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INVITED REVIEW

Utilization of whey in bakery products-A review

S Paul, S Kulkarni and RN Chauhan

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Abstract: Whey is a vast repository of valuable nutrients and serves as a key ingredient in the bakery sector. Whey being a significant by product of the dairy industry is necessary to be utilized to reduce the burden of its disposal on the environment, which is attributed to the high BOD of whey. Whey based ingredients find an ocean of application in foods. Whey based ingredients have superior functional properties attributable to whey proteins which results in enhancement of textural and chemical properties of products they are applied in. Whey proteins are used as fat replacers, emulsifiers, flavour and appearance enhancers etc in a wide category of bakery products. Whey based ingredients can negate the use of dough conditioners due to high water binding capacity which aids in developing improved texture and fine even crumb. Whey ingredients can boost levels of protein, calcium, potassium and other minerals in bakery products. With increasing demand for clean label products and low fat products with the same textural attributes as full fat ones the demand for whey ingredients is surging. In this article we review the applications of whey based ingredients in bakery products.

Keywords: Bakery; Dough; Functional; Texture; Whey

Introduction

Whey is a well known ingredient in the bakery industry for a long time because of its flavour enhancing and tenderizing qualities. Incorporation of whey ingredients have been attempted in a wide range of bakery products. The inclusion of whey solids in bakery recipes in the form of liquid whey concentrates, whey powder, lactose, whey protein concentrate and isolated whey proteins enhances the quality and acceptability of baked products by adding to their functionality and nutritive value.

Southern Regional Station, ICAR-National Dairy Research Institute, Adugodi, Bangalore- 560 030, India

S Paul(✉)
Southern Regional Station, ICAR-National Dairy Research Institute, Adugodi, Bangalore- 560 030, India
E-mail: sayantanp63@gmail.com

Whey: Production and properties

Whey is produced as a side stream from cheese or casein manufacture and can be concentrated to produce various whey protein ingredients. Whey protein concentrates (WPCs) can contain 35–80% protein and whey protein isolate contains more than 90% protein. Whey proteins are globular and exist as discrete molecules with varying numbers of disulphide crosslinks. They are very heat sensitive and engage in thiol disulphide Interchange. (Gallagher 2005)

Incorporation of whey in bakery products

Whey solids when incorporated into bakery products offer the following benefits:

- Enhances sensory attributes
- Improves nutritional profile with complete and bioavailable amino acids
- Contributes high calcium content: 100 grams of dry sweet whey contains about 770 mg of calcium and 100 grams of dry acid whey contains about 2,280 mg of calcium
- Contributes to a healthful image and clean label
- Whey protein concentrate (WPC) aids in the dispersion of shortening, which can reduce the shortening level in some formulas or increase the effectiveness of shortening in others
- WPC provides structure in bakery products through the formation of heat-set irreversible gels
- Enhances water binding in dough, which can also improve machinability

• Improves moisture retention of a finished product, which enhances consumers' perception of freshness

• Improves stable foaming and whipping for products such as angel food cake and meringues

• Can replace egg albumin in many products, such as yellow and sponge cakes, which may reduce costs, enhance microbiological safety and improve health image of product

• Lowers fat absorption in many fried products

• Contributes to browning and crust development

• WPC can be used as a prebake glaze on pastries and biscuits to produce excellent color and gloss in bakery products

• Delivers a bland, slightly sweet flavor that enables other flavors, such as chocolate and various spices, to develop to their full potential

• In sourdough breads, the distinctive flavor of acid whey can enhance the flavor contributed by the fermentation

Whey in the form of whole whey or concentrated or dried form has been widely used in the manufacture of several bakery products like varieties of bread, biscuits, cakes etc.

Whey and whey products serve both as protein supplements and baking aids in baked products (Sienkiewicz and Riedel, 1990). The use of whey increases the dietetic value of baked products by introducing calcium, thiamin and riboflavin. Both whey and whey powder exert, moreover, a positive influence on the important properties of baked products such as - increase in product volume, increase in yield, improvement in crumb consistency, structure (texture, porosity), appearance (browning), fresh-keeping quality and the flavor. Their use also leads to a reduction in the quantity of flour which is necessary for the baking process. In baked goods lactose is extensively used for promoting browning reactions without being fermented by baker's yeast (Zall 1992). Owing to the high percentage of lactose, whey solids can be substituted for dextrose and sucrose in many baking formulae (Alesch, 1958).

Three principal methods of application of whey in different products have been identified:

-partial or full replacement of water used for the particular recipe,

-partial or full replacement of the fat-free milk dry matter used,

-replacement of eggs by whey protein.

(Bunnies and Timm, 1983)

Utilization of whey in biscuits and cakes

Dried form

A protein rich biscuit with a keeping quality of as long as one year and readily acceptable to children in South East Asia and New Zealand was reported by Chapman (1967). This biscuit was made from dried skim milk (46 percent), anhydrous butter fat (15 percent), cheese (15 percent), whey powder (10 percent), sucrose (10.5 percent) and water (3.5 percent), minerals, vitamins and flavoring substances were added before molding and vacuum drying.

Concentrated form

Concentrated whey with 40 percent total solids was used at 2.5 and 10 percent levels, in manufacture of "Banan" a spiced biscuit with superior porosity, softness and good storage qualities (Shilovskaya, 1974). Concentrated whey was successfully used in the preparation of rusks and soup sticks (Malik and Kulkarni, 2009). Poonam(2007) reported that concentrated whey can be successfully used in the production of buns with increased Protein Efficiency Ratio(PER).

Demineralized and delactosed form

Ash and Colmey (1976) reported that bakery products like cakes, biscuits, muffins, pan-cakes, doughnuts and white bread can be fortified with partly demineralised and delactosed whey containing 32 percent lactose (by wt.) 54 percent protein, 10 percent minerals, 4 percent moisture and only trace amounts of fat to supplement dried skim milks.

