets, fermented products, etc. face challenges due to its unique chemical composition, which hinders its organoleptic acceptance. Even though there are lots of difficulties in processing camel milk, developing food products using camel milk remains an interesting topic. Several camel milk food products are being investigated, and some are commercially available, such as pasteurized and sterilized camel milk, camel milk powder, cheese, butter, yoghurt, various sweets, etc. However, these products need to be improved in terms of quality and sensory appeal so that they are at least comparable to those of bovine milk. Therefore, further extensive research and studies are required to improve the technological aspects of camel milk and its products.

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RESEARCH ARTICLE

Development of lactose hydrolyzed milk using micro fluidization assisted crude β-galactosidase enzyme of *Lactobacillus acidophilus*

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Abstract: The objectives of this study were to screen maximum β-galactosidase enzyme producing *Lactobacillus* strain out of seven commercial lactobacilli and development of lactose hydrolyzed milk using micro fluidization assisted crude β-galactosidase enzyme from selected maximum β-galactosidase enzyme producing *Lactobacillus* strain. From all the screening methods *L. acidophilus* ATCC 4356 culture was found to possess the maximum β-galactosidase enzyme, so this culture was selected for further studies. Crude β-galactosidase enzyme extract (CEE) was obtained from *L. acidophilus* ATCC 4356 using micro fluidization as cell disruption method. A total of 37.41 (µmol/ mL/ min) enzyme activity was obtained from crude extract. Lactose hydrolysis in milk was done using different concentrations (0.5, 1 and 1.5%) CEE of *L. acidophilus* ATCC 4356. CEE@1.5% showed highest 39.96% hydrolysis of lactose when compared with other CEE added milk after 8 h. There was significant difference found when sensory scores were recorded for lactose hydrolyzed milk obtained by using crude enzyme extracts concentrations @1.5% CEE of *L. acidophilus* ATCC 4356 and 1% commercial enzyme. Lactose hydrolyzed milk was successfully developed using crude enzyme extract and being an economical, innovative and therapeutic product, large scale production of the product can be taken up by large players of the field in future.

Keywords: β-galactosidase, Micro fluidization, Lactose hydrolyzed milk, Sensory analysis, Lactic acid bacteria

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Introduction

Lactose intolerance is a very common disease where any individual is unable to hydrolyze lactose (Vasiljevic and Jelen, 2001; Singroha et al. 2014; Singhroha et al. 2017; Szilagyi, and Ishayek, 2018). It is generally initiated by the deficiency of a specific enzyme β-galactosidase. β-galactosidase enzyme is also known for its various applications in dairy industry. β-galactosidase coverts lactose into glucose and galactose and it is also known for its ability to catalyze transglycosylation reactions (Oliveira et al. 2011).

In dairy industry, various applications of β-galactosidase have been reported like prevention of lactose crystallization, to increase the sweetness of the milk products, to produce low lactose food products and for cheese whey utilization by which water pollution can be controlled (Sani et al. 1999; Kaur et al. 2017; Joon et al. 2018). As we know, enzymes which are utilized for lactose free milk production are highly purified in nature. High purification of proteins generally makes the cost of enzymes higher. In this way, the expense of low lactose milk is nearly 80% greater than the standard un-hydrolyzed milk (Bury and Jelen, 2000).

One of the benefits of employing *Lactobacillus* strains as a source of β -galactosidase is to catalyze lactose hydrolysis. Since they are Generally Recognized As Safe (GRAS) organisms, so their enzymes can be utilized in milk products (Sani et al. 1999; Mishra et al. 2011).

β-galactosidase is an enzyme found inside the cell. For obtaining enzyme mechanical, enzyme disruption or chemical permeabilization of the cell membrane methods are generally utilized. The effectiveness of these methods for disruptions differs in terms of microorganism's genera and strains. In general, rupturing cells employing various cell disruption procedures can dramatically boost β-galactosidase activity in the media. In literature, disruption of yeasts is mainly focused; whereas less information on the disruption of lactobacilli. More lactose and lactose-containing dairy products will be manufactured, if a cost-effective lactose hydrolysis technology is developed and a suitable microbe source is discovered. Crude enzymatic extract

is one of the economical methods for lactose hydrolysis as enzyme purifying step is eliminated which is highly costly.

The objectives of the present study were to screen maximum β-galactosidase enzyme producing *Lactobacillus* strain out of seven commercial lactobacilli and development of lactose hydrolyzed milk using micro fluidization assisted crude β-galactosidase enzyme from selected lactobacillus strain.

Materials and Methods

Materials

Raw milk is the major ingredient used for the manufacturing of lactose hydrolyzed milk. Fresh, hygienic, good quality raw milk was procured from Experimental dairy plant, GADVASU Ludhiana. Commercial lactobacilli cultures for screening and extraction of β-galactosidase, were procured from ATCC through Hi media, Mumbai (Table 1.). Stock cultures were preserved at -80º C. Before any assay, strains were revived by transferring stock cultures into MRS medium and incubated at 37º C for 24 hours. Purity of each culture was ascertained by doing Gram staining and catalase test. The storage of cultures was done below 5ºC between transfers.

Screening of *Lactobacillus* isolates for their ability to produce β-galactosidase by Ortho-Nitrophenyl-β-galactoside (ONPG) Discs method

This test was used for the rapid detection of β-galactosidase activity by different lactobacilli cultures. Here, the lactose fermenters were identified. The 3 discs of ONPG were put in the sterile test tubes. To this, 5 ml of the 0.85% NaCl solution was added to these test tubes. One colony from each *Lactobacillus* strain was added to the respective tubes. Then tubes were maintained at temperature of 37±2°C in incubator. After every hour, the presence of yellow color was seen and observed till 6 hrs. Then color intensity was observed directly after 24 h of incubation.

Fermentation pattern of *Lactobacillus* isolates in milk

The curd formation ability of all 7 lactobacilli was observed. For this, 10 ml of milk was taken in centrifuge tube and 1% of each *Lactobacillus* strain was inoculated in the milk and incubated at 37°C till pH reach to 4.6 or below. Changes in acidity and pH were observed up to 6 hrs.

Titratable acidity and pH

The titratable acidity was observed by method of Indian Standards (1981). pH value of the fermented milk samples has been determined using laboratory pH meter (*Mettler Toledo)*. pH meter was previously calibrated using buffers 4.0, 7.0 and 10.0 and then used for subsequent samples.

Enzyme extraction by micro fluidization method

Method of Choi et al. (1997) was used with slight modifications. The microbe was fermented in 500ml of MRS-Lac broth. This cell biomass was centrifuged at 12000*g for 10 minutes at 4°C. Pellet was obtained and 5.0 ml of 0.05 M Na- phosphate buffer (pH 6.8) was added to this pellet and the suspension was vortexed vigorously. After the washing procedure the pellet was again centrifuged at 12,000*g for 10 minutes. Pellet was again suspended in 300ml of 0.05 M Na- phosphate buffer (pH 6.8). Cell disruption was done with the help of the micro fluidizer (Microfluidics M-110P, Newton, USA) pass for three times at 15,000 Pa. Supernatant was used for the enzyme assay after centrifugation at 12,000g for 10 minutes at 4 °C.

Enzyme assay

For analysis of activity, 300 µL of cell suspension was taken in a test tube. To this, 2.7 ml of phosphate buffer was added and 600 µL ONPG substrate was added. The tubes were immersed in a steam bath at 37°C for 15 minutes. After 15 min reaction was stopped by the addition of 2.25 ml of 1M Na_2CO_3 to the reaction mixture. Absorbance values were taken at 420 nm. One unit was described as the enzyme required to liberate 1 µmol of ONP from its substrate each minute under the same assay situations.

Manufacture of lactose hydrolyzed milk

For the research purpose, lactose hydrolyzed milk samples from raw milks were made under aseptic condition as shown in Figure 1.

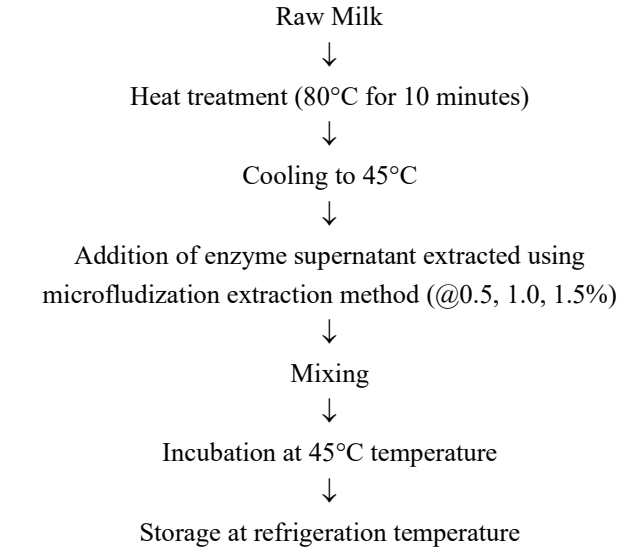

Figure 1. Flow chart for preparation of lactose hydrolyzed milk

Lactose estimation of milk

Lactose content of the samples was estimated as per Lane and Eynon method as described in Indian Standards (1981).

Glucose and galactose estimation of lactose hydrolyzed milk

Glucose and galactose content released after hydrolysis of lactose in the milk samples was estimated as per Nickerson et al. (1976).

Sensory evaluation of lactose hydrolyzed milk

The lactose hydrolyzed milk prepared by adding micro fluidization extracted crude enzyme @ 1.5% in previously boiled and cooled to 45°C milk and was placed in an incubator for a time period of 8 hours of incubation. After this, the hydrolyzed milk was stored in refrigeration condition i.e., 4±2°C. The product was then evaluated by a sensory trained panel for overall quality and acceptability. The product was served as coded (A-Control, B-1.5% CEE added, C-1% commercial enzyme added) and randomly arranged. The panel of seven judges evaluated the milk samples in terms of color and appearance, odor, flavor and taste and body (consistency) on the 100 point score card by BIS (IS: 7768, 1975). The sample for this purpose was brought to temperature condition of 10°C for sensory evaluation. The score given by evaluators were further considered for judging the final acceptability of milk samples (Makwana et al. 2019).

Statistical analysis

Under the supervision of a statistician, data gathered from numerous experiments during the screening and comparative analysis process were analyzed for two-way analysis of variance (ANOVA) and t-test using SAS 9.3 version. Microsoft excel was used to calculate the mean, standard error of l data, when needed.

Results and Discussion

Assessing purity of commercial *Lactobacillus* strains

During this study *L. acidophilus* ATCC 314, *L. plantarum* ATCC 8014, *L. sakei* ATCC 15521, *L. gasseri* ATCC 19992, *L. rhamnosus* ATCC 7469, *L. casei* ATCC 393 and *L. acidophilus* ATCC 4356 were used (Table 1.). All the lactobacilli were found to be Gram positive, rod shaped bacteria under microscope. All the lactobacilli were catalase negative.

Table 1: List of commercial lactobacilli purchased

S.No.	Name of Lactic acid bacteria
1	*Lactobacillus acidophilus* ATCC 314
2	*Lactiplantibacillus plantarum* ATCC 8014
3	*Lactobacillus sakei* ATCC 15521
4	*Lactobacillus gasseri* ATCC 19992
5	*Lacticaseibacillus rhamnosus* ATCC 7469
6	*Lacticaseibacillus casei* ATCC 393
7	*Lactobacillus acidophilus* ATCC 4356

Fig. 2 Ortho-Nitrophenyl-β-galactoside (ONPG) Disc Method for identification of β-galactosidase producing lactobacilli, where 1 tube: *L. acidophilus* ATCC 4356 and 2 tube: Negative control

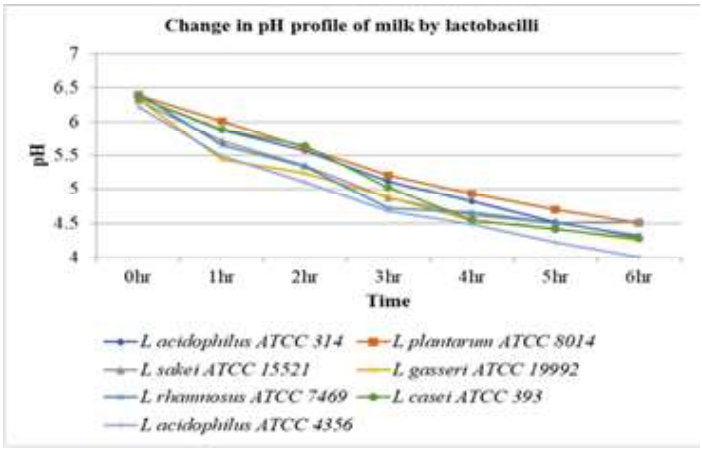

Fig. 3 Change in pH profile of milk by different lactobacilli

Screening by Ortho-Nitrophenyl-β-galactoside (ONPG) discs method

β-galactosidase enzyme not only acts on lactose but also on the other substrates like ONPG. When ONPG reacts with β-galactosidase it leads to the formation of substrates i.e., galactose and ONP. The ONP is a yellow chromogenic compound, thus, ONPG when used for screening it forms deep yellow color.

In our study all lactobacilli were screened by ONPG disc method and detected that the mix color changed to yellow color after some time of incubation. This indicated that all lactobacilli strains have the potential to hydrolyze ONPG into ONP (Figure 2). Favier et al. (1997) used a similar method and screened *Bifidobacteria* for β-galactosidase activity. In one of the study, *S. thermophilus* RD 102 *and S. thermophilus* RD 104 showed yellow color after incubation, thus confirmed β-galactosidase activity. The formation of color rapidly and slowly indicates its ability to form β-galactosidase production in terms of time (Iyer et al. 2010). *B. subtilis* VUVD001 showed similar results i.e., the formation of

deep yellow color in the Ortho-Nitrophenyl-β-galactoside (ONPG) disc method (Venkateswarulu et al. 2020).

Fermentation ability of *Lactobacillus* isolates

The curd formation ability of all 7 lactobacilli was observed by observing changes in acidity and pH after every hour up to 6 h duration. From the Figure 3, it can be observed that after 6 h of incubation maximum pH decrease in milk was observed by *L. acidophilus* ATCC 4356 (4.01), followed by *L. gasseri* ATCC 19992 (4.25), *L. casei* ATCC 393 (4.28), *L. acidophilus* ATCC 314 (4.3), *L. rhamnosus* ATCC 7469 (4.32), *L. plantarum* ATCC 8014 (4.51). The minimum pH decrease in milk was observed by *L. sakei* ATCC 15521 (4.53).

Similar pattern was observed for acidity increase (Figure 4) by all selected lactobacilli in milk medium except *L. acidophilus* ATCC 314 (0.723) which has shown the minimum increase in acidity after 6 h of incubation. After 6 h incubation, maximum acidity increase in milk was observed by *L. acidophilus* ATCC 4356 (0.881), followed by *L. gasseri* ATCC 19992 (0.871), *L. rhamnosus* ATCC 7469 (0.865), *L. casei* ATCC 393 (0.854), *L. plantarum* ATCC 8014 (0.822). The above pH and acid profile of all selected lactobacilli suggest that they all possess β-galactosidase enzyme as they all were able to ferment milk within 6 h of incubation time.

From all the above screening methods *L. acidophilus* ATCC 4356 culture was found to possess the maximum β-galactosidase enzyme, so this culture was selected for further studies.

β-galactosidase Enzyme activity after cell disruption using Micro fluidizer from *L. acidophilus* ATCC 4356

Cell disruption is necessary for the extraction of subcellular ingredients, and it has a considerable impact on subsequent extraction and purification processes. It is necessary to choose appropriate ways for breaking down cellular structures for the isolation of subcellular products. Here β-galactosidase extraction was done using micro fluidizer form previously selected *L. acidophilus* ATCC 4356 culture. Total 37.41+0.52 µmol/ mL/ min enzyme activity was obtained from crude extract

Comparison of addition of different crude β-galactosidase extract of *L. acidophilus* ATCC 4356 on glucose and galactose production in milk

Glucose and galactose production by crude enzyme extract (CEE) of *Lactobacillus acidophilus* ATCC 4356 in previously boiled and cooled milk to 37º C at different time intervals (0, 4, 6 and 8 h) with numerous concentrations of enzyme (0.5, 1, and 1.5%) were shown in the Table 2.

Comparison of addition of crude extract of β-galactosidase on hydrolysis of lactose in milk

Lactose hydrolysis by crude enzyme extract (CEE) of *Lactobacillus acidophilus* ATCC 4356 in previously boiled and cooled milk to 37º C at different time intervals (0, 4, 6 and 8 h) with different enzyme concentrations (0.5, 1, and 1.5%) were represented in the Table 3.

From the Table 3, it can be easily interpreted that the lactose hydrolysis (%) by commercial enzyme was significantly higher than other crude enzyme extract (CEE) used i.e., 80.64% after 8 h of incubation; whereas in the case of all 3 milk samples added with CEE of *L. acidophilus* ATCC 4356, CEE@1.5% showed highest 39.96% hydrolysis after 8 hrs. The hydrolysis rate by

Table 2: Comparison of addition of different crude enzyme extracts of *L. acidophilus* ATCC 4356 on Glucose and Galactose production in milk

Sample	Time			
	2 Hours	4 Hours	6 Hours	8 Hours
0.5% CEE of *L acidophilus* ATCC 4356	0.0857± 0.251[a]	1.918± 0.089[a]	2.54± 0.261[a]	3.31± 0.361[a]
1.0% CEE of *L acidophilus* ATCC 4356	0.114± 0.291[a]	2.661± 0.225[b]	3.15± 0.340[b]	3.9267± 0.197[b]
1.5% CEE of *L acidophilus* ATCC 4356	0.159± 0.341[a]	3.328± 0.169[c]	3.63± 0.232[c]	4.449± 0.170[c]
1% Commercial Enzyme	4.234± 0.105[b]	7.31± 0.225[d]	8.2312± 0.227[d]	9.11± 0.468[d]

Different alphabets ([a, b, c, d]) shows significant difference ($p \leq 0.01$) between the samples during the study

Table 3: Effect of crude extract of *L. acidophilus* ATCC 4356 β-galactosidase supernatant on hydrolysis of lactose

Sample	Time			
	2 Hours	4 Hours	6 Hours	8 Hours
0.5% CEE of *L. acidophilus* ATCC 4356	8.96± 0.251[a]	17.02± 0.089[a]	22.57± 0.261[a]	29.52± 0.361[a]
1.0% CEE of *L. acidophilus* ATCC 4356	10.24± 0.291[b]	23.44± 0.225[b]	26.59± 0.340[b]	33.56± 0.197[b]
1.5% CEE of *L. acidophilus* ATCC 4356	11.51± 0.341[c]	28.03± 0.169[c]	33.07± 0.231[c]	39.96± 0.170[c]
1% Commercial Enzyme	36.11± 0.105[d]	64.31± 0.225[d]	71.29± 0.227[d]	80.64± 0.468[d]

Different alphabets ([a, b, c, d]) shows significant difference ($p \leq 0.01$) between the samples during the study

CEE varies from 11.516 to 39.96%. The average rate of hydrolysis by the 1.5% CEE was observed 28.146% lactose hydrolysis. When the 8 h incubation was observed by 1.0% and 0.5% CEE the 33.56% and 29.52% lactose hydrolysis was observed. It was also observed that lactose reduction (%) by 0.5% CEE, 1.0% CEE and 1.5% CEE were significantly increased at different incubation periods of (2, 4, 6, and 8 hours). Statistically, time taken for hydrolysis was directly correlated with concentration of enzyme used (P d" 0.05) in milk.

Horner found the four times increase in the enzyme concentration lead to doubling of the hydroxylation of milk after 12 hours. This was examined by the commercial β-galactosidases of *Kluyveromyces* at 2ºC for 3 days (Horner et al. 2011). The hydrolysis of lactose was increased when enzyme concentrations increased (AKGUeL, 2012).

Sensory analysis of lactose hydrolyzed milk using crude enzyme extract of *L. acidophilus* ATCC 4356

When hydrolysis of milk occurs the lactose is fragmented into smaller molecules glucose and galactose is formed. This lead to an increase in glucose content in the milk and it tastes sweeter than raw milk. This is all because glucose is five degrees sweeter than lactose, whereas galactose is four times sweeter than lactose and both act as natural sweetener and thus provide sweet taste to milk.

Three combinations were decided (Control (A) Crude extract combinations (B), and commercial enzyme (C)) in this study. The hydrolyzed milk subjected to sensory should be free from unwanted flavor and other unwanted material. Lactose hydrolyzed milk samples were judged and graded on the basis of 100 point score card as per IS (IS:7768, 1975) for various sensory attributes: -

i) Color and Appearance,

ii) Odour (aroma),

iii) Flavour & taste and

iv) Body (consistency)

v) Overall acceptability

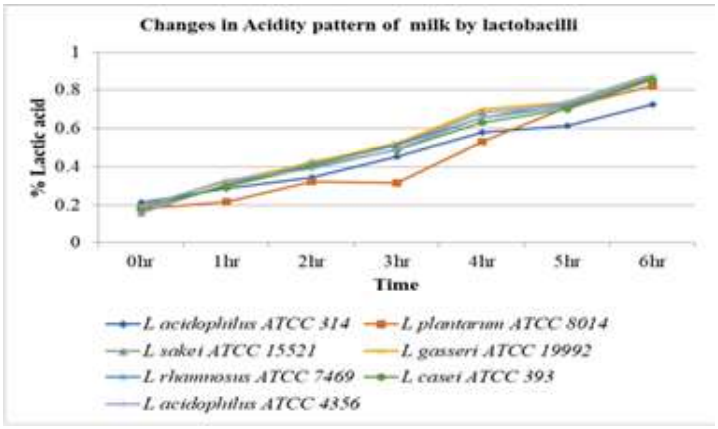

Fig. 4 Change in acidity pattern of milk by different lactobacilli

The alterations in the color and appearance scores of lactose hydrolyzed milk samples are shown in Table 4. Minimum score was recorded by the sample B (8.3) i.e., 1.5% CEE of *Lb. acidophilus* ATCC 4356. The mean color and appearance score was 8.433. Throughout, samples A and C showed the highest score and least by B.

The score card exhibited by the attribute odor of lactose hydrolyzed milk is shown in Table 4. The average odor score secured during analysis was 17.8. During sensory scoring by the penalists, Sample C (18.4) shown the highest score followed by B (18.0) and A (17.0). The mean odor score ranged from 17.0 to 18.4.

Attribute flavor and taste score results of lactose hydrolyzed milk were given in Table 4. During the sensory evaluation, Sample C (37.67) exhibited the highest score followed by B (35.7). Lowest score was observed by sample A (33) i.e., Control sample. The average flavor and taste score were 37.67.

The alterations in body (consistency) score results of milk samples were shown in Table 4. The mean body (consistency) score was 26.56. The sensory evaluators had analyzed Sample C having maximum score followed by B (26.8). The least score was recorded by sample A (25.5). The mean overall acceptability score was 86.89. During the sensory test, Samples C (90.67) had shown the maximum score followed by B (87.0). Lowest score was

Table 4: Sensory scores of lactose hydrolyzed milk using crude enzyme extract of *L. acidophilus* ATCC 4356

Sample	Attributes				
	Color and appearance (Max score 10)	Odor (Max score 20)	Flavor and taste (Max score 40)	Body (Max score 30)	Overall Acceptability (Max score 100)
A (Control)	8.5± 0.115^{a}	17± 0.311^{a}	33± 0.907^{a}	25.5± 0.208^{a}	83±0.371^{a}
B (1.5% CEE of *Lb. acidophilus* ATCC 4356)	8.3± 0.221^{b}	18± .260^{b}	35.7± 0.577^{b}	26.8±1.311^{b}	87±0.982^{b}
C (1% of Commercial)	8.5± 0.127^{a}	18.4± .508^{c}	37.67± 0.577^{c}	27.4± 0.585^{b}	90.67±0.692^{c}

Different alphabets ($^{a, b, c}$) shows significant difference ($p \leq 0.01$) between the samples during the study

observed in Sample A (83.0) i.e., Control sample. The overall acceptability score varied from 83.0 to 90.67.

Sensory attributes of ultra-pasteurized (UP) lactose-free milk having variable composition in term of fat and it was compared with normal milk. They also conducted the consumer survey. The low fat milk was regarded as lack of freshness and lower values on the sensory score. The ultra-pasteurized lactose-free milk had high cooked, processed and sweet flavor (Adhikari et al. 2010).

Also, Nielson with other scientists researched on variable storage condition effects on the shelf life of hydrolyzed-lactose UHT milk which was assessed with use of proteomics. Lactose was broken up to 40% with ultra and nano-filtration before the hydrolysis procedure. The stored milk was evaluated by the sensory characteristics of the product. The lactose-reduced milk was found to be bitter with increasing time period. This happened because of amount of peptides released with enzymatic or non-enzymatic pathway and heat and storage induction respectively. Here, the controlled sample taken was conventional boiled milk (Nielson et al. 2017).

Conclusions

Considering the fact that there is lack of scientific literature available with relevance to lactose hydrolyzed milk prepared from economical enzyme sources, current study was undertaken. In conclusions, technology for extraction of crude β-galactosidase extract from *L. acidophilus* ATCC 4356 by using micro fluidization extraction method had been optimized successfully. Also, lactose hydrolyzed milk was successfully developed using crude extracted enzyme and being an economical, innovative and therapeutic product, large scale production of the product can be taken up by large players of the field.

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Compliance with Ethical Standards

This article does not contain any studies with human or animal subjects.

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RESEARCH ARTICLE

Storage studies on *Low calorie burfi* incorporated with *Sucralose* and *Costus speciosus* extract

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Abstract: In the present study, changes in compositional, physico-chemical, sensory and microbial properties (SPC, coliform count and yeast and mould count) of *low calorie burfi* packed in low density polyethylene (LDPE) film was monitored. The changes were studied at 37 ± 1ºC and 4±1ºC on every 7th day of storage till the products became unacceptable. The sucralose content in *low calorie burfi* was estimated and found to be 150 ppm. The pH was found to significantly ($p<0.05$) decreased during the storage period whereas acidity, FFA, TBA value and soluble nitrogen contents increased significantly ($p<0.05$). The standard plate counts and yeast and mould counts were found to increased progressively during storage whereas coliform count was absent in the product throughout the storage period. Shelf life study showed that developed *low calorie burfi* had a shelf life of 20 days under refrigeration and 7 days at ambient temperature.

Keywords: Costus speciosus, Sucralose, Low calorie burfi

Introduction

In the present scenario, consumers are becoming more health conscious and the demand for health foods has been increasing rapidly. There has been a considerable interest in extending the use of herbal extracts in dairy foods, fruits juice based products and pharmaceuticals (Mann et al. 2018; Idowu et al. 2021). Further, now it has become important to look for an economical as well as therapeutically effective treatment especially for developed and developing countries. During the search for alternate antidiabetic foods, it was found that some of the herbs have potential antidiabetic activity. *Costus speciosus* plant known as insulin plant (diosgenin compound), belongs to *Costaceae* family, and is a medicinal plant. In recent times, these plant leaves are commonly being incorporated in various food products because of its exceptional health benefits. *Burfi* is one of the most popular *khoa* based sweet in India. Once confined to household production, *burfi* is gaining an international market in recent years owing to its delicious taste, flavour and texture (Aneja et al. 2002). *Khoa* has a unique adaptability in terms of flavour, body and texture to blend with a wide range of ingredients resulting in the development of a wide range of varieties of *burfi*. Several varieties of *burfi* are available in the market such as plain or *mawa*/*khoa burfi*, fruit and nut, cashew *burfi*, chocolate, saffron and *rava burfi* (Sarkar et al. 2002; Prasad et al. 2017; Prasad et al. 2018). It is prepared from a mixture of *pindi khoa* and sugar, heating to near homogenous consistency. Beating and whipping operations prior to cooling are sometimes practiced to obtain a product with smooth texture and closely knit body. It is white to light cream in colour with firm body and smooth texture with very fine grains (Patil and Pal, 2005). However, high levels of sugar are used in the preparation of burfi contributes to multiple health-related issues. Therefore, various low-calorie sweeteners such as saccharin, acesulfame K, aspartame and sucralose have been permitted in the dairy products like khoa, burfi, and rasogolla (FSSAI, 2012). Shelf-life of *burfi* is most important from manufacturing and consumer point of view. To make *burfi* as commercially viable product, it should have sufficient shelf-life. The growth of the microorganisms brings about various changes in the product and spoils the taste of the product during storage. Moreover, dairy products containing plant based extracts and low calorie sweetners in different packaging materials could be a major factor that affects its storage stability under different storage temperatures.

Khan et al. (2008) studied the changes in quality of groundnut *burfi* packed in polypropylene (PP, 75 μ) and metallized polyester

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(12 µ) low density/linear low density (MP, 75 µ) during storage in order to assess the shelf life. The samples without sorbic acid spoiled within 30 days of storage due to mold growth and fermented odour. Sachdeva and Rajorhia (1982) studied the chemical and microbiological changes in plain *burfi* during storage at room and refrigerated temperatures using two packaging materials viz., parchment paper and tin containers. The shelf life of *burfi* when stored in parchment paper was found 10 days at 30°C and 50 days at 5.0±1°C whereas *burfi* packed in tin containers had a shelf life of more than 105 days at 5.0±1°C. *Burfi* with high moisture develops a hard structure and crystallization of sugar during long storage (Anon, 1979; 2012). Vijayalakshmi et al. (2005) reported that a free O_2 absorber coupled with high – barrier materials like metalized films/foil laminates gave more than 45 days' shelf life to *burfi* at 27 °C. Prasad et al. (2017) investigated the effect of different packaging materials and essential oils on storage stability of burfi and reported that burfi containing mixed essential oils (Turmeric/ginger/cardamom) and packed in HDPE boxes had shown shelf life of 25 days at 4± 1°C. However, limited reports are available on the storage stability of sucralose added burfi in presence of *costous specious* extract. Hence the present study was aimed to evaluate the storage stability of *low calorie burfi* at two different storage temperatures i.e. refrigeration and room temperature.

Materials and Methods

Preparation of *low calorie burfi*

The *low calorie burfi* was prepared with c*ostus speciosus* extract (63.12 ppm) and sucralose (151.85) as per the optimized procedure described in Anupama et al. (2020). Samples were moulded into flat round shaped pieces and wrapped in parchment paper which was further packaged in LLDPE pouch. After packaging, pouches were stored at room temperature 37°C and refrigerated temperature 4°C.

In the preparation of traditional *burfi,* sucralose was replaced with sucrose (35 per cent w/v of milk) and no extract was added.

Estimation of sucralose in *low calorie burfi*

Sucralose content in *low calorie burfi* was quantified as per the procedure described by Arora (2010). Briefly, 1.75g of *low calorie burfi* was weighed and ultra-sonificated at 40°C for 20 min. After cooling to room temperature, two millillitres of Carrez solution No. 1, Carrez solution No. 2 and one millilitre of HPLC grade methanol was added. Then solutions were allowed to stand at room temperature for 10 min, and subsequently filtered using Whatman No.1 filter paper. The filtrate containing sucralose was analysed by HPTLC. Ten microlitres of the standard sucralose solution in methanol (1µg/10µl) and 10 µl of the sample filtrate was applied at a distance of 1-1.5 cm from the sides of silica gel 60 F_{254} (Kiesel gel 60 F_{254}) HPTLC aluminium sheets (which have been previously activated for 30 min in an oven at 100°C). The spots applied were dried simultaneously using a hot air blower. The plates were then developed in a vertical chamber consisting of dichloromethane: methanol (4:1), as a developing solvent system, till a distance of 1 cm remained from the top edge of the plate. The separation was accomplished within 15 min. The developed plates were then removed from the chamber and dried using a hot air blower, and sprayed with 15 per cent (v/v) methanolic sulphuric acid and dried again. Subsequently the plates were heated for 10 min at 100°C. At this temperature, sucralose appears as charred spots, having an R_f value between 0.40- 0.60. The charred spots obtained were scanned and quantified using the known amount of standard sucralose solution spot by Bio-Rad quantity one software.

Preparation of standard curve

Standard sucralose solution was prepared by dissolving 10 mg of sucralose in 100 ml of HPLC grade methanol to give a concentration of 1.0 µg/10 µl. The standard solutions at concentration of 2.5 µl, 5.0 µl, 7.5 µl, 10 µl and 12.5 µl was applied on the HPTLC silica gel plates at a distance of one centimetre (previously activated at 100°C for 30 min in an oven). The plates were then dried, developed, sprayed, scanned and quantified to get the standard curve for sucralose. The standard curve was plotted using the Bio-rad software.

Physico-chemical analysis of *burfi*

The acidity of *burfi* was determined by method described in BIS (IS: 1166-1968) for condensed milk. The pH of *burfi* was measured using Systronic digital pH meter, Model 335. The method prescribed by Deeth et al. (1975) was used to estimate the FFA content of *burfi*. The extent of oxidation of fat in *burfi* was measured in terms of TBA value. TBA value was expressed as absorbance (OD) at 532 nm. The soluble nitrogen contents of *burfi* sample were determined by the procedure outlined by Kosikowaski (1982).

Microbiological Analysis of *low calorie burfi*

Standard plate count, Coliforms and Yeast and mould count of *burfi* samples were estimated by pour plate technique, as described in IS: SP: 1224 (part I and II), 1981.

Sensory Evaluation of *low calorie burfi*

For the organoleptic evaluation of *burfi*, judges who were familiar with desirable attributes of *burfi* were selected. The selection criterion was kept as the judges were a regular consumer of the dairy sweets as well as their similar behavior between sensory evaluation sessions. The *burfi* samples were tempered at room temperature for 1-2 hour before judging. Sensory evaluation of the samples was conducted in isolated booths illuminated with incandescent light and maintained at 28±2 °C.

Statistical analysis

Two different burfi samples viz. traditional burfi and low calorie burfi were analysed and compared for changes under both room and refrigerated temperatures during storage. Data pertaining to physico–chemical and microbiological qualities was analysed statistically using repeated measures of ANOVA and the sensory qualities was analysed using Friedman's test and Mann-Whitney u-test.

Quantification of sucralose in *low calorie burfi*

HPTLC plates of sucralose standard and sucralose content in *low calorie burfi* are presented in Fig 1 (a). The peak responses curves obtained for the standard sucralose and sucralose in low calorie burfi are delineated in Fig 1(b) and (c). The quantitative analysis of standard sucralose was performed over silica gel HPTLC plates HPTLC. The regression equation was drawn from the obtained data and it is presented in equation 1. The coefficient of correlation for the regression equation was 0.996.

Results and Discussion

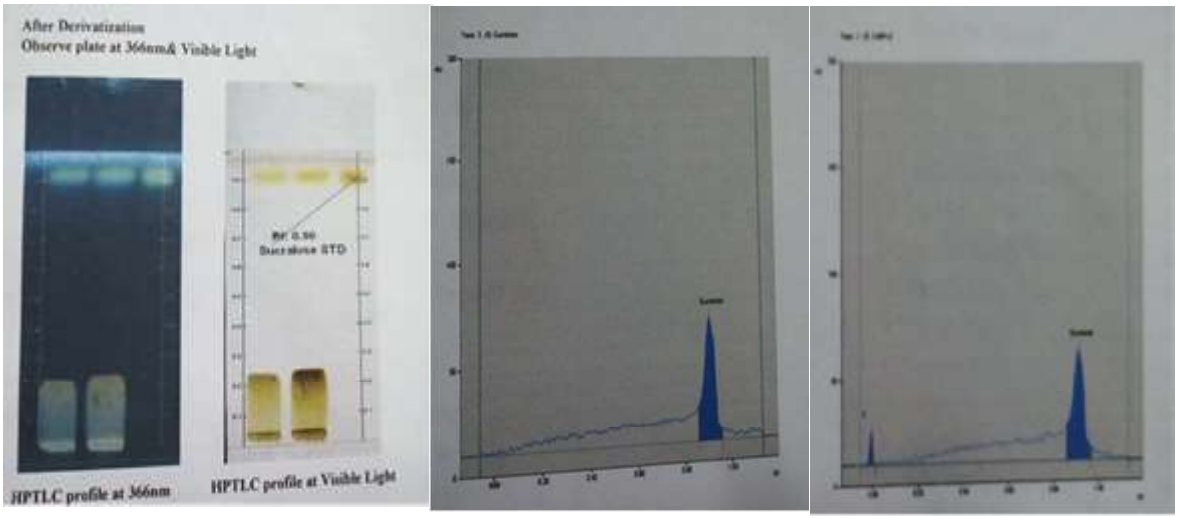

Fig 1 (a). HPTLC plates of sucralose standard and sucralose content in *Low calorie burfi*
(b): Peak response of sucralose standard
Fig 1(c): Peak response of sucralose in *Low calorie burfi*

Table 1. Effect of storage on physico-chemical properties of traditional and *Low calorie burfi* at 37±1°C

Sample	Days of storage	
	0th day	7th day (spoiled)
	Changes in moisture	
Traditional burfi	13.11±0.16^{ax}	12.25±0.18^{bx}
Low calorie burfi	18.033±0.16^{ay}	17.26±0.18^{by}
	Changes in pH	
Traditional burfi	6.42±0.08^{ax}	5.31±0.08^{bx}
Low calorie burfi	6.40±0.08^{ay}	5.18±0.05^{by}
	Changes in acidity (% Lactic acid)	
Traditional burfi	0.36±0.01^{ax}	0.47±0.01^{bx}
Low calorie burfi	0.35±0.01^{ay}	0.42±0.1^{by}
	Changes in FFA (µeq/g)	
Traditional burfi	11.05±0.11^{ax}	13.27±0.019^{bx}
Low calorie burfi	12.46±0.09^{ay}	14.25±0.19^{by}
	Changes in TBA	
Traditional burfi	0.18±0.01^{ax}	0.27±0.05^{bx}
Low calorie burfi	0.21±0.06^{ay}	0.27±0.05^{by}
	Changes in tyrosin value (mg/100g)	
Traditional burfi	7.70±0.20^{ax}	7.77±0.21^{bx}
Low calorie burfi	8.77±0.21^{ay}	9.82±0.18^{by}

Figures are mean ± standard error of three replications, $^{a-d}$ Means with different superscript vary significantly within a row, $^{x-y}$ Mean with different superscripts vary significantly within a column

Y = 763.7+ 1680.08 * X————————————Equation I

Concentration of sucralose in the product was calculated from the regression equation and it was found out to be 150 ppm which is under maximum limit of sucralose (750 ppm) in traditional milk products as per regulations of FSSAI, 2012. The lower detection level of sucralose than the added amount (i.e. 151.85) could be because of accuracy of the detection for the adopted method. The recovery percentage of sucralose in the low calorie burfi was observed to be 98.78% which is in range for the recovery % (96-99.2%) suggested by George et al. (2010) for the followed method.

Effect of storage on physico-chemical properties

The storage changes taking place in the composition of *burfi* samples during storage at room and refrigerated temperature are presented in Table 1 and Table 2 respectively. It can be seen from Table 1 that, moisture content of *low calorie burfi* significantly (P<0.01) decreased from an initial moisture content of 18.03% to 17.26% during the storage period up to 7 days and thereafter the product was unacceptable due to visible mould growth. It can be seen that there was a progressive significantly decreasing trend (p<0.05) in the moisture content of the product when stored at refrigerated temperature from 18.03% to16.58% (Table 3). After 21st day of storage at refrigeration temperature the product was unacceptable due to quality changes in the product. During storage period, the moisture content of *low calorie burfi* decreased rapidly at room temperature as compared to refrigerated temperature. Similar pattern in decline of moisture level for khoa and khoa products has been reported by several researchers (Londhe et al. 2012; Jha et al. 2013 & 2014; Singh et al. 2021). The decrease in moisture content during refrigerated storage might be due to drying at low temperature (7±2°C) and surface evaporation (Sharma et al. 2003). Sachdeva and Rajorhia (1982) also reported a decrease of moisture content during storage of *burfi* at 30±2 °C and 7±2 °C. A decline in the pH was observed in low calorie burfi with values ranging from 6.4 to 5.49 with significant decrease (p<0.05) at refrigeration temperature (Table 2). At room temperature storage, pH varied from 6.40 to 5.18 in low calorie burfi samples with significant decrease (p<0.01) from 0th day to 7th day. This can be correlated with the findings of Londhe et al. (2012) wherein pH of lal peda samples packed in paper board boxes and stored at 30 °C decreased from 6.3 to 5.2 in 20 days.

It can be seen that the acidity of *low calorie burfi* was significantly influenced by storage period when stored at room temperature (Table 1). During storage of *low calorie burfi* at room temperature a significant (P<0.01) increase in acidity content from 0.35% LA at 0thday to 0.42 % LA at 7th day was observed. Similar results were also observed when the product was stored at refrigerated temperatures (Table 2). During storage of *low calorie burfi* at refrigerated temperature a significant (P<0.01) increase in acidity content from 0.35% LA at 0thday to 0.49 % LA at 21st day was observed. It can be seen that the titratable acidity of *low calorie burfi* increased at faster rate during storage period

Table 2 Effect of storage on physico-chemical properties of traditional and *low calorie burfi* at 4±1°C

Sample	*Days of storage*			
	0th day	*7th day*	*14th day*	*21st day(spoiled)*
		Changes in moisture		
Traditional burfi	13.11±0.16^{ax}	12.45±12.25^{bx}	12.25±0.18^{cx}	11.35±0.14^{dx}
Low calorie burfi	18.03±0.16^{ay}	17.88±0.13^{by}	17.26±0.18^{cy}	16.58±0.14^{dy}
		Changes in pH		
Traditional burfi	6.42±0.08^{ax}	6.18±0.05^{bx}	5.61±0.05^{cx}	5.24±0.01^{dx}
Low calorie burfi	6.40±0.08^{ay}	6.18±0.08^{by}	5.68±0.01^{cy}	5.49±0.01^{dy}
		Changes in acidity (% lactic acid)		
Traditional burfi	0.36±0.01^{ax}	0.39±0.01^{bx}	0.47±0.11^{cx}	0.53±0.01^{dx}
Low calorie burfi	0.35±0.01^{ay}	0.37±0.01^{by}	0.42±0.01^{cy}	0.49±0.01^{dy}
		Change in FFA (µeq/g)		
Traditional burfi	11.05±0.09^{ax}	13.39±0.13^{bx}	12.74±0.08^{cx}	13.55±0.09^{dx}
Low calorie burfi	12.46±0.09^{ay}	13.00±0.13^{by}	13.52±0.08^{cy}	14.53±0.09^{dy}
Changes in TBA				
Traditional burfi	0.18±0.04^{ax}	0.25±0.01^{cx}	0.27±0.08^{cx}	0.29±0.01^{dx}
Low calorie burfi	0.21±0.01^{ay}	0.24±0.10^{cy}	0.27±0.01^{cy}	0.29±0.01^{dy}
		Changes in tyrosin value (mg/100g)		
Traditional burfi	7.70±0.21^{ax}	8.87±0.11^{bx}	9.43±0.12^{cx}	10.56±0.16^{dx}
Low calorie burfi	8.77±0.21^{ay}	9.42±0.11^{by}	10.33±0.12^{cy}	11.50±0.16^{dy}

Figures are mean ± standard error of three replications, $^{a-d}$ Means with different superscript vary significantly within a row, $^{x-y}$ Mean with different superscripts vary significantly within a co

at room temperature as compared to refrigerated temperature. The acidity development could be attributed to production of acids like formic acid, acetic acid, lactic acids and other organic acids. Similar trend was reported by Londhe et al. (2012) who recorded an increased acidity of *Lal* peda samples stored at 30 °C from 0.75 per cent to 1.52 per cent in 20 days. Maillard reaction also produces many organic acids which are also responsible for increment in acidity (Goyal and Shrinivasan, 1988). Similar findings were reported by Sachdeva and Rajorhia (1982) in *burfi* during storage. Increase in titratable acidity was also observed during storage of *khoa* (Choudhary et al. 2019).

