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INVITED REVIEW

An overview of mechanization in *chhana* production

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Abstract: *Chhana*, regarded as the Indian counter part of soft cottage cheese is a heat acid coagulated milk product that serves as the base for a variety of milk products such as *Rasogolla, sandesh, paneer, cham cham, chhana podo, chhana kheer, kheermohan* etc. Process mechanization of *chhana* and *chhana* based sweets is of huge scope due to the growing momentum in consumer demand for Traditional Indian milk products. Strengthening the knowledge base available for the technology of production of *chhana* is required to upgrade the existing systems with process automation and control. The main objective of all new technological interventions and improved mechanization systems for *chhana* developed so far is on yielding *chhana* of defined moisture content, soft texture and uniform quality along with reducing the cost and production time. Development of an improved mechanized systems for the rapid and hygienic production of *chhana* at small scale level is an urgent need of the hour. Mechanizations in various stages of *chhana* production can be integrated to the continuous system of *chhana* production. This article highlights the technological interventions and mechanization in the manufacture of *chhana.* In addition, the effect of coagulants in the manufacture of *chhana*, nutritive value and textural attributes of *chhana* are also discussed.

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Introduction

In India about 50-55 % of the milk produced in the country is utilized for the production of traditional milk products. It implies the prominent role of traditional milk products in the economy of our country. Indigenous milk products have influenced the economic, social, cultural, nutritional and religious status of the people in the country. The potential of these traditional dairy products resulting in large production volume is attributed to simple technology, low investment, low cost of production, simple infrastructure, low operational overheads and the most interesting fact of high profit margins and established markets (Patel and Bhadania, 2012). Signiûcant R&D efforts has resulted into optimization of processing variable for mechanized production. There is urgent need to exploit the mass appeal over the product having a high profit margins as well as high export potential and to modernize this sector with innovations, mechanizations and automations to have large scale commercial production of high quality products with long shelf life.

Chhana is regarded as the Indian counter part of soft cottage cheese. *Chhana* is a heat acid coagulated product having marble white colour, spongy texture with mild acidic flavour. It is used as a base material for manufacturing a large variety of sweets such as *rasogolla, Sandesh, rasomalai , chum chum* and *chhana murki* . Cow milk is preferred for manufacturing *chhana* as the product obtained is soft with smooth texture and velvety body which are highly desirable attributes for making *chhana* -based sweetmeats particularly *rasogolla* (Minz and Singh, 2016). According to the Food Safety and Standards Regulation (FSSR 2011), *chhana* means “product obtained from cow or buffalo milk or combination thereof, by precipitation with sour milk, lactic acid, or citric acid. It shall contain not more than 70 % moisture and the fat content should not be less than 50 % expressed on dry matter”. Milk solids can also be used in *chhana* production. About 6% of the total milk production in India is converted to *chhana* through coagulation (Sahu and Das, 2007)

Dairy technologists foreseeing the potential of *chhana* and its products had undergone few works in recent years for the characterization of quality attributes of *chhana*, effect of coagulants on texture of *chhana*, sensory quality of *chhana* prepared from goat milk, development of continuous commercial manufacturing methods, development of kneader and ball former of *chhana* in *rasogolla* production etc. The mass appeal enjoyed by the diversified range of products prepared from base materials like *khoa* and *chhana* across the country enhances the commercial value of Traditional Indian Dairy Products. With progressive increase in volume of milk handled by the organized sector of the dairy industry, increasing commercial interest in *chhana* and other indigenous dairy products has been apparent in recent times. The huge demand for traditional dairy products across the country projects the need for improved process mechanization and new technological interventions in the respective area.

Design and development of mechanized systems for rapid and hygienic production of *chhana* and *chhana* based sweets at small scale level is an inevitable requirement of present day. Investigations carried out in the mechanization of *chhana* production always aimed at yielding product of defined moisture content, soft texture and uniform quality along with reducing the cost and production time. The technological aspects such as effect of type of coagulant, time-temperature combination and dilution on the texture, yield and quality of *chhana* produced were studied through various works on *chhana* production. The knowledge base attained through those studies can be used as inputs in the future attempts to develop improved mechanized systems of *chhana* production.

Factors affecting quality of *chhana*

Temperature, pH of coagulation, method of straining, fat level of milk and homogenization are some of the factors that affect physical quality of *chhana* (Kundu and De, 1972). Generally, cow milk is preferred for *chhana* preparation because it produces good quality *rasogolla*, with soft, smooth and spongy texture. *Chhana* produced from buffalo milk is hard and greasy and cannot be used for *Rasogolla* preparation (De and Ray, 1954). Several attempts were made to optimize the process parameters in order to utilize buffalo milk for production of *chhana* in the preparation of sweets as buffalo milk constitutes more than 55% of total milk production of India (IDF, 2008). Aneja et al. (1982) suggested that dilution of buffalo milk with 25% water prior to coagulation improved the softness of *chhana*. Even though yielded limited success the other suggested measures include coagulation at low temperature, addition of sodium citrates (Jagtiani et al. 1960), homogenization (Soni et al.1980; Ahmed et al. 1981). Significant improvements in buffalo milk *chhana* was observed with altering certain processing parameters like dilution of milk with 25% water, adjustment of fat content in milk to about 4.0 to 4.5 %, adding of 0.05 % sodium citrate prior to boiling, homogenization at 140 kg/ cm^2 and coagulation with 1.0 % citric or lactic acid solution. The body, texture, and flavor of sweets manufactured from *chhana* was improved with the use of calcium lactate as coagulant (Rajorhia, 1987). Maximum yield of good quality *chhana* could be obtained at 80°C temperature in a minimum of 15 sec (Aneja et al. 1982). Kundu and De (1972) reported that in order to obtain desirable body and texture of *chhana* the pH of coagulation should be 5.4, the temperature of coagulation should be 82°C and the coagulation time range from 0.5 to 1 min. The influence of process of homogenization is highly significant in retaining higher moisture content in the product as well as in reducing the hardness value to a minimum. The process results in a considerable increase in % moisture content, yield, and milk solids recovery.

Effect of different coagulants on the quality attribute of *chhana*

The technological knowhow regarding the coagulation step in the manufacture of *chhana* is therefore inevitable for the mechanization and continuous production of *chhana*. Studies have revealed the effect of different coagulants in the production of *chhana*. Banker et al. (2016) reported that *chhana* prepared by using 1% citric acid recorded significantly highest yield followed by 2 % lactic acid. The superior scores for overall appeal and color of the product was for *chhana* prepared from 1% citric acid and 2% lactic acid. Attempts were also made to prepare *chhana* from non-conventional organic coagulants like tartaric acid, fumaric acid, and acetic acid. It was found that the organic acid coagulants resulted in a chewy, gummy, hardy, elastic and cohesive *chhana* which is more suited for preparation of dry *chhana* sweets (Bandyopadhyay et al. 2006). Lactic acid used as milk coagulant recorded higher yield, total solids, fat, acidity, fat protein ratio, milk solid: sugar ratio but lower sucrose content compared to other coagulants in sandesh preparation (Singh and Ray, 1977). Technological interventions by the utilization of buffalo milk in preparation of *chhana* and *rasogolla* were also attempted by several workers as buffalo milk contributes more than 57% of total milk production of India (IDF, 2008). Kumar et al. (2015) reported that *chhana* prepared from admixture of sweet cream butter milk and buffalo milk using citric acid as coagulant was found most suitable for *rasogolla* making on the basis of textural and sensory characteristics. Sandesh of acceptable quality also was prepared from buffalo milk by standardized procedure (Sanyal et al. 2011).

Nutritional value of *chhana* and its enhancement in *chhana* based sweets

Chhana is an ample source of fat and protein. *Chhana* has a superior nutritive value attributed to the presence of whey proteins that are rich sources of essential amino acids. It is also a good source of fat soluble vitamins A and D and some minerals especially calcium and phosphorous. About 90% of fat & protein, 50% ash &10 % lactose of the original milk is retained in *chhana*. It possesses a nutty flavor with slightly sour and sweet taste

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which makes it palatable to Indian palate. It is an ideal food for expectant and nourishing mothers, infants, growing children, adolescents and adults. The high protein and fat content along with low sugar content makes it a suitable food product for diabetic patients (De, 1980). Preparation and optimization of procedure for *chhana* podo was also done using buffalo, coconut and soy milk by response surface methodology. Soy milk contains a higher protein content than buffalo milk that adds to the nutritive value of product. The product can be successfully prepared and highly rated in organoleptic evaluation (Kumar and Singh, 2017). Herbal Sandesh was prepared with incorporation of different herbs possessing good antioxidant property. The study determined the total oxidative status by Randox method (Bandyopadhyay et al. 2007).

Textural attributes for *chhana* and *chhana* based sweets

Springiness of *chhana* is an important textural attribute in order to ascertain the syrup retention power and sponginess of *chhana*. Springiness of *chhana* can also be better defined as the extent to which *chhana* ball regained its shape when an applied force was removed from it. It also aids to establish distinct rheological differences between cow's and buffalo's milk *chhana* and to study the extent to which buffalo milk could, be adapted for *chhana* making. Gera (1978) developed an instrument for measuring the springiness of *chhana.* Springiness was observed the maximum at pH 5.7 for mixed and buffalo milk *chhana,* but pH 5.1 for cow milk. Springiness and softness of *chhana* were influenced by the temperature of coagulation. At higher temperature of coagulation, higher viscosity was evident whereas density remained unaffected. The hardness and viscosity of *chhana* were increased at lower pH of coagulation (4.6). Density of *chhana* was highest at pH 4.6. Worker also observed that higher viscosity of *chhana* was obtained with the use of citric acid as a coagulant. The type of coagulant causes slight variation in the compactness of *chhana.* The relationships between the composition, texture and microstructure of *chhana* and *rasogolla* were analyzed. *Chhana* contained a significantly higher proportion of fat, protein, lactose and minerals than *Rasogolla*. Cooking of *chhana* in 60% sucrose solution introduced sucrose and changed the texture and structure to that typical of *rasogolla*. As *chhana* was transformed to *rasogolla*, the Instron textural properties, hardness, gumminess and chewiness, fell significantly, whereas springiness increased dramatically (Adhikari et al. 1992). Textural attributes of *chhana* kheer prepared using three artificial sweeteners was studied by Gautam and his coworkers (2013). Aspartame and acesulfame-K at the level of 0.015% and sucralose at the level of 0.05% were used. Results demonstrated that increase in levels of acesulfame-K resulted in superior texture scores. The effects of time and temperature on the moisture content, oven spring, colour, texture and crumb grain characteristics of *chhana* podo during baking was studied (Kumari et al. 2015)

Shelf life and Packaging

Chhana has a shelf life of 3 days at 24°C and 6 days at 10°C. Refrigerated storage of *chhana* is needed to maintain the quality of *chhana* in most of the seasons across the country. Jagtap et al. (1973) reported that the keeping quality of *chhana* under ordinary packing is on average 2, 3, and 12 days at 37°C, 24°C, and 7°C respectively. Butter paper coated with sodium propionate solution prior to packaging can also extend the shelf life of *chhana.* Development of suitable packaging is essential for extending the shelf life of *chhana* and *chhana* based products . Heat sealable laminates, LDPE or transparent cellulose film and poster paper or aluminum foil may be used for packaging and storage of *chhana.* Oxygen scavengers, antimicrobial films, and gas impermeable packages emerged as a part of advancements in active packaging aids in keeping food fresh and safe. It retains food taste, color and preserves nutritive value of food products by effectively removing oxygen from the interior packaging environment.

Mechanizations in *chhana* production

The traditional method of *chhana* production is batch wise, manual, labor intensive, unhygienic and time consuming. Several studies were conducted for mechanization of various steps in *chhana* and *chhana* based sweets production. Mechanizations incorporated by various workers needs to be integrated for development of continuous system for *chhana* production.

Mechanized designs for coagulation step in *chhana* production

A continuous coagulator based on the principle of Transverse Jet Mixer-Reactor (T.J.M.R.) with di/D ratio of 0.125 was designed and developed by Patel (1998). Curdling occurs within 60 cm length of mixing point after instantaneous mixing of heated milk and acid. It has significantly helped in reducing the time required for coagulum formation. A laboratory scale continuous *Chhana* making machine with milk handling capacity of 60 l/h was designed by Singh (1994). Coiled tube heat exchangers were used for heating and cooling of milk. The acidulant flow rate through an electrically heated 'U'-tube type heat exchanger was 0.2 l/min. Both milk and acidulant were fed perpendicular to the axis of the coagulation column from the bottom and coagulation was achieved at 70°C in the coagulation column (diameter 45 mm and height 800 mm).

A continuous heat acid coagulation unit with a vaccum assisted strainer was developed for *chhana* production. The moisture content of the *chhana* obtained in the unit was 0.583 kg per kg milk whereas the yield of *chhana*was 0.203 kg per kg milk, when the total milk solid in the milk used for coagulation was 0.141 kg per kg milk. The total milk solid recovery in *chhana* was 0.602 kg per kg milk solid when holding time of *chhana* was 135s and 680 mm Hg vacuum was maintained inside the vacuum chamber. The major advantages of the developed set up includes reduction in

time and energy, good solids recovery, improvement in quality and reduction in microbial contamination (Sahu and Das, 2009).

Mechanized designs for draining of whey in *chhana* production process

Batch wise whey drainage using muslin cloth manually is a time consuming and unhygienic operation. So, mechanization is required for the manufacture of uniform quality traditional dairy products rapidly and hygienically. Draining of whey from coagulated curd was performed in two stages in the continuous *chhana* making machine developed at NDRI, Karnal by Aneja et al. (1977). First and second stages comprised of a stationary double jacketed inclined sieve and a slow moving conveyor covered with a muslin cloth respectively. *Chhana* with 55% moisture content was obtained at the end of the process. In order to facilitate the continuous dewatering of *Chhana* coagulum, an endless conveyor filter was developed by Sinha (2000). For continuous dewatering the use of endless conveyor with nylon filter of 28, 40 and 50 mesh size with dewatering period of 10, 20 and 30 min, were studied. The milk flow rate was found to be 125-135 l/h for acid flow rate of 45 l/h (0.6% citric acid solution) in order to achieve a pH of 5.4 in whey. The average moisture content was found to be 56.14 percent and average penetrometer reading was found to be 134.37 at measuring temperature of 37 °C. The filter medium with 50 mesh with10 min holding was found to be most suitable to produce *chhana* of optimum quality on this system.

Mechanized system for compaction of *chhana* into paneer

Impact type device for continuous production of paneer from *chhana* was developed by Das and Das (2009). Channa was filled in rectangular cages made screen for pressing, and the cages were subjected to impact forces. The overall amount of energy imparted to *chhana* during impacts was associated with moisture loss, increased hardness of pressed *chhana*, and the solid extracted from pressed *chhana* by whey. The rate of change in moisture content, hardness, and solid degradation with the imparted energy was observed to obey the kinetics of first order reaction. An impact type prototype has provision to remove compacted *chhana* blocks at regular intervals. Number of other researchers have made efforts for mechanized production of paneer (Halder et al. 2011; Halder et al. 2012; Chitranayak et al. 2017a; Chitranayak et al. 2017b)

Production of *chhana* from ultra-filtrated retentates

Chhana was successfully manufactured from ultra-filtrated retentates. Ultrafiltration aids in concentration of milk and removal of most of soluble solids. Study focused on ultrafiltration behavior of pasteurized whole milk versus severely heated whole milk, the flux, energy requirement for concentration and retention coefficients. An increase of 31.4% in the yield of *chhana* on product basis and of 16.4% on dry matter basis was achieved (Sachdeva and Reuter, 1991). Only 4.35 kg of milk was used to produce 1 kg *chhana* by the ultrafiltration method against 5.7 kg of milk by the conventional method. Process automation and control are the benefits of the process.

Mechanized designs for production of *chhana* based sweets

Choudhary et al. (2002) developed an equipment for continuous production of *chhana* balls for production of *Rasogolla* and other similar products. The system comprises of two parts: *chhana* kneading and ball shaping unit. *Chhana* obtained after heat acid coagulation of milk is kneaded in a screw arrangement. From this unit, the homogenously kneaded *chhana* is cold extruded in cylindrical shape through a die. A knife cutter cuts the cylindrical *chhana* mass into small pieces. The cylindrical cut *chhana* pieces fall into the ball-forming unit's inlet hopper. The cylindrical *chhana* pieces are rolled into spherical ball by a cylindrical gyration unit. Gyration unit has mechanism that to provides translatory motion in the horizontal and vertical axis while preventing the device from spinning. Capacity of this unit is 50 *Chhana* balls per minute or 3000 balls per hour (Choudhary et al. 2006).

Systamatic efforts were made by Karunanithy et al. (2007 a, b & c) to mechanize unit operations in *rasogolla* making for its continuous production. Kneading of *chhana* is an important step in preparation of *rasogolla*. In conventional method it is carried out by hands. *Chhana* has to be manually kneaded and consistent quality of *rasogolla* sometimes becomes difficult to achive. A kneader was developed for kneading of *chhana*. Experiments were conducted in four peripheral velocity (53.41, 66.76, 80.11 and 93.46 cm / s) using three different rotors. Kneading efficiency was determined in terms of degree of mixing, mixing index uniformity, increase in temperature and diameter expansion. Best results were obtained for kneading of *chhana* at 93.46 cm / s peripheral speed. The kneaded *Chhana* was formed manually into 2.5 g lumps. About 150 lumps (375 g) for producing spherical balls were fed into the ball former. The oscillator required 15 min to convert lumps into spherical balls. The variable parameters were : SS smooth, perforated and acrylic surface with 200 and 250 strokes / min, 5 and 10 cm stroke lengths, 0% and 5% slope as ball forming zones. Spherical form by sphericity was judged. Results established that good quality *chhana* ball could be prepared at 200 strokes / min, a stroke length of 5 cm on the flat SS surface of 0 percent slope. Sphericity values of *chhana* ball and *rasogolla* was 0.880 and 0.876, respectively using the mechanized system. On basis of parameters such as percentage of absorbed sugar syrup (121.28%), porosity (44.13%), expressible juice (49.43%), volume expansion (2.644) and overall acceptability (88.60), quality of *rasogolla* were found best at 200 strokes/min and 2.5 cm stroke length with 0% slope in SS plain surface. *Rasogolla* prepared using the mechanized system were comparable to the control and market samples.

Kumar and Das (2003) optimized the processing parameters viz. mixing, kneading and cooking of *chhana* and sugar mixture for the mechanized production of *sandesh* from cow milk. Kumar and Das (2007) subsequently developed a single-screw vented extruder for cooking of *chhana* and sugar mixture that can be integrated with the mechanized method for the continuous production of *sandesh* from cow milk. Studies undertaken on production of *rasogolla* by pressure cooker method revealed the influence of factors like moisture content, fat level and pH in milk or *chhana* on production of *rasogolla*. Initial moisture content in *chhana* is a critical factor in determining the quality of *rasogolla*. In order to produce a good quality *rasogolla* the moisture content in *chhana* should be between 50 to 60%. There is an increase in loss of fat in cooking syrup with respect to the increase of fat in milk or *chhana*, even though the rate of fat loss was less compared to traditional method (Bhattacharya and Raj, 1980). Studies were also conducted on optimization of process parameters for the preparation of *rasogolla* under atmospheric pressure conditions using genetic algorithm. The relation between the fourteen independent parameters that influence the quality of *rasogolla* and dependent parameters were found using neural network modeling (Mohanta and Srivastava, 2014).

Conclusions

Design of various improved mechanized systems for production of *chhana* and *chhana* based sweets in small scale level have huge scope and relevance as it facilitates the rapid, hygienic and effortless production of uniform quality soft textured *chhana* and *chhana* based sweets. Since majority of the production of *chhana* is in unorganized sector, mechanization is a need of the hour to preserve the hygiene, quality and texture of *chhana* and *chhana* based sweets. The available knowledge base can provide inputs to the future research in mechanization of *chhana* and *chhana* based products. However, elaborate studies are required to develop mechanized systems for rapid and hygienic production of *chhana* at small scale level. A concerted effort for mechanization in *chhana* production incorporating the recent upgraded technological knowhow is an inevitable area of future research.

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RESEARCH ARTICLE

Measurement and prediction of thermal properties of *pantoa* during deep-fat frying

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Abstract: Thermal properties of *pantoa* were determined during deep-fat frying (DFF) in sunflower oil at 125-145°C. Thermal conductivity (k: 0.357 to 0.199 $Wm^{-1}K^{-1}$), thermal diffusivity (α: 0.105×10^{-6} to 0.140×10^{-6} m^2s^{-1}) and volumetric specific heat (C_p: 3.275 to 1.401 $MJm^{-3}K^{-1}$) were measured simultaneously using dual probe heat pulse technique. Thermal conductivity and volumetric specific heat of *pantoa* decreased as the moisture content decreased, while thermal diffusivity increased with the decrease in moisture content. Empirical models were also developed as a function of moisture content, fat content and temperature of frying of *pantoa*. It was found that moisture content was inversely related to fat content during the progress of frying. The thermal properties and the developed models would be useful in the mathematical modeling of heat transfer in *pantoa* during DFF.

Keywords: Dairy product, Deep-fat frying, *Pantoa*, Thermal conductivity, Thermal diffusivity, Volumetric specific heat

Introduction

Nowadays, 'sweetmeats' in which heat-desiccated milk solids (*khoa*) or heat-acid coagulated milk solids (*chhana*) play an important role are very popular. *Pantoa*, a traditional sweetmeat based on *chhana*, finds its origin in the Eastern parts of India. It is similar to *gulabjamun* of North India in flavour and appearance but differ in its textural attributes. *Pantoa* is prepared by blending *chhana*, sometimes admixed with *khoa*, with *maida* (refined wheat flour), baking powder and other optional ingredients followed by deep-fat frying (DFF) in *ghee* or vegetable oil and soaking in sugar syrup. *Pantoa* is typically characterized by a light to dark brown crust and creamish white soft core. The product has optimum sweet nutty flavour with a firm body and moderately chewy texture (Neethu et al. 2015).

The thermal properties of the food material vary continuously due to moisture loss, oil uptake and temperature change during DFF. Thermal conductivity, thermal diffusivity and specific heat are the primary thermal properties of food materials. Thermal conductivity can be defined as the amount of heat transferred by conduction through unit cross section area per unit time due to unit thermal gradient existing perpendicular to the area. The importance of thermal conductivity is to predict or control the heat flux in foods during processing operations such as frying, cooking, drying, pasteurization, sterilization, freezing, etc. Thermal diffusivity indicates how fast heat propagates through a material while heating, frying, canning or cooling and is used to calculate time-temperature distribution in the material. Specific heat is the ability of a food product to store heat relative to its ability to conduct (loss or gain) heat. It is based on how much energy is needed to raise the temperature. Thermal properties of foods are important for the design of optimal processing systems, modeling processes, prediction and control of changes that occur in foods during thermal processing and in calculation of energy requirement. Sensory attributes of foods and energy savings during processing are also affected by thermal properties in addition to processing and preservation.

Experimental studies on determination of thermal properties of fried foods such as meat balls (Ateba and Mittal 1994), tortilla chips (Moreira et al. 1995b), sausages (Dincer and Yildiz, 1996), potato (Sahin et al. 1999), pastry (Williams and Mittal 1999), shrimp (Ngadi et al. 2000), chicken slabs (Vélez-Ruiz et al. 2002), *tofu* (Baik and Mittal 2003) and donuts (Vélez-Ruiz and Sosa-Morales 2003) were stated in literature. However, no study has been reported on thermal properties of *pantoa* during DFF. Information on thermal properties of *pantoa* is significant for modeling and simulation of heat transfer during DFF. Therefore, the present

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study was carried out to determine the thermal properties of *pantoa* during DFF and to develop empirical models for the thermal properties of *pantoa* as a function of frying conditions, moisture and fat contents.

Materials and Methods

Preparation of pantoa

Pantoa was made according to the procedure outlined by Neethu et al. (2015). *Khoa* and *chhana* were blended in 4:5 ratio along with other ingredients, such as, refined wheat flour (3%), semolina (3%), arrowroot powder (3%), ground sugar (0.7%) and baking powder (0.3%) to a smooth and homogeneous dough. *Khoa* and *chhana* were prepared according to the method described by Rajorhia and Srinivasan (1979) and Kumari et al. (2015), respectively. Exactly 15 g of dough was taken and rolled into balls and deep-fried in sunflower oil at temperatures of 125, 135 and 145°C for 8 min in a mini-master electric fryer (Model: CMF-6/I/E, Continental Equipment India Pvt. Ltd., Bengaluru).

Analysis of samples

During the progress of frying, *pantoa* samples were taken once in 60 s for the analysis of moisture content, fat content and thermal properties.

Determination of moisture content

Fried *pantoa* ball was mashed using pestle and mortar. Exactly 5 g of the mashed *pantoa* was taken and the moisture content of *pantoa* was determined using the method prescribed for hard cheese (AOAC, 2000).

Estimation of fat content

About 1 g of the ground sample was transferred to 100 mL beaker. The fat content was determined using the Mojonnier fat extraction apparatus as explained in BIS (1981). Ten mL concentrated hydrochloric acid was added to the samples for digestion followed by the addition of 10 mL of ethyl alcohol. Fat was extracted with 25 mL each of diethyl ether and petroleum ether. The extraction was repeated 3 times and the superficial layer was decanted to a beaker of predetermined weight. The solvents were evaporated completely in a water bath. The residue obtained was dried in hot air oven at 100±2°C for 1 hour.

Measurement of temperature

The thermocouple probes were used to measure the temperature of the frying oil and core temperature of *pantoa* which in turn was connected to a digital temperature indicator (Sigma Automation, Bengaluru, India).

Measurement of thermal properties

KD2 Pro thermal properties analyzer (Decagon Devices Inc., Pullman, Washington, USA) with dual needle SH-1 sensor was used to determine the thermal conductivity, thermal diffusivity, volumetric specific heat and thermal resistivity simultaneously. The device works on the principle of line heat source probe theory of transient heat transfer analysis. One needle has a line heat source and the other is a thermocouple. The gap between the thermocouple and the needle is filled by an epoxy material of high thermal conductivity. When electric current is passed through the heater, the probe gets heated at a constant rate and the temperature of the probe inserted at the centre of the food sample was monitored over time. The thermal properties are calculated by observing the dissipation of heat from a line heat source at a known voltage. The length and diameter of the needle are 30 and 1.3 mm, respectively with the spacing of 6 mm. The measurement ranges are: thermal conductivity: 0.02 to 2 $Wm^{-1}K^{-1}$; thermal diffusivity: 0.1 to 1 mm^2s^{-1} and volumetric specific heat: 0.5 to 4 $MJm^{-3}K^{-1}$ with the accuracy of 10%. The SH-1 is well-suited for solid and granular materials.

The thermal properties of *pantoa* were measured after cooling the samples to ambient temperature. The probe was pierced into the centre of *pantoa* and kept undisturbed while measuring. The probe was calibrated using a two-hole Delrin block method, before conducting the actual trials.

Statistical analysis and modeling of thermal properties

All the experiments for each treatment were conducted in triplicate. Two-way analysis of variance (ANOVA) was done to determine if there is a significant effect of different frying temperature (p d" 0.05) on thermal properties using SPSS (v.15.0) (SPSS Inc., Chicago, IL, USA). Statistical analysis was conducted to study the effects of frying time and temperature, moisture content and fat content on thermal properties. A multiple linear regression model was formulated for predicting the thermal properties of *pantoa* in similar conditions. Adjusted coefficient of determination (Adjusted R^2) was used to evaluate the adequacy of the models.

Results and Discussion

Relationship between moisture content and fat content

The correlation between moisture and fat contents of *pantoa* during frying is shown in Fig. 1. With the progress of frying time the moisture content decreased whereas the fat content increased with increase in frying temperatures. Thus, the moisture and fat contents were negatively correlated. Similar trend was observed by other researchers (Gamble et al. 1987; Rice and Gamble, 1989; Moreira et al. 1995a; Shih et al. 2001; Garayo and Moreira, 2002; Vélez-Ruiz et al. 2002; Nema and Prasad, 2004; Tungsangprateep and Jindal, 2004; Budžaki and Šeruga, 2005; Tan and Mittal 2006).

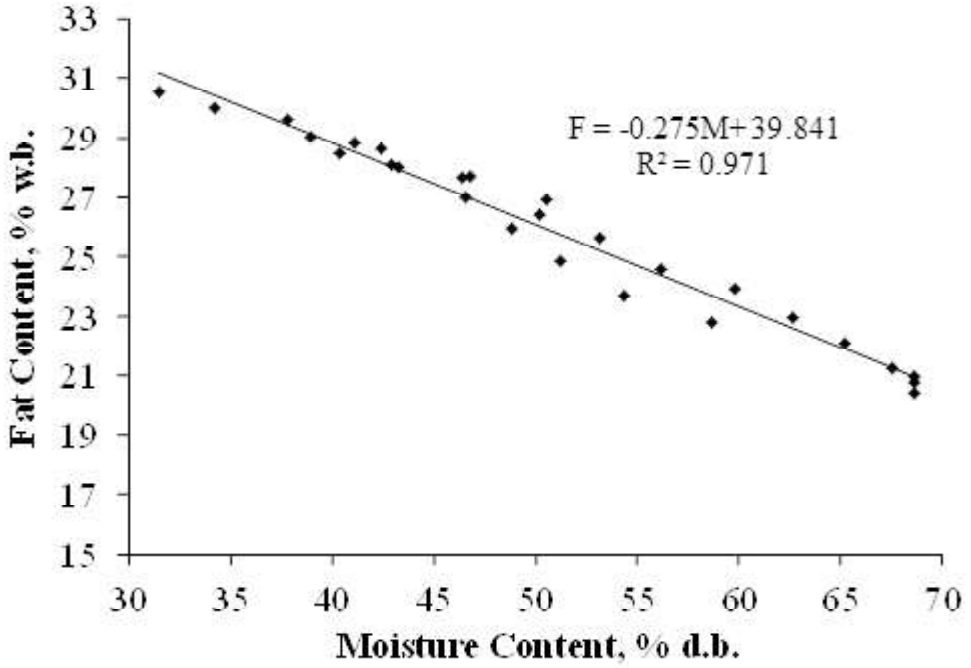

Fig. 1 Relationship between moisture content and fat content during DFF of *pantoa*

Fat content was predicted as a function of moisture content as given in Eq. 1.

$$F = -0.275M + 39.841 \quad \text{Eq. (1)}$$

where 'F' is the fat content (% w.b.) and 'M' is the moisture content (% d.b.). The correlation coefficient between moisture and fat contents was -0.986.

As soon as the *pantoa* was immersed in hot oil, surface heating occurred by natural convection between the hot oil and the product. Then, the moisture was released as a stream of steam bubbles and the moisture content started to drop rapidly. The outer surface became dry with the formation of crust providing a diffusion gradient. The oil gets absorbed into the crust and migrated into the core. Similar mechanism was observed in potato chips during frying (Nema and Prasad 2004).

Thermal conductivity (k)

Fig. 2 depicts the thermal conductivity of *pantoa* samples measured at different frying time-temperature combinations. As frying time and temperature increased, the thermal conductivity decreased. After 480 s of frying, it decreased to 0.232, 0.231and 0.214 $Wm^{-1}K^{-1}$ at 125, 135 and 145ºC, respectively, from 0.355 $Wm^{-1}K^{-1}$. The drop in moisture content, increased oil uptake and higher porosity might be the reason for decrease in 'k' values with progress of frying. Similarly, thermal conductivity of *chhana* varied from 0.331 to 0.442 $Wm^{-1}K^{-1}$ in the temperature range of 40-47°C, moisture 52-59%, fat 20-25% and protein 17-25%. It increased with increase in temperature, moisture and protein content in *chhana*. The thermal conductivity was inversely proportional to fat content (Nayak and Sawhney, 2004). The thermal conductivity of *khoa* with moisture content of 32-68% and temperature range of 10-90°C varied between 0.259 and 0.473 $Wm^{-1}K^{-1}$. Thermal conductivity of *paneer*, with the moisture content of 41-57% and temperature range of 6-40°C was found to be in the range of 0.212 to 0.353 $Wm^{-1}K^{-1}$ (Jayakumar, 1998). Our results were consistent with the findings of Moreira et al. (1995b) for tortilla chips. The thermal conductivity of tortilla chips reduced from 0.23 to 0.09 $Wm^{-1}K^{-1}$ when fried at 190ºC in soybean oil. Baik and Mittal (2003) observed that the 'k' of *tofu* ranged from 0.24 to 0.43 $Wm^{-1}K^{-1}$ at 5-80ºC. Fat acted as insulator and reduced heat conduction, thereby lowering the 'k' values (Ngadi et al., 2000). Heldman and Lund (1992) postulated that thermal conductivity was a function of moisture and structure of a food product.

Multiple linear regression analysis was done to formulate a model for thermal conductivity of *pantoa* incorporating frying time, frying temperature, moisture content and fat content as independent variables. The tests of significance for frying temperature and time were insignificant, and hence, were removed from the model. In fact, the adjusted R^2 of the multiple regression model improved, when temperature and time were excluded from the model (Eq. 2). The underlying basis for the lack of temperature effect could be due to the influence of 'k' of water. This was also stated by Sweat (1975), who concluded from several models that temperature had little effect on 'k'. The reduced multiple linear regression model for thermal conductivity of *pantoa* as a function of moisture and fat contents is presented below.

$$k = 0.513 - 0.012F + 0.001M \quad (\text{Adjusted } R^2=0.965) \quad \text{Eq. (2)}$$

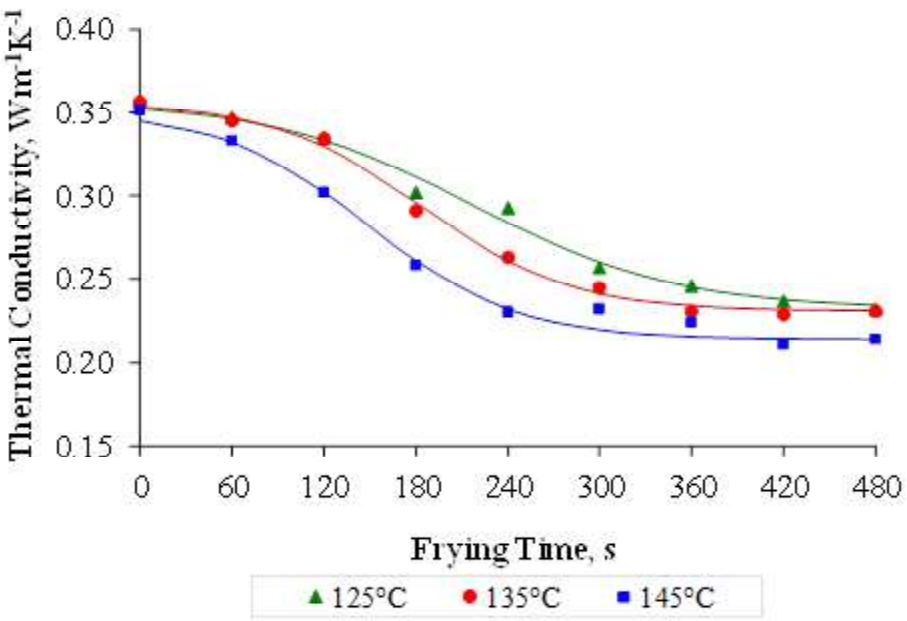

Fig. 2 Changes in thermal conductivity of *pantoa* at different frying conditions

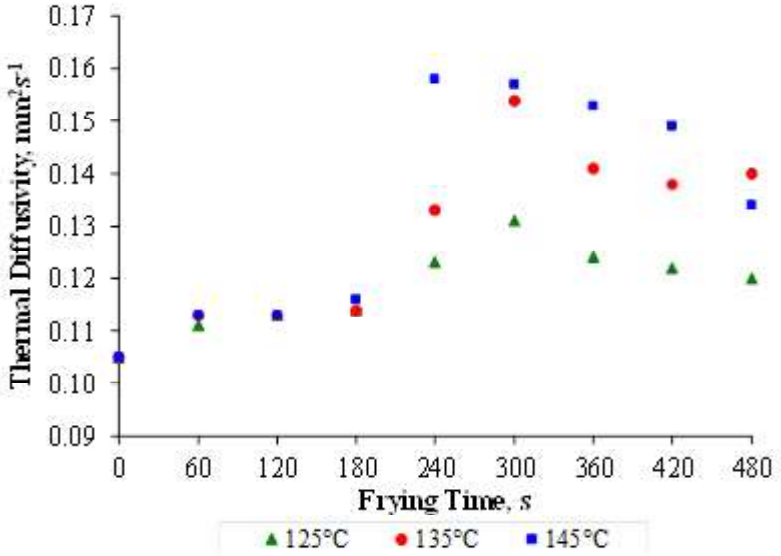

Fig. 3 Changes in thermal diffusivity of *pantoa* at different frying conditions

where 'k' was the thermal conductivity ($Wm^{-1}K^{-1}$), 'F' was the fat content (% w.b.) and 'M' was the moisture content (% d.b.). A better and elaborative model was developed (Eq. 3) by considering the core temperature of *pantoa*, oil temperature, fat and moisture contents.

$$k=0.324+0.002M-0.001CT-0.001OT \quad \text{(Adjusted } R^2 = 0.981; R^2 = 0.983) \quad (3)$$

where 'k' was the thermal conductivity ($Wm^{-1}K^{-1}$), 'M' was the moisture content (% d.b.) and 'CT' and 'OT' were the core and oil temperatures, respectively. Among all the factors, moisture content of the product had the strongest influence on 'k' value.

It is well-known that the thermal conductivity of water is the highest among all food constituents such as fat, protein, carbohydrate and ash while that of fat is the lowest. The thermal conductivity of water is nearly 4 times as that of fat at the same temperature (Choi and Okos, 1986; Rahman, 1995). There was negative correlation between thermal conductivity and fat content. This could be the reason for decrease in thermal conductivity of *pantoa* during DFF.

Thermal diffusivity (α)

The thermal diffusivity of *pantoa* determined at different frying time-temperatures is illustrated in Fig. 3. The thermal diffusivity of *pantoa* increased as frying temperature increased. Thermal

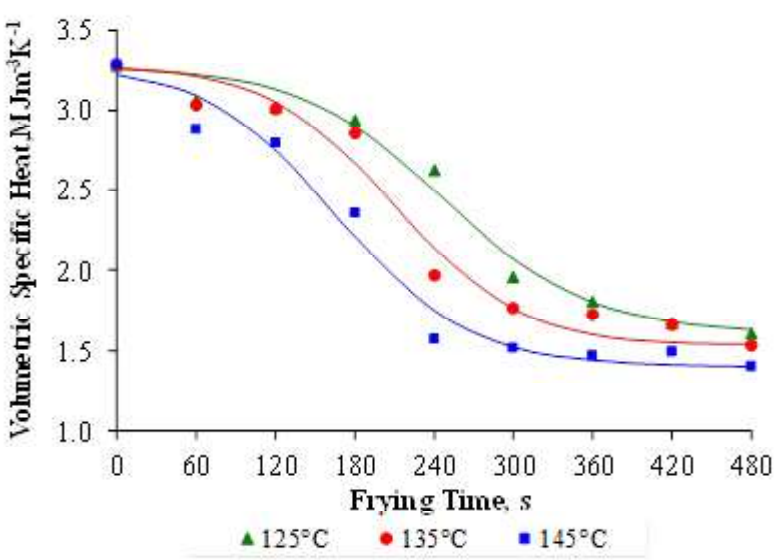

Fig. 4 Changes in volumetric specific heat of *pantoa* at different frying conditions

diffusivity increased from the initial value of 0.105 mm^2s^{-1} to 0.120, 0.140 and 0.134 mm^2s^{-1} up to 300 s, respectively at 125, 135 and 145ºC (Fig. 3). Thereafter, it decreased gradually till the end of frying regardless of the temperature. The changes in thermal diffusivity followed second order polynomial equation with R^2=0.82 at 125ºC, R^2=0.72 at 1235ºC and R^2=0.77 at 145ºC and mean RMSE value of 0.008. These results were coherent to the results of Vélez-Ruiz et al. (2002) during frying of chicken slabs at 130-150ºC. However, in the case of donuts, an increase in the 'α' value with frying temperature up to 120 s of frying was reported (Vélez-Ruiz and Sosa-Morales, 2003). The average 'α' values of *pantoa* were in the range observed during frying of pastry at 150ºC (0.102-0.156 mm^2s^{-1}) as reported by Williams and Mittal (1999). Andersland and Anderson (1978) postulated that product with high thermal diffusivity experienced an increase in temperature faster than that of with low thermal diffusivity.

Volumetric specific heat (C_p)

The volumetric specific heat of *pantoa* samples measured at different frying temperature and time combinations is depicted in Fig. 4. The volumetric specific heat decreased to 1.607, 1.530 and 1.401 $MJm^{-3}K^{-1}$ from the initial value of 3.282 $MJm^{-3}K^{-1}$ at 125, 135 and 145ºC, respectively. Also, the 'C_p' value decreased with frying temperature as reported by Vélez-Ruiz and Sosa-Morales (2003) for donuts. In general, C_p showed a trend similar to that of thermal conductivity. The decrease in C_p value with time was attributed to the fall in moistness of fried *pantoa*. In contradiction to that Baik and Mittal (2003) reported that the specific heat of *tofu* increased with increase in temperature. As *tofu* was heated, the mean kinetic energy of the molecules present also increased. This in turn increased the collisions between molecules which imparted energy for their rotation. Rotation of molecules in turn increased the internal energy thereby, increasing the specific heat. However, the moisture content of *tofu* had higher effect on C_p than temperature. The C_p of *pantoa* samples fried at all temperatures was modeled using multiple linear regression analysis incorporating frying time, frying temperature, moisture content and fat content as independent factors. Since the temperature effect was insignificant, it was excluded from the model (Eq. 4), and the final model is shown below.

$$C_p = 10.967 - 0.2605F - 0.032M - 0.001t \quad \text{(Adjusted } R^2\text{=0.921)} \qquad \text{Eq. (4)}$$

where C_p was the volumetric specific heat ($MJm^{-3}K^{-1}$), 'F' was the fat content (% w.b.), 'M' was the moisture content (% d.b.) and 't' was the frying time (s). A better and elaborative model was developed (Eq. 5) by considering the core temperature of *pantoa*, oil temperature, fat and moisture contents.

$$Cp=6.017-0.011CT-0.115F \quad \text{(Adjusted } R^2\text{=0.941; } R^2\text{=0.945)} \qquad \text{Eq. (5)}$$

where C_p was the volumetric specific heat ($MJm^{-3}K^{-1}$), 'F' was the fat content (% w.b.) and 'CT' was the core temperature. Amongst the factors, fat content was found to have a profound effect on the C_p of *pantoa*.