Egg substitute

In a review study conducted by Cocup and Sanderson (1987) it was found that whey protein concentrates with a protein content of 35 – 55% have found application in replacing whole egg as prebake glazes on biscuits and pastry. It was concluded that whey can be processed to promote properties with application in the shelf life extension of the baked products by retarding mold spoilage and replacing chemical additives.

Arunepanlop et al. (1996) conducted a study to investigate the effects of partial replacement of egg white proteins (EWP) with whey protein isolate (WPI) on the appearance, structure, texture and sensory properties of angel food cakes. EWP replacement cakes were generally inferior to 100% EWP control cakes, whereas EWP replacement cakes with xanthan gum as additive were similar to 100% EWP control cakes with respect to appearance, texture and sensory properties. EWP replacement cakes with methyl cellulose exhibited air cell size distribution that was similar to that of control cakes.

Puranik and Gupta (2017) developed a technology for the manufacture of egg less cake mixes using whey protein concentrate, in lieu of egg in cake formulation .It was observed

that the cakes had excellent physical (porosity and volume) and sensory characteristics.

Sugar substitute

Sviridenko and Smurygin (1990) studied the use of hydrolysed whey concentrates, in syrup form, as substitutes for sugar and other sweeteners in biscuits and cakes. These syrups are considered to be effective sugar substitutes with respect to their functional properties (sweetness, solubility and ability to be fermented by microorganisms) and are cheaper than beet sugar.

Liquid whey

In a study conducted by Sharma and Khetarpaul (1996) rice and dehulled bengal gram flour were mixed in ratios of 60:40, 70:30 and 80:20 (w/w). 100 g of the blend was then mixed with whey (105 ml) and fermented at 35°C for 18 h. The fermented slurries were used for the manufacture of biscuits and were evaluated for organoleptic characteristics. Biscuits with the fermented 70:30 blend were the most acceptable. As a result of incorporation of the fermented blend, the contents of antinutrients, such as phytic acid and polyphenols, were considerably reduced. Awasthi and Yadav (2004) studied the effect of incorporation of liquid dairy by products (Chhana whey and Skimmilk) on the chemical characteristics and then on sensory characteristics of soy fortified biscuits and reported that both skimmilk and whey increased the moisture, ash and crude fibre content of biscuits fortified with defatted soy flour. It also increased calcium, iron, phosphorus, sugar content and non enzymatic browning of soy fortified biscuits.

Whey protein concentrate

Because of high functionality of whey proteins, WPC has been used in the production of functional bakery products like high protein products and fat replaced products (Kamaliya and Subhash, 2005).

Mathur (1979) reported that WPC can also Substitute egg white for the manufacture of meringues and macaroons. Arunepanlop *et al*. (1996) reported that whey protein isolate can replace up to 25% of egg white protein without adversely affecting physical and sensory properties of angel food cakes.

In a study conducted by Narender (2007) high protein biscuits were developed by incorporating WPC up to 30% was well accepted because of improved sensory attributes. The improved color in the product was attributed to the maillard reaction due to the interaction of the lactose and the proteins in WPC. The biscuits were found to have three times more protein content. High protein cake developed by incorporating WPC up to 30 % showed improved sensory characteristics and functional properties.

In a study bakery products like milk cake were prepared by incorporating different levels i.e., 15, 20 and 25% of WPC gels and these products were subjected for sensory evaluation by panel of judges. The cake prepared by incorporating 20 % WPC gels had higher score than the control with respect to colour and appearance, flavour and mouthfeel. The product also had better scores for colour of crumb and grain formation thus indicating that the added WPC gel improved the sensory attributes for better acceptability.

Conforti and Lupano (2004) studied the functional properties of biscuits with WPC and honey. The presence of WPC with high protein content produced a decrease in the firmness and consistency and a increase in the cohesiveness of dough. Also the fracture stress of biscuits decreased with the incorporation of WPC. Gallaghar *et al*. (2005) investigated the effects of WPC and sodium caseinate on short dough biscuit formulation. Both protein powders increased the hardness of biscuits and hardness increased as the level of protein powder was increased. WPC resulted in biscuit shrinkage during baking, increased surface browning and also higher moisture and water activity reported during storage period of 24 hours and 8 weeks.

Whey protein concentrate and skim milk powder in combination was added as egg substitute in muffins and it resulted in lower calorific value of muffins apart from lower water activity and higher specific volume than the control which had egg protein. (Singh, 2017)

Utilization of whey in bread

Bread is the food produced by baking a dough obtained by mixing wheat flour, salt, and potable water, leavened by specific microorganisms of bread fermentation such as *Saccharomyces cerevisiae* (Collado, 2003).

There are many types of bread depending upon local demand like whole flour bread, brown bread, bread fortified with vitamins and minerals, milk bread and bread for diabetics (Rao, 2005). Bread also differs from one country to another such as rolls, pan bread, pita bread, French bread, toast bread, baguette, etc. (Collado, 2003). Since classical times, bread has continued to play an important role in the human diet. It is an important stable source of nutrients and energy and a source of complex carbohydrates. The major constituents of some wheaten bread have been illustrated in the Table 1. (Southgate, 2003)

The principal attributes of bread are - loaf volume, crumb softness, grain uniformity, silkiness of texture, crust color, flavor and aroma, softness retention and nutritive value (Collado, 2003).

Bread is a highly perishable product and has a shelf life of 3-5 days at room temperature, 1-2 weeks at refrigeration temperature and 3 months at freezer temperature. The three most common forms of bread deterioration are staling, moisture loss and

microbial spoilage (Seiler, 1984). The reason why molds are important spoilage organisms in bread is that the food matrix has a relatively high moisture content and water activity (water activity= 0.94-0.97) with a pH of about 6. The bread most prone to spoilage by molds is sliced, prepacked and wrapped bread (Seiler, 1984).