Effect of storage on FFA content

Lipolysis, regardless of cause seriously degrades the quality of the stored product by imparting off flavours and is also responsible for the development of rancidity. In stored dairy products, lipolysis by microbial lipase is of the greatest significance (Downey and Murphy, 1970). The influence of storage period on FFA content of *low calorie stored burfi* at room temperature as shown in Table 1 reveals that the FFA values of *low calorie burfi* stored increased from 12.46 % to14.25 % during 7 days of storage and this increase was observed in *low calorie burfi* in 21days of storage at refrigeration temperature. The FFA values of both traditional *burfi* and *low calorie burfi* samples increased significantly ($p<0.01$) at both storage temperatures. During storage of *low calorie burfi at* 37±1ºC, a significant increase in FFA content up to 7th day was observed and thereafter both the burfi samples found to be unacceptable due to visible mould growth. This increase in FFA content may be due to the higher SPC and Yeast and mould counts observed at higher temperatures as evident in Table 5 and Table 6. This increase in FFA content could be attributed to hydrolysis of fat which is primarily affected by the growth of yeasts and molds. In the present investigation also the increase in FFA could be due to increase in yeast and mold count. A similar trend of increase in FFA content during storage was noticed in *burfi* by Tiwari (2013). Vijaykhder and Patel (1983) also reported an increase in free fatty acids in *Peda* during storage at ambient temperature (25-29ºC), using polyethylene bags of various densities.

Effect of storage on soluble nitrogen content

Soluble nitrogen is the measure of water soluble nitrogenous portion of protein. This may result from the degradation of proteins because of proteolysis and hence it serves as an important constituent for monitoring the proteolysis in fermented milk products like chesses. In heat desiccated products such as *burfi*, it may serve as an indicator of storage related deterioration of milk proteins and some minor solubilization of micellar proteins due to vigorous heat and agitation employed in the process of manufacture of *burfi*. Tabulated values revealed that the soluble nitrogen of *low calorie burfi* samples (Table 1) stored at room temperature was significantly ($P<0.01$) affected by storage period. As the storage period advanced, soluble nitrogen increased in *low calorie burfi*. It can be seen that soluble nitrogen content of fresh *low calorie burfi* was significantly ($P<0.01$) increased from 8.77 % at 0th day to 9.82 at the 7th day of storage at ambient temperature. At refrigerated temperature of storage, a similarly the soluble nitrogen content of low calorie burfi increased significantly ($p<0.01$) from 8.77% to 11.50% in 21 days. The higher soluble nitrogen content observed could be attributed to the heat treatment employed. The phenomenon of the heat treatment on degradative changes in protein is well established (Jenness and Patton, 1969). On the other hand, survival of heat resistant groups of bacteria and heat stable enzymes capable of protein breakdown could be also considered for proportionately higher soluble nitrogen content during storage. The increase in soluble nitrogen content on storage might be the direct consequence of degradation of protein content of *low calorie burfi*. Kumar (2010) reported the tyrosine value of khoa samples to be 12.02 mg/100 g of khoa. A lesser tyrosine value in standardized product implies to a lower level of protein breakdown as compared to other treatments which can be correlated to lower heat treatment and lesser microbial count.

Effect of storage on TBA value

TBA determination is one of several analytical methods for the evaluation of the degree of oxidation of oils and fats. 2-thiobarbituric acid forms red-coloured products with malonaldehyde, some polyunsaturated aldehydes, dioxolanes and furan derivatives. The intensity of colouration is correlated with the rancidity degree of fats and oils. It can be revealed from the Table 2 that, TBA content of *low calorie burfi* was significantly ($P<0.01$) increased from an initial value of 0.21 to 0.29 over a period of 21 days at refrigeration temperature, whereas the increase in TBA values noted from 0.21 to 0.27 over a period of 7 days for *Low calorie burfi* at room temperature. At both temperatures the TBA content of *low calorie burfi* significantly increased during the storage period. However, it can be concluded that the TBA values of *low calorie burfi* during storage period increased more rapidly at room temperature than at refrigeration temperature. The increase in TBA values might be due to oxidation of milk fat of *low calorie burfi* during storage. Increase in TBA values during storage were also noticed by Sachdeva and Rajorhia (1982) in *burfi*. In yet another study conducted by Jha et al. (2014), the initial TBA value of 0.179 and 0.184 (absorbance at 532 nm) for *lal*/brown peda samples increased to 0.274 (at 4 °C) and to 0.281 (at 37 °C) in 31 and 9 days respectively.

Effect of storage on sensory properties

The sensory attributes have profound effect on the consumer's preference. Different food products undergo deterioration in sensory profile as a consequence of various chemical and biochemical changes that progress during storage. The effect of

storage period on sensory attributes of *low calorie burfi* stored at ambient temperature is presented in Table 3. The mean value presented revealed that flavour score of *low calorie burfi* was significantly (P<0.01) reduced during the storage period. During storage of *low calorie burfi*, flavour score up to 7th day was observed and thereafter the product became unacceptable due to visible mould growth. From the Table 3 and Table 4, it can be seen that the flavour score decreased rapidly at room temperature as compared to refrigerated temperature during storage. The decrease in flavour score could be attributed to slight loss of freshness, which is inherent with any food product.

In fresh product, the compounds formed during browning reactions are responsible for the typical flavour of the product, but as storage period progresses, the chemical reactions disturbed the delicate balance of the compounds. The findings of the present study are in accordance with the result reported by Londhe et al. (2012) for brown *peda* and Sharma et al. (2003) in *Malai Peda* samples during storage study. Similar observations were recorded on stored *kalakand* by Rao and Goyal (2007) and stored *doda burfi* by Chawla et al. (2015).

It can be seen from Table 3 that body and texture score of *low calorie burfi* was significantly (P<0.01) influenced by the storage period at room temperature. During storage of *low calorie burfi,* the body and texture score decreased significantly from 7.0 at 0th day to 6.53 at the 7th day of storage. Similarly, at the refrigerated temperature, the body and texture score of *low calorie burfi* was found significantly (P<0.01) decreased during storage period (Table 4). The initial mean body and texture score of 7.33% at 0thday decreased to 6.0% at 21st day of storage (Table 4). The decrease in body and texture score of samples was observed in both the samples kept at different temperatures and it was much faster in the product kept at room temperature compared to the samples kept at refrigeration temperatures. A similar decrease in body and texture scores were observed in *multigrain halwa* samples kept at ambient and refrigerated conditions of storage (Itagi et al. 2011).

At room temperature the integrity of the grains remained intact, but the grains became harder and chewier becoming conspicuous in the product as the moisture content reduces. At refrigerated temperature the product became dry, hard, sandy and brittle which might be ascribed to the loss of moisture due to addition of sucralose. This is because of dynamic structural and conformational changes, which may or may not be dependent on changes in moisture content (Navajeevan and Rao, 2005) and can be attributed to decline in hydrophilic groups. Therefore, body and texture was considered as important criteria for determining the acceptability of *low calorie burfi* during storage study particularly at refrigerated temperature. The mean values presented reveals that colour and appearance score of *low calorie burfi* was significantly (P<0.01) decreased during the storage period. During storage of *low calorie burfi* at room temperature, decreased in colour and appearance score from 7.83±0.16 at 0th day to 6.98±0.10 at the 7th day of storage was observed. From the Table 3 and Table 4 it can be observed that the changes in colour and appearance scores decreased rapidly at room temperature than at refrigeration temperature. The decline in scores during storage of *low calorie burfi* can be attributed to microbial, chemical and textural changes in the product. During storage the samples became drier in appearance and lacked the greasy appearance desired in good quality *burfi* which resulted in a steady decrease in colour and appearance scores. The colour and appearance of the product became dull and darker with dry appearance. Moreover, in the present study, evaporation of moisture during storage might have aggravated the appearance of the *low calorie burfi* as presence of moisture enlivens the appearance of the product by reflecting incident light. Londhe et al. (2012) reported decrease in colour and appearance score during storage study of brown *Peda* at 30±2 ºC using different packaging materials. These results are in accordance with those observed by Chawla et al. (2015) who also noted a decrease in colour and appearance scores of *doda burfi* on storage.

The overall acceptability of stored samples depends upon several factors like degree of proteolysis, extent of lipolysis, flavour

Table 3. Effect of storage on the sensory quality of traditional and *Low calorie burfi* at 37±1°C

Attribute	*Sample*	*Days of storage*		
		0th day	*7th day*	*Chi square value*
Flavour	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ab}	6.00**
	Low calorie burfi	8.0±0.001^{a}	7.83±0.21^{ab}	6.00**
Body and Texture	Traditional burfi	7.33±0.21^{a}	7.0±0.22^{ab}	6.00**
	Low calorie burfi	7.83±0.16^{a}	6.53±0.16^{ab}	6.00**
Colour and appearance	Traditional burfi	7.67±0.21^{a}	7.83±0.16^{ab}	6.00**
	Low calorie burfi	6.50±0.22^{a}	6.98±0.10^{ab}	6.00**
Sweetness	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ab}	6.00**
	Low calorie burfi	8.0±0.01^{a}	8.50±0.01^{ab}	6.00**
Overall acceptability	Traditional burfi	7.33±0.21^{a}	7.53±0.22^{ab}	6.00**
	Low calorie burfi	7.67±0.21^{a}	7.98±0.21^{ab}	6.00**

Figures are mean ± standard error of three replications, **-Significant at one per cent level (p<0.01), $^{a-d}$ Means with different superscript vary significantly within a row

changes and microbial activity. Statistical analysis indicated a significant (P<0.01) difference among the treatment, viz. type of *burfi* and storage period for both the temperatures studied viz. 37±1 °C and 4±1°C. As observed from the Table 3 and Table 4, the overall acceptability score decreased rapidly at room temperature compared to refrigeration temperature. The decline in overall acceptability scores of *low calorie burfi* was due to changes in flavour and body and texture characteristics. The influence of storage period and temperature of storage was significant for changes in flavour and body and texture and thus, overall acceptability scores. All deteriorative changes, i.e. oxidative, proteolytic, lipolytic, browning, acid development, microbial and textural changes were collectively reflected in sensory quality and thus led to unacceptability of the stored product after a definite period. Low temperature always promotes a longer shelf life of many products and the same was confirmed in this study. This could be attributed to the lower rate of lipid oxidation and non-enzymatic browning reactions as well as reduced rate of unwanted microbial growth which decreases the shelf life of products stored at elevated temperatures (Rossini et al. 2011).

Effect of storage on microbial quality

Most of the milk products are perishable commodities. The perishability of milk products is mostly ruled by microbiological quality of that product. According to BIS (IS: 5550:2005) standards laid down for Burfi, the standard plate count should not be more than 30,000/g and the yeast and mould count not more than 10/g burfi. The microbial count influences the acceptability and hence, shelf life of any product affecting its colour and appearance, flavour and body and texture of the product. The shelf life of product like *low calorie burfi* depends on the growth of microorganisms in the product during storage. Most of the physico-chemical changes as like FFA content, soluble nitrogen, acidity development, change in pH etc., are affected by the presence and growth of various microorganisms. Increase in FFA and soluble nitrogen content signifies lipolytic and proteolytic activity caused by microorganisms. Therefore, the stored samples of *low calorie burfi* were subjected to microbiological analysis for standard plate count (SPC), yeast and mold count (YMC) and coliform count. The influence of period of storage temperature 37±1° C on the SPC of *low calorie burfi* is presented in Table 5. The mean value

Table 4. Effect of storage on the sensory quality of traditional and *Low calorie burfi* at 4±1°C

Attribute	*Sample*	*Days of storage*			
		0th day	*7th day*	*14th day*	*Chi square value*
Flavour	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ac}	5.83±0.30^{bc}	8.45**
	Low calorie burfi	8.0±0.001^{a}	7.83±0.21^{ac}	5.83±0.30^{bc}	10.57**
Body and Texture	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ac}	6.17±0.30^{bc}	7.17**
	Low calorie burfi	7.83±0.16^{a}	7.83±0.16^{ac}	7.67±0.21^{bc}	0.50^{ns}
Colour and appearance	Traditional burfi	7.67±0.21^{a}	7.83±0.16^{ac}	7.33±0.21^{bc}	3.50^{ns}
	Low calorie burfi	6.50±0.22^{a}	6.50±0.22^{ac}	6.33±0.21^{bc}	4.0^{ns}
Sweetness	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ac}	7.50±0.22^{bc}	5.50^{ns}
	Low calorie burfi	8.0±0.01^{a}	8.00±0.01^{ac}	7.67±0.21^{bc}	4.0^{ns}
Overall acceptability	Traditional burfi	7.33±0.21^{a}	7.50±0.22^{ac}	7.33±0.21^{bc}	0.66^{ns}
	Low calorie burfi	7.67±0.21^{a}	7.67±0.21^{ac}	7.67±0.21^{bc}	0.23^{ns}

Figures are mean ± standard error of three replications, **-Significant at one per cent level (p<0.01), $^{a-d}$ Means with different superscript vary significantly within a row

Table 5 Effect of storage on microbial quality of traditional and *Low calorie burfi* at 37±1°C

Sample	*Days of storage*	
	0th day	*7th day*
	Standard plate count (log_{10}cfu/g)	
Traditional burfi	2.47±0.06^{ax}	4.46±0.14^{bx}
Low calorie burfi	2.49±0.06^{ay}	5.08±0.14^{cy}
	Coliform count (log_{10}cfu/g)	
Traditional burfi	Absent	Absent
Low calorie burfi	Absent	Absent
	Yeast and mold count (log_{10}cfu/g)	
Traditional burfi	Absent	1.93±0.05^{by}
Low calorie burfi	Absent	1.32±0.05^{cy}

Figures are mean ± standard error of three replications, $^{a-d}$ Means with different superscript vary significantly within a row, $^{x-y}$ Mean with different superscripts vary significantly within a column.

Table 6. Effect of storage on microbial quality of traditional and *Low calorie burfi* at 4±1°C

Sample	*Days of storage*			
	0th day	*7th day*	*14th day*	*21st day(spoiled)*
		Standard plate count (log_{10}cfu/g)		
Traditional burfi	2.47±0.06^{ax}	3.58±0.06^{bx}	4.46±0.11^{cx}	4.76±0.10^{dx}
Low calorie burfi	2.49±0.06^{ay}	3.73±0.06^{by}	4.58±0.11^{cy}	5.08±0.10^{dy}
		Coliform count (log_{10}cfu/g)		
Traditional burfi	Absent	Absent	Absent	Absent
Low calorie burfi	Absent	Absent	Absent	Absent
		Yeast and mold count (log_{10}cfu/g)		
Traditional burfi	Absent	0.91±0.03^{bx}	1.45±0.06^{cx}	1.93±0.39^{dx}
Low calorie burfi	Absent	0.92±0.31^{by}	1.11±0.06^{cy}	1.42±0.03^{dy}

presented reveals that SPC of *low calorie burfi* was significantly (Pd"0.01) influenced by storage period. The initial mean SPC of 2.49±0.06 log cfu/g at 0thday increased to 5.08±0.14 log cfu/g at the 7th day of storage in *low calorie burfi* at 37±1°C. During storage of *low calorie burfi* at refrigerated temperature also, a significant (Pd"0.01) increase in SPC up to 21st day was observed and thereafter the product was found unacceptable due to visible mold growth. From the Table 5 & 6, it can be seen that the SPC increases rapidly at room temperature compared to refrigeration temperature during storage period. Jha et al. (2015) reported that SPC of lal peda samples increased from initial value of 4.60 to 6.38 log_{10} cfu/g in 30 days when stored at 10 °C. Londhe et al. (2012) reported an increase of 2.6 to 4.3 log_{10} cfu/g for SPC in *lal* peda in 20 days when stored at 30 °C. The coliform count was absent in *low calorie burfi* both temperatures stored at 4 ± 1°C and 37±1°C during the storage period of 21 and 7 days respectively. Gautam et al. (2012) reported that coliform count was absent in retort processed chhana kheer during 90 days storage period. For most of the intermediate moisture Indian dairy foods such as *Peda*, *Burfi*, *Kalakand,* etc. mould growth tends to be a major problem and often most important single factor limiting their shelf life. The influence of period of storage at room temperature (37±1°C) on the yeast and mold count of *low calorie burfi* is presented in Table 5. The mean values presented reveals that Yeast and mould count of *low calorie burfi* was found nil on 0th day of storage. During further storage of *low calorie burfi*, increase in yeast and mold count on 7thday was observed and thereafter the product was found unacceptable due to visible mold growth. The influence of period of storage at refrigeration temperature (4±2°C) on the yeast and mold count of *low calorie burfi* is presented in Table 6. The mean values presented reveals that yeast and mould count of *low calorie burfi* was found nil in 0th day of storage. During further storage of *low calorie burfi*, increase in yeast and mold count up to 21st day was observed and the product was found unacceptable due to visible mold growth. From the Table 5 and Table 6, it can be seen that the yeast and mold count increased rapidly with storage period at room temperature compared to refrigerated temperature. The colonies obtained in the present study at room temperature storage were white and green colonies. The numbers of the fungal colonies obtained during present investigation are similar to various workers who had analysed the milk products like *Peda, burfi* and *Kalakand.* The results are in harmony with findings of Ghayal et al. 2014 in dietetic rabri. Jha et al. (2013) reported an increase in yeast and mold count of peda samples from 2.70 to 3.16 log_{10} cfu/g in 30 days of storage at 10°C. The product was found to be free from coliforms during storage at both temperatures.

Conclusion

Low calorie burfi packed in low density polyethylene (LDPE) has shown shelf life of 7 and 20 days at storage at room temperature (37±1°C) and refrigerated temperature (4±1°C) respectively. The sucralose content estimated by HPTLC had shown recovery of ~98%. The combination of *costus specious extract* and sucralose had not shown marked influence on the sensory parameters. However further investigations are required for establishing scientific knowledge on antidiabetic properties of *costus specious* extract added low calorie burfi.

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RESEARCH ARTICLE

Application of response surface methodology in preparation of low-fat paneer from recombined milk

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Abstract: The objective of current study was to optimize the level of milk fat, milk SNF (MSNF) and whey protein concentrate – 80 (WPC) to prepare low-fat paneer from recombined milk using response surface methodology. Milk fat, MSNF and WPC were used as variables while sensory attributes of paneer viz., flavour, body & texture, colour & appearance and overall acceptability, as well as compositional characteristics viz., moisture content and fat on dry matter basis were used as responses. On the basis of the results, RSM suggested the levels of milk fat, MSNF and WPC to be 2.14%, 10.72% and 0.75% respectively with desirability of 0.92. The experimental paneer was prepared as per the suggestions from RSM and compared with control paneer prepared from standardized milk containing 4.5% milk fat and 8.5% MSNF. The chemical composition of experimental paneer was significantly different from control paneer while rheological and sensory characteristics were statistically similar. Hence, RSM can be a useful tool for optimization of low-fat recombined milk paneer.

Keywords: Low-fat paneer; Recombined milk; Whey protein concentrate; response surface methodology; sensory parameters

Introduction

Paneer, a popular heat and acid coagulated traditional Indian dairy product, is an unripened variety of soft cheese. Paneer provides an easy means of conserving and preserving valuable milk solids mostly milk protein and fat. The paneer market in India exhibited strong increase in sales between 2015 and 2020. The average composition of good quality paneer is approximately 53.0-55.0 per cent moisture, 23.0-26.0 per cent fat, 17.0-18.0 per cent protein, 2.0-2.5 per cent carbohydrate and 1.5-2.0 per cent minerals (Kanawjia and Singh, 2000). The biological value (BV) of protein in paneer is in the range of 80 to 86 (Shrivastava and Goyal, 2007). Ideally, paneer should have a marble white appearance with a firm, cohesive and spongy body and a close-knit texture. It should have a clean, pleasing, boiled milk flavor.

Whey protein concentrate (WPC) is obtained from whey, a by-product obtained during manufacture of paneer and cheese, by using different methods like membrane technology. WPC has been used successfully in several dairy products for years. Whey protein concentrates (34.0-80.0 per cent protein) is the most commonly used ingredients for dairy products. It possesses wide range of functional properties such as foaming, solubility, emulsification ability, gelling and water binding. Whey products such as whey protein concentrates contribute to creaminess, texture, water binding, gelling, emulsification, viscosification, opacity and adhesion in a variety of food systems. Their high nutritional quality and unique range of functional properties make them valuable ingredient in a wide range of low-fat products. Their multifunctional characteristics provide several fat-like attributes (Johnson, 2008). It is widely being used in food sector for enriching various food formulations like frozen desserts, geriatric foods, yoghurt, processed cheese, infant formulae and in traditional dairy products and various bakery applications (Vijaykumar et al. 2020). Utilization of WPC improves viscosity, gelation and water holding capacity and thereby it can be explored in some of the products like soup, yoghurt, Khoa (Shree et al. 2017). The nutritional contribution of whey in this formulation such as whey protein concentrates provides high quality protein, calcium and a variety of health-enhancing components (Bounous,

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2000). Besides the nutritional and functional properties, whey protein also carries a bio-protective agent which enhances anti-inflammatory property, protection of intestinal flora with antibacterial or preservative effects and passive immunity against enteric, respiratory bacteria and viruses (Berber et al. 2015).

Data collection and processing is essential for development of new product and improvement in existing ones. Response surface methodology (RSM) plays a significant role in it. RSM is useful to optimize factors and produce best results. It is a robust and critical tool for data analysis which looks into an adequate approximation relationship between input and output variables and determines the best operating circumstances for a system (Myers et al. 2004).

With increased awareness towards health and nutrition, consumers are now moving to low-fat diet to avoid the risk of obesity, coronary heart disease, atherosclerosis, hypertension and tissue injury (Madadlou et al. 2005). High fat diet is also linked with psychiatric disorders (Jeong et al. 2019). Fat, being a costliest constituent in milk, increases the cost of final product and make the product unaffordable by low income group people. Paneer is a protein rich product and fulfills the protein requirement of most vegetarian people of India. However, reduction in fat content of paneer affects sensory and rheological characteristics of paneer. Therefore, development of a process for preparation of low-fat paneer with desired sensory and rheological characteristics is need of the hour

The market demand for paneer is increasing continuously and paneer is consumed throughout a year. Hence, there is also a need to develop a process to prepare paneer from recombined milk to meet the market demand during lean season. Therefore, in current investigation, reduced-fat paneer has been prepared using recombined milk.

Materials and Methods

Skimmed milk powder of Sagar brand, containing 1% fat and 95% MSNF, as well as fresh cream, containing 25% fat and 6.8% MSNF was used for recombination. Citric acid (edible grade), supplied by Loba-Chemical Pvt. Ltd., Mumbai was used as a coagulant. Whey protein concentrate-80 (WPC), containing 77.8 per cent protein, supplied by Saisukrithkar supplements Pvt. Ltd., Bengaluru was used as fat replacer. Paneer was packed in 12 µ polyester + 50 µ LD/LLDPE laminated pouches.

The fat and total solids content in milk; moisture, fat, protein and ash content in paneer as well as pH and acidity of paneer was estimated by methods described by FSSAI (2015). Lactose content was calculated by difference of all constituents in paneer.

Preparation of paneer

Low-fat paneer from recombined milk was prepared in the laboratory using method described by Aneja et al. (2002) with minor modifications. A calculated quantity of water, cream, skimmed milk powder and whey protein concentrate-80 were weighed accurately. Skimmed milk powder (SMP) and whey protein concentrate (WPC) were dry blended. Water and cream were mixed at 45°C and the mixture was heated to 55°C followed by addition of SMP and WPC mixture at slow rate. The recombined milk was then heated to 90°C and held for 10 min for the denaturation of whey protein. It was then cooled to 80°C and coagulated with pre-heated (80°C) 1% citric acid solution. The whey was drained with the help of muslin cloth and coagulated mass was pressed for 30 min. The paneer was then dipped in pasteurized chilled water (4°C) for 20 min and packed in laminated pouched followed by refrigerated storage (6±1°C).

Texture Profile Analysis: Compression testing of paneer samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962) using 5.0 KN probe which moved at a speed of 20.0 mm/min. The paneer samples were taken for texture measurement after tempering the same at 23±1°C for 1 h. All the textural measurements were conducted in a room maintained at 23±1°C temperature and 65±1% RH. Cubic samples of the experimental paneer, with edges of 20 mm, were placed in the compression support plate in uniform direction. The cubic samples were compressed up to 70% of their initial size. Five paneer samples were used for each experimental paneer under study and the average value of these readings was reported.

Sensory evaluation of paneer: Each block of paneer was cut into approximately 25 g rectangular pieces. The paneer samples were tempered to 15±2°C before judging. Sensory analysis of paneer samples was performed in isolated booths illuminated with incandescent light maintained at 23±2°C. The sensory panel (n=9) was composed of staff members and post-graduate students working in the institution. The paneer samples were evaluated using 9-point hedonic scale as described in Indian Standards (IS: 15346, 2003).

Statistical Analysis

The minimum and maximum levels of milk fat, milk SNF and WPC-80 were selected as 1 and 3%, 9 and 11% as well as 0.5 and 1.0% respectively, on the basis of preliminary trials. A central composite rotatable design (CCRD) of the response surface methodology (RSM) technique was adopted for the optimization of milk fat, milk SNF and WPC-80. The CCRD of three factors contained 20 combinations, including lower and upper limits, along with their responses for sensory parameters as well as moisture and FDM are displayed in Table 1. The data generated for different responses were analyzed using Design Expert® software (13.0.2 version) (Stat-Ease, Inc., 2021 E. Hennepin Avenue, Minnepolis, USA). A general polynomial equation given below was fitted for each response.

$Y=a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + \text{Error term}$

where Y represents the predicted response; a_0 the constant coefficient; a_{11}, a_{22} and a_{33} denote quadratic coefficients; a_{12}, a_{23} and a_{13} denote interaction coefficients; x_1, x_2 and x_3 denote milk fat, milk SNF and WPC content in milk.

Adequacy of the model was evaluated using coefficient of determination (R^2) and statistical significance was examined by F value. The effect of independent variables and individual responses was described at $P<0.01$ and $P<0.05$. t-test for two samples assuming equal variance was applied using Microsoft Excel for comparison of predicted values with the actual values of the responses. The variation between control and low-fat recombined milk paneer samples was analyzed using independent t-test.

Results and Discussion

The optimization of the level of milk fat, milk SNf and rate of addition of WPC was carried out on the basis of sensory characteristics of low-fat recombined milk paneer viz. flavour, body & texture, colour & appearance and overall acceptability as well as its moisture content and fat content on dry matter basis. The successive regression analysis of the responses produced the quadratic models for each response. The variation in the experimental data of fitted quadratic model was given by coefficient of determination (R^2) which ranged between 86 per cent and 96 per cent (Table 2). The model F-value of the fitted quadratic model for all responses was found to be significant. The sufficient accuracy for predicting all response variables of lo-fat recombined milk paneer prepared from any combinations of variables within the range was evaluated by non-significant lack of fit. These indicate that the obtained quadratic model fitted the data strongly. The signal to noise ration called *Adequate precision value (APV)* for a well fitted model should be more than four. This measure also fulfilled for the obtained mode with APVs ranging between 6.023 and 11.025. All these results firmly recommended that the model could be used to develop low fat recombined milk paneer.

Effect of different variables on flavour

Flavour is a combination of taste, smell and mouthfeel. It is a major factor in sensory analysis and consumer acceptance of

Table 1: Design matrix showing factors and their responses for the development of low-fat recombined milk paneer

Run	A: Milk fat content (%)	B: MSNF content (%)	C: Rate of addition of WPC (%)	Response 1: Flavour	Response 2: Body & Texture	Response 3: Colour & Appearance	Response 4: Overall Acceptability	Response 5: Moisture	Response 6: Fat on Dry Matter basis
1	2	10	0.75	7.69	7.81	8.15	7.93	58.61	19.51
2	0.32	10	0.75	6.12	6.55	7.51	6.71	63.15	4.15
3	1	11	1	6.56	6.98	7.73	7.16	62.59	10.11
4	3.68	10	0.75	7.98	8.05	8.15	8.03	58.75	27.07
5	3	9	0.5	7.85	7.94	8.1	7.96	54.87	27.57
6	2	10	0.75	7.72	7.84	8.12	7.95	58.39	19.62
7	2	10	0.75	7.7	7.85	8.11	7.94	58.66	19.35
8	2	10	0.75	7.66	7.86	8.16	7.95	58.31	19.47
9	2	11.68	0.75	7.56	7.61	8.03	7.76	62.11	16.49
10	2	8.32	0.75	7.26	7.38	7.91	7.56	66.42	23.78
11	3	11	0.5	7.91	7.58	7.95	7.81	56.42	23.05
12	2	10	0.75	7.73	7.84	8.11	7.91	59.02	19.16
13	3	11	1	7.41	7.12	7.82	7.51	65.12	22.07
14	2	10	0.33	7.62	7.05	7.89	7.43	53.26	20.43
15	1	9	1	6.91	6.84	7.84	7.08	64.95	13.29
16	2	10	0.75	7.71	7.8	8.12	7.95	58.14	19.72
17	1	9	0.5	6.61	6.52	7.82	6.99	55.21	14.39
18	2	10	1.17	7.11	7.29	7.89	7.42	67.06	18.67
19	3	9	1	7.16	7.25	7.96	7.46	64.78	26.27
20	1	11	0.5	7.21	7.51	7.88	7.49	56.24	10.79

any food product. The flavour score of experimental paneer samples ranged between 6.12 and 7.98. The minimum flavour score was obtained by paneer sample when fat content, milk SNF content and rate of addition of WPC in milk were 0.32%, 10% and 0.75% respectively while maximum flavour score was obtained when fat content, milk SNF content and rate of addition of WPC in milk were 3.68%, 10% and 0.75% respectively (Table 1). The paneer sample containing minimum fat content in milk displayed minimum flavour score and vice-versa which could be attributed to the rich flavour imparted by milk fat. The RSM estimated equation in terms of actual factors for predicting the effect of different variables on flavour is presented in table 3. The partial coefficients of flavour in terms of linear order, second order and interaction of two independent parameters are presented in the regression analysis data (Table 2). Milk fat had significant ($P<0.05$) positive impact while WPC had significant ($P<0.05$) negative impact on flavour score of paneer while MSNF failed to influence it. Addition of WPC led to increase in moisture content of paneer which resulted in flat flavour. At quadratic level, all the three parameters had significant ($P<0.05$) negative impact on flavour score while the interaction of the parameters had no significant impact on it. Higher milk fat content led to slight lipolytic flavour while higher milk SNF and WPC resulted in flat flavour. Khan et al. (2012) and Vashishta et al. (2019) also reported similar findings. Increasing fat from 3% to 6% increased flavour score of paneer samples (Sivakumar et al. 2011). The flavour scores of soft cheese (Soryal et al. 2005) as well as Kefalograviera-type cheese (Katsiari et al. 2002) were directly proportional to fat content of milk. Addition of WPC increased flavour score up to certain level followed by reduction in it (Mishra et al. 2022).

Effect of different variables on body and texture

Body and texture is also an important sensory parameter for paneer. The body and texture score for the experimental paneer samples ranged between 6.52 and 8.05. The paneer sample displayed minimum body and texture score was prepared from milk containing 1% milk fat, 9% MSNF and 0.5% WPC and the paneer sample contained 4.15% FDM while the paneer sample having maximum body and texture score was prepared from milk containing 3.68% milk fat, 10% MSNF and 0.75% WPC and the paneer sample contained 27.07% FDM (Table 1). The paneer sample with lower fat content was criticized for chewy body while the paneer sample with higher fat content was appreciated for good cohesive and firm body. The RSM estimated equation in terms of actual factors for predicting the effect of different variables on body and texture is presented in table 3. The partial coefficients of body and texture in terms of linear order, second order and interaction of two independent parameters are presented in the regression analysis data (Table 2). Milk fat and

Table 2: Regression coefficients and ANOVA fitted quadratic model for the responses of low-fat recombined milk paneer

Partial Coefficients	Flavour	Body & texture	Colour & appearance	Overall acceptability	Moisture	FDM
Intercept	7.70	7.84	8.13	7.94	58.59	19.47
A-Milk fat	0.45*	0.33*	0.12*	0.31*	-0.38	6.51*
B-MSNF	0.08	0.08	-0.01	0.06	-0.49	-2.03*
C-WPC	-0.18*	0.17*	-0.03	0.18*	4.24*	-0.51*
AB	0.01	-0.20*	-0.03	-0.09	0.40	-0.24
AC	-0.11	-0.12	-0.02	-0.07	0.32	-0.06
BC	-0.10	-0.08	-0.02	-0.03	-0.58	0.09
A^2	-0.24*	-0.20*	-0.10*	-0.21*	0.44	1.35*
B^2	-0.11*	-0.14*	-0.05*	-0.10*	1.62*	-0.25*
C^2	-0.13*	-0.25*	-0.08*	-0.19*	0.16	0.04
			Model fit statistics			
Lack of fit	0.0641	0.0612	0.0582	0.0551	0.0632	0.0651
Model F value	18.21	15.66	12.11	16.43	18.75	16.23
R^2	0.91	0.86	0.96	0.94	0.89	0.94
APV	11.025	6.147	6.023	7.236	9.12	7.26

Table 3: Regression equations for predicting sensory score, moisture and FDM of low-fat paneer recombined milk paneer

Property	Equation	R^2
Flavour	$7.70+0.45A+0.08B-0.18C+0.01AB-0.11AC-0.10BC-0.24A^2-0.11B^2-0.13C^2$	0.91
Body & texture	$7.84+0.33A+0.08B+0.17C-0.20AB-0.12AC-0.02BC-0.10A^2-0.05B^2-0.08C^2$	0.86
Colour & appearance	$8.13+0.12A-0.01B-0.03C-0.03AB-0.02AC-0.02BC-0.10A^2-0.05B^2-0.08C^2$	0.96
Overall acceptability	$7.94+0.31A+0.06B+0.18C+0.09AB-0.07AC-0.03BC-0.21A^2-0.10B^2-0.19C^2$	0.94
Moisture	$58.59-0.38A-0.49B+4.24C+0.40AB+0.32AC-0.58BC+0.44A^2+1.62B^2+0.16C^2$	0.86
FDM	$19.47+6.51A-2.03B-0.51C-0.24AB-0.06AC+0.09BC-1.35A^2+0.25B^2+0.04C^2$	0.94

WPC had significantly (P<0.05) increased body and texture score of paneer samples while MSNF had no significant impact. Looking to the interaction effect, the interaction of milk fat and MSNF showed significant (P<0.05) decrease in body and texture while the interaction of milk fat and WPC as well as of MSNF and WPC had no significant impact on it. At quadratic level, all the three factors had significant (P<0.05) negative impact on body and texture score of paneer samples. Soryal et al. (2005) and Katsiary et al. (2002) reported improvement in body and texture of soft cheese and Kefalograviera-type cheese respectively with increase in fat and SNF content of milk. Dwivedi et al. (2010), Khan and Pal (2011) and Chauhan et al. (2017) also observed similar results in paneer. Mishra et al (2022) reported similar results for goat-milk chhana.

Effect of different variables on colour and appearance

Colour and appearance is the first sensory parameter to be observed by the judges. The colour and appearance score for paneer samples ranged between 7.51 and 8.16. The paneer samples with minimum score for colour and appearance was prepared from milk containing 0.32% milk fat, 10% MSNF and 0.75% WPC while that with maximum score was prepared from milk containing 2% milk fat, 10% MSNF and 0.75% WPC (Table 1). The paneer sample with minimum colour and appearance score was criticized for dull appearance while the one with maximum score had comparatively bright white colour. Looking into the impact of different variables on colour and appearance, only milk fat had significant (P<0.05) influence while MSNF and WPC had no influence on the same at linear level. Interaction of different variables also failed to influence colour and appearance. Higher level of milk fat resulted in a typical objectionable glossiness while that of milk SNF and WPC led to dullness in the appearance (Table 2). Dwivedi et al. (2010), Khan et al. (2012) and Chauhan et al. (2017) also found similar results in paneer.

Effect of different variables on overall acceptability

Overall acceptability is the sum of all the sensory parameters. Overall acceptability score for paneer samples ranged between 6.71 and 8.03. The paneer samples with minimum score for overall acceptability was prepared from milk containing 0.32% milk fat, 10% MSNF and 0.75% WPC while that with maximum score was prepared from milk containing 3.68% milk fat, 10% MSNF and 0.75% WPC (Table 1). Milk fat and WPC had significant (P<0.05) effect on overall acceptability while MSNF had no impact on it at linear level. Interaction of different variables also had no significant impact while all factors had significant (P<0.05) negative effect on overall acceptability of paneer (Table 2). Dwivedi et al. (2010), Khan et al. (2012) and Chauhan et al. (2017) also found similar results in paneer. Mishra et al. (2022) also observed rise in overall acceptability of goat-milk chhana upto certain level of WPC followed by reduction in it.

Effect of different variables on moisture content of paneer

Moisture content of low-fat paneer should not be more than 60% (w/w) (FSSAI, 2006). Good quality paneer contains around 56-58% moisture, when WPC is incorporated. Moisture content of paneer samples ranged from 53.26% to 67.06%. The paneer sample containing minimum moisture was prepared from milk containing 2.0% milk fat, 10.0% MSNF and 0.33% WPC while the one containing maximum moisture was prepared from milk containing 2% milk fat, 10% MSNF and 1.17% WPC (Table 1). The hydrophilic

Table 4: Goals set for constraints to optimize the low-fat paneer prepared from recombined milk paneer

Constraint	Goal	Lower limit	Upper limit
Milk fat, %	In range	1	3
Milk SNF, %	In range	9	11
WPC, %	In range	0.5	1.0
Flavour	Maximize	6.12	7.98
Body & texture	Maximize	6.52	8.05
Colour & appearance	Maximize	7.51	8.16
Overall acceptability	Maximize	6.71	8.03
Moisture	Range – 55 to 59	53.26	67.06
FDM	Range – 16 to 19	4.15	27.57

Table 5: Comparison of predicted values and observed values for low-fat recombined milk paneer

Attributes	Predicted value	Observed value	p-value
Flavour	7.76	7.74	NS
Body & texture	7.85	7.88	NS
Colour & appearance	8.11	8.09	NS
Overall acceptability	7.91	7.95	NS
Moisture	58.54	58.89	NS
FDM	18.92	18.98	NS

nature of added WPC as well as native milk protein could be attributed for the variation in moisture content. Milk fat and milk SNF had no significant effect on moisture content of paneer while WPC had significant (P<0.05) positive impact on it. At quadratic level, only milk SNF had significant (P<0.05) positive impact on moisture content of paneer (Table 2). Moisture content of Grana Padeno cheese reduced with increase in fat content in milk (Pretto et al. 2013). Pazzola et al. (2019) reported increase in moisture content of cheese with increase in protein content of milk while Soryal et al. (2004) observed increase in moisture content of Domiati cheese with increase in SNF content of milk. Moisture content of soft cheese was directly proportional to protein content of milk (Soryal et al. 2005). Several other researchers observed similar results in paneer (Dwivedi et al. 2010; Khan et al. 2012) respectively. Increase in the level of WPC from 0.25% to 0.75% also increased moisture content of chhana from 62.05% to 69.72% (Mishra et al. 2022).

Effect of different variables on FDM content of paneer

Fat content on dry matter basis should not be more than 20% (w/w) for low-fat paneer (FSSAI, 2006). The FDM content of paneer samples ranged from 4.15% to 27.57%. The paneer samples containing minimum FDM was prepared from milk containing 0.32% milk fat, 10% MSNF and 0.75% WPC while that with maximum FDM was prepared from milk containing 3.0% milk fat, 9.0% MSNF and 0.5% WPC (Table 1). Milk fat significantly (P<0.05) increased FDM content of paneer while milk SNF and WPC significantly (P<0.05) decreased FDM content of paneer at linear level. At quadratic level, Milk fat had significantly (P<0.05) impact while milk SNF had significantly (P<0.05) positive impact on FDM content of paneer. Fat content of paneer is directly proportional to fat content of milk upto certain level, beyond that there is tendency to increase fat loss in whey. An increase in fat content of milk increased FDM content of cheddar cheese (Lucas et al. 2006) and soft cheese (Soryal et al. 2005). Garem et al. (2000), Dwivedi at al. (2010) and Dikshit et al. (2015) observed similar results in low-fat paneer and recombined milk paneer respectively. Mishra et al. (2022) also observed reduction in FDM content with increase in the level of WPC.

The level of optimized contents of milk fat, MSNF and WPC to be used in low-fat recombined milk paneer was carried out using numerical optimization technique. The criteria used for optimization are summarized in Table 4. All factors as well as moisture and FDM content were kept in range while sensory parameters were kept at maximum during the optimization process. RSM suggested levels of milk fat, MSNF and WPC to be 2.14%, 10.72% and 0.75% respectively with desirability of 0.92. Low-fat recombined milk paneer was prepared at desired optimum level of milk fat, MSNF and WPC as suggested by RSM. The predicted values for flavour, body & texture, colour & appearance, overall acceptability, moisture and FDM content for the experimental paneer were 7.76, 7.85, 8.11, 7.91, 58.54% and 18.92% respectively (Table 5). It is evident from the table that the observed values were not significantly (P>0.05) different from predicted values with respect to all attributes. Therefore, it was confirmed that the selected level of combination of milk fat, MSNF and WPC is best for preparation of low-fat recombined milk paneer with optimum sensory as well as compositional attributes.