A relatively high specific heat of water could be reason for high specific heat of *pantoa* dough due to the presence of moisture (Mohsenin, 1980). A negative relationship was found between fat content and volumetric specific heat of *pantoa*.

Conclusions

Thermal properties of *pantoa* during DFF were determined. Both thermal conductivity and volumetric specific heat decreased with respect to the frying temperature and time, whereas the thermal diffusivity increased with increase in frying temperature up to

270 s, 270 s and 240 s at 125, 135 and 145 ºC, respectively and then decreased gradually with respect to frying time. The thermal conductivity, thermal diffusivity and volumetric specific heat varied from 0.357 to 0.199 $Wm^{-1}K^{-1}$, 0.105×10^{-6} to 0.140×10^{-6} m^2s^{-1} and 3.275 to 1.401 $MJm^{-3}K^{-1}$, respectively. Moisture content and fat content were inversely proportional during frying. Empirical models were developed for thermal conductivity and volumetric specific heat as function of moisture content, fat content and temperature. The thermal properties and the developed models would be useful in the mathematical modeling of heat transfer in *pantoa* during DFF.

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RESEARCH ARTICLE

Physico-chemical analysis of calcium enriched herbal ice-cream

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Abstract: Calcium enriched herbal ice cream (CEHI) was developed under the current study using calcium gluconate (CG) as a calcium source, *Bacopa monniera* as herbs with health beneficial properties. The optimized product was analyzed for its sensory, textural and microbial characteristics. The developed calcium enriched herbal ice cream has shown overall acceptability score of 7.5 (Like moderately) which is a good indication of the higher organoleptic quality for frozen products. The prepared product is having a higher hardness (42 N) compared to conventional ice cream (30 N). The color of the CEHI sample decreased in whiteness ($P < 0.05$) compared to the control sample. Melting rate of experimental ice cream and control ice cream are 0.75 and 0.56 g/min respectively and had shown a significant difference ($P < 0.01$). Viable count of calcium enriched herbal ice cream had shown no significant difference when compared to control ice cream ($p>0.05$). There was no detection for yeasts and mold for calcium-enriched herbal ice cream and the control

Keywords: Herbal ice cream, Physico-chemical, Textural and Microbial analysis

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Introduction

Ice cream is a frozen dairy food made by freezing a pasteurized mix with agitation to incorporate air and to ensure uniformity of consistency. The mix is composed of a combination of milk products, sugar, and stabilizer/emulsifier all of the edible material (Marshall et al. 2003). Ice-cream is a complete food in which constituents are completely digested which makes a desirable food for children and persons who need to put on weight (Arbuckle, 1996). Nowadays, consumers are becoming health conscious and the demand for dietetic/health foods has been increasing. There has been considerable interest in extending the use of herbs in dairy foods, fruits juice-based products and pharmaceuticals (Singh, 2010). Herbs can be incorporated into ice cream and have the potential for preventive and remedial purposes. Herbal ice cream is gaining wider popularity over normal ice cream due to its functional, nutritional and pharmacological activities (Kumar et al. 2013). Medicinal properties of herbal ice cream are antiseptic, anti-microbial, anti-viral and antidiabetic. Ice cream is a widely enjoyed palatable food product that provides a useful source of dietary calcium in the diet and can be fortified to provide additional amounts without changing flavor and color. India is known for its traditionally well-practiced knowledge of herbal medicines. In spite of having a large number of medicinal plants and well-practiced knowledge of herbal medicines, the share of India in the global market is not up to the mark (Rajkumar and Singh, 2009).

Bacopa monniera is an Ayurvedic medicine, clinically used for memory enhancing, epilepsy, insomnia and as a mild sedative (Chunekar, 1960). *Bacopa monniera* is considered as a nerve tonic in Indian traditional medicine (Chopra et al. 1969). It was used in traditional medicine to treat various nervous disorders, as a brain tonic to enhance memory, learning, improves concentration and to provide relief to patients with anxiety. It is also used in digestive complaints, for skin disorders and as an antiepileptic, antipyretic, and analgesic (Satyavati et al. 1976). High Performance Liquid Chromatography (HPLC) (Baig et al. 2019 a and Haratifar et al. 2014) and methods based on UV detection have been developed for the determination of bacosides in B. monniera (Shrikumar et al. 2004). Calcium, an important

mineral component of the human diet and chiefly available in milk and milk products, seafood, legumes, and some vegetables. Over the past years, the economic burden of osteoporosis is increasing, the clinical implications of calcium deficiency are being better recognized, and deficiency of vitamin D (important for calcium absorption) was being documented in tropical countries. Calcium absorption from dairy products can be easily done by the intestine compared to calcium from vegetables and cereals (Weaver et al. 1999). Dairy products like ice cream, desserts, cottage cheese, cream cheese, yogurt, yogurt drinks, and sour cream can be added with calcium due to their higher bioavailability (Gerstner, 2002).

Texture is an important factor as it influences how a sample of ice cream reacts within a person's mouth. The resistance of ice cream to the mechanical forces imparted by the tongue, upper palate and teeth will dictate the overall perception of ice cream texture (Yilsay et al. 2006). A coarse texture is a most frequently cited defect in ice cream (Marshall and Arbuckle, 1996). As this defect becomes pronounced, a gritty or icy mouthfeel is followed by a relatively cold sensation in the mouth caused by excessively large ice crystals. To achieve small initial ice crystals, the ice cream mix must be rapidly subcooled to the point of maximal nucleation rate (Hartel, 1996). This allows the greatest number of ice crystals to form and the least amount of ice crystal growth in the freezer. Upon extrusion from the freezer, ice cream must immediately be hardened to minimize recrystallization. The temperature and rate of hardening determine the final ice crystal size and the physical and sensory properties of the product (Sutton and Bracey, 1996). The texture defect icy is eminent as ice crystals grow throughout storage. The present study aims at the utilization of ice-cream as a carrier of calcium (calcium gluconate) and Brahmi (ethanol extraction) for added nutritional benefits to the consumers with objectives like sensory, textural characters and microbial quality were found out.

Materials and Methods

The skim milk and cream were procured from KVASU Dairy Plant, Mannuthy used for product preparation. Food grade calcium gluconate (CG) was procured from Nice chemicals Ltd, Cochin. Ice cream mix was prepared using a batch freezer according to Marshall et al. (2003).

Preparation of calcium enriched herbal ice cream

Preparation of calcium enriched herbal ice cream was done according to Baig et al. (2019 b). Skim milk (0.5% Fat, 8.7% SNF) and cream (40% Fat, 5% SNF) was taken and standardized to 10 % fat and 11 % SNF (Solid non fat), subjected to pasteurization (80ºC/25 sec). After pasteurization, product was cooled to 60ºC and dry ingredients including CG (2.2g/L mix), sugar (15%) and stabilizers/emulsifiers (0.5%) were added. BME (100 mg/L) was added to the ice cream mix. Thorough mixing was done by using egg beater and then subjected to two-stage homogenization at 60ºC (13.8MPa and 3.45 MPa). After homogenization, the ice cream mix was kept for aging at 4ºC/24 hrs. Then the ice cream mix was frozen and incorporation of air was done by batch freezer at -4ºC/ 7 min. The product so obtained was filled in polystyrene cups and placed in hardening room for hardening (-24ºC/ 6 h). Finally, the product was taken from the hardening room and stored in a freezer at -15ºC. Response surface methodology (RSM) was used to optimize the levels of two ingredients (CG and BME) according to Baig et al. (2019 b). Central Composite Rotatable Design (CCRD) using two 8 variables and five responses comprising of sensory attributes was used for computation of optimized solution. All the responses fitted well into the quadratic equation with R^2>0.60. The optimum levels of CG and BME are 217.34 mg/100 mL and 10 mg/ 100 mL for preparation of experimental ice cream. Flow chart of product preparation is shown in Fig 1.

Sensory evaluation of calcium enriched herbal ice cream

The sensory characteristics of the ice cream were judged by 10 panelists according to the method modified from Bodyfelt et al. (1998). Judges were selected based on their availability and willingness to participate in the study. Hardened ice cream samples were tested at a serving temperature of -10ºC. Freshly calcium enriched herbal ice cream prepared was evaluated for its sensory characteristics such as color, flavor, body, and texture, melting property and overall acceptability scores based on 9-point Hedonic scale (Amerine et al. 1965). Panelists were asked to note any defects or undesirable characteristics. Physicochemical and sensory analyses were carried out 2 weeks after the production of ice cream samples.

Textural analysis

Various textural characteristics such as hardness, cohesiveness, springiness, gumminess, and chewiness were measured for sample ice cream and for control ice cream, using Stable microsystems TA HD Plus textural analyzer, fitted with 25 kg load cell. Experiments were carried out using a cylindrical probe (10 mm diameter) combined with Exponent lite Texture Expert Software. The ice cream samples were carefully filled up in 100 ml ice cream cup so that no air pockets remained within the sample. It was then stored for minimum 24 hrs at -21 ± 2º C temperature for hardening. The probe was kept at -21ºC for 30 min before starting the experiment in order to reduce variations due to the temperature difference between the probe and ice cream sample. The experiments were carried out after maintaining room temperature (20 ºC). Ice cream cup was kept for 5 min and then hardness was measured (6 readings minimum). The hardness was determined as the peak compression force during penetration (Fig.2)

Following the conditions were employed while testing the texture of ice cream samples:

Sample: Ice cream has taken in a polystyrene cup

Compression: Ice cream samples were compressed to 75 percent of its height

Load cell: P/5 10 mm diameter stainless-steel cylindrical probe

Probe speed: A probe speed of 1 mm/sec during the test and 2 mm/sec for pre- and post-test was used throughout the study

Testing temperature: Ice cream sample is taken at -20ºC tempered to -10ºC.

Interpretation of texture profile parameters from texture profile curve: A typical force-deformation curve for double cycled compression is given in Fig. 2

Hardness - Maximum force recorded during the first compression cycle (g)

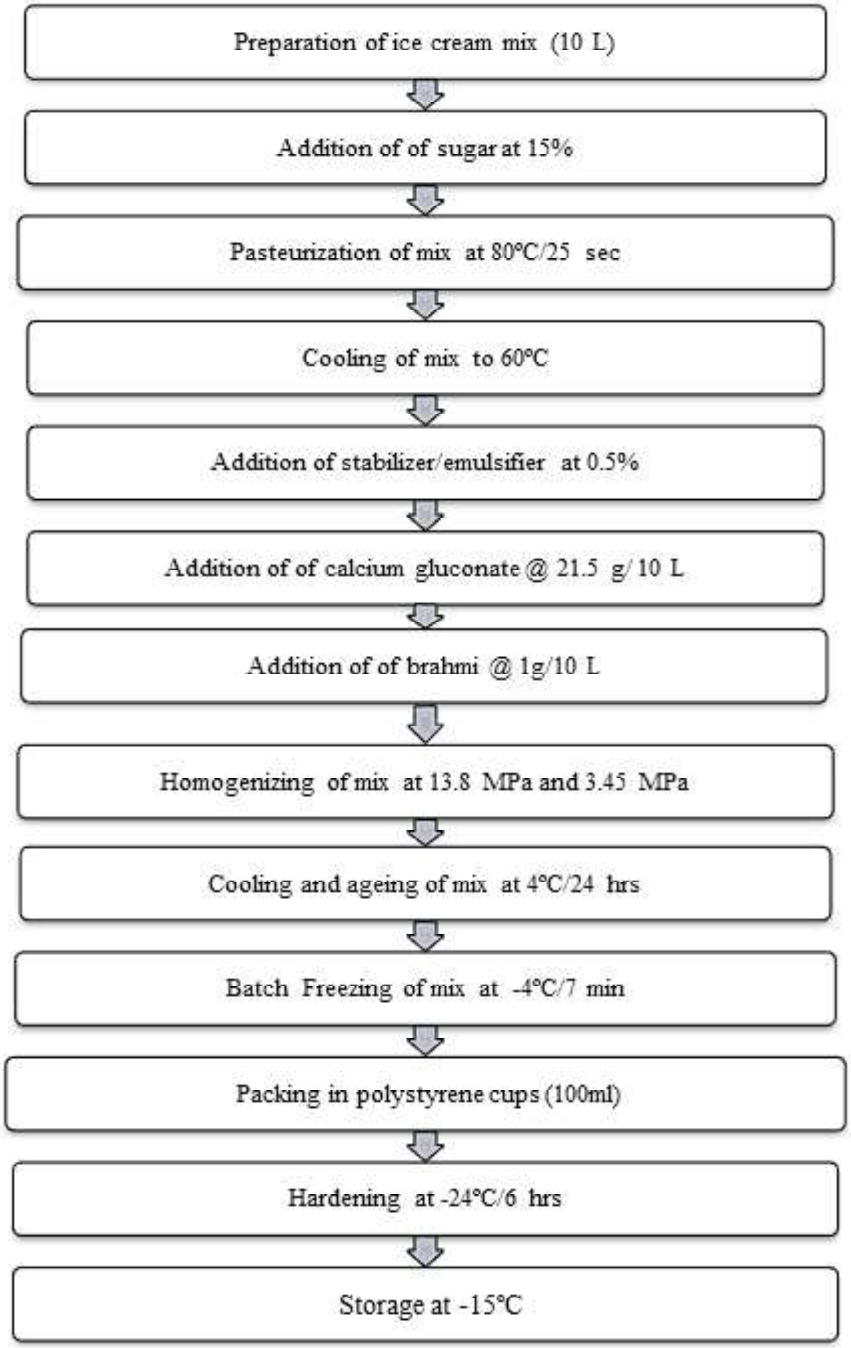


Fig 1. Flow chart of preparation of calcium enriched herbal ice cream

Springiness - Width of the downstroke in curve A_2 (mm)

Gumminess - Hardness x Cohesiveness (g)

Chewiness - Gumminess x Springiness (g.mm)

Colour

Colour measurement was done by using color Flex (Hunter Associates Laboratory, Inc., Reston VA, USA) color measurement system equipped with dual beam xenon flash lamp and universal software. The instrument was calibrated prior to sample measurements with standard black and white tiles as prescribed by the supplier. The results were represented by the L^*, a^*, and b^* notation. It is a 3-D color presentation method in which L^* is the lightness of color and equals 0 for black and 100 white. The a^* represents the amount of red (0 to 60) or green (0 to 60) while b^* represents the yellowness (0 to 60) or blueness (0 to -60)

Microbiological quality

Total. colony count of ice cream sample was estimated by pour plate technique, as described in IS 2802: 1964 Coliform count of each ice cream sample was estimated by IS2802:1964.

Melting rate (g/min)

According to Cruz et al. (2009), the melting time of ice cream is related to its stability after overrun and indicates the extent of the stabilization and partial coalescence of fat. Furthermore, an increase in coalesced fat provides greater resistance to ûow of the liquid phase resulting in slower melting (Muse & Hartel, 2004). Melting rate is a selective term and it depends upon the melting temperature and time. The melting rate was determined by the following procedure: ice cream sample of known weight was placed on a wire mesh, which was placed on a pre-weighed measuring cylinder (100 ml) with a glass funnel (10 cm dia.). The whole assembly was kept undisturbed at 25±1ºC for 45 min. The weight of the melted samples collected in the measuring cylinder was noted and the melting rate was determined by the formula given below:

$$Melting\ rate\ (g/min) = \frac{\text{Weight of melted ice cream (g)}}{time\ (min)} \times 100$$

The process optimization of calcium enriched herbal ice cream was carried out by using Central Composite Response Design (CCRD) of Response Surface Methodology (RSM). CG and *Bacopa monniera* extract were two variables selected for this purpose and responses noted were sensory, color, hardness and melting quality. The data generated during the study were analyzed statistically using Design expert version 8.0.7.1. ANOVA was applied to the data of sensor score of ice cream with different level of ingredients.

Results and Discussion

Sensory characteristics

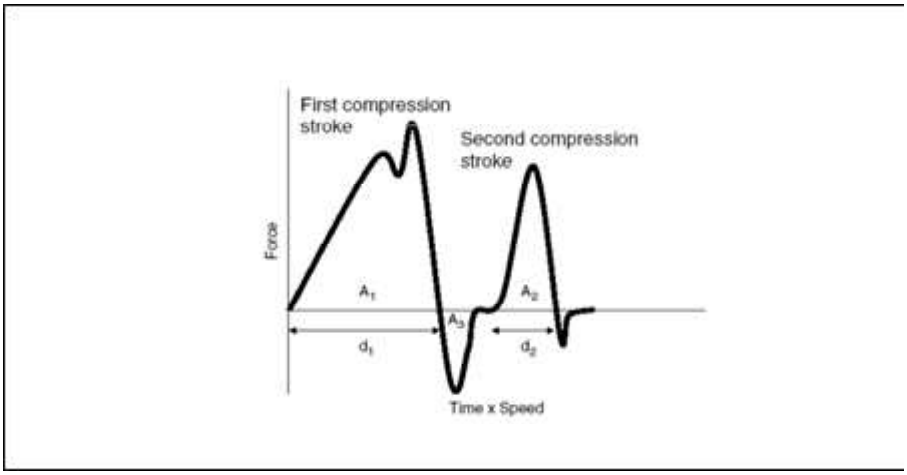
The sensory profile of traditional ice cream (control) was compared with two coded samples (A and B) of CEHI prepared from the same lot of ice cream mix, were assessed by a panel of judges using the principle of triangle test. The obtained results were presented in table 1 and Fig 3.The sensory response to the ice cream samples was affected by the variation in calcium level and BME level. The sensory attribute of the textural appearance corresponded to whether an ice cream sample cut smoothly or crumbled by using a spoon. Similarly, graininess was related to whether the surface had a typical appearance on the product or the granular icy appearance. Although these attributes are similar, the sensory panel found differences ($P < 0.05$) for the CEHI sample with a control sample when scoring the samples for appearance. Panelists found crumbled during cutting or scooping CEHI sample. The appearance term, air holes, described whether or not discrete air pockets were observed as the panelist scraped the surface of the ice cream with a spoon. Although all of the samples had the same amount of air incorporated into them (90% overrun), the panelists scored the higher for CEHI samples as having more

Table 1 Sensory attributes of calcium enriched herbal ice cream compared to control ice cream

Sensory attributes	Type of ice cream			Chi-square value
	Traditional ice cream	A	B	
Appearance	8.8±0.08[b]	7.61±0.06[a]	7.69±0.04[a]	5.25**
Flavor	8.75±0.08[bc]	7.3±0.06[a]	7.2±0.06[ac]	7.40**
Body and texture	8.62±0.07[a]	8.33±0.07[a]	8.40±0.06[a]	ns
Overall acceptability	8.6±0.07[bc]	7.72±0.06[ac]	7.65±0.07[a]	6.30**

Figures are mean ± standard error of three replications, **-Significant at one percent level ($p<0.01$), ns- non-significant ($p>0.05$) [a-b] mean scores with the same superscript within rows did not differ from each other, A and B–calcium enriched herbal ice cream

Fig 2. Force versus time x speed curve from the compression test



(Source: Everard et al. 2007).

Fig.3 Sensory attributes of calcium enriched herbal ice cream from two lots of samples compared to control ice cream

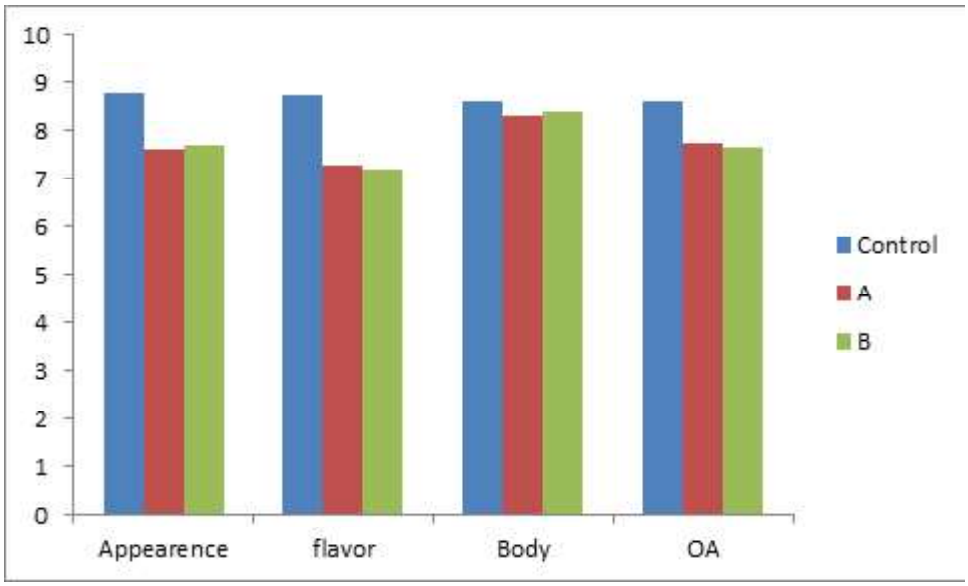


Table 2 Color values of calcium enriched herbal ice cream compared to control ice cream

Color value	CEHI	Control ice cream	t-value
L	69.4± 0.1	89.1± 0.4	18.5**
a	24.5± 0.6	0.8± 0.2	21.4**
b	5.6± 0.3	7.8± 0.1	ns

Figures are mean ± standard error of three replications, **-Significant at one percent level ($p<0.01$), ns- non-significant ($p>0.05$)

of these visible, discrete air pockets than the control samples (Table 1). The appearance term, stickiness, referred to the adhesion of the product to itself. The scores for the appearance attributes of stickiness and glossiness increased in CEHI compared to control ice cream. Pinto (2006) also noted that calcium imparts smooth texture to the product thereby resulting in enhanced glossiness of the ice cream. All of the ice cream samples were formulated to have relative sweetness values of 15 [contained the sweetness equivalent of 15% sucrose (wt/wt)]. Correspondingly, no significant ($P > 0.05$) differences in sweetness were observed as the calcium content of the ice cream was increased from 145 to 165 mg/100ml. Singh et al. (2008) have reported that there is no difference in the control yogurt and CG enriched fruit yogurt up to 260 mg/100 mL when compared to four different characteristics viz., flavor, appearance and overall acceptability. The flavor scores were lower for CEHI samples ($P < 0.05$) as the BME imparts a bitter taste. Similar findings reported by Russo and Borrille, (2005) that Increasing the BME adversely affected the flavor of the product because of the presence of phenolics, which are bitter in taste and thereby decrease the flavor score. BME concentration had significantly reduced overall acceptability, affected the sweetness of the product because of

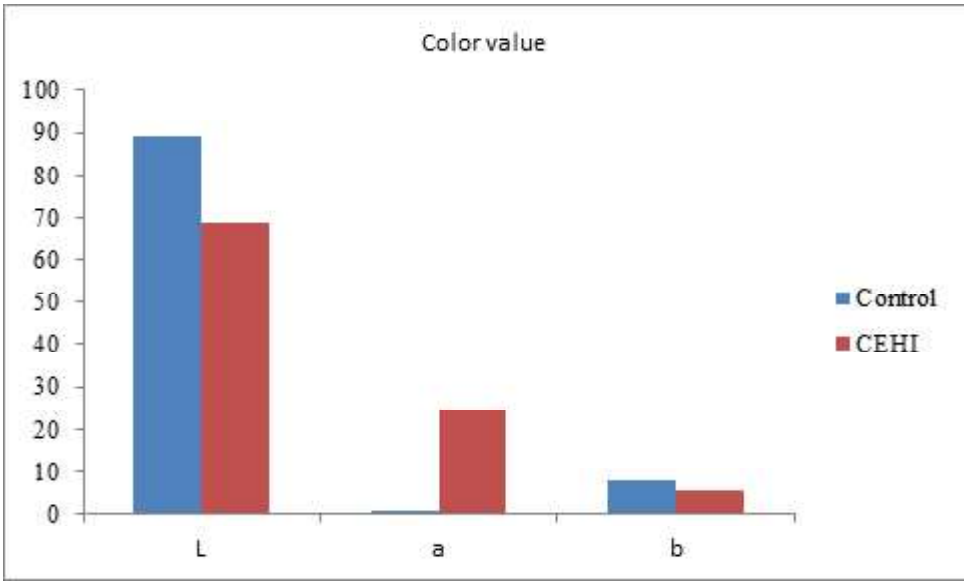


Fig 4. Color values of calcium enriched herbal ice cream compared to control ice cream where L is lightness (0 for black; 100 for white), a* is for red (0 to 60) or green (0 to 60) and b* represents the yellowness (0 to 60) or blueness (0 to -60)

Table 3 Hardness and melting rate of calcium enriched herbal ice cream compared to control ice cream

Property	CEHI	Control ice cream	t- value
Hardness (N)	42.76± 0.87	30.47± 1.03	9.65**
Melting rate (g/min)	0.75±1.60	0.56±1.40	5.94**

Figures are the Mean ± Standard Error of six replications, **-Significant at one percent level (p<0.01), ns- non-significant (p>0.05)

Table 4 Microbial count of calcium enriched herbal ice cream compared with control ice cream

Parameter	Calcium enriched herbal ice cream (log_{10} CFU/g)	Control ice cream (log_{10} cfu/g)	t-value
Viable count	11.848±0.02	11.854±0.03	0.532^{ns}
Coliform count	Nil	Nil	-

Figures are the Mean ± Standard Error of six replications, ns- non-significant (p>0.05)

the increased perceived bitterness and astringency owing to the presence of phenolics in the extract. The developed calcium enriched herbal ice cream has shown overall acceptability score of 7.5 (Like moderately) which is a well indication of the higher organoleptic quality for frozen products. Though the developed product had bitter taste due to the frozen condition makes it to accept the product moderately. The sensory analysis not only was more sensitive to the textural differences between ice cream samples but also distinguished ice creams based on flavor, which is the final indicator of ice cream quality. The perception of sweetness was affected by the increase in CG content; however it couldn't mask the bitter flavour from BME.

Effect of calcium and BME on the color of ice cream

The color of the CEHI sample decreased in whiteness ($P < 0.05$) compared to the control sample. Correspondingly, CEHI samples were less red and greener due to BME, as reflected by increasing a* values (Table 2). According to Baig et al. (2019 b), BME contains phenolics which imparted a bitter taste and green color to the ice cream. The b* value is higher in CEHI samples which corresponded to the sample being more yellow and less blue (Table 2). The obtained results were shown in Fig 4.

Hardness and melting rate

Ice cream with desirable melting quality begins to show definite melting within 15-20 mins. When kept at room temperature and melted product should flow readily and form a homogenous fluid with the appearance like that of the unfrozen mix (Marshal et al. 2003). The textural attributes of the sample were compared with that of control ice cream. Structure development in ice cream often is attributed to the macromolecules present in the ice cream mix-such as milk fat, protein, and complex carbohydrates. The strengthening of the protein network produces a uniform and stable emulsion and reduces the formation of ice crystals during storage (El-Nagar et al. 2002). The hardness of experimental ice cream and control ice cream are 42 N and 30 N (Table 3) respectively and had shown a significant difference ($p < 0.01$). According to Yonis et al. (2013) hardness of CG fortified banana yogurt shown a significant increase of hardness compared with the control. Due to the interaction between calcium salt and

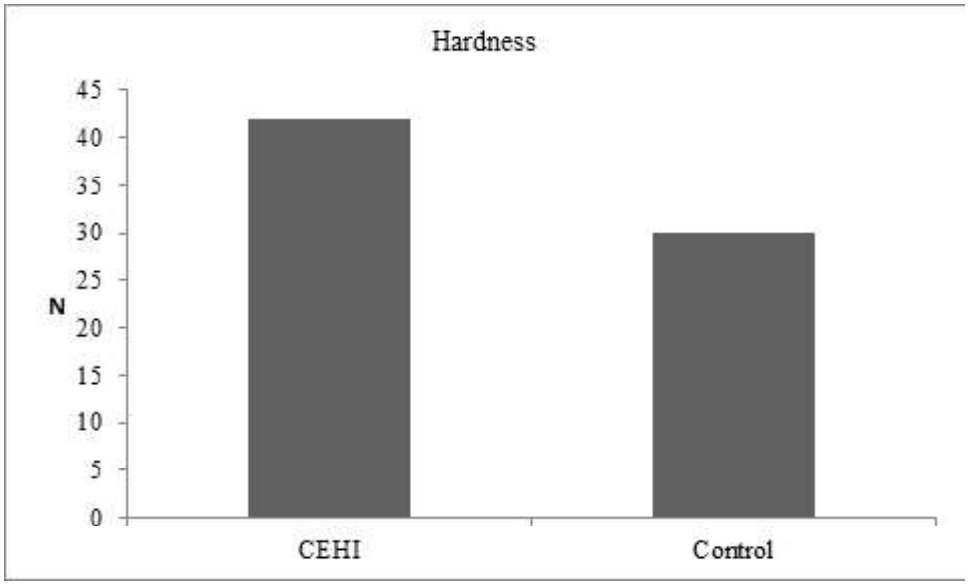


Fig 5. Hardness of calcium enriched herbal ice cream compare with control ice cream where N is newton

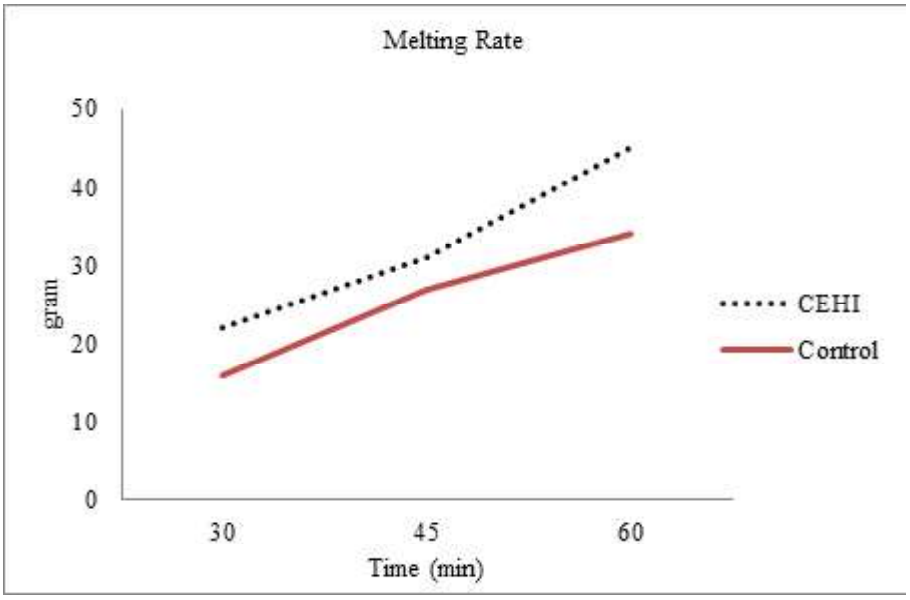


Fig 6. Melting rate of calcium enriched herbal ice cream compared with control ice cream

protein matrix present in the ice cream mix, it makes ice cream slight harder than usual. Melting rate of experimental ice cream and control ice cream are 0.75 and 0.56 g/min (Table 3) respectively and had shown a significant difference ($p< 0.01$). Calcium had a negative effect on the melting rate. On increasing calcium in ice cream, the melting rate decreased. Similar findings of the effect of calcium on melting quality of ice cream have been reported Marshal, (2003). The obtained results were shown in Fig 4 and Fig 5.

Microbial Quality

The microbiological quality of the Calcium enriched herbal ice cream was analyzed by enumerating the viable cells and coliform results are presented in Table 4.In the present study viable count of calcium enriched herbal ice cream (Table 4) had shown no significant difference when compared to control ice cream ($p>0.05$). There was no detection for yeasts and mold for calcium-enriched herbal ice cream and the control. These results indicate that the manufacture of calcium enriched herbal ice cream was carried out under proper hygienic conditions, resulting in the elimination of the contamination with such undesirable bacteria. According to Younus et al. (2002) absence of coliform bacteria in fresh control and CG fortified banana stirred yogurt was due to pasteurization of premix prior to incubation.

Conclusions

Sensory parameters play a major role in determining the acceptability of a food product as well as the ultimate purchase decision of consumers. In this study, it was found that CG is suitable for enrichment of CG without any stabilizer for

neutralizing calcium salt. BME alone imparted light green color and bitter taste to ice cream without altering the body characters. Beyond the level (10 mg/ 100 g) BME had shown lower acceptability in terms of color, flavor and overall acceptability. The effect of CG at had shown no negative effect on melting rate and improved the glossiness of the product. In this newly developed product, additional calcium fortification was done to meet the 30% RDA of calcium and reduce the chances of calcium deficiency diseases.

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RESEARCH ARTICLE

Efficiency of bronopol and kathon in preservation of milk and milk products samples stored for analytical purpose

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Abstract: Formalin (0.4%) is the only legally permitted preservative by FSSAI for preservation of milk and milk product samples which are kept for analytical analysis. However, formalin is reported to interfere with the estimation of milk fat which results in lower fat values than original. In the present investigation, the efficiency of bronopol and kathon was evaluated in preservation of milk and milk products. Bronopol and kathon both are broad spectrum antimicrobial compounds. Kathon (0.40%) and bronopol (0.40%) did not have significant effect on fat, SNF and protein content. However, lactose content increased significantly in milk samples added with kathon. A preservative formulation (combination of kathon and bronopol) was standardized and addition of 0.6% it, all microbiological counts were nil in first dilution as well as there was no significant effect on fat, SNF, lactose and protein content of milk. Moreover, on addition of this preservative formulation 1.0 1.2 and 1.2 % in khoya, paneer and dahi samples respectively, there was a significant reduction of microbiological counts of khoya, paneer and dahi. The optimized formulation (combination of bronopol and kathon) was found to be stable at 37 °C as well as refrigeration temperature for 120 days in amber colour bottle based on microbiological study.

Keywords: Bronopol, Combination of preservatives, Kathon,

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Introduction

India is the largest milk producer country in the world accounting 176.3 MT (2017-18) milk per annum. About 70% milk from total milk production is consumed in the form of liquid milk and remaining is utilized for various milk products. The great demand and scarce availability of milk and milk products, has led to the worldwide problem of food adulteration. According to Food Safety and Standard Act (FSSAI, 2006), adulteration of food is defined as "the addition or subtraction of any substance to or from food so that the natural composition and quality of food substance is affected". Among the various foods, milk and milk products are highly prone to adulteration. Thus, in India, the Food Safety and Standard Authority (FSSAI) has introduced Act (FSSAI, 2006) and Rules (FSSR, 2011) with the aim of eradicating the anti-social evil of food adulteration. It appoints Food Safety Officers for carrying out chemical examination of milk and milk products. Milk and milk products are perishable in nature and deteriorates rapidly, thus at the time of drawing of sample preservative is added to keep sample fit for analysis over a longer period of time. As per FSSAI (2006), formalin (0.4%) is the only legally permitted preservative for keeping samples fit for analysis for long duration.

Several preservatives have been earlier used so farfor the preservation of milk and milk products. In several studies, hydrogen peroxide (Giolitti, 1949), mercury chloride (Wolfschoon, 1978) sodium omadine (Wilson, 1983), potassium dichromate (Armandola, 1969), Kathon, Bronopol, sodium azide, sodium omadine, Dowicil, Triclosan, hydrogen peroxide, paraben (Bumbadiya et al., 2017) were used at different concentration for preservation of milk and milk products. According to Bumbadiya et al., 2017, based on the germicidal action of different preservatives against various groups of microorganism (bacteria, coliform and yeast & mould counts), bronopol and kathonpreservative are suitable for preservation of milk for analytical purpose at 0.1% and 0.4% concentration respectively. Bronopol known as 2-Bromo-2-nitropropane-1, 3-diol and has a broad spectrum bactericide effective against both gram-positive and gram-negative bacteria. Bronopol shows antibacterial activity by its interaction with essential thiols within the cell. Such

interaction is lead to the oxidation of thiols through a radical intermediate (Shepherd et al., 1988). The active ingredients of kathon are 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. This preservative is a highly effective, broad-spectrum biocide with excellent compatibility and stability and low toxicity at in-use levels. It is effective against gram positive and gram-negative bacteria along with yeast and mold. Kathon results in the lethal loss of protein thiols via: direct electrophilic attack, generation of a secondary electrophile by disulfide exchange and tautomerization to a thioacyl chloride and intracellular generation of free radicals as a result of severe metabolic disruption (Dole et al., 1989).

Although formalin is the only legally permitted preservative that shows the maximum suitability in most analytical tests, there are still a few limitations as reported by several researchers. Formalin is reported to interfere with the assessment of various constituents in milk especially milk fat. Formalin is known to harden the protein with the formation of high molecular weight polymers (Fraenkel and Olcott 1946). This cross-linked matrix is not properly dissolved in Gerber and Rose-Gottlieb method and entrapped amount of milk fat, which results in lower fat values. As per study, the fat content of milk sample reduced from 6.00% to 5.45% after storage of 1 year with 0.4% formalin at ambient temperature (Singh et al., 2016). It also affects the analysis of other compositional parameters of milk. The protein content of formalin (0.4%) added milk by Dye binding method showed immediate decline around 21% and 13% in cow and buffalo milk respectively after six month (Bajaj and Rai 1992). When Lowry method used, Protein content of cow & buffalo milk decreased from 3.55 to 2.77 % & 4.50 to 3.92 %, respectively in 0.4% formalin preserved milk at 30°C for 6 month (Bajaj and Rai 1993). However, no any effect on protein content for was observed by using kjeldahl method with 0.4% formalin preserved milk at 7°C up to 21 days (Suvartan, 2015). Apart from these, lactose (Bajaj and Rai 1993) and TS (Bajaj and Rai 1992) not significantly changed in formalin preserved milk samples. As no single preservative can satisfy the entire necessary requirement, there is need to find out suitable alternative to formalin. Therefore, in the present investigation attempt has been made to understand the feasibility of bronopol and kathon as an alternative preservative to formalin for the preservation of milk, paneer, khoya and dahi samples meant for chemical analysis.

Materials and Methods

Chemicals and reagents

Iso amyl Alcohol was purchased from S D Fine-Chem. Ltd., Mumbai, India. Boric Acid, Folin's Reagent, Ethyl alcohol (95%), Isopropanol, Sulphuric acid and Hydro chloric acid were purchased from Merck. Copper Sulphate Penta Hydrate, Phenolphthalein Indicator, Sodium Carbonate and Sodium Hydroxide were purchased from SRL Chemicals. Methyl Red and Methylene Blue were purchased from Qualigens Fine Chemicals. Bronopol and Kathon preservatives were purchased from Sigma-Aldrich Inc., St. Louis, USA. Tartric Acid, M-17 Agar, Tri-Chloro Acetic Acid, Nutrient Agar, Potato Dextrose Agar, Violet Red Bile Agar were purchased from Hi Media, Germany.

Collection of milk, paneer, Khoya and Dahi samples

Pooled raw cow milk samples were collected from Livestock Research Centre of ICAR-National Dairy Research Institute, Haryana, India. Paneer and khoya samples were collected from the Experimental Dairy of ICAR-National Dairy Research Institute, Karnal, Haryana. Dahi was prepared in laboratory by using the standard method of IS: 9617 (1980)

To understand the effect of bronopol and kathon on compositional parameters of milk, both are added at 0.1% concentration in milk individually and samples were subjected for compositional analysis (Fat, SNF, Protein and Lactose content). From this part of study, preservatives with minimum or no effect on were selected and further used for preparation of combination of preservatives.

Preparation of combination of preservative using bronopol and kathon and its effect on antimicrobial activity and compositional parameters of milk, paneer, khoya and dahi

Three different combinations (A, B and C) were prepared using different ratios of kathon and bronopol. From combination A to C, there was a decrease in concentration of Kathon and an increase in concentration of bronopol. A patent has been filed for combination of preservative (Patent application no. 201911032383 dated 09.08.2019) at Indian patent office. All three combinations were added to milk at different concentrations (0.2-0.6%) and antimicrobial activity was elucidated by assessing the milk samples for Total plate count (TPC), Lactic acid bacterial count (LAB), Coliform count, Yeast and mould count. All samples were stored at 37°C for 24 hrs for action of preservatives. After 24 hrs, samples were subjected to microbiological analysis (total plate count, lactic acid bacterial count, coliform count and yeast and mould count). Control milk samples without preservatives were also analyzed for TPC, LAB count, Coliform count and Yeast and mould count. Along with this, three combinations (A, B and C) were added in milk and its effect on fat, SNF, lactose and protein content were analyzed for optimization of its level in milk.

From this part of study, a combination of bronopol and kathon was selected. The optimized combination was added in paneer and khoya at different concentrations (0.2-0.6%) and antimicrobial activity was elucidated by assessing the milk samples for Total plate count (TPC), Lactic acid bacterial count (LAB), Coliform count, Yeast and mould count. Paneer samples (100 g) were prepared by cutting cubes of approximately 0.8-1.0 cubic cm size

with kitchen knife. The samples were placed in screw capped wide mouth autoclaved bottles andto those prepared samples; optimized preservative formulation (0.6%, 0.8%, 1.0% and 1.2%) was added by spraying (using sprayer of 5 mL capacity). After closing the bottles with caps, thorough mixing was done by inverting the bottles 8-10 times. In khoya (100 g), optimized preservative formulation (0.6%, 0.8%, 1.0% and 1.2%) was added by direct method (directly from pipette) and mixed properly by kneading. The prepared sample was then filled into screw capped wide mouth autoclaved bottles. In Dahi (100 g), optimized preservative formulation (0.6%, 0.8%, 1.0% and 1.2%) was added by direct method (directly from pipette) and mixed properly by spatula. All samples were stored at 37°C for 24 hrs for action of preservative. After 24 hrs, samples were subjected to microbiological analysis (total plate count, lactic acid bacterial count, coliform count and yeast and mould count.).

Evaluation of Stability of optimized formulation for milk

The optimized formulation (combination of bronopol and kathon) was stored under refrigeration (4-7°C) and room temperature (37°C) in transparent and amber color glass bottles. After every 15 days the antimicrobial activity was elucidated by assessing the milk samples for Total plate count (TPC), Lactic acid bacterial count (LAB), Coliform count, Yeast and mould count.

Compositional analysis

Estimation of fat in milk was done by Gerber method as given in BIS (IS: 1224-1 1977). For the assessment of SNF of milk samples, the procedure given in BIS (IS: 10083-1982, amendment 1-1997) was used. Protein content of milk sample was evaluated by (AOAC, 2000) method for estimation of total nitrogen content. For detection of Lactose content, the procedure given in BIS (IS: 1497-2 1961) was used.The raw milk, paneer and khoyasamples were examined for microbiological parameters according to the methods described in IS: SP 18 (Part I, 1980).