Liquid whey – fermented or unfermented – concentrated or unconcentrated - or whey solids or whey proteins could be used as one of the ingredients for bread manufacture for effective utilization of whey. Whey-based ingredients in bread:

• Enhance crust browning

• Improve toasting qualities

• Enhance crumb structure (provide a fine, even crumb without additional dough conditioners)

• Have the potential to slow staling of bread, thus increasing shelf-life

• Enhance bread flavor

The following parameters provide a guideline for selecting a whey-based ingredient for application in a bread formulation:

• In order to optimize loaf volume,the whey-based ingredient shouldbe low in lactose, high in protein and the protein should be significantly denatured.

• Optimum usage levels vary, but 2-3% is a good starting point to obtain maximum benefits.

• Water absorption is lower for whey ingredients than for flour, with water absorption increasing as protein denaturation levels increase; therefore water requirements may need to be adjusted depending on the whey ingredient used.

• Time required to mix dough to maximum consistency (resistance) may increase (mixogram time to peak).

• If the whey-based ingredient is high in lactose, adjustments in the process or other ingredients may be needed to maintain yeast growth and carbon dioxide production.

• Baking time and temperatures may require adjustment because crust color might develop more rapidly with whey-based ingredients.

Whole whey

Whole whey was utilized in liquid form successfully as a replacement of water (Czerwinski et al. 1974). Fermented curd whey for usage as additive in bread manufacture was developed by Skudra et al. (1998). Curd whey fermented by using pure cultures of *L. bulgaricus* and *L. acidophilus* was can be used as an additive in bread making to prevent the spoilage of wheat bread by *Bacillus mesenthericus* (Skudra et al. 1998). Whey fermented along with milk and wheat flour was prepared by Gelinas et al. (1995) for use in pan bread formulation.

Concentrated form

Acidified and concentrated rennet whey (45 – 60% TS) was successfully used up to 5% level for production of bread of acceptable quality (Chramcov, 1977). Precipitated whey protein (PWP) upto 75% was successfully used in the formulation of Iranian Lavash flat bread. The increased supplementation of PWP resulted in increase in sensory scores of the samples (Jooyandeh, 2009). Divya (2010) reported that concentrated whey had a considerable positive impact on the loaf volume. Jayalakshmi (2011) reported that even lactose hydrolysed whey had an influence in raising the loaf volume.

Dried form

Whey in dried form along with other nutritive ingredients was utilized in production of protein enriched bread or buns for feeding elderly persons (Vukobratovic and Beleslin, 1991). Powdered whey was also utilised along with wheat germ etc. to prepare enriched bread (Vukobratovic and Beleslin, 1991). A preconcentrate of cottage cheese whey containing protein as high as 80 – 90 % prepared by UF vacuum evaporation and spray drying was developed for fortification of bread (Anon, 1975). Use of whey protein along with wheat, soya and yeast proteins was also reported by Titcomb and Juers (1976). Riemsdijk et al. (2011) reported the use of mesoscopically structured whey protein for the preparation of gluten free bread. Whey protein particles were prepared during phase separation by cold gelation of soluble whey protein aggregates. The addition of 2.4% whey protein particle suspension to wheat starch resulted in a dough that could be baked into a leavened bread with specific volume upto 3.7 mg/l and a bubble size comparable with a normal bread. Ultra-

Table1. Major constituents of wheat breads (per 100 g)

Bread type	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Dietary fiber (g)	Energy (kJ)	Country of origin
Whole meal	38.3	9.2	2.5	41.6	5.8	914	UK
Multigrain Bread	33	13	4	43	7	1005	Egypt
Brown	39.5	8.5	2	44.3	3.5	927	UK
White	40.3	9.5	2	41.5	3.3	899	UK

filtered cheese whey powder was reported to have the best effect on the sensory quality of Taftoon bread (Rostami, 2013).

Paste form

Pashchenko et al. (2003) described a technological process for manufacturing a whey paste which contained on DM basis protein 10%, lactose 56 – 65%, salts 5.7 – 6% and water 30 – 35%. This paste was suitable not only for use in bread making but also in the manufacture of other bakery products.

Demineralized and delactosed form

Ash and Colmey (1976) reported that bakery products such as white bread can be fortified with partly demineralised and delactosed whey containing 32% lactose, 54% protein, 10% minerals, and 4% moisture.

Margarine substitute

Pashchenko (2003) has described the use of a combination of concentrates of whey or albumin milk (with a high alpha – lactalbumin content) with a lipoprotein concentrate, prepared from protein – bearing oilseeds such as rapeseed, as a substitute for margarine in the manufacture of bread and other bakery products. Advantages of this mixture compared with margarine included a higher proportion of polyunsaturated fatty acids, shorter processing times in bread making, and improved flavour, aroma and volume characteristics, as well as its lower cost.

Effect on dough quality

Whey solids because of good water binding quality are expected to affect the dough quality like stability, proofing time etc. Kadharmestan et al. (1998) reported that fortification of wheat flour with 10 % whey protein concentrate resulted in wet and sticky bread dough, but imparted improved handling properties. The stability and mechanical tolerance index of the French type bread dough were also found to improve by addition of 1% whey solids. Use of whey and whey solids also had an effect on the dough fermentation time or proofing.

Imbs et al. (1974) reported that when water was replaced with liquid whey in bread formulation, it not only resulted in better kneading of dough but also improved yeast fermentation. Similarly use of 20 – 30% whey was found to reduce the total processing time by 12 – 13% (Silagadze, 1980). Yousif et al. (1998) found that that incorporation of liquid whey or acid whey or dried whey (@0.85 – 3.5% solids) improved the rheological properties of dough and also enhanced absorption and resulted in improved dough development time. Prior fermentation of whey before using in the formulation was also found to help leavening.

Gelinas et al. (1995) reported that incorporation of cultured mixture of milk; whey and wheat flour in a pan bread formulation did not reduce proof time, but affected dough mixing stability. Sanina et al. (1996) after performing an analysis using a statistical tool generalized Lagrange multiplier method, established optimum whey concentration as 16.6% and moisture as 46.2% in dough for getting good quality of bread.