Analysis of low-fat recombined milk paneer

Table 6: Comparison of low-fat recombined milk paneer with control paneer

Parameter	Control paneer	Reduced-fat paneer	t-value
Chemical composition			
Moisture, %	55.18	58.89	31.632**
Fat, %	25.30	11.17	30.341**
FDM, % on DMB	52.92	18.98	24.491**
Protein, %	18.11	23.99	7.307*
Lactose, %	2.52	2.69	NS
Ash, %	1.89	2.29	9.115**
Rheological characteristics			
Hardness, N	18.952	17.407	NS
Cohesiveness	0.438	0.426	NS
Springiness, mm	6.978	7.279	NS
Gumminess, N	6.986	7.841	NS
Chewiness, N.mm	48.748	57.074	NS
Adhesiveness, N	0.355	0.422	NS
Sensory characteristics			
Flavour	8.51	8.39	NS
Body & texture	8.32	8.16	NS
Colour & appearance	8.42	8.39	NS
Overall acceptability	8.43	8.29	NS

Low-fat recombined milk paneer was analyzed along with traditional paneer for its compositional parameters as well as rheological and sensory characteristics and analyzed statistically using t-test (Table 6). Fat and FDM content of low-fat recombined milk paneer were significantly ($P<0.01$) lower than that of control paneer while moisture ($P<0.01$), protein ($P<0.05$) and ash ($P<0.05$) content of experimental paneer were significantly higher than those of control paneer. Protein content of paneer increased with reduction in fat content. Fat and protein are two major constituents in paneer and their content is inversely proportional to each other (Lobato-calleros et al. 2001). The experimental as well as control paneer samples were also analyzed for rheological and sensory characteristics and found no significant difference.

Conclusion

Low-fat recombined milk paneer was prepared using response surface methodology and the levels of milk fat, MSNF and WPC were optimized in order to obtain sensorially acceptable product with similar rheological characteristics. The final product also met the legal requirement for chemical composition. At linear level, milk fat improved sensory profile and increased FDM content while milk SNF showed reduction in FDM content while WPC had negative influence on flavour but positive influence on body and texture as well as overall acceptability. It also showed increase in moisture content and reduction in FDM content. At quadratic level, all the three parameters had negative impact on sensory profile while milk fat increased FDM content and milk SNF increased moisture content but reduced FDM content. Hence, response surface methodology can be used as an important application for development of low-fat recombined milk paneer or any other similar product.

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RESEARCH ARTICLE

Development of grape pulp enriched low calorie ice cream made with aspartame and maltodextrin

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Abstract: An attempt has been made to increase anti-oxidants presence at higher levels in grape pulp by incorporating in ice cream to enhance the functional properties and nutritional quality. Ice cream was prepared using grape pulp at 8 to 12 % levels in ice cream mix prior to freezing. Inclusion of grape pulp at augmented levels resulted in significant (P<0.05) changes in physico-chemical properties and antioxidant activity of ice cream. The percentage values of fat and protein in grape pulp enriched low calorie ice cream ranged from 9.73±0.02 to 9.43±0.04 and 3.54±0.01 to 3.95±0.04 respectively. The mean carbohydrate percentage of ice cream were ranged from 10.91±0.07 to 9.75±0.20 in treatments Cm to G_3 respectively, which was significantly higher (Pd"0.05) in Cm than in other treatments. The mean total solids percentage of grape pulp enriched low calorie ice cream was in the range from 25.75±0.06 to 24.06±0.21. A significant (P<0.05) higher antioxidant activity and over run per cent were found in the G_3 samples (55.16±0.98 % inhibition of DPPH) and 86.80±0.37 respectively than remaining formulations. The samples with 10% grape pulp incorporated low calorie ice cream was found to have highest overall acceptability scores (8.46±0.06) with enhanced functional properties and nutritional value.

Key words: Ice cream, Aspartame, Maltodextrin, Low calorie, Grape pulp, Antioxidant activity, Sensory Characteristics.

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Introduction

Frozen dairy desserts can be used successfully to deliver unique additional and nutritional benefits to consumers beyond the basic nutrition. New varieties of ice cream are coming out targeting the health conscious consumers, and also new manufacturing processes giving more value for money spent by consumers (Sasikala et al. 2020). At present focus on nutritional enrichment has shifted from the provision of nutrient deficiency to the pursuit of optimal health and dietary intake. The consumers are now more interested in healthy foods and looking for foods that have added beneficial compounds such as antioxidants, phenolics and phytosterols. At present focus on nutritional enrichment has shifted from the provision of nutrient deficiency to the pursuit of optimal health and dietary intake. The consumers are now more interested in healthy foods and looking for foods that have added beneficial compounds such as antioxidants, phenolics and phytosterols. Consumers can only be expected to consider consuming functional foods if they are perceived as healthier than their conventional counterparts (Urala and Lahteenmaki, 2003). However, consumers' acceptance of functional foods does not depend only on their interest in health. In order to gain the health benefits derived from functional foods consumption, consumers need to include them as part of their usual diet for a relatively long period of time (Sarubin, 2000), and consequently the sensory properties of functional foods should not discourage sustained consumption. The addition of many functional ingredients results in the appearance of off-flavors that decreases the sensory quality of the product (Urala and Lahteenmaki, 2004). It has been reported that functional food consumers are committed to the health benefits of these products and might be willing to have a food product with an unpleasant taste since they consider it as a marker of the health benefit of the product (Reineccius, 2000 and Baixauli et al. 2008). However, several authors have stressed that consumers are hardly willing to compromise on the taste of functional foods for eventual health benefits (Tuorila and Cardelo, 2002; Cardello and Schutz, 2003; Verbeke, 2006; Ares et al. 2009). Thus producers have to add functional ingredients to food products to attract the attention of health conscious consumers (Shaviklo et al. 2011). Grapes contain a variety of phytochemicals, like phenolic acids, stilbenes, anthocyanins, and proanthocyanidins, all of which act as strong

antioxidants (Yang et al. 2009). Increasing preference of consumers towards natural ingredients has tempted the ice cream manufactures to search for new innovations in components having favourable health effects. Therefore, during the development of functional foods, it is extremely important to rely on methodologies that enable the identification of consumers' sensory and hedonic perception of the products. Clinical data has shown that the antioxidant potential of grape phytochemicals is twenty and fifty fold greater than vitamins E and C respectively which is arising from increased levels of polyphenol proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin present in GSE (Yilmaz and Toledo, 2004). Mechanism of antioxidant action of grape phytochemicals includes oxygen radical scavenging activity (Bagchi et al. 2000), stimulation of the enzymatic production of nitric oxide and inhibition of nitrositive stress (Roychowdhury et al. 2001). Thus, the objective of this study was to develop grape pulp added low calorie functional ice cream to improve the nutritional and sensory characteristics.

Materials and Methods

Fresh chilled raw cow milk and cream was procured from College Experimental Station, College of Dairy Technology, Tirupati. Aspartame was procured from Niutang Chemical Plant Co. Ltd., Niutang Town, China, whereas, maltodextrin used as bulking agent was procured from Vintop Products Pvt. Ltd., Mysore Road, Bangalore, and Karnataka. Other ingredients such as skim milk powder, stabilizer, black grapes and vanilla essence were purchased from the local market.

Preparation of low calorie ice cream

In the present study, low calorie ice cream (control) was prepared using 10% fat, 11% MSNF, 900 ppm aspartame, 2% maltodextrin, 0.3% stabilizer and emulsifier and 0.2% vanilla flavour used. Liquid ingredients (milk and cream) were mixed and heated to 49°C. Thereafter, dry ingredients (skim milk powder, maltodextrin and stabilizer) were added. The ice cream mix was then pasteurized at 68°C for 30 minutes, homogenized. The mixture was cooled to 30°C. Calculated quantity of aspartame was first dissolved in small quantity water and mixed with the mixture properly. This mixture was for ageing at 0 to 4°C for 4 hrs. After addition of vanilla essence, the mix was subjected to freezing at -4 to -5°C, filled in 100 ml polystyrene cups and kept for hardening at -23°C.

Preparation of grape pulp

Good quality well ripened black grapes were purchased from local market of Tirupati. The grapes were debaunched and thoroughly rinsed in tap water followed by with distilled water. The whole fruits were pureed well using a fruit pulper and then filtered through a wire mesh to obtain pure pulp. The pulp was pasteurized (80 to 90°C) and then concentrated to maintain the total soluble solids 15° brix and then cooled at 4°C for ice cream preparation.

Preparation of grape pulp enriched low calorie ice cream

Grape pulp enriched low calorie ice cream was prepared by incorporating grape pulp is presented in Fig 1. The mixes were homogenized at 2000/500 psi and ice cream mix were kept for ageing at 4°C for 4 hours and for freezing at -4°C. After packing of the ice cream were kept for hardening and storage at -23°C.

Analysis of ice cream

The ice cream was evaluated for physico-chemical and sensory characteristics. The fat content of the ice cream were determined by the standard method as suggested in ISI Hand Book (1989) for ice cream mixes using 5 g ice cream mix sample. The total nitrogen in the sample was determined by Macro-Kjeldahl method (AOAC, 2000). Ash content of ice cream samples was determined by procedure described in IS: 1547-1985. Total solids content of the ice cream mix was determined by gravimetric method (IS:2802-1964). The total carbohydrate content in the samples was determined by difference i.e. the sum of moisture, protein, fat and total ash percent was subtracted from 100. The

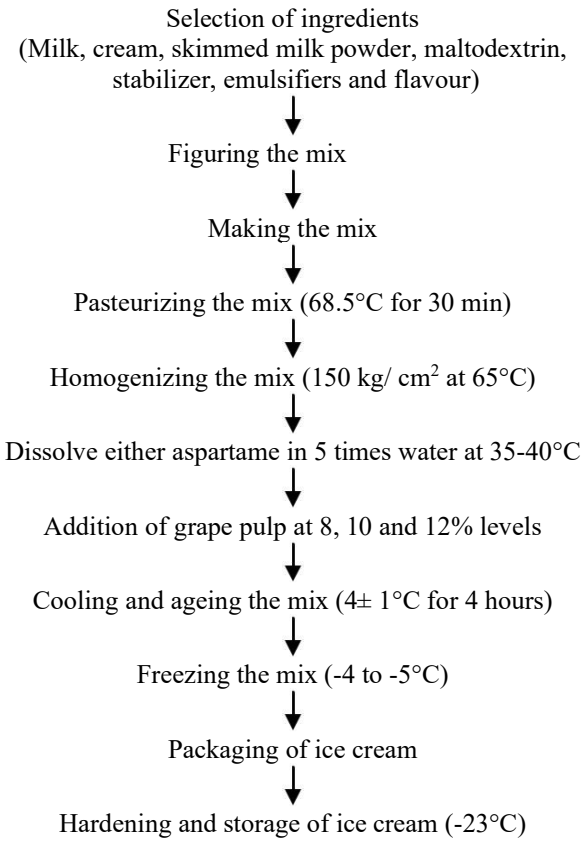

Fig 1. Flow chart for preparation of grape pulp enriched low calorie ice cream

titratable acidity of the ice cream was determined by the standard method suggested in ISI Hand Book (1989). The pH of ice cream mix was determined after ageing using a digital pH meter (Elico Pvt. Ltd., Hyderabad) (AOAC, 2000).

The viscosity of ice cream mix was determined by the method of Lowenstein and Haddad (1972) using a Brookfield Viscometer, Model LTD2T, (Brookfield Engineering Laboratories, Chennai). The overrun in ice cream was determined as per the method of Marshall et al. (2003). The penetration value of the hardened frozen product was measured using cone penetrometer. The melting rate was determined as per the procedure given below by Specter and Setser, (1994).

Sensory evaluation

The acceptability of low calorie ice cream (aspartame) enriched with grape pulp was studied by conducting sensory evaluation with the help of panel of trained judges were assessed by using 9 point hedonic scale.

Statistical analysis

The results obtained during the course of investigation were subjected to statistical analysis using the software OPSTAT, as proposed by Sheoran et al. (1998).

Results and Discussion

Utilization of fruits in milk products for value addition is great challenge to dairy processing industry. Nowadays consumers prefer value added milk products. There is a large scope in dairy processing industry for conversion of milk into innovative fruit based milk products. Ice cream is rich in macronutrients i.e. carbohydrates, fats, proteins, and some micronutrients i.e. vitamin A, E and calcium. However, commercially available ice creams are generally poor in natural antioxidants like vitamin C, colours and phenols. Grape is a rich source of antioxidants including phenolic, flavonoid, and anthocyanin. To improve the functional and nutritional attributes of low calorie ice cream a trail has been conducted by addition of grape pulp levels (G_1) 8, (G_2) 10 and (G_3) 12 percent level with control low calorie ice cream without grape pulp (Cm).

Compositional and physico-chemical analysis of different levels of grape pulp addition in low calorie ice cream

Perusal of the Table 1 reveals that fat content of ice cream greatly influences the compositional, physico-chemical and sensory properties of ice cream. Fat imparts rich flavour, soft body and smooth texture and also important in acceptance of ice cream in terms of consumer's sensory perception. The percentage values of fat in grape pulp enriched low calorie ice cream ranged from 9.73±0.02 to 9.43±0.04. Grape pulp ice cream prepared by using 8 % pulp addition was having highest percent fat (9.73±0.02) content. The present findings illustrated that control sample (Cm) had the highest (10.19±0.03) fat per cent than remaining three low calorie ice cream samples added with grape pulp. Irrespective of treatments grape pulp enriched low calorie ice cream had low fat percent. The decrease in the fat content of ice cream with increasing levels of grape pulp is ascribed due to low fat content of grape pulp. The results are in agreement to Bajwa et al. (2003), Murtaza et al. (2004) and Goraya and Bajwa (2015) who incorporated strawberry pulp, fig pulp and amla products respectively. The mean protein percentage of low calorie ice cream prepared using grape pulp for different treatments were 3.63±0.01, 3.58±0.03 and 3.54±0.01, while control low calorie ice cream had a protein percentage of 3.95±0.04. The protein content of ice cream in all treatments with grape pulp was significantly (Pd"0.05) lower than control (3.95±0.04 per cent). The observations revealed that as the pulp level in the low calorie ice cream increased, the protein content was decreased; the reason might be due to low protein content of grape pulp. Similarly, Bajwa et al. (2003) and Murtaza et al. (2004) observed significant effect on protein content in different treatments of ice cream.

The mean carbohydrate percentage of ice cream were ranged from 10.91±0.01 to 9.75±0.20 in treatments Cm to G_3 respectively, which was significantly higher (Pd"0.05) in Cm than in other treatments. It was observed that the decrease in the carbohydrate content of ice cream with increase in levels of grape pulp.

The mean ash percentage of ice cream in all treatments with Cm was significantly higher than G_1, G_2 and G_3. Addition of grape pulp significantly affected the ash percentage of ice cream. It was observed that the decrease in the ash content of ice cream with increasing levels of grape pulp due to the high moisture content of grape pulp.

The mean total solids percentage of grape pulp enriched low calorie ice cream was in the range from 25.75±0.06 to 24.06±0.21. It showed that there was significantly higher (Pd"0.05) in Cm than in other treatments. However, the variation in total solids content due to different treatments was significant, although decrease in total solids content with an increase in grape pulp was noticed. Similar observations were recorded by Goraya and Bajwa (2015) who reported that the decrease in total solids content of ice cream with an increase in level of jamun juice, orange and pineapple juice, respectively.

The data on the pH and acidity of the ice cream samples as influenced by different levels of grape pulp are presented in Table 1. The mean pH of ice cream was ranged from 6.49±0.01 to 6.31±0.01 and acidity values from 0.25±0.01 to 0.33±0.00 in treatments Cm to G_3 respectively. However, grape pulp affects the titratable acidity of low calorie ice cream, as the acidity values of Cm ice cream is lower than the low calorie ice cream prepared with different levels grape pulp. Grape pulp addition at increased levels caused a significant increased in acidity and decrease in

the pH of ice cream samples, due to the presence of tartaric acid and phenolic substances in the grape pulp. The addition of phenolic rich substances, like elagic acid and gallic acid, enhanced acidity due to the acidic nature of these components. The results are in accordance with Pinto et al. (2004) who observed that acidity increased with addition of ginger juice.

The mean antioxidant activity values were 32.03±0.04, 36.73±0.04, 43.52±0.05 and 55.16±0.98 in treatments Cm to G_3 respectively, which is significantly (Pd"0.05) different between the treatments. The antioxidant activity of low calorie ice cream enriched with grape pulp showed an excellent ability in antioxidant activity (36.73±0.04 to 55.16±0.98 percent), while it was 32.03±0.04 percent in the case of Cm ice cream. The antioxidant activity increased significantly (Pd"0.05) and was greater at 12 percent level than 8 percent level. However, it increased progressively with increased amount of grape pulp was due to more phenols and tannins infusing into the ice cream matrix. Thus, the enrichment with grape pulp increases health benefits by increasing antioxidant activity. Therefore, grape pulp used as good source of antioxidants for making ice cream with good nutritional and functional properties. The results are similar with (Goraya and Bajwa, 2015) reported that the processed amla (Indian gooseberry) incorporated ice cream samples were also found to have higher antioxidant activity, total phenols and tannins than control due to more total phenols and tannins infusing from the amla into the ice cream matrix.

Physical properties of different levels of grape pulp addition in low calorie ice cream

Perusal of the data presented in Table 2 reveals that the mean viscosity (Cp) values of control (Cm) and grape pulp enriched samples G_1 (8 %), G_2 (10 %), G_3 (12 %) mixes before ageing were 154.40±3.76, 145.00±2.98, 143.70±2.23 and 134.70±4.02 respectively. The corresponding mean viscosity (Cp) of mix after ageing at 4°C values were 291.00±4.95, 240.06±4.28, 229.00±3.92 and 214.80±3.29 respectively. It may be seen from the Table that the viscosity of the control mix (Cp) was significantly higher (Pd"0.05) in comparison to the other treatments for both the viscosities. Viscosities in low calorie ice cream prepared with different levels of grape pulp were observed to be decreasing with increasing levels of grape pulp and are significantly different from one another. The above observations indicated that, as the grape level decreases the viscosity of ice cream mix this might be due to grape pulp contain high moisture content contributing a low level of viscosity in grape pulp enriched low calorie ice cream mix.

The mean overrun percentage values were 77.60±0.44, 81.20±0.37, 84.60±0.40 and 86.80±0.37 in treatments Cm to G_3 respectively, which differ significantly (Pd"0.05) among the treatments. It was also observed from the table that overrun of G_3 was higher than Cm and that of G_1 and G_2 lower than G_3. Addition of grape pulp significantly affected the overrun of the ice cream. The reason might be due to a decrease in viscosity with increase in the level of grape pulp as was observed in the increasing overrun. Table 2 shows mean penetration value (mm/5s) observed that there was increasing in penetrometer reading means decreasing the hardness of sample with increasing levels of grape pulp (8 to 12% i.e. G_1 to G_3) respectively, which was significantly different (Pd"0.05) between the treatments. The penetration values were in the range from 71.60±0.99 in Cm to 91.60±1.05 in G_3. However, the low calorie ice cream containing 12% grape pulp was found to give a softer frozen dessert compared to Cm as well as G_1 and G_2, though very slightly. However, the hardness could depend on the overall structure of the product. It is observed a slight increase in penetrometer reading (that is decrease in hardness) by increased addition of grape pulp.

Effect of different levels of grape pulp addition in low calorie ice cream on the first dripping time and melting rate

The values given in the Table 3 (Fig 2 and 3) indicates that there was significantly lower in G_3 than in other treatments for first dripping time, in case of melting rate showed that there was significantly lower in Cm than in other treatments. It is observed from table that the first dripping time decreased and melting rate was increased with increasing level of grape pulp because of pulp contains high moisture content which caused decrease in viscosity and thus enhanced the melting resistance. Sakurai et al. (1996) found that ice creams with high overruns melted quickly whereas those with low overruns began to melt slowly and had a good melting resistance. As the ice cream melts, heat is transferred from the warm air surrounding the product into the ice cream to melt the ice crystals. They explained that initially the ice melts at the exterior of the ice cream and there is a local cooling effect (in the vicinity of the melting ice). The water from the melting ice diffuses into the viscous unfrozen serum phase and this diluted solution then flows downwards (due to gravity) through the structural elements that are destabilized fat globules, air cells and remaining ice crystals etc. to drip through the screen on which the ice cream rests. Meltdown is an important property of ice cream that affects its sensory quality. The melting resistance of ice cream increased progressively with increased levels of grape juice and thereby gradually decreased the melting rate. Maximum meltdown rate was observed for first 50 min after which it decreased progressively and followed a stationary curve for all samples of ice cream. The meltdown of ice cream is influenced by its composition, the amount of air incorporated, additives, the nature of ice crystals and by the network of fat globules formed during freezing (Koxholt et al. 2001). In a study by (Sakurai et al. 1996), it was found that ice creams with high overruns melted quickly whereas those with low overruns began to melt slowly and had a good melting resistance. As the ice cream melts, heat is transferred from the warm air surrounding the product into the ice cream to melt the ice crystals. Further, it was observed that initially the ice melts at the exterior of the ice

Table 1 Compositional and physico-chemical analysis of different levels of grape pulp addition in low calorie ice cream.

Treatments	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)	Total solids (%)	pH	Acidity (%LA)	Antioxidant activity (% inhibition of DPPH)
Cm	10.19^{a}± 0.03	3.95^{a}± 0.04	10.91^{a}± 0.07	0.72^{a}± 0.01	25.75^{a}± 0.06	6.49^{a}± 0.01	0.25^{c}± 0.01	32.03^{d}± 0.04
G_1	9.73^{b}± 0.02	3.63^{b}± 0.01	10.60^{ab}± 0.01	0.69^{a}± 0.01	25.07^{b}± 0.14	6.44^{b}± 0.01	0.29^{b}± 0.01	36.73^{c}± 0.04
G_2	9.57^{c}± 0.03	3.58^{b}± 0.03	10.18^{bc}± 0.09	0.65^{b}±0.01	24.67^{b}±0.19	6.39^{c}± 0.01	0.31^{b}± 0.01	43.52^{b}± 0.05
G_3	9.43^{d}± 0.04	3.54^{b}± 0.01	9.75^{c}± 0.20	0.60^{c}± 0.01	24.06^{c}± 0.21	6.31^{d}± 0.01	0.33^{a}± 0.00	55.16^{a}± 0.98
CD (P≤0.05)	0.10	0.09	0.43	0.03	0.49	0.04	0.02	0.08

Cm (control): 900 ppm aspartame + 2% of maltodextrin added in ice-cream;
Values mentioned above are mean ± SE; (n=5);
abcd: Means in the same column with different superscripts differ significantly (Pd”0.05)

Table 2 Physical properties of different levels of grape pulp addition in low calorie ice Cream

Treatments	Viscosity(before ageing at 37°C)	Viscosity(after ageing at 4°C) value (mm/5s)	Over run (%)	Penetration
Cm	154.40^{a}± 3.76	291.0^{a}± 4.95	77.60^{d}± 0.44	71.60^{d}± 0.99
G_1	145.00^{ab}± 2.983	240.06^{b}± 4.28	81.20^{c}± 0.37	77.24^{c}± 0.62
G_2	143.70^{bc}± 2.23	229.00^{b}± 3.92	84.60^{b}± 0.40	84.20^{b}± 1.03
G_3	134.70^{c}± 4.02	214.80^{b}± 3.29	86.80^{a}± 0.37	91.60^{a}± 1.05
CD (P≤0.05)	9.900	32.99	1.195	2.83

Values mentioned above are mean ± SE; (n=5);
abcd: Means in the same column with different superscripts differ significantly (Pd”0.05)

Table 3 Effect of different levels of grape pulp addition in low calorie ice cream on the first dripping time and melting rate

Treatments	FDT	Melting rate % (Time in minutes)									
		10	20	30	40	50	60	70	80	90	100
Cm	10.600a±0.509	8.139a±0.42	19.375a±0.273	29.065a±0.564	37.12 b±0.773	43.59 c±0.567	47.631c±0.423	53.901d±0.567	57.404b±0.284	58.518c±0.380	59.886c±0.218
G1	9.800a±0.489	5.449b±0.16	15.07b±0.433	26.014b±0.377	35.104b±1.005	47.608b±0.657	55.502b±0.375	58.336c±0.406	59.324b±0.412	60.471c±0.400	62.295c±0.663
G2	5.800b±0.663	5.985b±0.09	15.60 b±0.436	27.098b±0.515	37.479b±1.271	49.538b±0.91	57.40 b±0.676	66.067b±1.543	68.869a±1.637	70.957b±1.679	71.514b±1.424
G3	5.400b±0.509	7.320a±0.43	18.564a±0.681	30.606a±0.716	42.501a±0.702	55.164a±2.010	66.620a±1.728	70.455a±1.755	71.723a±1.503	74.541a±0.777	76.464a±0.837
CD (P≤0.05)	2.426	0.957	1.435	1.669	3.684	3.554	2.909	3.657	3.416	2.900	2.690

Values mentioned above is mean ± SE, (n=5)
abcd: means in the same column with different superscripts differ significantly (P≤0.05).

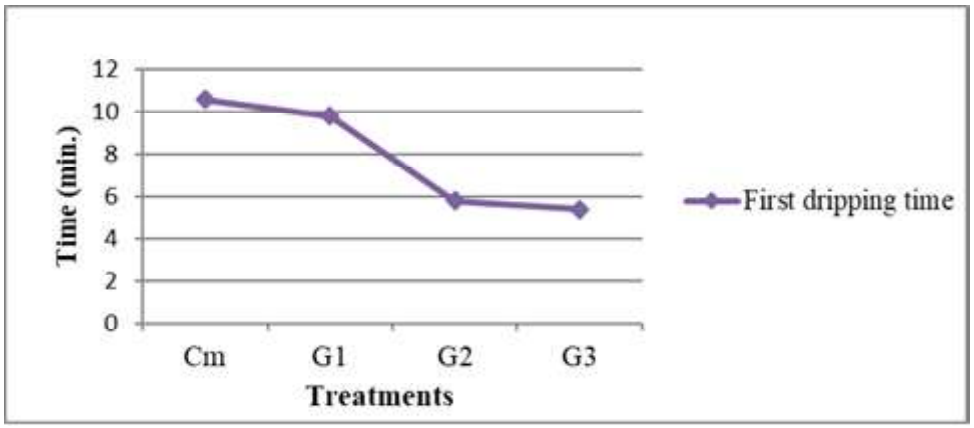

Fig. 2 Effect of different levels grape pulp on first dripping for low calorie ice cream prepared with aspartame and maltodextrin**.**

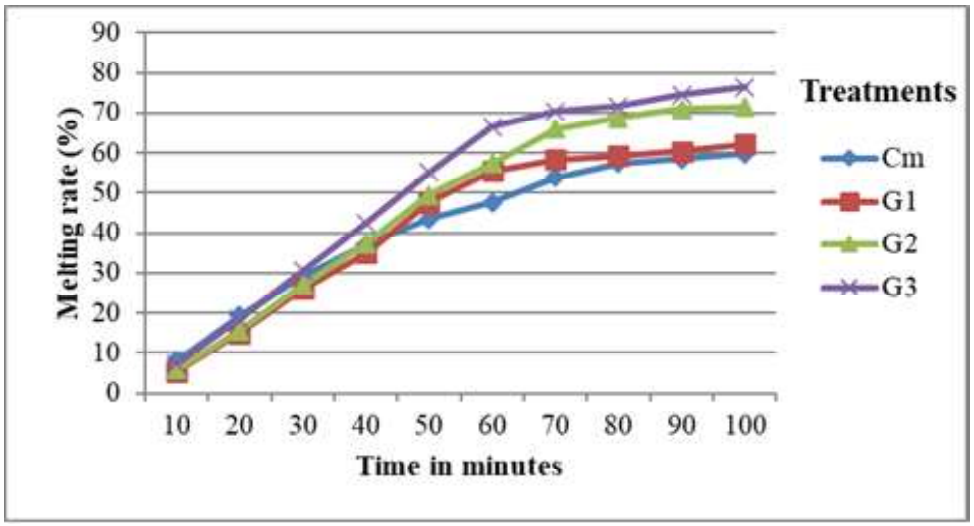

Fig. 3 Effect of different levels of grape pulp addition in low calorie ice cream on the first dripping time and melting rate

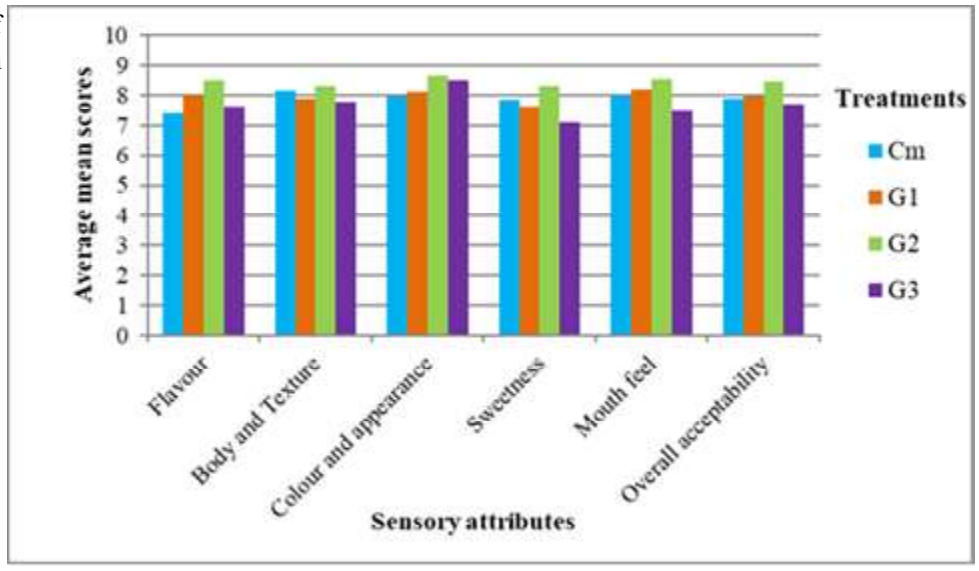

Fig. 4 Sensory scores for selection of grape pulp level for low calorie ice cream

cream and there is a local cooling effect (in the vicinity of the melting ice). The water from the melting ice diffuses into the viscous unfrozen serum phase and this diluted solution then flows downwards (due to gravity) through the structural elements that are destabilized fat globules, air cells, and remaining ice crystals etc. to drip through the screen on which the ice cream rests. The use of Konjac flour alone or combined with ê-carrageenen as stabilizer retarded the meltdown of ice cream samples with respect to the control (Akesowan, 2008). Pinto et al. (2004) reported that 40-45% of ice cream melted during

Table 4 Average sensory scores of grape pulp added low calorie ice cream

Treatments	Flavour	Body and texture	Colour and appearance	Sweetness	Mouth feel	Overall acceptability	Comments
Cm	7.41c± 0.28	8.15[a]± 0.04	7.96d± 0.05	7.85b± 0.03	8.01c± 0.04	7.87b± 0.12	Acceptable sweetness
G1	8.02ab± 0.08	7.91b± 0.08	8.12c± 0.05	7.61c± 0.06	8.20b± 0.02	7.97b± 0.10	Low level of sour taste
G2	8.49a± 0.03	8.31a± 0.03	8.63a± 0.01	8.32a± 0.03	8.53a± 0.02	8.46a± 0.06	Acceptable level of sour taste
G3	7.64bc± 0.19	7.77b± 0.07	8.50b± 0.03	7.09d± 0.04	7.48d± 0.07	7.69b± 0.23	High level of sour taste
CD(P≤0.05)	0.53	0.18	0.12	0.14	0.13	0.43	-

Values mentioned above are mean ± SE; (n=5)
abcd: Means in the same column with different superscripts differ significantly (P≤0.05).

first 40 min and also found that addition of ginger shreds at higher levels decreased the meltdown rate. Meltdown rate was also significantly affected by strawberry inclusion at different levels (Bajwa et al. 2003). Melting resistance of ice cream was significantly affected by 10 and 15% addition of pumpkin, black mulberry and red grape pulp (Gafour et al. 2007).

Sensory evaluation of grape pulp added low calorie ice cream

The ice cream prepared from the addition of grape pulp were analyzed for sensory quality parameters like flavour, body and texture, colour and appearance, sweetness mouth feel and overall acceptability on nine point hedonic scales. The sensory scores presented in Table 4 (Fig.4) indicated that flavour of control sample and ice cream with 10% addition were good compared to other samples. Flavour parameter tells about the presence of grape flavour, an increase in addition of grape pulp the flavour score decreased significantly the reason might be due to sourness increases causing ice cream unacceptable at 12 % addition. A 12 % addition makes texture unacceptable. The mean body and texture scores of ice cream were 8.15±0.04, 7.91±0.08, 8.31±0.03 and 7.77±0.07 in treatments Cm to G_3 respectively, which was significantly higher (P≤0.05) in G_2 than in other treatments. It was observed from above finding that 10 % grape pulp ice cream developed a superior body and texture whereas the lowest noticed for ice cream prepared 12 percent grape pulp. The mean colour and appearance scores of ice cream were 7.96±0.05, 8.12±0.05, 8.63±0.01 and 8.50±0.03 in treatments Cm to G_3 respectively, which was significantly higher (Pd"0.05) in G_2 than in other treatments. It is observed that the colour has been changed from white to light purple colour as the level of grape pulp was increased the colour and appearance score decreased. Appearance parameters tell about the looks of the ice cream physically, it becomes light purple on addition of grape pulp.

The mean sweetness scores of ice cream were 7.85±0.03, 7.61±0.06, 8.32±0.03 and 7.09±0.04 respectively, which was significantly higher (P≤0.05) in G_2 than in other treatments. A significant decrease in sweetness scores observed as grape pulp level increases in ice-cream. The mean mouth feel scores were 8.01±0.04, 8.20±0.02, 8.53±0.02 and 7.48±0.07 respectively, which was significantly (P≤0.05) higher in G_2 than in other treatments. Ice cream containing 12 % grape pulp obtained lower acceptance. This was because of sourness of samples at higher levels. The overall acceptability scores were significantly (Pd≤0.05) higher in G_2 ice cream than in other treatments and similar findings were noticed by Goraya and Bajwa (2015) that 10 percent amla candy was optimal for incorporation in ice cream. Based on the sensory attributes of the above study confirming that addition 10% grape pulp enriched low calorie ice cream was better acceptable since it has optimum flavour and mouth feel as compare to other treatments

Conclusions

The addition of grape pulp to low calorie ice cream improved the appearance and flavour of low calorie ice cream, giving it a good natural colour and flavour. To improve the functional property of low calorie ice cream (Cm) added at three different levels of grape pulp (8, 10 and 12 percent) compared with the control low calorie ice cream. The compositional, physico-chemical and sensory attributes of the product have been studied. The overall acceptability scores were highest (8.46) for 10 percent grape pulp with 900 ppm aspartame and 2 percent maltodextrin added low calorie ice cream (G_2). So, low calorie ice cream can be prepared and enriched by addition of grape pulp with improved colour, flavour and enriched with antioxidants.

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RESEARCH ARTICLE

Production, survival, and storage study of freeze and spray dried *Lactococcus lactis* using whey as protectant

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Abstract: The present research was hypothesized that preservation of bacterial cell with higher viability by drying methodologies is a challenging process as *L. lactis ssp. lactis*, is heat sensitive bacteria. Thus, drying conditions must be mild enough to avoid damaging them but sufficiently efficient to yield a powder with moisture content below 4%, which is desirable for storage stability. To overcome the lack of moisture sweet whey was used as protective medium for freeze and spray drying of *L. lactis ssp. lactis.* The present study was designed to evaluate the survival and storage stability of freeze and spray dried *Lactococcus lactis ssp. lactis*, using sweet whey as protective medium up to 90 days at -20°C storage temperature. The percent survivability for freeze and spray dried powders of *Lactococcus lactis ssp. lactis* was found 60% and 36% respectively. A significant decrease of more than one log count for freeze dried culture and two log count decrease in case of spray dried culture powder was observed after 90 days of storage period. The moisture content and storage temperature played a crucial role in storage stability of both the powders, which was observed within the acceptable limit. the present study culminates that storage temperature and moisture content of bacterial powders are the key factors influencing its viability. In addition, higher survivability and storage stability of freeze-dried powder (60%) as compared to spray dried bacterial cells (36%) develops new technological route to improve cell survivability by spray drying and further during storage.

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Introduction

Drying is the most proficient technique for long term preservation of bacterial cultures in food and dairy industry. Along with a stable and extended shelf life of bacteria, drying ensures ease of storage, handling, transport, and their subsequent use in functional food applications. Among different drying methods, freeze drying (FD) is the best process known for preservation of bacteria instead of damaging their viability and highly viable cells for long-term storage period, although it's a laborious and comparatively expensive process. (Broeckx et al. 2016, Huang et al. 2013, Kupletskaya and Netrusov, 2011, Morgan et al. 2006). Sublimation is the strategic factor, ensues in three phases containing a freezing stage subsequently two step drying processes under high vacuum (Alonso, 2004). However, due to higher cost of freeze drying have limited use in large-scale processes. Spray drying (SD) has advantages over other in relative ease in operation, shorter process time, large scale production and relatively cheaper production cost (Huang et al. 2016, Schuck et al. 2013). Moreover, lower bacterial survivability of bacterial culture due to exposure to high temperature (upto 200°C) which damage the cell integrity, is the main limiting condition of spray drying in food and dairy industry (Peighambardoust et al. 2011). In relation to that lactic acid bacteria are heat sensitive, therefore drying conditions must be adjusted to avoid any kind of cellular damage although suitably competent to yield a powder with lower moisture to improve its storage stability. To overcome this challenge extensive research has been carried out which improve culture viability and subsequent storage during drying processes. Various factors have been reported which induce bacterial tolerance and protect bacteria against spray drying, such as pretreatment of bacteria with sub-lethal doses of stress, using a protective matrix as a drying medium and modification in drying conditions (Desmond et al. 2001; De Castro-Cislaghi et al. 2012; Perdana et al. 2014 and Schuck et al. 2013). Freeze drying mainly cause cellular damages due to the formation of crystals and osmotic stresses, thus to protect cells against such damages a wide range of cryoprotectants such as skim milk, sugars can be added to the drying media prior to

freeze-drying. Dairy-based ingredients have often been reported to act as efficient protective agents during drying because of their compatibility with bacterial cell and the possibility to promote their efficiency (Lee and Marco, 2015). Whey is a by-product of cheese manufacturing has found to be an excellent protective agent (Huang et al. 2016, 2017; Maciel et al. 2014). A potentially good powder quality like solubility, flowability, and dispersibility is obtained using whey as it contains good amount of lactose and whey proteins in sweet whey, obtained which makes it an ideal medium for growing dairy bacteria (Lavari et al. 2014; Sadek et al. 2013). Moreover, these components have protective effects on bacterial cell against adverse stresses during spray drying process conditions, re-suspension and storage (Huan et al. 2016; Huang et al. 2014; Yonekura, Sun, Soukoulis, & Fisk, 2014; Huang and Chen, 2013; Paéz et al. 2012; Rajam et al. 2012; Mattila-Sandholm et al. 2002; Picot and Lacroix, 2004). Among the non-reducing disaccharides trehalose is the most investigated which accumulate in bacterial cell during drying process. The protective mechanism of trehalose is due to the stabilizing effect on membranes and proteins of cell by replacing the water around polar residues within these macromolecular structures and thus decreasing the membrane phase transition temperature (Morgan et al. 2006).

During storage several factors affect survival and fermentation activity, such as temperature, presence of light, oxygen and moisture content (Morgan et al. 2006). Higher survival is reported at lower storage temperatures as well as oxidation of the fatty acids of membrane lipids which is the most likely cause of death of microbial cells due to the increased lipid oxidation during storage (Bozaet al. 2004; Corcoran et al. 2004; Desmond et al. 2002; Silva et al. 2002). In dairy industry most of the research has been carried out on the drying of *Lactobacillus, Lactococcus* and various *Bifidobacteria* species. Different bacterial species, or even strains, show variable level of resistance towards various types of stress encounter during spray drying process. *Lactococcus lactis* which is a common starter in cheese industry has found to be better survival after spray-drying due to greater tolerance to heat and oxygen (Dijkstraet al. 2014; Lavariet al. 2015). In order to perform fermentation dried powder products should meet the criteria of more than 10^6 colony-forming units (CFU/g) (Kurmann, 1992). Limited reports are available on the aspect of freeze and spray dried *mesophilic Lactococcus lactis* ssp. *lactis* and its survivability during storage conditions. Therefore, keeping this in view the present study was designed to evaluate the survivability of freeze and spray dried *Lactococcus lactis* ssp. *lactis* NCDC97 culture using whey as protectant during drying conditions.

Materials and Methods

Bacterial culture and biomass production

Lactococcus lactis sp. *lactis* NCDC97 was obtained from National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal-132001, Haryana, INDIA, subsequently, maintained and sub-cultured overnight in M17 broth (Hi Media, Mumbai, India; pH 6.8) at 30ºC under static conditions. The fresh overnight grown culture (8 $\log_{10}$ CFU/mL) was transferred into 1L M17 broth under the same conditions. The preparation of starter culture biomass, 5L sterilized M17 broth was transferred into the 14 L bio-reactor (BioFlo® 320, Eppendorf Pvt. Ltd. Germany). The previously freshly grown 1L starter culture was inoculated into the 5L sterilized M17 media followed by incubated statically at 30°C for 18 h with constant agitation of 70 rpm. Overnight grown cells were harvested by pre-cooled (4°C) centrifugation (SIGMA 3-18K, Germany) at 5000 RPM for 10 min at 4°C followed by re-suspended in drying medium and cell count were adjusted to 10^{10} cells/mL. Drying medium was prepared by rehydrating sweet whey powder (AMUL, Anand, India) and trehalose (PubChem CID: 7427) (99.8% purity, Sigma–Aldrich, Australia) in deionized water (PubChem CID: 962) @ 5:1 adjusting final total solid up to 30%. The drying medium was also sterilized under recommended conditions (121°C for 10 min).

Freeze drying

To determine the effects of dehydration on viability of the bacteria, cell suspensions of *Lactococcus lactis* sp. *lactis* NCDC97 mixed with drying medium were frozen overnight at -20°C followed by freeze drying (Lyodel 0555, DELVAC Company Pvt. Ltd., Chennai) at -40°C under vacuum of 0.2 mbar.