Preparation of dilution blanks

The dilution blanks consisted of 0.85-0.9% sodium chloride in 9 ml portions in culture tubes. These were cotton plugged and autoclaved at 121^{0}C for 15 min.For milk, 1 ml was aseptically pipetted and transferred to9 ml dilution tube.Further, dilutions were made with 9 mL dilution blanks.Whereas, paneer and khoya samples were weighed (11 g) aseptically and transferred to sterile mortar. The samples were thoroughly mixed with sterile pestle and transferred to 99mL dilution blank. The dahi sample was properly mixed in the container with sterile spatula and 11 g of sample was transferred to 99 mL dilution blank. Further dilutions were made with 9 mL dilution blanks.

Total plate counts

Nutrient Agar was used to enumerate the total viable count. To rehydrate the medium 28 g of the dry medium was suspended in 1000 ml distilled water and boiled to dissolve the medium completely. It was then filled in conical flask and the mouths of the conical flasks were closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15-psi pressure (121^{0}C) for 15 min. One ml of diluted sample (suitable dilution) was transferred in each of the duplicate sterile petri dishes. To each plate, 12-15 ml of plate count agar, previously melted and cooled to 42 to 44^{0}C was added. The contents were mixed thoroughly by tilting and rotating the dish. The mixture was allowed to solidify. The plates were then inverted and incubated at 37.0±0.5^{0}C for 48 hours and colonies were counted as cfu/ml.

Coliform counts

Violate red bile agar (VRBA) was used to enumerate coliform count. To rehydrate the medium, 41.53 g was suspended in 1000 ml sterilized distilled water and boiled to dissolve the medium completely. Other steps were followed as method explained for nutrient agar preparation for total plate count. At last, once the agar has solidified, pour an additional 4ml of medium to maintain anaerobic condition.

Yeast and mold counts

Potato dextrose agar (PDA) was used to enumerate yeast and mold count. To rehydrate the medium, 39 g was suspended in 1000 ml distilled water and boiled to dissolve the medium completely. Other steps are followed as method explained for nutrient agar preparation for total plate count.

Lactic Acid Bacterial counts

M–17 agar was used to enumerate lactic acid bacterial (lactic streptococci) count. To rehydrate the medium, 42.25g was suspended in 1000 ml sterilized distilled water and boiled to dissolve the medium completely. One ml of diluted sample (suitable dilution) was transferred in each of the duplicate sterile petri dishes. Pour 12 to 15 ml nutrient medium into the plates and mix well. Allow it to solidify at room temperature. Incubate the plates at 37 ± 2°C for 48 – 72 hrs. Colony were counted and expressed as log cfu (Colony Forming Unit)/ml of sample.

Statistical Analysis

Data reported were expressed as mean values with standard errors of three replicates. In experiments, wherever required, two-way analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied for multiple sample comparison to test for any significant differences ($P<0.05$) in the mean values of all the groups as described by Snedecor and Cochran (1994), using the statistical program of Microsoft® Excel Version 5.0

(Microsoft Corporation, Redmond, WA, U.S.A.). Graphs were prepared in software Graph Pad Prism version 5.0 (Graph Pad Software, Suite 230 La Jolla, CA 92037, U.S.A.).

Results and Discussion

Effect of bronopol and kathon on compositional parameters of milk

In control raw milk the fat, SNF, protein and lactose content were found 4.37±0.32, 8.72±0.22, 3.74±0.13 and 4.50±0.14 % respectively. As presented in Table 1, on addition of kathon, no significant difference was observed in fat, SNF and protein content however, lactose content increased significantly. On addition of kathon, increase in lactose content is due to the reduction of fehling solution by reducing groups in kathon structure. On addition of bronopol, no significant difference in fat (Gerber method), SNF, protein and lactose content was observed.

Effect of bronopol and kathon concentration in combination of preservative on antimicrobial activity and compositional parameters of milk

In raw milk the log values of TPC, coliform count, yeast and mould count and LAB counts were found 6.46±0.32, 3.76±0.03, 2.62±0.11 and 5.98±0.09 log cfu/ml, respectively. After addition of the three combinations (A, B and C) at different concentration (0.2, 0.4, 0.5 and 0.6%), milk samples were kept at 37°C for 24 hrs for action of preservatives, milk samples were then again analyzed for total plate count (TPC), coliform count, yeast & mould count and lactic acid bacteria counts. It was evident from Table 2,3 and 4 that in all combination(A, B and C) at all concentration no count was found in coliform and yeast and mold in first dilution. In all combinations (A, B and C) at 0.2, 0.4 and 0.5% concentration the lactic acid bacteria and total plate counts were found in the range of 3.4 to 5.2 cfu/ml in first dilution, however at 0.6% concentration in all combinations (A, B and C), lactic acid bacteria counts and total plate counts were found nil in the first dilution. In all combinations (A, B and C) at all concentrations the coliform and yeast and mould counts were nil in the first dilution.

The effect of combination A, B and C on compositional profile of milk is presented in Table 5. It is evident that on addition of combination A, B, C in milk, there was no significant effect was observed on fat, SNF, protein content of milk. However, on addition of combination and B, the lactose content was found to be significantly higher in milk. However, on addition of combination C, there was no significant effect on lactose content of milk.

Effect of bronopol and kathon concentration in combination of preservative on antimicrobial activity of paneer, khoya and dahi

As evident from above, the combination C was found to be effective in preservation of milk. Therefore, the combination C was added at various concentrations (0.6-1.5%) in paneer and khoya samples to understand the effect on antimicrobial activity. Incontrol paneer sample (without preservative), the $\log_{10}$ values of TPC, LAB counts, Coliform count and yeast and mould counts were observed to be 5.4±0.36, 4.6±.0.23, 3.5±0.32 and 2.9±0.24 log cfu/ml respectively. After addition of different concentrations 0.6, 0.8, 1.0 and 1.2% of combination C,paneer samples were kept at 37p C for 24 hrs for the action of preservative. The samples were then analyzed for the total plate counts, lactic acid bacteria counts, coliform countform counts and yeast and mould counts.

As shown in Table 6. On increasing concentration of combination C, there was decrease in microbial counts and at 1.2 %

Table 1 Effect of bronopol and kathon on compositional parameters of milk

Particular	Compositional Parameter			
	Fat%	SNF%	Protein%	Lactose%
Raw Milk	4.37±0.32[a]	8.72±0.22[a]	3.74±0.13[a]	4.50±0.14[a]
Kathon (0.4%)	4.37±0.16[a]	8.72±0.21[a]	3.72±0.17[a]	4.72±0.09[a]
Bronopol (0.10%)	4.37±0.29[a]	8.75±0.14[a]	3.73±0.13[a]	4.48±0.15[a]

Data are presented as means ± S.E (n=3). Means with different superscript letters are significantly different (Pd”0.05) from each other

Table: 2 Effect of different concentration of combination A on growth of microorganisms in milk

Particular	With Preservative analysis($\log_{10}$ cfu/mL)			
Concentration (%)	0.2%	0.4%	0.5%	0.6%
TPC Count	4.31±0.09	4.13±0.10	3.41±0.41	0.0±0.0
Coliform Count	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Yeast & Mold Count	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LAB Count	4.34±0.06	4.22±0.02	3.95±0.06	0.0±0.0

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table: 3 Effect of different concentration of combination B on growth ofmicroorganisms in milk

Particular	With Preservative analysis(log_{10} cfu/mL)			
Concentration (%)	0.2%	0.4%	0.5%	0.6%
TPC	5.22±0.19	4.11±0.29	3.67±0.30	0.0±0.0
Coliform Count	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
YMC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LAB Count	5.22±0.19	4.52±0.07	3.83±0.14	0.0±0.0

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table 4 Effect of different concentration of combination C on growth of microorganisms in milk

Particular	With Preservative analysis(log_{10} cfu/mL)			
Concentration (%)	0.2%	0.4%	0.5%	0.6%
TPC	4.67±0.09	4.31±0.29	3.67±0.3	0.0±0.0
Coliform Count	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
YMC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LAB Count	4.281±0.11	4.08±0.1	3.39±0.05	0.0±0.0

Data are presented as means ± S.E (n=3), TNTC: too numerous to count

Table 5 Effect of 0.6% concentration of different combinations (A, B and C) on compositional parameters of milk

Particular	Compositional Parameter			
	Fat%	SNF%	Protein%	Lactose%
Control	4.46±0.12[a]	8.56±0.05[a]	3.73±0.13[a]	4.5±0.14[a]
Combination (A)	4.4±0.05[a]	8.50±0.09[a]	3.69±0.14[a]	4.71±0.12[b]
Combination (B)	4.43±0.15[a]	8.48±0.2[a]	3.71±0.17[a]	4.6±0.08[b]
Combination (C)	4.42±0.1[a]	8.52±0.13[a]	3.70±0.09[a]	4.52±0.18[a]

Data are presented as means ± S.E. (n=3). Means with different superscript letters are significantly different (Pd”0.05) from each other

Table 6 Efficiency of optimized formulation (combination of bronopol and kathon) in preservation of paneer samples

Particulars	Control (log_{10} cfu/mL)	Paneer samples added with optimized preservative(log_{10} cfu/mL)			
		0.6%	0.8%	1.0%	1.2%
TPC	5.4±0.36	4.06±0.35	2.66±0.08	2.03±0.14	0.0±0.03
LAB	4.6±.0.23	3.23±0.32	2.4±0.11	1.6±0.15	0.15±0.1
Coliform count	3.5±0.32	2.7±0.25	1.8±0.11	0.9±0.10	0.0±0.0
YMC	2.9±0.24	1.4±0.26	0.73±0.06	0.1±0.06	0.0±0.0

Data are presented as means ± S.E (n=3).

concentration of combination C all the microbiological counts were nil in first dilution.

In control khoya sample (without preservative), the log_{10} values of TPC, LAB counts, Coliform countform counts and yeast and mould counts were observed to be 4.4±0.4, 3.24±0.26, 3.2±0.2 and 2.2±0.1 cfu/mlrespectively. After addition of different concentrations 0.6, 0.8, 1.0 and 1.2% of combination C, khoya samples were kept at 37p C for 24 hrs for the action of preservative. The samples were then analyzed for the TPC, LAB counts, Coliform countand yeast and mould counts. As shown in Table 7. On increasing concentration of preservative formulation, there was decrease in microbial counts and at 1.0 and 1.2% concentration of optimized preservative formulation at all the microbiological counts were almost nil in first dilution.

In control dahi sample (without preservative), the log_{10} values of TPC, LAB counts, Coliform countand yeast and mould counts were observed to be 7.1±0.20, 6.2±0.11, 4.8±0.17 and 5.2±0.05 cfu/ml respectively. After addition of different concentrations 0.6, 0.8, 1.0 and 1.2% of optimized preservative formulation, dahi samples were kept at 37p C for 24 hrs for the action of preservative. The samples were then analyzed for the total plate counts, lactic acid bacteria counts, Coliform count form counts and yeast and mould counts.

Table 7 Efficiency of optimized formulation (combination of bronopoland kathon) in preservation of Khoya samples

Particulars	Control (log_{10} cfu/mL)	Khoya samples added with optimized preservative(log_{10} cfu/mL)			
		0.6%	0.8%	1.0%	1.2%
TPC	4.4±0.40	3.18±0.06	2.47±0.29	0.07±0.07	0.0±0.0
LAB	3.24±0.26	2.93±0.20	2.30±0.11	0.06±0.06	0.0±0.0
Coliform count	3.2±0.20	2.62±0.07	2.03±0.15	0.0±0.0	0.0±0.0
YMC	2.2±0.10	0.70±0.35	0.0±0.0	0.0±0.0	0.0±0.0

Data are presented as means ± S.E (n=3).

Table 8 Efficiency of optimized formulation (combination of bronopol and kathon) in preservation of Dahi samples

Particulars	Control (log_{10} cfu/mL)	Dahi samples added with optimized preservative(log_{10} cfu/mL)			
		0.6%	0.8%	1.0%	1.2%
TPC	7.1±0.20	6.53±0.08	4.3±0.15	2.13±0.08	0.05±0.03
LAB	6.2±0.11	5.33±0.08	3.02±0.34	0.2±0.11	0.01±0.01
Coliform count	4.8±0.17	3.7±0.43	2.53±0.08	0.93±0.14	0.0±0.0
YMC	5.2±0.05	4.2±0.25	2.3±0.25	0.33±0.12	0.0±0.0

Data are presented as means ± S.E (n=3)

Table 9 Efficiency of optimized formulation stored in transparent bottle at Refrigeration temperature(7°C) and room temperature (37°C)

Days	TPC		LAB		Coliform		YMC	
	7°C	37°C	7°C	37°C	7°C	37°C	7°C	37°C
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
30	3.11±0.21	3.90±0.12	4.18±0.23	3.25±0.09	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
45	4.08±0.07	3.57±0.17	4.43±0.19	3.68±0.15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
60	3.11±0.30	4.30±0.10	3.59±0.12	4.54±0.10	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
75	3.71±0.13	4.38±0.16	3.61±0.13	4.61±0.16	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
90	3.85±0.11	4.69±0.09	3.60±0.11	4.88±0.08	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
105	4.16±0.10	4.30±0.13	3.87±0.10	5.10±0.13	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
120	4.46±0.11	4.56±0.06	4.23±0.11	5.35±0.06	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

As shown in Table 8. On increasing concentration of preservative formulation, there was decrease in microbial counts and at 1.2 % concentration of optimized preservative formulation all the microbiological counts were almost nil in first dilution.

Evaluation of Stability of optimized formulation

The optimized combination C was stored under refrigeration (4-7°C) and room temperature (37°C) in transparent and amber colour glass bottles. After every 15 days, 0.60% concentration of formulation was added to milk, samples were kept at 37°C for 24 hrs for action of preservatives and then analysed for total plate count (TPC), coliform count, yeast & mould count and lactic acid bacteria counts in first dilution. As shown in Table 9, the optimized formulation C was not stable in transparent bottle both at refrigeration and room temperature. However on storing the optimized formulation C in amber color bottle, all TPC, coliform count, yeast and mould and LAB count were found to be nil the in first dilution up to 120 days.

Conclusions

The results of the present investigation conclude that the individually addition of kathon (0.40%) and bronopol (0.40%) did not have significant effect on fat, SNF and protein content. However, lactose content increased significantly in milk samples added with kathon. Among the three different combinations (A, B and C), the combination C was selected. On addition of 0.6% of combination C, all microbiological counts were nil in first dilution as well as there was no significant effect on fat, SNF, lactose and protein content of milk. Moreover, on addition of 1.0 1.2 and 1.2 % of combination C in khoya, paneer and dahi samples

respectively, there was a significant reduction IN ALL microbiological counts of khoya, paneer and dahi. The optimized formulation (combination of bronopol and kathon) was found to be stable at 37 °C as well as refrigeration temperature for 120 days in amber colour bottle based on microbiological study.

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RESEARCH ARTICLE

Chemical changes of *kradi* cheese stored at refrigeration temperature under vacuum and normal conditions

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Abstract: This study investigated changes in chemical properties of *kradi* cheese stored under vacuum and normal conditions at 5°C at weekly intervals for a period of fourteen weeks. The initial moisture content of 49.09 % in normal packed samples decreased to 48.34 % while in vacuum packed samples it decreased to 48.31 % at 14th week of storage. The drop in initial pH was small in both vacuum packed and normal packed samples. The decrease in acidity was small in vacuum packed samples than normal packed samples. Fat leakage was less under vacuum packaging. The initial free fatty acid content of 0.93 meq/g in normal packed samples increased to 17.9 meq/g while in vacuum packed samples it increased to 21.31 meq/g on 14th week of storage. The initial tyrosine value of 0.013 µg 5ml^{-1} in normal packed samples increased to 5.25 µg 5ml^{-1} while in vacuum packed samples it increased to 4.93 µg 5ml^{-1} on 14th week of storage. The vacuum packaging retarded increase in acidity in comparison to normal packaging.

Keywords: Free fatty acids, *Kradi* cheese, Moisture loss, Refrigerated storage, Tyrosine value, Vacuum packaging

Introduction

Kradi, semisoft, white, unripened fresh cheese (Punoo et al. 2018a, 2018b and 2018c) is a famous traditional milk product of Jammu and Kashmir, India manufactured primarily by the tribal population of Gujjar and Bakarwal community. It is sold unpackaged, undergoes moisture loss and increase in acidity. Despite this, due to increased demand for this cheese, the demand for modern packaging is increasing at production units. The annual production is 21 thousand kilogram during 2017 and sales of product has increased by 15% in 2016-17. Although the sale of *kradi* cheese is increasing annually but no studies has been reported with respect to chemical changes during storage. The study of chemical changes in this product is necessary from food safety point of view for clearing the concerns of consumers.

During storage of cheese biochemical changes involve proteolysis and lipolysis mainly by rennet enzymes or bacteria (Fox and Mc Sweeney, 2004). The chemical degradation of cheese is not desired during storage. Lipolysis involves accumulation of free fatty acids (FFA) with most of the FFA being released from triglycerides.

Vacuum Packaging has developed much attention in production of dairy products for keeping safe from harm, prolonging the shelf life, retarding the activity of bacteria and reducing the use of preservatives. Descriptive sensory analysis, physico chemical, microbiological, textural and microstructural properties of *kradi* cheese have been reported (Punoo, et al. 2018c, Punoo, et al. 2018a; Punoo, et al. 2018b).

As compared to normal packaging, vacuum packaging can reduce chemical deterioration. The alteration in packaging condition by vacuum can either accelerate or inhibit the biochemical changes during storage of *kradi* cheese. Thus vacuum packaging can be a way of preserving the chemical quality of *kradi* cheese. Therefore present study was aimed at assessment of chemical changes of *kradi* cheese throughout its storage at different periods at refrigeration temperature under vacuum and normal packaging conditions.

Materials and Methods

Preparation of *Kradi* cheese

Kradi cheese was made as per the method described (Punoo et al. 2018a). The fresh product was packed in multilayer laminates (5-layer natural PFP, 20x20 cm size and 105-175 micron thickness

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of film) under vacuum and normal packaging conditions and stored at refrigeration temperature (5±1°C). The products stored were evaluated at weekly intervals to monitor changes in various chemical properties for a period of fourteen weeks.

Chemical analysis

Moisture

The moisture content in *kradi* cheese was analyzed by the method as described in IS: SP: 18 (Part XI) 1981.

Titratable acidity

Titratable acidity of *kradi* cheese was determined by the method recommended by the Association of Official Analytical Chemists (AOAC, 1984).

pH

Ten g of the grated sample was mixed with 10 ml of glass distilled water and slurry was prepared thereof in a mortar. The pH of slurry was determined by using double junction electrode pH Scan 2+, Eutech Instrument Pvt. Ltd., Singapore.

Fat leakage

Fat leakage of kradi cheese samples was determined by the method of Nilson and La Clair (1976).

Free fatty acids (FFA)

The total free fatty acids content of the *kradi* cheese was estimated by the method delineated by Deeth and Fitz-Gerald (1976).

Tyrosine value

The tyrosine content as a measure of proteolysis in *Kradi* was calorimetrically determined according to the method of Hull (1947). Five grams of sample were weighed and taken in a clean dry test tube, added with 10 ml of distilled water followed by addition of 10 ml of 0.72 N TCA. The test tubes were then stoppered shaken vigorously and incubated at 27°C for 10 min. The precipitated proteins were filtered through Whatman No. 42 filter paper and the tyrosine content in the filtrate was determined calorimetrically. To 5.0 ml of this TCA soluble filtrate, 2.0 ml of distilled water, 10 ml of sodium carbonate reagent (75.0 g anhydrous Na_2CO_2 and 10.0 g sodium tetra phosphate dissolved in glass distilled water and diluted to 500 ml) followed by 2.0 ml of the Folin's phenol reagent (Folin's phenol reagent diluted 1: 2 with distilled water) was added, mixed well and incubated again at 27°C for 10 min for colour development. This solution was used as a blank. The intensity of blue colour so developed was measured at 650 nm using UV visible spectrophotometer. A tyrosine standard curve was prepared using concentrations of 0, 10, up to 100 µg /5ml in TCA filtrate. A regression equation was obtained using the absorbance of the standard solutions against their tyrosine concentration (µg 5 ml^{-1}). The tyrosine equivalent in *Kradi* based on its concentration in the final 5 ml TCA soluble filtrate obtained from 5 g sample used for producing blue colour was expressed in terms of tyrosine equivalent (mg 100 ml^{-1}).

Statistical analysis

The data obtained during the present investigation was compared by one-way analysis of variance (ANOVA) with the application of SYSTAT software, version 6.0.1 copyright © 1996, SPSS INC. Significant difference ($p<0.05$) among treatments were detected using Duncan's multiple range tests.

Results and Discussion

The loss of moisture during storage is a common observation for cheeses. Level of moisture in the product plays a significant role on quality of the product during storage as far as bacterial activity, browning reaction and the acceptability of *kradi* is concerned. Therefore, packaging materials and packaging techniques are carefully selected to check the moisture loss during storage. The initial moisture content of 49.09 % in air packed *kradi* samples (Table 1) decreased to 48.34 % while in vacuum packed samples it decreased to 48.31 % on 14th week of storage. There was more loss of moisture under normal packaging conditions compared to vacuum conditions in saloio cheese stored at 8°C for 60 days (Pantaleao et al. 2007). Both packaging conditions and storage periods had no significant effect on the moisture content of *kradi* samples stored at 5°C (Table 2). The initial pH of 5.4 in normal packed *kradi* samples dropped to 4.82 while in vacuum packed samples it dropped to 4.80 on 14th week at 5°C of storage temperature. The effect of packaging conditions and the effect of storage periods was non significant on pH of samples (Table 2). Fuentes, L. et al. 2015, reported significant decrease in pH of pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The initial acidity of 0.54 % lactic acid (LA) in normal packed samples increased to 1.14 % LA on 14th week of storage while in vacuum packed samples it increased to 1.05 % LA. The higher development of acidity under normal packaging conditions could be attributed to increased activity of lactic acid bacteria under normal compared to vacuum conditions. The effect of packaging conditions and storage periods was highly significant (pd"0.01) on the acidity of samples (Table 2). Since vacuum packaging did not favoured increase in acidity of *Kradi* cheese, therefore vacuum packaging can be more desirable from consumers point of view as the sourness in *kradi* cheese because of acidity development will be less. A slower increase in acidity for white cheese under vacuum compared to normal conditions stored at 4-5°C for 90 days was reported (Cinbas and Kilic, 2006). Free-oil formation, also called 'oiling off' or 'fat leakage' is the tendency of liquid fat to separate from melted cheese and accumulate in pockets or pools, particularly at the surface of

cheese. The excessive free oil and limited free oil are considered serious defects in cheese. Table 1, depict the trend and values for fat leakage of kradi packed in two types of atmospheres (vacuum and normal) and stored at 5°C for fourteen week period. At 5°C of storage temperature, the initial fat leakage of 0.16 (cm^2), in normal packed samples increased to 0.81(cm^2) after 14th week of storage while in vacuum packed samples it increased to 0.71 (cm^2). Similar observations were also recorded by Kindstedt and Rippe (1988) who observed that refrigerated storage of mozzarella cheese increased the amount of free oil. Gobbetti et al. 2002, reported an increase in flowability during 60 days ripening of caciocavllo pugilese cheese. ANOVA (Table 2) revealed that both packages as well as storage periods had no significant effect on the fat leakage of samples stored at 5°C. Slower fat leakage under vacuum packaging is good for storage of product. The initial FFA of 0.93 meq/g in air packed *kradi* samples increased to 17.9 meq/g on 14th week of storage while in vacuum packed samples it increased to 21.31 meq/g. The slower rate of lipolysis at 5°C indicates that rate of lipolysis was slower under refrigeration conditions. A slower rate of lipolysis for white cheese under vacuum compared to normal conditions stored at 4-5°C for 90 days was reported (Cinbas & Kilic., 2006). Ahuja and Goyal (2013) reported that increase in concentrations of free fatty acids (FFA) of vacuum packed paneer tikka samples during refrigerated vacuum storage for a period of 30 days and observed influence of storage intervals on the FFA values was more significant (ñ<0.01) than types of packages (ñ<0.05) during storage. Fuentes et al. 2015, reported that concentrations of free fatty acids (FFA) did not change (P > 0.05) in pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The camembert cheese manufactured using tibetan kefir co-culture underwent lipolysis (Mei et al. 2015). The packages and storage periods had no

Table 1 Effect of vacuum packaging on chemical characteristics[1] of *kradi* cheese stored at 5±1°C

Period of Storage (weeks)	Chemical characteristics											
	Moisture %		pH		Acidity (LA %)		Fat Leakage (cm^2)		Free fatty acids (µeq/g)		Tyrosine value (µg $5ml^{-1}$)	
	VP	NP	VP	NP	VP	NP	VP	NP	VP	NP	VP	NP
0	49.09	49.09	5.40	5.40	0.54	0.54	0.16	0.16	0.93	0.93	0.01	0.01
1	49.00	48.95	5.33	5.36	0.56	0.57	0.16	0.16	2.00	2.66	0.14	0.29
2	48.90	48.85	5.23	5.30	0.58	0.62	0.2	0.22	2.60	4.00	0.44	0.73
3	48.85	48.78	5.18	5.24	0.62	0.65	0.24	0.28	3.33	4.66	0.55	1.45
4	48.76	48.73	5.13	5.18	0.65	0.68	0.28	0.34	4.06	7.06	0.86	2.15
5	48.74	48.69	5.08	5.14	0.67	0.77	0.32	0.4	4.73	8.60	1.56	2.71
6	48.71	48.65	5.05	5.10	0.69	0.77	0.36	0.46	5.66	9.66	1.95	2.99
7	48.68	48.65	5.02	5.06	0.71	0.84	0.4	0.52	6.66	11.33	2.43	3.50
8	48.60	48.62	5.00	5.02	0.73	0.91	0.44	0.58	7.66	12.33	2.71	3.82
9	48.58	48.56	4.99	4.98	0.75	0.97	0.48	0.62	8.22	13.00	2.98	4.05
10	48.56	48.51	4.98	4.96	0.76	1.02	0.52	0.66	9.00	13.66	3.38	4.36
11	48.52	48.47	4.96	4.88	0.78	1.07	0.54	0.71	11.66	15.12	3.70	4.59
12	48.47	48.42	4.94	4.85	0.81	1.11	0.56	0.74	14.33	16.49	3.81	4.79
13	48.43	48.39	4.92	4.82	0.84	1.14	0.58	0.77	16.21	17.9	4.21	5.11
14	48.39	48.34	4.88	4.80	0.87	1.17	0.62	0.81	17.92	19.11	4.43	5.25

[1] Values are mean of three trials NP=Normal packaging VP=Vacuum pack

Table 2 Analysis of variance for chemical characteristics of *kradi* cheese stored at 5±1°C

Attributes	df (between packaging system)	Mean sum of squares		F-value
		Packaging System	Time interval	
Moisture %	1	0.01 ns	0.04 ns	0.25
pH	1	$-1.11x10^{-16}$ ns	0.03 ns	$-3.6x10^{-15}$
Acidity %	1	0.16**	0.02**	5.59
Fat leakage	1	0.08 ns	0.03 ns	2.16
Free fatty acids	1	57.51 ns	30.03 ns	1.91
Tyrosine value	1	5.32 ns	2.75 ns	1.93

** Significant at 1%, * Significant at 5%, ns Non-significant

significant effect on the FFA of kradi samples (Table 2). The initial tyrosine value of 0.013 µg 5ml^{-1} in air packed *kradi* samples increased to 5.25 µg 5ml^{-1} while in vacuum packed samples it increased to 4.93 µg 5ml^{-1} on 14th week of storage. The slower rate of proteolysis at 5^{o}C indicates that proteolytic enzymes were less active under refrigeration conditions. Slower rate of proteolysis was reported for white cheese (Turkish cheese) under vacuum compared to non-vacuum conditions stored at 4-5^{o}C for 90 days (Cinbas and Kilic, 2006). Ahuja and Goyal (2013) reported that increase in concentrations of tyrosine content of vacuum packed paneer tikka samples during refrigerated vacuum storage for a period of 30 days and observed duration of storage as well as type of package significantly affected tyrosine content. Fuentes et al. 2015, reported that proteolysis did not showed significant changes during storage in pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The camembert cheese manufactured using tibetan kefir co-culture underwent proteolysis (Mei et al. 2015). The effect of packaging conditions and storage periods was non significant on the tyrosine value of kradi samples (Table 2). The chemical analysis was discontinued after14 weeks of storage under normal packaging and vacuum packaging as the samples expired due to off flavor development.

Conclusions

This study revealed that vacuum packaging of *kradi* cheese prevented loss of moisture, drop in pH was low and increase in acidity was also slow besides a lesser fat leakage. Proteolysis and lipolysis were slowed down by vacuum packaging. The increase in acidity of product was retarded under vacuum packaging which is a very good characteristic from consumer's point of view. Chemical quality of product was better maintained at refrigeration temperature of 5^{o}C. This study also reveals that although chemical changes occurred both under normal and vacuum packaging, but the magnitude of changes under vacuum packaging was less. This study supports that both normal and vacuum packaging are desirable for packaging of kradi cheese. However from a technological point of view, vacuum packaging could be an alternative to conventional packaging for fresh *kradi* cheese production designed for long storage. Therefore vacuum packaging of *kradi* cheese can extend the shelf life of this traditional regional product and can guarantee the consumers a quality product.

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RESEARCH ARTICLE

Studies on the preparation of antioxidant rich ber (*Zizyphusmauritiana* Lamk.) powder burfi with coconut sugar as natural sweetener

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Abstract: Efforts were made to formulate an antioxidant rich, low sugarburfi. The burfi was enriched with different levels of ber fruit powder (5%, 10% and 15% w/w) and coconut sugar (28% w/w). Sensory scores revealed that burfi with 10% ber powder had highest overall acceptability. The optimizedber powderburfi was compared with market samples and control burfi and the physicochemical property, antioxidant activity and total phenolic content varied significantly($P<0.05$). Addition of ber powder significantly($P<0.05$) increased iron content, vitamin C content, antioxidant activity and total phenolic content of optimized burfi followed by the effect of coconut sugar incorporation. The addition of coconut sugar showed significant ($P<0.05$) decrease in total sugar content. From the study, it was concluded that the incorporation of berpowder and coconut sugar was a great success and will be helpful for developingother value added dairy confectioneries.

Keywords: Antioxidant activity, Ber powder, Coconut sugar, Burfi, Vitamin C

Introduction

Ber (*Zizyphusmauritiana*Lamk.) is a very old fruit of India. It is also commonly called as Jujube around the world. It is an underutilized fruit in India.Bercomes from the genus *Zizyphus* of the family Rhamnaceae. The most common cultivated species of ber fruit are *Zizyphusjujuba* and *Zizyphusmaurtiana*.*Zizyphusmauritiana*is commonly grown all over thenorthwest of India and in the arid parts of South India(Azam et al. 2001; Kumar et al. 2011).It is called as the king of arid zone fruits (Yamadagni 1985).Ber fruits are good source of vitamin C, total phenolics ranging from 19.54 to 99.49 mg/100g and 172 to 328.6 mgGAE/100g, respectively (Koley et al. 2011).They are rich in mineralslike calcium, phosphorus and iron (Yamadagni1985; Shobaand Bharathi2007). The major phenolics reported are caffeic acid, p-hydroxybenzoic acid,ferulic acid and p-coumaric acid (Tanmay et al. 2011; Memon et al. 2012)which justifies for its significant antioxidant activity (Krishna and Parashar 2012).

Ber fruit is seasonal as well as nutritious, so it needs to be preserved. However, the techniques to be used for preservation must sustain or improve their nutritional quality (Hsu et al. 2003). Out of the numerous processing techniques, spray drying was found to most suitable. It is a technique useful for increasing the shelf life of the fruit. The spray drying process can form good quality powder with less water activity so that it can be easy to store and transport. The most common carrier agent used for fruit juices are maltodextrins (Cano-Chuca et al. 2005).

Coconut sugar is a natural sweetener with wonderful taste and nutritional content. It is however, less known to people. Coconut sap is converted to coconut sugar and is becoming popular worldwide because it is natural, minimal processed and healthy. One of the most important health claims is its glycemic index (GI). Foods with low GI are vital for diabetes, obesity, heart disease and hypertension (Jenkins et al. 1981). A research published that the GI of coconut sap sugar was reported to be 35, i.e. in low category (Trinidad et al. 2010). Coconut sugar consists of minerals such as iron, zinc, calcium, phosphorus, magnesium and potassium, accompanied bysome short chain fatty acids. It is also good in vitamins such as vitamin C and vitamin B complex, polyphenols, antioxidants, dietary fibre and inulin (Hebbar et al. 2015; Secretaria et al. 2007).

Burfi is one of the most popular khoa based milk product appreciated all over India. It is prepared by evaporating milk in an open pan to obtain a semi-solid product called khoa(BIS 1970).

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There are many types of burfi present in market viz. simple, mawa, fruit, cashewnut, almond, besan, khajoor etc. Due to its attractiveness and wide acceptancethroughout India, many forms of burfi with numerous ingredients and flavours have been developed. Inventive researches are being done with burfi like herbal burfi prepared by Goyal and Shamsher (2015), Prasad et al. (2017), burfi with essential oil formulated by Prasad et al. (2018) and aloe-verapeda by Srikanth et al. (2017).

Keeping the above points in mind, the study was aimed to formulate a value- added product with high nutritional content, good antioxidant properties and low sugar content. Looking to the functional, nutritional and low sugar aspect, ber fruit powder and coconut sugar was found to be suitable for incorporation in milk based product, burfi. Hence, our present investigation was undertaken with the objective of evaluating the best ber powder burfi and the effect of incorporation of spray dried ber powder and coconut sugar on various physicochemical and antioxidant attributes of burfi samples.

Materials and Methods

Materials

Full cream milk and coconut sugar were purchased from the local market of Varanasi, India. Ber fruits were acquired from horticultural farm of Banaras Hindu University, Varanasi and the cultivar was identified by the experts of the Department of Horticulture. They identified the cultivar as Banarsi Karaka. All chemicals were obtained from Hi-media Laboratories Limited, Mumbai, India.

Production of ber fruit powder

The flowchart of the whole process is given in Figure 1. The process conditions (Inlet air temperature and maltodextrin concentration) were optimized by Response Surface Methodology using Minitab 17.1.0 software to get the finest quality ofber fruit powder. The slurry was spray dried in a lab-scale spray dryer (Model: Spray Mate - JISL Instruments Private Limited, Mumbai, India) with an inlet air temperature of 166.21°C, outlet air temperature of 80 °C, feed rate of 18 rpm, air pressure of 2 kg/cm^2 and aspirator speed of 1250 rpm. The spray dried fruit juice powder was collected at the bottom of the cyclone jar. The samples were then transferred to aluminium laminated polyethylene packages of size 12 cm x 9 cm and sealed immediately.

Preparation of ber burfi

The buffalo milk was standardized to 6% fat and 9% SNF. Milk was concentrated in an open karahi (pan) at boiling temperature with continuous stirring and scraping till the final stage of semi-solid consistency (khoa) is reached. The coconut sugar was added @ 28% in the burfi. The temperature was further lowered to 88-90 °C and selected levels of spray dried ber powder (0, 5, 10 and 15%) were added. Finally, this mixture was heated at low temperature with slow stirring till the desired consistency of ber powderburfi was obtained. This mixture was then spread uniformly in a tray and allowed to cool. After it has been settled, pieces of rectangular blocks were cut by knives.

The proportions of ingredients produced by the levels of ber powder addition, were considered as treatments as given below.

Treatment details:

T_0 (Control)= 0% ber powder + 100% of khoa by weight

T_1 = 5% ber powder + 95% of khoa by weight

T_2 = 10% ber powder + 90% of khoa by weight

T_3 = 15% ber powder + 85% of khoa by weight

Sensory evaluation

The sensory attributes of ber powder burfiwere analyzed for colour and appearance, body and texture, flavour, sweetness and overall acceptability by a semi-trained panel of judges consisting of ten members selected from the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.To minimize the variances, the panellists were made familiar with the quality attributes and the flaws generally associated with burfi under study. The attributes were evaluated on the basis of 9-point hedonic scale.

Colouranalysis

The colour of ber powder burfi was measured in terms of the CIE L*, a*, b* values using Color Flex EZ spectrophotometer. L* represents the lightness (L*=0 for black and L*=100 for white), a* indicates red (+) to green (“) axis, and b* represents yellow (+) to blue (“) axis (Duangmal et al. 2008).

Texture analysis

Texture profile analysis on ber powder burfi samples was performed by using the texture analyser TA.XT plus, Exponent Lite (Stable Micro Systems, Surrey, UK). A 75mm compression platen was used under 50 kg load cell. Burfi samples were cut into 1 cm^3 cubes and subjected toa dual bite compression force by probe upto a 5.00mm distance at 1.0 mm/s test speed.

Proximate analysis

Moisture, fat, protein, ash, acidity, total solids, total sugar, iron and vitamin C were analyzed in ber powder burfi sample and spray dried ber fruit powder by following the AOAC (2000) methods.

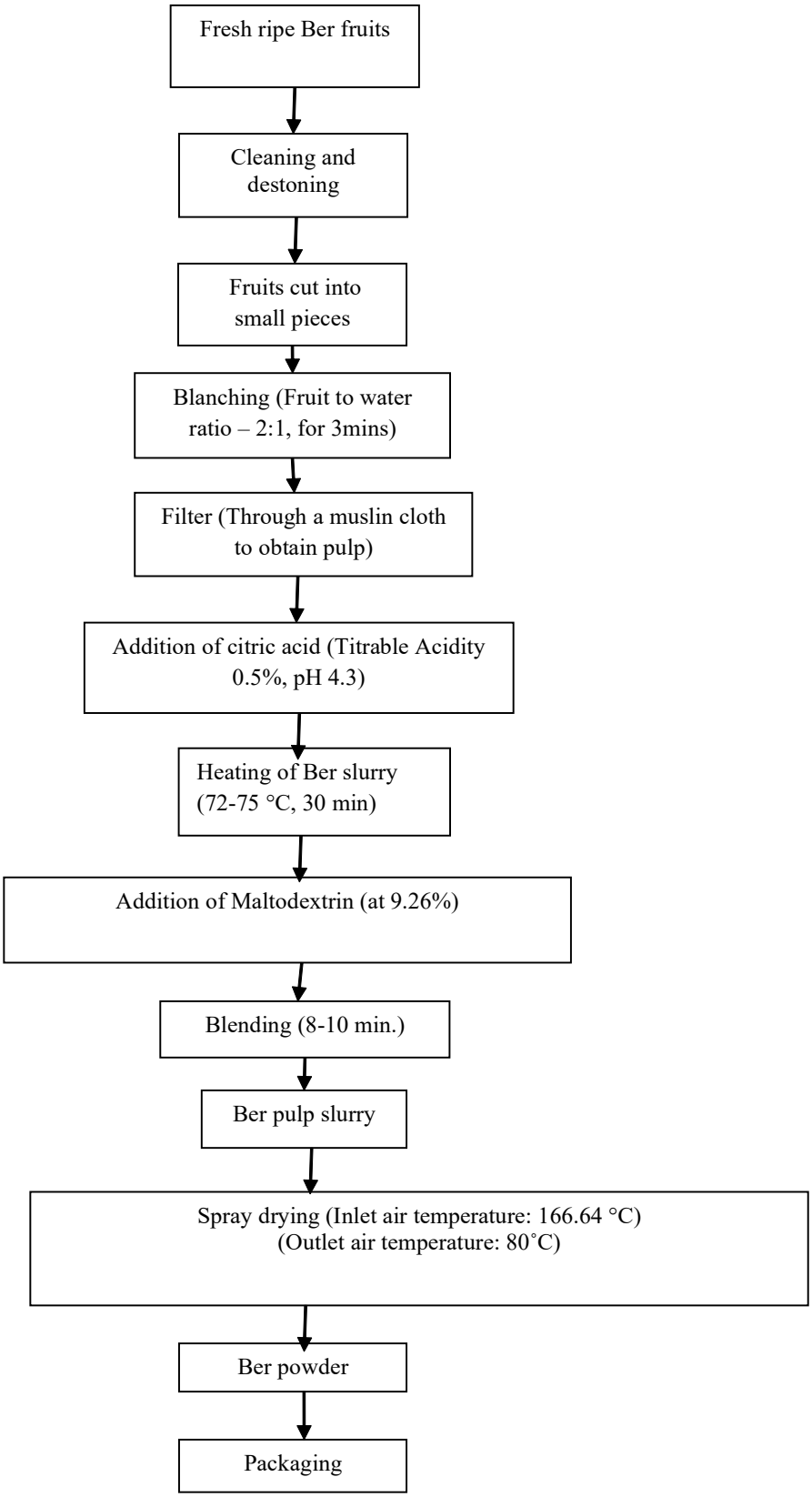

Fig. 1 Process flowchart of ber fruit powder production

Free radical scavenging activity

The free radical scavenging activity was determined by using DPPH assay (Brand-Williams et al. 1995). A 3.9 mL aliquot of a 0.0634mM of DPPH solution, in methanol (95%) was added to 0.1 mL of methanolic burfi sample extract or ber powder sample extract and shaken. The samples were kept in the dark room for 30 minutes after which absorbance was recorded at 515nm. The percentage inhibition of DPPH was calculated by the following formula: %inhibition =100 x(A_0 - A)/A_0 where $A_0$was the control absorbance at 515 nm and A was the final absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank.

Total phenolic content

Total phenolic content was evaluated byFolin–Ciocalteu method defined by Liu et al. (2008) with some modifications. 60 µL of burfi extract or ber powder extract , 300 µL of Folin–Ciocalteaureagent and after 3 minutes 750 µL of 20% sodium carbonate in water were added in 4.75 mL of water. The mixture was kept for about 30 minutes. The absorbance was then recorded at 765nm.For blank preparation, 60µL of distilled water was taken instead of sample. Total phenol content of sample was calculated using equation of standard curve and the results are expressed as mg of GAE.

Statistical analysis

The data acquired from various experiments were recorded as mean ± standard deviation (SD). Data was statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey's comparison test to find the significant differences among the mean values at 95% level of confidence using Minitab software ver. 17.0.1.

Results and Discussion

The results of sensory attributes, colour values and instrumental textural profile were discussed for different burfi samples (T_0, T_1, T_2 and T_3). The spray dried ber powder was analyzed for proximate parameters, antioxidant activity and total phenolic content (Table 1) which will further be used for the comparative analysis.