Constandache (2005) reported that when untreated whey protein concentrate (WPC) and heat treated whey protein concentrate (WPCHT) were incorporated in the dough along with sodium casienate by the replacement into wheat flour, it decreased proofing time, increased loaf volume and improved the texture. WPCHT resulted in improved hanging properties of dough, bread volume and overall baking performance.

Asghar (2009) investigated the effect of modified whey protein concentrate on the TPA characteristics of frozen dough made from flour with different protein contents and found that values of instrumental texture parameters were significantly affected by the addition of mWPC and there was a significant decrease in hardness, cohesiveness, gumminess and springiness with its addition in dough samples.

Ammar et al. (2011) reported the effect of addition of whey protein (i.e., acid whey, sweet whey, retentate of whey by Reverse Osmosis (R.O), the permeate, pasteurized acid whey, pasteurized sweet whey and whey protein concentrate) and soy protein (raw soy milk, sterilized soy milk, soy protein isolate) on the rheological properties of wheat dough using the studies of result conducted by a Farinograph. The addition of whey protein concentrated by R.O (retentate) exhibited the highest ability to increase water absorption, dough stability, dough development time and time to breakdown of the dough (72.8%, 8.7, 7.7 and 10.0 min, respectively) followed by pasteurized sweet whey and whey protein concentrate.

Meshkani et al.(2013) studied the effect of whey powder and carboxy methyl cellulose (CMC) on the rheological characteristics of bread dough by response surface methodology (RSM). The dough rheological properties (strength and elasticity) increased significantly on increasing the percentage of whey powder and CMC.

Alomari et al. (2011) reported that hydrolysed whey lactose syrup can be used as a sugar replacement for French type bread. The bread made by 25% sugar replacement was found to be better than the control bread on the basis of sensory scores. The bread dough made with 25% replacement of sugar by hydrolysed whey lactose syrup was found to have better rheological properties than the control bread dough with regards to stability, rate of absorption and mechanical tolerance indication.

Paul et al. (2016) observed that concentrated whey of Indian cottage cheese (paneer) retarded the proofing rate of the dough

which could be recovered by using higher proofing temperatures of 45 °C. The dough obtained by addition of whey was observed to be less gummy, more firm and sticky than control multigrain bread dough.

Effect on bread quality

Several studies indicated that use of whey has beneficial effects on bread quality like loaf volume, flavour, aroma, crumb quality etc. Replacement of 13-18% water in dough formulation with concentrated whey (20% TS) was found to improve bread volume (Preller, 1978). Similar effect was reported by Silagadze (1980) when whey was added @ 20-30%. Such a result was also observed by Erdogdu et al. (1996) on incorporation of commercial whey protein concentrate into dough. Similar observation was made by Kadharmestan et al. (1998) who reported increase in bread volume by 50 ml. Improvement in specific loaf volume was also reported by Yousif et al. (1998) as a result of addition of whey solids to formulation in the form of liquid whey or dried whey, and by Sudhakara (2006) using 20% WPC gels. But, according to Gelinas et al. (1995) use of cultured mixture of whey, milk and wheat flour reduced the specific volume of bread.

Addition of whey was found to enhance the porosity of bread (Imbs et al. 1974; Silagadze, 1980). It also resulted in lighter crumb color and improvement in crust quality (Imbs et al. 1974). Use of 20 % WPC gels in dough also improved sensory scores with respect to crust color and character of bread (Sudhakara, 2006), but fermented whey either in dilute form or concentrated form was found to adversely affect the same (Venskutonis, 1995). With regard to crumb quality, use of 2% whey concentrate (30% TS) gave a more elastic crumb than usual (Liepin et al. 1984) and use of 20% WPC gel the crumb colour and grain formation (Sudhakara, 2006). Use of powdered whey along with wheat germ, wheat bran and whole meal flour was found to result in excellent crumb quality (Vukobratovic and Beleslin, 1991). Cultured dairy ingredients including whey yielded a firmer crumb (Gelinas et al. 1995). Multigrain bread made by replacing water of the dough with 15% TS whey, resulted in firmer bread with browner crust colour than control. (Paul et al. 2019)

Enhancement of flavour of bread as a result of whey incorporation was reported by Preller (1978). Similar result was reported by Liepin et al. (1984) with the addition of fermented whey concentrate, and the improved aroma was attributed to volatile fatty acids, alcohols and benzaldehyde (Venskutonis, 1995). Whey has also been utilized to prepare flavour enhancers for bread. Gelinas and Lachance (1995) described preparation of concentrated cultured dairy ingredients with enhanced aroma levels. This involves inoculation of equal mixture of milk and whey (20 % TS) with *L. casei* var. *rhamnosus* and incubation for 24 h. This could be used as such in bread manufacture or the same can be dried and used as flavour enhancer (@ 1 – 2 % dry basis) in bread making process.

Morr (2003) reported that angel food cakes incorporated with WPC solution of 25ml/100ml and baked at variable atmospheric pressure exhibited almost the same physical, textural and sensory properties as that of the control angel food cake that is baked under atmospheric air pressure.

Effect on shelf life of bread

Numerous studies aimed at prolonging bread's period of freshness and suitability reveal that the process of aging can be retarded by adding to the dough certain additives as antistaling agents, such as fat, milk, whey, enzymatic or other preparations (Xu et al. 1992; Fik et al. 2012).

Some studies have indicated that whey proteins have an influence on the rate of staling and thereby on shelf life. Erdogdu et al. (1996) utilized heat treated acid whey protein in bread and found that it lowered the rate of staling of bread as measured by universal testing machine and differential scanning calorimetry enthalpy changes. Similar results were reported by Yousif et al. (1998) who utilized unconcentrated and concentrated whey in French type bread and reported retarded bread staling and increased keeping quality by 2 days.