Spray drying

Spray drying was carried out at laboratory-scale using Laboratory spray dryer (LSD-48 Spraymate, JISL Pvt. Ltd, India) with water evaporation capacity of 1L/ h. The *Lactococcus lactis* sp. *lactis* NCDC97 bacterial suspension was pneumatically atomized using a two-fluid nozzle with an orifice diameter of 0.7 mm. The inlet and outlet air temperature were at 150 ± 1°C, and 60 ± 3°C with a feed rate of 10 mL/ min, respectively.

Storage and their survivability study

The bacterial freeze-dried powder and spray dried powder were collected through single cyclone separator and stored at -20°C for 90 days in dark cryo-vials. The storage duration was chosen based on the typical duration used in previous shelf-life studies (Gandhi et al. 2013; Fonseca et al. 2000) and in many industrial settings. Total lactic count in terms of viable cells were expressed as colony forming unit (CFU). As per the method of Gardiner et al. 2000 briefly, powder samples were re-constituted by dissolving 0.1 g in 9.9 mL sterile peptone water (1% w/v, pH = 7.0 ± 0.1). Each diluted sample of *L. lactis* sp. *lactis* NCDC97 was poured onto M17 agar, mixed and incubated at 30°C for 48 h (aerobic conditions). Survival (%) of bacteria was expressed as a percentage of the live bacterial cells immediately after drying

(freeze and spray) was enumerated (before the powders were stored = zero storage time) using the following equation:

$$\% \text{ Survival} = \frac{Nt}{N0} X\ 100$$

Where, N_t (CFU/g) refer to the bacteria population after both method of drying, *viz.,* spray drying and freeze drying, and N_0 to the initial population before drying treatments.

Analysis of Moisture content and water activity (a_w)

Moisture content of dried powders was determined gravimetrically by oven drying at 102°C for 2h (AOAC, 1990). The water activity of powders was determined using an a_w-meter (Aqua Lab, Decagon Devices, WA, USA) at 25°C.

Powder morphology

The dried bacterial cultures (freeze and spray) morphology was observed under scanning electron microscopy ZEISS EVO SEM (Zeiss, Cambridge, U.K.) as protocol described by Fu et al. (2013). Briefly, dried culture powders were fixed on carbon tape and then sputter-coated with gold palladium. These powder samples were examined followed by micrographs were taken under the scanning electron microscopy ZEISS EVO SEM.

Statistical analysis

All the experiments were performed in triplicate and all data are reported as mean ± SEM. The p value of <0.05 was considered to be statistically significant. Data were subjected to analysis of variance (ANOVA) with Tukey's multiple comparisons. Statistical analysis was performed with Graph Pad Prism 6.0 for Windows software.

Results and Discussion

Total Lactic Count of dried cultures

The initial count of freeze and spray powders of *L. lactis* sp. *lactis* NCDC97 were 11.46 and 11.18 log CFU/g, respectively. Total lactic count of freeze-dried powder was 11.03 log CFU/g up to 45 days and significantly decreased to 10.16 log CFU/g after 90 days, whereas in case of spray dried powder, lactic counts were reduced from 11.18 to 10.71 after 30 days and further 9.47 log CFU/g after 90 days with a significant reduction (Table 1). The survival of freeze dried and spray dried culture (as per the mentioned equation) was around 60% and 36% respectively (Figure 1). Similar results were obtained by Gandhi et al. 2013.

Zayed and Roos, 2004, reported the 45% survival of *L. salivarius* when combination of skim milk and sugar was used as freeze drying medium and remained stable upto 50 days. Correspondingly, slight loss of viability occurred in spray-dried *L. rhamnosus* GG (reduction of 0.25 log unit) using skim milk as drying medium upto 6 weeks (Ananta et al. 2005). Loss in viability was higher in case of SD bacterial powder as compared to FD bacterial powder due to use of high temperatures during spray drying process, which can damage bacterial cells and subsequently reduce their viability (Ananta et al. 2005). Our study reported that the presence of high amounts of protein and phosphate salts in whey may have provided a supplementary defensive coating for the cells during drying and storage.

Moisture content and water activity (a_w)

In this study we observed minimal moisture content of spray dried powder of *L. lactis* sp. *lactis* NCDC97 was 3.7% under optimized conditions (inlet 155°C, outlet 60°C and feed rate 35 mL/ min) whereas, in case of freeze-dried powder it was obtained 2.6%, which was considered for optimum survival of bacteria during storage. The water activity (a_w) of freeze dried and spray dried powders were obtained 0.35 and 0.36 respectively which, is in considerable range.

Higher moisture content (4.1-8.6%) could result into reduced stability during storage (Champagne and gardner,2001; King et al. 1998) however, Bielecka and Majkowska, (2000) reported that excessive moisture content (10%) of spray dried powder was produced when the outlet air temperature was less than 60°C. Moreover, the moisture content of spray dried powder is significantly influenced by inlet-outlet drying air temperature, composition, and feed solution concentration (Anandharamakrishnan et al. 2007). The whey proteins used as cryoprotectant in drying medium led to formation of film around the particle surface, which favors the removal of moisture more rapidly, hence controlled the diffusion of water vapour from the interior of droplet to the surface during spray drying (Adhikari et al. 2009).

Table 1: Total lactic count (log CFU/g) in dried powder of L. lactis ssp. lactis NCDC 97culture during storage at -20±1oC for 90 days

Dried culture	0 day	15th day	30th day	45th day	60th day	75th day	90th day
Freeze dried	11.46 ± 0.19^{aA}	11.29 ± 0.11^{bA}	11.19 ± 0.21^{cA}	$11.03\pm0.27d^{A}$	$10.80\pm0.18e^{A}$	10.62 ± 0.22^{fA}	10.16 ± 0.15^{gA}
Spray dried	11.18 ± 0.20^{aA}	11.11 ± 0.19^{bA}	10.72 ± 0.21^{cB}	10.46 ± 0.30^{dB}	10.02 ± 0.13^{eB}	9.72 ± 0.13^{fB}	9.47 ± 0.28^{gB}

$^{A, B}$ Mean (±SE) values with different superscript with in a column differ signiûcantly ($p<0.05$)
^{a,b,c,d,e,f,g} Mean (±SE) values with different superscript with in a row differ signiûcantly ($p<0.05$)

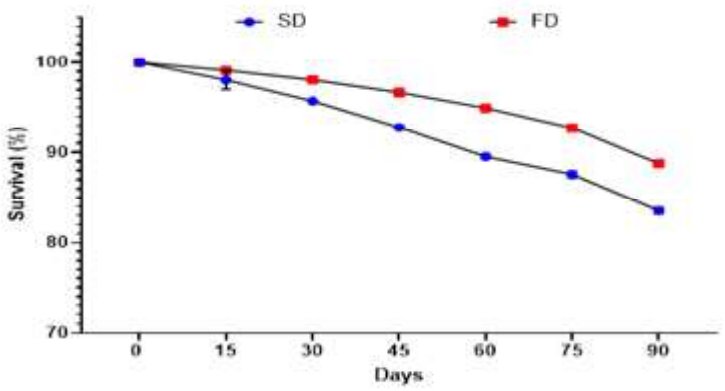

Fig. 1 Total lactic count (CFU/g) of freeze and spray dried *Lactococcus lactis* sp. *lactis* NCDC 97. (Mean ± SEM; FD= Freeze dried; SD= Spray dried)

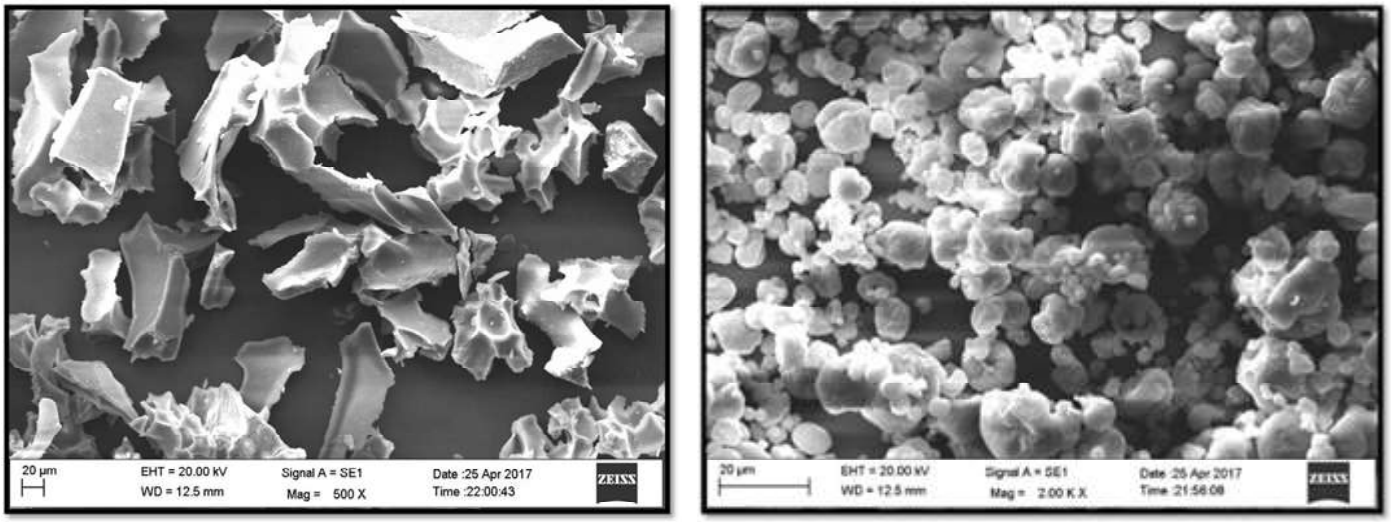

Fig. 2 SEM micrographs of freeze dried (A) and spray dried (B) powder of *Lactococcus lactis* sp. *lactis* NCDC 97

The a_w and residual water contents in dried powders are affected by hygroscopicity and water binding ability of the drying matrix components (Barbosa-Canovas *et al*, 2005). Poddar et al. (2014) observed that the a_w of spray-dried *Lb. paracasei CRL* 431 was lower than 0.33. In order to reduce detrimental effects on bacterial cells, the drying medium can be supplemented with an antioxidant such as ascorbic acid and monosodium glutamate in spray-drying medium to improved culture viability during powder storage (Sunny-Roberts and Knorr, 2009).

Powder morphology

Images obtained by scanning electron microscopy of the prepared powders of *L. lactis* sp. *lactis* NCDC97 by freeze and spray drying is shown in figure 2 (A & B), respectively. The SEM images of freeze-dried bacterial cells shows irregular shape of particles with variations in range. The freeze-dried material retained its solid amorphous form and the structure resembled as broken glass or flake-like structure. The bacterial cells can be seen as randomly distributed throughout the wall matrix.

The SEM image of spray dried powder clearly depicts that the whey protein, skim milk and sugars used in drying medium formed a protective coating around the bacterial cells which helped them to survive in drying conditions (Gaiani et al. 2007 and Sadek et al. 2014). During spray drying whey proteins encounters high temperature which causes their denaturation and results in wrinkled rough surface after spray drying (Holt et al. 1999). No bacteria were observed on the surface of micro-particles and no visible surface fissures or cracks confirmed good structural integrity and low gas permeability e.g., oxygen, water vapour that provides better protection to bacterial cells (Fritzen-Freire et al. 2012). The micro structural aspects of spray dried products are significantly affected by parameters such as the drying rates, composition, viscosity of the drying carrier aliquots and the atomization (Kim et al. 2009).This structure of freeze dried powder is due to the direct sublimation of ice into water vapor during freeze-drying operation (Ezhilarasi et al. 2013, Rajam et al.2015).

Conclusion

The results obtained in the present study concludes that storage temperature and powder moisture content of bacterial powders

are the key factors influencing its viability. In addition, higher survivability and storage stability of freeze-dried powder (60%) as compared to spray dried bacterial cells (36%) develops new technological route to improve cell survivability by spray drying and further during storage. At a molecular level the clear mechanism of bacterial cell adaptation and its interaction with whey protein during spray drying and storage could be investigated. Moreover, as the drying processes were done at laboratory scale further its feasibility should be explored on an industrial scale.

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Conflict of interests

The authors declare that there are no conflicts of interest.

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RESEARCH ARTICLE

Assessment of bioactive components of essential oils for antimicrobial activity in the dairy food matrix

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Abstract: Essential oils as a whole have been widely investigated for their antimicrobial properties against spoilage microorganisms and pathogens. However limited information is available about the inhibitory activities of components of EO in foods and more specifically in dairy food matrices. The widespread components of common EOs were evaluated for their antibacterial activity in media and skim milk. Amongst the EO components, trans-cinnamaldehyde and eugenol were the most effective on the basis of disk assay and minimum bactericidal concentrations in milk. The MBC of these EOs was unaffected by the conditions of the incubation temperature of milk. However the MBC was decreased in paneer slurry against *L. monocytogenes* and *E. coli* O157:H7 when incubated at 7 °C than 37 °C. In the dairy spread, trans-cinnamaldehyde and eugenol were effective in inhibiting TBC, coliform, and yeast & mold count at 0.26 mg/g without affecting sensory properties. The microbial load in paneer made from EO added to milk (10 mg/ml) significantly inhibited microbial growth during storage. In addition eugenol has shown antioxidant activity in milk through ABTS radical scavenging assay.

Keywords: Cinnamaldehyde, Eugenol, Channa-based dairy spread, Essential oil

Introduction

Consumers nowadays demand minimally processed foods that are nutritionally rich, free of chemical preservatives and microbiologically safe for human consumption. As a result, research has focused on reducing the intensity of heat treatment, developing alternatives to heat processing techniques, and replacing conventional chemical preservatives with natural ones that can preserve the food product freshness without compromising food safety (Ait-Ouazzou et al. 2011, Badola et al. 2018, Mitropoulou et al. 2022).

Essential oils (EOs) are aromatic and volatile hydrophobic compounds that can be extracted from any part of the plant. For several decades, EOs have been known for their antimicrobial activity and are now widely used in cosmetics, pharmaceuticals, food preservation, and food additives (Rota et al. 2004, Chen et al. 2014). Due to their safe application and the image of being natural many EOs currently generally as recognized as safe (GRAS). Chemically, EOs are complex natural mixtures that are composed of more than 20-80 individual constituents, each of which is present at significantly varying concentrations (Saraiva et al. 2021). The major widespread constituents of EOs thymol, linalool, citral, p-cymene, carvacrol, eugenol, etc. Numerous studies have investigated the antimicrobial activity of EOs as a whole against foodborne pathogens and these properties are attributed mainly to constituents of EO (Mith et al. 2014, Cho et al. 2020). However, the chemical composition of whole essential oils is highly varying and is dependent on several factors such as environmental conditions, extraction method, and stage of harvesting. Concerning this challenge, few studies have explored the use of purified major constituents of EO in food preservation (Ait-Ouazzou et al. 2011). Since the constituents in an EO have different chemical structures and may have different chemical properties such as solubility, volatility, and oxidative stability which in turn affect the antimicrobial properties of the whole EO (Chen et al. 2014). The major individual compounds can be easily procured in large quantities and according to desired specifications (Das et al. 2021). Moreover, a reproducible control of spoilage and pathogens in foods can be achieved with a steady supply of essential oils with major compounds at the same concentrations tested (Ju et al. 2020). The compounds selected in this study are the major constituents of commonly available EOs. Studying microbial sensitivity in liquid and solid food matrices as models might facilitate optimization for the final application of essential oil constituents (Gutierrez et al. 2009).

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This study was carried out to evaluate the antibacterial activity of widespread constituents of EOs eugenol, carvacrol, cinnamaldehyde, menthol, thymol, vanillin, and p-cymene in skim milk against pathogenic and spoilage bacteria. Further, paneer and chhana-based dairy spread were used as dairy product food models to assess the application potential of those effective EO components *in situ*.

Materials and Methods

Essential oils

Eugenol, carvacrol, cinnamaldehyde, menthol, thymol, vanillin, and p-cymene were purchased from Sigma-Aldrich, St. Louis, USA.

Bacterial strains

L. monocytogenes ATCC 15313, *E. coli* O157:H7, *S. aureus* MTCC 3160, *E. faecium* NCDC 211, *B. cereus* NCDC 66, *B. subtilis* NCDC 215, *S. typhi* NCDC 13 and *E. faecalis* NCDC 223 were used in this study. These bacterial cultures are part of the National Collection of Dairy Cultures, NDRI, Karnal. All bacterial strains were maintained at 4 °C on tryptic soy broth (TSB) medium (Himedia, India).

Disk diffusion assay

As a preliminary step, the agar disc diffusion technique was performed to screen antibacterial activity against test bacterial strains (Rota et al. 2004). A 15 ml of TSA agar (Himedia, India) was poured into sterile Petri plates and allowed to solidify and then overlaid with 5 ml of TSA soft agar (Agar 0.75%) inoculated with 10^6 CFU/ml of each tested bacterium. The paper disk impregnated with 15 µl of EO was placed on the surface of the agar. The plates were incubated overnight at the appropriate temperature, and the diameter of the resulting zone of inhibition was measured in millimeters.

Minimum bactericidal concentration (MBC) test

The minimum bactericidal concentration (MBC) in sterilized skim milk was determined using the microdilution method (Cava-Roda et al. 2012). Briefly, 100 µl of sterilized milk was dispensed into the wells of the 96-well flat bottom plate. A 100 µl of sterilized milk containing EO was loaded into the first well, and subsequent serial double dilutions were carried out to obtain concentrations ranging between 32-0.0125 mg/ml. To each well, another 100 µl of sterilized skim milk spiked with a tested bacterium (10^6 CFU/ml) was added and mixed for 30 seconds in a microplate shaker. The microplate was incubated for 24 h at an appropriate temperature without shaking. After incubation 25 µl sample from each well was spot inoculated on a TSA plate and growth was observed after incubation at 37 °C for 24 h. The concentration of compound at which no visible growth was observed was defined as MBC of EO.

Challenge studies

Sterilized milk, 30 ml without or with different concentrations of EO was transferred to sterilized tubes with cap. The test bacterium was inoculated at 1% from cell suspension containing 10^6 CFU/ml. Subsequently samples were transferred to sterilized 100 ml sample bottle and capped. For the survivor counts serially diluted samples were pour plated on tryptic soy agar and after 37 °C/24-48 h the CFU/g was recorded.

A portion of sterilized paneer (32 g) was blended with sterile water (16 g) to obtain slurry by eliminating natural microbiota. Subsequently, samples were transferred to sterilized 100 ml sample bottles with a cap. The samples were experimentally inoculated with test bacterial strains (10^6 CFU/ml) and desired concentration of EO was added and MBC was determined as described above.

Activity against natural microflora in paneer and chhana based dairy spread

For paneer, EO was added to raw milk at a level of 10 mg/ml and mixed at 15000 rpm/5 min using Ultra-Turrax T25 (IKA Labortechnik). The temperature of the milk was increased to 90 °C with no hold and the coagulation of milk was done at 70 °C by the addition of citric acid solution (1% w/v). The coagulum was collected in a muslin cloth and pressed for 15 min under 1.5-2 kg/cm^2 pressure. Following this coagulum was immersed in chilled water for 1 h which was then packed in LDPE films and stored at 7 °C. Similarly, the control paneer was prepared from milk without EO addition. The strict hygiene was observed during paneer production. The microbiological analysis was conducted at regular intervals. Pour plate methods were performed for total bacteria with plate count agar, coliform with VRBA, yeast and mold with potato dextrose agar and *Staphylococcus* sp. with Baird Parker agar with egg yolk and potassium tellurite as supplement. Subsequently, CFU/g was calculated using dilution factors.

The chhana based dairy spread was manufactured from cow's milk by following the flow chart described in (Amitraj et al. 2016). The mixture after adding all the ingredients (chhana, maltodextrin, skimmed milk powder, whey powder, emulsifying salt, emulsifier, EMC, edible salt mixed with a known amount of water) the EO was added and mixed for 40-45 seconds using a domestic blender. The mixture after preheating to 70 °C was immediately homogenized without pressure and heat treated at 80 °C for 5 minutes. When the product was still hot packed in polystyrene cups with air-tight screw caps and stored at 5 °C. Batches without EO were prepared similarly. Total aerobic count, coliform, and yeast & mold counts were determined at different intervals by the plate count technique.

Sensory evaluation

The EO-added products were subjected to sensory evaluation by a panel of judges. The changes caused by the incorporation of EO were assessed through the difference-from-control where only product without EO addition was used as control. Each panelist compared the smell and taste, and the results were quantified on 9 points hedonic scale for paneer. For the spread, the organoleptic scheme consisted of flavor (45 points), body and texture (35 points), spreadability (10 points), and color (10 points) (Amitraj et al. 2016).

Antioxidant activity

The antioxidant activity was determined by ABTS (2, 2-azino-bis 3-ethyl benzothiazoline-6-sulphonic acid) assay as described by (Re et al. 1999). Before use, the absorbance of ABTS solution was adjusted to 0.7±0.02 at 734 nm (Specord-200, Analytik Jena, Germany). Three ml of ABTS radical solution was added with 30 µl sample and absorbance was read at 734 nm at 1 min interval for 6 min.

$$\text{Inhibition (\%)} = 1 - \frac{Absorbance\ (sample)}{Absorbance\ (control)} \times 100$$

The total antioxidant activity of experimental samples determined from the standard curve and expressed as Trolox equivalent antioxidant capacity (TEAC).

Statistical analysis

Experiments were performed in triplicates and the results were analyzed with SPSS software for analysis of variance (ANOVA) followed by Tukey's comparison test to determine the significant differences ($P < 0.05$) amongst the mean values.

Results and Discussion

The preliminary screening of EO for antibacterial activity was carried out against different spoilage and pathogenic microorganisms by disk diffusion method. Figure 1 shows the the diameter of inhibition zone including diameter of paper disk (6 mm diameter of the disc). By generating an inhibition zone, each EO components demonstrated inhibitory effects against the microorganisms under study. Cinnamaldehyde and eugenol showed the largest inhibition zone values, which ranged from 12 to 33 mm. The diameter of the zone that was generated by menthol, vanillin and *p*-cymene was less than 12 mm. However the disk diffusion used in study is not highly quantitative and inappropriate for hydrophobic compounds like EO which lack uniform diffusion in the media (Ait-Ouazzou et al. 2011).

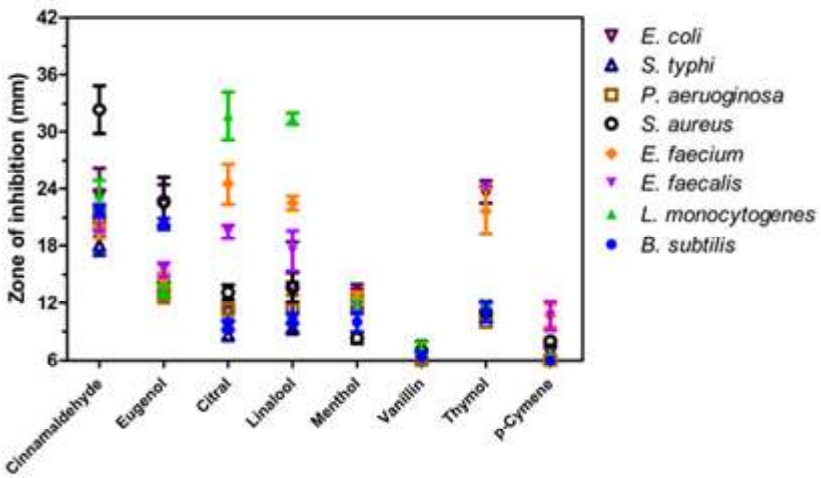

Fig 1: Antibacterial activity of EO components using paper disc diffusion method

Values are given as mean ± SD (n=3).

Table 1. MBCs of EO components in skim milk (mg/ml)

Essential oil	*B. cereus*	*B. subtilis*	*L. monocytogenes*	*E. faecalis*	*E. faecium*	*S. aureus*	*P. aeruginosa*	*S. typhi*	*E. coli*
Cinnamaldehyde	20	4	4	10	4	4	4	6	4
Eugenol	30	8	4	4	8	4	15	9	8
Thymol	15	10	4	8	2	20	25	10	4
Citral	25	20	20	15	10	4	40	20	20
Linalool	50	20	20	50	10	20	40	20	10
Vanillin	50	20	20	50	50	20	20	20	15
p-Cymene	50	5	50	50	40	50	40	50	40
Menthol	50	20	50	50	50	50	50	50	50

To obtain more precise data on the antimicrobial activity of EO component, MBC values were obtained in skim milk against each test bacterium and are shown in Table 1. Based on the MBC values cinnamaldehyde and eugenol, out of eight EO components, had the strongest antibacterial effect aganist *B. subtilis, L. monocytogenes, E. fecalis, E. fecium, S. aureus, P. aeruginosa, S. typhi* and *E. coli,* with MBC values ranging 4-10 mg/ml. Thymol and citral had moderate antibacterial activity as measured by MBC values ranging from 4-25 mg/ml. Vanillin, *p*-cymene, linalool and menthol, on the other hand had lower antibacterial activity owing to higher MBC values (20-50 mg/ml). These results are in agreement with other published reports (Friedman et al. 2002, Ananda Baskaran et al. 2009, Yuan et al. 2019) which emphasize the stronger activity of cinnamaldehyde and eugenol against a wide range of microorganisms including food borne pathogens and spoilage bacteria. Thus, further research was conducted on cinnamaldehyde and eugenol, which exhibited the strongest antibacterial activity in this study.

Table 2 depicts the MBC values that were observed in paneer and semi-skimmed milk at different storage temperatures. The concentrations of 4-12 mg/ml eugenol and 12 mg/ml cinnamaldehyde in skimmed milk were sufficient for complete bactericidal activity against *L. monocytogenes* and *E. coli*. The storage temperature conditions did not influence the MBC of these EOs. These finding in this regard confirm the observations of Cava et al. (2007) that storage temperature had no effect on the MBC of clove and cinnamon EOs in skim milk against *L. monocytogenes.* On the other hand MBC of eugenol for *E. coli* O157:H7 in paneer decreased to 80 mg/ml at 7 °C from 100 mg/ml at 37 °C. In a previous study the bactericidal activity of LC-EO against *L. monocytogenes* increased when the storage temperature of tofu was decreased from 37 °C to 7 °C (Liu and Yang, 2012). This suggests that various dairy products that require low temperatures could benefit from the use of EO in conjunction with refrigeration temperature.

The results of time kill assay using eugenol and cinnamaldehyde in sterilized milk is shown in Figure 2. At respective MBC concentrations of eugenol and cinnamaldehyde the target bacteria were completely inactivated at 1 hour and 3 hour incubation, respectively. Eugenol appeared faster in killing *L. monocytogenes* and *E. coli* than cinnamaldehyde. However the MBC values of eugenol and cinnamaldehyde are 5 times higher in paneer than skimmed milk indicating food matrix effect on the antibacterial efficacy. The higher MBC values of EO up to 10 times in food than in vitro are recurrent in several studies (Cava-Roda et al. 2012). Nevertheless addition of EO to food product at concentration equal to MBC might mainly negatively impact the sensory scores of the food product (Saraiva et al. 2021). Therefore this study also highlight the importance of evaluating efficacy in simultaneous studies in vitro and in real foods to arrive at the balance between sensory scores and microbiological quality.

In view of stronger activity of cinnamaldehyde and eugenol the effectiveness were evaluated in chhana based dairy spread. The table 3 shows the sensory evaluations of chhana based dairy spread with and without EO. With the addition of EO at 0.26 mg/g the scores of sensory were not affected significantly. However EO at a concentration of 0.52 mg/g caused the negative effect on the flavor and overall acceptability ($P<0.05$) of spread. Overall the cheese spread with EO at a concentration of 0.26 mg/g was acceptable concerning the sensory scores.

As shown in Figure 3 the growth of total plate count, mold and yeasts were inhibited as a result of addition of EO components. In control, the counts were found to increase with progress in the storage period. The addition of eugenol or cinnamaldehyde (0.26 mg/g) caused the total plate count, yeast and mold counts to decrease significantly ($P<0.05$). For instance on week 6 the count of total plate count, molds and yeast in control was 3.97 and 1.86 log CFU/g, respectively. The total plate count in cinnamaldehyde and eugenol added spread samples reached 2.39 and 2.61 log CFU/g, respectively. Eugenol incorporated spread samples showed yeast and mold count 1.3 log CFU/g and cinnamaldehyde was more effective to prevent the growth of yeast and molds with counts below 1 log CFU/g throughout the storage period ($P<0.05$). According to previous published reports the essential oils as antimicrobial agents suppress the growth of microorganisms including spoilage causing yeast and mold (Balaguer et al. 2013, Ju et al. 2020, Mitropoulou et al. 2022). Makhal et al. (2014) reported that the addition of thymol to cottage cheese at 40 ppm increased the shelf life by decreasing the psychrotrophs and yeast and mold counts. Likewise, Badola et al. (2018) observed that curry leaf EO (0.15 ml/kg) and Clove bud EO (0.25 ml/kg) were effective to keep the microbiological counts within the limits of FSSAI in Indian traditional confection, burfi. Overall the addition of cinnamaldehyde and eugenol can enhance the microbiological quality of chhana-based dairy spread and improve the shelf life.

When added to milk EO exhibited protective effect on paneer during storage against various microbial populations (Figure 4). According to earlier reports, the typical concentration for spices and herbs used in food system ranges from 0.05 to 0.1% (Licon et al. 2020). TBC of control samples increased from initial counts of 3.02 to 7.21 log CFU/g after 21 days, but in cinnamaldehyde and eugenol containing samples 3.3 and 6.49 log CFU/g, respectively ($P<0.05$). Similarly, yeast and mold counts compared to control there was a significant reduction in cinnamaldehyde and eugenol treated paneer samples ($P<0.05$). Coliform counts in paneer was inhibited by eugenol and remained below 1 log CFU/g in cinnamaldehyde containing samples. The final increments of *Staphylococcus* sp counts in cinnamaldehyde treated samples was 1.69 log CFU/g was significantly less than eugenol and control samples 4.5 log CFU/g. There are reports in the literature on application EO as an ingredient to milk and to control microbial counts in the product such as cheese (Zantar et al. 2014, Hachana

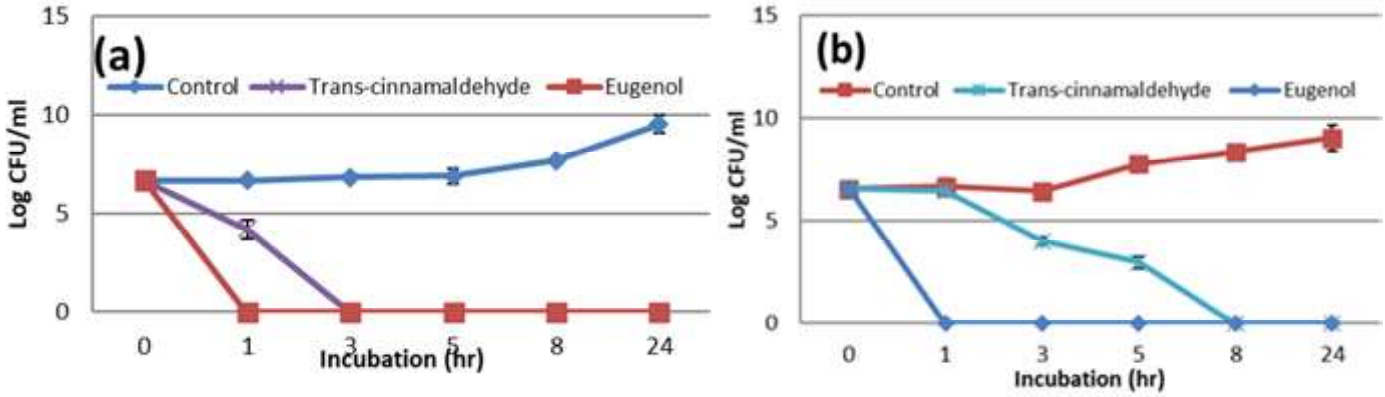

Fig 2: Time kill curves obtained after treating EO at MBC concentrations in skim milk(a) E. coli O157:H7, b) L. monocytogenes)

Table 2: Minimum bactericidal concentrations (mg/ml) in milk and paneer incubated at 7 and 37 °C

EO	Test organism	7 °C for 14 days		37 °C for 24 h	
		Milk	Paneer	Milk	Paneer
Cinnamaldehyde	*L. monocytogenes*	12	12	12	20
	E. coli O157:H7	12	80	12	100
Eugenol	*L. monocytogenes*	12	12	12	20
	E. coli O157:H7	4	80	4	100

Table 3: Sensory scores of chhana-based dairy spread incorporated with EO components

Parameter	Control	Eugenol (mg/g)		Cinnamaldehyde (mg/g)	
		0.26	0.52	0.26	0.52
Flavor	42.00±0.71[a]	40.00±0.71[a]	32.25±1.64[b]	39.25±2.48[a]	35.50±0.50[b]
Body and texture	33.0±0.31[a]	32.5±0.41[a]	32.0±0.50[a]	32.0±0.25[a]	32.2±0.12[a]
Colour and appearance	8.2±0.24[a]	8.1±0.03[a]	8.2±0.02[a]	8.3±0.13[a]	8.1±0.21[a]
Spreadability	7.5±0.50[a]	7.5±0.13[a]	7.4±0.30[a]	7.4±0.20[a]	7.4±0.15[a]
Overall acceptability	90.7±1.76[a]	88.1±1.28[a]	79.85±2.46[b]	89.95±3.06[a]	83.2±0.98[b]

Superscript in the treatment column indicates significant difference with control ($P<0.05$)
Values are given as mean ± SD (n=3)

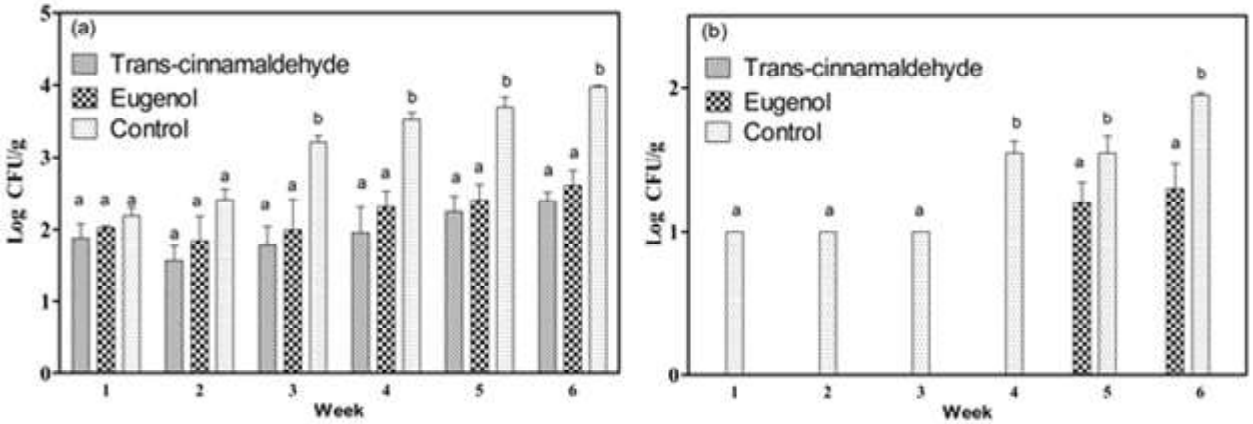

Fig 3: Effect of EO addition (0.26 mg/g) on the microflora of chhana-based dairy spread during storage at 7 °C (a) total plate count (b) yeast and mold count

et al. 2019, Ahmed et al. 2021). Thyme, basil and *M. officinalis* EOs transferrence upto 53% from milk to cheese and up to 3.3 log reduction in microbial counts in cheese has been earlier reported (Licon et al. 2020). The presence of spicy flavor of EO decreased the sensory scores of paneer prepared from milk with EO (10 mg/ml), which were slightly liked on a 9-point hedonic scale. Nevertheless, paneer usually is eaten with condiments or in other dishes and some changes to the EO flavor notes and quantification

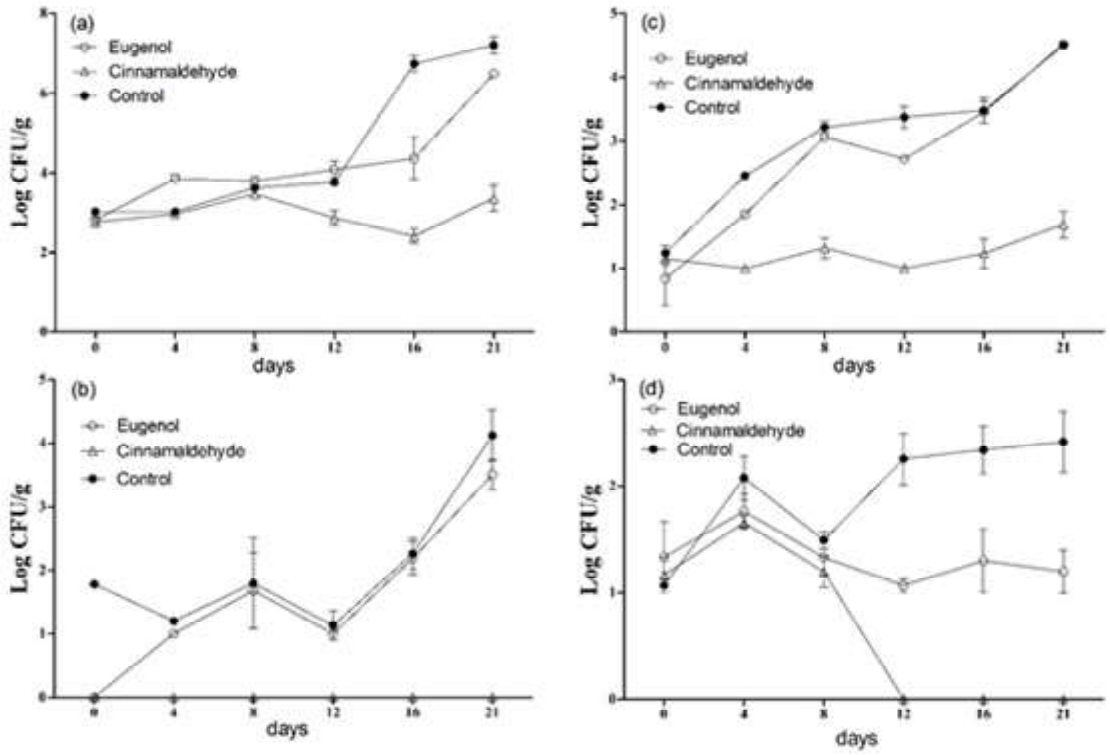

Fig 4: Effect of EO addition on the microflora of paneer made from milk added with EO (1%) during storage at 7 °C. (a) Total plate count (b) coliform count (c) *Staphylococcus* sp. (d) yeast and mold count

of transferrence make EO usable (Mitropoulou et al. 2022). To summarize the addition of EO to milk followed by paneer making showed transference to paneer and can exhibit preservative effect during storage and a concentration below 10 mg/ml is suggested for paneer.

The oxygenated terpenes and phenylpropanoids are believed to exhibit strong antibacterial activity than hydrocarbons. According to the findings of this study, eugenol and cinnamaldehyde belongs to terpenes and phenylpropanoids had consistently strong activity than hydrocarbons (Cava-Roda et al. 2012). The difference in the antibacterial activity of EO components could be attributed to the distinction in their chemical structure (Cho et al. 2020). The functional groups interact with the plasma membrane and play important role in disruption of cytoplasmic membrane as well as coagulation of cell contents of target microorganisms (Lv et al. 2011, Das et al. 2021). However, the mechanism of antibacterial action of pure compounds of EO is not well established in the literature (Licon et al. 2020).

The Figure 5 shows the antioxidant ability of EO components expressed as Trolox equivalent antioxidant capacity. Only milk had low lowest antioxidant potential which was significantly increased in the presence of EO components. In milk added with eugenol at 0.1 to 1 mg/ml the TEAC increased from 1229.93 to 1438.24 µM respectively, indicate antioxidant activity in a dose dependent manner (P<0.05). The only milk antioxidant activity 1033.5 µM TE is reported to be improved by the addition of various plant biomolecules depending on chemical characteristics (Alenisan et al. 2017). The TEAC values resulted by eugenol supplementation are comparatively higher than cinnamaldehyde. Eugenol has been reported to possess considerable radical scavenging, reducing ability and antileishmanial activities than cinnamaldehyde (Sharma et al. 2017). The high antioxidant activities of eugenol is a result of free hydroxyl group on aromatic ring which is absent in cinnamaldehyde.

Conclusions

Amongst different components of EOs investigated in the present study cinnamaldehyde and eugenol exhibited the greatest antibacterial activity. Cinnamaldehyde and eugenol have good potential for antimicrobial application to control microbial counts in dairy products such as chhana based dairy spread and paneer. Eugenol possess better antioxidant activity as compared to cinnamaldehyde. Future studies should investigate different dairy products as strong flavour may make it challenging to use in dairy products. Further the effect of addition of EO on the physico-chemical, textural and sensory properties of dairy products needs a deeper study.

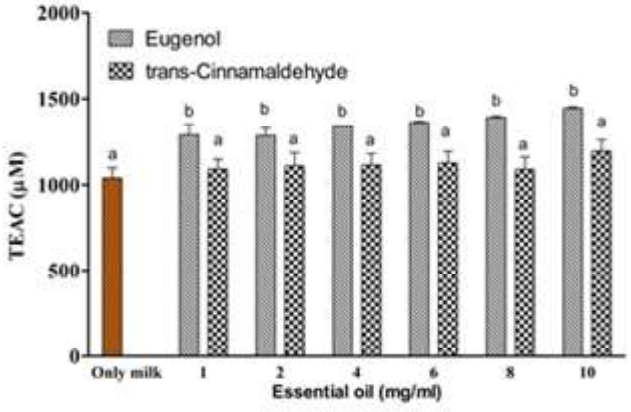

Fig 5: Antioxidant activity of milk with or without EO addition

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RESEARCH ARTICLE

Application of Taguchi orthogonal array design to optimize microencapsulation of zinc by spray-drying

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Abstract: Zinc is an essential trace element for the body to maintain normal health and perform important functions. As zinc is not stored in the body, it needs to be replenished through our daily diet. Microencapsulation technique can be used to encapsulate the functional ingredient such as zinc by masking its metallic taste and enhancing its bioavailability. Among the microencapsulation techniques, spray-drying is the most economical and scalable method. Zinc was microencapsulated by spray-drying using maltodextrin, HI-CAP® 100 and WPI along with gum Arabic as wall materials. The spray-drying conditions were optimized using Taguchi L_{18} orthogonal array design using encapsulation efficiency and bulk density as response factors. The influence of wall materials, wall material to zinc ratio and inlet air temperature was evaluated. Microcapsules prepared with HI-CAP® 100 in the ratio of 20:1 at 185°C showed maximum encapsulation efficiency, whereas microcapsules with HI-CAP® 100 in the ratio of 10:1 at 185°C had maximum bulk density. The microencapsulated zinc powder had bulk density of 437.40-541.20 kg/m^3 and encapsulation efficiency of 76.86-92.65%. Validation experiments confirmed that Taguchi orthogonal array design was successful in optimizing microencapsulation of zinc. Microencapsulated zinc can be fortified in various delivery systems such as milk and milk products.