The optimized ber powder burfi was determined on the basis of sensory evaluation. The optimized burfi was then compared with market and control burfi (Table 5). The means were compared using Tukey's Test with confidence level of 95%. The differences were indicated as superscripts.

Sensory attributes

The sensory mean score for colour and appearance of ber powder burfi varied from 7.35 to 8.57, body and texture from 7.23 to 8.66, flavour from 7.21 to 8.83, sweetness from 7.31 to 8.48 and overall acceptability from 7.41 to 8.63. It is evident from the data presented in Table 2 that most of the samples prepared are significantly different ($P<0.05$) from the control sample. The overall acceptability score for the ber powder burfi prepared with 10 % ber powder was found to be most satisfactory by the panelist. This optimized burfi was finally used for further analysis in the study. This shows that ber powder incorporation improved the sensory properties of the burfi samples up to 10 % level, but further increase led to the reduction of sensory score. These results are in similarity with Bankar et al. (2013) and Khapre et al. (2015), who studied the preparation of pineapple burfi and fig fruit powder burfi respectively. Kim and Lee (2012) worked on jujube (ber) powder muffins and recommended10 % level of jujube powder with respect to overall preference score.

Colour values

The lightness, redness and yellowness values of ber powder burfi are presented in Table 3. Lightness index ranged from 44.24 to 47.34 which is a low value. This could be due to the addition of coconut sugar which has imparted brown colour to burfi. Lightness value decreased in significant manner ($P<0.05$) and was found lowest for 15% level of incorporation due to higher percentage of ber powder. The redness-greenness index ranged from 7.28 to 8.07 and increased significantly with increased levels of powder. They ellowness-blueness index ranged from 16.25 to17.63 and decreased significantly. Similar results were reported by Tanuja et al. (2017) in apple pomace incorporated burfi andKim and Lee (2012) in jujube powder muffins.

Instrumental texture parameters

The measured textural parameters of the ber powder burfi samples are given in Table 4. The value of hardness ranged from 5292.76 to 7018.78 g, adhesiveness -5.05 to -80.03 g.sec, springiness 0.23 to 0.35, cohesiveness from 0.26 to 0.34, gumminess from 1438.62 to 2205.97 and chewiness from 365.05 to 764.71.

Hardness is the most assessed characteristic in determining the texture of burfi. Addition of ber powder increased the hardness of burfi significantly ($P<0.05$). This may be due to the lower moisture content of ber powder. The result here is in agreement with walnut powder burfi (Satav et al. 2014). Similar findings were reported byJha et al.(2014) in lalpeda.

The sensory stickiness is due to adhesiveness. The adhesiveness of ber powder burfidecreased significantly($P<0.05$) because of low moisture. The result was similar to that of lalpedareported by Jha et al. (2014).Rasane et al. (2012) reported variation in adhesiveness of market samples of burfi due to different sugar levels. A higher adhesiveness value is due to higher moisture content in peda (Londhe and Pal 2008). Cohesiveness and springiness were not much affected but showed a decreasing trend. It may be attributed with the loss of moisture content and increasing totalsolids. Gupta et al. (1990) reported that cohesiveness of khoa decreased with increasing total solids.

Gumminess and chewiness also decreased significantly (P<0.05). The textural parameters were greatly influenced by the moisture content and total solids. Similar findings were reported by Tanuja et al. (2017) in apple pomace burfi.

Effect of incorporating ber powder and coconut sugar on the physicochemical parameters of burfi and its comparison with the market burfi

The moisture content of burfi samples varied from 17.65 to 21.37%(Table 5) and decreased significantly (P<0.05).The optimized burfi had the lowest moisture content of 17.65%. This was because of the addition of spray dried ber powder, which had less moisture (Table 1).Results are in similarity with Kim and Lee (2012). The moisture content of the control burfi is also less than market burfi, this may be due to the fiber content of coconut sugar (Trinidad et al. 2010).

The fat content of burfi samples ranged from 19.46 to 22.60% was not much influenced by the coconut sugar addition. But it is lowest in optimized burfi i.e. 19.46%, may be because of ber powder having very minimum fat content and also due to khoa percentage has decreased.

The protein content, titratable acidity and ash content were not much affected by addition of coconut sugar powder and ranged from 13.20 to 15.48%, 0.24 to 0.36% and 2.70 to 3.14% respectively. However titratable acidity is affected by the ber powder addition and is highest in case of optimized burfi due to the acidity ofber fruit powder (Table 1). Also the protein content and ash content decreased in optimized burfidue to low percentage of khoa. Some results here are in agreement with Bankar et al.(2013) and Goyal and Shamsher (2015) while they worked on pineapple burfi and herbal burfi, respectively.

Total solids is significantly (P<0.05) influenced by addition of coconut sugar due to its minerals and fiber content (Philippine Coconut Authority) (Trinidad et al. 2010). It is highest for the control burfi, 84.25% and lowest for the optimized burfi, 80.29%. The result here is in similarity with probiotic ice cream which

Table 1 Physicochemical and antioxidant composition of spray dried ber powder

Parameters	Composition
Moisture (%)	4.85±0.05
Fat (%)	0.06±0.06
Ash (%)	0.98±0.02
Acidity (% citric acid)	0.83±0.05
Total Sugar (%)	24.00±2.00
Iron (mg/100g)	12.80±0.08
Vitamin C (mg/100g)	91.62±0.32
DPPH activity (%)	90.50±2.26
Total phenolic content (mgGAE/100g)	1133.33±2.86

Values are mean ± standard deviation (n = 3)

Table 2 Mean sensory score for burfi prepared by different levels of ber powder

Level of incorporation (%)	Colour and appearance	Body and texture	Flavour	Sweetness	Overall acceptability
0 (T_0)	7.80 ± 0.07^{b}	7.23 ± 0.13^{c}	7.52 ± 0.08^{c}	7.82 ± 0.01^{bc}	7.58 ± 0.10^{c}
5 (T_1)	8.11 ± 0.19^{b}	7.51 ± 0.07^{d}	8.03 ± 0.06^{b}	8.30 ± 0.17^{ab}	7.97 ± 0.12^{b}
10 (T_2)	8.57 ± 0.25^{a}	8.66 ± 0.12^{a}	8.83 ± 0.20^{a}	8.48 ± 0.40^{a}	8.63 ± 0.10^{a}
15 (T_3)	7.35 ± 0.10^{c}	7.80 ± 0.01^{b}	7.21 ± 0.12^{c}	7.31 ± 0.11^{c}	7.41 ± 0.10^{c}

Values are mean ± standard deviation (n = 3)
Means in the same column that do not share a letter differ significantly (P<0.05)

Table 3 Colour values for burfi prepared by different levels of ber powder

Level of incorporation (%)	L*	a*	b*
0 (T_0)	47.34 ± 0.10^{a}	7.28 ± 0.08^{c}	17.63 ± 0.08^{a}
5 (T_1)	47.09 ± 0.02^{b}	7.47 ± 0.08^{c}	17.24 ± 0.12^{b}
10 (T_2)	44.38 ± 0.01^{c}	7.80 ± 0.10^{b}	16.25 ± 0.02^{c}
15 (T_3)	44.24 ± 0.04^{c}	8.07 ± 0.03^{a}	16.35 ± 0.05^{c}

Values are mean ± standard deviation (n = 3)
Means in the same column that do not share a letter differ significantly (P<0.05)

Table 4 Instrumental texture profile for burfi prepared by different levels of ber powder

Level of incorporation (%)	Hardness (g)	Adhesiveness	Springiness (g.sec)	Cohesiveness	Gumminess	Chewiness
0 (T_0)	5292.76±2.01^{d}	-30.50±0.25^{b}	0.25±0.03^{b}	0.27±0.02^{b}	1438.62±2.01^{d}	365.05±0.05^{d}
5 (T_1)	6546.72±3.00^{c}	-5.05±0.10^{d}	0.35±0.01^{a}	0.34±0.03^{a}	2205.97±2.01^{a}	764.71±0.03^{a}
10 (T_2)	6812.11±2.11^{b}	-17.88±0.31^{c}	0.32±0.06^{ab}	0.29±0.01^{ab}	1987.20±1.02^{b}	635.27±0.07^{b}
15 (T_3)	7018.78±3.00^{a}	-80.03±0.97^{a}	0.23±0.02^{b}	0.26±0.01^{b}	1851.94±0.98^{c}	425.52±0.31^{c}

Values are mean ± standard deviation (n = 3)
Means in the same column that do not share a letter differ significantly (P<0.05)

Table 5 Physicochemical and antioxidant parameters of different burfi samples

Parameters	Market burfi	Control burfi (T_0)	Optimized burfi (T_2)
Moisture(%)	21.37 ±0.06^{a}	19.42±0.04^{b}	17.65 ± 0.01^{c}
Fat (%)	21.50 ±0.02^{b}	22.60 ±0.01^{a}	19.46 ±0.01^{c}
Protein (%)	15.13±0.22^{a}	15.48 ± 0.02^{a}	13.20 ± 0.17^{b}
Acidity (% lactic acid)	0.24±0.02^{b}	0.25±0.006^{b}	0.36±0.01^{a}
Ash (%)	3.14±0.03^{a}	3.10±0.10^{a}	2.70±0.20^{b}
Total solids (%)	82.25 ±0.01^{b}	84.25 ±0.05^{a}	80.29 ±0.02^{c}
Total sugar (%)	54.13 ±0.03^{a}	36.04±0.19^{c}	36.86±0.02^{b}
Iron (mg/100g)	0.00 ± 0.00^{c}	2.55±0.04^{b}	5.50±0.05^{a}
Vitamin C (mg/100g)	0.00 ± 0.03^{c}	6.80 ± 0.11^{b}	18.98 ± 0.19^{a}
DPPH activity (%)	6.07 ± 0.35^{c}	15.46 ± 0.78^{b}	40.88 ± 0.56^{a}
Total phenolic content (mgGAE/100g)	110.27 ± 1.30^{c}	285.32± 2.01^{b}	406.72± 2.09^{a}

Values are mean ± standard deviation (n = 3)
Means in the same row that do not share a letter differ significantly (P<0.05)

showed increased total solids when the concentration of coconut sugar was increased as studied by Low et al. (2015).

Total sugar concentration showed a higher significant (P<0.05) difference among the three burfi samples. Total sugar was highest for market burfi, 54.13% and lowest for the control burfi, 36.04%. The addition of coconut sugar has pointedly contributed to the above findings. It is due to reason that coconut sugar has a total sugar concentration of 23.77-71.89% and sucrose of 49.41% as studied by Phaichamnanet al.(2010) and Somawiharja et al.(2018), respectively which is relatively lesser than the refined sugar which has a sucrose level of 99.80% (USDA). Iron content is also significantly affected by the addition of both coconut powder and ber powder (Table 1) because both are iron rich, therefore enriching burfi which does not have iron in it. The iron concentration ranged from 0.00 to 5.50 mg/100g.

The vitamin C content ranged from 0 to 18.98 mg/100g. Both coconut sugar and ber powder have contributed significantly(P<0.05) to the highest content of vitamin C in optimized burfi.

The DPPH free radical scavenging activity was also influenced by coconut sugar and ber powder. It ranged from 6.07 to 40.88%. The ber powder incorporation had the maximum significant (P<0.05) effect on the free radical capacity of optimized burfi. Ber fruit powder has free radical (DPPH) capacity of 90.50% as shown in Table 1. These findings are in resemblance with Kavitha and Kuna (2014) who formulated ber RTS beverage. Likewise,ber juice vinegar was formed and due to its high antioxidant activity it was called as functional vinegar (Vithlani and Patel 2010).

Coconut sugar also influenced the free radical capacity significantly(P<0.05). The results in the current findings are in close similarity with Low et al. (2015) who studied the antioxidant activity of probiotic ice cream by incorporating different levels of cane sugar and coconut palm sugar.

Total phenolic content of optimized burfi was significantly higher (P<0.05) as compared to market burfi and control burfi i.e. 406.72, 110.27 and 285.32 mgGAE/100g, respectively. Both coconut sugar and ber powder have influenced phenolic content. The total phenolic content and antioxidant activity are related to each other as they have hydroxyl group in their chemical structure (Tawaha et al. 2007). Results here are comparable with Victor and Orsat (2018) who studied that palm sugar has appreciable amount of antioxidant activity and total phenolic content. Also, Koley et al. (2011) reported that 12 commercial cultivars of *Z. mauritiana* are good source of ascorbic acid, total phenolics, flavonoids, and total antioxidant activity. Further, it was also observed that even though no fruit powder or polyphenolic substrate was added to market burfi, yet it showed some amount of anti-oxidative activity, viz., total phenolic content of 110.27 mgGAE/100g and DPPH inhibition of 6.07%. This could be due to association with free

sulphahydral groups and maillard browning products which were formed during preparation of khoa (Prasad et al. 2017). Similarly, Oh et al. (2013) reported maillard browning products have DPPH free radical scavenging activity.

Conclusions

Ber fruit powder is rich in vitamin C and has good antioxidant properties. With the results mentioned above, it can be concluded that the ber powder burfi with 10% level of ber powder and 28% coconut sugar was considered best. Both coconut sugar and ber powder have significantly enhanced the physicochemical properties, free radical scavenging activity and total phenolic content of burfi. Therefore, with such an outcome our attempt to formulate a value-added burfi with good antioxidant property and low sugar has been successfully attained.

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RESEARCH ARTICLE

Factors affecting milk yield, milk composition and physico-chemical parameters of ghee in Murrah buffaloes of Punjab region

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Abstract: The objective of this study was to find out the effects of season, stage of lactation, production status and parity on milk yield, milk composition and physico-chemical parameters of ghee in sixty Murrah buffaloes. Milk samples were collected from individual animal during morning in both summer (May - August) and winter (November - February) season. The results indicated that milk yield was significantly influenced by the stage of lactation, production status, and parity. The average fat percentage was significantly affected by season, stage of lactation, production status and parity. However, the protein percentage was affected by the production status and stage of lactation. The Reichert-meissl (RM) value of buffalo ghee was significantly influenced by the season, production status and lactation stage, however, Polenske value (PV) by the season, production status and parity. An effect of season, lactation stage, production status and parity on butyro-refractometer (BR) reading was significant.

Keywords: Murrah buffalo, Milk yield, Milk composition, Physico-chemical parameters of ghee

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Introduction

Buffaloes are considered as the major dairy animal of India belongs to the genus *Bubalus bubalis*. The current buffalo population in India as per latest 19th livestock census is 108.7 million which accounts for 21.23% of the total livestock population. Currently there are 51.05 million milch buffaloes which contribute 53% (67.68 million tonnes) of the total milk produced in the country (19th Livestock Census, 2012). Punjab had around 4.75% buffalo population of the country and bestowed with high milk producing breeds such as Murrah and Nili Ravi (19th Livestock Census, 2012). Murrah is one of the superior breeds of Indian buffaloes with a population of 20.49 million, which constitutes about 19.45% of the total buffalo population and is known for high productivity in the country (Chitra et al. 2018). Besides milk production, buffaloes contribute significantly towards meat production, draft power, dung for manure and fuel. To enhance productivity of a dairy animal, it is necessary to develop an understanding of the factors affecting its milk production and composition. The variation in milk production is a regular phenomenon in all milking animals; broadly the factors which are responsible for such variations can be divided into genetic (breeds, stage of lactation) and non-genetic (season, amount and quality of feed, parity, period of calving etc) factors (Bernabucci et al. 2002).

The physico-chemical quality of ghee (clarified butterfat) is usually assessed by analyzing certain characteristics such as Reichert-Meissl (RM) value, Polenske value, Butyro-refractometer (BR) reading, Iodine value and Saponification value. These analytical characteristics are mostly the reflections of the fatty acid composition of the milk lipid. For instance, RM value is substantially a measure of the lower chain volatile water soluble fatty acids i.e butyric acid ($C_{4:0}$) contributes about 3/4th and caproic acid ($C_{6:0}$) about 1/4th to this value whereas Polenske value is a measure of lower chain volatile water insoluble fatty acids i.e caprylic acid ($C_{8:0}$) contributes about 1/4th and capric acid ($C_{10:0}$) about 3/4th to this value. Butyro-refractometer (BR) reading, which measures the index of refraction between air and the liquid fat and varies with the nature of the fat, is usually determined at 40^{o}C. However, the composition of milk as well as fatty acid composition is largely affected by various factors i.e., lactation stage, lactation number, breed, season and environmental factors

(Yadav et al. 2013). The available information on various factors influencing the composition of milk and physicochemical properties of milk fat/ ghee is scanty in Indian buffaloes. Thus the present investigation was undertaken with the objective to study the effect of various factors affecting the milk yield, milk composition and chemical parameters of ghee prepared from Murrah buffalo's milk.

Materials and Methods

Sample collection and analysis

Sixty Murrah buffaloes from the Livestock farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU, Punjab, India) were kept under farm management. Diet of buffaloes was met through green fodder (*ad lib.)* and concentrate according to milk production. All the experimental animals were offered identical ration to meet production/maintenance requirement to negate the feeding effect on milk production. The animals had free access to water throughout the day. Samples of feed sample offered in two different seasons (May – August and November - February) were collected and ground to pass through 1 mm sieve and then analyzed for fat, protein, acid detergent fibre (ADF) and neutral detergent fibre (NDF) content (Robertson and Van Soest, 1981).

The buffaloes were grouped according to production status (high yielders, >15 kg/day; medium yielders, 8-15 kg/day; low yielders, <8 kg/day), stage of lactation (early - up to 100 days of postpartum; mid – 101 to 200 days; late – more than 201 days of postpartum) and parity (1st to 6th lactation). Milk samples were collected from individual animal during morning in both summer (May - August) and winter (November - February) season. Milk yield was recorded after complete milking. Contents of fat, solid-not-fat (SNF) and protein in milk samples were analyzed by a MilkoScreen (Indifoss Analytical Pvt Ltd, Ahmedabad, India). Cream was separated from the each of the individual milk samples by centrifugal method in the cream separator. Cream samples were then converted in to ghee by direct cream method as described by De (2005). Ghee samples were stored in refrigerator (4^{o}C) till for further analysis. Ghee samples were analyzed for Reichert Meissl (RM) and Polenske value as per the standard procedure described in IS: 3508 (ISI, 1966). Butyro refractometer (BR) reading of ghee was measured using digital butyro refractometer (Atago Co Ltd, Tokyo, Japan).

Statistical analysis

The data was analyzed in a factorial design (Snedecor and Cochran, 1994) by using the software package SPSS version 16 (SPSS, 1996) and differences in mean was assessed by using Tukey's b test.

Results and Discussion

The chemical composition of feed offered to buffaloes in different season is represented in the Table 1. The buffaloes were offered 18.5 and 12.8 kg of dry matter in summer and winter, respectively. The roughage to concentrate ratio was 65:35 and 57:43 per cent in summer and winter, respectively. The roughage portion constituted available green fodder (48%) and silage (17%). The crude protein and fat content were higher in the winter fodder, whereas NDF and ADF content were higher in the summer fodder.

Effect of season on milk yield, milk composition and physico-chemical parameters of ghee

The seasonal differences can attributed to the differences in quality and quantity of available fodder and climatological variation in different seasons which have direct impact on production traits. The data in Table 2 revealed that milk yield of lactating buffalo was not affected by the season. However, fat percent was observed to be significantly higher ($P<0.05$) during winter than summer season. Pawar et al. (2012) observed that milk fat percentage of buffaloes calved in winter season (November – February) was significantly ($P\leq0.05$) higher than that of the animals calved in summer (March – June) season, due to environmental effects. The content of protein, SNF and daily yield of fat and protein were not affected by the season. Sikka et al. (2004) observed that season of calving did not show any significant difference in protein content of buffalo milk.

The RM and Polenske values of buffalo ghee were observed to be higher ($P<0.05$) during summer than winter season. Mor et al. (2018) also reported that RM and Polenske values of pure buffalo ghee clarified at 110^{o}C vary significantly with season. BR reading of buffalo ghee was found to be lower ($P<0.05$) in summer

Table 1. Composition (% dry matter) of the feed offered to buffaloes in different seasons

Parameter	Season	
	Summer	Winter
Dry matter, kg /day	18.5	12.8
Roughage : Concentrate ratio, %	65:35	57:43
Crude Protein, %	13.0	18.1
Fat, %	2.4	3.13
NDF, %	54.12	44.5
ADF, %	34.01	31.2

Results are expressed as mean values, n=5

compared to winter season. For buffalo milk fat, the lowest BR reading (40.40) was observed in January but highest (41.20) was observed in the July (Kumar et al. 2017). Mor et al. reported that BR reading of buffalo ghee clarified at 110°C was highest in the month of February - March (42.22±0.004) and lowest in month of August -September (41.29±0.004). From the results it can be concluded that effect of season was visible in BR reading, RM and Polenske value of ghee. This may be due to the change in feeding as well as some seasonal variations like heat and humidity, wherein behavior of animals with respect to feed intake and digestion was affected.

Effect of production status on milk yield, milk composition and physico-chemical parameters of ghee

The daily milk yield varied from 4.43 (low yielders) to 13.31 kg (high yielders). The fat and protein content of milk followed the reverse trend and was observed to be highest in milk of low producing animals (Table 3). However, SNF content was not affected by production status of animals. Khan et al. (2011) also reported that high yielders (7.36%) had the lowest fat contents, followed by moderate (7.46%) and low yielders (7.58%). However, SNF content did not show any significant difference among the groups. The daily yield of fat and protein was observed to be higher (P<0.05) from animals yielding higher milk. Higher fat and protein yield were observed in high milk producing buffaloes than medium and low producing buffaloes.

RM value of buffalo ghee varied from 30.09 (low yielders) to 35.25 (high yielders) (Table 3). RM value was found to be higher (P<0.05) in high yielders than medium and low yielders. The polenske value of ghee was observed to be highest (P<0.05) in medium yielders and lowest (P<0.05) in low yielders. The BR reading increased with decrease in milk yield of animals and values were found to be higher in low yielders than high and medium yielders.

Effect of stage of lactation on milk yield, milk composition and physico-chemical parameters of ghee

Average milk yield was 10.45 kg/day during early lactation, decreased up to 6.5 kg/day during late lactation (Table 4). With

Table 2. Effect of season on milk yield, milk composition and physico-chemical parameters of ghee

Parameter	Season#		
	Summer	Winter	PSE
Milk yield kg/day	8.57	8.42	0.21
Fat, %	7.84[a]	8.73[b]	0.27
Protein, %	4.55	4.65	0.076
SNF, %	11.22	10.85	0.16
Fat yield, kg/day	0.65	0.70	0.026
Protein yield, kg/day	0.38	0.38	0.01
RM value	34.51[b]	31.96[a]	0.42
Polenske value	1.18[b]	0.85[a]	0.023
BR reading	40.03[a]	41.12[b]	0.11

Mean values with different superscripts in a row differ significantly (P<0.05), n=60. #Irrespective of lactation number, stage of lactation and production status. PSE – Pooled standard error

Table 3. Effect of production status on milk yield, milk composition and physico-chemical parameters of ghee

Parameter	Production status#			
	High yielders	Medium yielders	Low yielders	PSE
Milk yield kg/day	13.31[c]	7.74[b]	4.43[a]	0.26
Fat, %	7.54[a]	8.39[b]	8.93[c]	0.34
Protein, %	4.38[a]	4.53[a]	4.80[b]	0.10
SNF, %	11.09	11.02	11.00	0.20
Fat yield, kg/day	0.99[c]	0.64[b]	0.38[a]	0.033
Protein yield, kg/day	0.58[c]	0.35[b]	0.21[a]	0.013
RM value	35.25[c]	32.66[b]	30.09[a]	0.53
Polenske value	0.92[b]	1.03[c]	0.82[a]	0.029
BR reading	40.08[a]	40.59[b]	41.05[c]	0.014

Mean values with different superscripts in a row differ significantly (P<0.05). #Irrespective of lactation number, stage of lactation and season. PSE – Pooled standard error

the advancement of lactation stage, daily milk yield decreased significantly. Results of the present study is in agreement with study of Yadav et al. (2013) who reported that effect of lactation stage on milk yield of Murrah buffaloes was significant which varied from 9.41 (1-12 weeks) to 4.36 kg/day (> 48 weeks). The fat percentage of buffalo milk increased with advancement of stage of lactation and values were higher (P<0.05) during late than mid and early lactation. Sethi et al. (1994) reported that lactation stage significantly affected the fat content of buffalo milk. Milk fat content increased with a concomitant decrease in milk yield during advance lactation (Yadav et al. 2013). The increase in total lipid contents may be due to the activity of fatty acid synthesizing enzymes particularly acetyl CoA carboxylase which is a regulatory enzyme in the fatty acid synthesis might have slightly increased in late lactation than early and mid lactation (Sharma et al. 2000). Protein percentage also increased with progress of lactation stage, however, significant difference was not observed between early and mid lactation. Similar result was also reported by Sharma et al. (2000) for fat and protein content of buffalo milk. The results (Table 4) depicted that SNF content, yield of fat and protein remain almost unchanged throughout the lactation period. These results are in good agreement with those reported by Sharma et al. (2000).

The RM value of ghee decreased with the advancement of lactation stage and values were higher (P<0.05) during early than mid and late lactation (Table 4). However, Sharma et al. (2000) reported that short chain fatty acids ($C_{4:0}$ and $C_{6:0}$) of Murrah buffalo milk was higher during mid than early and late lactation. Polenske values were lower in early than mid and late lactation, however, significant difference was not observed. Our results are in good agreement with study of Sharma et al. (2000) who reported that short chain fatty acids ($C_{8:0}$ and $C_{10:0}$) content were lower in early than mid and late lactation. BR reading of ghee increased with advancement of lactation period and values were higher (P<0.01) in late than early and mid lactation. Sharma et al. (2000) also observed that total unsaturated fatty acids of buffalo's milk fat were higher during late than early and mid lactations.

Effect of parity on milk yield, milk composition and physico-chemical parameters of ghee

Table 4. Effect of stage of lactation on milk yield, milk composition and physico-chemical parameters of ghee

	Stage of lactation[#]			
Parameter	Early lactation	Mid lactation	Late lactation	PSE
Milk yield, kg/day	10.45^{c}	7.94^{b}	6.50^{a}	0.24
Fat, %	7.24^{a}	8.26^{b}	8.75^{b}	0.32
Protein, %	4.46^{a}	4.47^{a}	4.75^{b}	0.09
SNF, %	11.03	10.82	11.26	0.19
Fat yield, kg/day	0.66	0.69	0.68	0.031
Protein yield, kg/day	0.39	0.38	0.39	0.012
RM value	34.67^{c}	32.69^{b}	31.24^{a}	0.50
Polenske value	0.99	1.05	1.01	0.027
BR reading	40.33^{a}	40.83^{b}	41.45^{c}	0.14

Mean values with different superscripts in a row differ significantly (P<0.05). [#]Irrespective of season, lactation number and production status. PSE – Pooled standard error

Table 5. Effect of parity on milk yield, milk composition and physico-chemical parameters of ghee

Parameter	Parity[#]						
	1	2	3	4	5	6	PSE
Milk yield, kg/day	7.85^{ab}	8.08^{ab}	7.63^{a}	7.32^{a}	8.67^{b}	8.74^{b}	0.33
Fat, %	7.54^{a}	7.75^{ab}	8.85^{b}	8.60^{ab}	8.66^{ab}	8.33^{ab}	0.44
Protein, %	4.58	4.53	4.62	4.61	4.65	4.61	0.12
SNF, %	11.00	10.74	11.18	11.11	11.05	11.12	0.26
Fat yield, kg/day	0.59	0.66	0.69	0.68	0.70	0.71	0.42
Protein yield, kg/day	0.37	0.39	0.37	0.38	0.39	0.39	0.015
RM value	33.14	33.27	32.56	33.81	32.88	33.73	0.68
Polenske value	0.96^{ab}	0.88^{a}	1.02^{b}	1.02^{b}	1.05^{bc}	1.16^{c}	0.037
BR reading	40.86^{b}	40.80^{b}	40.54^{b}	40.63^{b}	41.12^{b}	39.50^{a}	0.18

Mean values with different superscripts in a row differ significantly (P<0.05). [#]Irrespective of season, stage of lactation and production status. PSE – Pooled standard error

Effect of parity on milk yield, milk composition and chemical parameters of ghee is represented in the Table 5. The daily milk yield varied from 7.32 (3rd) to 8.74 kg (6th) and it was significantly influenced by the parity but no systematic trend on daily milk yield could be observed. Study corroborates with earlier findings (Yadav et al. 2013) revealing that significant effect of parity on milk yield exists in Murrah buffaloes. Most of the workers have reported increase in milk yield up to fifth parity and decline thereafter (Lee and Kim, 2006; Yadav et al. 2013). However, Bashir et al. (2015) reported that average milk yield increased with parity, peaked in the third lactation and declined in the later parities. Bath et al. (1985) suggested 20% of the increase in milk production with advancing lactations (age) due to increased body weight and 80% increase is due to the effect of recurring pregnancy in cattle. The fat percentage of buffalo milk increased from first to third lactation and then declined in subsequent lactations. Similar result was also reported by Sodhi et al. (2008) in Murrah buffaloes. Verma et al. (2017) reported that milk fat percentage varied significantly among the parities with no consistent increase over the advancement of the parities. The content of protein and SNF and daily yield of fat and protein were not differed significantly in different parities. Similar results were reported by Sodhi et al. (2008) for the protein and lactose content in buffalo milk. Milk protein level did not vary significantly over the parities till sixth parity (Yadav et al. 2013). Sikka et al. (2004) reported that no significant difference was found in milk protein in relation to the variation in parity of buffaloes.

RM value of ghee did not differ significantly ($p>0.05$) in different parity, however it varied from 32.56 to 33.81. The Polenske value was observed to be highest ($p<0.05$) of ghee obtained from animals in 6th parity and low in 1st parity. BR reading varied from 39.5 (6th) to 41.1 (5th). There was no significant difference in BR reading was observed till 5th parity and later decreased during 6th parity.

Conclusions

From study, it was found that effect of season, lactation stage, production status and parity were visible in milk yield, milk composition and chemical parameters of buffalo ghee. Average milk yield was significantly influenced by the stage of lactation, production status, and parity. The fat percentage was significantly affected by season, stage of lactation, production status and parity. However, the protein percentage was affected by the production status and stage of lactation. The RM value of buffalo ghee was significantly influenced by the season, production status and lactation stage, however, polenske value by the season, production status and parity. An effect of season, lactation stage, production status and parity on BR reading was significant. The values obtained for different physico-chemical constants of ghee were within the limit specified by Food Safety and Standard (Food Products Standards and Food Additives) Regulations, 2011 (FSSA, 2006) irrespective of season, lactation stage, production status and parity.

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RESEARCH ARTICLE

Iron fortification of shrikhand using *Murraya koenigii* leaves extract

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Abstract: In the present study, a novel shrikhand enriched with vitamin C and iron was developed using curry leaves extract. Encapsulation, which is the latest trend in delivering the phytochemicals through food products has increased the acceptability of the product. The treatment S_3 which had the curry leaves extract of highest concentration 3:10 W/V had a good score on sensory evaluation due to the wall material - sodium alginate which acted as a barrier between the product and the extract without influencing the acceptability of shrikhand with its strong off-flavor. The nutrient composition of iron-fortified shrikhand for energy, carbohydrate, fat, protein, moisture and ash was 199.65 Kcal, 23.67 g, 9.62 g, 4.55 g, 61.26 g and 0.85 g per 100 g of the product, respective vitamin C and iron content of product was of 18.86 mg/100g and 2.26 mg/100g.

Keywords: Curry leaves, Encapsulation, Iron, Shrikhand, Vitamin C

Introduction

Food can be defined as a substance that is composed of macronutrients - carbohydrates, proteins and fat and the necessary micronutrients including vitamins and minerals that are vital for an organism to sustain itself (The Editors of Encyclopædia Britannica 2018). Fruits and vegetables have long been considered as a major source of micronutrients. However, dairy products have now been evidenced to possess several minerals and trace elements that contribute to a healthy system and has been included in several nutritional food guidelines (Painter et al. 2002). Indian ayurvedic scripts since 6000 BC refers to the consumption of fermented milk products (Brothwell and Brothwell ,1998). Consumption of fermented products by people around the globe increases especially during the hot climate (Nicholls et al.1939). These products are mainly desirable due to their high acidity, which keeps the product away from harmful pathogens. Since milk contains a reasonably high nutritional quality and has higher bioavailability, milk has conventionally been recommended as a nutritional food for daily consumption (Claeys et al. 2013). On the other hand, milk lacks some essential minerals and vitamins such as iron and vitamin C (Fernandez, 2017).

Shrikhand is a fermented dairy product of Indian origin and the name is derived from the Sanskrit word 'Shikharani' that refers to a delicacy prepared with curd, fruits, nuts, sweeteners, and flavoring agents. Shrikhand is used as a dessert in several places in India including Gujarat, Maharastra, Karnataka and certain parts of South India (Aneja et al. 2002). The macronutrients contained in Shrikhand comprises of 10% fats, 78% carbohydrates and 11.5% proteins and has a high water (moisture) content of 39% with an acidic pH range of 4.2 to 4.4 (Kulkarni et al. 2006; Boghra et al. 2000)

Murraya koenigii (curry leaves) have always been an integral part of the Indian Ayurvedic system of medicine and have been reported as an appetizer, carminative, anti-inflammatory agent, antibacterial agent (Husain 1992). Dinesh et al. (2015) evaluated the ascorbic acid content in selected Indian spices including curry leaves. The findings reported that curry leaves have 22.53 mg/100g of ascorbic acid present in them. Singh et al. (2014) compared the nutrition and mineral profile of curry leaves in fresh and dried form. It was observed to be 0.93 mg/100g of iron when it is fresh and 12 mg/100g when it is dehydrated. *Murraya koenigii*, in Indian dialects, curry leaves or karipatta belongs to the family Rutaceae that represents more than 150 genera and 1600 species (Satyavati et al. 1987). As it has a high nutrition

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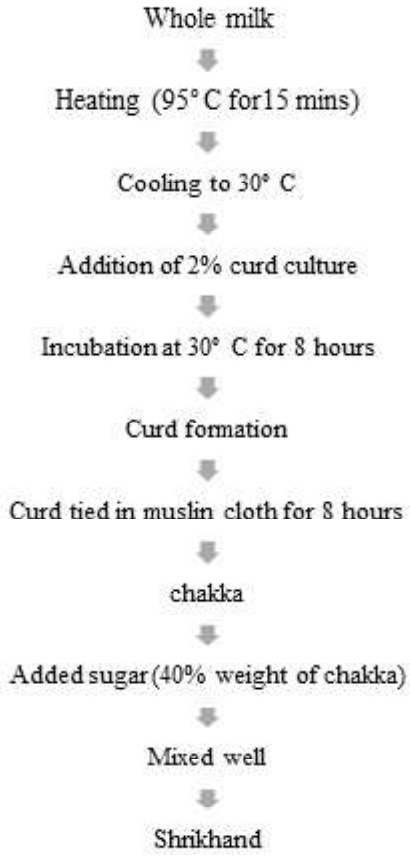

Fig. 1 Production of shrikhand (flow chart)

profile it can be used as an ideal supplement to fermented dairy products.

Encapsulation is defined as a technique that used to protect a component from external environment. The protective layer serves as a barrier between the encapsulated component and environment without altering the nature of the component. Sodium alginate is a commonly used material that is most compatible with all kinds of encapsulation. Usually, they are used as a combination with other components for the encapsulation of probiotic cultures (Burgainet al. 2011) and since it can absorb water and facilitates easy manipulation, sodium alginate is used as a material for encapsulation in the food industry (Goh et al. 2012). It is known for its gelling, stabilizing and thickening properties. So, in the present study, it is intended to encapsulate the curry leaves extract using sodium alginate and is added to the dairy product.

Materials and Methods

Preparation of curry leaves powder

The methods used by Das et al. (2011) with slight modifications was followed. The twigs were removed and the leaves alone were collected, washed in distilled water and dried in a solar dryer at 50-60° C until the moisture got removed. Once the moisture was completely removed, they were pulverized to a fine powder with a sieve and stored in a desiccator for further use.

Preparation of curry leaves extract

Methods used by El-Amin et al. (2013) with slight modifications was followed. The powdered sample was taken in a beaker with different solvents with a 1:10; 2:10 and 3:10 (W/V) ratio and subjected to continuous stirring at room temperature with a magnetic stirrer for 6 hours. Then the extract was filtered using Whatman filter paper, sealed and stored in the refrigerator for further use.

Preparation of shrikhand

The method followed by Swapna and Chavannavar (2013) was adapted for the preparation of shrikhand shown in Figure 1 .

Preparation of alginate and gelling solution

The alginate and gelling solution were prepared based on the protocol by Valenzuela et al. (2014) with slight modifications. 2g

of sodium alginate powder (food grade) was dissolved in 100 mL of distilled water and stirred continuously for 30 mins at room temperature to produce 2% (W/V) alginate solution. The gelling solution was prepared by dissolving 2.8 g of calcium chloride in 40 mL of distilled water.

Preparation of alginate encapsulates with curry leaves extract

The aqueous curry leaf extract was added to 2% food grade sodium alginate powder. It was mixed completely with a magnetic stirrer. The extract was loaded into a syringe and dripped down into the calcium chloride bath (gelling solution). The beads formed were washed and stored at 4°C for future use.

Standardization and optimization of the curry leaves encapsulates in shrikhand

Plain shrikhand was prepared. The curry leaves extracts that were extracted with different concentrations and encapsulated with sodium alginate were added.

The experimental model is illustrated below in Table 1.

Sensory evaluation of the product

Sensory evaluation of the product was carried out by trained judges on a 9-point Hedonic scale with sensory attributes viz. taste, odor, mouthfeel, color, and overall acceptability at the College of Food and Dairy Technology, Chennai – 52.

Proximate analysis of the product

The moisture protein, fat, and ash were determined by the method suggested in FSSAI manual (2016), sections 14 and 15 (Milk and milk products). The crude fiber content was determined by the method suggested in AOAC 20th edition (2016) 926.09. The Nitrogen Free Extract (NFE) and energy were calculated by the difference method suggested in ISI: SP: 18 part XI, (1989) provided below.

% carbohydrates (NFE) = {100- (Moisture + Total ash + Total protein + Fat)}

Energy = {(protein x 4) + (carbohydrate x 4) + (fat x 9)}

Analysis of vitamin C and iron

The method suggested in AOAC 20th edition (2016) 926.09 was followed for the estimation of iron as (Fe) iron and the procedures recommended in FSSAI manual (2016) 2 (Fruits and vegetable product) were followed for the estimation of ascorbic acid (vitamin C).

Statistical analysis

The data collected on various parameters were statistically analyzed using IBM SPSS version 23. One way Analysis of variance (ANOVA) at 5% level of significance was used to evaluate all the results as per the standard method listed in the procedure of Snedecore and Cochran, (1980).

Results and Discussion

Optimizing the concentration of curry leaves extract encapsulated in alginate encapsulates on shrikhand

The mean ± SE of taste, odour, mouth feel, colour and overall acceptability scores of shrikhand topped with alginate encapsulates viz. S_0, S_1, S_2 and S_3 has been represented in Table 2.

In the present study, a significant ($P<0.05$) difference occurred in sensory attributes between the control (S_0) and all the other treatments viz. S_1, S_2 and S_3.

The acceptability of the shrikhand topped with alginate encapsulates is measured in terms of sensory attributes such as taste, odour, mouthfeel, colour, and overall acceptability.

Sensory evaluation of shrikhand topped with alginate encapsulates was carried out separately for each treatment (S_1, S_2, and S_3) with 1:10, 2:10 and 3:10 W/V of curry leaves extract respectively encapsulated with alginate as a wall material and with control S_0 (plain shrikhand without the encapsulates). The optimization of the product was predicted based on the sensory score given by the judges. Shrikhand topped with alginate encapsulates of different concentrations of curry leaves extract selected from each treatment was evaluated based on the sensory attributes to select the best one.

Statistical analysis revealed there is a highly significant (P d" 0.01) difference concerning taste, odour, colour and overall

Table 1 Experimental model for the product

Product	Experimental model	Code
Shrikhand	Plain shrikhand (Control)	S_0
	Plain shrikhand* + alginate encapsulates** with (1:10) W/V of curry leaves extract	S_1
	Plain shrikhand* + alginate encapsulates** with (2:10) W/V of curry leaves extract	S_2
	Plain shrikhand*+ alginate encapsulates** with (3:10) W/V of curry leaves extract	S_3

(*) 95 g of the dairy product

(**) 5 g of the encapsulates as a topping

acceptability and also a significant ($0.01 < P \leq 0.05$) difference with regard to mouthfeel between the treatments.

The order of sensory attribute test was identified as ($S_0 > S_3 > S_2 > S_1$) for taste, ($S_0 > S_2 > S_3 > S_1$) odour, ($S_0 > S_1 > S_2 > S_3$) mouthfeel, ($S_0 > S_3 > S_2 > S_1$) colour and ($S_0 > S_3 > S_1 > S_2$) overall acceptability.

The treatment S_3 was significantly superior over other treatments (S_1, S_2). The shrikhand S_3 topped with alginate encapsulates with 3:10 W/V of curry leaves extract had better overall acceptability (7.80) following the plain shrikhand S_0 (8.39) without encapsulates. The results indicate that the addition of alginate encapsulates over shrikhand has influenced the sensory scores with respect to taste, odour, mouth feel, colour and overall acceptability.

Proximate analysis of the product

Table 3 shows the comparison of mean ± SE proximate analysis of control (S_0) and shrikhand topped with alginate encapsulates (S_3). The results obtained for energy, carbohydrate, fat, protein, moisture, ash and crude fiber for plain shrikhand (S_0) and shrikhand topped with alginate encapsulates (S_3) are as follows, energy (212.50 ± 0.49) and (199.65 ± 0.27), carbohydrate (26.96 ± 0.12) and (23.67 ± 0.06), fat (9.61 ±0.007) and (9.62 ± 0.004), protein (4.52 ± 0.008) and (4.55 ± 0.008), moisture (58.16 ± 0.12) and (61.26 ± 0.06), ash (0.82 ± 0.003) and (0.85 ± 0.003). The crude fiber was found to be below the detection level in both S_0 and S_3.