Using whey proteins along with powdered whey, gelatin, potato flakes and acetic acid, Sukara (1988) reported that the bread made from the formulation remained wholesome and tasteful for eating even after seven days. In a review study conducted by Cocup and Sanderson (1987), it was concluded that whey could be processed to acquire properties helpful in the shelf life extension of bread and fermented baked products.

Effect on nutritive value of bread

Silagadze (1980) noted that by addition of 20 – 30% whey to bread formulations, nutritive value of bread improved. Incorporation of whey protein concentrate at 10% levels was found to increase the protein content of bread up to 20.2% and also elevated the proportion of essential amino acids (Kadharmestan et al. 1998). Vukobratovic (1992) discussed the nutritional value of several products including bread enriched with wheat germs, whey powder, milk powder, soya flour, wheat bran and gluten flour.

Utilization of Whey In other bakery products

Cakes

In cakes, more protein is needed for crumb strength. The finished structure of a cake is dependent upon the gelatinization of starch and denaturation of protein. The addition of sugar in cakes increases the gelation temperature of gluten so the finished structure of the cake cannot be obtained without the addition of a protein with a lower gelation temperature. Whole eggs and egg whites are added to achieve this desired structure. Successful

Table 2 Recommended level of addition as % of total formula

	Sweet Whey (%)	WPC34 to WPC50 (%)	WPC80 (%)	Demineralized whey,modified whey (%)
White Bread	1-5	1-4	1-3	2-6
Sweet Rolls	2-5	1-4	1-3	2-6
Cookies and Biscuits	1-5	1-5	1-4	2-5
Crackers	1-5	1-4	1-3	2-6
Pizza Doughs	1-5	1-4	1-3	2-6
Fillings	1-6	1-4	1-3	1-6
Icing and Fillings	1-3	1-2	1-2	1-3
Low fat, low sugar baked goods	2-10	3-9	3-5	2-10

application of WPC as egg replacers in cakes is inversely related to the sugar and fat level in the cake system. Egg whites in cakes can be partially or totally replaced by WPCs with high protein content (WPC80). The higher the sugar and the lower the fat, the harder it is to make an acceptable cake with a complete replacement of whole egg with WPC. The higher protein WPCs generally are required for cake applications because of the requirement for gelation. The WPC34, WPC50, and WPC80 products are well suited to partially replace the functions of whole egg in a cake application. WPC80 is better suited for egg white replacement. WPCs can provide body and viscosity to cake batters to help entrap air and retain carbon dioxide produced by the leavening system. They can also help in retaining moisture in cakes. Another ingredient that can be replaced in a baked product is fat. The addition of WPC80 (at a 2% level) to a low fat pound cake formula can result in a higher volume, softer product that is preferred over both a full fat control and a low-fat control (no WPC80) in moistness, flavor and overall characteristics.

Cookies

Replacement of whole egg in a soft cookie with a WPC is also possible. In less aerated products such as cookies, replacement of skim milk powder or egg is easily accomplished. Whey added to cookies is an economical source of dairy solids. Both WPC34 and WPC80 have been found to improve the color, thickness and chewiness of cookies. In reduced fat cookies, combinations of WPC80, modified starch, emulsifiers and water are able to replace whole eggs and shortening. This addition results in batters with similar spread and baked cookies with similar texture, flavor and overall preference when compared to the control. Egg whites in formulas for scones and crepes can be replaced with WPC80. The substitution is made on an equal protein basis.

The resulting products are similar to control products in overall acceptance, but they are generally tender in texture.

Crackers

In contrast to cookies, crackers contain little or no sugar. They are formulated with higher protein flours, often a mixture of soft and hard wheat. The functional requirements for WPC in crackers are similar to that in breads. WPC has been used to replace flour in yeast leavened crackers. WPC34 gives superior results over WPC75 (when using WPC75, less than 5% of the flour can be replaced). The longer the fermentation time, the more satisfactory is the cracker.

Pie-Crusts

Whey or lactose can be added to pie crusts. Whey at approximately 2-3% of lactose, 6-8% of flour weight aid in emulsifying the shortening. This allows for a reduction in shortening without sacrificing the tender, flaky texture. Bakers also report improvements in color and flavor of the baked crust

Bakery mixes

Bakery mixes are generally one of three different types: complete mix, dough base and dough concentrate. Dough bases or partial mixes require that the end user add water as well as oil or shortening and eggs. Dough concentrates are specifically designed for continuous, high throughput, automated production. Used for fat reduction, high solubility, water binding, and moisture retention, they blend well with other ingredients in a bakery dry mix. Mild flavor is another attribute of WPCs that typically blends well with baked products. The bland, dairy flavor of WPC enhances many of the browning type flavors that develop during baking. The added browning that results due to the lactose content also contributes to an appealing surface color.

Bakery Glazes

Bakery glazes based upon whey protein concentrates and caseinates have many advantages over traditional glazes made from whole eggs and water. The whey-based glaze is microbiologically stable and free of salmonella which is more prevalent in egg based glazes. Although good sanitation practices are always necessary, whey based glazes are less prone to microbial contamination. This type of glaze gives good adhesion of toppings such as seeds and crushed grains as a top spray on proofed bread or rolls.

Biscuits

Concentrated whey (both sweet and sour) upto 12.5 % can be used in the manufacture of multigrain biscuits (Chauhan, 2015). The whey replaced water in the dough. The biscuits made by using whey had better palatability, higher TS and higher hardness than control samples. The shelf life of biscuits made by using whey was 16 months at 30°C.

WPC can be used to fortify biscuits. Upto 4% fortification was acceptable in terms of sensory characteristics. WPC incorporation of biscuits resulted in overall shrinkage of biscuits. (Wani, 2015.)

The recommended quantities of whey as a percentage of total formula for various bakery items are given in Table-2.