Keywords: Microencapsulation, Optimization, Spray-drying, Taguchi orthogonal array, Zinc

Introduction

Minerals are the vital inorganic nutrients required by human body for its normal functioning and growth. Among these, zinc is an essential trace element needed by the body to maintain normal health and perform vital functions such as cell growth, wound healing, immune system function, bone mineralization, blood clotting, cognitive functions and intellectual development (Maret and Sandstead 2006). Zinc is a cofactor to more than 300 enzymes and a powerful therapeutic tool to manage a long list of illness (Polekkad et al. 2021). As zinc is not stored in the body, it needs to be replenished through our daily diet. Zinc deficiency in humans is known to be a major malnutritional problem. According to International Zinc Association (IZA), nearly 1.9 billion people are suffering worldwide due to zinc deficiency (International Zinc Association 2018). Foods that are rich source of zinc include chicken, oysters, tofu, beef, pork, lentils, nuts, hemp seeds, yoghurt, oatmeal and mushrooms. With most of the vegetarian diet consisting of whole grains and plant proteins, the vegan populations are under the risk of zinc deficiency. Though some of the vegetarian foods are rich in zinc, its absorption from composite foods is a problem due to the presence of phytate (Oberleas and Harland 1981). To alleviate the problem of zinc deficiency, it could be fortified in suitable food systems. However, zinc cannot be added directly to food as it has a bitter, unpleasant and metallic after-taste, which is not liked by the consumers (Öner et al. 1988).

Microencapsulation is a delivery technology that can be used to encapsulate and mask the metallic taste of functional ingredients such as zinc (Ré 1998). In addition, microencapsulation also helps in controlled-release of bioactives, which enables their better absorption (Singh et al. 2010). In food systems, encapsulation by physical methods is mostly preferred for protection and effective delivery of nutrients (Madene et al. 2006). Studies demonstrated that microencapsulation of minerals was useful in preventing the deteriorative reactions in food matrix,

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since the minerals could not act as catalysts (Gupta et al. 2015). Microencapsulation should ensure the reduction of reaction of minerals with other ingredients, its proper release and dietary uptake. The stability of microencapsulated mineral depends on the reaction between wall and core materials (Gharsallaoui et al. 2007). Therefore, depending on the stability, bioavailability and compatibility with the selected technique, wall materials required for encapsulation are selected (Augustin and Sanguansri 2008).

Spray-drying is an economical and scalable method of encapsulation used in the food industry, which transforms the dispersion of liquid feed into dried capsules. It has already been used to microencapsulate nutrients due to its low processing time and high throughput (Rezvankhah et al. 2019). It effectively microencapsulates all active ingredients and reduces their chemical and biological degradation. The properties of spray-dried microencapsulated minerals depend on the type of polymer and its concentration (Oneda and Ré 2003). Spray-dried powders have instantaneous solubility and superior functional and reconstitutional properties. The desired properties of spray-dried powders can be achieved by regulating the process parameters. However, microencapsulation is influenced by spray-drying conditions such as inlet temperature of drying air, the ratio of wall material to active ingredient, flow rate of feed, total solids content in feed, etc. For successful microencapsulation of zinc, appropriate wall material or a combination of wall materials and spray-drying conditions need to be optimized.

The Taguchi orthogonal array optimization is a robust design that considers both the controllable and noise factors. The tools used in the Taguchi design are signal-to-noise (S/N) ratio (measures quality) and orthogonal arrays (accommodate design parameters) (Ghani et al. 2004). This method can be used to optimize the spray-drying conditions for microencapsulation of zinc for production of the microencapsulated zinc powder. To alleviate the problem of zinc deficiency, zinc could be fortified in suitable foods. Milk is considered as a potential vehicle for fortification. Hence, fortification of milk and milk products with zinc is an effective method to alleviate this problem. However, zinc cannot be added directly to milk as it has a bitter, unpleasant and metallic after taste, which is not liked by consumers (Öner et al. 1988).

Thus, the objective of the study was to establish spray-drying as a successful method for encapsulation of zinc and to optimize microencapsulation of zinc using Taguchi orthogonal array design.

Materials and Methods

Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) was procured from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Whey protein isolate (WPI) was procured from Nakoda Dairy, Bengaluru, India. HI-CAP® 100 was supplied *on gratis* by Ingredion India Pvt. Ltd. Mumbai, India. Gum Arabic and all other chemicals used in this study were of analytical grade, and were purchased from HiMedia Laboratories, Mumbai, India.

Preparation of dispersion

Dispersions of zinc sulphate heptahydrate were made using different wall materials such as maltodextrin, HI-CAP® 100 and WPI along with gum Arabic. The wall material to zinc ratio were 10:1 and 20:1, and the total solids (TS) content of the dispersions was maintained at 30.5%. To prepare the dispersions, zinc sulphate was dissolved in double distilled water, and the wall materials were blended using a magnetic stirrer (Model: CMAG HS7, IKA, Staufen, Germany). The dispersions were homogenized using high shear mixer (Model: Unidrive X 1000D, CAT Scientific, Staufen, Germany) at 15,000 rpm for 15 min.

Microencapsulation process

Microencapsulation of zinc was carried out in a co-current flow spray-dryer (Model: LU-222 Advanced, Labultima, Mumbai, India), equipped with two-fluid nozzle atomizer, drying chamber, cyclone separators, hot air system, pre-filters and bag filters. The feed flow rate of 6 mL/min was adjusted by peristaltic pump, and the inlet drying air temperatures were 170, 185 and 200°C. The outlet air temperature was kept at 80-85°C. The aspirator flow rate was maintained at 60 Nm^3/h. Hot air was used as heating medium with co-current flow mode and the powder was collected from the chamber and cyclone, and mixed.

Experimental design and optimization

Wall material to zinc ratio, wall material and inlet air temperature were selected as the process factors for microencapsulation. Taguchi L_{18} ($3^2 \times 2^1$) mixed orthogonal array design was used to optimize the spray-drying conditions using Minitab 17 software (Minitab Inc., Pennsylvania, USA) (Table 1). This method uses a loss function, which is converted into S/N ratio (η). The S/N ratio is the logarithmic function of target value. The targets are maximum encapsulation efficiency and bulk density of the microcapsules as they are generally desirable properties of food powders. Larger-the-better was used because they are important characteristics of microcapsules as given in Equation (1).

$$\eta = S/N = -10 \log \left[\frac{1}{n} \sum_{i=1}^{n} \frac{1}{X_i^2} \right] \quad \text{(Haq et al. 2008)} \qquad (1)$$

where, 'X_i' is the observed value at the i^{th} response and 'n' is the total number of observations in the experiment. ANOVA was run using Minitab 17 software (Minitab Inc., Pennsylvania, USA) to evaluate the effect of individual factors at 5% level of significance. The significance of process factors was assessed by relative

comparison of 'F' values, and the contribution rate of each factor indicating the degree of influence on process performance was also calculated. Once the optimal levels of the process factors were selected, a confirmation experiment was conducted to validate the optimized conditions. For predicting the optimum spray-drying conditions, the estimated S/N ratio () was computed using Equation (2).

$\hat{\eta}=\eta_m+\sum_{i=1}^{q}(\eta_i-\eta_m)$ (Haq et al. 2008) (2)

where, 'ηm' is the mean S/N ratio, 'q' is the number of significant factors and '$\bar{\eta}$' is the average S/N ratio corresponding to i^{th} significant factor on j^{th} level.

In order to evaluate the accuracy of optimization, the confidence intervals (*C.I.*) for encapsulation efficiency and bulk density were computed using Equations (3) and (4) (Haq et al. 2008).

$$C.I.=\sqrt{F_{\alpha(1,f_e)}V_e\left[\frac{1}{\eta_{eff}}+\frac{1}{R}\right]} \quad (3)$$

where, $F_{\alpha(1,fe)}$ is the 'F' ratio at 95% confidence, 'á' is the significance level, 'f_e' is the error degrees of freedom, 'V_e' is the error variance, 'η_{eff}' is the effective number of replications, 'R' is the number of replications.

$$\eta_{eff}=\frac{N}{1+T_{dof}} \quad (4)$$

where, 'N' is the total number of experiments and 'Tdof ' is the total degrees of freedom of the main factor. The regression models were established between the process and response variables. The error percentage between Taguchi design and regression models was compared.

Analysis of microcapsules

Mineral contents

Exactly 2 g of microencapsulated powder was taken in a pre-weighed silica crucible. The sample was decarbonized on a flame, and transferred to muffle furnace for ignition at 550-600°C for 5 h. Then the sample was cooled and weighed quickly. Exactly 15 mL of 5 N HCl was added into the silica crucible and the contents were boiled for 15 min. The solution obtained was filtered through Whatman No.41 filter paper into a volumetric flask. The volume

Table 1. Taguchi orthogonal array design $L_{18}(3^2\times2^1)$.

Trial	Process factors			
	A Wall material to zinc ratio	B Wall material	C Inlet air temperature (°C)	Designation
1	10:1	Maltodextrin	170	$A_1B_1C_1$
2	20:1	Maltodextrin	170	$A_2B_1C_1$
3	10:1	Maltodextrin	185	$A_1B_1C_2$
4	20:1	Maltodextrin	185	$A_2B_1C_2$
5	10:1	Maltodextrin	200	$A_1B_1C_3$
6	20:1	Maltodextrin	200	$A_2B_1C_3$
7	10:1	HI-CAP® 100	170	$A_1B_2C_1$
8	20:1	HI-CAP® 100	170	$A_2B_2C_1$
9	10:1	HI-CAP® 100	185	$A_1B_2C_2$
10	20:1	HI-CAP® 100	185	$A_2B_2C_2$
11	10:1	HI-CAP® 100	200	$A_1B_2C_3$
12	20:1	HI-CAP® 100	200	$A_2B_2C_3$
13	10:1	WPI	170	$A_1B_3C_1$
14	20:1	WPI	170	$A_2B_3C_1$
15	10:1	WPI	185	$A_1B_3C_2$
16	20:1	WPI	185	$A_2B_3C_2$
17	10:1	WPI	200	$A_1B_3C_3$
18	20:1	WPI	200	$A_2B_3C_3$

was then made up to 100 mL by repeated washing of crucible. The zinc content was estimated in inductively coupled plasma optical emission spectrometer (ICP-OES) (Model: Optima 8000, Perkin Elmer, Shelton, USA) at a wavelength of 206.20 nm using zinc oxide as standard solution. The equipment was operated in radial spectrophotometric view with the sample volume uptake of 1 mL/min.

Encapsulation efficiency

Encapsulation efficiency of microcapsules was evaluated by the method reported by Abbasi and Azari (2011) with slight modifications. Exactly 30.5 g of microcapsules was added to 100 mL deionized water. Then 10 mL of this solution was taken in a cellulose membrane bag (MW cut-off 12,400 Da), and it was immersed in deionized water with gentle agitation, where non-encapsulated zinc was leached out. After 6 h, the contents were taken from the bag and the zinc content was estimated using ICP-OES as described above. Encapsulation efficiency of microcapsules was calculated using Equation (5).

$$\text{Encapsulation efficiency} = \frac{\text{Bound zinc}}{\text{Total zinc}} \times 100 \quad (5)$$

Bulk density

Bulk density of the powder prepared under various conditions was determined through ASTM D7481-09 (2009). Exactly 100 g of sample was taken in a graduated cylinder and weighed. The cylinder was mildly tapped thrice, and the volume was measured (Equation 6).

$$\text{Bulk density (kg/m}^3) = \frac{\text{Weight of microencapsules}}{\text{Apparent volume}} \quad (6)$$

Results and Discussion

Optimization of spray-drying conditions

Taguchi orthogonal array design was used to optimize the spray-drying conditions for achieving maximum encapsulation efficiency and bulk density of zinc microcapsules. The encapsulation efficiency and bulk density of microcapsules were determined experimentally for 18 combinations listed in the design (Table 2). It is evident that the encapsulation efficiency ranged between 76.86 and 92.65%, while the bulk density lied in the range of 390.0-541.2 kg/m^3. It was found that microencapsulation efficiency was highly impacted (p<0.001) by factors such as type of wall materials, ratio of wall material to core material and inlet air temperature. The encapsulation efficiency was most strongly impacted by the ratio of wall material used, with the mean values for 10:1 and 20:1 ratios being 82.15% and 86.53%, respectively. The type of wall material and temperature also influenced the encapsulation efficiency, although to a lesser extent than the ratio of wall material used. According to Hogan et al. (2001), the ratio of core to wall material had greater impact on the microencapsulation efficiency of powders. The authors concluded that a higher proportion of wall material can increase the rate of formation and thickness of the semi-permeable

Table 2. Taguchi orthogonal experimental design responses with S/N

Designation	Encapsulation efficiency (%)	Bulk density (kg/m^3)	S/N ratio for encapsulation efficiency (dB)	S/N ratio for bulk density (dB)
$A_1B_1C_1$	76.86	502.3	38.04	54.02
$A_2B_1C_1$	84.37	476.7	38.52	53.56
$A_1B_1C_2$	82.80	536.4	38.36	54.59
$A_2B_1C_2$	88.00	490.1	38.89	53.80
$A_1B_1C_3$	79.42	515.8	38.11	54.25
$A_2B_1C_3$	86.54	487.3	38.74	53.75
$A_1B_2C_1$	85.00	515.4	38.59	54.24
$A_2B_2C_1$	87.73	485.6	38.86	53.73
$A_1B_2C_2$	87.60	541.2	38.85	54.67
$A_2B_2C_2$	92.65	505.8	39.34	54.08
$A_1B_2C_3$	86.83	524.1	38.77	54.39
$A_2B_2C_3$	88.90	492.4	38.98	53.85
$A_1B_3C_1$	79.83	412.7	37.71	52.31
$A_2B_3C_1$	83.62	390.0	38.24	51.82
$A_1B_3C_2$	82.60	437.4	38.15	52.82
$A_2B_3C_2$	87.90	408.6	38.58	52.22
$A_1B_3C_3$	80.20	417.2	37.97	52.40
$A_2B_3C_3$	84.07	398.7	38.49	52.01

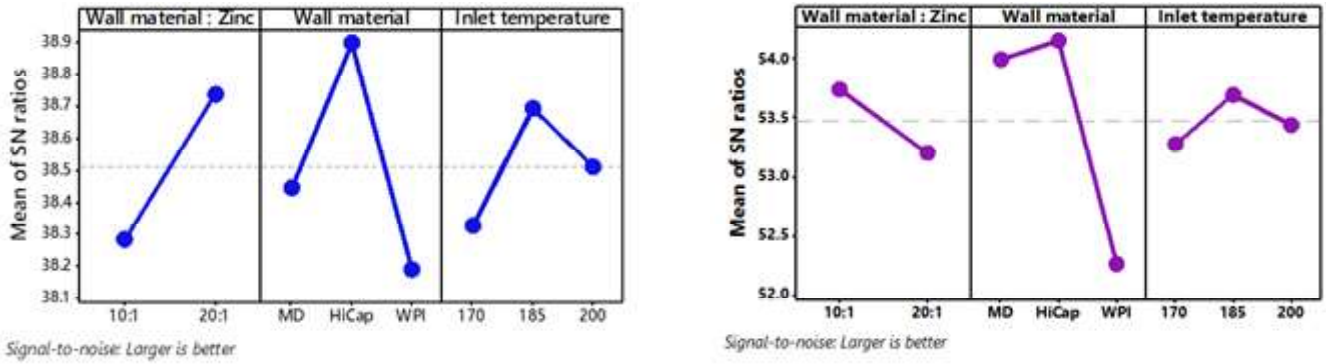

Fig. 1 Effect of parameters on mean S/N ratio for (a) encapsulation efficiency and (b) bulk density

membrane surrounding the core material, leading to increased encapsulation of the active ingredient inside the microcapsules. This finding is in consistent with the results on encapsulation efficiency, which demonstrated that increasing the ratio of wall material resulted in higher encapsulation efficiency.

High ratios of wall materials are often used in microencapsulation processes. For instance, Turasan et al. (2015) employed wall to core material ratios of 40:1, 20:1, and 10:1 for encapsulating rosemary oil in whey protein concentrate and maltodextrin through spray-drying. The authors observed that the best wall to core material ratio for achieving high encapsulation efficiency was 20:1. Similarly, Wu et al. (2014) used wall to core material ratios ranging from 5:1 to 25:1 for microencapsulating sulforaphane through spray-drying and determined that the optimal ratio for encapsulation as 20:1.

The highest encapsulation efficiency (mean 88.12%) was observed with HI-CAP® 100, whereas WPI exhibited the lowest efficiency (mean 81.24%). These findings are consistent with those of Gupta et al. (2015), who reported microencapsulation efficiency of 91.58% for iron using 1:10 ratio of wall material. Furthermore, the authors observed that modified starch provided higher encapsulation efficiency compared to maltodextrin-based microcapsules. Notably, HI-CAP®100 is a modified starch specifically developed for encapsulation purposes. Additionally, an increase in air temperature was found to increase the efficiency of zinc encapsulation, as it led to faster drying rate of droplets and the formation of particle crust, which locked the core material (zinc) inside the dry matrix (Liu et al. 2016).

The average bulk density of zinc microcapsules produced using maltodextrin, HI-CAP® 100, and WPI as wall materials were 536.40, 541.20, and 437.40 kg/m^3, respectively. The wall material type had the greatest impact (p<0.001) on bulk density, followed by the ratio of wall material (p<0.001) and drying air temperature (p<0.001). The higher bulk density of HICAP® 100-based microcapsules was attributed to their higher molecular weight, ordered and arranged particulates. Conversely, the lower bulk density of WPI-based zinc microcapsules was due to high occluded air content. The emulsification and membrane forming ability of WPI was believed to lead to ballooning or puffing of the microcapsules during drying, which increased the particle size by increasing the occluded air content and reduced the bulk density (Walton 2000). Samborska et al. (2015) investigated the use of maltodextrin as wall material in microencapsulation of honey by spray drying and determined its impact on bulk density of the dried powder. Results showed that the bulk density ranged between 330 and 550 kg/m^3, with a decrease observed as the drying air temperature increased, could be due to the production of larger microcapsules (Tonon et al. 2011). On the other hand, an increase in the wall material ratio resulted in reduction of bulk density, indicating that zinc had higher density compared to the three wall materials used. As bulk density is a crucial factor in packaging, transportation, marketing, and storage, HI-CAP® 100 based zinc microcapsules would be the optimal choice.

Signal-to-noise (S/N) ratio

Optimization of the process factors was done using S/N ratio, which is indicative of the deviation of the responses from the desired value. The mean S/N ratio for encapsulation efficiency and bulk density were computed as 38.51 and 53.47 dB, respectively.

Analysis of the effect of each process factor (wall material to zinc ratio, type of wall material and inlet air temperature) on encapsulation efficiency and bulk density was performed using S/N ratio responses. By calculating the difference between the highest and the lowest S/N ratio, the delta value was obtained. The process factor with the highest delta value was ranked first (I), and so on. Thus, the optimum levels of process factors for maximum encapsulation efficiency and bulk density of the microcapsules were obtained.

The influence of process conditions on the response factors is graphically illustrated using S/N ratio in Figs. 1 a & b. The best spray-drying process conditions to achieve maximum encapsulation efficiency and bulk density could be visualized from these graphs (Figs. 1 a&b). Accordingly, the levels and S/N

ratio of the process factors yielding maximum encapsulation efficiency were identified as factor A (Level 2, S/N=38.74 dB), factor B (Level 2, S/N=38.90 dB) and factor C (Level 2, S/N=38.69 dB). In real numbers, the optimum spray-drying process conditions for maximizing the microcapsules encapsulation efficiency were wall material to zinc ratio of 20:1, HI-CAP® 100 as wall material and inlet drying air temperature of 185°C. Similarly for maximum bulk density of zinc microcapsules, the optimal conditions were wall material to zinc ratio of 10:1, HI-CAP® 100 as wall material and inlet drying air temperature as185°C. A comparable result was documented by Pal and Chattacharjee (2018) regarding the encapsulation efficiency of spray-dried marigold flowers enriched with lutein. Additionally, it was observed that the bulk density showed an increasing-decreasing pattern when the temperature of the inlet air used for drying was increased from 170 to 200°C. The highest bulk density was achieved at 185°C. Jafari et al. (2019) employed the Taguchi orthogonal array design to determine the optimal levels of maltodextrin, modified starch, whey protein concentrate, and GA as wall materials for microencapsulating vitamin D_3-enriched whey powder. The results showed that the combination of all wall materials produced the highest yield of powder when the inlet air temperature was set at 170°C. Also, the results were in accordance with Patel et al. (2022), who employed the Taguchi orthogonal array design (L_{18}) to optimize the microencapsulation of curcumin. The results showed that the best conditions for microencapsulation of curcumin were inlet drying air temperature of 185°C, feed rate of 6 mL/min, and HI-CAP® 100 as the wall material. These conditions resulted in moisture content of 4.65%, encapsulation efficiency of 82.42%, and bulk density of 358.40 kg/m^3.

Analysis of variance (ANOVA)

ANOVA was done to evaluate the significance of each process factor on response variables. The results of ANOVA are shown in Table 3. All the three factors had statistically significant effect on encapsulation efficiency and bulk density. However, the type of wall material was observed to be the most significant factor, whereas inlet air temperature of spray-drying was considered as less significant factor to achieve both maximum encapsulation efficiency and bulk density. Moghbeli et al. (2020) used the Taguchi design to optimize the type of drying aid, pH, and inlet air temperature on the moisture content and bulk density of spray-dried powder. The results indicated that temperature had the most significant impact on the responses. Similar results were reported by Patel et al. (2022) for microencapsulation of curcumin.

Regression analysis

The linear and quadratic predictive models for encapsulation efficiency and bulk density are presented in Equations (7-10). The relationship between the observed and predicted encapsulation efficiency and bulk density are shown as Fig. 2. The quadratic relationships between experimental and predicted data were adequate and satisfactory with adj. R^2 above 0.8, while simple linear relationships were not satisfactory. Thus, the encapsulation efficiency and bulk density of zinc microcapsules were predicted based on the spray-drying conditions.

$$\text{Encapsulation efficiency} = 69.30 + 4.38 \times \text{wallmaterial:zinc} - 1.21 \times \text{wallmaterial} + 0.059 \times \text{inletairtemperature} \quad (\text{adj. } R^2 = 0.281) \tag{7}$$

$$\text{Bulk density} = 555 - 29.70 \times \text{wallmaterial:zinc} - 45.33 \times \text{wallmaterial} + 0.293 \times \text{inlet air temperature} \quad (\text{adj. } R^2 = 0.612) \tag{8}$$

$$\text{Encapsulation efficiency} = -355 + 4.38 \times \text{wall material:zinc} + 21.46 \times \text{wallmaterial} + 4.46 \times \text{inletairtemperature} - 5.67 \times \text{wallmaterial}^2 - 0.0119 \times \text{inlet air temperature}^2 \quad (\text{adj. } R^2 = 0.963) \tag{9}$$

$$\text{Bulk density} = -2398 - 39.83 \times \text{wallmaterial:zinc} + 165.67 \times \text{wallmaterial} + 30.55 \times \text{inletairtemperature} - 54.65 \times \text{wallmaterial}^2 - 0.08 \times \text{inletairtemperature}^2 + 5.07 \times \text{wallmaterial:zinc} \times \text{wall material} \quad (\text{adj. } R^2 = 0.993) \tag{10}$$

Table 3. ANOVA for encapsulation efficiency and bulk density

Factor	DOF	SS	MSS	F ratio	Contribution (%)
		Encapsulation efficiency			
Wall material : zinc	1	86.417	86.4170	143.82	31.13
Wall material	2	146.027	73.0136	121.52	52.60
Inlet air temperature	2	37.951	18.9756	31.58	13.67
Error	12	7.210	0.6009		2.60
Total	17	277.606			100
		Bulk density			
Wall material : zinc	1	3969.4	3969.4	176.03	9.35
Wall material	2	36607.8	18303.9	811.71	86.27
Inlet air temperature	2	1586.6	793.3	35.18	3.74
Error	12	270.6	22.5		0.64
Total	17	42434.4			100

**Bold values indicate the most influential factor*

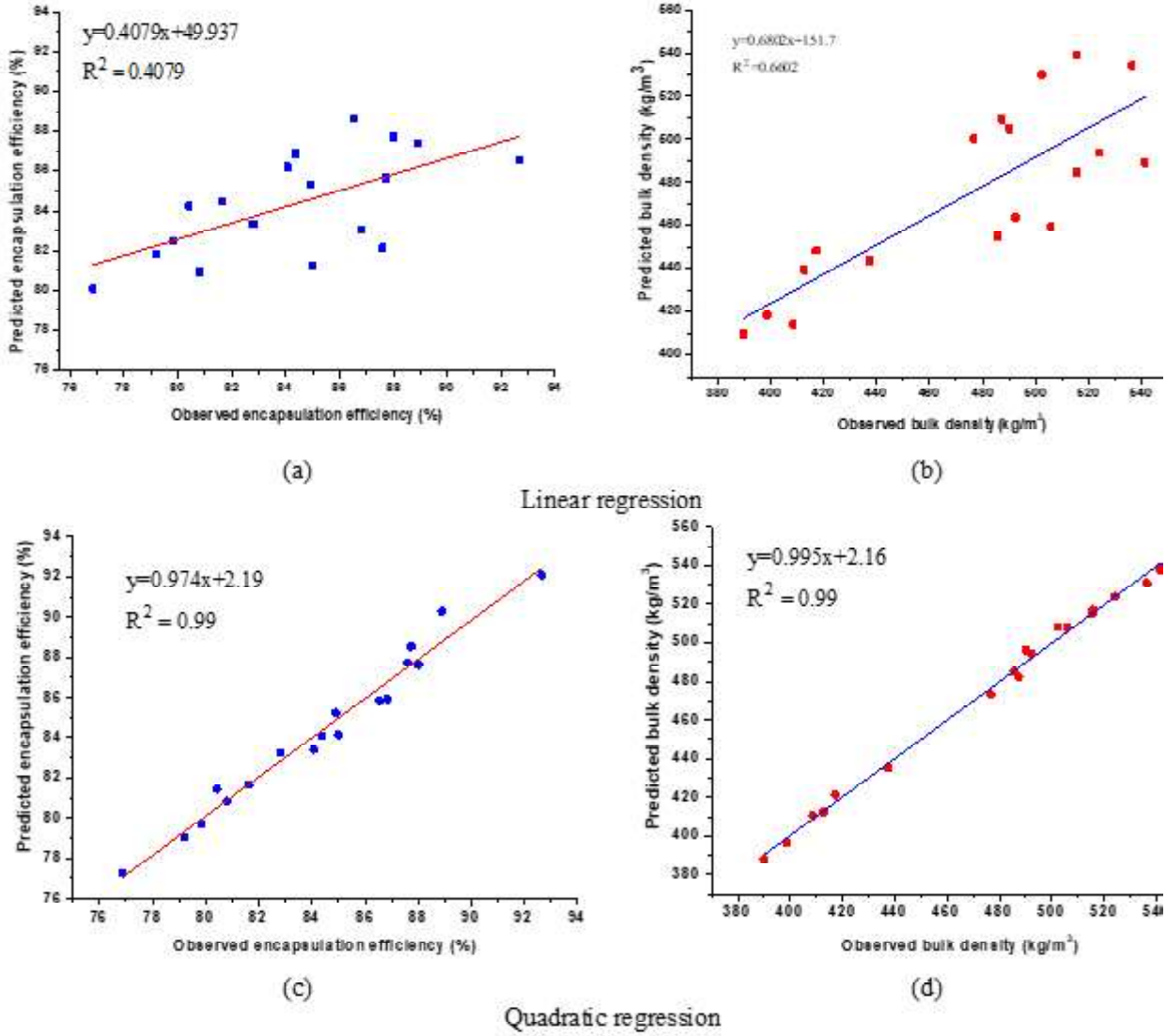

Fig. 2. Relationship between experimental and predicted values of encapsulation efficiency and bulk density using linear (a and b) and quadratic regression (c and d)

Prediction of optimum encapsulation efficiency and bulk density

For the prediction of optimum encapsulation efficiency and bulk density, Equations (11) and (12) were used.

$$Encapsulation\ \ efficiency_{Opt} = \overline{A}_2 + \overline{B}_2 + \overline{C}_2 - 2\mu \quad (11)$$

$$Bulk\ \ density_{Opt} = \overline{A}_1 + \overline{B}_2 + \overline{C}_2 - 2\mu \quad (12)$$

The confidence intervals of encapsulation efficiency and bulk density were calculated using Equations (3) and (4) as ±1.38 and ±8.44, respectively. The experimental values of both responses were within the limits of confidence interval at significance level of 0.05.

Validation

For validation, experiments were repeated thrice at the optimum and random levels and the predicted values from Taguchi design and predictive regression models were compared (Table 4). As the error obtained was less than 20% (acceptable limits), the selection of levels of spray-drying conditions (independent factors) was appropriate.

Conclusions

Spray-drying was found successful for microencapsulation of zinc with encapsulation efficiency of 92% and bulk density of

Table 4. Predicted values and confirmation test results by Taguchi method and regression analysis

Level	Expt.	Taguchi method		Linear regression		Quadratic regression	
		Pred.	Error (%)	Pred.	Error (%)	Pred.	Error (%)
			Encapsulation efficiency				
$A_2B_2C_2$(Optimum)	92.43	92.10	0.36	86.53	6.38	92.09	0.66
$A_1B_1C_1$ (Random)	79.77	79.70	0.09	82.48	3.40	79.70	0.39
			Bulk density				
$A_1B_2C_2$ (Optimum)	539.20	537.97	0.23	489.17	9.28	537.87	0.25
$A_2B_1C_1$ (Random)	474.65	476.07	0.30	500.40	5.43	473.52	0.24

541 kg/m^3. Taguchi orthogonal array technique was used to optimize the spray-drying conditions for microencapsulation of zinc. The optimized conditions for maximum encapsulation efficiency were Hicap-100 as wall material, 20:1 as wall material: zinc ratio and inlet air temperature of 185°C, whereas optimized conditions for maximum bulk density were HiCap-100 as wall material, 10:1 as wall material: zinc ratio and inlet air temperature of 185°C. The influence of wall materials, wall material to zinc ratio and inlet air temperature was evaluated. It was established that Taguchi orthogonal array design is a successful method for the optimization of microencapsulation of zinc and HI-CAP® 100 was found to be a suitable wall material for encapsulation. Microencapsulated zinc can be fortified in various delivery systems such as milk and milk products.

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RESEARCH ARTICLE

Unravelling the relationship between udder morphometric traits and milk production, composition and clinical mastitis in Karan Fries cattle via principal component analysis

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Abstract: The present study was aimed at reduction in dimensionality using principal component analysis of 16 linear udder type traits and to identify those components having strongest relationship with milk production traits and clinical mastitis in Karan Fries cattle. Kaiser statistic for sampling adequacy with a lower limit of 0.50, and Bartlett's sphericity test was used to determine the adequacy of variables for use in factor analysis. Components were chosen based on auto values greater than one and scree test. The relationship of these principal components with milk production traits was analysed using the general linear model and effect of the components on the incidence of clinical mastitis were analysed through binomial logistic regression model. The general mean value of KMO was obtained as 0.695, indicated the existence of true factors. Five principal components were extracted using Kaiser Rule criterion contributing 70.11% of the cumulative variance between the linear udder type traits. The communality ranged from 0.277 (udder balance) to 0.879 (distance between rear teats) for all these 16 different udder type traits. The relation between principal component 1 and 305-day milk yield was positive and significant with a non-significant effect of 305-day milk-fat (305 DF), milk-protein (305 DP) and Solids not fat yield (305 DSNF). The principal component 1 was also found to have a significant effect on incidence of clinical mastitis. Results of PCA suggest that the use of orthogonal synthetic variables principal component one (PC1), two (PC2) and three (PC3) provided a means of reduction in the number of linear udder type traits to be recorded in Karan Fries cattle which could explain the whole udder biometric traits. The PC1 can be used in breeding programmes as a means to explain the mammary system for better milk production with lesser incidence of clinical mastitis in Karan Fries dairy cows.

Keywords: Clinical Mastitis; Karan Fries cattle; Milk production traits; Principal component analysis; Udder type traits

Introduction

Linear type classification is an important aspect of selection of highly productive animals based on their morphologic traits, making it a key component in decision making process in the herds (Posadas et al. 2008). A higher 305-day milk yield and productive life in the herd can be achieved by selecting the animals based on final score of linear type traits along with the conformation traits of mammary system (Kern et al. 2014). Moreover, the unified score card for dairy cows that describes ideal dairy conformation gives significant weightage to udder traits i.e. 40% of the total score (Stamschror, 2000). In terms of milk storage capacity, udder anatomy may be a significant factor in determining milk production (Sabuncuoglu and Coban, 2007). Wilmink (1996) also recommended that udder conformation traits should be considered, over and above milk performance in dairy cattle selection. The relationship between udder characteristics and milk yield can be a key tool for selecting dairy animals (Mingoas et al. 2017). A positive significant genetic and phenotypic correlation between udder type traits and milk yield was reported both in zebu (Tapki and Guzey, 2013; Dubey et al. 2014; Khan and Khan, 2016) as well as in crossbred cows (Waghmore and Siddiqui, 2000; Singh et al. 2010; Patel et al. 2016). In high yielding dairy cows, the most prevalent (90%) and challenging disease is mastitis which imposes enormous economic losses in dairy herds of developing and developed countries causing $35 billion annual economic losses worldwide (Varshney and Mukherjee, 2002; Donovan et al. 2005; Sharma and Sindhu, 2007). Over and above the pathological reasons, udder biometric traits of dairy cow add to the occurrence of mastitis (Chrystal et al. 2001; Amin et al. 2002).

Karan Fries, a synthetic strain of cattle (Tharparkar X Holstein Friesian), was evolved at National Dairy Research Institute, Karnal

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during 1982. Karan Fries cattle were evolved to have high milk yield and to maintain heat tolerance, disease resistance characters of indigenous cattle (Gurnani et al. 1986).

Principal component analysis (PCA), a statistical procedure, transforms a set of possibly correlated variables into a set of linearly uncorrelated variables (principal components), which retain most of the original variability (Hotelling, 1933; Lukibisi et al. 2008; Hair, 2009). Principal component analysis (PCA) has been used as a tool to evaluate the body morphometric traits of an animal (Salako, 2006; Sadak et al. 2006). Thus, morphological classification by means of PCA will support selection for multiple economic traits and also improve the management of animals (Yunusa et al. 2013).

The literature on inclusive study of all the vital udder conformation traits of Karan Fries cattle along with their dimension reduction is scarce. Furthermore, scanty reports are available to demonstrate associations between various udder biometric traits and the occurrence of mastitis in crossbred dairy cattle (Singh et al. 2014; Danish et al. 2018). Hence, present investigation was undertaken to study the different udder biometric traits, relationships among the traits, develop un-observable components (latent) to define which of these traits best represent udder conformation in Karan fries cows and to explore their relation with milk production, composition and clinical mastitis.

Materials and methods

Animals

The study was conducted on 254 lactating Karan Fries (Bos taurus X Bos indicus) cows maintained at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India during 2016-2018.

The farm is situated in the north eastern semi-arid areas of Haryana (between 30° and 31°N and 76°50' to 77°30'E), having mean maximum temperature ranges from 37°C to 47°C and minimum temperature ranges from 3°C to 25°C. The farm experiences more than 800 mm of annual rainfall. Animals under this study were maintained in a loose housing system inside the farm. Paddock for the animals was large, open and brick paved with herring bone system containing drainage in between covered and open space having adequate slope for better drainage. The animals were machine milked, three times a day (morning 4 a.m., 12 noon and evening 4 p.m.) followed by the recording of milk yield. The average per day milk production of Karan Fries cows was 13.07 kg. Limited concentrates and ad libitum green fodders were offered to meet the nutrient requirement of the lactating animals. The feeding management practices and feed ingredients (ICAR 2013) were similar for all lactating animals in the herd.

Udder and teat morphometric traits

The data on nine udder morphometric traits and seven teat morphometric traits were recorded. The animals were summoned one hour before the routine milking for recording of the traits. The traits included udder morphometric traits such as rear udder height (RUH), rear udder width (RUW), udder width (UW), fore udder attachment (FUA), udder circumference (UC), udder balance (UB), central ligament (CL), udder depth (UD), udder length (UL) and teat morphometric traits such as fore and rear teat length (FTL and RTL), teat diameter (TD), distance between fore and rear teat (DFR), distance between right and left teat (DLR), shortest distance from fore teat end to floor (SDF), shortest distance from rear teat end to floor (SDR) (Table 1). Udder and teat measurements were done by using meter long measuring tape whereas, teat diameter was measured using vernier caliper. The udder and teat measurements of the animals under the study were carried out during December, 2016 to June, 2018. Norms relating to the ethical treatment of animals throughout the whole operation were strictly followed. The udder measurements were recorded by a qualified veterinarian. All the measurements were recorded once in straight animal standing on a level ground and by the same person to avoid between-recorder effects.

The data on 305-days milk yield, 305-days milk-fat yield, 305-days milk-protein yield and 305-days solids not fat yield of each animal were collected from Livestock Record Unit of Institute.

Mastitis data

Incidences of clinical mastitis among the lactating Karan Fries cattle were recorded from treatment register of the Animal Health Complex of Livestock Research Centre, ICAR-NDRI, Karnal. Animals were regularly screened by rapid California Mastitis Test (CMT), and results were recorded. The data on clinical mastitis was of a binomial distribution since the scoring of clinical mastitis was dichotomous (either healthy or infected). If a cow got infected with mastitis at least once in the studied lactation, a response of "1" was attributed to the record. A healthy record with a response of "0" was created for a cow that was never treated for the disease.

To improve consistency of the structure of database and subsequent analysis, the records of cows without pedigree, dates of birth, end of lactation and without production records were removed from the data. For cows with more than one classification over the productive life, only the first was considered. The udder and teat morphometric traits were adjusted for the significant effect of parity, season and stage of lactation. Further analysis was done with the data adjusted for these non-genetic factors.

The principal component analysis incorporated 16 linear type traits using the correlation matrix between the traits to make sure that all traits are standardized in the analysis (Vucasinovick et al. 1997). The matrix of partial correlations, Kaiser statistic for sampling adequacy (MSA) with a lower limit of 0.50, and Bartlett's sphericity test were used to determine the degree of interrelations

between variables and adequacy for use in the principal component analysis. Bartlett test (Bartlett, 1950) was performed to check whether the data set of 254 animals with 16 traits could be factored or not. Maxwell (1959) suggested that the test should be used prior to the application of principal component analysis. The following formula was used to compute Bartlett's test of sphericity:

$\chi^2 = [(n-1)1/6(2p + 5) \log_e$ œ#R œ#]

where, n, sample size; p, number of variables; œ#Rœ#, determinant of correlation matrix. It follows χ^2 distribution with [p (p–1)/2] degree of freedom.

Components were selected on the basis of values greater than one and scree test (graph) (Cattell, 1966). The point where the graph begins to turn into horizontal is considered indicative of the maximum number of components to be extracted (Hair, 2009). Varimax rotation was used for rotation of principal components through the transformation of the components to almost a simple structure. Components were rotated via varimax rotation to assist interpretation because of the reduction of ambiguities in non-rotated solutions (Hair, 2009). The value of 0.50 was used to conclude a significant correlation between traits and components.

The statistical analyses were performed by means of the SPSS (2001) statistical package by the maximum likelihood method to decrease the dimensionality and lessen the information in a group of p original variables $Z_1, Z_2 ... Z_p$, to a new group of variables $Y_1(F_1), Y_2(F_2) ... Y_p(F_p)$. In this analysis few of the first components hold greater part of the variability of the original variables (Cruz and Regazzi, 1997).

Relationship of these principal components with milk production traits were analyzed using general linear model in the GLM procedure of SPSS (2001):

Effect of the principal components on incidence of clinical mastitis were analyzed through binomial logistic regression model with the SPSS software statistical package (Version 23). The following dichotomous logistic model was used:

$$\text{logit}(p) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_{16} X_{16}$$

Where,

logit(p)βo == Log[p/ (1-p)] = Odds of occurring clinical mastitis in the animalsInterception at y-axis
β1…16 = Partial regression coefficients
X1...16 = Predictor variables i.e., udder and teat type traits

The multivariate analysis for binomial logistic regression considered incidence of clinical mastitis as a categorical response variable, expressed on a scale of 1 and 0. The model included continuous traits (linear udder and teat morphometric traits). The data were analyzed by logistic regression procedure as well as odds ratios (ORs) with a 95% confidence interval. The reference category was set according to preference. Differences were interpreted as significant if P d" 0.05.

Results and Discussion

The means of linear udder type trait measurements varied between 1.00 cm for udder balance and 142.85 cm for udder circumference (Table 1). Whereas, the means of teat type trait measurements varied between 2.23 cm for teat diameter and 47.01 cm for shortest distance from front teat end to floor (Table 1). The descriptive statistics for all the udder and teat conformation traits are given in Table 1. The means of udder and teat conformation traits showed that Karan Fries cows were having intermediate udder and teat measurements.

The phenotypic correlations among different udder and teat conformation traits are presented in Table 2. The magnitude of correlation coefficient ranges between -0.491 (SDR, shortest distance from rear teat end to the floor and UW, udder width) to 0.879 (SDR, shortest distance from rear teat end to the floor and SDF, Shortest distance from fore teat end to the floor). Among the total 78 correlations (in all combinations), 44 were significant, of which 21 were positive correlations (Table 2).