On comparing the obtained figures of nutritional profile, the 0.03 g raise in protein on S_3 may be due to the presence of curry

Table 2 Sensory analysis for shrikhand topped with alginate encapsulates encapsulated with curry leaves extract of different concentration (Mean ± SE)[@]

Sensory attributes (9-point hedonic scale)	Concentration of alginate encapsulates topped in shrikhand				
	S_0	S_1	S_2	S_3	F value
Taste	$8.57^{c} \pm 0.10$	$7.87^{a} \pm 0.08$	$7.75^{a} \pm 0.12$	$8.00^{a} \pm 0.17$	7.21^{**}
Odour	$8.29^{c} \pm 0.07$	$7.45^{a} \pm 0.17$	$8.04^{bc} \pm 0.18$	$7.66^{ab} \pm 0.15$	5.83^{**}
Mouth feel	$8.33^{c} \pm 0.15$	$8.12^{bc} \pm 0.22$	$7.75^{ab} \pm 0.12$	$7.58^{a} \pm 0.15$	4.15^{*}
Colour	$8.41^{c} \pm 0.12$	$7.50^{a} \pm 0.11$	$7.50^{a} \pm 0.14$	$7.95^{b} \pm 0.17$	9.68^{**}
Overall acceptability	$8.39^{b} \pm 0.04$	$7.73^{a} \pm 0.05$	$7.76^{a} \pm 0.04$	$7.80^{a} \pm 0.04$	33.99^{**}

Data are presented in Mean ± SE, n=6 (Different superscripts in a row differ significantly)

** - Highly Significant ($P \leq 0.01$)

* - Significant ($0.01 < P \leq 0.05$)

NS – Not Significant ($P > 0.05$)

S_0 - Plain shrikhand

S_1 - Plain shrikhand+5 g of 1:10 (W/V) alginate encapsulates

S_2 - Plain shrikhand +5 g of 2:10 (W/V) alginate encapsulates

S_3 - Plain shrikhand +5 g of 3:10 (W/V) alginate encapsulates

Table 3 Proximate analysis of product (Mean ± SE)[@]

Parameter	Unit	S_0	S_3
Energy	Kcal/100g	212.50 ± 0.49	199.65 ± 0.27
Carbohydrate	g/100g	26.96 ± 0.12	23.67 ± 0.06
Fat	g/100g	9.61 ± 0.007	9.62 ± 0.004
Protein	g/100g	4.52 ± 0.008	4.55 ± 0.008
Moisture	g/100g	58.16 ± 0.12	61.26 ± 0.06
Ash	g/100g	0.82 ± 0.003	0.85 ± 0.003
Crude Fiber	g/100g	BDL	BDL

BDL – Below the Detection Level

Data are presented in Mean ± SE, n=6

Table 4 Analysis of vitamin C and iron

Parameter	Unit	Y_0	Y_3
Vitamin C	mg/100g	BDL	18.86 ± 0.05
Iron	mg/100g	BDL	2.26 ± 0.01

BDL – Below the Detection Level

Data are presented in Mean ± SE, n=6

leaves extracts on the encapsulates which has 11.8% of the protein in it (Zhang et al. 2011). The 0.01 g increase in fat in the product S_3 may be due to the wall material (alginate) as reported by a previous study (Reyes-Tisnado et al. 2005) where 2.11 g/100g of fat was obtained post encapsulation. An increase in moisture content on product S_3 was noticed due to the addition of encapsulates which hold the aqueous curry leaves extract. It may also be due to the addition of alginate beads which has 11.10 % moisture as reported by (Reyes-Tisnado et al. 2005). Though there is an increase in the moisture content of product S_3 there occurs a slight decrease in carbohydrate and energy content of the products compared to the plain shrikhand S_0.

Analysis of vitamin C and iron

Table 4 shows the comparison of mean ± SE vitamin C and iron of control (S_0) and shrikhand topped with alginate encapsulates (S_3).

The results obtained for the vitamin C and iron for plain shrikhand (S_0) and shrikhand topped with alginate encapsulates (S_3) are as follows - vitamin C (BDL and 18.86 ± 0.005) iron (BDL and 2.26 ± 0.01) respectively.

On interpreting the obtained data on vitamin C and iron, iron and vitamin C were not detected in plain shrikhand (S0) due to the commonly known fact that milk and milk products lack in vitamin C and iron (Fernandez, 2017).

On the other hand, the shrikhand topped with alginate encapsulates that has curry leaves extract shows a remarkable enrichment on its vitamin C and iron profile by increasing the content to 18.86 ± 0.05 mg/100g and 2.26 ± 0.01 mg/100g respectively. However, the vitamin C content was lower and the iron content was higher when compared to the findings of other researchers (Bahuguna and Vijayalakshmi, 2018). The iron content was slightly higher than that obtained by Ranjitha and Sudha (2016).

Conclusions

The curry leaves extract extracted with 3:10 W/V of curry leaves powder and encapsulated using alginate added to shrikhand (S_3) and found to be acceptable by sensory evaluation. Nutrition analysis on S_0 and S_3 was found to be good in all the parameters. The product topped with alginate encapsulates S_3 was found to have an increased level of vitamin C and iron. From the present study, it is concluded that curry leaves extract is rich in vitamin C and iron and can be used in dairy products by encapsulating them which serves as a barrier between the product and the extract.

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RESEARCH ARTICLE

Nutritive value of ghee residue incorporated bakery product

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Abstract: The present study entitled "Nutritive value of ghee residue incorporated bakery product" was carried out with the objectives to utilize the ghee residue for the preparation of bakery products. The products prepared were cake and muffins by utilization of ghee residue incorporated in different proportions and served as treatments T_1, T_2, T_3 and T_4 respectively and T_0 served as control. Estimation of fat, carbohydrate, protein, energy, calcium was found by AOAC (2005) methods. Sensory evaluation was carried out using the nine-point Hedonic scale. The nutritive value of the prepared products was determined using food composition tables. Data obtained were statistically analysed by using analysis of variance (ANOVA), (t) test and critical difference (CD) techniques. On the basis of findings, it was observed that in case of Cake and muffins both 60 percent refined flour and 40 percent ghee residue incorporation level scored the best with regard to colour, body and texture, taste and flavour and overall acceptability. In case of cake, the calcium (71.35/100g), protein (23.48g/100g) and fat (89.96g/100g) were increased as compared to control while in muffins calcium (68.01mg/100g), protein (21.48g/100g) and fat (90.72g/100g) were increased as compared to control. Cost of the products on the basis of raw ingredients per 100g ranged between Rs 29.00 for cake, Rs 31.00 for muffins. It was concluded that ghee residue can be incorporated in the preparation of different products for improving the nutrient content.

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Introduction

The utilization of different bakery and confectionary products is in demand now a days due to the changes in food habit of people. Cake is one of the relished and palatable baked products which is prepared from refined flour, sugar, shortening, baking powder, egg, essence as principal ingredients and Muffins are sweet, spongy breakfast or evening snack food prepared traditionally from refined flour, sugar, oil/fat, milk and eggs (Kaur et al.2017). Preparation of plain cakes from refined flour is the conventional practice (Giami et al. 2004) however cake and muffins prepared with ghee residue have not been done so far.

India is the global leader in the production of milk and ghee residue (GR) and its production is more than 3 MT/annum (Varma and Raju, 2008). Ghee residue is a nutritious by-product of the dairy industry, obtained during the "creamery-butter" method of ghee manufacture. It is a good source of energy as well as protein and calcium (Arumugam et al. 1989). Nutritive value of the ghee residues and its complete analysis revealed the mean value of moisture content, crude protein, crude fibre, ether extract, nitrogen free extract and total ash contents of ghee residue were 12.10, 19.86, 3.49, 47.12, 25.63 and 3.90 percent respectively. Fatty acid profile of ghee residue revealed that the palmitic acid registered the highest percentage (38.88) among saturated fatty acids and the oleic acid accounted for the highest percentage (25.15) among unsaturated fatty acids. Linoleic, linolenic, eicosatetraenoic and docosahexaenoic acid content of ghee residue were 2.02, 0.79, 0.36 and 0.25 per cent respectively. Amino acid profile of ghee residue revealed that the lysine and methionine, content were 0.99 and 0.61 per cent, respectively. Threonine and arginine levels are found to be at 1.44 and 0.76 per cent, respectively. The glutamic acid recorded the highest percentage (5.26), while cystine registered the lowest percentage (0.35) among amino acids in ghee residue. Thus, it could be concluded that ghee residue is a rich source of fat, protein, unsaturated fatty acids and amino acids (Ramesh et al. 2018).

An optimized process for the extraction of protein from ghee residue was suggested by (Munirathnamma et al. 2017) which

signifies its competence to be an excellent food item for human nutrition. Ghee residue can also be a good source for overcoming protein-energy malnutrition (Dua et al. 2018). In India, the annual production of milk is 165.4 million tons as per NDDB statistics 2016-17. About 30 - 35% of the milk produced in India is converted in to ghee (Gandhi et al. 2013).

Ghee residue obtained at household level of ghee production is generally consumed after mixing with cooked rice or by spreading it over chapattis. However, in large ghee manufacturing and refining plants. It is thrown away as a waste product, losing a huge quantity of nutrients in terms of fat, protein and minerals. Ghee residue thus by virtue of its chemical composition, physical characteristics and long shelf life permitting its collection and centralized handling has great potential and is more emendable to exploit its utilization (Rao and Gopinath, 2001).

The aim of this study, therefore was to assess nutritional and sensory properties of ghee residue incorporated in cake and muffins with the aim of encouraging the use of these under-utilized products in developing value-added products with nutraceutical potential.

Materials and Methods

Fresh cream was collected from the local market of Allahabad and Varanasi each time as per the requirement. Ghee residue was obtained by heating fresh cream. Other ingredients (refined flour, milk, salt, sugar, egg and butter etc.) were purchased from the local market of Allahabad.

Preparation of Ghee residue

Ghee residue was obtained by heating cream at 50-60ºC for 30-40 min or medium flame until and unless the ghee and ghee residue get separated, as given by (Aneja et al. 2002)

Development of products

Two products namely Cake and Muffins were prepared by ghee residue and other ingredients. The products were prepared by using standard recipe.

Treatment and replication of the products

Table 1 shows refined flour was replaced with ghee residue for preparation of bakery products namely cake and muffins. The whole experiment was replicated four times with four treatments.

Details of Treatments

1. Cake: -

- Control (T_0): Cake was prepared with only standard ingredients without any incorporation of ghee residue.
- Treatment(T_1): Cake was prepared with refined flour and ghee residue in a ratio of 90:10.
- Treatment (T_2): Cake was prepared with refined flour and ghee residue in a ratio of 80:20.
- Treatment (T_3): Cake was prepared with refined flour and ghee residue in a ratio of 70:30.
- Treatment (T_4): Cake was prepared with refined flour and ghee residue in a ratio of 60:40.

2. Muffins: -

- Control (T_0): Muffin was prepared with only standard ingredients with any incorporation of ghee residue.
- Treatment(T_1): Muffin was prepared with refined flour and ghee residue in a ratio of 90:10.
- Treatment (T_2): Muffin was prepared with refined flour and ghee residue in a ratio of 80:20.
- Treatment (T_3): Muffin was prepared with refined flour and ghee residue in a ratio of 70:30.
- Treatment (T_4): Muffin was prepared with refined flour and ghee residue in a ratio of 60:40.

Sensory evaluation of developed products

The panel of judges assessed the coded cake and muffins samples at random, according to the methodology described by (Meilgaard et al.1999). Sensory evaluation of samples was carried out with a 10-member panel (ages 22 to 45 year) who were scientists, students and technical staff of Department of Food & Nutrition, Ethelind School of Home Science SHUATS Allahabad. The panellists had a good knowledge on the sensory evaluation of dairy products and participated previously in such evaluations. Sensory evaluation of the samples was carried out in the sensory evaluation room under appropriate fluorescent lighting. Each panellist was asked to taste the samples and rate the sensory parameters on a 9-point hedonic scale. According to the 9-point structured hedonic scale, the acceptance test was carried out for the attributes of colour and appearance, body and texture, flavour and overall acceptability. For all the attributes, 9-point scale was defined as that the highest value indicates the highest degree of preference.

Chemical analysis of developed products

Products prepared were chemically analysed by the standardized procedure of AOAC (2005). The proximate components viz., moisture, ash, carbohydrate, fat, protein and calcium were determined by using standard procedure prescribed by AOAC (2005) each sample were replicated three times.

Cost of developed products

The prevailing prices of the ingredients used in the preparation of the products were used to calculate their actual cost.

Statistical analysis

The data obtained from sensory evaluation were statistically analysed by using analysis of variance technique (one-way

classification). Significant difference between the treatments was determined by using CD (critical difference) test.

Packaging of Developed Product

Food grade 3-ply laminated film of polyethylene/aluminium foil/ polyethylene (PE/Al foil/PE) of thickness 80µ procured from local market of Varanasi, U.P were used as packaging material for packaging of ghee residue incorporated cake and muffins. Each packet weight about 200gm.

Results and Discussion

Optimization of basic formulation for the preparation of ghee residue incorporated cake and muffins

The basic formulation of the cake and muffins was optimised for the different levels of ghee residue, Other ingredients (refined flour, milk, salt, sugar, egg and butter etc.) For this purpose, different proportions of ghee residue and refined flour viz. 10:90, 20:80, 30:70 and 40:60 were used for preparation of cake and muffins keeping the fixed level of other ingredients (as per the standard) to optimize the levels of ghee residue and refined flour. The 0:100 combination served as control. The products were judged on the basis of various sensory attributes. The results are presented in table 3and 5. Results showed that with increase in the levels of ghee residue a non-significant ($P>0.05$) change was observed up to 40:60 level thereafter, a significant ($P<0.05$) decrease was observed. This could be due to the brown colour of the ghee residue and so could be incorporated up to 40% level. This is in accordance with the study of Borawake and Bhosale (1996), where it was observed that increasing levels of replacement of fat with GR decreased the colour and appearance scores in nankhatai type cookies and sponge cakes. The scores for flavour increased non-significantly ($P>0.05$) with the increase in the content of ghee residue. This is probably because of the high flavour potential of GR due to the presence of high FFA, carbonyls and lactones (Galhotra and Wadhwa 1991a and 1991b). Overall acceptability of cake and muffins containing Ghee residue: refined flour up to 40:60 level had comparable scores. The overall acceptability scores decreased as the level of replacement increased but the difference was statistically not significant. Similar results were seen by Subbulakshmi et al. (1990), where the mean score obtained for the control was 2.16 which increased to 2.34 and 2.40 in 50 and 100 percent ghee residue substituted biscuits and cakes respectively on a three point scale. Based on above observations, 40% ghee residue and 60% refined flour proportion was selected for further studies.

Composition of ghee residue

It is evident from the results (Table 2) that the ghee residue contained 893.50±3.95 kcal energy, 50.25±0.33g fat, 25.07±0.46g protein, 13.28±0.08% moisture, 8.24±0.43g ash, 0.91±0.03mg calcium and trace amount of total carbohydrate and the data showed non-significant ($P>0.05$) change. The Comparative values of ghee residue per 100g as given by Arumugam *et al.* (1989) are moisture 13.4, protein 25.8g, fat 50.8g, energy 900kcal, calcium 0.88mg

Sensory characteristics of cake

The table 3 shows that the mean sensory scores of cake in relation to colour which indicates that T_4 (8.25±0.19) had the highest score followed by T_3 (8.15±0.20), T_2 (7.8±0.22), T_1 (7.35±0.16) and T_0 (6.85±0.15) respectively. Scoring shows that the treatment T_4 was liked very much while T_3, T_2, T_1 and T_0 were moderately liked by the panel of judges. The body and texture of cake clearly

Table 1 Details of control and treatment combinations

Treatment products	Treatments %										
	T_0		T_1		T_2		T_3		T_4		Replications
	R. F	GR	R.F	G.R	R. F	G.R	R. F	GR	R. F	GR	
Cake	100	-	90	10	80	20	70	30	60	40	4
Muffins	100	-	90	10	80	20	70	30	60	40	4

R.F: Refined Flour, G.R: Ghee Residue

Table 2 Nutrient composition of ghee residue per 100 g

Nutrients	Nutritive Value
Energy kcal/100g	893.50±3.95
fat g/100g	50.25±0.33
Protein g/100g	25.07±0.46
Moisture%	13.28±0.08
Ash g/100g	8.24±0.43
Calcium mg/100g	0.91±0.03
Total carbohydrate g/100g	Traces

indicates that the treatment T_4 (8.6±0.1) had the highest score for the body and texture of Cake followed by T_3 (8.3±0.11), T_2 (7.8±0.22), T_1 (7.5±0.18) and T_0 (6.85±0.11) respectively. The effect of ghee residue on the taste & flavor of cake indicates that treatment T_2 (8.65±0.25) held the maximum scores as compared to control T_0 (7.6±0.37), T_1 (7.7±0.33), T_3 (8.6±0.37) and T_4 (8.4±0.46).The mean scores of cake in relation to overall acceptability indicates that the treatment T_4 (8.43±0.08) scored maximum followed by treatment T_3 (8.36±0.10), T_2 (7.59±0.16), T_1 (7.51±0.078) and T_0 (7.09±0,09).

Nutrients content in control and treated samples of cake

Table 4 shows that highest protein, calcium and fat was found in T_4 as 23.48±0.21g, 71.35±0.64mg and 89.96±0.81g respectively followed by T_3, T_2, T_1 and T_0. Energy (1560.2±13.99kcal) and carbohydrate (173.3±1.55g) content was found to be highest in T_0. Energy and Carbohydrate content decreased as the incorporation of ghee residue increased. Therefore, it can be concluded that with increase in amount of ghee residue in Cake the percentage of protein, fat and calcium increased. Energy and Carbohydrate content decreased by increasing the level of Ghee residue since refined flour contain 356.64kcal energy per 100g (Dutta et al. 2018) so with the decrease in refined flour content the energy value of cake decreases significantly.

Sensory characteristics of Muffins

The data illustrated in the Table 5, indicates the effect of ghee residue on the colour and appearance of muffin*s* indicates that the treatment T_4 (8.55±0.08) got the highest sensory score for the colour of muffins followed by T_3(7.8±0.07), T_2(7.55±0.10), T_1(6.85±0.29), and T_0(6.15±0.23). It is concluded that the concentration of ghee residue influences the appearance of products. The mean sensory score to the effect of addition of ghee residue to the body and texture of muffins shows that the treatment T_4 scored the maximum marks of (8.35±0.22) followed by T_2 (8±0.15), T_3 (7.85±0.17), T_1 (7.25±0.24) and T_0 (6.2±0.27) respectively. Therefore, it is concluded that the treatment 4 were liked moderately while T_2 was liked very much by the panel of

Table 3 The average sensory scores of different parameters in control and treated sample of *'*Cake*'*

Treatments	Color and appearance	Body and Texture	Taste and flavor	Overall acceptability
T_0(Control)	6.85±0.15	6.85±0.11	7.6±0.37	7.09±0.09
T_1	7.35±0.16	7.5±0.18	7.7±0.33	7.51±0.08
T_2	7.8±0.22	7.8±0.22	8.65±0.25	7.59±0.16
T_3	8.15±0.20	8.3±0.11	8.6±0.37	8.36±0.10
T_4	8.25±0.19	8.6±0.1	8.4±0.46	8.43±0.08
F-calculated	17.74(S)	32.5(S)	0.744 (NS)	77.2 (S)
C.D	0.19	0.42	-	0.10

S = Significant, ± = S.E NS = Non- Significant

Table 4 Average nutrients content in control and treated samples of "cake"

Nutrients (per 100g)	Control T_0	Treatments			
		T_1	T_2	T_3	T_4
Energy(kcal)	1560.2±13.99	1502.5±13.48	1445±12.96	1387.5±12.44	1329.6±11.93
Carbohydrate(g)	173.3±1.55	155.97±1.40	138.64±1.24	121.31±1.08	103.38±0.93
Fat(g)	88.4±0.79	88.29±0.79	88.18±0.78	89.07±0.80	89.96±0.81
Calcium(mg)	65±0.58	66.58±0.59	68.17±0.61	69.76±0.58	71.35±0.64
Protein(g)	17.6±0.16	19.07±0.17	20.54±0.18	22.01±0.19	23.48±0.21

Table 5 Average sensory scores of different parameters in control and treated sample of *'*Muffins'

Treatments	Color and appearance	Body and Texture	Taste and flavor	Overall acceptability
T_0(Control)	6.15±0.23	6.2±0.27	6.5±0.16	6.2±0.53
T_1	6.85±0.29	7.25±0.24	7.2±0.12	7±0.16
T_2	7.55±0.10	8±0.15	7.7±0.20	7.7±0.31
T_3	7.8±0.07	7.85±0.17	8±0.21	7.8±0.08
T_4	8.55±0.08	8.35±0.22	8.2±0.23	8.4±0.17
F-calculated	24.27(S)	20.12(S)	25.64(S)	27.85(S)
C.D	0.662	0.668	0.48	0.546

S= Significant, NS= Non- Significant, ± = S.E

Table 6 Average nutrients content in control and treated samples of "Muffins"

Nutrients (per 100g)	Control T_0	T_1	T_2	T_3	T_4
		Treatments			
Energy(kcal)	1533.22±13.75	1495.3±13.41	1438.42±12.90	1379.15±12.37	1324.72±11.88
Carbohydrate(g)	175.5±1.57	158.17±1.42	140.84±1.26	123.51±1.11	105.58±0.95
Fat(g)	84.18±0.75.	87.29±0.78	88.18±0.79	89.07±0.80	90.72±0.81
Calcium(mg)	62±0.55	64.67±0.58	65.03±0.58	66.12±0.59	68.01±0.61
Protein(g)	18.4±0.16	19.25±0.17	20.36±0.18	20.87±0.18	21.48±0.19

Table 7 Cost of "Cake" per 100 g of raw ingredients

Treatment	T_0		T_1		T_2		T_3		T_4	
Ingredients	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)
Refined Flour (22/kg.)	100	2.2	90	1.98	80	1.76	70	1.54	60	1.32
Ghee Residue (200/kg.)	-	-	10	2	20	4	30	6	40	8
Sugar (40/kg.)	100	4	90	3.6	80	3.2	70	2.8	60	2.4
Butter (20/100g)	100	20	90	18	80	16	70	14	60	12
Egg (1/pc)	1pc	5	1pc	5	1pc	5	1pc	5	1pc	5
	31.20		30.58		29.96		29.34		28.72	

Table 8 Cost of "Muffins" per 100 g of raw ingredients

Treatment	T_0		T_1		T_2		T_3		T_4	
Ingredients	Quantity (g./ml.)	Cost (Rs.)	Quantity (g/ml)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)	Quantity (g./ml.)	Cost (Rs.)
Refined Flour (22/kg.)	100	2.2	90	1.98	80	1.76	70	1.54	60	1.32
Ghee Residue (200/kg.)	-	-	10	2	20	4	30	6	40	8
Sugar (40/kg.)	100	4	90	3.6	80	3.2	70	2.8	60	2.4
Butter (20/100g)	100	20	90	18	80	16	70	14	60	12
Milk (40/kg)	50ml	2	50ml	2	50ml	2	50ml	2	50ml	2
Egg (1/pc)	1pc	5	1pc	5	1pc	5	1pc	5	1pc	5
Total		33.20		32.58		31.96		31.34		30.72

judges. The sensory mean scores of muffins in relation to taste and flavor indicates that T_4 (8.2±0.23) had the highest score followed by T_3 (8±0.21), T_2 (7.7±0.20), T_1 (7.2±0.12) and T_0 (6.5±0.16) respectively. The mean sensory scores of Muffins in relation to overall acceptability indicates that T_4 (8.4±0.17) had the highest score followed by T_3 (7.8±0.08), T_2 (7.7±0.31), T_1 (7±0.16) and T_0 (6.2±0.53) respectively.

Nutrients content in control and treated samples of Muffins

The Table 6, indicates that the nutritive value of muffins with and without utilization of ghee residue at different levels i.e. 10 percent, 20 percent, 30 percent and 40 percent of T_1, T_2, T_3 and T_4 respectively. Result revealed that highest protein, calcium and fat was found in T_4 in the order of 21.48±0.19g, 68.01±0.61mg, and 90.72±0.8g respectively followed by T_3, T_2, T_1 and T_0. Energy (1533.22±13.75kcal) and carbohydrate (175.5±1.57g) content was found to be highest in T_0. Energy and Carbohydrate content decreased by increasing the level of Ghee residue.

Cost of developed products (per 100g)

Table 7 shows that total cost of cake for T_0 was 31.20 Rs/100g, T_1 was 30.58 Rs/100g, T_2 was 29.96 Rs/100g, T_3 was 29.34 Rs/100g and T_4 was 28.72 Rs/100g. It is therefore, concluded that the treatment T_0 has highest cost followed by T_1 T_2 T_3 and T_4. The cost of the developed food product is decrease due to incorporation of ghee residue at different levels and table 8 shows that total cost muffins for T_0 was 33.20 Rs/100g, T_1 was 32.58 Rs/100g, T_2 was 31.96 Rs/100g, T_3 was 31.34 Rs/100g and T_4 was 30.72 Rs/100g. It is therefore, concluded that the treatment T_0 has highest cost followed by T_1 T_2 T_3 and T_4. The cost of the developed food product is decrease due to incorporation of ghee residue at different levels.

Conclusions

It is concluded that ghee residue can suitably be incorporated in prepared cake and muffins bakery products. The product was

accepted with regard to sensory characteristics. Both the products prepared with treatment T_4 using 40 percent ghee residue and 60 percent refined wheat flour scored the best in term of colour and appearance, body and texture, taste and flavour and overall acceptability. Nutritional composition of the prepared bakery products regarding fat, protein and calcium were increased as the level of incorporation of ghee residue increased.

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RESEARCH ARTICLE

Antioxidant activities, proteolytic activity and growth behavior of *Lactobacillus* cultures during fermentation of goat milk

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Abstract: In the study, five *Lactobacillus* cultures i.e. *Lb. fermentum* (M3), *Lb. casei* (NK9), *Lb. rhamnosus* (M8), *Lb. rhamnosus* (M9) and *Lb. paracasei* (M11) were used for the fermentation of goat milk. Lactobacillus cultures were evaluated for their growth behavior on pH, acidity and *Lactobacillus* counts for different time periods. We found that pH reduction by *Lactobacillus* cultures in goat milk medium was ranged from pH 6.25 at 0 h to pH 3.06 after 48h at 37ºC, for acidity produced by *Lactobacillus* cultures was ranged from 0.28 in 0 h to 3.21%LA after 48 h at 37ºC and for lactic counts (log CFU/ml) were ranged from 3.87 log CFU/ml at 0 h to 9.03 log CFU/ml after 36 h incubation at 37ºC. Then, antioxidant activities (ABTS assay, Hydroxyl free radical scavenging assay and Superoxide free radical scavenging assay) as well as proteolytic activity (OPA method) were analyzed for different time periods and found that antioxidant activity (ABTS assay) of *Lactobacillus* cultures was in the range of 42.14 to 53.27%, hydroxyl free radical scavenging activity of *Lactobacillus* cultures was found in the range of 35.32 to 53.43%, superoxide free radical scavenging activity of *Lactobacillus* cultures was in the range of 24.34% to 50.99% and proteolytic (OPA) activity of *Lactobacillus* cultures was found in the range of 7.30 to 9.96 mg/ml.

Keywords: Antioxidant activity, *Capra aegagrus hircus*, Fermented Goat milk, Proteolytic

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Introduction

In Mesopotamia, part of today's the Middle East, Goats (*Capra aegagrus hircus*) were the first species to be domesticated as livestock about 8000 BC. For centuries, humans have been used goats for many purposes (milk and meat) in all continents (Zervas and Tsiplakou, 2011). Goat milk production is an important part of the economy in many countries such as Spain, Switzerland, Italy, France, Turkey and New Zealand. On a global basis, different varieties of cheese, yoghurt, ice cream, fluid milk and milk powder are produced from goat milk. Goat milk production in the country has also increased from 3.6 to 4.7 million tons during the same period with an annual growth rate of 2.6 Per cent. The country stands first in goat milk production and is the second largest in goat meat production in the world by sharing 29 Per cent and 12 Per cent production, respectively (CIRG, 2015-2016).

Goat milk is nutritional and therapeutic food. Goat milk differs from cow or human milk in the context of higher digestibility, distinct alkalinity, higher buffering capacity and certain therapeutic in medicine and human nutrition (Park, 2009). Goat milk proteins may be digested more freely and their amino acids absorbed more efficiently than those of cow milk. Goat milk is considered to form a softer, more friable curd when acidified, which may be related to lower contents of αs1-casein and in the milk (Zenebe et al. 2014). Goat milk is good for ulcer treatment because it has better buffering capacity due to higher non-protein nitrogen (NPN) than cow milk (Park, 2009). The protein content of goat milk is quite similar to that of cow milk, although the caseins content in goat milk is slightly higher, and there is great homology between major proteins. However, β-casein is the major protein in goat milk (50 Per cent of total caseins), which is in contrast to cow milk where β-casein and αs1-casein are almost equally abundant, 37 Per cent and 30 Per cent, respectively (da Costa et al. 2014).

Many of the Lactic Acid Bacteria (LAB) were isolated from goat milk. Badis et al. (2004) isolated 725 lactic acid bacteria from raw goat milk of four Algerian races. They were phenotypically classified as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*. Da Silva et al. (2016) too isolated riboflavin and folate producing 179 lactic acid bacteria from goat milk and cheeses from these predominance species are

Streptococcus thermophilus, *Weissella paramensenteroides*, and *Lactococcus lactis*. 84% of isolates were able to produce folate, 8% riboflavin and 8% both vitamins. Goat milk fermented with different LAB increase the biologically active peptides from corresponding sequences of the precursor protein. The health benefits of the bioactive peptides may be attributed to their demonstrated antimicrobial, antioxidant, antihypertensive, antithrombotic, immune-modulatory and opioid activities (Sharma et al. 2017: Parmar et al. 2018).

Generally, chronic diseases and ageing phenomena are relevant to the imbalance in free radical levels in the body. An excess of free radicals can cause lethal cellular effects by oxidizing membrane lipids, cellular proteins, DNA, and enzymes, thus shutting down cellular respiration (Rahal et al. 2014; Urso and Clarkson, 2003). To prevent foods from undergoing deterioration and to provide protection against serious oxidative related diseases, it is important to prevent the peroxidation of lipids and the formation of free radicals generating in the living body and foodstuffs. Lipid oxidation is inhibited by antioxidant agents (Chen et al., 2018; Kondyli et al. 2007). Artificial antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and n-propyl gallate, exhibit strong antioxidant activity against several oxidation systems. However, the use of artificial antioxidants in foodstuffs is restricted or prohibited in some countries because of the potential risks for the living body. Antioxidants from natural sources are receiving increased attention. Food-derived peptides have been demonstrated to be the natural antioxidants without marked adverse effects. An increasing number of food protein hydrolysates and antioxidant peptides have been found to exhibit antioxidant activity (Samaranayaka and Li-Chan, 2011). Goat milk casein (GMC) differs greatly from bovine casein in content, peptide chain length, and Amino acid sequences (Kondyli et al. 2007; Ceballos et al. 2009). The study aims to evaluate the antioxidant activities, proteolytic activity and growth behaviour of Lactobacillus cultures during fermentation of goat milk.

Materials and Methods

Materials and culture collection

In this study, Most of the bacteriological grade media, molecular biology grade chemicals and reagents were purchased either from Hi-media (Mumbai), Merck (Germany), Sigma (USA), Bio-Rad, Promega, Ameresco, MP Biomedicals. *Lb. fermentum* (M3)*, Lb. casei* (NK9)*, Lb. rhamnosus* (M8)*, Lb. rhamnosus* (M9) and *Lb. paracasei* (M11) were procured from the Culture Collection of Dairy Microbiology Department, Sheth M. C. College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. Sterilized reconstituted skim milk with 12% Total solids was used for the propagation of lactic culture and further stored at 5 ± 2 °C.

Procurement of Goat milk (*Capra aegagrus hircus*)

Goat milk of Surti breed (*Capra aegagrus hircus*) was procured from Instructional Livestock Farm Complex (ILFC), Veterinary College, AAU, Anand during the study.

Determination of pH, acidity and lactic counts of fermented goat milk

All the cultures were activated by growing in sterilized goat milk. The activated cultures were added to 100 ml of sterilized goat milk flasks at a rate of 2%. After mixing them thoroughly, culture flasks were incubated at 37ºC for different time intervals i.e. 0, 6, 12, 24, 36 and 48 h. Then flasks were taken out for the determination of pH, titratable acidity and lactic counts.

Determination of pH

pH of fermented goat milk was determined as per the procedure described in Indian Standard (1961) with a calibrated pH meter (OAKTON pH700, India). Well mixed 10 ml fermented goat milk sample was put into a beaker and then pH was measured by immersing the pH meter probe into the fermented milk sample. Standard buffer solution of pH 4, 7 and 9 was used to calibrate the pH meter before measuring the sample.

Determination of titratable acidity

The titratable acidity was estimated by the procedure described in Indian Standard (1960). 10 ml sample was taken after each interval of 0, 6, 12, 24, 36 and 48 h into porcelain dish and an equal volume of lukewarm distilled water was added to it, then 1.0 ml phenolphthalein indicator was added and the contents of dish were titrated against 0.1 [N] NaOH till the appearance of light pink colour, which persisted for 30 seconds in the solution. Titratable acidity was calculated by the following formula:

Acidity (% Lactic acid) =9 × V × NX

Where,

V = Volume (ml) of 0.1 [N] NaOH required for the titration,

N = Normality of NaOH solution and

X = Volume of milk (ml) taken for the titration

Determination of lactic counts

Lactobacillus counts of fermented goat milk samples were determined as per the method described by IDF standards (146:2003). 1.0 ml sample was taken out from the tubes and added to 9 ml phosphate buffer tubes. Similarly, as per the required number of serial dilutions were prepared. 1.0 ml diluted sample from appropriate tubes was transferred to labelled sterile Petri plates (performed in duplicates), then 15-20 ml of melted and

cooled (45ºC) MRS agar was poured to each Petri plates. The content was mixed thoroughly by tilting and rotating the plates and allowed it to solidify and then add second layer (5-7 ml) of the same agar was poured completely over the solidified medium. Again allowed it to solidify, then incubated at 37ºC for 24 h in the inverted position. Typical colonies were calculated and the counts were expressed as log CFU/ml.

Assessment of antioxidant activities (ABTS assay, Hydroxyl free radical scavenging assay and Superoxide free radical scavenging assay) of Lactobacillus cultures

Sample preparation

Fresh goat milk was collected. Then filtered through a muslin cloth and sterilized at 121°C (250°F) at 15 psi (pounds per square inch) for 15 minutes and stored at 5±1ºC. The pure culture was activated by inoculating in sterilized goat milk at the rate of 2% and incubated at 37ºC for 24 h. Further, sampling was carried out by inoculating each culture in 10 ml sterilized goat milk at the rate of 2% and incubated for 0 and 48 h at 37ºC. After incubation of 0, 12, 24, 36 and 48 h, the fermented goat milk samples were centrifuged at 14,000 rpm for 30 min at 4ºC (Eppendorf centrifuge, US). The supernatants were collected and filtered using 0.22µm syringe filter (Millex®-HV, MERK, Ireland) and stored further for evaluating the antioxidant activities.

ABTS assay (2, 2-Azino-bis (3-ethylbenzothaizoline 6-sulfonic acid) assay) of fermented goat milk

The radical scavenging capacity of *Lactobacillus* cultures was based on the capability of a compound to scavenge the stable ABTS radical. This assay was evaluated as per the method described by Hati et al. (2013) with some modification. The ABTS working solution was prepared in dark bottle by mixing 88 µL of 140 mM potassium persulphate with 5 mL of 7 mM ABTS stock solution and incubating overnight for the generation of radicals and was diluted with phosphate buffer saline (PBS) to adjust the absorbance at 734 nm to 0.7 ± 0.02. An aliquot of 200 µL of product supernatant was mixed with 2.3 ml ABTS in PBS solution and both were mixed for 10 sec. The decrease in absorbance at 734 nm was recorded over for 10 min at 10 sec (A_{Sample}) using Spectrophotometer (Systronics PC based double beam Spectrophotometer 2202, India). Unfermented goat milk supernatant was used as a control sample ($A_{Control}$). Free radical scavenging capacity of the peptides was measured using the following equation:

ABTS radical scavenging activity (%) = $(A_{Control} - A_{Sample} / A_{Control})$ x 100

Hydroxyl free radical scavenging assay of fermented goat milk

Antioxidant activity was carried out by using Hydroxyl free radical scavenging ability of the peptides following Li et al. (2008) with some modification. A mixture of 3 ml phenanthroline (0.75mM) and 1.5 ml $FeSO_4$ (0.75mM) in phosphate buffer (pH 7.4) was prepared, and after adding 2 ml H_2O_2 (0.01%) and 1 ml supernatant, then the mixture was incubated at 37ºC for 1 hour. Ultimately, the absorbance was evaluated at 536 nm. Hydroxyl free radical scavenging capacity of the peptides was evaluated by the following equation:

Hydroxyl radical scavenging activity (%) = $[(A_S - A_1) / (A_0 - A_1)]$ x 100

Where A_S is the absorbance of the sample; A_1 is the absorbance of the control containing 1, 10-phenanthroline, $FeSO_4$ and H_2O_2; and A_0 is the absorbance of the blank containing 1, 10-phenanthroline and $FeSO_4$.

Superoxide free radical scavenging assay of fermented goat milk

This method is based on the ability of peptides to scavenge O_2^- through the production of a chromophoric compound during the reaction. This assay was followed by Liu et al. (2010) with some modification. In this method, 800 µl of supernatant along with 800 µl of Tris-HCl buffer (0.05M, pH 8.3) was placed in a clean test tube; thereupon, 400 µl of pyrogallol solution (1.5mM) was added. Finally, the absorption of the mixture was measured at 320 nm for 5 minutes at 25ºC. Butylated hydroxytoluene (at 0-1mg/mL, final concentration) was used as a positive control (AC) to evaluate the superoxide free radical scavenging capacity of the sample (AS):

Superoxide radical scavenging (%) = $[(\Delta AC/min) - (\Delta AS/min)] / (\Delta AC/min)$ x 100

Assessment of proteolytic activity

The proteolytic activity of *Lactobacillus* cultures was optimized by measuring the peptide content through O-phthalaldehyde (OPA) method (Hati et al., 2015; Solanki et al., 2017). *Lactobacillus* cultures were activated by growing in sterilized goat milk. The activated cultures were added to 10 ml tubes of sterilized goat milk at the 2% rate of inoculation and incubated at different times intervals (0, 6, 12, 24, 36 and 48 h). Then the samples were taken out for the evaluation of peptide content (Proteolytic activity) after each interval. The degree of proteolysis during fermentation of milk was determined by measuring the release of free NH_3 groups following the O-phthalaldehyde (OPA) method. An aliquot of 3 ml from each fermented goat milk sample was mixed with 5 ml of 0.75% trichloroacetic acid (TCA) and vortexed for 1 min and then the mixture was filtered using Whatman no. 42 filter papers (UK). The filtrate (200 µl) was added to 3 ml of OPA reagent and after incubation at room temperature (20ºC) for 2 min, the absorbance of the solution was measured by a Spectrophotometer (Systronics PC based double beam Spectrophotometer 2202, India) at 340 nm.

Statistical analysis

According to the statistical methods, all the study parameters were analyzed. Every experiment of the study was performed at least in triplicates with the results expressed as means (Average) ± standard deviations (SD). Statistical designs and software were used to analyze the experimental data. Using 5.0% level of significance and analysis of variance (ANOVA), the significant difference between the treatments was evaluated (Steel and Torrie, 1980).

Result and Discussion

Growth behaviour of *Lactobacillus* cultures in goat milk medium

The each *Lactobacillus* culture was inoculated at the rate of 2% in sterilized goat milk and then pH, titratable acidity (% Lactic acid) and *Lactobacillus* counts were evaluated at different time intervals (0, 6, 12, 24, 36 and 48 h) at 37ºC. The pH reduction, titratable acidity (%LA) and *Lactobacillus* counts of individual *Lactobacillus* culture were determined.

pH

pH reduction by *Lactobacillus* cultures in goat milk was ranged from pH 6.25 at 0 h to pH 3.06 after 48h at 37ºC. M8 showed maximum pH reduction (pH 3.06) followed by M3 (pH 3.11), NK9 (pH 3.18), M9 (pH 3.32) and M11 (pH 3.42) after 48 h at 37ºC (Fig. 1).

Titratable acidity

Titratable acidity was determined by calculating the amount of acidity developed up to 48 h of incubation by the formula given in (2.3.2). The acidity produced by *Lactobacillus* cultures was ranged from 0.28 in 0 h to 3.21%LA after 48 h at 37ºC. During the growth in sterilized goat milk, M8 showed highest titratable acidity (3.18%LA), followed by M3 (3.17%LA), NK9 (3.05%LA), M9 (2.73%LA) and M11 (2.08%LA) after 48 h at 37ºC (Fig. 2).

***Lactobacillus* counts**

Lactobacillus counts (log CFU/ml) of all the *Lactobacillus* cultures were evaluated for 0, 6, 12, 24, 36 and 48 h at 37ºC. Overall lactic counts (log CFU/ml) were ranged from 3.87 log CFU/ml at 0 h to 9.03 log CFU/ml after 36 h incubation at 37ºC (Fig. 3). Different cultures treatment and time periods showed significant behaviour but the interaction of both varied non-significantly. Non-significant increases in viable counts were observed among the five *Lactobacillus* up to 24 h and growth was significantly ($P<0.05$) decrease after 48 h at 37ºC in the study. M8 exhibited maximum growth (9.03 log CFU/ml), followed by M11 (9.00 log CFU/ml) up to 36 h than decrease after 48 h at 37ºC. NK9 (8.98 log CFU/ml), M9 (8.62 log CFU/ml) and M3 (8.61 log CFU/ml) up to 24 h than decrease after 48 h at 37ºC. In one study, Parmar et al. (2018) studied the growth and acidification of five selected lactic acid bacteria in heat-treated goat milk. Among five *Lactobacillus* cultures (*L. rhamnosu*s (NK2), *L. casei* (NK9), *L. fermentum* (M5), *L. paracasei* (M16) and *L. fermentum* TDS030603 (MTCC 25067) (LF)) studied, during the growth in heat-treated goat milk, M5 showed highest titratable acidity (3.25%LA), followed by LF (3.17%LA), NK2 (3.13%LA), NK9 (2.88%LA) and M16 (1.72%LA) after 48 h at 37ºC. Maximum pH reduction showed M5 (pH 3.10) followed by NK9 (pH 3.14), NK2 (pH 3.25), LF (pH 3.28) and M16 exhibited lowest pH reduction (pH 4.20) after 48 h at 37ºC. Significant increases in viable counts were observed among the five lactic acid bacteria up to 12 h than growth was significantly ($P<0.05$) decrease after 48 h at 37ºC in the study. M16 exhibited maximum growth (9.35 log CFU/ml), followed by LF (8.82 log CFU/ml), NK9 (8.80 log CFU/ml), NK2 (8.65 log CFU/ml) and M5 (8.18 log CFU/ml) up to 12 h than decrease after 48 h at 37ºC except M5 as we found.