Conclusion

Whey ingredients have the ability to provide emulsification, foaming or whipping, good solubility, thickening, browning, gelation, nutritional fortification, water binding properties and also a clean flavour in bakery products. The functionality of bakery products is enhanced by the application of whey based ingredients. There is a multitude of opportunity, both functional and nutritional, for the use of whey in bakery products.

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RESEARCH ARTICLE

Moisture sorption characteristics of *Mohanthal*, a chickpea flour (besan) based traditional dairy product

MB Chaudhary[1], K Jayaraj Rao[1] and Asha Ashok[2]

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Abstract: Moisture sorption characteristics of *Mohanthal,* a traditional dairy product from Gujarat were investigated at temperature of 5–45 °C and water activities (a_w) of 0.11–0.97. The sorption isotherms obtained were sigmoid in nature. Nine different mathematical models were found to effectively describe the moisture sorption data on the basis of regression analyses and goodness of fit. Each model was statistically evaluated by means of percent root mean square and coefficient of determination. The GAB model gave the best fit in the entire range of a_w. Temperature dependence of the GAB constants and good fit were determined. Using Caurie's model, the properties of sorbed water such as monolayer moisture content, number of adsorbed monolayers, density of sorbed water, bound water content and surface area of adsorption which decreased with an increase in temperature, were calculated. The monolayer moisture content ranged from 1.82 to 6.25 g/100 g solids in Mohanthal.

Keywords: *Mohanthal*; Modeling; Sorption; Temperature

Introduction

The study of moisture sorption isotherm finds its application in the food product process development (Delgado and Sun, 2002).The moisture sorption isotherm is a graphical representation of the relationship between moisture content of a product and a_w at a particular temperature. Moisture sorption isotherms give vital information about sorption behaviour and interaction of various components of a food with water (Kaymak-Ertekin and Gedik, 2004). The information from moisture sorption data reveals deteriorative reactions in foods. The sorption isotherms study of several western dairy products has been established, but limited study on traditional Indian dairy products is reported. Moisture sorption isotherms of numerous traditional dairy products including *pedha* (Kumar et al. 2012), *chhana podo* (Rao et al. 2006), *dudh churpi* (Hossain et al. 2002) and *khoa* (Sawhney and Cheryan, 1988) have been established. The objectives of this study were to provide reliable experimental data of the sorption characteristics of *Mohanthal.* The moisture sorption characteristics of *Mohanthal* were *studied at* 5ºC, 25ºC and 45ºC. The water binding mechanisms were investigated and simple mathematical models which describe the sorption data are suggested.

Mohanthal is one of the region specific traditional products popular in Gujarat. *Mohanthal* is prepared using ingredients such as chickpea flour (besan), ghee and sugar while *khoa* is used as optional ingredient for improving flavour of product. *Mohanthal* is characterized as light brown to medium brown colour, pleasant, nutty, roasted and caramelized flavor, soft bodied but enough firmer and cohesive to cut in rectangular pieces which holds its shape without becoming floury (Chaudhary et al. 2020).

Materials and Methods

Preparation and Chemical analysis of *Mohanthal*

For Preparation of *Mohanthal*, *dhrabo* was prepared by mixing 100 g of fresh besan with small quantity of milk (10 g) and ghee (20 g). The ingredients were mixed well till the mixture attains a bread crumb consistency. After proper mixing, the mixture was kept undisturbed for half an hour. Ghee (103 g) was taken in a separate vessel and heated till it melts completely. Then *dhrabo* was added to the molten ghee and heated to 145-148 ºC till the mixture develops a light brown colour and pleasant roasted flavor and then 28 g khoa is added. The ghee-besan-khoa mixture was then allowed to cool to 100 ºC. Ninety eight gram sugar in syrup form (75 ºBrix) was added to the prepared mixture and continuous

[1]Dairy Technology Section, SRS of ICAR-National Dairy Research Institute, Bengaluru, India

[2]Department of Dairy Engineering, College of Dairy Science and Technology, Thiruvananthapuram, India

MB Chaudhary (✉)
Dairy Technology Section, SRS of ICAR-National Dairy Research Institute, Bengaluru, India
Email:madhav.chaudhary5@gmail.com

mixing was done till it attains a semi solid consistency. Finally, the product was spread on a greased dish and garnished with nuts and cardamom. The product was then allowed to cool by keeping it overnight. Next day, the product was cut into pieces and used for further study.

The gross chemical composition of *Mohanthal* was determined using different methods: moisture by gravimetric method (BIS, 1981), fat by Mojonnier method (IS: (SP: 18) Part XI (1981)), protein by Micro Kjeldahl method BIS (1981), lactose and sugars BIS (1981) and ash (AOAC, 2005) and other carbohydrates (by difference).

Measurement of sorption equilibrium

The sorption apparatus used in the equilibrium studies by Wolf et al. (1985) was modified for use in the present study. Wide mouthed glass bottles (200 ml) with vapour-tight lids were used as sorbostats. Inside each sorbostat there was a support for weighing bottle, in which the sample material was exposed to the humid atmosphere in the containers. Nine salt solutions in the a_w range of 0.113 to 0.985, namely lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, sodium bromide, sodium chloride, potassium chloride, potassium nitrate and potassium sulphate, chosen according to Greenspan (1977) acted as sorbate source.

The salts were dissolved in distilled water at 100 ºC and cooled to different test temperatures (5, 25 and 45ºC) for crystallization to form saturated slushes. Approximately 1 g finely ground paste of freshly made *Mohanthal* was placed into the tared weighing bottle which was kept on the glass bead support into the sorbostat. The weighing bottles in triplicate were equilibrated for 24 h with the surroundings within the sorbostats before the samples were put into them. Approximately 5 mg of potassium sorbate was added to each sample to prevent mould growth. The sorbostats were tightly closed and placed in incubators at different temperatures. The samples were weighed periodically till the equilibrium was attained. The moisture content in the samples were determined by gravimetric method (Helrich, 1990), and expressed as g water per 100 g solids.