Rear udder height had significant negative correlations with udder length, udder width, udder circumference and distance between fore and rear teats. Udder depth had significant negative correlations with udder length, udder width, udder circumference, central ligament, distance between front and rear teat, distance between rear teats and significant positive correlations with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. Udder length had significant positive correlations with udder width, udder circumference,

Table1. Descriptive statistics of udder biometric traits in Karan Fries cows

Trait	Mean
1.Udder type traits	
Rear udder height (cm)	23.47±0.33
Udder depth (cm)	53.73±0.36
Udder balance (cm)	1.00±0.35
Udder length (cm)	60.20±0.46
Udder width (cm)	71.06±0.53
Udder circumference (cm)	142.85±1.59
Central ligament (cm)	3.12±0.09
2.Teat type traits	
Fore teat length (cm)	5.30±0.09
Distance between front and rear teats (cm)	6.70±0.27
Distance between rear teats (cm)	8.84±0.31
Shortest distance from front teat end to floor (cm)	47.01±0.35
Shortest distance from rear teat end to floor (cm)	46.96±0.35
Teat diameter (cm)	2.23±0.02

distance between front and rear teat, distance between rear teats, teat diameter, and significant negative correlations with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. Udder width had significant positive correlations with udder circumference, fore teat length, distance between front and rear teats, distance between rear teats, teat diameter and significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. Udder circumference had significant positive correlations with distance between front and rear teat and distance between rear teats; significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. A significant positive correlation was observed between central ligament and fore teat length and significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. Fore teat length had significant positive correlations with distance between front and rear teat, distance between rear teats, teat diameter and significant negative correlation with shortest distance from front teat end to floor. Distance between fore and rear teat had significant positive correlations with the distance between rear teats; significant negative correlation with the shortest distance from fore teat end to the floor and the shortest distance from rear teat end to the floor. Distance between rear teats had a significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to the floor. Shortest distance from fore teat end to the floor also had significant positive correlations with the shortest distance from rear teat end to the floor.

The PCA analysis was applied to 16 udder and teat conformation traits in Karan Fries cows. The general mean value of KMO test gave measure of sampling adequacy (MSA) as 0.695, a level pointing to the existence of significant correlations between linear type traits and the existence of true factors. The estimate of KMO also indicates suitability of the data for PCA analysis. The KMO analysis excluded 3 traits (fore udder attachment, rear udder width, rear teat length) with KMO lesser than 0.5. KMO-MSA score greater than 0.5 is essential for suitable PCA analysis to continue (Kaiser, 1974; Hair, 2009). The estimate of KMO-MSA revealed proportion of the variance in different udder biometric traits caused by underlying components (Kaiser, 1958). Bartlett's test of sphericity was used to test overall significance of the correlation matrix for the udder type traits. Chi-square value for the test was estimated as 1222.095, which was highly significant ($P<0.001$). As the correlation matrix was found to be non identity matrix, it signified the validity of the PCA analysis of udder biometric traits data.

Five principal components were extracted out of the total 13 principal components by means of Kaiser Rule criterion (Johnson and Wichern, 1982) which finds out the number of components having eigen values greater than 1 (Table 3). Principal components were also chosen based on scree test (graph) (Cattell, 1966). In the scree plot, the point where the graph begins to become horizontal is considered indicative of the maximum number of components to be extracted (Hair, 2009), components having eigen values up to the point "bent of elbow" are usually considered (Fig. 1). The scree plot also indicated extraction of five components (Fig. 1). The identified five components could explain 70.11% of the cumulative variance between the linear udder type traits (Table 3). PCA determines the variability of individual traits and how these traits contribute towards the total morpho structural variance of animal (Mavule et al. 2013). The first principal

Table 2. Phenotypic Correlation among 13 different udder biometric traits in Karan Fries cows

	RUH	UD	UB	UL	UW	UC	CL	FTL	DFR	DRT	SDF	SDR	TD
RUH	1												
UD	.047	1											
UB	-.007	.089	1										
UL	-.165**	-.286**	-.010	1									
UW	-.192**	-.398**	.018	.563**	1								
UC	-.341**	-.160*	.015	.273**	.300**	1							
CL	-.002	-.195**	-.099	-.041	.081	.114	1						
FTL	-.030	-.022	.040	.007	.164**	-.001	.163**	1					
DFR	-.157*	-.155*	.065	.206**	.264**	.167**	-.016	.150*	1				
DRT	-.053	-.201**	.019	.275**	.411**	.141*	.045	.143*	.749**	1			
SDF	.001	.687**	.026	-.298**	-.369**	-.187**	-.317**	-.194**	-.179**	-.192**	1		
SDR	.059	.710**	.039	-.434**	-.491**	-.225**	-.242**	-.093	-.224**	-.307**	.879**	1	
TD	-.020	.003	.144*	.155*	.194**	.041	-.070	.355**	.070	.107	-.105	-.060	1

The lower triangle shows the phenotypic correlation among the different udder type traits with superscripts showing their respective level of significance i.e. ** means $p<0.01$ and * means $p<0.05$ respectively. FTL, Fore teat length; SDF, Shortest distance from front teat end to floor; RUH, Rear udder height;SDR, Shortest distance from rear teat end to floor; DFR, Distance between front and rear teat; TD,Teat diameter; DRT, Distance between rear teats; UD, Udder depth; CL, Central ligament; UW,Udder width; UC, Udder circumference; UB, Udder balance; UL, Udder length

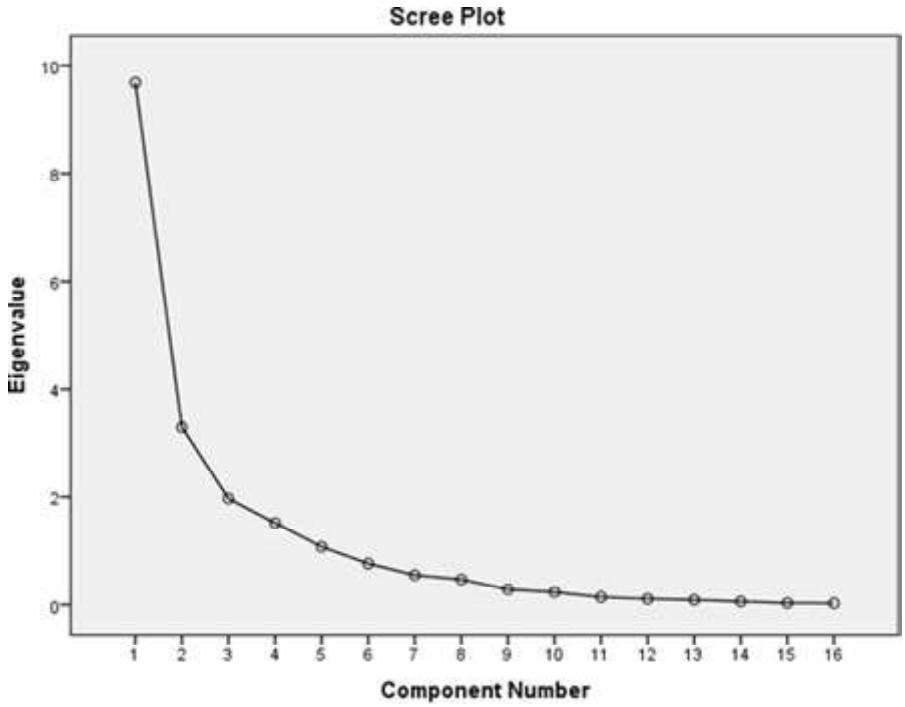

Fig. 1. Scree plot showing component number with eigen values

Table 3. Total variance explained by different components in Karan Fries cows

Component	Initial eigen values			Extraction sums of squared loadings			Rotation sums of Squared loadings
	Total	% of variance	Cumulative%	Total	% of variance	Cumulative%	Total
1	3.702	28.475	28.475	3.702	28.475	28.475	3.053
2	1.708	13.140	41.615	1.708	13.140	41.615	1.837
3	1.368	10.521	52.135	1.368	10.521	52.135	1.539
4	1.235	9.499	61.635	1.235	9.499	61.635	1.445
5	1.102	8.475	70.110	1.102	8.475	70.110	1.241
6	.937	7.208	77.318				
7	.723	5.562	82.880				
8	.626	4.818	87.698				
9	.542	4.167	91.864				
10	.438	3.370	95.234				
11	.313	2.410	97.645				
12	.216	1.664	99.309				
13	.090	.691	100.000				

n, number of Karan Fries cows

component (PC1) accounted for 28.475% of the variation (Table 3). It was represented by significant positive high loading of udder depth, shortest distance from front teat end to floor and shortest distance from rear teat end to floor (Table 5). The first principal component seemed to be explaining the maximum of udder and teat conformation traits in Karan Fries cows.

The second principal component (PC2) explained 13.14% of total variance (Table 3) with a high loading of distance between front and rear teats and distance between rear teats (Table 5). The third principal component (PC3) explained 10.521% of the variance (Table 3) and showed high component loading for rear udder height (Table 5). The fourth principal component (PC4) accounted for 9.499% of total variability (Table 3) with comparatively higher loading for fore teat length and teat diameter (Table 5). The fifth principal component (PC5) accounted for 8.475% of total variability (Table 3) with comparatively higher loading for central ligament (Table 5).

The PC1 gave different weights with positive sign to all the biometric traits except to udder length, udder width, fore teat length, distance between front and rear teat, distance between rear teats and teat diameter. The PC2 gave negative coefficient value for udder depth, udder balance, udder circumference,

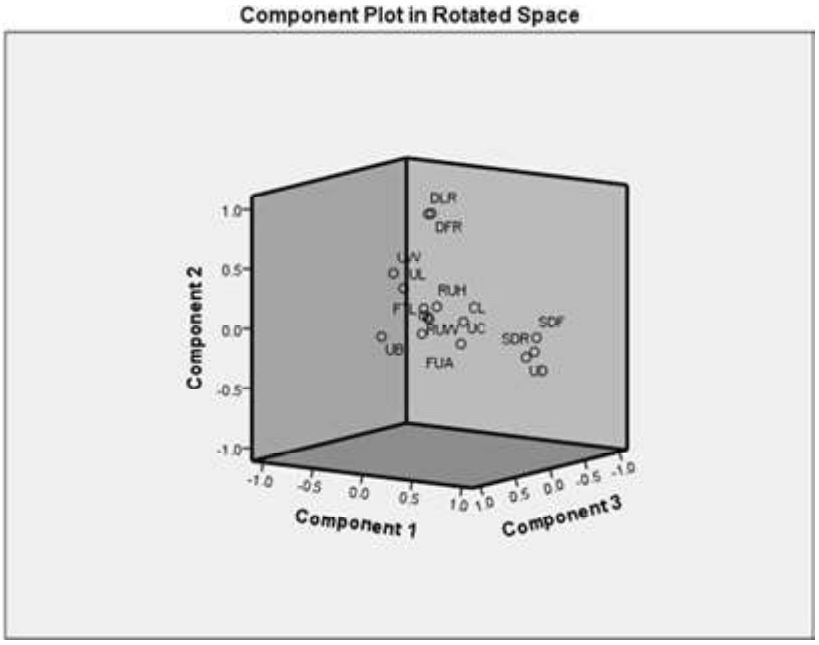

Fig. 2. Component plot in rotated space.

FUA, Fore udder attachment; FTL, Fore teat length; SDF, Shortest distance from front teat end to floor; RUH, Rear udder height; RUW, Rear udder width; RTL, Rear teat length; SDR, Shortest distance from rear teat end to floor; DFR, Distance between front and rear teat; TD, Teat diameter; DRT, Distance between rear teats; UD, Udder depth; CL, Central ligament; UW, Udder width; UC, Udder circumference; UB, Udder balance; UL, Udder length.

Table 4. Communalities and unique factor of various udder biometric traits in Karan Fries cows

Trait	Communalities	UniqueFactor
Rear udder height	0.666	0.334
Udder depth	0.719	0.281
Udder balance	0.277	0.723
Udder length	0.627	0.373
Udder width	0.621	0.379
Udder circumference	0.622	0.378
Central ligament	0.680	0.32
Fore teat length	0.742	0.258
Distance between front and rear teats	0.860	0.14
Distance between rear teats	0.879	0.121
Shortest distance from front teat end to floor	0.846	0.154
Shortest distance from rear teat end to floor	0.876	0.124
Teat diameter	0.700	0.3

shortest distance from front teat end to floor, shortest distance from rear teat end to floor and teat diameter. The PC3 gave negative coefficient value for udder depth, udder balance, udder circumference, central ligament, fore teat length and shortest distance from rear teat end to floor. The PC4 gave negative coefficient value for central ligament, shortest distance from front teat end to floor and shortest distance from rear teat end to floor. The PC5 gave negative coefficient value for rear udder height and fore teat length (Table 5).

The communality ranged from 0.277 (udder balance) to 0.879 (distance between rear teats) and unique factors ranged from 0.121 (distance between rear teats) to 0.723 (udder balance) for all these 13 different udder type traits (Table 4). The lower estimates of communality of udder balance inferring that this trait is less effective in explaining variation shared with the other traits. Distance between rear teats and shortest distance from rear teat end to floor had the highest communalities, confirming its equilibrium position between udder and teat conformation traits.

Varimax rotation was applied to find the squared correlations between variables and components as it maximizes sum of the variances of the squared loadings (Fernandez, 2002). Coefficients of the PCA of rotated component matrix of the five extracted principal components are presented in Table 5. Correlation between a principal component and the original traits correspond to the weight of each trait. The trait with a higher weight represents the corresponding principal component more efficiently. Each principal component can be interpreted physiologically or biologically based on the sign and magnitude of the component weight of each trait (Vukasinovic et al. 1997).

Among all the PCs, the component weights varied from -0.763 to 0.918 for udder circumference and shortest distance from rear teat end to floor, respectively (Table 5). The higher significant weights in the first component were for udder depth, shortest distance from front teat end to floor and shortest distance from rear teat end to floor. All these traits were related to the mammary system so this component was called mammary system. The component weights for PC1 varied from -0.568 to 0.918 for the udder width and shortest distance from rear teat end to floor, respectively (Table 5).

The component weights for PC2 varied from -0.138 to 0.916 for the shortest distance from rear teat end to floor and distance between front and rear teat, respectively (Table 5). This principal component was called teat morphometry system as the higher significant component weights in second component were for the distance between front and rear teats and distance between rear teats which are a part of teat morphometric traits (Table 5). The component weights varied from -0.763 (Udder circumference) to 0.810 (Rear udder height) for the third component (Table 5). The higher significant component weight in the third principal component was for rear udder height related to the rear udder (Table 5).

The component weights varied from -0.116 (shortest distance from front teat end to floor) to 0.807 (teat diameter) for principal component 4 (Table 5). The higher significant component weights in principal component 4 were for fore teat length and teat diameter related to teat morphometric traits (Table 5).

For principal component 5, the component weights varied from -0.363 (fore teat length) to 0.785 (central ligament) (Table 5). The higher significant component weight in principal component 5 was for central ligament which is related to the rear udder (Table 5).

In general, three well-defined factors were formed (Fig. 2). Principal component 1, 2 and 3 had 28.475%, 13.140% and 10.521% of the

Table 5. Estimates of component weights for udder biometric traits using varimax rotation

Trait	Principal component				
	1	2	3	4	5
Rear udder height	0.067	0.050	0.810	0.006	-0.042
Udder depth	0.832	-0.063	-0.008	0.100	0.114
Udder balance	0.052	-0.001	-0.038	0.364	0.374
Udder length	-0.531	0.161	0.369	0.062	0.423
Udder width	-0.568	0.284	0.368	0.199	0.208
Udder circumference	0.181	-0.061	-0.763	0.013	0.057
Central ligament	0.229	0.023	-0.089	-0.053	0.785
Fore teat length	-0.029	0.156	-0.002	0.765	-0.363
Distance between front and rear teat	-0.086	0.916	0.104	0.046	0.004
Distance between rear teats	-0.195	0.913	0.045	0.061	0.029
Shortest distance from front teat end to floor	0.877	-0.051	0.020	-0.116	0.245
Shortest distance from rear teat end to floor	0.918	-0.138	-0.061	-0.016	0.102
Teat diameter	-0.081	-0.002	0.039	0.807	0.201

Table 6. Extracted component and their respective descriptions obtained from linear udder type traits in Sahiwal cows

Factor	Name	Characterization of the component
1	Mammary System	Deeper udder with optimum distance from front and rearTeat end to floor
2	Teat morphometricTraits	Optimum distance between front-rear teat and rear teats
3	Rear udder	Optimum rear udder height

Table 7. Linear regression coefficients (b) of components and their respective standard error on milk production traits

Phenotypic measure	Linear regression coefficients (b)				
	Component 1	Component 2	Component 3	Component 4	Component 5
305 DMY	1.017±0.010*	-0.001±0.002	0.003±0.001	-0.001±0.002	0.003±0.002
305 DF	-2.646±1.041	1.335±0.813	1.155±0.149	1.090±0.351	-1.737±0.948
305 DP	1.939±0.986	0.882±0.772	-1.179±0.097	-1.513±0.035	-1.074±0.900
305 DSNF	2.941±0.862	-1.972±0.139	-1.764±0.620	1.365±0.528	1.436±0.330

305 DMY = 305-day milk yield, 305 DF = 305-day milk-fat yield, 305 DP = 305-day milk protein yield, 305 DSNF = 305-day Solids not fat yield

common variance respectively, explaining 52.135% of the total variance between the linear udder morphometric traits.

If the traits of principal component 1 are included in the selection programme (Table 6), cows are expected to have a deep and voluminous udder, with optimal distance from front and rear teat end to floor which is vital for superior milk production as well as better udder health. Animals with deeper udder will have additional area for mammary tissue within the udder. As the mammary tissues are the primary source to synthesize milk, thus the animals with deeper udders are expected to produce higher quantity of milk.

When principal component 2 is used for selection (Table 6), the cows should have optimum distance between front and rear teats as well as optimum distance between rear teats. The teats should not be too close or too far from each other as they create in compatibilities for machine milking and handling. Including the traits of principal component 3 in selection decisions (Table 6), cows are expected to have an optimum rear udder height. This will prevent broken/very pendulous suspension of udder and in turn less susceptible to udder damage.

The relation between principal component 1 and 305-day milk yield was positive and significant with a non-significant effect of 305-day milk-fat (305 DF), milk-protein (305 DP) and Solids not fat yield (305 DSNF) (Table 7). The principal component 1 was also found to have a significant effect on incidence of clinical mastitis (Table 8). Cows with deep and voluminous udder, with optimum distance from front and rear teat end to floor are associated with higher milk production and lesser incidence of clinical mastitis. It means that selection for this combination of udder and teat type traits could bring associated increase in 305-day milk yield as well as lesser incidence of clinical mastitis.

The present investigation was focused to study the different udder biometric traits, relationships among the traits, develop unobservable components (latent) to define which of these traits best represent udder conformation in Karan fries cows and to explore their relation with milk production, composition and clinical mastitis. The means of rear udder height, udder circumference, udder depth, udder balance and central ligament of Karan Fries cattle in this study were in accordance with Dubey et al. 2014 and Deng et al. (2012). The mean of udder width and udder length were in agreement with Patel et al. (2016). Among the teat type traits studied, the means of fore teat length, teat diameter, distance between front-rear teats, distance between rear teats, shortest distance from front teat end to floor and shortest distance from rear teat end to floor are in concurrence with Singh et al. (2014) who studied the same in Holstein Friesian × Sahiwal crossbred dairy cows.

Rear udder height had significant negative correlations with udder length, udder width, udder circumference and distance between fore-rear teats. These findings are in agreement with Dubey et al. 2014 and Mingoas et al. (2017) in zebu cows. The positive genetic correlation between udder depth, udder cleft (central ligament) and front teat distance reported in the present study were in accordance with Sorensen et al. (2000). Khan and Khan (2016) reported positive genetic correlation between udder depth, length, width and circumference which is in agreement with the present study. Udder balance had significant positive correlations with teat diameter which is also supported by Dubey et al. 2014. Udder length had significant positive correlations with udder width, udder circumference, distance between front and rear teat, distance between rear teats, teat diameter, and significant negative correlations with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor which is in accordance with Sezenler et al. (2016) and Mingoas et al. (2017). Udder width had significant positive correlations with udder circumference, fore teat length, distance between front and rear teats, distance between rear teats, teat diameter and significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. The current findings are supported by Ahlawat et al. (2008); Singhai et al. (2013) and Sezenler et al. (2016). A significant positive correlation was observed between central ligament and fore teat length which is in concurrence with Sorensen et al. (2000). Central ligament also had significant negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to floor. These findings are supported by Nakov et al. (2014). Distance between fore and rear teat had significant positive correlations with the distance between rear teats; significant negative correlation with the shortest distance from fore teat end to the floor and the shortest distance from rear teat end to the floor. Distance between rear teats had a significant

Table 8. Effect of principal components on incidence of clinical mastitis in Karan Fries cattle (Healthy animals=64.5% and Mastitic animals= 35.5%)

S.No.	Components	Wald $\chi 2$	Exp(B)	95% C.I.	
				Lower	Upper
1	Principal Component 1	6.088*	1.018	0.994	1.042
2	Principal Component 2	3.009	1.015	0.968	1.064
3	Principal Component 3	0.013	0.994	0.903	1.095
4	Principal Component 4	2.114	0.900	0.828	0.979
5	Principal Component 5	0.364	0.935	0.867	1.009

negative correlation with shortest distance from fore teat end to the floor and shortest distance from rear teat end to the floor. Shortest distance from fore teat end to the floor also had significant positive correlations with the shortest distance from rear teat end to the floor. All these findings are in concordance with Nakov et al. (2014).

KMO-MSA score greater than 0.5 is essential for suitable PCA analysis to continue (Kaiser, 1974; Hair, 2009). The general mean value of KMO test gave measure of sampling adequacy (MSA) as 0.695, which revealed that proportion of the variance in different udder biometric traits caused by underlying components (Kaiser, 1958). The present finding is supported by Corrales et al. (2011) and Kern et al. (2014) who accounted sampling adequacy of 0.75 and 0.79 for linear type traits in Holstein Cattle, respectively.

Five principal components were extracted out of the total 13 principal components by means of Kaiser Rule criterion (Johnson and Wichern, 1982) which find out the number of components having eigen values greater than 1. In a study of linear type traits in Brazilian Holstein cattle, four factors were extracted using factor analysis by (Kern et al. 2014). In Holstein cows of Colombia (Corrales et al. 2011) seven factors were identified with auto values greater than one. Previous studies using principal components established higher number of factors to be extracted for linear type traits. The different number of extracted factors in those studies might be due to differences in the statistical methods, as well as populations.

A relation between principal component 1 and 305-day milk yield was positive and significant with a non-significant effect of 305-day milk-fat (305 DF), milk-protein (305 DP) and Solids not fat yield (305 DSNF). The principal component 1 was also found to have a significant effect on incidence of clinical mastitis. The genetic correlations showed that higher yielding cows have relatively deeper udders in Holstein dairy cattle (Bohlouli et al. 2015). Positive and highly significant correlations were observed between udder depth and 305-day milk yield in zebu cows (Mingoas et al. 2017) as well as in Holstein cows (Zwertvaegher et al. 2012; Corrales et al. 2011). Deeper udders showed the highest somatic cell count in Holstein cows (Nemcova et al. 2007). Whereas, in Holstein Friesian × Sahiwal crossbred dairy cows, deeper udder showed higher susceptibility to mastitis (Singh et al. 2014). The deeper udders may hamper easy movement of the cow and raise the risks of udder and teat lesions (Mein et al. 2004). The deeper udders also has higher predisposition to become soiled and, thus, being contaminated with environmental pathogens (Lopez-Benavides et al. 2005). Consequently, deeper udders were observed to have more risk of developing mastitis. Bhutto et al. (2010) and Bardakcioglu et al. (2011) accounted elevated predisposition to intra mammary infection and higher milk SCC with the teats placed closer to the floor. An increasing proportion of teat lesions were observed alongside decreasing teat tip to floor distance which results in higher occurrence of mastitis in Holstein Friesian (Breen et al. 2009) and Holstein Friesian × Sahiwal crossbred dairy cows (Singh et al. 2014).

Furthermore, lower udders with the teats placed closer to floor had additional liner slips and required longer milking time. The liner slips give rise to a sudden and rapid loss of vacuum that might move the pathogens located at the teat opening or inside the streak canal into the teat cistern and bring about new infections (Mein et al. 2004). The longer milking time due to lesser teat end to floor distance causes roughness of the teat end which consecutively results in higher probability of new intra mammary infections (Neijenhuis et al. 2000, 2001).

Principal component 1 including the mammary system traits may be more important for milk production compared to other factors such as those of teat morphometric traits as well as rear udder.

Conclusion

A positive and significant correlation was observed among different linear udder and teat type traits suggesting high predictability among these traits, and also making them acquiescent for analysis through PCA. The principal component analysis has resulted in the reduction in dimensionality of the group of linear type traits studied, forming three orthogonal synthetic variables which could be used in explaining the whole udder and teat conformation in Karan Fries cattle. The communalities estimate specified the fact that udder balance could not put in effectively to explain udder conformation, at the same time the remaining traits contribute effectively, thus these traits could be considered to explain the udder conformation of the Karan Fries cows. The Component 1, that included udder depth, shortest distance from front teat end to floor and shortest distance from rear teat end to floor, had a positive association with 305-day milk yield and incidence of clinical mastitis, signifying that this component can be used in breeding programme for phenotypic selection of females to get better milk production together with lesser incidence of clinical mastitis.

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RESEARCH ARTICLE

Genetic and non-genetic factors affecting calf survivability in Gir crossbreds

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Abstract: Data on survivability traits of Gir crossbred calves, maintained at RCDP on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra was collected for a period of 15 years (2005-2019) to study effect of genetic and non-genetic factors on survivability traits of calf. Traits studied were survivability at day 0-3, 4-15, 16-30, 3-90, 91-180 and 181-365 days. To determine the effects of genetic and non-genetic factors on survivability traits, least squares analysis of variance (Harvey, 1990) was applied, considering the fixed effects like genetic group, season and period of birth, parity of dam and birth weight of calves. The least square means of survivability per cent of Gir crossbred calves at 0-3, 4-15, 16-30, 31-90, 91-180 and 181-365 days were 96.17±0.80, 86.17±2.12, 89.23±2.03, 80.39±2.89, 83.41±3.02 and 95.61±1.79 per cent, respectively. The significant (P<0.05) effect of period of birth was found on calf survivability trait at different ages viz. days 31-90 and days 181-365. Parity of dam had non-significant effect on calf survivability traits of the Gir crossbred calves. Significant (P<0.05) effect of season of birth on calf survivability was observed only at 0-3 days age survivability trait. Significant (P<0.05) effect of sex of calves on survivability traits was recorded at 0-3, 4-15, 16-30 and 31-90 days. In the present study, birth weight of calf had significant (P<0.01) effect on calf survivability at 0-3 days after birth. The non-significant effect was reported among the survivability patterns of calves with different genetic groups.

Keywords: Calf survivability, Genetic factors, Non-genetic factors

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Introduction

Calves are the future progeny of livestock sector. Future of dairy herd solely depends upon successful rearing of new born calves and heifers. Healthy calves are not only required for sustenance of dairy herd but also necessary for preserving authenticated germplasm. In a dairy farm, a high survival rate aids in increasing selection pressure, which is one of the most important elements determining genetic gain and profitable returns (Sreedhar and Sreenivas, 2015). In rural dairy farms, the growth performance of calves revealed poor health condition, which indicates that lack of awareness among farmers on scientific management of calves (Tiwari et al. 2007). Dairy cattle mortality is important not only with regards to financial losses, but also in terms of animal health and welfare. For genetic improvement, efforts need to be increasing the intensity of selection, which becomes possible by increasing the herd size and the number of offspring reaching the milking herd in the next generation.

Although calf and heifer mortality reported to be relatively low, but they arise ethical issues and their economic impact on cattle breeding is substantial. Higher young stock or heifer mortality results in more replacement and veterinarian costs and reduced possibility for selection and genetic gain. As replacement costs increase with age, losses at higher ages up to first calving are even more economically important than early losses. Concerns related to animal health and welfare are increasing day by day, and hence these aspects should be considered in future animal breeding strategies. Thus, necessary measures can be taken by improving management practices and by studying the genetic factors deciding the ability of calf to survive. It is thus essential to make, calf survival traits as integral part in the definition of an overall breeding objective in cattle. In dairy cattle breeding, genetic improvement of survival traits and welfare of newborn calves have been not paid attention for a long time. The reason for this is that calves have traditionally been viewed as a cost rather than an investment by farmers. Time, labor, and money spent on raising healthy calves do not yield immediate results, but are only quantifiable over time. Calf health, welfare, and survival data are limited and not frequently collected, with the exception of features that can be linked to the dam of the calf, such as calving ease and stillbirth, which are included in routine

collection schemes. This results in newborn calves that have little information available at the time of birth and then disappear for two years only to come back after first calving. However, genetic studies regarding the losses from birth to calving are not enough and little information is available about survival traits of calves. Looking to this present study was planned to study the effect of genetic and non-genetic factors on calf survivability.

Materials and Methods

Data on mortality of Gir crossbred calves, maintained at the Research cum Development Project on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri were collected for a period of 15 years (2005-2019). Data were classified as period of birth, parity of dam, season of birth, sex of calf and birth weight group of calf and genetic group of calves. The year of birth was classified as 3 periods of birth, each period comprising of 5 years. Each year was divided into 3 seasons of birth viz. rainy (June to September), winter (October to January) and summer (February to May). Parity of dam was classified as 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and above. Sex of calf was grouped as male and female calves. The birth weight of calf has been grouped into 5 classes as d" 15 kg, > 15 kg tod" 20 kg, > 20 kg to d" 25 kg, > 25 kg to d" 30 kg and > 30 kg. On the basis of exotic inheritance of calves, the data was distributed into 5 genetic groups as G_1 (50% HF + 50% Gir), G_2 (50% HF + 25% Jersey + 25% Gir), G_3 (50% HF + 12.5% Jersey + 37.5% Gir), G_4 (50% Jersey + 50% Gir) and G_5 (75% HF + 25% Gir). The traits considered for the study were survivability from 0 to 3, 4 to 15, 16 to 30, 31 to 90, 91 to 180 and 181 to 365 days. The mean, standard error and coefficient of variation of all traits were estimated by using standard statistical procedures (Snedecor and Cochran, 1994). The data of all traits were normalized using mean and standard deviation of traits. To determine the effects of genetic and non-genetic factors on survivability traits, least squares analysis of variance (Harvey, 1990) was applied considering the fixed effects like genetic groups of calves, season and period of birth, parity of dam and birth weight of calves. The random effect of sire was also included in the model. The following model was used:

$$Yijklmno = \mu + Si + GGj + POBk + SOBl + PYm + BWn + eijklmno$$

Where,

$Y_{ijklmno}$ = o^{th} survivability observation of the calf which is progeny of i^{th} sire, having j^{th} genetic group, born in k^{th} period and l^{th} season, having m^{th} parity of dam, n^{th} birth weight of the calf.

μ = overall mean

S_i = random effect of i^{th} sire

GG_j = effect of j^{th} genetic group (j = 1, 2, 3, 4, 5)

POB_k = effect of k^{th} period of birth (k = 1, 2, 3)

SOB_l = effect of l^{th} season of birth (l = 1, 2, 3)

PY_m = effect of m^{th} parity of dam (m = 1, 2, 3, 4, 5, 6, 7)

BW_n = effect of n^{th} birth weight of calf (n = 1, 2, 3, 4, 5)

$e_{ijklmno}$ = random error associated with each observation assumed to be NID (0, ó$_e^2$)

Results and Discussion

Measurement of central tendency and dispersion of raw data

The mean values along with their standard deviations (S.D.) and coefficient of variation (C.V.) of calf survivability traits of Gir crossbred calves at different ages are presented in Table 1.

Causes of calf mortality

The highest (37.11%) mortality was reported due to gastroenteritis / diarrhoea followed by respiratory disease mainly pneumonia (25.76%), hepatitis (10.91%), pyrexia (8.87%), tympany / bloat (3.05%) and other causes were 14.26 per cent.

However, Kharkar et al. (2017) studied mortality percentage in Jersey and Sahiwal crosses, and found that highest mortality in calves were due to gastroenteritis (32.81%) followed by pneumonia (18.75%), septicaemia (12.50%) and other causes (15.63%). whereas, Mishra et al. (2015) reported that mortality in calves were mainly due to diarrhoea and respiratory diseases.

Table 1. Statistical analysis of calf survivability traits

Traits	Mean	S.D.	C.V.(%)
S_1	0.98	0.12	12.39
S_2	0.88	0.32	36.15
S_3	0.92	0.28	30.35
S_4	0.83	0.38	45.95
S_5	0.86	0.35	40.90
S_6	0.96	0.19	19.61

(Mean obtained based on 1389 observations)

Occurrence of calf survivability

The least square means of survivability per cent of Gir crossbred calves at days 0-3, 4-15, 16-30, 31-90, 91-180 and 181-365 were 96.17±0.80, 86.17±2.12, 89.23±2.03, 80.39±2.89, 83.41±3.02 and 95.61±1.79, per cent respectively (Table No. 2 and 3).

Effect of genetic and non-genetic factors on different calf survivability traits

The significant (P<0.05) effect of period of birth was found on calf survivability trait at different ages viz. D 31-90 and D 181-365. Significant effect of period of birth on calf survivability was also noticed by Singh and Gurnani (2003), Mishra et al. (2015) and Kharkar et al. (2017) in different breeds of cattle. The parity of dam had non-significant effect on calf survivability traits of the Gir crossbred calves. Similar to present findings, Hansen et al. (2003) and Gulliksen et al. (2009) observed that calf mortality was not influenced by parity of dam in different cattle breeds. Study in Jersey × Sahiwal crosses by Kharkar et al. (2017) also revealed that parity of dam not showed any significant effect on calf mortality. Conversely, Norberg et al. (2013) and Mishra et al. (2015) observed significant effect of parity on calf mortality at different ages after birth of crossbred calves. The significant (P<0.05) effect of season of birth on calf survivability was observed at D 0-3 age survivability trait. Findings of the present study showed that highest survivability was observed in calves born in winter (97.30%) followed by rainy (95.92%) and summer (95.28%) season at 0-3 days age period. Gulliksen et al. (2009) and Panmei et al. (2014) observed that the calf mortality was significantly (P<0.05) influenced by season of birth of calves in different breeds of cattle. Significant (P<0.05) effect of sex on survivability was recorded at 0-3, 4-15, 16-30 and 31-90 days. Similarly, several research workers Kulkarni and Bansod (2001), Kumar et al. (2002) and Mishra et al. (2015) reported that highest mortality was observed in male calves. Birth weight of calf had significant (P<0.01) effect on calf survivability at 0-3 days after birth. Calves having low birth weight (<15Kg) having more risk of mortality than calves with high birth weight. Survivability per cent increases with increasing birth weight at different ages of calves. These findings were supported by research workers like Riley et al. (2004), Henderson et al. (2011) and Bunter et al. (2014) revealed that low birth weight and poor vigor at the time of birth,

Table 2. Least squares means of calf survivability at 0-3, 4-15 and 16-30 days in Gir crossbreds

Parameters	Survivability per cent at D 0-3	Survivability per cent at D 4-15	Survivability per cent at D 16-30
Overall mean	96.17±0.80 (1389)	86.17±2.12 (1368)	89.23±2.03 (1210)
	Period of Birth (POB)		
P_1	96.22±1.23 (380)	88.61±3.22 (376)	92.58±3.03 (347)
P_2	95.94±1.01 (489)	88.67±2.65 (481)	90.79±2.53 (441)
P_3	96.35±1.42 (520)	81.23±3.69 (511)	84.31±3.53 (422)
	Parity of Dam (PY)		
PY_1	96.65±1.02 (315)	86.55±2.65 (310)	89.58±2.54 (270)
PY_2	95.19±1.07 (274)	85.03±2.82 (268)	90.67±2.69 (236)
PY_3	96.53±1.13 (210)	87.22±2.96 (208)	87.61±2.83 (187)
PY_4	95.99±1.18 (178)	84.81±3.08 (176)	89.61±2.97 (154)
PY_5	96.31±1.25 (150)	87.47±3.27 (148)	86.49±3.08 (134)
PY_6	96.61±1.38 (115)	82.99±3.60 (114)	90.25±3.45 (99)
PY_7	95.89±1.27 (147)	89.12±3.31 (144)	90.38±3.10 (130)
	Season of Birth (SOB)		
S_1	95.92^{b}±0.96 (397)	86.61±2.54 (390)	89.09±2.41 (350)
S_2	97.30^{a}±0.90 (536)	87.72±2.36 (532)	87.41±2.25 (467)
S_3	95.28^{b}±0.96 (456)	84.18±2.53 (446)	91.17±2.43 (393)
		Sex of Calf	
Male	95.35^{b}±0.90 (492)	81.55^{b}±2.38 (480)	85.62^{b}±2.31 (401)
Female	96.99^{a}±0.86 (897)	90.79^{a}±2.26 (888)	92.83^{a}±2.15 (809)
	Birth Weight (BW) of Calf		
B_1	86.53^{b}±1.95 (48)	75.95±5.36 (42)	83.41±5.52 (31)
B_2	97.81^{a}±0.90 (439)	86.99±2.35 (434)	90.92±2.22 (382)
B_3	98.10^{a}±0.87 (461)	90.12±2.26 (456)	90.18±2.10 (411)
B_4	98.61^{a}±0.96 (330)	89.88±2.50 (326)	92.00±2.35 (291)
B_5	99.79^{a}±1.38 (111)	87.92±3.56 (110)	89.63±3.41 (95)

(Means with different superscripts differ significantly (P<0.05) from each other, values in parenthesis are number of observations)

Table 3. Least squares means of calf survivability at 31-90, 91-180 and 181-365 days in Gir crossbreds

Parameters	Survivability per cent at D 31-90	Survivability per cent at D 91-180	Survivability per cent at D 181-365
Overall mean	80.39±2.89 (1108)	83.41±3.02 (915)	95.61±1.79 (784)
	Period of Birth (POB)		
P_1	91.98[a]±4.21 (331)	85.36±4.40 (289)	97.55[a]±2.65 (246)
P_2	82.35[a]±3.56 (412)	85.60±3.75 (347)	98.97[a]±2.28 (301)
P_3	66.84[b]±5.01 (365)	79.26±5.46 (279)	90.32[b]±3.28 (237)
	Parity of Dam (PY)		
PY_1	79.42±3.57 (247)	82.31±3.73 (204)	95.78±2.22 (168)
PY_2	77.46±3.79 (221)	83.53±3.96 (179)	96.98±2.37 (154)
PY_3	78.71±3.98 (169)	84.88±4.17 (139)	95.12±2.48 (122)
PY_4	84.77±4.17 (142)	77.40±4.37 (122)	96.16±2.61 (100)
PY_5	78.84±4.39 (119)	80.85±4.65 (95)	93.88±2.80 (79)
PY_6	82.31±4.85 (91)	85.10±4.98 (78)	95.87±2.90 (69)
PY_7	81.22±4.37 (119)	89.79±4.56 (98)	95.48±2.64 (92)
	Season of Birth (SOB)		
S_1	79.72±3.41 (321)	84.08±3.59 (260)	96.50±2.13 (224)
S_2	79.02±3.20 (417)	84.16±3.36 (338)	95.26±1.99 (291)
S_3	82.43±3.42 (370)	81.27±3.57 (317)	95.07±2.13 (269)
	Sex of Calf		
Male	74.53[b]±3.32 (355)	83.44±3.51 (274)	95.90±2.1 (235)
Female	86.25[a]±3.02 (753)	83.38±3.17 (641)	95.32±1.88 (549)
	Birth Weight (BW) of Calf		
B_1	80.70±8.05 (26)	74.11±8.45 (21)	94.16±5.38 (16)
B_2	83.34±3.11 (353)	83.94±3.20 (300)	95.42±1.87 (259)
B_3	81.93±2.97 (375)	84.77±3.08 (313)	96.76±1.83 (268)
B_4	79.84±3.26 (269)	84.47±3.41 (217)	95.85±2.03 (184)
B_5	76.14±4.81 (85)	89.74±5.22 (64)	95.85±3.09 (57)

(Means with different superscripts differ significantly (P<0.05) from each other, values in parenthesis are number of observations)

correlated with occurrence of high mortality rates in different cattle breeds. The random effect of sire was significant (P<0.05) on calf survivability trait at 31-90 day. Similarly, Dechow et al. (2012) reported that effect of sire had significant effect on incidence of calf mortality and early lactation culling, particularly in herds with adverse cow survival environments. The significant effect of sire on calf survivability trait shows that this trait may be improved genetically by selection of sires.

Comparison of survivability patterns of calves with different genetic groups

The non-significant (P<0.05) effect was observed among the survivability patterns of calves in different ages with different genetic groups of Gir crossbred. However, the JG group had higher survivability per cent followed by FJG, F, FG and lower survival was noticed in R group. Similarly, Dhakal et al. (2013) found that genetic group had no significant effect on neonatal calf mortality in first or later parities in Holstein, Jersey and crossbred calves. Davis et al. (2020) conducted research in beef x dairy crossbred calves and studied the genetic parameters and sire breeding values for young stock survival.

Conclusions

The calf survivability was significantly influenced by the period of birth, season of birth, sex, birth weight and sire which indicated that the selection of sire and improvement in management could improve the survivability of calves in Gir crossbred cattle.