Similar kind of result was also found by Solanki et al. (2017) that studied the growth and acidification of nine selected lactic acid bacteria in heat treated camel milk. Among nine lactic acid cultures (*Lb. rhamnosus* (NS4 and NS6), *Lb. acidophilus* (298), *Lb. helveticus* (V3), *Lb. acidophilus* (015), *Str. thermophilus* (MD2), *Lb. bulgaricus* (09), *Lactococcus lactis ssp. lactis* (NK6) and Lb. *fermentum* (LBF)) studied, during the growth in heat treated camel milk, V3 showed highest titratable acidity (2.548%LA), followed by 09 (2.487%LA), LBF (2.450%LA), 015 (2.422%LA), NS6 (1.732%LA), 298 (1.333%LA), NS4 (1.221%LA), MD2 (0.836%LA) and NK6 (0.785%LA) after 48 h at 37ºC. Maximum pH reduction showed V3 (pH 3.16) followed by 09 (pH 3.17), LBF (pH 3.27), 015 (pH 3.30), NS6 (pH 3.44), 298 (pH 3.55), NS4 (pH 4.00), MD2 (pH 4.49) and NK6 exhibited lowest pH reduction (pH 4.79) after 48 h at 37ºC. Maximum lactic count (log CFU/ml) exhibited by NS6 (11.62 log CFU/ml) followed by 09 (11.33 log CFU/ml), NK6 (10.51 log CFU/ml), MD2 (10.40 log CFU/ml), V3 (10.20 log CFU/ml), NS4 (10.15 log CFU/ml), 015 (10.00 log CFU/ml), 298 (10.00 log CFU/ml) and LBF (9.40 log CFU/ml).

In another study, Hati et al. (2013) studied the growth and acidification of eight selected lactic acid bacteria in skim and soy milk. Among eight lactic cultures (*S. thermophilus* MD2, *L. helveticus* V3, *L. rhamnosus* NS6, *L. rhamnosus* NS4, *L. bulgaricus* NCDC 09, *L. acidophilus* NCDC 15, *L. acidophilus* NCDC 298 and *L. helveticus* NCDC 292) studied, *L. bulgaricus* NCDC 09 and *S. thermophilus* MD2 decreased the pH of skim and soy milk in greater extent. Acid production (i.e. titratable acidity) by *L. bulgaricus* NCDC 09 and *L. helveticus* V3 was higher than other strains. Higher viable counts were observed in *S. thermophiles* MD2 and *L. helveticus* V3. All the tested lactic acid bacteria performed better in skim milk as compared to soy milk. Hati et al. (2015) studied the growth performance of *Lactobacillus*

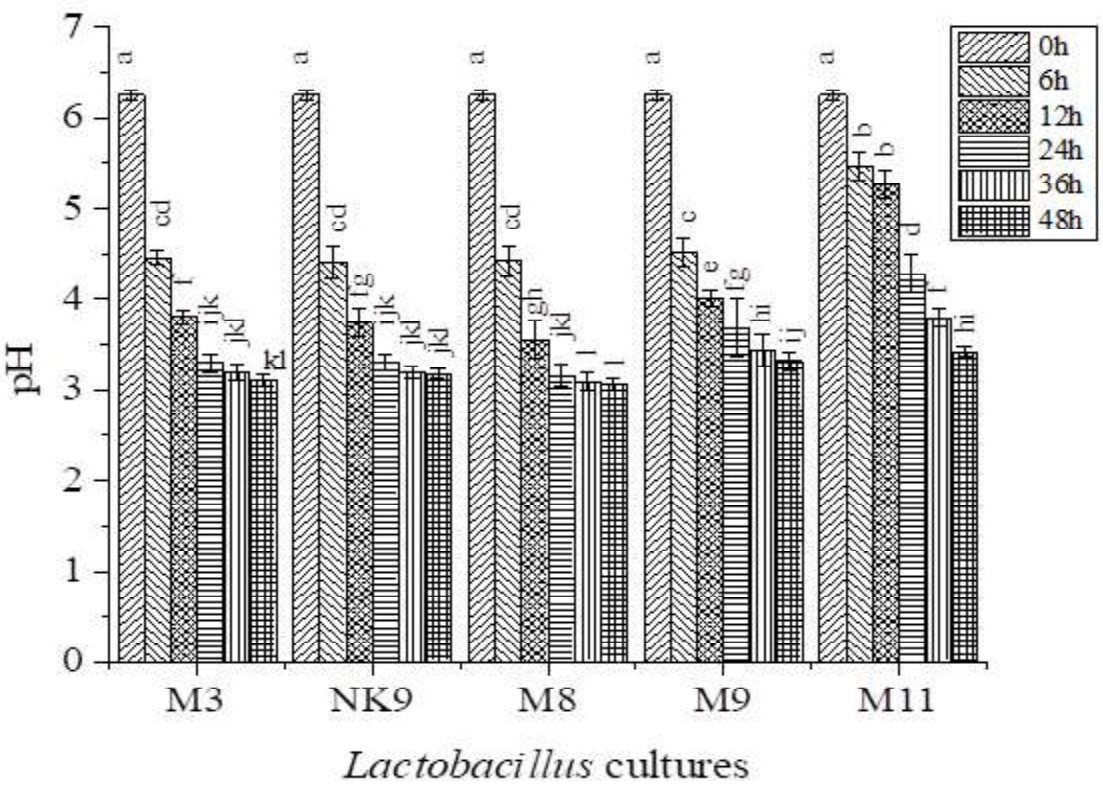

Fig. 1 Changes in pH of goat milk by *Lactobacillus* cultures at different incubation hours

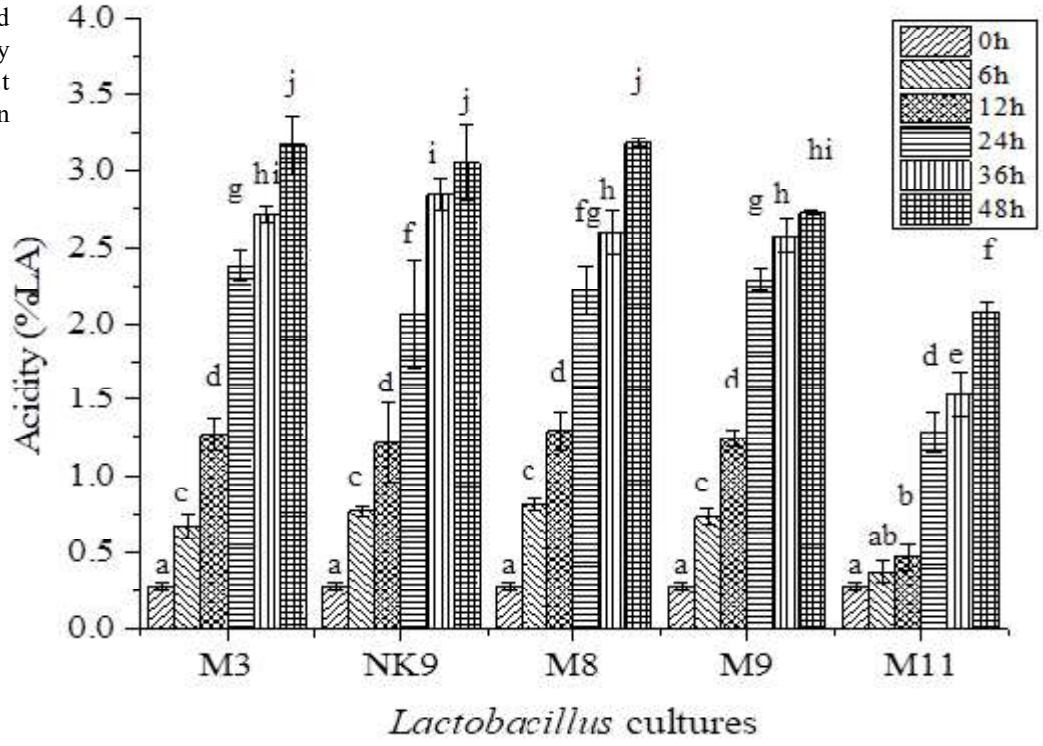

Fig. 2 Changes in lactic acid production (%LA) by *Lactobacillus* cultures at different incubation hours in goat milk medium

rhamnosus (NS4 and NS6), *Lactobacillus helveticus* MTCC 5463 (V3), *Lactobacillus delbruckii* (09), *Enterococcus feacalis* (ND3), *Enterococcus feacalis* (ND11) and *Lactobacillus rhamnosus* (SH8) by determining viable counts (log CFU/ml) and production of *Lactobacillus* acid measured by decline in pH in skim milk inoculated at the rate of 1% and incubated at 37ºC for 12 h. It was observed that NS4 lowered down the pH at a maximum level compared to V3, ND3 and SH8. However, it was also observed

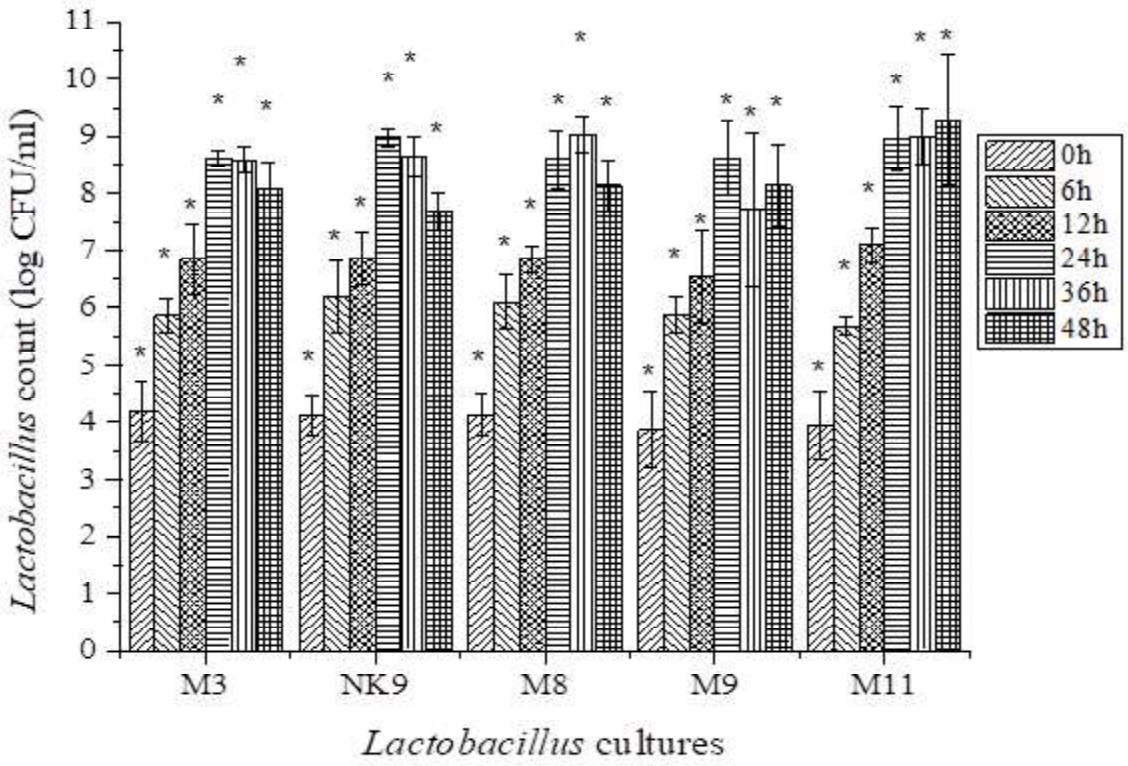

Fig.3 *Lactobacillus* counts of different cultures at different incubation hours in goat milk medium

[#]Values with different superscripts differ significantly ($p < 0.05$), Hydroxyl free radical scavenging activity (%) Mean ± SD.

that NS4 produced maximum acidity compared to V3, ND3, SH8 and I4. Viable counts of all the cultures were measured after 12 h of incubation at 37ºC. From the study, it was also found that NS4 gives the highest viable cell counts 10.68 log CFU/ml than the other bacterial isolates at this specified growth conditions. V3 also showed higher viable cell counts and NS6 was relatively exhibited lesser bacterial counts compared to other isolates in MRS agar medium. It was concluded that viable cell counts, pH and acidity varies due to the use of different strains (Hati et al., 2015) as similar to our study.

Antioxidant activity (ABTS assay) of fermented goat milk

The antioxidant activity generally indicates the relative ability of antioxidants to scavenge the free radicals generated in the aqueous phase. The ABTS is generated by reacting a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt (Hati et al. 2013). From Table 1, it had been observed that antioxidant activity was differing significantly (P<0.05) with incubation periods. Also, there was a significant difference (P<0.05) observed within the cultures. Also, it was found that the antioxidant activity of all the five *Lactobacillus* cultures was increased significantly with the time of incubation.

The antioxidant activity (ABTS assay) of *Lactobacillus* cultures was found in the range of 42.14 to 53.27%. NK9 had exhibited highest antioxidant activity (53.27%), followed by M8 (51.99%), M9 (50.55%), M3 (45.85%) and M11 (42.14%) after 48 h at 37ºC.

Studies revealed the agreement with our work that Rahmawati and Suntornsuk (2016) found the antioxidant activity of goat milk yoghurt increase or constant during fermentation proceed or storage at 4R”C up to 21 days. The value of ABTS activity in per cent inhibition was almost 19% and then decrease after 21 days of storage. Similarly, Moreno-Montoro et al. (2017) evaluated the antioxidant activity of fermented goat milk. Different fractions of whey i.e. whey, cation exchange membrane retentate R, permeate P of two fermented skimmed goat milk (ultra-filtered (UF) goat milk fermented with the classical starter bacteria or with the classical starter plus the *Lactobacillus plantarum* C4 probiotic strain) were assessed. The maximum value reaches was up to 0.4 µmol Trolox equivalents per mL for ABTS radicals. In one study, Freire et al. (2017) evaluated ABTS activity of fermented goat milk beverage by *Lactobacillus rhamnosus* and *Streptococcus thermophilus* with or without addition of grape pomace on gut microbiota and showed that Antioxidant activity of formulation-1 (Goat milk, Sugar and Grape juice) was 418.02 ± 16.14 mmol TE g^{-1} and formulation-2 (Goat milk, Sugar, Grape pomace extract and Grape juice) was 743.78 ± 23.88 mmol TE g^{-1}. Li et al. (2013) evaluated the antioxidant activities of goat milk casein and goat milk casein hydrolysates (hydrolysed by using a combination of neutral and alkaline proteases). They found that half-maximal inhibitory concentration (IC_{50}) value of ABTS

activity of goat milk casein was 71.251 ± 2.747 µg/ml and for goat milk casein hydrolysates was 0.449 ± 0.027 µg/ml. It indicating that goat milk casein hydrolysates is a good antioxidant compound with strong free radical scavenging activity compared with goat milk casein. One possible reason is that some peptides of goat milk casein hydrolysates are electron donors, which could react with free radicals, convert them to more stable products, and terminate the radical chain reaction.

Hydroxyl free radical scavenging activity of fermented goat milk

Hydroxyl radicals are reactive oxygen species that begin peroxidation of lipid membranes. Hydroxyl radicals are one of the most damaging free radicals in the body and can be an important mediator of damage to cell structures, nucleic acids, lipids and proteins. Hydroxyl free radical scavenging assay measures the relative ability of an antioxidant to scavenge the free radical generated in the aqueous phase (Li et al. 2008).

Hydroxyl free radical scavenging activity of all the five *Lactobacillus* cultures was presented in Table 2. From Table 2, it had been observed that hydroxyl free radical scavenging activity was differing significantly (P<0.05) with incubation periods. Also, there was a significant difference (P<0.05) observed within the cultures. Also, it was found that the hydroxyl free radical scavenging activity of all the five *Lactobacillus* cultures was increased significantly with the time of incubation. The hydroxyl free radical scavenging activity of *Lactobacillus* cultures was found in the range of 35.32 to 53.43%. M8 had exhibited highest hydroxyl free radical scavenging activity (53.43%), followed by M9 (50.98%), NK9 (48.88%), M3 (46.93%) and M11 (35.32%) after 48 h at 37ºC. It was observed that the percentage of hydroxyl free radical was increased with an increase in the incubation time from 0 to 48 h.

Similar kind of observation was shown by Shu et al. (2018) reported hydroxyl free radical scavenging activity in goat milk fermented by *Lactobacillus casei* L61 and added the optimal additive amounts were 0.99% (w/v) calcium lactate, 0.21% (w/v) glucose, and 0.29% (w/v) casein peptone. They found that the hydroxyl free radical scavenging rate increased significantly ($p < 0.001$) from 56.50 ± 0.57% to 88.01 ± 0.69%. In another study, Chen et al. (2019) investigated the hydroxyl free radicals scavenging activity of cheddar cheese during its ripening. They found that the activity of hydroxyl free radicals scavenging in cheese increased significantly in the first 4 weeks (P< 0.05), and reached the maximum of 43.86%, 46.19%, 46.66% and 47.43%. In the 20th and 24th weeks, all of them show the significant decrease (P<0.05). But in the last 3 weeks, no significant differences were observed (P>0.05). In the 16th week, there were no significant differences (P>0.05) between mixed and individual probiotic cheese, however, the value of mixed one was significantly higher than that of control cheese (P<0.05). In our study, we also found the same observation with the significant increase of hydroxyl free radicals-scavenging activity than the control (0 h).

Superoxide free radical scavenging activity of fermented goat milk

Superoxide free radical scavenging assay measures the relative ability of an antioxidant to scavenge the free radical generated in the aqueous phase. The original pyrogallol (1,2,3-trihydroxy benzene) method, which was developed specifically for superoxide dismutase, is widely used for measuring superoxide-scavenging of other antioxidants spectrophotometrically at 320 nm (Liu et al. 2010).

Superoxide free radical scavenging activity of all the five *Lactobacillus* cultures was depicted in Table 3. From Table 3, it had been observed that superoxide free radical scavenging activity was differing significantly (P<0.05) with incubation periods. Also, there was a significant difference (P<0.05) observed within the cultures. Also, it was found that the superoxide free

Table 1 Antioxidant activity (%) of *Lactobacillus* cultures (ABTS assay)

Lactobacillus Cultures	0h	12h	24h	36h	48h
M3	2.51±0.76^{a}	10.19±1.34^{b}	20.87±2.00^{d}	31.62±1.76^{f}	45.85±4.07^{i}
NK9	2.49±0.77^{a}	15.45±0.50^{c}	29.79±1.77^{ef}	44.41±1.70^{hi}	53.27±1.54^{j}
M8	2.48±0.76^{a}	15.06±1.91^{c}	20.87±2.00^{d}	40.48±1.58^{g}	51.99±1.01^{j}
M9	2.49±0.76^{a}	14.67±1.84^{c}	26.74±2.77^{e}	33.33±1.93^{f}	50.55±1.34^{j}
M11	2.49±0.77^{a}	04.98±1.04^{a}	15.06±2.00^{c}	23.03±1.02^{d}	42.14±7.16^{gh}

Table 2 Hydroxyl free radical scavenging activity (%) of *Lactobacillus* cultures

Lactobacillus Cultures	0h	12h	24h	36h	48h
M3	0.31±0.22^{a}	07.70±2.50^{b}	32.91±0.85^{e}	40.15±1.50^{hi}	46.93±0.81^{lm}
NK9	0.31±0.21^{a}	11.32±1.07^{c}	33.08±0.85^{ef}	43.20±1.45^{jk}	48.88±4.72^{mn}
M8	0.33±0.22^{a}	05.96±1.61^{b}	37.88±2.00^{gh}	40.82±0.71^{ij}	53.43±0.86^{o}
M9	0.31±0.21^{a}	11.04±1.12^{c}	38.80±1.77^{hi}	44.83±0.70^{kl}	50.98±1.54^{n}
M11	0.32±0.21^{a}	01.77±1.05^{a}	19.95±0.71^{d}	35.57±2.80^{fg}	35.82±1.96^{g}

radical scavenging activity of all the five *Lactobacillus* cultures was increased significantly with the time of incubation.

The superoxide free radical scavenging activity of *Lactobacillus* cultures was found in the range of 24.34% to 50.99%. NK9 had exhibited highest superoxide free radical scavenging activity (50.99%), followed by M3 (48.68%), M8 (41.14%), M9 (36.13%) and M11 (24.34%) after 48 h at 37ºC. It was observed that the percentage of superoxide free radical was increased with an increase in the incubation time from 0 to 48 h. At 0 h, there was no superoxide free radical scavenging activity found in fermented goat milk produced by *Lactobacillus* cultures.

Similarly kind of observation reported by Shu et al. (2017). They optimized rate of addition of prebiotic was inulin (0.6%), xylo-oligosaccharide (0.6%), galacto-oligosaccharide (0.6%) and fructo-oligosaccharide (0.4%) and value of superoxide free radical were 21.09%, 18.20%, 27.61% and 29.92%, respectively. In another study, superoxide radical scavenging activity of fermented goat milk product was evaluated by Liu et al. (2016). They compared the antioxidant properties of cow milk, goat milk, cow milk kefir and goat milk kefir and showed that maximum (4.0 mg/ml) dose level of kefir gives better superoxide radical scavenging activity i.e. almost 70% for both kefir compare to milk. This same kind of observation found in our case because fermentation was increase superoxide radical scavenging activity compares to control. In one study, Shu et al. (2016) optimized the different condition for best superoxide free radical scavenging activity for goat milk casein hydrolysates by alcalase. The value of superoxide free radical scavenging activity at different condition was like, at temperature 50ºC (36.08%), at pH 8 (85.36%), at enzyme to substrate ratio 1.5% (43.01%), at 150 min hydrolysis time (46.13%) and also found increase in superoxide free radical scavenging activity with time of hydrolysis as we seen in our study.

Proteolytic (OPA) activity of fermented goat milk

The proteolytic (OPA) activity of all the five *Lactobacillus* cultures was presented in Table 4. From Table 4, it had been observed that proteolytic (OPA) activity was differing significantly ($P<0.05$) with incubation periods. Also, there was a significant difference ($P<0.05$) observed within the cultures. It was found that the proteolytic (OPA) activity of all the five *Lactobacillus* cultures was increased non-significantly with the time of incubation.

The proteolytic (OPA) activity of *Lactobacillus* cultures was found in the range of 7.30 to 9.96 mg/ml. M9 had exhibited highest proteolytic (OPA) activity (9.96 mg/ml), followed by M3 (9.08 mg/ml), NK9 (8.62 mg/ml), M8 (8.37 mg/ml) and M11 (7.30 mg/ml) after 48 h at 37ºC. It was also observed that the proteolysis was increased with an increase in the incubation time from 0 to 48 h.

Basically, Increase in proteolytic activity with the different incubation periods was directly proportional to the number of amino acids required by the *Lactobacillus* cultures during their growth phases based upon which the release of free NH_3 groups. It was also reported that the extent of proteolysis showed to be the time and strain-dependent (Donkar et al. 2007). In agreement of our work, Parmar et al. (2018) evaluated the proteolytic activity of goat milk fermented by NK9 (*Lb. casei*) and LF (*Lb. fermentum* TDS030603 (MTCC25067)). They optimized the rate of inoculation (1.0, 1.5 and 2.0 %) and incubation period (0, 6, 12, 24 and 48 h) by OPA method. They found that 2% inoculation rate and 48 h of incubation gives the best proteolytic activity for both cultures i.e. for NK9 (7.598 mg/ml) and LF (9.709 mg/ml). Karthikeyan et al. (2018) isolated eight LAB from goat milk. They screened that isolates on the basis of proteolytic activity on skim milk agar. After its genotypic and phenotypic evaluation, found that *Lb.*

Table 3 Superoxide free radical scavenging activity (%) of *Lactobacillus* cultures

Lactobacillus Cultures	0h	12h	24h	36h	48h
M3	0.00±0.00^{a}	0.00±0.00^{a}	16.43±2.84^{c}	32.07±1.84^{f}	48.68±1.05^{i}
NK9	0.00±0.00^{a}	0.43±0.75^{a}	21.29±2.67^{d}	29.73±0.69^{f}	50.99±2.30^{i}
M8	0.00±0.00^{a}	0.88±0.88^{a}	10.80±2.62^{b}	25.82±1.86^{e}	41.14±2.64^{h}
M9	0.00±0.00^{a}	0.17±0.29^{a}	08.74±2.93^{b}	17.55±1.10^{c}	36.13±2.81^{g}
M11	0.00±0.00^{a}	0.00±0.00^{a}	02.73±2.38^{a}	15.84±2.22^{c}	24.34±1.83^{e}

$^{\#}$Values with different superscripts differ significantly ($p < 0.05$), Superoxide free radical scavenging activity (%) Mean ± SD.

Table 4 Proteolytic activity (mg/ml) of *Lactobacillus* cultures (OPA activity)

Lactobacillus Cultures	0h	12h	24h	36h	48h
M3	2.49±0.03*	4.69±0.37*	6.40±0.55*	7.05±0.37*	9.08±0.93*
NK9	2.30±0.15*	5.49±0.82*	6.76±0.43*	7.81±0.78*	8.62±0.93*
M8	2.40±0.11*	5.06±0.72*	6.11±0.54*	6.93±0.52*	8.37±1.04*
M9	2.33±0.11*	5.72±1.12*	7.35±0.33*	9.11±1.28*	9.96±0.67*
M11	2.37±0.02*	4.01±0.27*	5.03±0.85*	6.34±0.48*	7.30±0.69*

*Values with different superscripts differ non-significantly ($p < 0.05$), Proteolytic activity (mg/ml) Mean ± SD.

delbrueckii had the best proteolytic activity (maximum 3 mm zone was hydrolysed). In order to isolate LAB from spontaneous fermented local goat milk, which had proteolytic activity and used for further fermentation of goat milk. Based on proteolytic activity, the isolates composed of two bacteria species namely *Lactobacillus plantarum* (YN 1.1, YN 1.3, YN 1.8 and isolates YN 2.25) and another bacteria was *Lactobacillus pentosus* (YN 1.6). Five isolates had proteolytic activity, where three of them had the best proteolytic activity namely *Lactobacillus plantarum* YN 1.1, *Lactobacillus plantarum* YN 1.3 and *Lactobacillus pentosus* YN 1.6 (Yelnetty et al., 2014). In a study, nineteen *Lactobacillus* isolated from Algerian goat's milk, 13 belonging to *Lb. plantarum*, three to *Lb. pentosus*, two to *Lb. rhamnosus* and one to *Lb. fermentum*, were examined *in vitro* used as adjunct culture in dairy products. The strains were tested for their proteolytic activity, sensory and safety properties. Strains LbMS16 and LbMS21 *Lb. plantarum* and LbMF25 *Lb. rhamnosus* presented the highest proteolytic activity (Ahmed and Bousmaha-Marroki, 2014).

Conclusions

Lb. rhamnosus (M8) showed highest pH reduction, maximum acidity and *Lactobacillus* counts i.e. 3.06, 3.18%LA and 9.03 log CFU/ml, respectively during the fermentation of goat milk after 48h at 37^0C than other cultures. Antioxidant activity (ABTS assay) of *Lactobacillus* cultures were found in the range of 42.14 to 53.27% and maximum antioxidant activity was shown by *Lb. casei* (NK9) culture. The hydroxyl free radical scavenging activity of *Lactobacillus* cultures was found in the range of 35.32 to 53.43% and highest was observed in *Lb. rhamnosus* (M8) culture. The superoxide free radical scavenging activity of *Lactobacillus* cultures was found in the range of 24.34% to 50.99% and the maximum was presented by *Lb. casei* (NK9) culture. The proteolytic (OPA) activity of *Lactobacillus* cultures was found in the range of 7.30 to 9.96 mg/ml and the maximum was exhibited by *Lb. rhamnosus* (M9). However, M8, NK9 and M9 could be used for the development of functional fermented goat milk.

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RESEARCH ARTICLE

Application of soft computing models for prediction of subclinical mastitis in indigenous breed of dairy cattle in India

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Abstract: Mastitis is an important problem in dairy cattle. Soft computing models i.e. Adaptive Neuro Fuzzy Inference System (ANFIS) can be possible way out for detecting this disease. Therefore, the present study was undertaken for predicting the subclinical mastitis in indigenous breed such as Sahiwal cows and Murrah buffaloes. The selected eight parameters for study were Milk pH, electrical conductivity, temperature (udder, milk and skin), milk yield, dielectric constant and milk somatic cell counts. Animals were judged healthy and infected as per milk somatic cell counts. Accordingly, animals were classified into three categories, i.e., healthy, subclinical and clinical mastitis animals. Data generated were utilized for developing ANFIS models to identify healthy versus mastitis animals. Also, Multiple Linear Regression (MLR) models were developed for comparing classification accuracy of proposed models using Root Mean Square Error (RMSE) technique. ANFIS models were found to be superior as compared to MLR models for both the breed with RMSE 0.23 (Shahiwal cows) and 0.20 (Murrah buffaloes) as compare to MLR model 4.88 (Shahiwal cows) and 4.08 (Murrah buffaloes). Hence, it is deduced that ANFIS can be used as a suitable technique for detecting mastitis in indigenous breed of dairy cattle.

Keywords: Adaptive Neuro Fuzzy Inference System, Mastitis, Murrah buffaloes, Sahiwal cows, Soft computing models, Subclinical mastitis

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Introduction

With an increasing trend in milk production the incidence of mastitis in dairy cattle and buffaloes has also increased, incurring great loss in terms of economic loss and future productivity of dairy animals (Barth, et al. 2000). It is evident from the studies conducted in the United States of America that economic loss associated with mastitis on dairy farms are approximately US $200 per cow/year leading to annual loss of US $2 billion for dairy industry (Bogni, et al. 2011). In India, annual economic loss incurred by dairy industry on account of udder infections has been estimated to be Rs 6264.18 crores and out of which loss of Rs 4515.54 (70-80%) has been attributed to subclinical mastitis (Dang, et al. 2010). In another report from India (De Mol and Woldt, 2001; Dua, 2001; Gaddi, et al. 2016, Srivastava, et al. 2015) the annual economic loss due to mastitis has been calculated as Rs7165.51 crores, Losses being almost same for cows (Rs 3775.58) and buffaloes (Rs 3637.78). Subclinical mastitis has been estimated to account for 57.93% (Rs 4293.69) of the total economic loss due to mastitis. Hence, there is an urgent need to identify certain diagnostic tools to detect mastitis at its earliest stage. The changes in the ion concentration of mastitis milk induce some variations in the electrochemical properties of milk and have the potential to be used as an effective technique for early prediction of mastitis (Jacceh, 2003; Mammadova and Keskin, 2013). The present study was therefore designed to study the mastitis induced variation in electro chemical properties in conjunction with other milk quality parameters in indigenous breed of dairy cattle by application of Adaptive Neuro Fuzzy Inference System (ANFIS).

Evidently, there was no study to identify mastitis in indigenous dairy cattle i.e. Sahiwal cows and Murrah buffaloes using ANFIS models. Moreover, the ANFIS models developed for exotic cattle were based on individual parameter. Hence, in this paper, these models have been developed on the basis of Milk pH, electrical conductivity, temperature (udder, milk and skin), milk yield, dielectric constant and milk somatic cell counts for detection of mastitis in the dairy cattle. These models would not only help in rapid detection of raw milk quality but also facilitate timely isolation of animals susceptible to subclinical mastitis, for proper treatment to avoid the economic losses. The classifying ability

of above proposed models has been evaluated in comparison with that of conventional Multiple Linear Regression (MLR) models, which have also been developed in this study.

Materials and Methods

A total no of 670 milk samples were collected from hundred lactating Sahiwal cows. Simultaneously 517 milk samples were collected from Murrah buffaloes for the investigations purpose. The breeds were maintained by Livestock Research Centre at ICAR – National Dairy Research Institute, Karnal, India for a period of one year, i.e., March, 2013 - February, 2014. Milk samples were collected twice a day, i.e., in the forenoon (11.30 AM) and in the evening (4.30 PM). Milk samples were tested for mastitis by analyzing the milk somatic cell counts using microscopic method (Mammadova and Keskin, 2015). Animals having milk SCC below 200000 per ml were categorized as healthy animals, whereas, SCC in the range of 200000 - 500000 per ml were considered to be subclinical mastitis animals (Alhussien and Dang, 2018). The remaining animals with milk SCC more than 500000 per ml were positioned in clinical mastitis category. Further, other parameters like pH and Electrical conductivity and Dielectric Constant were determined by precision handheld pH meter (Haana instrument, range 0-14), EC meter (Oriental Instrument Ltd. Japan range 0-13 mS/cm) and dielectric constant meter (PSAW454 frequency range 9-10 MHz) respectively. The temperature of milk was measured by regular thermometer (glass thermometer range 0-100 ºC) whereas, temperature of skin and udder of animals were measured with infrared thermometer. Milk yield was recorded at the time of sampling collection using weighing balance. Further, SCC were determined by microscopic method (Mammadova and Keskin, 2015) in which milk was heated to 40°C in a water-bath, and set aside at that temperature for 15 minutes before cooling it to 20°C by stirring gently. About 0.01 ml of milk was spread on a 1cm^2 (0.5 cm ′ 2 cm) area of a microscopic slide and was dried in a horizontal position. Furthermore, The data generated above were utilized for models development (ANFIS and MLR) using Milk pH, electrical conductivity, temperature (udder, milk and skin), milk yield and dielectric constant as a input variables whereas milk

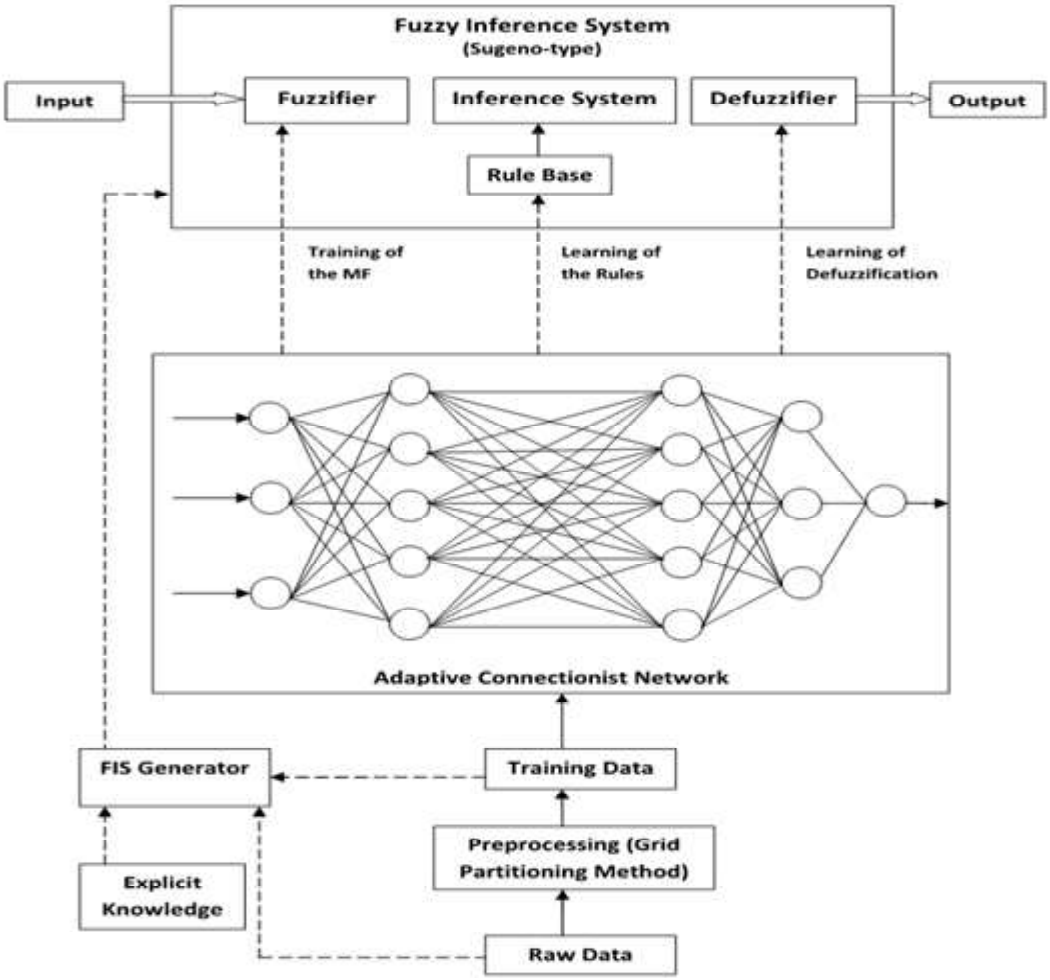

Figure 1 Schematic diagram of ANFIS model

somatic cell counts is used as output variable in dairy cattle i.e. Sahiwal cows and Murrah buffaloes.

Adaptive neuro fuzzy inference system model

ANFIS is fusion of neural network and Fuzzy Inference System (FIS). The connectionist networks are used to determine the parameter of the FIS. It combines both the fuzzy logic (FL) and neural network (Seegers, et al. 2003; Skrzypek, et al. 2004). FL was an exodus from classical Boolean logic as it implements soft linguistic variables on a continuous range of truth values, which allows transitional values to be defined between conventional binary. FL application solves the problem with three steps: first it convert numerical values to a set of fuzzy values, an inference system based on fuzzy if-then rules and defuzzification (Sharma, et al. 2014) as shown in Figure1.

ANFIS model can be of two types, *i.e.*, Mamdani and Sugeno. The Sugeno-type system was used in this study. The architecture of ANFIS has following five layers to accomplish the tuning process of the fuzzy modelling system (Veleva, et al. 2010).

Layer 1: Every node in this layer is an adaptive node with a node function which is called Membership Function (MF). Parameters of membership functions are known as premise or antecedent parameters.

Layer 2: Every node in this layer is a fixed node, which multiplies the incoming signals and sends the product out. Each node represents the firing strength of a fuzzy rule.

Layer 3: In this layer every node calculates the ratio of the one firing strength to the sum of all rules' firing strengths. The outputs of this layer are called normalized firing strengths.

Layer 4: In this layer every node is an adaptive node (*i.e.*, linear combination of input variables). Parameters in this layer are referred to as subsequent parameters.

Layer 5: The single node in this layer is a fixed node that computes the overall output as the summation of all incoming signals.

This five-layer network architecture, ANFIS being a hybrid model puts the fuzzy model into the framework of adaptive networks that computes gradient vectors systematically. The Fuzzy Toolbox under MATLAB software was used for all simulation experiments. The 'trial and error' method using error back propagation with three data partitioning schemes i.e. 70:30, 80:20 and 90:10 and different combinations of parameters such as epochs, range of influence, Squash factor, accept ratio and reject ratio is used to reach optimum model configuration in all the simulation experiments. The structure of rule node is formed by the linguistic fuzzy rule if –than model that is self generated by the system. The number of rules node is dependent on the n numbers of inputs and m numbers of linguistic fuzzy terms.

Multiple linear regression models

Multiple Linear Regression (MLR), which is known simply as multiple regression, is a statistical technique that fits linear equation between a dependent variable and two or more independent variables. A good MLR model is able to explain most of the variance of the dependent variable with the minimum number of independent variables. The MLR equation is given by Eq.1:

$$Y = \beta_0 + \beta_1 X_1 + \cdots + \beta_n X_n \quad \text{Eq. (1)}$$

where Y is dependent variable (SCC), β_0 is the Y intercept (constant), β_i is coefficient of i^{th} independent variable, X_i. These variables have been shown in Table 1.

Models development

For the models development, the generated data were divided into two subsets, viz., training set and test set. The training set was used for model training while the test set was used for model validation. Three data partitioning schemes such as 70:30, 80:20 and 90:10 (training set : testing set) were explored for model development. The network was best trained with 10, 50 and 100 epochs; learning rate as 0.01 and error goal as 0.001, which was determined emperically. Each training experiment was conducted ten times with different combinations of the parameters such as, data partitioning scheme, epochs, range of influence, Squash Factor, accept ratio and reject ratio. For the model selection, the architecture of connectionist model was decided by 'trial and error' procedure. The MATLAB programming environment has been used for training and simulation experiments. SAS 9.3 was used for developing MLR models.

Model performance analysis

The performance of neural network models, ANFIS and MLR models was evaluated in terms of Root Mean Square percent error (%RMSE) at 70:30, 80:20 and 90:10 partitioning schemes. The RMSE, calculated using formula as given below.

Root mean square error

The prediction error and RMSE have been calculated using the following equation 2 and 3.

$$\text{Prediction Error\%} = \frac{\text{Actual value - Predicted value}}{\text{Actual value}} \times 100$$

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\text{Prediction Error})^2} \quad (3)$$

Results and Discussion

The Mean ± SE values of milk pH, electrical conductivity, dielectric constant, temperature of skin, udder and milk of animal in normal, subclinical and clinical mastitis cases in Sahiwal cows and Murrah buffaloes have been presented in table1. There is a considerable variation in all the quality characteristics or the traits among the healthy, subclinical and clinical mastitis cases in both the group of animals. A difference has been noticed in the values of all the traits in the Sahiwal cows and the Murrah buffaloes, which could be attributed to the differences in compositional aspects and the salt balance of cow and buffalo milk, which has resulted in the variation in the electrochemical properties and other quality parameters of milk in both the species as shown in table 1.

The data above generated were used for proposed model development. For the ANFIS models development, the MATLAB Toolbox ANFIS interface was used. A total of 7 inputs i.e. milk pH, electrical conductivity, milk yield, dielectric constant, temperature (milk, udder and skin) were used for training (70%, 80%, and 90% of all data) and milk SCC as a output data were entered into the system for model training and testing. Inputs were fuzzified using the FL technique. Rules and an inference system were prepared by the system using the artificial neural networks technique. Each training experiment was conducted ten times with different combinations of the parameters such as epochs, range of influence, Squash Factor, accept ratio and reject ratio. The Sugeno inference method was used here as a mining method. The Sugeno inference method is similar to the Mamdani inference method used in the fuzzy expert systems, except that the output data membership functions can be either constant or linear in the Sugeno method. The membership function of the outputs in the established model was defined as constant.