Mathematical analysis

The moisture sorption isotherms of foods were described by numerous mathematical models with two or more parameters. All the equations were converted into linear form, and the moisture sorption data were analyzed and fitted to different equations either in the whole range of isotherm or part of it. Temperature dependence of isotherm parameters for the Caurie model was also studied. The following equations were fitted to the data on the basis of correlation and regression analyses (Chirife and Iglesias, 1978). The data were fitted using Microsoft Excel.

The parameters of the equations were estimated by fitting the mathematical model to the experimental data. Goodness of fitting is to check the accuracy of prediction of the moisture adsorption isotherm by a mathematical model. Several statistical criteria were tried in the present study to check the goodness of fit (Table 2)

Determination of sorbed water properties

The different properties of sorbed water that were determined are:

a) Number of adsorbed monolayers (N), which was calculated using the formula (Caurie, 1981):

$$N = \frac{M_0}{C_c}$$

Where, M_0 was the monolayer moisture content (g/kg solids), calculated from Caurie's model, and C_c was the Caurie's constant

b) Bound water content was expressed as $M_0 \times N$

c) Assuming Caurie's constant (C_c) to be equivalent to the density of adsorbed water in the monolayer, the surface area of adsorption (A) was calculated using the formula (Sahu and Jha, 2008):

$$A = \frac{M_0}{C_c \times d \times 10^8}$$

Where, d was the diameter of a water molecule (3.673×10^{-10} m)

Results and Discussion

Mohanthal prepared under optimized conditions contained (w/w) 10.20 % moisture, 31.05 % fat, 7.88 % protein, 1.70 % lactose, 26.02 % sucrose, 0.98 % ash and 22.17 % other carbohydrates. The moisture adsorption isotherms of *Mohanthal* were generated at three different temperatures *viz. 5*, 25 and 45 °C in the a_w range of 0.11-0.97. The equilibrium moisture contents so obtained are presented in Table 3. It can be seen from the table that Equilibrium Moisture Content (EMC) ranged from 0.1885 to 31.0283, 1.1652 to 40.3623 and 4.9109 to 54.3668 g/100g of solids at 45, 25 and 5 °C, respectively. The EMCs was found to decrease with increase in temperature at a particular a_w. The EMCs were plotted against a_w to obtain an isotherm. Moisture sorption isotherms of *Mohanthal* are depicted in Fig. 1.

As per Brunauer-Deming-Deming-Teller (BDDT) classification, most of the food products exhibits type II sigmoid shaped sorption isotherm. Dairy products such as *pedha*, *dudh churpi* and *khoa* also exhibit similar results (Biradar et al. 1985; Hossain et al. 2002;

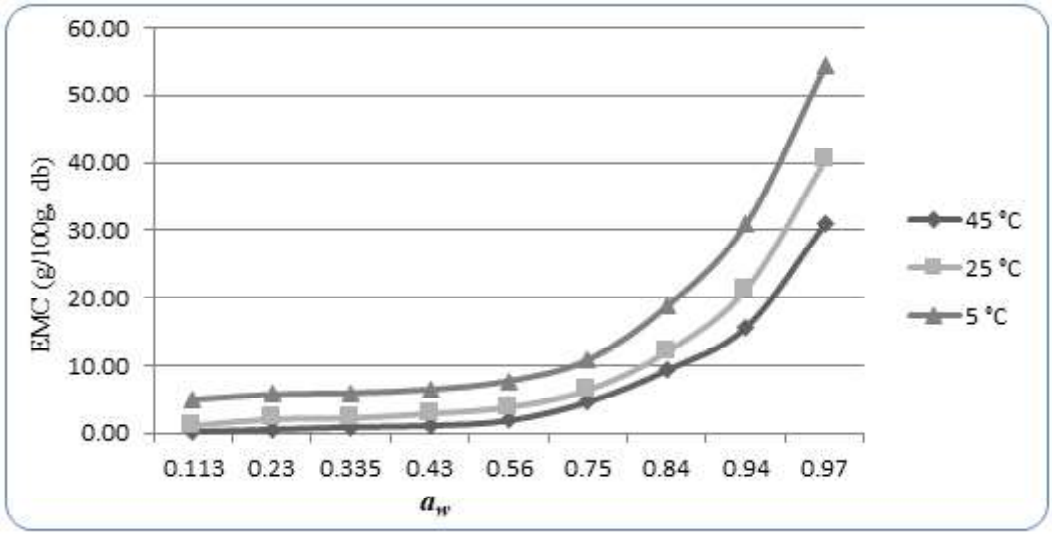

Fig. 1 Moisture Sorption Isotherm of *Mohanthal* at different temperature

Sawhney and Cheryan, 1988). In the multilayer sorption region i.e. of low and intermediate water activities, the moisture content increased linearly with a_w, whereas in capillary condensation region i.e. of high a_w, the moisture content rapidly increased with a_w. The EMC increased gradually at lower a_w activities followed by a steep rise above 0.7 a_w as shown in Fig. 1. *Mohanthal* consist primarily of sugar, bengal gram carbohydrates and protein, ghee (milk fat) and very minor quantity of milk protein and lactose. At low a_w values, protein are the preferred sorption sites, hence the sorption in the low *aw* range (around monolayer level) is presumably due to protein. In addition, at low a_w it is possible to have a local dissolution of sugar, a swelling of proteins and appearance of new active sites (Falade and Aworh, 2004). In the intermediate aw range, sorption occurs mostly on less active sites. The steep rise in sorption at high water activities (above 0.85) could be due to solubilization of sugars (Pallavi et al. 2015).

Mathematical description of the desorption isotherms

In total about 9 mathematical models consisting of two and three parameters were tried to fit the sorption data of the *Mohanthal* at all temperatures and the goodness of fit was chosen by multiple statistical criterion.