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Table 4. Least square means of calf survivability traits at different age periods as affected by genetic groups in Gir crossbreds

Parameters	No. of observations	Survivability per cent at D 0-3	Survivability per cent at D 4-15	Survivability per cent at D 16-30	Survivability per cent at D 31-90	Survivability per cent at D 91-180	Survivability per cent at D 181-365
Overall mean	1389	96.17±0.80	86.17±2.12	89.23±2.03	80.39±2.89	83.41±3.02	95.61±1.79
		(1389)	(1368)	(1210)	(1108)	(915)	(784)
G_1(FG–50% HF + 50% Gir)	274	95.48±3.10	82.49±7.99	90.93±7.22	91.67±10.07	77.51±10.65	95.37±6.12
		(274)	(271)	(244)	(232)	(192)	(168)
G_2(FJG – 50% HF + 25% J + 25% Gir)	573	98.69±1.71	94.13±4.43	88.08±4.07	74.70±5.73	86.38±6.38	93.84±3.52
		(573)	(563)	(490)	(437)	(360)	(294)
G_3(R – 50% HF + 12.5% J + 37.5% Gir)	170	93.77±2.71	81.23±7.08	82.73±6.62	75.08±9.40	77.35±10.13	96.32±6.00
		(170)	(168)	(153)	(141)	(125)	(109)
G_4(JG – 50% J + 50% Gir)	167	96.11±3.74	82.99±9.65	89.08±8.79	90.41±11.86	93.52±13.03	96.04±7.23
		(167)	(165)	(149)	(134)	(106)	(101)
G_5(F - 75% HF + 25% Gir)	205	96.79±1.91	90.01±4.96	95.31±4.62	70.09±6.60	82.28±7.49	96.47±4.27
		(205)	(201)	(174)	(164)	(132)	(112)

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RESEARCH ARTICLE

Nutritional value and energy balance of pearl millet fodder as influenced by different nutrient management practices

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Abstract: Fodder deficiency and its poor-quality leads to lower productivity of Indian cattle. To improve productivity and nutritional value of fodder pearl millet, the present study was undertaken during *kharif* season of 2019-20 at Agronomy research farm, ICAR-NDRI, Karnal, and laid out in Randomized Block Design with eight treatments, *viz*. T_1: Absolute control; T_2: 100% RDF; T_3: 100% RDF + Cow urine foliar spray (CU); T_4: 100% RDF + PGPR; T_5: 100% RDF + PGPR + Cow urine foliar spray (CU); T_6: 75% RDF + Cow urine foliar spray (CU); T_7: 75% RDF + PGPR and T_8: 75% RDF + PGPR + Cow urine foliar spray (CU) with three replications. The results showed that the fodder nutritional value and digestibility *viz*., total digestible nutrients, digestible dry matter, digestible crude protein and dry matter intake increase by 28.46, 13.10, 40.26 and 8.45% respectively, with T_5 treatment than absolute control. The higher energy fraction such as digestible energy (11.54 MJ/Kg), metabolisable energy (9.47 MJ/Kg), digestible feed energy (9.27 MJ/Kg), net energy (3.07 MJ/Kg) and net energy for lactation (5.29 MJ/Kg) was observed with the application of 100% RDF+PGPR+CU (T_5), which was found statistically at par with applications of 100% RDF+PGPR and both were found significantly higher over rest of the treatments. Quality fodder strengthen and sustain the performance of livestock in terms of health and milk production.

Keywords: Energy, Fodder, Milk, Nutritional Value, Pearl millet

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Introduction

Gross Value Added (GVA) of livestock sector is about Rs. 11,14,249 crores at current prices during FY 2020-21 and it is about 30.87% of agricultural and allied sector, and 6.17% of total GVA (Anonymous 2022). India has 535.78 million livestock population, and it has increased by 4.63% (20th livestock census) as compared to previous livestock census (Anonymous 2022). Green fodder is an essential component for livestock production, and ever-increasing livestock population has tremendous pressure on total available feed and fodder resources. Quality fodder can curtail the cost of feeding livestock because feeding contributes to about 65 to 70% of total cost of livestock farming (Kumar et al. 2023). Currently, India is facing a net deficit of green fodder by 35.6%, dry fodder (straw) by 10.95% and concentrates by 44% (Kushwaha et al. 2018). Deficiency of quality fodder and feed for livestock leads to decrease in their production level, and has an impact on their health, which ultimately influences return from livestock sector (Surve et al. 2011). Among the different fodder crops pearl millet (*Pennisetum glaucum*) is a gifted crop of the tropical and sub-tropical regions that provide food, fodder and stover (dry straw) to millions of families of poor farmer and their livestock. Nutrient management is an important aspect to achieve sustainable crop production. Scenario from green revolution era, shows that productivity of cereals increased largely with the use of high yielding variety, intensive agronomic practices and indiscriminate use of chemical fertilizers at higher rate with little or no use of organic source of nutrients to plant, that creates adverse effects on soil *viz.,* inadequacy in one or more nutrients and deterioration of soil fertility which leads to stagnating or even declining of crop productivity and quality (Shormy et al. 2013). Deficiency of nutrients in soils leads to the production of mineral deficient foods and fodder. However, animal and humans depending on such fodder and foods have also shown symptoms of nutrients deficiency (Shukla et al. 2015). Judicious use of inorganic and organic sources of nutrients may sustain and enhance the fodder quality.

Among different organic source of nutrients for plant, cow urine and Plant Growth Promoting *Rhizobacteria* (PGPR) are excellent and important for agriculture uses. Cow urine contains; nitrogen,

phosphorus, potassium, sulphur, sodium, manganese, iron, carbolic acid, silicon, chlorine, enzymes and hormones (Saunders 1982). PGPR, is a consortium of bacteria that actively colonize around plant roots and enhances plant growth and yield (Wu et al. 2005). PGPR strains belongs to a wide range of genera *viz*., *Pseudomonas, Azospirillum, Bacillus, Serratia* and *Azotobacter* (Bashan et al. 2004). The beneficial effects of PGPR are due to their ability to produce various organic compounds *viz.*, auxins, gibberellins, cytokinin, ethylene, organic acids, siderophores, nitrogen fixation, solubilization of insoluble inorganic soil phosphate to available form, sulphur oxidation, extra cellular production of antibiotics, increase in root permeability and enhancement of essential plant nutrients uptake (Enebak and Carey 2000 and Pal et al. 1999). Considering the above facts, the present study was executed to find out a suitable combination of nutrient source to enhance the fodder quality of pearl millet.

Materials and Methods

Description of experimental site

This study was conducted during *kharif* season of 2019-20 at Agronomy research farm, ICAR-NDRI, Karnal, Haryana, India, located at 29º45' North latitude and 76º58' East longitude and at an altitude of 245m above mean sea level. The area has a semi-arid climate, with a mean annual rainfall of 707 mm, and 70-80% of the rainfall is received during the months of July-September and rest during winter and spring seasons. The mean minimum and maximum temperature during study period was 20.49°C and 34.54°C, respectively. The soil of experimental site was clay loam in texture (Piper 1942) with pH of 7.35, Electrical conductivity of 0.37 dS/m (Jackson 1967), organic carbon of 0.49% (Walkley and Black's 1934), available nitrogen of 215 kg/ha (Subbiah and Asija 1956), available phosphorus of 24.70 kg/ha (Olsen et al. 1954), and available potassium of 285 kg/ha (Jackson 1967).

Treatment details and input application

The experiment was laid out in simple Randomized Block Design with eight treatments *viz*., T_1: Absolute control; T_2: 100% Recommended dose of fertiliser (RDF); T_3: 100% RDF + Cow urine foliar spray (CU); T_4: 100% RDF + PGPR; T_5: 100% RDF + PGPR+ Cow urine foliar spray (CU); T_6: 75% RDF + Cow urine foliar spray (CU); T_7: 75% RDF + PGPR; T_8: 75% RDF + PGPR + Cow urine foliar spray (CU). Each treatment had three replications. The land preparation involved one deep ploughing each with disc plough, disc harrow and thereafter planking. As per treatments, recommended dose of fertilizers (80:30:30 kg/ha, N: P_2O_5: K_2O, respectively) were applied. The half of N and full doses of P_2O_5 and K_2O were applied before last ploughing operation during land preparation. The remaining half of the nitrogen was top-dressed as two doses 1st at 25 days after sowing (DAS) and 2nd at 40 DAS as per the treatment. Other package of practices was followed as per standard procedure for fodder pearl millet cultivation. The PGPR (100 ml/10kg seeds) liquid culture was diluted in water, and applied on seeds. Thereafter, inoculated seeds were dried in shade for 60-90 minutes, after drying seeds were manually sown. Nutrified variety of fodder pearl millet (*Pennisetum glaucum*) was sown using 10 kg seed per hectare with maintaining row to row spacing of 30 cm and plant to plant spacing of 10 cm. As per treatments, cow urine (10%) foliar spray was applied at 30 and 45 days after sowing.

Fodder sample collection and their quality analyses

The crop was harvested manually at 50 % flowering stage. Net plot area was harvested separately from each plot. Fresh chopped plant samples were collected and subjected to analysis of different quality parameters. The oven-dried fodder samples were ground to pass through 40 mesh sieves using a Macro-Wiley Mill, stored in air tight containers, and were used for chemical analysis. Fibre

Table 1 Estimation of data according to different standard equations

Particulars	Equation	References
TDN (%)	(-1.291 × ADF%) + 101.35	Horrocks and Vallentine (1999)
DDM (%)	88.9 – (0.779 × ADF%, dry matter basis)	Horrocks and Vallentine (1999)
DMI (% of body weight)	(120/NDF%, dry matter basis)	Horrocks and Vallentine (1999)
NE_1 (MJ kg^{-1})	(1.044 – (0.0119 × ADF%) × 2.205 × 4.184	Horrocks and Vallentine (1999)
RFV (%)	DDM% × DMI% × 0.775	Horrocks and Vallentine (1999)
RFQ	DMI% × TDN%/1.23	Undersander et al. (2002)
DE (MJ Kg^{-1})	0.27+ [0.0428 × DDM%] × 4.184	Fonnesbeck et al. (1984)
ME (MJ Kg^{-1})	DE (MJ Kg^{-1}) × 0.821	Gonzalez and Everitt (1982)
DCP (%)	(0.929 × CP%) – 3.52	Demarquilly and Weiss (1970)
DFE (MJ Kg^{-1})	[{TDN (%) × 4.4}/100] × 4.184	Bull (1981)
NE (MJ Kg^{-1})	[{(TDN % × 3.65)-100}/188.3] × 6.9	Riviere (1977)

Note: TDN: Total digestible nutrients; DDM: Digestible dry matter; DMI: Dry matter intake; NE_1: Net energy for lactation; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; RFV: Relative feed value; RFQ: Relative feed quality; DE: Digestible energy; ME: Metabolizable energy; DCP: Digestible crude protein; DFE: Digestible feed energy and NE: Net energy.

$$\text{TDN yield (q/ha)} = \frac{\text{TDN content (\%)} \times \text{Dry matter yield (q/ha)}}{100}$$

fractions *viz.*, NDF and ADF were determined as per Van Soest et al. (1991). Another data was estimated according to following equations (Table 1):

Statistical data analysis

All data recorded were analysed with the help of analysis of variance (Gomez and Gomez 1984). Significance among treatments mean differences for various parameters were analysed by least significant differences (LSD) at 0.05 probability level.

Results and Discussion

Dry matter yield

Study indicated (fig. 1) that dry matter yield of fodder pearl millet was significantly influenced with different nutrient management practices and recorded significantly higher dry matter yield (113.35 q ha^{-1}) at harvest with T_5 treatment, which was found statistically at par with T_4 treatment 100% RDF+PGPR and both were significantly higher over rest of the treatments. Balanced and regular supply of essential plant nutrients, PGPR produce phytohormones (Enebak and Carey 2000) and cow urine supply enzyme and hormones (Saunders 1982) that attributed to stimulate plant physiological processes leads to increase leaf area index that responsible for higher interception of solar radiation and produce more photosynthates and nutrients acquired, resulted in to increase dry matter assimilation in different part of plant leads to increase dry matter yield. Further, higher biomass production and dry matter content attributed to increase dry matter yield. The similar results also reported by Chattha et al. (2017).

fibre fraction

The chemical analysis of fodder pearl millet showed that fibre fractions was significantly influenced with different nutrient management practices (fig. 1) and found decreasing trend of these parameters with increased fertility levels. Significantly lowest neutral detergent fibre (63.99%) and acid detergent fibre (39.50%) was observed with the application of 100% RDF+PGPR+CU. However, it remained at par with the treatment that received 100% RDF+PGPR and both were found significantly lower over rest of the treatments. Fibre fraction *viz*., neutral detergent fibre (NDF) and acid detergent fibre (ADF) content recorded lowest with

Table 2 Effect of nutrient management practices on mean* nutritional value, digestibility, intake and relative feed value (RFV) of pearl millet fodder

Treatment	TDN%	TDN yield (q/ha)	DDM%	DMI%	RFV	DCP%
Absolute control	39.20	21.83	51.40	1.73	68.89	3.97
100% RDF	46.77	46.27	55.96	1.83	79.19	4.83
100% RDF + CU	46.99	47.16	56.10	1.83	79.56	5.00
100% RDF+PGPR	50.09	55.99	57.97	1.87	84.07	5.28
100% RDF+PGPR+CU	50.35	57.09	58.13	1.88	84.50	5.57
75% RDF+ CU	42.54	35.02	53.42	1.76	73.07	4.35
75% RDF+ PGPR	45.52	42.84	55.21	1.80	77.10	4.58
75% RDF+PGPR+CU	45.96	44.21	55.48	1.81	77.80	4.71
SEm (±)	0.53	1.24	0.32	0.01	0.90	0.11
CD (P=0.05)	1.59	3.77	0.96	0.04	2.74	0.33

Note: *Mean of three replications; PGPR: Plant growth promoting rhizobacteria; CU: Cow urine; RDF: Recommended dose of fertiliser; SEM: Standard error of mean and CD: Critical difference

Fig. 1 Effect of nutrient management practices on NDF, ADF content and dry matter yield of pearl millet

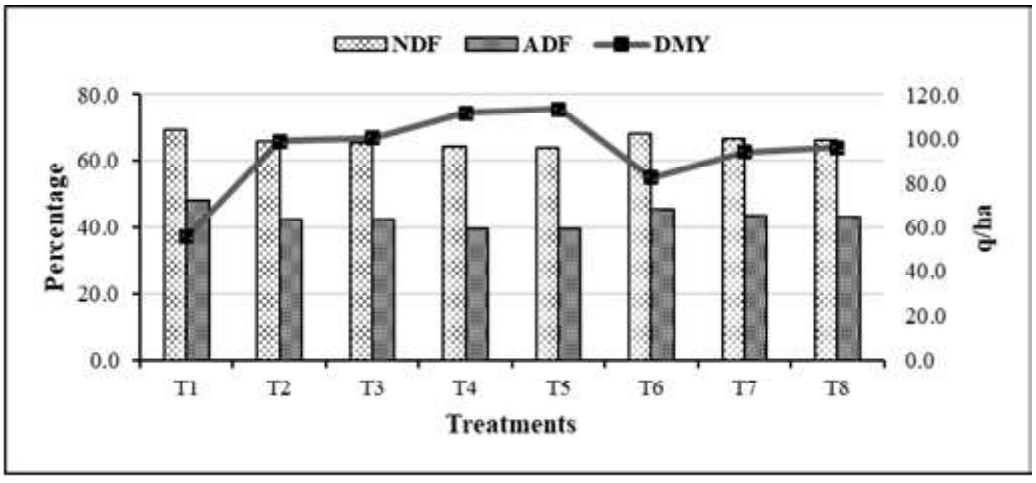

Table 3 Effect of nutrient management practices on mean* energy fraction and relative fodder quality (RFQ) of pearl millet fodder

Treatment	DE (MJ/Kg)	ME (MJ/Kg)	DFE (MJ/Kg)	NE (MJ/Kg)	NEL (MJ/Kg)	RFQ
Absolute control	10.33	8.48	7.22	1.58	4.35	55.11
100% RDF	11.15	9.16	8.61	2.59	4.99	69.42
100% RDF + CU	11.18	9.18	8.65	2.62	5.01	69.92
100% RDF+PGPR	11.51	9.45	9.22	3.04	5.27	76.21
100% RDF+PGPR+CU	11.54	9.47	9.27	3.07	5.29	76.80
75% RDF+ CU	10.70	8.78	7.83	2.03	4.63	61.06
75% RDF+ PGPR	11.02	9.04	8.38	2.42	4.88	66.68
75% RDF+PGPR+CU	11.06	9.08	8.46	2.48	4.92	67.62
SEm(±)	0.06	0.05	0.10	0.07	0.04	1.17
CD (P=0.05)	0.17	0.14	0.29	0.21	0.14	3.55

Note: *Mean of three replications

100% RDF+PGPR+CU followed by 100% RDF+PGPR treatments, due to higher nitrogen level in plant tissue that increase metabolism of carbohydrates leads to decrease cell wall constituents/carbohydrates (Iqbal et al. 2017). Less fibre fraction attributed to increase cell soluble contents in plant. These results are in line reported by Kushwaha et al. (2018).

Nutritional value and digestibility

A critical examination of data presented in Table 2 indicated that among the different nutrient management practices, the significantly higher total digestible nutrients (50.35%), total digestible nutrients yield (57.09 q/ha), Digestible dry matter (58.13%) and relative feed value (84.50) was observed with the application of 100% RDF+PGPR+CU, which was found statistically at par with applications of 100% RDF+PGPR and both were found significantly higher over rest of the treatments. While, significantly highest dry matter intake (1.88%) and digestible crude protein (5.57%) was observed with the application of 100% RDF+PGPR+CU over rest of the treatments. The treatment supplied with 100% RDF+PGPR+CU increases total digestible nutrients by 0.52, 7.14, 7.67 and 28.46%; digestible dry matter by 0.27, 3.61, 3.87 and 13.10%; dry matter intake by 0.24, 2.51, 2.73 and 8.45%, and digestible crude protein by 5.45, 11.33, 15.18 and 40.24% over 100% RDF+PGPR, 100% RDF+CU, 100% RDF and absolute control, respectively.

The total digestible nutrients (TDN) refer to the nutrients, which, are available for animals and their availabilities are related to the ADF content of the fodder. The TDN contents decrease as ADF content increases in fodder, which means animals are not able to uses the nutrients present in the offered fodder (Lithourgidis et al. 2006). As ADF content increases resultant increase cellulose and lignin content of fodder leads to decrease DDM. Lower value of ADF content with application of 100% RDF+PGPR+CU attributed to higher value of TDN and DDM. Higher TDN value and dry fodder yield attributed to higher yield of TDN. The NDF content inversely correlated with dry matter intake (DMI), which means that when NDF content of fodder increase the DMI will be decrease because NDF contains; cellulose, hemicellulose and lignin that have lower digestibility and take more time to digest and passes through the gastrointestinal tract of ruminants (Horrocks and Vallentine 1999). The relative feed value (RFV) is an index that predict the energy value of the fodder and their intake. Higher value of DMI and DDM attributed to increase relative feed value. The digestible crude protein (DCP) content increase with increasing crude protein content of fodder. The TDN, DMI, DDM, RFV and DCP values increase with increasing N fertilization which, increase N concentration in plant part (Albayrak and Turk 2011). The similar results were also reported by Albayrak and Turk (2011) and Bhakar et al. (2020).

Energy balance

A critical examination of data presented in Table 3 indicated that among different nutrient management practices, the significantly higher digestible energy (11.54 MJ/Kg), metabolisable energy (9.47 MJ/Kg), digestible feed energy (9.27 MJ/Kg), net energy (3.07 MJ/Kg), net energy for lactation (5.29 MJ/Kg) and relative forage quality (76.80) was observed with the application of 100% RDF+PGPR+CU, which was found statistically at par with applications of 100% RDF+PGPR and both were found significantly higher over rest of the treatments. The treatment supplied with 100% RDF+PGPR+CU increases digestible and metabolizable energy by 0.24, 3.25, 3.47 and 11.66%; digestible feed energy by 0.52, 7.14, 7.67 and 28.46%; net energy for lactation by 0.42, 5.70, 6.11 and 21.82%; and relative forage quality by 0.77, 9.85, 10.63 and 39.35% over 100% RDF+PGPR, 100% RDF+CU, 100% RDF and absolute control, respectively.

Digestible energy (DE) is the fraction of total fodder energy that remain after excretion in the faeces. DE is positively correlated with DDM content of fodder. Metabolisable energy (ME) is the fraction of DE energy that remain after some losses in urine and as methane (CH_4) gas released by microbes of rumen and hind gut, which, are positively correlated with DE of fodder. Net energy (NE) is the fraction of ME energy that remain after heat losses in metabolism and digestion process, which is the actually energy that available for animal uses. NE and DFE are positively correlated with TDN content of fodder. Net energy of lactation (NE_l) is

energy requirements for maintenance and milk production that are negatively correlated with ADF content of fodder. NE_1 values increase with increasing N fertilization (Albayrak and Turk 2011). Higher DMI and TDN contents of pearl millet fodder attributed to increase relative feed quality. These results are in agreement to result reported by Albayrak and Turk (2011).

Conclusion

Study concludes that application of organic sources of nutrients *viz.*, PGPR and cow urine foliar spray with inorganic fertiliser shows positive effect on nutritional and energy value of pearl millet fodder. 100% RDF+PGPR+CU (T_5) remained productive as well as profitable in term of nutritional and energy value. For future line of work, as like pearl millet, different cereal fodder crops can be explored location wise along with proper dose and sources (inorganic and organic) of nutrients for better quality.

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RESEARCH ARTICLE

Performance of dairy processing firms in India- An empirical analysis across size and experience categories

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Abstract: The Indian dairy processing sector is characterized by the co-existence of organized and un-organized sectors, while the latter is predominant. For the progressive development of the dairy processing industry, the participation of the organized sector needs to be encouraged. In order to make this industry attractive for investing, its productivity, efficiency, and profitability need to be higher. Keeping these facts in view, this study investigated the profitability of the organized dairy processing sector in India using firm-level secondary data for 1991-2017. Comparative performance analysis was done across different categories of firms according to size and experience. There was a significant increase in profit margin over the period, while it varied significantly across different size categories of firms but not across firms having different years of experience.

Keywords: Capital Intensity, Dairy Industry, Labor Productivity, Profitability, Size

Introduction

The dairy sector in India has shown impressive growth over the years due to the successful implementation of various dairy development programs, including Operation Flood. This has made India the world's largest milk producer, with an annual production of 187.7 million tonnes, and per capita availability of 394 grams per day during 2018-19 (Economic survey, 2019-20). Additionally, dairying plays an essential role in the country's socio-economic development as a source of livelihood, food, and nutrition for millions of people. The development of the dairy sector in India is inclusive as the 80 million households engaged in milk production consist primarily of small and marginal farmers and landless. Besides, the dairy sector has solid forward linkages with industries and backward linkage with rural people, which promotes the balanced development of the economy. Despite these facts, the Indian dairy sector lacks on the processing front.

In India, both organized and unorganized sectors are involved in milk processing. The organized dairy industry comprises cooperatives and private dairy plants, including multinationals. The unorganized sector consists of milkmen, dairies (small processing units), and small vendors who collect milk from local producers and sell it in urban and peri-urban areas either as liquid milk or milk products. Out of the total milk production, 52 per cent is the marketable surplus available for sale to consumers in the urban centers after meeting the consumption needs of producers and non-producers in the rural area. From the available market surplus, 40 per cent is handled by the organized sector namely, dairy co-operatives (20%), private dairies (19%), and producer companies (1%), and the unorganized sector handles the remaining 60 per cent. Despite the use of low-level technology and low benchmark quality control measures by the unorganized sector, value addition from this sector is of great economic significance (Singh and Datta, 2010). It is expected that this trend in milk handled by private players, co-operatives, producer companies and the unorganized sector may change to 30, 20, 2 and 8 per cent, respectively in 2023-24 (GoI, 2018).

In the recent past, the share of the organized sector in dairy processing has been increasing because of liberalization and policy changes in the Milk and Milk Products Order (MMPO) that encourage private investment and demand-driven growth of the sector. The growth rate in the number of units and growth in gross value added of the dairy processing sector was higher (5.87 % and 14.35 %, respectively) compared to the food industry as a whole (1.58 % and 10.51 %, respectively) during 1980-2001 (Ali et al. 2007). This sector's growth is driven mainly by three factors, such as growth in population, urbanization, and an increase in per capita income.The growth in processing provides farmers with broader market access and remunerative price. To

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meet the increasing demand and compete with the global players, India needs to focus on manufacturing value-added products with enhanced shelf-life, improved packaging, and prescribed quality standards. The high profitability of investment in this sector is going to attract investment from private players. In this context, the present study attempts to find out the past trends in the profitability of dairy firms and the factors determining the profitability.

Data and Methods

Description of data

The study is based on secondary data from the Prowess database of Centre for Monitoring Indian Economy Private Limited, Mumbai (CMIE). It is a query-based database that contains firm-level calendar year financial data of active business entities, including registered companies. For the present study, data of 138 dairy processing firms was filtered from the abovementioned database for 27 years (1991-2017). Data availability on a particular variable varies across firms for different years, resulting in unbalanced panel data formation. The information on total income, expenses, assets, sales, production, and unit prices were collected along with general characteristics of firms like size and experience. As some cases were missing, information was generated from aggregates. For example, 'the number of employees' information was given, but sufficient observations were unavailable to conduct any analysis. Hence, number of employees' information (termed as efficient units of labor) was calculated by dividing the total salary expense of the firm (from the prowess database) by the average wage rate of the industry (obtained from the Annual Survey of Industries database) as in Balakrishnan et al (2000).

A firm's performance may be affected by its size and experience. A study conducted by Majumdar (1997) using firm-level data revealed that larger firms were more productive and less profitable, whereas older firms were more profitable and less productive, which is attributed to the institutional framework of the Indian economy and industrial policy instruments, such as inter-alia, restrictive entry policies. Hence, a comparison of performance indicators across size and experience categories over the years was made to understand if there is a significant difference across distinct categories. According to size, firms were classified into large, medium, and small size categories based on the decile classification of firms given in the database. This classification was based on the last three-year averages (TE 2018) of the total income and assets. Accordingly, there were 31.88 per cent large firms, 44.93 per cent medium firms and 23.19 per cent small firms. The experience is calculated by taking the difference between the year of study (2018) and the year of incorporation of the firm. Based on years of experience, the firms were classified into three groups, viz., firms established less than 20 years ago were termed as low experience firms, 21-40 years ago as moderate experience firms, and more than 40 years ago as high experience firms. The database consists of firms established as early as 1940 to the latest 2018. The firms with low experience were more than one-third of the total firms (37.68%). The share of moderate and high-experience firms was about 54 per cent and 8 per cent, respectively.

Estimation of trends in profitability

For the present study, the profit margin was considered as an indicator of profitability. It is nothing but the profit over sales. Different studies have estimated profit margins according to data availability (Kambhampati and Parikh 2003; Kalirajan and Bhinde 2005). The present study calculated profit margin as the ratio of PAT and sales, as in Kalirajan and Bhinde (2005).

Profit Margin = Profit after tax (PAT)/Sales

PAT and sales are values in monetary terms (rupees in million). The performance indicators of the firms were taken as PAT, labour productivity, capital-output ratio and cost per unit sale. The whole study period was divided into sub-periods with equal intervals of 9 years, viz., 1991-99, 2000-2008 and 2009-2017 to know if there was any shift in trend or fluctuations during different sub-periods which may be attributed to changes in the policy environment and other external factors. To find out the growth rate of each indicator compound annual growth rate was worked out by fitting the exponential function ($\mathbf{Y=ab^te^t}$) where the compound annual growth rate can be calculated as [(Antilog of b)-1]*100. The data series was deflated before estimating the growth rate using respective indices to reflect real change.

Determinants of profitability at the firm level

To find the factors affecting profit margin, a panel data regression was carried out. Panel data has the advantage of having two dimensions, i.e., across firms and over time. Thus, it adds more variability by having more observations and considering heterogeneity across firms. Extreme observations that affect the sample statistics as well as data of firms which were facing loss for the entire period of study were removed to avoid any bias arising due to them. Hence, the final dataset for panel data analysis consisted of unbalanced data of firms for the period 1991-2017.

To determine factors affecting profit margin following factors were included as explanatory variables as in Kambhampati and Parikh (2003 and 2005):

Profit Margin= *f* (Share of Sales, Labor productivity, Capital-output ratio, cost per unit sale, time trend)

Where, share of sales indicates the market power of a firm which was calculated by taking the ratio of firms' sale to industry sales for a particular year. The labour productivity was calculated as sales per efficient unit of labour as in Balakrishnan et al. (2000). The Capital- output ratio was estimated as Gross Fixed Assets

(GFA) per rupee of sales. The sales series was deflated using the wholesale price index for dairy products at 2004-05 prices, and the GFA series was deflated with an implicit deflator at 2004-05 prices. The cost per unit sale is an indicator of cost efficiency, and it was calculated by taking the ratio of operational cost per rupee of sales. The time trend was included to consider the effect on profitability due to improvement in knowledge, experience, and technology over the years.

A linear panel data regression was run with fixed (FE) and random effects (RE). FE model control individual heterogeneity or time-invariant individual-specific characteristics that may or may not influence the predictors/outcome variables and hence, reduce the possible bias by estimating the net effect of predictors on the outcome variable. FE is appropriate when the individual-specific intercept may correlate with one or more regressors. At the same time, in RE model, it is assumed that the intercept of an individual unit is a random drawing from a much larger population with a constant mean value. It is appropriate when the intercept of each cross-sectional unit is uncorrelated with the regressors (Gujarati, 2004). The choice between two models was made using the Hausman test, which tests if the unique error term is correlated with the regressors with the null hypothesis that they are not correlated.

Results and Discussion

The trend in performance indicators over time

The values of different performance indicators over time for the Indian dairy industry are given in Table 1. All values are in ratio form in rupees per annum. The overall profit margin (1991-2017) was 1.5 per cent of the sale value, and it increased significantly at a compound growth rate of 0.164 per cent exhibiting improvement in the profitability of the dairy industry in India. The profit margin, during 1991-99 was negative, i.e., for per unit sale there was 0.5 per cent loss. In the following sub-periods, there was an increase in profit margin to the extent of 0.016 in 2000-08 and 0.020 in 2009-17.

Annual changes in profit margin can be seen from Figure 1, which shows that the profit margin has improved over time. During the first decade, the profit margin was continuously declining. The possible reason for the same may be significant trade policy reforms in the early 1990s that favored privatization and liberalization of all sectors, including the dairy industry. Delicensing of the dairy industry attracted a large number of domestic and private players, including multinational companies, to make investments in the sector. The increase in competition due to the rise in the number of plants might have squeezed the profit margin. Further, the establishment of WTO and reductions in quantitative restrictions on imports might have increased competition from other countries also.

The next indicator studied was labor productivity. As shown in Table 1, the overall average labour productivity in the dairy industry was observed as Rs 7.42 million per efficient units of labour in a year which has increased significantly at a compound growth rate of 2.36 per cent per annum during 1991-2017. During 1991-99, annual average labour productivity was 5.16 million rupees per labour and it increased in subsequent sub-periods to the tune of Rs 7.34 and Rs 8.23 million during 2000-08 and 2009-17, respectively. There was a steady growth in labour productivity due to the automation of dairy processing that

Fig. 1 Trend in Profit margin, 1991-2017

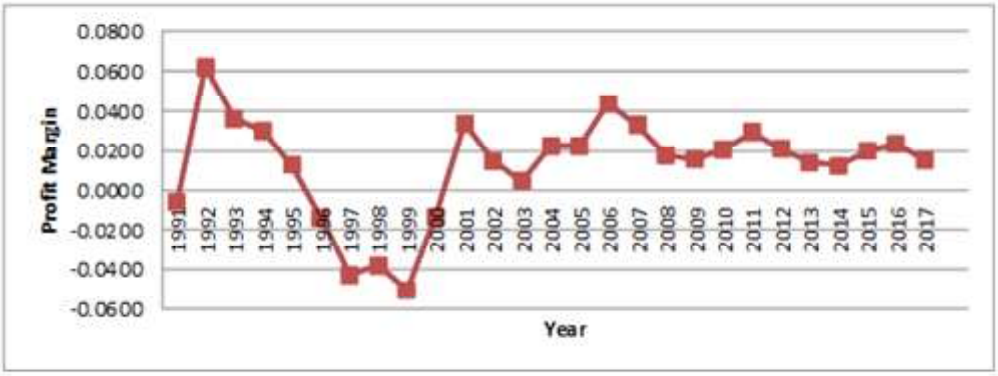

Table 1. Trend in performance indicators of Indian dairy industry (1991-2017)

Sub-periods	Profit margin (Ratio in Rs)	Labour Productivity (Rs. Million / EU)	Capital Output ratio (Ratio in Rs)	Cost per unit sale (Ratio in Rs)
1991-99	-0.005	5.16	0.52	0.966
2000-08	0.016	7.34	0.71	0.954
2009-17	0.020	8.23	0.53	0.954
Overall	0.015	7.42	0.59	0.956
CGR (%)	0.164**	2.36***	0.59	-0.081

CGR is calculated for the period 1991-2017;***, **, *indicates significance at 1% level,5% level and 10% level of probability

subsequently reduced the number of labor required to handle per unit of milk, but at the same time, the quality, as well as the value of product, increased. The analysis of aggregate data from the Annual Survey of Industries (ASI) indicates that, during 1991-2017, the number of persons engaged per factory decreased at a compound growth rate (CGR) of 2.18 per cent. Ohlan (2014) also reported that the number of employees per factory has shown a sharp decline with a CGR of -4.03 per cent during 1980-2008, exhibiting the labor substitution by capital.

Another performance indicator that reveals capital intensity is the capital-output ratio. It is the extent of capital used per unit output. It can be seen from the table that the overall capital-output ratio was 0.59 in the Indian dairy industry, which means the value of capital used was 59 per cent of the value of output. During the last 27 years (1991-2017), this ratio has not increased significantly and, at the same time, fluctuated too much. The capital-output ratio has shown a fluctuating trend, and it increased from 0.52 in 1991-99 to 0.71 in 2000-08, then declined in 2009-17 to 0.53. Non-significant growth in the capital-output ratio indicates that capital used per unit of output has remained the same since capital intensity increased in proportion to the increase in output.

Besides, the overall cost per unit sale ratio was 0.96, which denotes that for producing one-rupee-worth output, the operational cost accrued to the firm was 0.96 Rupees. If we compare the performance of Indian dairy industry in terms of cost reduction there was not much improvement as indicated by the growth rate, i.e., during the study period cost per unit sale was reduced by only 0.08 per cent and it was statistically non-significant. It might be because a major share of operation cost is constituted by raw material expenses that cannot be reduced without affecting production. Besides, due to increased competition, firms might be allocating more money for advertising, research, and development, improved and attractive packaging etc.

Trends in performance indicators across categories according to size and experience

The changes in performance indicators over time across sizes and experience categories can be seen from Table 2. Large firms were found to have the highest profit margin (0.018) followed by medium (0.016), while the small firms were in loss intermittently with an overall profit margin of -0.006 during 1991-2017. It might be because, as firm size increases, firms might benefit from economies of scale, leading to a decrease in average cost.

If we examine the growth in profit margin, small firms grew at a faster rate (0.99 % per annum), while growth in profit margin was statistically non-significant in the case of medium (0.14 %) and large (0.01 %) firms. This may be because small firms were operating with much loss when compared to the other two categories of firms; hence they might have tried to make the firms profitable. However, the other two categories were already in a comparatively better position in terms of PAT, and their focus might be to sustain profitability rather than increase profits. A similar trend was observed in the case of labor productivity also. It was found highest in the case of large firms (8.16), followed by medium (6.76) and small (6.11) firms. The higher labor productivity of larger firms might be attributed to the higher capital intensity so that they might employ less labor. Labor productivity improved over time, implying that the labour is being replaced by capital irrespective of firm size. Examination of trend growth in labour productivity revealed that it was growing significantly in the case of large (2.13%) and medium (2.55%) firms. It may be because these two categories of firms might be replacing labour with capital at a faster rate when compared to small category firms for which the growth was found non-significant.

Table 2: Performance Indicators across size and experience categories

Classification	Parameters	Profit margin	Labour productivity	Capital-output ratio	Cost per unit sale
Size					
Large	Mean	0.018	8.16	0.39	0.955
	CGR	0.005	2.13***	1.63**	-0.039
Medium	Mean	0.016	6.76	0.67	0.957
	CGR	0.138	2.55***	0.86	0.017
Small	Mean	-0.006	6.11	1.17	0.955
	CGR	0.988***	2.37	2.2	-0.360***
Experience category					
Low Experience(< 20 years)	Mean	0.012	9.23	0.58	0.957
	CGR	0.125	4.40***	2.05	-0.105
Moderate Experience (21-40 years)	Mean	0.013	6.63	0.6	0.957
	CGR	0.1	0.97***	2.21	-0.052
High Experience(>40 years)	Mean	0.03	5.29	0.54	0.949
	CGR	0.492***	1.475	0.33	-0.059

*** and **indicates significance at 1% level and 5% level of probability

However, capital-output ratio was highest in the case of small firms (1.17) followed by medium (0.67) and large firms (0.39). The capital intensity declines as the size increases. It might be because large firms may be utilizing capital more productively than smaller firms, or the capacity utilization of large firms might be higher than smaller firms. Calculation of the growth rate in the capital-output ratio indicated that it had grown significantly in the case of large firms (1.63%). In contrast, it was not statistically significant for small and medium firms. Hence it could be understood that large firms accumulated a substantial amount of capital over the years.

Lastly, the cost per unit sale was almost the same across different size categories (0.955, 0.957, and 0.955, respectively, for large, medium, and small firms). This reveals that firms incur almost the same operational cost per unit sale irrespective of size. The reason might be that the raw material expense constitutes the major expense share in operational costs, and for per unit production of output, the requirement of raw material (milk) might be the same for all firm sizes. But there is a chance of difference in total cost per unit production, which may be least in the case of larger firms due to economies of scale. Other than that rate of growth in cost per unit sale per annum was negative and significant in the case of small firms (-0.36 %) while growth was insignificant in the case of large (-0.04 %) and medium (0.01 %) firms. So we can conclude that small firms significantly reduced operational costs over the years. This might have attributed to significant improvement in profit margin in the case of small firms, as mentioned earlier.

Further, it is assumed that firms established recently may be using capital-intensive technologies compared to those established much before; hence, there are chances of differences in performance in terms of profit margin and other economic indicators for firms established in different time periods. Therefore, tabular analysis was undertaken for three experience categories, and the results are presented in Table 2. It could be observed that profitability increases as the experience of the firm increases (0.012, 0.013, and 0.030 respectively for low, medium, and high Experience firms). The reason may be apparent if we check the trend in other indicators. If we see the trend in labor productivity, we can find a reversible trend to profit margin, which means labour productivity decreases with an increase in experience (9.23, 6.63, and 5.29 respectively for low, moderate, and high experience firms). It may be due to the use of comparatively capital- intensive technologies by firms with low experience, which are new that require a smaller number of employees; hence per unit output of employees may be high. At the same time comparison of capital-output ratio across different experience categories revealed that moderate experience firms (0.60) had comparatively high capital per unit output than low (0.58) and high experience (0.54) firms. Hence, we can conclude that relatively low-experience firms use more capital per unit output than high-experience firms. Similarly, cost per unit sale was found to be lowest in the case of high-experience firms (0.949) and similar in the case of low and moderate-experience firms. From the table, we can find that firms belonging to low and moderate experience categories are more or less similar for all indicators except labor productivity. After analyzing the trend of all factors, we can conclude that the higher profit margin of high-experience firms may be due to use of low capital per unit output and low cost per unit sale compared to less experienced firms.

Analysis of the growth rate of profit margin revealed that high-experience firms registered high and significant (0.492) growth while other two categories reported almost the same growth rate (0.125 % and 0.100 % respectively for firms upto 20 years and 21-40 years), but insignificant. Labour productivity has shown an increasing trend over the years irrespective of categories. At the same time growth in labor productivity was found highest in the case of low-experience firms (4.4%) while moderate-experience firms also reported significant growth of 0.967 per cent. It may be because of the adoption of more capital-intensive technologies by these firms over the years. In the case of high-experience firms, the growth was insignificant. The capital-output ratio fluctuated over the years. It has shown an increasing trend between the first and second sub-period, then decreased in the third sub-period for all categories. The growth rate in capital–output ratio was found insignificant for all the categories. In general, cost per unit sale has shown a declining trend across all experience categories which may be due to the cost reduction strategies adopted by the firms through experience and learning over the years. It has decreased from 0.974 to 0.956 in the case of low experience, 0.968 to 0.954 in the case of moderate experience, and 0.952 to 0.947 in the case of high experience firms between 1991-99 and 2009-17. The growth rate in cost per unit sale was found to be negative in all cases but insignificant. So it can be concluded that operational cost per unit sale remained constant over the period for all experience categories.

Table 3. Summary statistics of variables

Size	Profit Margin	Share of sales	Labour Productivity	Capital Intensity	Cost per unit sale
Large	0.018	0.043	8.158	0.389	0.955
	(0.052)	(0.077)	(8.085)	(0.629)	(0.070)
Medium	0.005	0.050	7.688	0.898	0.954
	(0.131)	(0.059)	(13.349)	(3.676)	(0.116)
Small	-0.006	0.002	6.111	1.172	0.955
	(0.100)	(0.004)	(8.736)	(1.688)	(0.124)

Factors affecting profit margin across firm size categories

Having discussed the trends in selected performance indicators across size and experience of firms, to know the determinants of profit margin at firm level, panel data regression was undertaken as per size. The summary statistics of different size categories of firms are provided in Table 3.

To examine size-wise variation in determinants, analysis for each size category was undertaken for the time period, 1991-2017. From Table 4, it can be seen that numbers of firms in large, medium and small category were 44, 59 and 17 respectively. Further investigation of co-efficients across size categories indicates that in the case of Large firms, capital-output ratio, cost per unit sale and trend were found to affect profitability significantly. It is evident from the co-efficient of capital –output ratio that use of more capital per unit output reduces profit margin. The extent of reduction in profit margin for unit increase in the ratio was 0.091 units. As expected cost per unit sale negatively affects profit margin which means unit increase in cost per unit sale decreases the profit margin by -0.244 units, while profit margin increases by 0.001 units per year due to technological progress. In the case of medium firms, cost per unit sale and trend were the two variables that were found to be significant. A unit increase in cost per unit sale decreases the profit margin by -0.249 units while the trend affects the profit margin positively. Profit margin increases by 0.004 units per year in the case of medium firms due to technological progress. In the case of small firms, labor productivity and cost per unit sale were the two variables that affected the profit margin significantly. As the cost per unit sale increased by one-unit profit margin decreased by 0.63 units which were comparatively higher to the other two category firms, while labour productivity contributes positively to profit margin. That means as labor productivity improves by one-unit profit margin increase by 0.004 units. Hence, we can conclude that cost per unit sale was the most important variable that found to affect profit margin of all size categories as well as overall dairy industry. Therefore, any strategies adopt by the firms to reduce the cost may improve profit margins. The improvement in profit margin due to trend was observed to be significant only in the case of large and medium firms.