Performance results of ANFIS models developed with different data partitioning schemes (*i.e.*, 70:30, 80:20 and 90:10) to detect mastitis milk in Sahiwal cows and Murrah buffaloes, are presented in Table 2. The model performance was selected based on minimum average testing error. The least value of testing error, i.e., 0.23 was found with data partitioning scheme as 90:10 with 0.5 range of influence, 1.25 squash factor 0.9 accept ratio, 0.15 reject ratio and 50 epochs in Sahiwal cows whereas in Murrah buffaloes, the least value of testing error was found 0.20 with data partitioning scheme as 90:10 with 0.5 range of influence, 2 squash factor 0.5 accept ratio, 0.15 reject ratio and 50 epochs. Mammadova and Keskin (2015) evaluated two mastitis detection models and reported that ANN model yielded a sensitivity rate of 80%, a specificity rate of 91%, and an error rate of 64%. These values were 55%, 91%, and 35% for the ANFIS model. It showed that error rate in ANN method is higher than the ANFIS. Similar study was done by Cavero et al. (2006) and concluded that Fuzzy logic is a useful tool to develop a detection model for mastitis. A noticeable decrease in the error rate can be made possible by means of more informative parameters. Comparable results were obtained by Krieter et al (2007) but with lower values than reported by Mammadova and Keskin (2015).

Multiple linear regression model performance

The Multiple linear regression models were also developed with the same data partitioning schemes as used for constructing the ANFIS models described in previous section, *i.e.*, 70:30, 80:20 and 90:10. The MLR equations thus developed for Sahiwal cows and Murrah buffaloes are shown in Table 3.

The accuracy of fit was checked by calculating the RMSE between experimental and predicted values of SCC. The lower the value of RMSE the better was the goodness of fit. Generally, a good description of data is considered, on an average, with RMSE to be smaller than 7%. The mastitis detection accuracy of MLR models varied between 4.08% and 22.90%.

ANFIS vis-a-vis conventional regression models

There are different models available for detecting the mastitis like Conventional Regression Models (MLR, Logistic regression

Table 1 Mean ± SE of different milk parameters recorded in normal, subclinical and clinical mastitis Sahiwal cows and Murrah buffaloes

Traits	Milk pH	Electrical-conductivity (mS/cm)	Dielectric constant at (25°C)	Skin temperature (°C)	Udder temperature (°C)	Milk temperature (°C)	Milk Yield (Kg)	SCC x 10^5
				Sahiwal cows (N*= 670)				
Normal	6.51^{a}±0.03	4.36^{a}± 0.03	66.05^{a}±0.30	32.21^{a}±0.14	33.56^{a}± 0.13	33.86^{a}± 0.11	6.33^{a}± 0.09	1.31^{a}± 0.04
Sub-clinical	6.80^{b}±0.02	4.91^{b}±0.04	71.78^{b}±0.19	32.35^{a}±0.12	33.66^{a}±0.11	33.62^{a}± 0.12	6.07^{a}± 0.13	3.37^{b}± 0.07
Clinical	7.00^{b}±0.01	5.73^{c}±0.07	82.03^{c}±0.24	33.80^{b}±0.10	34.95^{b}±0.09	34.44^{b}± 0.08	5.52^{b}± 0.22	15.46^{c}±1.30
				Murrah buffaloes (N*=517)				
Normal	6.48^{a}±0.02	3.54^{a}± 0.02	65.92^{a}±0.20	32.42^{a}±0.10	33.48^{a}± 0.07	33.15^{a}± 0.01	7.53^{a}± 0.09	1.06^{a}± 0.03
Sub-clinical	6.62^{b}±0.05	4.16^{b}± 0.09	69.96^{b}±0.35	32.78^{a}±0.11	33.75^{a}± 0.19	33.48^{a}± 0.53	7.44^{a}± 0.10	3.31^{b}±0.13
Clinical	7.03^{c}±0.03	5.79^{c}± 0.23	74.63^{c}±1.42	34.75^{b}±0.12	34.32^{b}±0.13	35.77^{b}± 0.16	7.02^{b}± 0.13	21.39^{c}±2.69

N*= 670 milk samples collected from Sahiwal cows, N*= 517 milk samples collected from Murrah buffaloes

Superscripts within column differ significantly from each other (P >0.05)

Table 2 ANFIS model based on error back propagation to classify healthy and infected Sahiwal cows and Murrah buffaloes with different data partitioning scheme

	70:30					80:20					90:10				
						Sahiwal cows (N*= 670)									
Epochs	Range of Influence	Squash Factor	Accept Ratio	Reject Ratio	RMSE %	Range of Influence	Squash Factor	Accept Ratio	Reject Ratio	RMSE %	Range of Influence	Squash Factor	Accept Ratio	Reject Ratio	RMSE %
10	0.80	1.00	0.50	0.15	0.84	0.90	1.00	0.10	0.15	0.83	0.50	1.25	0.50	0.15	0.51
	0.80	0.90	0.50	0.10	0.25	0.50	2.00	0.50	0.15	0.42	0.90	2.00	0.50	0.15	0.35
	0.90	2.00	0.50	0.15	0.36	0.50	2.50	0.50	0.15	0.37	0.50	2.00	0.10	0.15	0.47
50	0.90	2.00	0.50	0.15	0.34	0.90	2.00	0.50	0.15	0.34	0.50	1.25	0.90	0.15	0.23
	0.80	0.90	0.50	0.10	0.70	0.90	1.00	0.10	0.15	0.74	0.90	2.00	0.50	0.15	0.32
	0.50	1.00	0.50	0.15	0.48	0.50	2.50	0.50	0.15	0.35	0.90	2.50	0.10	0.01	0.31
100	0.90	2.00	0.50	0.15	0.35	0.50	1.25	0.50	0.15	0.60	0.50	1.25	0.50	0.15	0.41
	0.90	1.50	0.80	0.01	0.43	0.90	1.00	0.10	0.15	0.64	0.90	1.00	0.10	0.15	0.26
	0.80	0.90	0.50	0.10	0.80	0.50	2.00	0.50	0.15	0.32	0.50	2.00	0.50	0.15	0.26
						Murrah buffaloes (N*= 517)									
10	0.50	1.25	0.50	0.15	0.28	0.50	1.25	0.50	0.15	0.25	0.50	1.25	0.50	0.15	0.24
	0.90	1.25	0.50	0.15	0.23	0.90	1.25	0.50	0.15	0.24	0.70	1.25	0.50	0.15	0.23
	0.50	2.00	0.50	0.15	0.28	0.70	1.25	0.50	0.15	0.22	0.50	1.25	0.80	0.15	0.24
50	0.50	1.25	0.50	0.15	0.26	0.50	1.25	0.50	0.15	0.27	0.50	1.25	0.50	0.15	0.27
	0.90	1.25	0.50	0.15	0.26	0.50	1.25	0.10	0.15	0.24	0.50	1.25	0.10	0.15	0.26
	0.50	2.00	0.50	0.15	0.20	0.50	1.25	0.10	0.1	0.24	0.50	2.00	0.50	0.15	0.20
100	0.50	1.25	0.50	0.15	0.28	0.50	1.25	0.50	0.15	0.28	0.90	1.25	0.50	0.15	0.27
	0.90	1.25	0.50	0.15	0.27	0.90	1.25	0.50	0.15	0.27	0.70	1.25	0.50	0.15	0.25
	0.50	2.00	0.50	0.15	0.26	0.50	1.25	0.10	0.1	0.30	0.50	1.00	0.90	0.15	0.25

Table 3 Multiple linear regression equations for Sahiwal cows and Murrah buffaloes

Data partitioning scheme	MLR Equation
	Sahiwal cows
70:30	SCC=0.147pH+3.36EC+0.60UT+0.27MT+0.71ST+0.08MY+0.786DC- 129.99
80:20	SCC=1.29pH+3.24EC-1.39UT+0.50MT+1.96ST+0.134MY+ 0.91DC- 118.33
90:10	SCC=2.11pH+4.37EC-1.61UT+0.61MT+2.71ST+ 0.29MY+ 0.76DC- 139.89
	Murrah buffaloes
70:30	SCC=0.059pH+5.53EC+0.034UT+0.179MT+0.063ST+0.29MY+0.65DC-73.46
80:20	SCC=0.126pH+5.50EC-0.04UT+0.21MT+ 0.025ST+0.25MY+0.53DC-61.15
90:10	SCC=0.198pH+5.41EC+0.079UT+0.263MT-0.035ST+0.20MY +0.4433DC-60.25

EC= Electrical conductivity, UT= Udder temperature, MT= Milk temperature, ST= skin temperature, MY= Milk yield, DC=Dielectric constant

Table 4 Comparison of developed ANFIS vis-a-vis MLR model for different data partitioning schemes of Sahiwal cows and Murrah buffaloes

Data Partitioning Scheme	RMSE %	
	*ANFIS model	MLR model
	Sahiwal cows	
70:30	0.34	4.88
80:20	0.32	11.39
90:10	0.23	8.38
	Murrah buffaloes	
70:30	0.20	22.90
80:20	0.22	4.72
90:10	0.23	4.08

*The values of RMSE% in ANFIS model are from table 2

etc.,), ANN, ANFIS, Support Vector Machine (SVM) and others. The comparative performance of ANFIS models *vis-à-vis* MLR models developed in this paper was made in terms of RMSE on test set and results are presented in (Table 4). The percent root mean square error of ANFIS models in Sahiwal cows varied between 0.23 to 0.84 whereas that of MLR models ranged between 4.88 and 11.39. In case of Murrah buffaloes, percent root mean square error varied between 0.20 to 0.28 whereas that of MLR models ranged between 4.08 and 22.90. Mammadova and Keskin (2013) have also used binary logistic regression model and found that the error rate of logistic regression model was 57% and finally concluded that SVM technique has the potential to perform better than such traditional statistical methods as logistic regression. The above presented results indicate that ANFIS is far better than Conventional Regression Model (MLR).

Conclusions

In this paper, several soft computing models (ANFIS) have been developed and validated to identify healthy vs. mastitis Sahiwal cows and Murrah buffaloes on the basis of Milk pH, electrical conductivity, temperature (udder, milk and skin), milk yield, dielectric constant and milk Somatic cell counts of normal and mastitis milk. The error back propagation training algorithm with Bayesian regularization scheme and several combinations of different values of network parameters was empirically investigated. The performance of ANFIS models was compared to that of classical multiple linear regression models. The comparative analysis of the results thus obtained revealed that ANFIS models was more superior than multiple linear regression models

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RESEARCH ARTICLE

Prevalence subclinical mastitis in small-scale dairy farms under grazing or in total confinement in the central highlands of Mexico

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Abstract: Mastitis is the most frequent disease in dairy farms worldwide, causing severe economic loss; being subclinical mastitis the most important as it is a silent disease. The objective of this work was to compare the udder health status in cows under limited grazing against cows in total confinement in small-scale dairy systems. Ten small-scale dairy farms participated in the study, with five farms that had implemented grazing of their pastures and five farms that continued the conventional management of total confinement of their herds and feeding based in cut-and-carry pasture, straws, agricultural by products, and concentrates. The highest yielding five cows from each farm were selected for the study. The California Mastitis Test (CMT) performed every 2 weeks, from June 17 to July 15 on all participating cows, and Somatic cell counts (SCC) was determinedon the farm pooled milk. Results for CMT were subjected to a Chi-squared test, and SCC to analysis of variance after $\log_{10}$ transformation. There were significant differences both for CMT and SCC with lower values for cows under restricted grazing than in total confinement. Restricted grazing of dairy cows in small-scale dairy systems result in better udder health, and therefore better quality milk.

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Keywords: California Mastitis Test; highlands; Mexico, Somatic Cell Count; Subclinical mastitis; Small-scale dairy systems,

Introduction

Dairy production farming in Mexico is carried out under diverse agro-ecological conditions in three main production systems., large scale intensive farms, dual purpose dairy production in the tropical lowlands and small-scale family dairy systems that takes place in all the temperate, arid and semi-arid regions of the country.

Small-scale dairy systems are farms with limited land endowments and herds between 3 and 35 cows plus replacements (Fadul-Pacheco et al. 2013) that rely mostly on family labour (Posadas-Domínguez et al. 2014); providing occupation and incomes that enable dairy families to overcome poverty (Espinoza-Ortega et al. 2007).

Conventional feeding of herds in these farms is heterogeneous, comprising maize straw, purchased lucerne hay, irrigated pastures under cut-and-carry, and large amounts of commercial concentrates (Martínez-García et al. 2015) that result in high production costs that jeopardize the economic viability of these farms (Fadul-Pacheco et al. 2013).

An alternative promoted is grazing of pastures that has been successful in reducing feeding costs and improving the profitability of farms (Prospero-Bernal et al. 2017). Grazing may also reduce the incidence of some diseases such as mastitis since the cows can maintain clean udders (Abramsén et al. 2014).

Mastitis is the inflammation of the mammary glands, a common ailment in dairy cows all over the world, of which subclinical mastitis is of utmost importance (Bangar et al. 2015) as it is a 'silent disease' without evident external signs, that causes a reduction in milk yields and therefore it affects incomes. Subclinical mastitis is caused mainly by pathogens in the herd environment or as a result of an injury.

The high prevalence of subclinical mastitis makes it the most important disease affecting dairy production, causing severe economic losses, representing up to 30% of total expenditures in

dairy farms all over the world (Abrahmsén et al. 2002; Wellenberg et al. 2002; Manjarrez-López et al. 2012; Frössling et al. 2017; Hachana and Tibbini., 2018).

There are reports in Mexico of an incidence of sublclinical mastitis due to type of milking up to 57% (Manajarrez-López et al. 2012), but prevalence results are also affected by the hygienic conditions during milking (Ávila-Tellez et al. 2002).

Some reports mention a higher incidence of subclinical mastitis in cows under confinement compared to cows under grazing (Carrasco-Rodriguez et al. 2014); although these authors report inadequate management at dry-off and a lack of disinfection of teats after milkings. Also, Abramsén et al. (2014) report a higher incidence of subclinical mastitis in confined cows compared to grazing cows.

The objective of this work was to assess subclinical mastitis in small-scale dairy farms under conventional total confinement on concrete floors, in comparison with cows from farms that have implemented grazing of their herds for 8 h a day.

Materials and Methods

Study area

The study was undertaken in the municipality of Aculco in the central highlands of Mexico, located between coordinates 20° 06' and 20° 17' N and at 99° 40' and 100° 00' W; with a sub-humid temperate climate and mean altitude of 2440 m (Celis-Alvarez et al. 2016). Mean temperature is 13.2 °C with frosts in winter and rainfall is between 800 and 1000 mm with rains in summer (May to October) and a dry season from November to April.

Small-scale dairy systems have herds between 3 and 35 cows plus replacements, small land holdings, and rely on family labour Fadul-Pacheco et al. (2013).

Selection of dairy farms

The study followed a participatory livestock research approach (Conroy, 2005) with 10 participating farmers, of whom five keep their herds under conventional total confinement in pens with cut-and-carry pastures, and five farms that have implemented day grazing of their pastures (between morning and afternoon milkings) for 8 h/day, and confinement overnight in similar concrete floor pens as in the total confinement herds.

A total of 10 small scale dairy farms (5 each from conventional confinement in pens with cut-and-carry pastures and grazing system) were selected from the study area, that were initially selected by a snow-ball sampling procedure in the project to which this work belongs (Prospero-Bernal et al. 2017). Table 1 shows the characteristics of participating farms.

Five cows with the higher average daily milk yield from each participating farm were selected for sampling.

Milking was by hand, between 4:00 and 6:30h in the morning and between 16:00 and 18:00 h in the afternoon. Hygiene routines were the same in all farms: farmers washed their hands before milking, and cleaned the udder with water and a cloth, and foremilk examined. Cows were milked always by the same person.

Duration of the study

The study took place for 45 days during the rainy season. Milk samples were collected thrice at fortnightly intervals at morning milking in the case of grazing herds and at evening milking in confined and stall fed herds. Since all farmers milk at similar times in the region, for logistical purposes, by random allocation, grazing farms were sampled in the morning milking, and confinement farms in the afternoon milking.

For the detection of subclinical mastitis California Mastitis test (CMT) was performed on collected milk samples. The CMT scores given were; negative, traces, 1, 2 and 3.

Pooled samples of the selected five cows from each farm were refrigerated after every milking. The samples were then homogenised and warmed to 37 °C in a water bath and somatic cell counts (SCC) were determined using a DeLaval DCC Automatic Somatic Cell Counter.

Statistical analyses

A *Student "t"* test for independent samples was performed to compare California Mastitis Test scores (in percentage) between the two treatments (grazing vs. confinement (Schefler, 1979).

Log_{10} transformation of SSC values were analysed by ANOVA under a split-plot design (Kaps and Lamberson, 2004) with the model:

$$Y_{ijkl} = \mu + r_i + S_j + E_k + p_l + Tp_{jl} + e_{ijk}$$

Where:

μ = General mean

r = Effect of replicates (farms), i = 5

S = Effect of feeding strategy (confinement or grazing) (Main Plot) j = 1, 2

E = Error term for Main Plots [r(T)ij]

p = Effect of sampling periods (split - plot) k = 1, ..., 3

Sp = Interaction term between treatments and measurement periods

e = Error term for split plots and the interaction

Results and Discussion

Table 2 shows the results for the analysis of CMT scores. There were 34.6% more negative cases in grazing cows than cows in confinement, although given the nature of the variable (percent of quarters with a given score), the *Student "t"* test could not detect significance (P>0.05).

However there was a significant trend (P<0.10) for a lower number of quarters with score 2 in grazing cows, and a significantly lower (P<0.05) proportion of cases with CMT score 3 in grazing cows compared with cows under permanent confinement.

Eighty percent of quarters of cows under day grazing had negative or trace CMT scores, compared to only 64% under confinement.

Day time grazing, even when restricted to only 8 h/day, resulted in cleaner udders and less contact with manure contaminated floors in pens which is critical in the rainy season. Besides stage of lactation, rain and dry seasons are determinant in the presence of mastitis (Bradley and Green 2004).

Lack of adequate bedding in pens in small-scale dairy systems mean difficult conditions to keep pens clean from manure, representing an infection source for cows since the main source for infections are pathogens in the cow environment (Phuektes et al. 2001).

Ávila-Téllez et al. (2002), from a study of mastitis in sub-tropical dual purpose dairy systems in Mexico also with restricted grazing, reported 57% incidence of subclinical mastitis, higher incidence than results from the work herein reported both for day grazing as for confined cows. These differences might be due to the higher ambient temperatures in the subtropics that favour microbial proliferation.

Subclinical mastitis is a worldwide problem for dairy farmers, and in line with results from this study, Abrahmsen et al. (2014) in Uganda reported 73.5% incidence of mastitis in confined cows compared with a significantly lower rate (53.8%) for grazing cows.

A high CMT score indicates a high incidence of infected quarters; directly related with somatic cell counts as shown in Table 3. Cows in confinement had 70% more somatic cells/ml of milk than cows under day grazing, again due to cleaner udder conditions in cows under day grazing compared with soiled floors under confinement. Inadequate hygienic conditions are known as risk factors that enable mammary gland infections (Manjarrez-López et al. 2012).

On the contrary, Carrasco-Rodríguez et al. (2014) studying mastitis in cows from the Carora breed in Venezuela under grazing and total confinement reported a higher incidence of mastitis (32.7%)

Table 1 Characteristics of participating farms

Pasture management Strategy	Grazing						Cut-and-carry					
Farm	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Farm size (ha)	6.3	7.8	13.0	9.0	12.5	9.7	4.5	2.0	3.5	2.5	7.8	4.1
Total pastures (ha)	1.5	3.0	4.0	2.0	2.5	2.6	1.5	1.0	1.5	1.0	3.0	1.6
Milking cows	7.0	7.0	8.0	6.0	17.0	9.0	17.0	10.0	8.0	13.0	7.0	11.0
Dry cows	1.0	1.0	2.0	2.0	5.0	2.2	3.0	1.0	1.0	2.0	1.0	1.6
Milk yield (L/cow/day)	15.3	13.9	17.0	14.9	16.4	15.5	13.8	18.0	11.0	14.9	13.9	14.3
Milk fat (g/kg milk)	33.0	38.0	35.0	34.0	34.0	34.8	35.0	33.0	32.0	35.0	38.0	34.6
Protein (g/kg milk)	32.0	32.0	33.0	33.0	32.5	32.5	32.0	33.0	32.0	33.0	32.0	32.0

Table 2 Incidence of subclinical mastitis (%) in small-scale dairy farms under day grazing or total confinement

Variable	Negative	Traces	1	2	3
Grazing	67.7	18.7	13	4.6	2.0[a]
Confinement	50.3	14.3	16.3	11.7	7.3[b]
SEM	6.09	3.25	2.93	8.6	2
P-value	0.187	0.34	0.147	0.067	0.043

SEM= Estándar error of the mean, [a,b] (P<0.05)

Table 3 Somatic cell counts (SCC) for different feeding strategy (cells/ml milk)

Grazing	Confinement	SEM_M	SEM_p
4,40,000	7,43,000	261.82**	51.80**

SEM_S = Standard error for housing management (Main Plots), EEMp= Standard error for sampling periods (split-plots) ** P<0.01

in cows under grazing that on permanent confinement (19.9%). Authors discuss that differences were mainly due to adequate milking practices in confinement farms, while in grazing farms hygiene at milking was deficient.

Table 3 shows the results for somatic cell counts, with highly significant differences ($P<0.05$) between housing managements with higher SCC for cows under permanent confinement compared with lower cell counts for cows under day grazing.

Mean SSC in cows from grazing farms were 440,000 cells/ml, a value near the 400,000 cells/ml required by the intensive dairy industry for large farms; even though small-scale dairy farmers milk by hand and with deficient hygiene practices. These results mean that day grazing of dairy cows may be an option, not only to reduce feeding costs and increase profitability, but also to improve the quality of milk and the health and welfare of dairy cows in these small-scale dairy systems.

Conclusions

In conclusion, results indicated that even under restricted grazing for 8 h/day reduce the incidence and severity of subclinical mastitis in small-scale dairy systems. CMT scores showed a larger number of negative and trace scores and lower CMT scores 1, 2, and 3 under grazing than under confinement.

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RESEARCH ARTICLE

An observational study investigating uniformity of manual body condition scoring in dairy cows

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Abstract: Body condition scores (BCS) are very useful for dairy herd management; however, its reliability and consistency of recordings made by observers have been questioned. Moreover, regular high-quality manual body condition scoring of an individual cow is difficult as a routine practice on the farm. This study aimed to find out the agreement in BCS within- and between the observers. An observational study was conducted in which two observers independently scored BCS of 43 crossbred animals from one dairy hard. Cohen's kappa (κ) and spearman's rho (ρ) was computed to quantify the agreement between observer for overall BCS measurement and only kappa test for different BCS groups (high, medium and low). For overall BCS score ê of 0.62 to 0.71 ($p<0.001$) and 0.68 to 0.74 ($p<0.001$) were obtained for intra observer 1 and 2 respectively, such values would be interpreted as moderate agreement. Whereas, the inter-observer agreement (ê) was found to be low i.e. between 0.52 to 0.60 ($p<0.001$). However, the spearman's rho value was higher in all the cases, indicating a good correlation among the individual observers. Besides, kappa value (κ) for different BCS groups revealed the lowest agreement between observers (0.31 to 0.37, $p<0.001$) and within observers (0.34 to 0.59, $p<0.001$) for medium BCS group as compared to the other BCS groups. These findings suggest multiple observers should perform manual Body Condition Scoring for better accuracy in the outcome.

Keywords: BCS, Dairy cattle, Observation variation

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Introduction

Initially, BCS evaluation chart was developed by assessing the eight anatomical locations of the rear half of the cow within the areas of the loin, pelvis and tail head and prepared a scale ranges from 1 to 5, using 0.25-unit increments (Wildman et al. 1982, Edmonson et al. 1989). Several researchers from different countries had developed various scales by observing and palpating the animal body. Bewley and Schutz (2008) reviewed international BCS systems, in the UK and Ireland widely used scale ranges from 1-5 with 0.50 and 0.25 intervals, respectively. Likewise, in Australia and New Zealand most common and widely used scale ranges from 1-8 and 1-10 with an increment of 0.5 scale intervals. In India, Prasad (1994) has developed a modified Body Condition Scoring scale ranges from 1 to 6, with an increment of 0.5 for better accuracy. Evans, (1978) and Nicoll, (1981) have studied the factors causing variation in BCS and found 60 to 70 % was due to animal variation, 5 % from the evaluator and 10 % happened animal-evaluator variation. The quality of manual Body Condition Scoring depends on the observers and scoring protocol (Kristensen et al. 2006), where a trained person had consistency up to 58 to 67 % accuracy and 27 % precision by an untrained person (Ferguson et al. 1994). Inter-observer agreement was more reliable than the single observer conducted all BCS evaluation (Morin et al. 2017). Also, BCS (1-5 point scale) changes of 0.25 cannot realistically be detected, even with trained observers (Bewley et al. 2008; Bewley and Schutz, 2008). When comparing the methods for monitoring the BCS, Mazeris (2015) found an automated BCS system to be highly accurate to a human scorer, with 98% of scores being within a quarter-point. Previously, intra and inter observational agreements were done in 1 to 5 BCS scale with 0.25 unit increments (Kristensen et al. 2006; Morin et al. 2017) based on the scale of Ferguson et al. (1994). Our hypothesis of the present study was observational agreement within and between professionals on BCS in 1-6 scale with 0.5 unit increments.

Materials and Methods

Data collection

Two veterinarians were independently assessed BCS of a total of 43 cows. BCS was measured using a visual plus palpation

technique in 1 to 6 scales with 0.5 increments, which was established by Prasad (1994). The data were collected two consecutive days in a week for three weeks. All the crossbred Jersey cattle were divided into three BCS groups i.e. high (> 4.5/ above 4.5, N= 12), Medium (3 to 4.5, N= 22) and Low (< 3/ below 3, N= 9) by an expert. Both the veterinarians were well trained for manual body condition scoring of dairy cattle. Prasad (1994) have mentioned specific regions for body condition scoring in dairy cattle, which needs sound anatomical knowledge of the observer for better accuracy. The anatomical regions i.e. vertebral column, spinous processes, transverse processes, pin and hook bone, tail head region and ribs were considered, based on that scoring were done.

Statistical analysis

All statistical analyses were performed using IBM SPSS statistics software (version 22.0). However, we considered BCS as an ordinal variable because it classifies cows into different categories of body condition. The quality of manual body condition scoring of the two observers was quantified by the scoring agreement Cohen's kappa (κ) and the scoring correlation coefficient Spearman's rho (ρ). Cohen's kappa (κ) measures the pairwise agreement of two observers or one observer across 2 days, including the possibility of agreement occurring by chance. Spearman's rho (ρ) is a pairwise rank correlation and is defined as the statistical dependence between the rankings of 2 manual scores without interference from the systematic scoring difference. The ρ value ranges from 1 (i.e. a pair set of scores with identical ranks) to – 1 (i.e. a pair set of scores with opposite ranks). Both ê and ñ values were calculated for intra-observer agreement between the consecutive days and for inter-observer comparison of the 2-d average scores between observers 1 and 2.

Results and Discussion

The descriptive statistics of BCS observation by two veterinarians presented in Table 1, whereas intra and inter-observer agreement (ê) and Spearman correlation coefficients (ñ) of the overall manual BCS of two observers shown in Table 2. In the present study, two experienced veterinarians were engaged in BCS evaluation to increase homogeneity in inter-class variations. Both the observers assessed overall BCS with good intra observational agreement i.e., κ values were 0.62 to 0.71 ($p<0.001$) and 0.68 to 0.74 ($p<0.001$) respectively. However, inter-observer agreement was moderate and kappa value (κ) was significantly ($p<0.001$) varied in the range of 0.52 to 0.60. Additionally, intra and inter-observer correlation coefficient (Spearman rho, ρ) were calculated and it was significantly higher within observers ($\rho>0.95$) than between observers ($\rho > 0.88$) during the study period. However, Ferguson et al. (1994) also reported a high correlation among observers i.e. 0.76 to 0.85. The kappa value (κ) is widely used as an indicator of agreement and greater than 0.80 is representing excellent agreement in clinical observation (Ersboll et al. 2004). Kristensen et al. (2006) have found low to moderate Intra and inter-observer agreement on BCS measurement among veterinarians i.e. κ , ranges between 0.22 to 0.75 and 0.17 to 0.78 respectively. Whereas, Song and coworker (2019) have observed moderate intra-observer agreement (κ , 0.40 to 0.59 and 0.60 to 0.79) with lower inter-observer agreement (ê, 0.20 to 0.79). On the contrary, excellent intra (κ , 0.86) and inter-observer (κ , 0.76 to 0.85) agreement was observed for highly trained instructors by Kristensen et al. (2006) and Morin et al. (2017) respectively. In this present study, intra and inter observational variations were comparably narrower than Kristensen et al. (2006). However, the comparison was not error free with other studies as it uses different BCS scale i.e. 6 point scale with 0.5 increments. Furthermore, in our study, BCS observation values ranged from 1 to 6 due to wide variation in the BCS groups. On the contrary, Morin et al. (2017) found all BCS values ranged between 2.5 to 3.5, resulting in higher inter-observer agreement (κ) between observers than the present findings.

Further, the intra and inter-observer agreement (κ) were measured for three different BCS groups and represented in Table 3. In the high BCS group, Kappa (κ) values were ranges between 0.62 to

Table 1 Descriptive statistics of BCS measured by two different observers

Time	Observer	N	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
1st week	1	43	1.5	5.5	4.05	0.178	1.17	1.37
		43	1	6	3.95	0.191	1.25	1.58
	2	43	1.5	6	3.67	0.201	1.32	1.75
		43	1.5	5.5	3.59	0.189	1.24	1.54
2nd week	1	43	1.5	5.5	4.03	0.174	1.15	1.31
		43	1	6	3.93	0.185	1.20	1.47
	2	43	1.5	6	3.88	0.199	1.30	1.71
		43	1.5	5.5	3.74	0.188	1.24	1.53
3rd week	1	43	1.5	5.5	4.03	0.174	1.15	1.31
		43	1	6	3.95	0.194	1.27	1.62
	2	43	1.5	5.5	3.85	0.179	1.17	1.37
		43	1.5	6	3.74	0.183	1.20	1.44

Table 2 Intra and inter-observer agreements and correlation coefficient for manual BCS ($p< 0.001$)

Scoring agreement and correlation coefficient	scoring	1st week	2ndweek	3rd week
Intra observer 1	Cohen's kappa (κ)	0.62	0.68	0.71
	Spearman's rho (ρ)	0.95	0.96	0.98
Intra observer 2	Cohen's kappa (κ)	0.68	0.71	0.74
	Spearman's rho (ρ)	0.97	0.96	0.98
Inter observer	Cohen's kappa (κ)	0.52	0.55	0.60
	Spearman's rho (ρ)	0.88	0.90	0.91

Table 3 Description of Cohen's kappa (κ) values among Low BCS (L-BCS), Medium BCS (M-BCS) and High BCS (H-BCS) groups

Groups		Scoring agreement, Cohen's kappa (κ)		
		1st week	2nd week	3rd week
Intra observer r 1	L-BCS	0.64	0.82	0.66
	M-BCS	0.34	0.42	0.54
	H-BCS	0.70	0.71	0.88
Intra observer 2	L-BCS	0.82	0.83	0.85
	M-BCS	0.54	0.56	0.59
	H-BCS	0.62	0.75	0.74
Inter observer	L-BCS	0.53	0.52	0.68
	M-BCS	0.31	0.33	0.37
	H-BCS	0.57	0.60	0.58

Kappa ranges from 0 to 1
** 0 to 0.20 Slight agreement** 0.4 to 0.6 Moderate agreement** 0.8 to 1.0 Perfect agreement
$p< 0.001$ for all calculations of scoring agreement.

0.88 within observers and 0.57 to 0.60 in between observer, indicates moderate to high agreement by the two observers. Similarly, Kappa (κ) values were 0.64 to 0.85 within observers and 0.53 to 0.68 between observers for low BCS group. In contrast, there was more observational variation found in the medium BCS group where κ value ranges from 0.34 to 0.59 within observers and 0.31 to 0.37 in between observers, such values would be interpreted as fair agreement. The heterogeneity in observational agreement in the medium BCS group was due to lower anatomical variation among the animals, lead to less subjectivity in the judgment of evaluators and could be influenced by the previously observed cow (Bercovich et al. 2013).

Conclusions

From this present study, it can be concluded that the quality of the overall BCS assigned by two assessors was acceptable, as we found high observational uniformity or homogeneity for intraobserver BCS agreement with a high-rank correlation (ρ) and moderate BCS agreement (κ) between the observers. In addition, for different BCS groups, heterogeneity in observational agreement by the assessor was more pronounced in the medium BCS group than the other groups. Therefore, to minimize the observational error in manual BCS recommends multiple observers assessment on BCS of dairy cattle in the commercial dairy herds.

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RESEARCH ARTICLE

D^2 statistic technique used for analysis of genetic divergence among Gir crossbreds

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Abstract: D^2 analysis is used for selection of genetically divergent parents. It is used to measure degree of diversification and determines relative portion of each component trait to total divergence. The study aimed to estimate the genetic divergence among FG (50% HF+50% Gir), IFG (FG *Interse*), FJG (50% HF+25% Jersey+25% Gir), IFJG (FJG *Interse*) and R (50% HF+12.50% Jersey+37.50% Gir) crosses of Gir on the basis of age at first calving, first calving interval, lactation milk yield, lactation length and lactation milk yield per day of calving interval using Mahalanobis D^2 statistics. The genetic group differences were significant ($P<0.01$) for all traits separately and simultaneously (V test) based on five traits. The differences in the D^2 values among all the genetic groups combinations, were significant except IFG with R genetic group combination. The total D^2 values for AFC, 1st CI, LMY, LL and LMY/CI were 20.53, 0.71, 4.37, 4.16 and 0.26 respectively. The contribution of AFC to the total D^2 value was maximum as 68.37 per cent and lowest of LMY/CI as 0.87 per cent. Among the clusters formed on the basis of D^2 values, IFG, IFJG and R formed one cluster, whereas, FG and FJG formed second cluster. The magnitude of inter-cluster distance was greater than intra-cluster distance.

Keywords: D^2 analysis, Gir, canonical analysis and traits, Genetic divergence

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Introduction

Genetic diversity has been defined as the variety of alleles and genotypes present in a population, and this is reflected in morphological, physiological and behavioral differences between individuals and populations. Recently, loss of genetic diversity within indigenous livestock breeds has been a major concern. Every year many species and breeds of animals become extinct thereby decreasing the biodiversity and genetic variation of populations. Hence, need for sustainable management and conservation strategies for these animal genetic resources are necessary. Previous efforts on the phenotypic characterization of breeds of livestock have been restricted to the use of analysis of variance, whereas the current trend in livestock classification involves the use of multivariate statistical tools. This is because univariate statistical analysis, analyze each variable separately and do not explain how the populations under investigations differ when all measured variables are considered jointly. Multifactorial discriminant analyses have been found to be more suitable in assessing variation within a population and can discriminate different population types when all variables are considered jointly Yakubu et al. (2010). The best way to understand the potential of the available germplasm is by analysing its genetic diversity. For an outstanding breeding programme in the cattle improvement, diversity analysis greatly helps the breeder in the identification and proper choice of parents for specific breeding objectives. The present study was conducted to assess the genetic diversity among different genetic groups of Gir for further breeding programme. The Mahalanobis distance is a very useful statistic in multivariate analysis. It plays a fundamental and important role in statistics and data analysis with multiple measurements. It is one of the most common measures in chemometrics, or indeed multivariate statistics. Extending to multivariate situation, Mahalanobis proposed a distance from the centre of the data (Brereton 2015). Hence, this study was done for genetic group differentiation using Mahalanobis D^2 statistics by combining information on important characters and to know the divergence through relative contribution of characters in different combinations.

Materials and Methods

Data collection

Data for the present investigation were collected from history sheet of Gir crossbred cattle at Research Cum Development Project on Cattle, MPKV, Rahuri. The data pertained to 158 FG (50% HF+50% Gir), 212 IFG (FG *Interse*), 107 FJG (50% HF+25% Jersey+25% Gir), 324 IFJG (FJG *Interse*) and 71 R (50% HF+12.50% Jersey+37.50% Gir) crosses of Gir were distributed over a period of 44 years from 1972 to 2015. The traits considered were age at first calving, first calving interval, lactation milk yield, lactation length and lactation milk yield per day of calving interval. Cows with abnormal and incomplete records were excluded from the study. The lactation records of less than 200 days were considered as abnormal and were not included in the data.

Statistical analysis

As the data in the present study were non-orthogonal in nature with unequal subclass numbers, they were subjected to least squares analysis of variance without interactions using different models to examine the effect of genetic as well as non-genetic factors on various traits. Least-squares analysis of variance was conducted for the effect of period and season of birth/calving on age at first calving (AFC), first calving interval (1st CI), lactation milk yield (LMY), lactation length (LL) and lactation milk yield per day of calving interval (LMY/CI) by Harvey (1990). The data were corrected for the significant effects due to period and season of birth/calving as suggested by Gacula et al. (1968). Analysis of variance and covariance for the traits studied was done for testing the differences among the genetic groups for each character by F- test. Analysis of dispersion was done for the simultaneous test of differences between all the traits studied and for all the genetic groups. The significance of dispersion was observed using V-statistics test, Wilk's criteria were used as described by Rao (1952). The original mean values of the character were transformed to uncorrelated variables by using pivotal condensation method (Rao, 1952). The diversities between different genetic groups based on various characters were estimated by using Mahalanobis (1936) D^2 Statistics as per following formula:

$$D_P^2 = \sum_i^P \sum_j^P W^{ij} \left(\bar{X}_{i1} - \bar{X}_{i2} \right)\left(\bar{X}_{j1} - \bar{X}_{j2} \right)$$

Where,

D^2_p = D^2 based on p number of characters

W^{ij} = Inverse of estimated variance covariance matrix

$\bar{X}_{i1}$ = Mean for i^{th} character of genetic group 1

$\bar{X}_{i2}$ = Mean for i^{th} character of genetic group 2

$\bar{X}_{j1}$ = Mean for j^{th} character of genetic group 1 and

$\bar{X}_{j2}$ = Mean for j^{th} character of genetic group 2

The test of significance of D^2 values was done for any two out of five genetic groups by using statistics suggested by Rao (1952). The percentage contribution of each character to overall diversity was calculated on the basis of D^2 values and rank basis. For grouping of the genetic groups into various clusters, Tocher's method (Singh and Choudhary 1985) was used. To verify the clustering pattern canonical analysis (Rao 1948) was done. The data were analysed with the help of two programmes Harvey (1990) and SAS version 9.3 (2013). The CANDISC (SAS 2013) procedure was used to perform multivariate analysis that calculated the Mahalanobis distance between the five genetic groups and to compute canonical discriminant analysis to derive canonical functions. Based on the Mahalanobis distance dendogram was created using PROC CLUSTER (SAS 2013).

Results and Discussion

The analysis of variance indicated that the genetic group difference was significant ($P<0.01$) for each trait separately. The V-statistics revealed that the influence of genetic group on traits was significant ($P<0.01$) when all the five traits were considered together. Boujenane (2015) studied on multivariate characterization of Oulmes Zaer and Tidili cattle and reported that the multivariate test for differences between the breeds (Wilks' Lambda) was significant ($P<0.001$).

Relative diversities

The differences in D^2 values among all genetic group combinations were significant except between IFG with R group (Table 1). Mahalanobis squared distance values were maximum between FG and R group as 6.58. It's due to higher difference in performance between two groups for the traits under study. The maximum D^2 values among FG and R genetic groups might be due to the fact that cows of FG group calved in early age than cows of R group and also due to higher lactation milk yield in FG than R group. The higher performance of FG (F_1) might be due to heterotic effect. The inter-genetic D^2 value (0.16) between IFG and IFJG group was least. The minimum D^2 value among these groups is due to similarity in performance of these *interse* groups.