Finally, the co-efficients of random effects model for the overall industry showed that cost per unit sale and trend were the two variables that affected profit margin as in the case of fixed effects model but the magnitudes were different. We also mentioned that fixed effects model was appropriate for overall dairy industry but to introduce dummy variables random effects model was analysed thus it may suffer from bias due to omission of the individual effect. Large firm category was considered as the benchmark category, and the co-efficients of both medium and small dummies were significant. It indicates that the profit margins of medium and small-sized firms were significantly less than large firms by 0.015 units and 0.045 units. A study conducted by Kumar (2003) to analyze the economic performance of 30 dairy firms including the 5 MNC firms for the period 1991-92 to 2000-01 revealed that sales and value of the output of dairy processing firms increased during the period, and about 5.5 per cent of profit margin was due to a handful of well-performing large farms. So we can conclude that profit margin across different sizes of firms in dairy industry differed significantly and it increases with the size of the firm which may be due to economies of scale.

Factors affecting profit margin across firms having different years of experience

Table 4. Co-efficients of size wise panel data regression

Independent Variables	Size			Overall with size dummy
	Large	Medium	Small	
Share of Sales	-0.06422(0.136)	0.10788(0.094)	0.086(7.043)	0.08021(0.063)
Labour Productivity	0.00003(0.000)	-0.00003(0.000)	0.004**(0.002)	0.00001(0.000)
Capital output ratio	-0.09104***(0.026)	0.00087(0.001)	-0.012(0.014)	-0.00002(0.003)
Cost per unit sale	-0.24360***(0.086)		-0.24985***(0.090)	-0.631***(0.197) - 0.35798***(0.071)
Trend	0.00102*(0.001)	0.00367**(0.002)	0.003(0.004)	0.00139**(0.001)
Constant	0.26682***(0.083)	0.16633*(0.088)	0.502*(0.259)	0.33027***(0.070)
Medium dummy	—-	—-	—-	-0.01506*(0.009)
Small dummy	—-	—-	—-	-0.04486***(0.016)
No. of groups	44	59	17	120
No. of observations	532	506	106	1150
Model	FE	FE	FE	RE
Hausman test statistic	Chi2= 61.94; Prob>chi2=0.0	Chi2=13;Prob>chi2= 0.0234	Chi2=10.47;Prob> chi2=0.033	—-
F statistic	F(5,43)=2.73 Prob>F=0.032	F(5,58)=2.41 Prob>F=0.047	F(5,16)=35.26 Prob>F=0.000	Wald chi2= 43.36;Prob>chi2=0.000

Figures in parentheses are robust standard errors;*, **, ***indicates significance at 1% , 5% and 10% levels of probability

Table 5. Summary statistics of variables according to experience

Experience	Profit Margin	Share of sales	Labour Productivity	Capital Output ratio	Cost per unit sale
Upto 20(Low Experience)	0.011	0.022	9.32	0.58	0.957
	(0.072)	(0.039)	(9.11)	(0.99)	(0.100)
21-40(Moderate experience)	0.014	0.023	6.69	0.60	0.957
	(0.090)	(0.069)	(6.63)	(1.15)	(0.095)
>40(High experience)	0.016	0.211	5.94	0.56	0.945
	(0.066)	(0.224)	(6.19)	(0.64)	(0.076)

Figures in parentheses indicates the standard deviation

Table 6. Co-efficients of experience wise panel data regression

Independent Variables	Experience category			Overall with experience dummy
	Low	Moderate	High	
Share of sales	-0.2616(0.368)	0.460939(0.297)	-0.0136(0.045)	0.14307*(0.076)
Labour Productivity	0.0034***(0.001)	0.000002(0.000)	-0.0002(0.000)	0.00002(0.000)
Capital output ratio	-0.0002(0.011)	0.001037(0.001)	-0.0286(0.043)	-0.00022(0.003)
Cost per unit sale	-0.2777**(0.126)	-0.291118***(0.094)	-0.3377**(0.136)	-0.35033***(0.071)
Trend	0.0017**(0.001)	0.002247*(0.001)	0.0010(0.001)	0.00166**(0.016)
Constant	0.2104*(0.123)	0.231960***(0.096)	0.3404**(0.137)	0.30435***(0.000)
Upto 20	—-	—-	—-	-0.00449(0.010)
>40	—-	—-	—-	0.01516(0.009)
No. of observations	395	631	123	1150
No. of groups	42	69	9	120
Model	FE	FE	FE	RE
HausmanStatistic	Chi2=95.56; Prob>chi2=0.000	Chi2= 29.63; Prob>chi2=0.000	Chi2=12.42; Prob>chi2=0.0294	—-
F statistic	F(5,41)=26.62; Prob>F=0.000	F(5,68)=3.31; Prob>F=0.0098	F(5,8)=2.13; Prob>F=0.163	Waldchi2=36.17; Prob>chi2=0.000

Figures in parentheses are robust standard errors;***, **, *indicates significance at 1% level, 5% level and 10% level of probability

Studies revealed that there was a difference in performance in terms of the use of technology and inputs for firms established during different time periods that in turn have an effect on output and profits. Therefore, to find out the difference in determinants according to the year of incorporation of the firm, panel data regression was undertaken. The Hausman test indicated that the fixed effects model was appropriate. The summary statistics of the variables are presented in Table 5, and the analysis results are presented in Table 6.

It can be observed from Table 6 that the profitability of firms with low experience was affected mainly by labour productivity, cost per unit sale and trend. All other coefficients were found to be insignificant. Labour productivity found to affect profit margin positively, which means that as labour productivity increases by one-unit, profit margin may improve by 0.003 units. Cost per unit sale, on the other hand, affects profit margin negatively. As the cost per unit sale increases by one unit, the profit margin may decrease by 0.27 units. Trend, a proxy for technological progress, affects profit margin positively by 0.002 units per year. Cost per unit sale and trend were found to be significant for moderate experience firms also. As the cost per unit increases by one unit, the profit margin decreases by 0.29 units. Profit margin improved by 0.002 units per year due to technological progress. The model fitted was non-significant in case of high experience category, so the results cannot be interpreted. The coefficients obtained from the random effects model with dummies show no significant difference in profit margin between experience categories, even though we can observe a similar profit margin trend as in the tabular analysis case. That means compared to the moderate experience category, the profit margin of high-experience firms was higher by 0.016 units, for younger firms, it was less by 0.004 units, as observed from the co-efficients.

Conclusions

The demand for dairy products has been increasing over the years due to population increase, improvement in per-capita income, urbanization, etc. The dairy processing industry should be equipped to meet this rising demand for diversified dairy products. The profitability of this sector should be sufficient to attract new investment. In this context, this study examined the performance of the dairy processing industry in India using firm-level data obtained from CMIE. The study found that the profit

margin and labor productivity have increased significantly over the study period, while the growth in other performance indicators was not significant. The profit margin was found to be increasing with the increase in size and experience of the firms, while labour productivity was directly related to size but inversely related to experience. The lower profit margin among small firms and firms with low experience requires policy intervention to enhance their efficiency and productive capacity. The cost per unit of sale was the most determining factor that adversely affected the profit margin. At present, it is very high in all cases. Hence, any measures to reduce operational costs, including improving efficiency, will help to improve the profit margin.

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RESEARCH ARTICLE

Economics of milk production and its constraints: A case study of Himachal Pradesh

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Abstract: An attempt has been made in this investigation to work out the cost and returns from milk production across different milch species of animals. Tabular analysis was employed to work out the cost and returns while Garrett's Ranking Technique (GRT) was used to identify the major constraints in milk production. The multistage random sampling technique was adopted to select the sample of 60 dairy farmers. Average milk yield per day per animal was found to be significantly higher in the case of crossbred cows (7.06 litres) as compared to local cows (2.88 litres). Similarly, net maintenance cost per milch animal per day was found to be relatively higher in crossbred cows (₹ 200.76) as compared to local cows (₹ 113.76). The per litre cost of milk production was observed to be significantly higher in case of local cows (₹ 35.09) followed by crossbred cows (₹ 26.34). The net income per day was relatively higher in case of crossbred cows (₹ 64.62) as compared to local cows (₹ 4.29), while it was found lowest in the small herd size category for local cows (₹ 0.54). The net return from crossbred cows was more than that of local cows indicating that crossbred milking cow was more economical than the local cow in the study area. High cost of concentrate, unavailability of veterinary services and low milk productivity were the major constraints in milk production in the study area. Therefore, efforts should be made to impart knowledge to dairy farmers regarding advanced animal husbandry techniques through extension services.

Keywords: Average milk yield, Cobb-Douglas, Constraints, Extension services, Garrett's Ranking, Herd size, Milk production, Net maintenance cost

Introduction

India being a major agrarian economy has deep connection with dairy farming since the Vedic era. The dairy and livestock sector contributes 5.2 per cent to the country's GDP and employs more than 8 crore farmers directly including a very high proportion of small and marginal farmers (86.00%) (Economic Survey, 2021-22). Dairy farming in India occupies a prominent place in rural life and provides not only subsidiary occupation and nutritional standards but is also a source of organic manures and draught power (Kumari et al. 2020). It is quite interesting to note that India ranks first among the world's milk-producing nations with 23 percent of global production in the year 2022 (Economic Survey, 2021-22). Dairy is currently the top-ranking commodity in India, with a value of output of Rs. 8.39 lakh crore in 2019-20 which was higher than the combined output value of paddy and wheat during the same period (National Accounts Statistics, 2020-21). In India, the population of crossbred cows was 19.42 million in the year 2012 which increased to 25.67 million in the year 2019. In 2012, the population of indigenous cows was 48.13 million which increased to 48.51 million in the year 2019. The buffalo population was recorded at 51.05 million in the year 2012 which increased to 51.17 million in the year 2019. The population of crossbred cows, indigenous cows and buffaloes increased by 32.18, 0.79 and 0.24 per cent respectively from 2012 to 2019. While in Himachal Pradesh, the population of crossbred cows was 5.21 lakhs which increased to 6.12 lakhs in the year 2019. The population of indigenous cows in the year 2012 was 3.72 lakhs which decreased to 2.66 lakhs in the year 2019. In the year 2012, the buffalo population was 3.96 lakhs which decreased to 3.49 lakhs in the year 2019 (BAH&S, 2019). During 2012- 2019, the population of crossbred cows increased by 18.87 per cent while the population of indigenous cows and buffaloes decreased by 28.45 and 11.74 per cent respectively. Dairy farmers are drastically shifted their choice from indigenous breeds to the crossbred cow (Sharma et

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al. 2012 and Kumar et al. 2012) Rearing of livestock is an integral component of the rural economy in Himachal Pradesh which plays a vital role in improving the socio-economic conditions of rural masses (Tulachan and Neupane, 1999). In Himachal Pradesh, there is a dynamic relationship between common property resources (CPRs) such as forests, water, grazing land, livestock and crops (Economic survey of Himachal Pradesh, 2021-22). The region has a high potential for dairying because of its rich natural endowments. The study was conducted in the Shimla district of Himachal Pradesh one of the frontline districts in milk production. It holds the third position in the state in milk production with an annual output of 1.75 Lakh tonnes during 2018-19 (Statistical Abstract of Himachal Pradesh, 2018-19). Despite its increasing importance, very few studies have been carried out in the district so far for analysing dairy farming and studying intricate issues of the economics of milk production (Dogra AK, 2016). Economic analysis of milk production provides useful information with respect to the share of various input components in the maintenance cost of milch animals (Pathania and Sharma, 2016). Whether the per litre cost of milk production is profitable in dairy farming or not. The research can be of great importance to recognize the profitability of milk production in the region, as well as in other regions with similar agro-climatic conditions. It can also assist in the planning and decision-making process in determining the right plan of action for the development and expansion of dairy farming in the region.

Materials And Methods

Selection of Study area

In the present study, a multistage random sampling technique was employed for selecting the ultimate milk-producing households. Shimla district has been selected purposively for the study because it has a relatively large bovine population (2.85 lakhs) as compared to other districts of the state (Livestock Census, 2019). In terms of milk production, it is the third largest milk-producing district in the state with an annual output of 1.75 lakh tonnes which shared 12 % of the state's milk production during the year 2018-19 (Statistical Abstract of Himachal Pradesh, 2018-19). In the first stage, out of twelve blocks in Shimla, the three blocks namely; Mashobra, Rohru and Rampur were selected randomly. In the second stage, 2 gram-panchayats were selected randomly from each selected block and thereafter in the third stage, 2 villages were selected randomly from each selected gram-panchayat. Thereafter, 5 dairy farmers were drawn randomly from each selected village thus making a total sample of 60 dairy farmers.

Data Collection

The primary data were collected from the 60 sample households by conventional survey method using a well-structured schedule through personal interviews on various aspects of dairy enterprises from selected households for the year 2020-21. The collected data covered socio-economic characteristics, management practice, land-use patterns, labour use and availability, capital, output and the problems encountered by dairy farmers.

Analytical Procedures

Dairy farms based on herd size were categorized into three different categories namely; small (0-3.64 SAUs), medium (3.64-7.66 SAUs) and large (7.66 SAUs) using the cumulative square root frequency method of stratification (Singh and Mangat, 1996). Tabular analysis was applied to work out the costs and returns from milk production and the Garrett ranking approach was followed for identifying the major constraints faced by the farmers during milk production.

Standard Animal Units (SAUs)

Considering the differences in regional endowments of animal wealth and species, the dairy animals have been converted into SAUs using factors suggested by Sirohi et al. (2019) for the hilly region. In this study apart from labour utilization, the body weight of the animal was also taken into consideration for the estimation of the SAUs. Based on expert opinion, 60 % of the weight was given to labour utilization and 40 % to the body weights of animals for the final estimation. As the study area falls in the hilly region so Standard Animal Units for this region were used as given below in table 1.

Resource Productivities in Dairy Farming

To analyse the resource productivities of different farms for improving the economic conditions of the farmers and to measure the contribution of a specific factor in combination with other factors which are responsible for the change in the level of output, multiple regression analysis was used. The Cobb-Douglas type production function was used because the coefficient of determination (R^2) was recorded highest with significant t-ratios thus the model was well fitted to the data. The Cobb- Douglas production function was fitted to the data as follows:

$$Y = AX_1^{b_1} X_2^{b_2} X_3^{b3} X_4^{b_4} X_5^{b_5} \ldots\ldots X_6^{b_6} u_i$$

In logarithms, the function is of the following form:

$$\log Y = \log A + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + b_5 \log X_5$$

Where,

Y_i = Income from milk per animal per day (Rs.)

X_1 = Expenditure on green fodder per animal per day (Rs.)

X_2 = Expenditure on dry fodder per animal per day (Rs.)

X_3= Expenditure on concentrates per animal per day (Rs.)

X_4= Value of Human labour used per animal per day (Rs.)

X_5 = Miscellaneous expenses per animal per day (Rs.)

A = Intercept

u_i = Error term

Constraints in Dairy Farming

Garrett ranking approach was adopted for identifying the various constraints experienced by the farmers in existing dairy farming in the study area given by Garrett and Woodworth (1969). The prime advantage of this technique over simple frequency distribution is that the constraints are arranged based on their severity from the point of view of farmers (Zalkuwi et al. 2015). Further, in order to test the significant difference for the problem among selected farm categories, the Chi-Square test was used.

Results and Discussion

Costs and returns from milk production for local cows

It can be seen in Table 2 that the overall average daily net maintenance cost per milch local cow was worked out to be ₹ 113.76, while it was highest in the case of the large category (₹ 125.05) followed by medium (₹116.16) and small category (₹ 106.85). On average, feed cost accounted for 68.48 per cent of the gross cost in the overall category which was lower than 81.36 % as reported by Pathania and Sharma (2012) in Kangra and higher than 62.45 % of the gross cost as reported by Patil et al. (2019) in Karnataka, 55.01 per cent by Meena et al. (2010) in Rajasthan. The expenditure on dry fodder constituted the major feed cost across all the household categories which constituted 32.47 per cent of the gross cost. At overall level, labour cost accounted for 20.66 percent of the gross cost. The category-wise analysis has shown that the contribution of labour was 20.96, 21.14 and 19.13 per cent of the gross cost for small, medium and large farms, respectively. This finding is much higher than the previous studies of 12.79 per cent of labour cost as reported by Khoveio et al. (2012) in Nagaland and 8.26 per cent in Meghalaya (Singh and Chauhan 2015). The component-wise break-up of the cost of milk production indicated that for the overall category, the contribution of variable cost to the gross cost (91.47%) was much higher than the contribution of fixed costs (8.53%) which was worked out to be 91.31, 91.54 and 91.66 % for small, medium and large farms, respectively. The overall gross return per milch local cow was worked out to be ₹ 105.24 while it was ₹ 108.44, 118.32 and 127.46 for small, medium and large categories of sample farms, respectively. The gross return per milch cow was highest for the large farm category. The results revealed that milk productivity increased with the increase in herd size and it was found highest for large farms (3.23 litres) followed by medium (2.97 litres) and small farms (2.65 litres). The reason behind this could be large category farmers were feeding a higher quantity of concentrate as compared to medium and small dairy farmers. The per litre cost of milk production was found highest in the case of small (₹ 35.59) followed by medium (₹ 34.78) and large farm categories (₹ 34.58), respectively. The net returns per local cow were recorded as lowest in the case of the small (₹ 0.54) farm category followed by medium (₹ 1.89) and large (₹ 2.57) categories of sample farms, respectively. Small herd category dairy farmers were feeding less concentrate to cows which results in lower milk productivity that ultimately lower the net return from the milk. These findings are in accordance with the findings of Meena et al. (2010), Khoveio et al. (2012), Jaiswal et al. (2015) and Patil et al. (2019).

Cost and returns from milk production for crossbred cows

The overall gross maintenance cost for crossbreds was worked out to be ₹ 200.76 per day which varies from ₹ 189.53 per day for the small category to ₹ 218.73 per day for the large farm category (Table 3). Feed cost for the large herd size category (₹ 161.84) was higher as compared to medium (₹ 150.87) and small herd size category (₹ 139.93). These findings are in accordance with earlier studies carried out by Tanwar et al. (2012). The reason behind this could be increased awareness among large category farmers regarding the importance of proper feed for animals. The overall fixed cost was found to be ₹ 16.29 which varies from ₹ 16.06 for the small herd size category to ₹ 16.63 for the large herd size category. The overall fixed cost accounted for 8.11 per cent of the total gross costs. The percentage of the fixed cost was highest for the small herd size category (8.47 %) and lowest for the large herd size category (7.60%). The overall total variable cost was found to be ₹ 184.48 which varies from ₹ 173.47 for the small herd size category to for ₹ 202.10 the large herd size category. Thus, fixed cost accounted for about 8.11 per cent and variable cost accounted for about 91.89 per cent of the gross cost. Feed and fodder costs accounted for about 73.79 per cent of the total gross cost followed by labour costs of 15.95 per cent of the total gross cost. On the appraisal of per litre cost of milk production, it was found that the per litre cost of milk production was found to be 26.37 which varies from ₹ 25.96 per day for small to ₹ 26.69 per day for the small category. Therefore, it can be concluded that the cost of milk production was highest in the case of medium

Table 1: Standard animal units for Hill regions of India

Category of animals	Local Cow	Crossbred cow	Buffalo
Adult Male (≥ 3 years)	1.11	1.48	1.43
Adult female (≥3 years)	1.00	1.71	1.70
Young stock male (<1)	0.29	0.41	0.35
Young stock female (<1)	0.63	0.72	0.63
Young Stock male (>1)	0.55	0.71	0.73
Young Stock female (>1)	0.82	1.08	0.94
Heifer	0.98	1.24	1.09

Sirohi et al. (2019)

herd size categories. Though the total cost was highest for the large herd size category the cost per litre was highest for the medium herd size category. The overall gross return per milch crossbred cow was estimated to be ₹ 250.57 while it was ₹ 234.82, 256.11 and 276.23 for the small, medium and large categories in sampled farms, respectively. The gross return per milch cow was highest for the large farm category. The average daily milk yield was found to be 6.74, 7.12 and 7.68 litres for small, medium and large farms. The net returns per milch were recorded highest in the case of the large (₹ 72.85) farm category followed by medium (₹ 66.10) and small (₹59.83) categories of sample farms, respectively.

Resource productivities

The milk production function describes the input-output relationship in milk production. The cobb-Douglas production function for cattle milk was fitted and the results of regression analysis are presented in Table 4. The results revealed that the adjusted coefficient of determination value (R^2) was 0.78 indicating thereby that 78 per cent of the variation in the gross income was explained by the independent variables included in the production function model. The coefficients of green fodder and concentrate were positive and significant ($p<0.01$) with the coefficient values of 0.56 and 0.67, respectively, which shows that a one per cent increase in expenditure on green fodder and concentrate cause 0.56 and 0.67 per cent increase in gross returns keeping other factors constant. These results indicated greater bearing of green fodder and concentrate returns from milk production. This conforms with earlier studies carried out (Meena et al. 2012) in the Alwar district of Rajasthan. The labour was also positive and significant ($p<0.05$) with a coefficient value of 0.21, which showed that a one percent increase in expenditure on labour would increase gross income by 0.21 per cent respectively. The returns to scale were increasing (1.49) implying that doubling of input will result in enhancing the output by more than double. These findings are in accordance with the findings of Prusty et al. (2015) in Odisha and Pundir et al. (2018) in Gujarat.

Constraints encountered by the dairy farmers

At the overall level, the high cost of concentrate was the most severe problem and it was more prevalent in the small category with a mean Garrett score of 63.18 (Table 5). The findings of the

Table 2: Costs and Returns from Milk Production for Local Cows

(₹/animal/day)

Particular	Farm Category			
	Small	Medium	Large	Overall
Total Fixed Cost (TFC)	9.29	9.83	10.43	9.71
	(8.69)	(8.46)	(8.34)	(8.53)
Cost of green fodder (F_1)	20.55	23.33	26.06	22.63
	(19.23)	(20.08)	(20.84)	(19.89)
Cost of dry fodder (F_2)	35.78	36.99	39.56	36.94
	(33.49)	(31.84)	(31.64)	(32.47)
Cost of concentrates (F_3)	16.28	18.70	22.44	18.34
	(15.24)	(16.10)	(17.94)	(16.12)
Feed and Fodder Cost	72.61	79.02	88.06	77.90
($V_1=F_1+F_2+F_3$)	(67.96)	(68.03)	(70.42)	(68.48)
Imputed Cost of family labour (V_2)	22.40	24.56	23.92	23.51
	(20.96)	(21.14)	(19.13)	(20.66)
Miscellaneous cost (V_3)	2.55	2.75	2.64	2.64
	(2.39)	(2.37)	(2.11)	(2.32)
Total Variable Cost	97.56	106.33	114.62	104.05
($TVC=V_1+V_2+V_3$)	(91.31)	(91.54)	(91.66)	(91.47)
Gross Cost (A=TFC+TVC)	106.85	116.16	125.05	113.76
	(100.00)	(100.00)	(100.00)	(100.00)
Returns from Manure (B)	12.54	12.86	13.35	12.81
Net Cost (C=A-B)	94.31	103.30	111.70	100.94
Sale price of milk (¹ per litre)	36.13	36.67	37.15	36.52
Average milk production (litre/animal/day)	2.65	2.97	3.23	2.88
Gross Return (D)	95.74	108.91	119.99	105.24
Net Return (D-C)	1.43	5.61	8.29	4.29
Cost of milk production (₹ per litre)	35.59	34.78	34.58	35.09
Return (₹ per litre)	0.54	1.89	2.57	1.43

Figures in parentheses are percentage to total

Table 3: Cost and returns from milk production for crossbred cows (₹ /animal/day)

Particular	Farm Category			
	Small	Medium	Large	Overall
Total Fixed Cost (TFC)	16.06	16.38	16.63	16.29
	(8.47)	(8.00)	(7.60)	(8.11)
Cost of green fodder (F_1)	38.1	38.83	40.22	38.77
	(20.10)	(18.95)	(18.39)	(19.31)
Cost of dry fodder (F_2)	44.85	46.7	49.9	46.49
	(23.66)	(22.79)	(22.81)	(23.15)
Cost of concentrates (F_3)	56.98	65.34	71.72	62.89
	(30.06)	(31.89)	(32.79)	(31.32)
Feed and Fodder Cost	139.93	150.87	161.84	148.14
($V_1=F_1+F_2+F_3$)	(73.83)	(73.64)	(73.99)	(73.79)
Imputed Cost of family labour (V_2)	29.98	32.95	34.92	32.02
	(15.82)	(16.08)	(15.96)	(15.95)
Miscellaneous cost (V_3)	3.56	4.67	5.34	4.31
	(1.88)	(2.28)	(2.44)	(2.15)
Total Variable Cost (TVC=$V_1+V_2+V_3$)	173.47	188.49	202.10	184.48
	(91.53)	(92.00)	(92.40)	(91.89)
Gross Cost (A=TFC+TVC)	189.53	204.87	218.73	200.76
	(100.00)	(100.00)	(100.00)	(100.00)
Returns from Manure (B)	14.54	14.86	15.35	14.81
Net Cost (C=A-B)	174.99	190.01	203.38	185.95
Sale price of milk (₹ per litre)	34.84	35.97	36.25	35.53
Average milk production (litre/animal/day)	6.74	7.12	7.68	7.06
Gross Return (D)	234.82	256.11	276.23	250.57
Net Return (D-C)	59.83	66.10	72.85	64.62
Cost of milk production (₹ per litre)	25.96	26.69	26.48	26.34
Return (₹ per litre)	8.88	9.28	9.56	9.16

Figures in parentheses are percentage to total

Table 4: Resource productivities of dairy farms in Shimla

Particulars	Coefficients	Standard error	t stat
Intercept	1.062**	0.104	10.162
Value of green fodder) (X_1)	0.565**	0.206	2.746
Value of dry fodder (X_2)	0.011	0.064	0.172
Value of concentrate (X_3)	0.673**	0.103	6.534
Value of labour (X_4)	0.216*	0.090	2.400
Miscellaneous Expenses (X_5)	0.026	0.096	0.275
R^2	0.78		
Adjusted R^2	0.76		
Return to scale (Σbi)	1.49		
Sample size (n)	60		

Note: ** Significant at 1% level of significance and * Significant at 5% level of significance.

present study are in line with the findings of (Khoveio et al. 2012 and Lalrinsangpuii et al. 2016). The second most severe problem was the unavailability of veterinary services on time with a mean Garrett score of 57.28. It was observed that this problem was more prevalent in medium category farmers (71.04) followed by small and large category farmers with mean Garret scores of 59.31 and 23.73, respectively. These results are in agreement with the findings of Patil et al. (2009) and Dubey et al. (2013). Low milk productivity of milch animals was the third most severe problem faced by the dairy farmers on an overall basis with a mean Garret score of 51.89. This problem was found more frequently in the small category (59.74) followed by the medium (53.08) and small category (32.64) respectively. Dogra A (2016) and Kumar A (2020) also reported that the low milk productivity of milch animals was one of the major constraints in Himachal Pradesh. Next to this, lack of awareness about advanced animal husbandry techniques

Table 5: Constraints encountered by the dairy farmers

Particulars	Small		Medium		Large		Overall		Chi-Square (S«2)
	Score	Rank	Score	Rank	Score	Rank	Score	Rank	
Low Milk Productivity of milch animals	59.74	IV	53.08	III	32.64	V	52.22	III	8.23
Unavailability of veterinary services on time	59.31	II	71.04	II	23.73	IV	57.28	II	23.64**
Problem of heat detection	26.92	X	22.43	X	54.45	II	30.25	X	17.37*
High cost of concentrate feed	63.18	I	71.23	I	72.09	I	67.89	I	0.70
Lack of awareness about advanced animal husbandry techniques	56.27	III	58.39	IV	34.82	IV	53.15	IV	6.82
No sufficient water availability all the time	32.46	IX	30.61	IX	29.91	VI	31.28	IX	0.11
Lack of transport facilities	47.46	V	48.65	V	25.18	VIII	43.83	V	8.65
Low price offered for the milk	44.42	VIII	41.22	VI	42.27	III	42.80	VI	0.12
Lack of credit support	44.77	VII	35.78	VIII	27.18	VII	38.10	VIII	4.31
Inadequate knowledge about balanced feeding	47.00	VI	40.87	VII	14.09	X	38.62	VII	18.02*

Note: Figure is parentheses indicate standard error of the estimate. ** Significant at 1% level of significance and * Significant at 5% level of significance

among the dairy farmers (53.15), lack of transport facilities (43.83), low price of milk (42.80), inadequate knowledge about balanced feeding (38.62), lack of credit support (38.10), no sufficient water availability all the time (31.28) and the problem of heat detection with mean Garrett score 30.25 were the major problems encountered by the dairy farmers in the study area. These findings are in conformity with the findings of (Prusty and Tripathy, 2015). To test the significant difference for the problem among selected farm categories, the Chi-Square test was used. The chi-square value was found significant for the constraints like lower conception rate through AI (21.64), the problem of heat detection (17.37) and inadequate knowledge about balanced feeding (18.02) indicating that these constraints differed significantly among three farm categories.

In the present study on economic analysis of milk production, it was observed that the feed cost accounted for 73.79 per cent of the gross cost in crossbred cows and 68.48 per cent in local cows where concentrate formed the major constituent of the feed cost. These results are in agreement with the findings of Khoveio et al. (2012) and Mohapatra et al. (2021). The overall net returns per litre of milk were recorded highest for crossbred cows (¹ 9.16) followed by indigenous cows (₹ 1.43). These findings are in accordance with earlier studies carried out by Athare et al. (2019) and Kumari et al. (2020). The average milk production per day of milch crossbred and local cows was found to be 7.06 and 2.88 litres, respectively. The per litre cost of milk production was observed to be significantly higher in the case of local cows (¹ 35.09) followed by crossbred cows (₹ 26.34). The high per litre cost of milk may be a result of the high feed cost associated with low milk yield in the case of local cows. Therefore, there is a need to adopt scientific dairy farming practices and efforts should be made to upgrade the germplasm of indigenous cows to improve its productivity thus reducing the cost of milk production (Athare et al. 2019).

Conclusion

The study further observed that the high price of concentrate followed by, unavailability of veterinary services on time, low milk productivity of milch animals, and lack of awareness about advanced animal husbandry techniques were the major constraints faced by the dairy farmers in the study area. It can be concluded from the study that there is a knowledge gap among dairy farmers. Therefore, efforts should be made to bridge this knowledge gap and empowers the farmers with adequate information for effective and sustainable dairy farming through extension services.

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SHORT COMMUNICATION

Thermal imaging and physiological responses of crossbred goats under different housing system during hot humid season

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Abstract: In order to observe the effect of hot humid (July-August) season on physiological responses and skin surface temperature, eighteen adult female crossbred goats were selected divided equally into three groups i.e. Group I (control), Group II (modified shed) and Group III (open environment) based on their body weight. These animals were fed as per the standard feeding schedule followed at NDRI, Karnal. The average respiration rate of group I, II and III was 76.11±1.03, 67.22±0.06 and 70.49±0.61 per minute respectively during morning hours. The RR increased significantly during afternoon over morning values in all the groups due to increase of THI. The rectal temperature of goats during morning hours was 38.73±0.02, 38.57±0.03 and 38.68±0.03°C in group I, II and III respectively and no significant change in RT was observed during the afternoon. The overall lowest and highest skin temperature were recorded at the lower part of legs and ears respectively in group I and II, whereas in group III the highest skin temperatures was found to be at dorsal and lowest at ventral region of the body. The information generated may serve as normal reference values for crossbred goats under different housing conditions and may be useful for predicting the animal welfare and prognosis of diseases.

Keywords: Heat stress, Infrared thermography, Physiological responses

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Heat stress is one of the limiting factors worldwide for livestock production under changing climatic scenario. Goats are considered the best-suited animal among the farm animals to survive under tropical climatic conditions. It is well known fact that thermal stress negatively affects the productive and reproductive performance of goats by reducing feed intake, growth performance, milk production, immunity, reproductive efficiency and quality meat production. Hence, the efforts should be made for enhancing the adaptive capacity of goats to reproduce and perform better under extreme weather conditions. Tropical breeds of goat are well adapted to the different agro-climatic conditions where they have evolved and are thriving well on low quality roughages and extreme stressful conditions where high yielding exotic and crossbred animals succumb (Akinyi 2008). Therefore selection of goat breeds that are best suited to a broad range of environmental conditions is needed. Compared to another livestock species, goats acquire several unique morphological and physiological adaptive traits. Welfare of goats could be examined based on several indicators including behavioral, physiological, reproductive and productive responses. In order to understand the adaptive mechanisms of crossbred goats to heat stress, the present study was planned under different housing system during most stress period of the year i.e. hot humid season.

Eighteen adult crossbred (Alpine x Beetal) goats were selected from the Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal (Haryana) and further divided equally (6 in each group) into three groups. Group-I animals were kept under existing housing system, group-II was kept under modified shed with fan and group-III were kept in open environment. Karnal is situated at an altitude of 250 meter above mean sea level at latitude of (29°42"N) and longitude of (79°54"E). The maximum ambient temperature during summer goes up to 45°C and minimum temperature in winter comes down to 0°C with a diurnal variation of 15-20°C. The experiment was conducted during hot humid season (July and August) and physiological parameters viz. respiration rate (RR) and rectal temperature (RT) was recorded in morning (09.45- 10.15AM) and in afternoon (2.15-2.30PM). Physiological parameters were recorded using standard methods i.e. respiration rate by counting the flank movement and rectal temperature by using digital thermometer. Thermograph of

different group of animals were also taken using non contact infrared camera and body surface temperature were recorded from thermographs at different anatomical sites viz. head, dorsal and ventral region, ear, lower and upper leg of goats. The data was analyzed for mean, standard error and their significance. The environmental parameters in terms of dry and wet bulb temperature were recorded for calculation of temperature humidity index (THI) using the following formula.

THI=0.72 (Dbt+Wbt) + 40.6

Where: Dbt = dry bulb temperature in °C and Wbt = wet bulb temperature in °C

The average morning THI was lowest in modified shed (81.86) followed by control (83.53) and outside (85.28). Vaidya et al. (2017) also reported the almost similar values during July and August months. The mean ambient temperature and relative humidity recorded during the present study was higher than the thermoneutral zone established for sheep and goats by Ayo et al. (1998) and Silanikove (2000). THI is commonly used to measure the level of heat stress in dairy animals (Armstrong 1994).

Heat stress affects the physiological parameters of goat drastically that causes the huge economic loss (Panda et al. 2016). Physiological parameters viz. rectal temperature, respiration rate and heart rate are the best indicators for assessing the adaptability of goats (Al Dawood, 2017). Sanusi et al. (2011) reported the higher rectal temperature and pulse rate in heat stressed goats. Respiration rate is used as the reliable indicator of heat stress (Okoruwa 2014). During the present investigation, the average respiration rate of group I, II and III was 76.11±1.03, 67.22±0.06 and 70.49±0.61 breaths per minute respectively during forenoon. The RR was found to be lower in the group II animals which were maintained under fan in the shed followed by group III and group I. The RR increased significantly during afternoon over morning values in all the groups due to increase of THI. The results of the present study are in agreement with Hooper at al. (2018) and Alam et al. (2011) who reported higher respiration rate during heat stress in goats. Fahmy (1994) also reported higher respiration rate of goats during summer compared to winter and during walking by Khan and Ghosh (1989). When high relative humidity is combined with the high ambient temperature; there was a further increase in respiratory frequency of goats (Phulia et al. 2010; Hamzaoui et al. 2013). The severity of heat stress can

Table 1: Effect of different housing systems on physiological responses and skin temperatures (°C) at different anatomical sites of crossbred goats

Groups	Parameters	Forenoon	Afternoon	Overall Mean ± S.E
Control (Group-I)	Respiration Rate/min	76.11^{a}±1.03	89.23^{b}±0.85	82.67±1.45
	Rectal Temperature (°C)	38.74±0.02	38.83±0.44	38.78±0.41
	Skin temperature at different anatomical sites (°C)			
	Head	35.48±0.23	35.87±0.16	35.68±0.14
	Dorsal	35.87±0.64	38.50±1.94	37.18±1.05
	Ventral	34.25±0.33	35.52±0.79	34.88±0.45
	Ear	37.55±0.25	37.95±0.25	37.75±0.18
	Upper Leg	35.52±0.14	36.57±0.58	36.04±0.33
	Lower Leg	34.32±0.35	35.15±0.69	34.73±0.39
Modified shed(Group-II)	Respiration Rate/min	67.22^{a}±0.56	88.22^{b}±0.60	77.72±0.66
	Rectal Temperature (°C)	38.56±0.03	38.82±0.02	38.69±0.02
	Skin temperature at different anatomical sites (°C)			
	Head	34.42±0.35	34.90±0.17	34.66±0.20
	Dorsal	34.88±0.22	35.38±0.11	35.13±0.14
	Ventral	34.35±0.39	34.73±0.20	34.54±0.22
	Ear	37.02±0.23	37.47±0.42	37.24±0.24
	Upper Leg	34.83±0.26	35.42±0.21	35.13±0.18
	Lower Leg	33.47±0.32	34.75±0.11	34.11±0.25
Outside(Group-III)	Respiration Rate/min	70.48^{a}±0.61	91.98^{b}±0.73	81.23±0.76
	Rectal Temperature (°C)	38.66±0.02	38.67±0.31	38.67±0.16
	Skin temperature at different anatomical sites (°C)			
	Head	36.13± 0.63	40.08±1.12	38.11±0.85
	Dorsal	40.10±0.56	41.75±1.00	40.93±0.60
	Ventral	35.90±0.75	37.27±0.58	36.58±0.50
	Ear	38.28±0.43	39.95±0.28	39.12±0.35
	Upper Leg	36.87±0.66	38.93±0.71	37.90±0.56
	Lower Leg	36.30±0.73	38.73±1.17	37.52±0.75

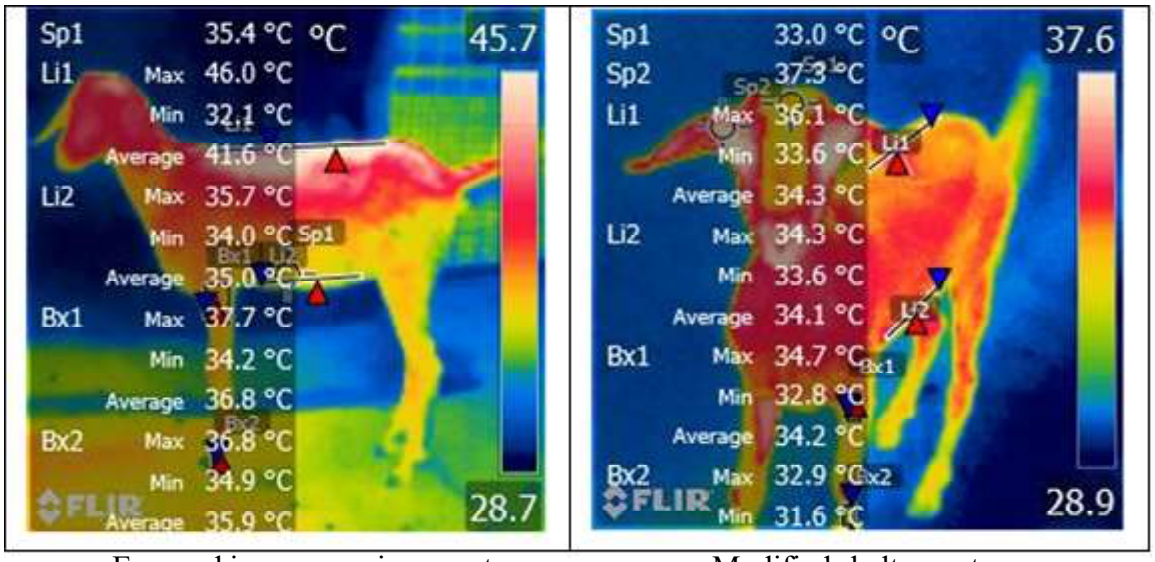

Fig. 1 Thermographs of crossbred goats in different housing systems

Exposed in open environment Modified shelter system

be categorized according to panting rate (breaths/min) (low: 40–60, medium: 60–80, high: 80–120, and severe: >200), as it appears to be the most accessible and easiest way for evaluating the impact of heat stress (Silanikove 2000). The increased respiration rate is probably indicating an effort of animals to maintain their normal body temperature by increasing their heat dissipation through evaporative cooling.

Measurement of rectal temperature is an important method for evaluation of physiological status of the farm animals as well as an ideal indicator of their adaptability to extreme climatic conditions (Sanusi et al. 2011). The rectal temperature of goats during forenoon was 38.73±0.02, 38.57±0.03 and 38.68±0.03°C in group I, II and III respectively. Slight increase in the rectal temperature of goats was observed during afternoon over forenoon values. The results of the present study are in accordance with Swenson and Reece (2006) who reported that the rectal temperature of goats varies from 38.3°C to 40.0°C and are used as an indicator of stress. No significant changes in rectal temperature of goats during present study in afternoon with the increase in THI is inconsistent with those of Fahmy (1994) and Marai et al. (2007), who reported that heat stress increased rectal temperatures in goats. The reason for the difference in the results might be due to different experimental conditions and/or breed and age of goats.

The skin surface temperature (ST) of goats was recorded at different anatomical sites during forenoon and afternoon. The skin surface temperature of all experimental groups of goats was numerically higher during afternoon compared to forenoon values. This higher skin temperature during afternoon is mainly due to increase in THI. Darcan et al. (2007) also reported the increase in skin temperature due to increase in THI. The lowest overall mean values of ST was recorded at the lower part of legs i.e. 34.73±0.39 °C, 34.11±0.25 °C in group I and II respectively, whereas in group III the lowest value (36.58±0.50 °C) was found at ventral region of the goats. These observations of lower skin temperature are in accordance with Phulia et al. 2010. Kelly (2006) also reported significant lower temperature of lower part of the legs. Whereas the highest skin surface temperature was recorded at ears and dorsal surface of the goats (Table 1). Ayo et al. (1998) and Minka and Ayo (2014) also reported the highest values on the ears region of the body. The skin temperature of head, upper part of leg was in between the temperature of ear/ dorsal and lower leg/ ventral region of the body (Table-1). These variations in the skin temperature at different anatomical sites might be due to the direct exposure of the body parts to the ambient conditions and the muscular activity of the different body parts.

Conclusion

IRT may be a useful non-invasive and accurate tool to detect the level of environmental stress and can also be used for diagnosis of inflammatory diseases. Further the results demonstrated the effects of high THI on the daily rhythmicity of physiological responses of crossbred goats. The information generated may also be beneficial for the evaluation of the welfare and productivity of small ruminants under hot humid conditions. Finally it can be stated that the crossbred goats must be protected from direct exposure to higher THI conditions.

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