D^2 values and character wise D^2 values with ranks

The genetic diversity among five genetic groups was measured by employing D^2 statistics and was grouped into two clusters using Tocher's method. The total D^2 value contributed by all five traits was 30.03 (Table 2). The per cent contribution of AFC to the total D^2 value was maximum as 68.37, whereas, LMY/CI contributed minimum as 0.87 in total diversity. These results resembled with

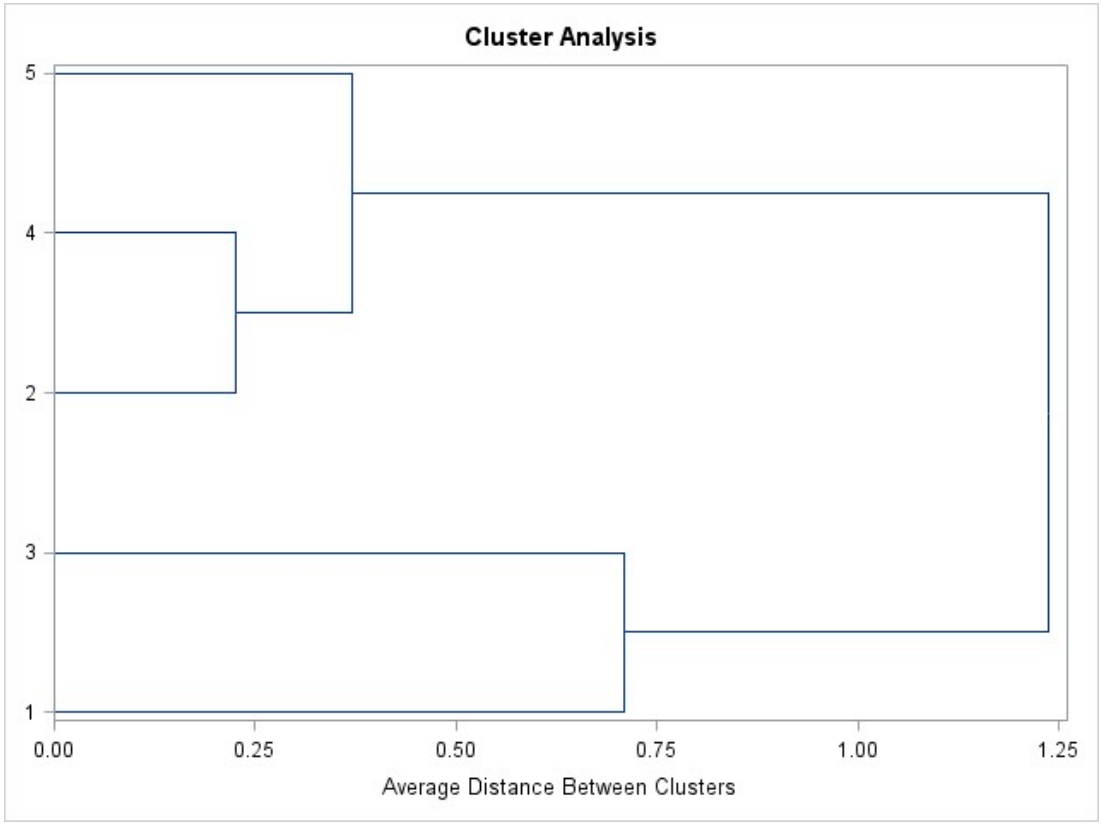

Fig.1 Dendogram showing cluster formation

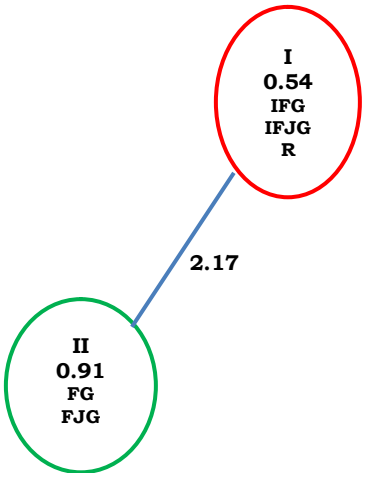

Fig. 2 Cluster diagram depicting intra and inter-cluster distances based on Tocher's method

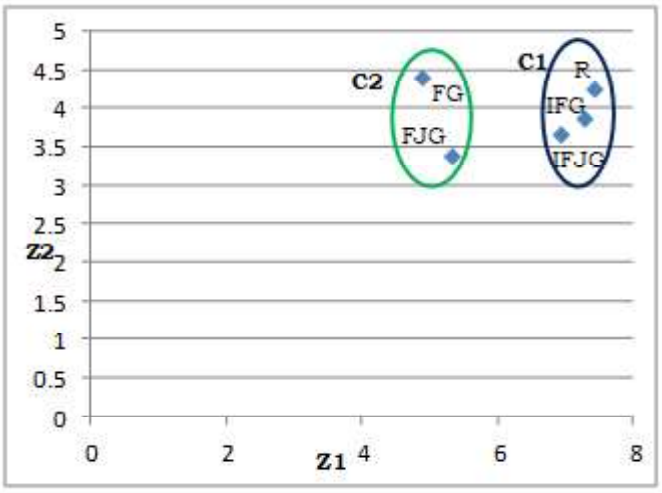

Fig.3 Clustering pattern plotted with 2-dimensional diagram (canonical method)

Singh and Bhat (1986) reported in HF × Sahiwal crosses who noted contribution of CI as 2.15 per cent. Lower contribution of FCI than the present results was reported by Singh and Parekh (1989) in Gir crossbreds as zero per cent. The contribution of age at first calving in FJG with R and FG with R genetic group combinations were maximum among all combinations. The highest D^2 values in AFC were between FJG and R (4.58) groups which indicate maximum difference in AFC in both groups. This might be due to higher Gir inheritance in R crossbred (37.50%) than FJG crossbred (25.00%). The minimum D^2 value for AFC was noticed between FG with FJG genetic group combination. The rank total indicated that LMY/CI had higher rank, whereas, AFC had the lowest rank. This indicates minimum per cent contribution of LMY/CI and maximum per cent contribution of AFC in overall divergence. The present results were in accordance with Sangwan and Singh (1995) observed in Hariana crosses who noted contribution of LMY/CI as zero per cent. The discriminant quantitative variable and Mahalanobis distance between two

Table 1 Analysis of variance for genetic group differences

Source of variation	d.f.	MS				
		AFC	1st CI	LMY	LL	LMY/CI
Between genetic groups	4	2279244.06**	80452.46**	10539149.38**	30845.91**	124.44**
Within genetic groups	867	16897.62	10935.10	587722.98	3410.13	3.25
			Simultaneous test of significance for the above traits			
Wilk's criterion			0.000162			
V-statistic			7556.74**			

** = P< 0.01

Table 2 Total D^2 values and character-wise D^2 values with ranks for each genetic group combination

GG Combination	Total D^2	AFC	1st CI	LMY	LL	LMY/CI
FG-IFG	5.87**	3.25 (1)	0.15 (4)	1.23 (2)	1.16 (3)	0.08 (5)
FG-FJG	0.83**	0.00 (5)	0.03 (3)	0.58 (1)	0.20 (2)	0.02 (4)
FG-IFJG	4.48**	2.07 (1)	0.24 (4)	1.08 (2)	1.07 (3)	0.02 (5)
FG-R	6.58**	4.50 (1)	0.08 (4)	1.18 (2)	0.75 (3)	0.07 (5)
IFG-FJG	3.89**	3.31 (1)	0.04 (4)	0.12 (3)	0.40 (2)	0.02 (5)
IFG-IFJG	0.16**	0.13 (1)	0.01 (3)	0.00 (4)	0.00 (5)	0.02 (2)
IFG-R	0.15	0.10 (1)	0.01 (3)	0.00 (4)	0.04 (2)	0.00 (5)
FJG-IFJG	2.64**	2.12 (1)	0.10 (3)	0.08 (4)	0.34 (2)	0.00 (5)
FJG-R	4.88**	4.58 (1)	0.01 (5)	0.10 (3)	0.17 (2)	0.02 (4)
IFJG-R	0.55**	0.47 (1)	0.04 (2)	0.00 (5)	0.03 (3)	0.01 (4)
Total D^2	30.03	20.53	0.71	4.37	4.16	0.26
% contribution as per D^2	100	68.37	2.36	14.55	13.85	0.87
Rank total		14	35	30	27	44
Number of times appearing in first ranking	-	9	0	1	0	0
% contribution as per first ranking	-	90	00	10	00	00

Values in parentheses are ranks; * = P<0.05; **= P<0.01

Table 3 Average intra and inter cluster distance for five genetic groups obtained on the basis of five traits

Clusters	I	II	III
	Genotypes		
	IFG, IFJG, R	FG, FJG	
	Intra and inter-cluster D^2 and D values		
I	0.29(0.54)	4.72(2.17)	
II		0.83(0.91)	0.00(0.00)

Figures in parenthesis indicate D (distance) values

N'Dama populations of agro-ecological zones were assessed using discriminant analysis by N'goran et al. (2018). They reported that the Mahalanobis pair wise distance between the two populations of N'Dama was equal to 3.69, which was significant (P<0.05). They further noticed that discriminant analysis showed N'Dama populations were separated following the two agro-ecological zones.

Cluster formation

Clustering of genetic groups under study are presented in Table 2 and Figures 1 to 3. Based on the D^2 values all the genetic groups were grouped into two clusters, signaling the presence of diversity for different traits. The cluster I had the three genetic groups (IFG, IFJG and R) and cluster II had two genetic groups (FG and FJG). Similar cluster pattern was observed by Jagtap et al. (1989) in Gir crossbreds showing FG, FJG and JFG in one cluster. Singh and Parekh (1989) in Gir crossbreds noticed that FG and FJG formed common cluster due to minimum genetic divergence between them.

Intra and inter-cluster distance

Intra and inter cluster divergence values (D^2) by Tocher's method between and within two clusters are presented in the Table 3 and Figures 1 to 3. The magnitude of inter-cluster distance was greater than intra-cluster distance. This showed distinct difference between grades belonging to two clusters. Lower inter cluster

Table 4 Mean values of the first 2 canonical variates (Z_1 and Z_2) for five genetic groups

Genetic group	Z_1	Z_2
FG	4.89	4.39
IFG	7.27	3.84
FJG	5.34	3.38
IFJG	6.93	3.66
R	7.45	4.24
Per cent contribution of first two roots		
Canonical root 1		92.08
Canonical root 2		6.83
Total		98.91

values between clusters are indication of close relationship and similarity for most traits in the genetic groups hence selection of parents from these clusters are to be avoided. The inter-cluster distance value between cluster I and cluster II was 2.17.

Canonical analysis

This method was used to plot the various grades into two dimensional picture by considering the mean values of the canonical variates (Z_1 and Z_2). The nearness observed between these grades on the basis of Z_1 and Z_2 values may be considered as more appropriate than distance observed on the basis of D^2 values. It's due to the fact that the first two roots explained 98.91 per cent of variability (Table 4). Canonical discriminant analysis was used by Boujenane (2015) to obtain the function of all body measurements necessary for the separation of Oulmes Zaer and Tidili cattle breeds. He revealed that the squared Mahalanobis distance between breed means was 11.77 ($P<0.001$).

Conclusions

D^2 analysis offers a reliable technique to estimate the divergence present in the population. This technique measures the forces of differentiation at intra cluster, inter cluster level and further helps in selection of genetically divergent parents for exploitation in hybridization programme based on their superior mean performance. The D^2 statistics indicated that reproductive trait age at first calving played an important role than all other traits studied in discriminatory analysis. The contribution of LMY/CI was least among all the traits studied. The IFG, IFJG and R crosses formed one cluster, whereas, FG and FJG formed second cluster with higher intra cluster distance.

Acknowledgement

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RESEARCH ARTICLE

A study on information needs of dairy farmers in hill region of Uttarakhand

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Abstract: Dairy farming is an indispensable economic activity in the rural hill region of Uttarakhand which is closely intervened with farming systems. Despite its relative importance contradicting facts appear in case of milk production and milk productivity in hill region Uttarakhand. The milk production of the state is 1.656 MT which contributes only 1.15 percent to dairy industry of the entire nation. There is huge information gap which is major problem in dairy farming. Moreover, information is said to be appropriate when are based upon local situation meeting their basic dairy needs. Against this backdrop, the present study was conducted to assess the information needs of dairy farmers. The study revealed that majority of the respondents had moderate information needs on fodder production (81.70%), animal health (66.7%) and input & record keeping reported by 66.67 percent respondents. All the respondents 'not needed' the information regarding preparation of milk products of dairy animals. Majority of the respondents (60.83%) had moderate overall information needs. It was further indicated that land holding, herd size, material possession, achievement motivation and scientific orientation were negatively and highly significantly correlated with information needs of dairy farmers

Keywords: Information needs, Dairy farmers, Hill region

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Introduction

Dairy farming is an integral part of agriculture in hill region of Uttarakhand where people have been performing it for their livelihood security. Every household keeps either a cow or a buffalo, irrespective of its economic viability. Dairying is carried out in millions of households across the state, providing employment to the marginal and landless farmers especially. Presently, Uttarakhand is coming up with a vast web of small-scale dairy and milk collection centers. Milch cow and buffalo are reared at all altitudes and they have high potential to develop dairy farming. The other important factors that promote dairy farming in the Uttarakhand Himalaya are vast forest (59.7%), grazing land (3.4%) and ample water (Sati, 2016). These factors make the state potential area for milk production but bigger question arises is why dairy farmers of Uttarakhand state are still contributing only 1.15 percent to dairy industry and the annual average income of livestock owners in the state is ¨ 13,560 (ULDB, 2001) only. At present, many public and private institutions in India are coming up with improved package of practices for dairy development but these improved practices do not reach dairy farmers. There lies a huge information gap which needs to be bridged. Sharma and Verma (2017) found that the farmers had low awareness about various aspects i.e. causes, management, control and preventive measures of abortion in dairy animals. Moreover, Singh et al. (2004) in Almora found that farmers are not aware of recent development in area of animal nutrition, particularly improved utilization of existing feed resources, strategic supplementation of roughage based diets, use of common salt and mineral mixture for improving animal health, production, reproduction, and feeding of colostrum to newly born calves. Dairy farmers lack information on many aspects of improved dairy practices and hence, it remains unprofitable for them. It is said in every sphere of life information is inevitable component which has to be acquired, stored, retrieved, processed and disseminated. Similarly, agriculture information is undoubtly important for agriculture development (Adio et al. 2016). It is observed that the information dissemination system is supply driven instead of being demand driven. The programs planned at the top aren't in tune with the needs of local community (Hedge, 2012). It emphasizes that dairy farmers are not getting right information at right time for taking right decision and reduce

uncertainties related to dairy farming. Simultaneously, information is said to be appropriate when are based upon local situation meeting their basic dairy needs. Hence, transfer of technology can never succeed without knowing the information needs of farmers. The assessment of information needs can only justify the relevance of plenty of information available.

Against this backdrop, the present study was conducted with the following objectives: (1) To assess the information needs of dairy farmers (2) To study relationship between socio-economic and psychological characteristics and information needs of dairy farmers.

Material and Methods

The present study was carried out in Almora district of Uttarakhand. Almora district was purposively selected as locale of the study because from livestock wealth and milk production point of view, it is a well-endowed district with highest milk production and dairy animal population among all hill districts of Uttarakhand. Dwarahat and Tarikhet block were randomly selected through Simple Random Sampling using chit method. Two villages were selected from each selected block through Simple Random Sampling using chit method for accessing information needs of dairy farmers. Total 120 respondents were selected from all the four villages through Probability Proportionate to Size (PPS) sampling. The data was collected with the help ofpre-tested well-structured interview schedule. Frequency, percentage, arithmetic mean, standard deviation, co-efficient of correlation and test of significance were used to analyze the data for meaningful interpretation.

Results and Discussion

Information needs of dairy farmers

Information needs of dairy farmers was assessed in eight key areas on three-point continuum i.e. most essential, essential and least essential with their respective score as 3, 2, and 1. The respondents were asked to indicate any of the three alternative responses against each selected modern practice. These eight key areas were made on the basis of literature review and expert consensus. The areas for taking responses on information need was taken from Gupta and Tripathi (2002) which was further modified based on expert review.

Information needs of dairy farmers related to breeds

The findings regarding to information needs of dairy farmers on breeds (Table 1) revealed that majority of the respondents reported information 'needed' on types of breeds (79.17%), selection of breeds (77.50%), morphology of animal (75.83%), resistance towards abiotic and biotic factors (75.83%) and milk production of animal (76.60%).

Information needs of dairy farmers related to breeding

The findings on information needs related to breeding reveal that all the respondents acknowledged information need on 'breeding of dairy animals' as 'not needed'. The responses were taken on four aspects viz., information on artificial insemination, and time of insemination, pregnancy diagnosis, and identification of heat symptoms. It was observed that none of the farmers lacked information on the mentioned breeding practices for dairying. This may be due the fact that breeding is considered as an important aspect of dairying in villages and so, all the information required for breeding was transferred generations after generations properly.

Information needs of dairy farmers related to feeding

The findings on information needs of dairy farmers related to feeding (Table 2) indicate that majority of the respondents 'needed' information on preparation of balanced ration (61.67%), feeding of newly born calf (85.83%), feeding of mineral mixture (77.50%), feeding of pregnant animals (80.00%) and feeding of sick animals (80.00%). It was found that feeding of newly born calf was area of maximum information need where only 5 percent farmers reported information as 'not needed'.

Information needs of dairy farmers related to fodder production

Fodder production is one the major constraint to dairy farming in hills of Uttarakhand. The findings related to information needs of dairy farmers on fodder production (Table 3) shows that majority of the respondents 'needed' information on improved varieties of fodder seeds and fodder trees (83.30%), time and method of sowing (83.30%), nutrient management of fodder crop (83.30%), irrigation and harvesting (83.30%) and rotation of fodder crops (82.50%). In case of silage preparation, it was reported 70.83 percent respondents acknowledged information as 'most needed' while 29.17 percent reported information as 'needed' indicating large information gap. Also, none of the respondent needed information regarding 'conservation/storage of fodder crops'. It may be due to the fact that they already perform various practices of storing odder crops and also, they have structures for storage of fodder as and when needed.

The results are in consonance with the findings of Sah and Fulzale (1999) who reported medium information needed on 'fodder rotation for round the year cultivation of green fodder'.

Information needs of dairy farmers related to health care practices

Data regarding information needs of dairy farmers on healthcare practices is depicted in Table 4. It was observed that majority of the respondents 'needed' information on common diseases of animals (70.83%), contagious disease and their symptoms (73.33%), vaccination schedule (71. 67%), first aid treatment

(75.00%), deficiency disease of animals and their symptoms (73.33%) whereas information on deworming (81.67%), information about ectoparasites and their control (87.50%) and care of sick animals (70.83%) was acknowledged as 'not needed'. Findings point out that information on all health care practices is either 'needed' or 'not needed' except 'information about common diseases of animals'.

Information needs of dairy farmers related to management practices

The findings regarding information needs of dairy farmers on management practices (Table 5) reveals that majority of the respondents 'not needed' information on housing plan (90.00%), care of newborn calves (95.00%), care at calving (95.00%), dehorning of calves (64.17%), castration (98.33%), clean milk production (85.00%) and milk testing techniques (75.00%). None of the respondents required information regarding weaning.S Findings on management practices point out that none of the respondent 'most needed' information on it. It was the area with least information gap which was observed to be most prevalent among respondents. Majority of the respondents marked different aspect of management practices in 'not needed' category.

Information needs of dairy farmers related to preparation of milk products

It was reported that all the respondents 'not needed' the information regarding 'preparation of milk products' of dairy animals. This may be due to the fact that they already knew about preparation of milk products or they were not interested in commercial production of milk products like ice-cream, sweets, etc.

Information needs of dairy farmers related to input supplies and record keeping

Data on information needs of dairy farmers on input supplies and record keeping (Table 6) reveals that majority of the respondents acknowledged information as 'needed' on information about credit facilities (57.50%), maintenance of records (69.17%). Findings on 'marketing of milk' reveal that none of the

Table 1 Distribution of respondents on the basis of information needs related to breeds (n=120)

S.No.	Components	Most Needed	Needed	Not Needed
1.	Types of breed	3 (2.50%)	95 (79.17%)	22 (18.33%)
2.	Selection of breed	25 (20.83%)	93 (77.50%)	2 (1.67%)
3.	Morphology of animal	27 (22.50%)	91 (75.83%)	2 (1.67%)
4.	Resistance towards abiotic and biotic factors	29 (24.17%)	91 (75.83%)	0 (0%)
5.	Milk Production of animal	28 (23.33%)	92 (76.60%)	0 (0%)

*Figures in parenthesis indicate the percentage

Table 2 Distribution of respondents on the basis of information needs related to feeding (n=120)

S. No.	Component	Most Needed	Needed	Not Needed
1.	Preparation of balanced ration	18 (15.00%)	74 (61.67%)	27 (22.50%)
2.	Feeding of newly born calf	11 (9.10%)	103 (85.83 %)	6 (5.00%)
3.	Feeding of mineral mixture	18 (15.00%)	93 (77.50%)	9 (7.50%)
4.	Feeding of pregnant animals	15 (12.50%)	96 (80.00%)	9 (7.50%)
5.	Feeding of sick animals	15 (12.50%)	96 (80.00%)	9 (7.50%)

*Figures in parenthesis indicate the percentage

Table 3 Distribution of respondents on the basis of information needs related to fodder production (n=120)

S. No.	Component	Most Needed	Needed	Not Needed
1.	Information about improved varieties of fodder seeds and fodder trees	16 (13.33%)	100 (83.30%)	4 (3.33%)
2.	Time and method of sowing	16 (13.33%)	100 (83.30%)	4 (3.33%)
3.	Nutrient management of fodder crop	16 (13.33%)	100 (83.30%)	3 (2.50%)
4.	Irrigation and harvesting	16 (13.33%)	100 (83.30%)	3 (2.50%)
5.	Rotation of fodder crops	18 (15.00%)	99 (82.50%)	3 (2.50%)
6.	Silage preparation	85 (70.83%)	35 (29.17%)	0 (0%)
7.	Conservation/storage of fodder crops	0 (0%)	0 (0%)	120(100%)

*Figures in parenthesis indicate the percentage

Table 4 Distribution of respondents on the basis of information needs related to health care practices (n=120)

S. No.	Component	Most Needed	Needed	Not Needed
1.	Information about common diseases of animals	33 (27.50%)	85 (70.83%)	2 (1.67%)
2.	Deworming	0 (0%)	22 (18.33%)	98 (81.67%)
3.	Information about ectoparasites and their control	0(0%)	51 (4.50%)	105 (87.50%)
4.	Care of sick animals	0 (0%)	35 (29.16%)	85 (70.83%)
5.	Contagious disease and their symptoms	0 (0%)	88 (73.33%)	32 (26.67%)
6.	Vaccination schedule	0 (0%)	86 (71. 67%)	34 (28.33%)
7.	First aid treatment	0 (0%)	90 (75.00%)	30 (25.00%)
8.	Deficiency disease of animals and their symptoms	0 (0%)	88 (73.33%)	32 (26.67%)

*Figures in parenthesis indicate the percentage

Table 5: Distribution of respondents on the basis of Information needs related to management practices (n=120)

S. No.	Component	Most Needed	Needed	Not Needed
1.	Housing plan	0 (0%)	12 (10.00%)	108 (90.00%)
2.	Care at calving	0 (0%)	6(5.00%)	114 (95.00%)
3.	Care of newborn calves	0 (0%)	6(5.00%)	114 (95.00%)
4.	Dehorning of calves	0 (0%)	43 (35.83%)	77 (64.17%)
5.	Castration	0 (0%)	2 (1. 67%)	118 (98.33%)
6.	Weaning	0 (0%)	0 (0%)	120 (100%)
7.	Clean milk production	0 (0%)	18 (15.00%)	102 (85.00%)
8.	Milk testing techniques	0 (0%)	30 (25.00%)	90 (75.00%)

*Figures in parenthesis indicate the percentage

Table 6 Distribution of respondents on the basis of information needs related to dairy farming related to input supplies and record keeping (n=120)

S. No.	Component	Most Needed	Needed	Not Needed
1.	Information about credit facilities	34 (28.33%)	69 (57.50%)	17 (14.17%)
2.	Maintenance of records	0 (0%)	83 (69.17%)	37 (30.83%)
3.	Marketing of milk	0 (0%)	0 (0%)	120 (100%)
4.	Information about government schemes and subsidies	90 (75.00%)	24 (20.00%)	6 (5.00%)

*Figures in parenthesis indicate the percentage

respondent lack information about it. It emphasizes that respondents are well aware of market for disposing of milk. This may be due the fact that milk cooperative was well functioning in the village. The findings on information needs regarding 'government schemes and subsidies' reveal that majority of the respondents (75.00%) 'most needed' information pointing out towards existing information gap on government scheme and subsidies.

Information regarding overall information needs

Findings on overall information needs indicates majority of the respondents (66.67%) had moderate overall information needs while 20.83 percent respondents had low information need followed by 12.50 percent respondents had high information need regarding dairy farming.

Relationship between independent variables and information needs of the dairy farmers

Data regarding relationship between independent variables and information needs of dairy reveals that land holding, herd size, material possession, achievement motivation and scientific orientation were negatively and highly significantly correlated with information needs of dairy farmers. It indicates that farmers having low land holding and small herd size had high need for information whereas farmers having large land holding and big herd size already had much information related to improve dairy farming. This may be due to the fact that farmers having small land holding never grew fodder in field due to space and so they need information in fodder production. Similarly, it was found that farmer having high material possession required less information related to dairy farming whereas farmers having low material possession required high information need. Moreover, lower achievement motivation of farmer towards dairy farming indicated higher information need and lower scientific orientation also indicated towards higher information need of farmer. This point out that information gaps is more for farmers having low achievement motivation.

The results find consonance with the findings of Devaki and Senthilkumar (2013) who reported that herd size had negative and significant relationship with information needs of dairy farmers. Similar results were found by Singh and Sharma (2016) who found significant relationship between information needs of dairy farmers and their herd size and age.

Conclusions

The study concludes that farmers needed moderate information on all the aspect of improved dairy practices. Dairy farmers in hills well acquainted with conservation/storage of fodder crops and deworming but they needed information on about preparation of silage, resistance of animals towards abiotic and biotic factors and milk production of different breeds. Furthermore, management practice is the area with least information gap.

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SHORT COMMUNICATION

Effectiveness of Public Private Partnership model of dairy farming in Haryana

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Abstract: A study was undertaken to assess effectiveness of Public Private Partnership (PPP) model in dairy farming in three districts representing three different agro climatic zones of Haryana namely Kaithal, Hissar and Mahendragarh during 2017-18. Total 225 respondents were selected out of which 45 were private functionaries, 30 public functionaries and 150 were farmers. Interview schedule was used to elicit the response from them. Ex-post facto research design was used for study. An effectiveness index was developed in order to assess the effectiveness of PPP model in dairy farming. Study revealed that Effectiveness index score of farmers about utility and access to technical services was highest (0.62) which shows that farmers are able to access the technical services in dairying effectively through PPP approach although the main role of Gopal (private extension functionary) was focused on delivering AI services. Farmer's effectiveness index score on profit maximization was 0.60 which show that farmers considered PPP to be effective in realizing maximum profit. Effectiveness index score of transparency was very low (0.48) which indicate that farmers expectation on transparency in service delivery was still very high. The findings suggest the further improvement of effectiveness of PPP model through focus on capacity building, better transport facility for Gopal, effective monitoring by public extension functionaries, periodic coordination meeting and village level veterinary camp.

Keywords: Effectiveness index, Field Extension functionaries, Public Private Partnership

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India is primarily an agrarian country wherein crop and dairying are the major farm enterprises. The dietary habit related to milk and milk products, endowed with diverse culture and festivals provide enormous opportunities for horizontal growth concerning 70 million farm families in the country. Although India has highest livestock population of 512.06 million (19th livestock census, 2012) in the world, milk productivity is abysmally low. In order to boost the milk productivity, Government has spearheaded many schemes and programmes but many of them could not ensure the desired level of performance. This could probably due to poor adoption of good management practices, difficulty in accessing appropriate technologies and constraints in extension delivery system (Hegde, 2012) at farm level, apart from developments in other sectors, changing priorities and emerging trends at national and global level (Ponnusamy and Pachaiyappan, 2018). Moreover, currently, dairy sector is facing high input costs for milk production, lack of infrastructure for handling, transport, processing and marketing. The demand for milk in India is projected to increase to 191.30 MT in 2020. It is essential to bring new insights in the extension programmes to motivate the farmers by ascertaining the push and pull factors of the impact on the ongoing and just completed development programmes in the dairy sector. Currently, Public Private Partnership is one of the best experimented strategies to achieve the specified goals within the time frame and modernize public services and infrastructure in agriculture/dairying, health, science and technology, education, infrastructure development and extension (Ponnusamy, 2013).PPP can be understood as collaborative effort between public and private sector in which each sector contributes to planning, resources, knowledge and capacities needed to accomplish mutual objectives. So it is necessary to evaluate the effectiveness of PPP model in dairy farming to assess the extent of effectiveness of PPP model which could fulfill the desired expectation. Hence by keeping all these in mind, present study was undertaken to assess the effectiveness of PPP model in dairy farming.

The present study was undertaken in Kaithal, Hissar and Mahendragarh districts which were selected to represent three different Agro-climatic zones of Haryana, where a PPP model is operating and providing service in the field of dairying during 2017-18. The study aimed at assessing effectiveness of PPP model

running in Haryana by collaborative effort of JK Trust which runs Gram VikasYojna and Haryana government. Interview schedule was used for collecting the primary data. The ex-post facto research design was followed for the study with 225 respondents, comprising 30 respondents from public extension functionaries, 45 respondents from private extension functionaries and 150 beneficiaries who were selected through proportionate random sampling to represent real population of study. An effectiveness index was developed to assess effectiveness of PPP model. The PPP effectiveness has multidimensional aspects. Effectiveness Index was developed by incorporating 32 indicators under dimensions related to PPP model i.e Utility and access to technical services, Access to Advisory services, Access to market, Cost reduction, Profit maximization, Transparency, Generation of employment and Risk reduction based on opinion of experts from the field of social science. Therefore, it is important to select indicators, which could represent the intended dimensions of the study. The availability of appropriate literature and through discussion with experts in relevant field played an important role in the identification of these indicators. It was decided to give specific weights (Scale Values) to each dimension of the effectiveness Index based on their perceived significance. The Normalized Rank Order Method suggested by *Guilford (1954)* was used for determining the scale values. As per the method, eight different indicators of effectiveness index were ranked by the group of judges according to their perceived significance in determining the effectiveness about PPP. Ranking was obtained from judges who are experts in the field of social science. For making indicator scale free following methods were applied:

$$U_{ij} = \frac{Xij - Min\ Xij}{Max\ Xij - Min\ Xij} \quad (1)$$

$$Uij = \frac{Min\ Xij - Xij}{Max\ Xij - Min\ Xij} \quad (2)$$

Where

i=1, 2, 3……n indicators

j= 1, 2,3……n dimensions of PPP model related to dairy

Xij= value of i^{th} indicator of j^{th} dimension

Equation (1) was applicable to indicators which was positively related to effectiveness of PPP and equation (2) was applicable to indicators which was negatively related to effectiveness of PPP.

The relevancy weightage (RW) and mean relevancy score (MRS) were calculated. The indicators with the statement having RW> 0.70 and MRS> 2.25 were considered for incorporating into effectiveness index. Effectiveness index was developed and it was found that score of dimensions ranged from 0 to 1. The responses of farmers were recorded on this effectiveness index.

The effectiveness of PPP in dairying was evaluated under following dimensions and depicted in Table 1.

Public private partnership is very helpful in providing utility and access to technical services. The details are depicted in table 1. Effectiveness index score of farmers about utility and access to technical services was highest (0.62) which shows that farmers are able to access the technical services in dairying effectively through PPP approach although the main role of Gopal was focused on delivering AI services. On the basis result obtained from analysis of data this can be further thought that instead of evaluating effectiveness of PPP in terms of AI services, the better option can be evaluating PPP in terms of number of calves born.

Farmers need advisory support like guidance of new technologies, new schemes and programmes to run their farm with maximum profit and minimum risk. The effectiveness index score of (0.53) shows that farmers had considered PPP as less effective in providing advisory services about various dairy related practices. When Gopal visit the client's farm he tends to provide advisory services when farmers seek the same. This can help Gopal to get more trust worthiness about their service delivery.

Effectiveness index score of access to market was (0.57) which shows that farmers considered PPP as moderately effective in providing access to market and various market related information like price of the products, customer availability and future scope for milk and milk products. Higher milk productivity and healthy calves production would enable farmers to get remunerative market.

Farmers did not consider PPP as much effective in reduction of cost in dairy farming. Effectiveness index score of farmers about cost reduction was 0.58 which indicates that PPP is moderately effective in reducing the cost as main role of Gopal remains in providing doorstep AI services. The cost reduction is mostly possible based on the interventions in nutrition and labour managementof dairy farming.

Farmers are having very limited resources and low level of input usage. So they need those technologies which can help them in maximizing the profit with least investment cost. Farmers' effectiveness index score on profit maximization was 0.60 which show that farmers considered PPP to be effective in profit maximization (Ponnusamy*et al*, 2017). However cost reduction and productivity enhancement are closely related to realize higher profit margin in dairy farming.

The lay inseminators often were reported to cheat the farmers especially with respect to semen quality. Therefore transparency in service delivery is very much essential. Effectiveness index

Table 1 Distribution of farmers as per scores obtained on the effectiveness Index

S. No.	Major indicators	Effectiveness Index score
1.	Utility and access to technical services	0.62
2.	Access to advisory services	0.53
3.	Access to market	0.57
4.	Cost reduction	0.58
5.	Profit maximization	0.60
6.	Transparency	0.48
7.	Generation of employment	0.51
8.	Risk reduction	0.54

Table2 Distribution of farmers as per the scores obtained on the effectiveness index for PPP representing the level of effectiveness

	Effectiveness about PPP among farmers (n=150)		
S. No.	Category	Frequency	Percentage
1	Low (<0.45)	37	24.66
2	Medium (0.45-0.60)	83	55.34
3	High (>0.60)	30	20.00

score of transparency was very low (0.48) which indicate that farmers expectation on transparency in service delivery is still very high**.** The monitoring mechanism in the PPP model should take care of building transparency aspects.

Effectiveness index score of generation of employment was 0.51 which indicate that farmers considered PPP to be less effective in providing employment to people if we consider overall employability but in term of educating unemployed youth as Gopal employability is effective. However, there was not much scope to generate more employment among farmers on the basis of this PPP approach.

Effectiveness index score of risk reduction was 0.54 which indicate that farmers do not consider PPP as very much effective in risk reduction in dairy farming, although it can able to reduce the risk to some extent due to sharing nature of PPP model. Compulsory insurance, diversification in composition of animals and farm enterprises and assured market price can reduce risk considerably.

Majority of the farmers (55.34%) were having medium level of effectiveness of PPP according to the effectiveness index, while 24.66 per cent belong to low level of effectiveness. This indicates that there is considerable scope to strengthen this PPP model in dairying as this will ultimately address the skilled manpower shortage in the animal husbandry sector. The major focus should be on capacity building of private extension functionaries on scientific dairy management practices, period coordination meeting between public and private players and incentives for better performance of private players which will further motivate them and enhance the productivity.

Conclusions

Main purpose of this study was to assess the effectiveness of PPP model in dairy farming. The findings indicated that the PPP model has inherent weakness especially the aspects related to the job security of private extension functionaries, accountability to the assigned tasks and lack of adequate mechanism to monitor the PPP activities at different levels. Moreover, their services are limited to only AI services and pregnancy diagnosis. So there is a tremendous scope for improving the effectiveness of extension delivery through PPP approach which needs further focus on capacity building, better transport facility, effective monitoring, periodic coordination meeting and village level veterinary camp.

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SHORT COMMUNICATION

A longitudinal study on the impact of species and age of animals on milk production in dairy animals

JK Chaudhary[1], MR Verma[2] and Shashank Kshandakar[3]

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Abstract: A longitudinal study was conducted to assess the impact of species and age of animals on milk production in dairy animals. The longitudinal data on milk yield of cattle and buffaloes have been recorded fortnightly interval up to 300 days for the period 2005 to 2014 from the dairy farm of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh. From the study, we found that the base line milk yield was significantly (p value<.0001) higher in cattle than buffaloes. The change in milk yield decreases with time and this decrement was more in buffaloes than cattle. After adjusting for baseline age, on average cattle baseline milk yield was about 1.05 units more than buffaloes and after adjusting for species, one year increase in age corresponds to 0.102 unit increases in baseline milk yield. Cattle milk yield change rate was about 0.0009 units greater than buffaloes after adjusting for baseline age and one year increase in age corresponds to 0.0012 units less in milk yield change rate after adjusting for species.

Keywords: Generalized estimating equations, Linear mixed model, Model, Random effect model, Significant

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The age of the lactating animal has important effect on the productive capacity of the animal. The effects of age differ from species to species and breed to breed. As the age of the animal increases, its body functions such as physiological and metabolic activities also increase up to a certain age (mature age) and then there is decline in these functions. For example, in terms of milk production, the productive capacity of the animal increases until body maturity is reached, and there after it decreases with advancing age. Age of the animal is an important factor which affects the mammary gland activity and rumen functions, which in turn affect the yield and composition of milk. For better understanding of the impact of species and age of animals on milk production in dairy animals, this work has been planned.

In the present study, the longitudinal data on milk yield (continuous data) of cattle and buffaloes have been recorded fortnightly interval up to 300 days for the period 2005 to 2014 from the dairy farm of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh. A total of 180 dairy animals (Cattle and Buffaloes) have been recorded for the study.

The analysis of continuous longitudinal data has been undertaken by using two stage model, linear mixed model and generalized estimating equations (GEE) models (Edwards, 1985 and Diggle *et.al.,*1994). The models were applied in the analysis of continuous longitudinal data on milk yield. The two stage model was fitted by using Proc t test and Proc reg. Linear mixed model and Generalized Estimating Equations (GEE) was performed by using the Proc mixed procedures in SAS 9.3 (Zeger et al. 1988). The parameters of interest were species and age of the animal.

The model structure were

a) Two stage model: We examined the effect of age and species on intercept (b_0) by using the following model

$$\hat{b}_0 = \alpha_0 + \alpha_1 species + \alpha_2 age + \varepsilon_0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.1)$$

and the effect of species and age on slope (b_1) by using the following model

$$\hat{b}_1 = \beta_0 + \beta_1 species + \beta_2 age + \varepsilon_{1.} \ldots\ldots\ldots\ldots\ldots\ldots (1.2)$$

Model for true baseline response $\mathbf{b_0}$: The model for species and age for base line response is given below:

$$\hat{b}_0 = \alpha_0 + \alpha_1 species + \alpha_2 age + \varepsilon_0 \ldots\ldots\ldots\ldots\ldots\ldots (1.3)$$

Model for change rate of the true baseline response $\mathbf{b_1}$: The model for species and age for change rate of true baseline response is given below:

$$\hat{b}_1 = \beta_0 + \beta_1 species + \beta_2 age + \varepsilon_{1.} \ldots\ldots\ldots\ldots\ldots\ldots (1.4)$$

b) Linear mixed model: A linear mixed model is an extension of a linear regression model to model longitudinal (correlated) data.

$$y = \alpha + x\beta + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Change in the milk yield associated with species and age

$$b_{i0} = \beta_0 + species_i \beta_{0,species} + age_i \beta_{0age} + a_{i0} \ldots\ldots (2.2)$$

$$b_{i1} = \beta_1 + species_i \beta_{1,species} + age_i \beta_{1,age} + a_{i1} \ldots\ldots (2.3)$$

The $\beta_0 species$, $\beta_0 age$ are the species effect and the age effect on the baseline milk yield level. Similarly $\beta_1 species$, $\beta_1 age$ are the species effect and age effect on the change rate of the true milk yield level.

Substituting the above expression into model

$$y_{ij} = b_{i0} + b_{i1} t_{ij} + \varepsilon_{ij} \ldots\ldots\ldots\ldots\ldots\ldots (2.4)$$

We got

$$y_{ij} = \beta_0 + species_i \beta_{0,species} + age_i \beta_{0,age} + \beta_1 t_{ij} + species_i t_{ij} \beta_{1,species} + age_i \beta_1 t_{ij,age} + a_{i0} + a_{i1} t_{ij} + \varepsilon_{ij} \quad (2.5)$$

Where y_{ij} is the j^{th} milk yield level measurement from subject i , t_{ij} is time from the beginning of the study (or baseline) and b_{i0} and b_{i1} are random variables. ε_{ij} are independent errors distributed as $N(0, \sigma_\varepsilon{}^2)$.

Generalized Estimating Equations (GEE) **:** When the variation pattern in data is so high that we cannot use the random effect model then in that case we can use the model to estimate the fixed effects $(\beta' s)$ and use GEE approach to calculate the standard errors for the fixed effect estimates. These SE estimates will be valid regardless of the validity of the random effects structure. So these SE estimates are robust.

$$y_{ij} = \beta_0 + \beta_1 t_{ij} + \beta_2, {}_{species_i} + \beta_{3,age_i} + \beta_{4,speciesi} t_{ij} + \beta_{5,agei} t_{ij} + b_{i0} + b_{i1} t_{ij} + \varepsilon_{ij}$$

……...(3.1)

The estimated value of $\hat{E}(\hat{b}_0)$ for cattle was 8.8910 and for buffaloes 7.9290 means the base line milk yield was more in cattle than buffaloes, and this difference was highly significant (p value<.0001). The estimated value of $\hat{E}(\hat{b}_1)$ for cattle was -0.0131 and for buffaloes -0.0133 means the change in milk yield decreases with time and this decrement was more in buffaloes than cattle. There is no significant difference (p value =0.6302) between cattle and buffaloes with respect to change rate in milk yield with time. The estimated value of variance ($S^2_{\hat{b}_0}$) for baseline milk yield was 16.926 for cattle which was more than variance of buffaloes (3.862) and there was highly significant difference (p value<0.0001) between the variance of cattle and buffaloes. The estimated value of variance $S^2_{\hat{b}_1}$ for change rate in milk yield with time was 0 .00017 for cattle which was more than variance of buffaloes (0.0001) and there was highly significant difference (p value<0.0001) between the variance of cattle and buffaloes. The estimated value of intercept $\hat{\alpha}_0$ for this model was 9.4303 with standard error 0.1952. The estimated value of $\hat{\alpha}_1$ for species variable was -1.0566 with standard error 0.1237 that indicates that after adjusting for baseline age, on average cattle

Table 1 Comparison of fit statistics among models

Model	AIC	BIC
Model (2.1)	17053.8	17066.6
Model (2.5)	17054.4	17067.2
Model (3.1)	17054.4	17067.2

baseline milk yield was about 1.05 units more than buffaloes. The estimated value of $\hat{\alpha}_2$ for age variable was 0.1023 with standard error 0.0221 that indicates that after adjusting for species, one year increase in age corresponds to 0.102 unit increases in baseline milk yield. The estimated value of intercept $\hat{\beta}_0$ for this model was -0.0078 with standard error 0.0006. The estimated value of $\hat{\beta}_1$ for species variable was 0.0009 with standard error 0.0004 that indicated that after adjusting for baseline age, cattle milk yield change rate is about 0.0009 units greater than buffaloes. The estimated value of $\hat{\beta}_2$ for age variable was -0.0012 with standard error 0.00007 that indicated that after adjusting for species, one year increase in age corresponds to 0.0012 units less in milk yield change rate.

Tekerli et al. (2000) reported that the cattle in 1st lactation produced lower milk yield but they were more persistence in milk production with respect to older parity animals. Madani et al.(2011) reported that milk yield in early calving group produced 20% and 24% less milk yield during second lactation as compared respectively to medium and late maturing group. Tekerli et al. (2000) concluded that primiparous cow produce lower milk yield but they are more persistence in milk production with respect to older parity animals. Rekik et al. (2003) concluded that the ascending phase of lactation is not affected by parity and calving season while decreasing phase of lactation curve affected by parity and calving season; Persistency of milk production and peak yield varies significantly with variable which affects milk production; In contrast to 1st lactation, 3rd lactation had the highest peak milk yield & total milk yield. Kumar et al. (2012) concluded that genetic group and lactation order had significant effect on lactation length, total milk yield and peak yield.

The choice of model, especially the fixed terms, depends on objective of the study. However, we can use Akaike information criterion (AIC) or Bayesian information criterion (BIC) to determine the random effects and the error structure**.** If we want a model with the most prediction power, we can consider a complicated model with AIC or BIC as a guide for model selection. Here it seems that model (3.1) is the winner among different models if we are looking for a model with the most prediction power (Table 1)

Conclusions

The present longitudinal study generated information about the impact of species and age of animals on milk production in dairy animals. The analysis involved determining the change in milk yield with time in dairy animals and to assess the milk yield change with respect to species and age of animal. The base line milk yield was more in cattle than buffaloes, and this difference was highly significant (p value<.0001). The change in milk yield decreases with time and this decrement was more in buffaloes than cattle.

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