The isotherm models, their derived constants, root mean square error (*RMS %*), coefficient of determination (R^2), chi-square (χ^2) and percent mean deviation (*P*) are summarized in Table 4. Since, R^2 alone is not a reliable statistical criterion for evaluation of the EMCs (Rao et al. 2006) in the present study we have been dependent on multiple criterion. Hence, among all, Modified Mizrahi, was found to be most suitable for fitting EMCs data in a_w range 0.11-0.97 followed by GAB model, Halsey, Caurie, Oswin, Mizrahi, Lewcki-I while BET model was found to be the best in the range of 0.10-0.44 as they were characterized by highest R^2 values and lowest *RMS%*, *P* and χ^2 values. Fig. 2 illustrates the differences between actual and predicted (GAB) sorption isotherm of the *Mohanthal*. In the present study, the monolayer moisture

Table 1 Mathematical models used for prediction of the moisture sorption isotherm

Models	Equations
BET	$\frac{a_w}{(1-a_w)M} = \frac{1}{M_0 C_b} + \frac{(C_b-1)}{M_0 C_b} a_w$
Lewicki	$M = A\left(\frac{1}{a_w} - 1\right)^{B-1}$
Oswin	$M = A\left(\frac{a_w}{(1-a_w)}\right)^{B}$
Kuhn	$M = -\frac{K_r}{\ln a_w} - B$
Smith	$M = A - B\ln(1-a_w)$
Cauri	$\ln\frac{1}{M} = -\ln\frac{1}{CM_0} + \frac{2C}{M_0}\ln\frac{1-a_w}{a_w}$
Mizrahi	$M = \frac{(Ba_w - A)}{(1-a_w)}$
Halsey	$a_w = \exp\left(\frac{-A}{RT}\frac{1}{M^r}\right)$
GAB	$M = \frac{M_0 K C a_w}{(1-Ka_w)(1+(C-1)Ka_w)}$
Modified Mizrahi	$M(a_w - 1) = A + Ba_w + Ca_w^2$

Where a_w represents water activity, M is equilibrium moisture content, M_0 is monolayer moisture content and A, B, K and C are the isotherm constants.

Table 2 Statistical criteria used to check the goodness of fit

Test name	Equation
Root mean square percent	$RMS\% = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{P_{obs} - P_{pre}}{P_{obs}}\right)^2}$
Coefficient of determination	$R^2 = 1 - \frac{SS_{res}}{SS_{tot}}$
Chi-square	$X^2 = \sum \frac{(O_i - E_i)^2}{E_i}$
Percent mean deviation modulus	$P = \frac{100}{n}\sum_{i=1}^{n}\left\|\frac{M_{i\,exp} - M_{i\,pre}}{M_{i\,exp}}\right\|$

Table 3 Equilibrium Moisture Content of *Mohanthal* at different temperatures

a_w	EMC (g/100g solids, db) Temperature (°C)		
	45 °C	25 °C	5 °C
0.11	0.188±0.001	1.165±0.005	4.910±0.008
0.23	0.536±0.004	2.134±0.007	5.690±0.021
0.34	0.874±0.006	2.272±0.018	5.888±0.010
0.43	1.090±0.001	2.946±0.013	6.417±0.040
0.56	1.850±0.010	3.873±0.006	7.687±0.023
0.75	4.594±0.003	6.431±0.010	10.803±0.006
0.84	9.320±0.011	12.053±0.002	18.777±0.020
0.94	15.698±0.001	21.338±0.002	30.964±0.029
0.97	31.028±0.066	40.362±0.024	54.366±0.030

content (M_0) exhibited by the BET equation was close to GAB at all the temperatures i.e. 5, 25 and 45 °C. The applicability of BET and estimation of M_0 from BET and GAB equations was also evaluated by several authors. Chiste et al. (2012) found that BET model applicability was better and estimated M_0 of 4.92 percent. Durakova and Menkov (2005) mentioned the significance of BET model in estimating M_0. Palou et al. (1997) reported that the estimated M_0 using BET and GAB models were significantly different. In the present study, although Kuhn models obtained reasonably high R^2 values, other statistical parameters like *RMS%*, *P* and $\chi 2$ also achieved higher values and did not meet the criterion. Bradley, Smith and Henderson found to poorly fit the sorption data of *Mohanthal*.

Effect of Temperature

The temperature dependence of desorption isotherms were found to be inconsistent among the different temperatures studied. As foods are exposed to a range of temperatures during storage and processing and also a_w changes with temperature for the same moisture content, knowing the effect of temperature on the sorption isotherm could help in monitoring the dynamic equilibrium between vapour and adsorbed phase and regulating the changes occurring during storage (Goula et al. 2008). They also suggested that, temperature shows significant effect on the mobility of water molecules and the dynamic equilibrium between vapour and adsorbed phases. In Fig. 2 effect of temperature on the sorption isotherms of *Mohanthal* is shown. It can be seen that for the same a_w, EMC were generally higher at lower temperatures as confirmed by the higher values at 5 °C as compared to 25 °C and 45 °C. Higher EMCs at 5 °C might be due to sugar crystallization that took place and moisture of *Mohanthal* was difficult to remove at very lower a_w

Palipane and Driscoll (1992) also reported that, at higher temperatures water molecules get activated to higher energy levels and break away from the water-binding sites of the food which results in lower EMC values. The negative temperature behavior on EMC has also been observed in other foods containing high protein and carbohydrates including sugar (Delgado and Sun, 2002). Saravacos (1995) also reported that a_w and sorption isotherms are affected by the composition of the food and temperature of the system. The general trend of decrease in EMC observed at higher temperature could be attributed to relatively protein, sugar and carbohydrates of *Mohanthal*. Proteins and carbohydrates have more water binding capacity at low temperatures as compare to high temperatures (Berlin et al.

Table 4 Mathematical models for moisture sorption isotherm of *Mohanthal*

Parameter	Constants	5 °C	25 °C	45 °C
	M_0	3.34	1.82	1.87
	C	19.19	13.03	1.093
BET	R^2	0.914	0.9954	0.952
(a_w=0.10-0.44)	χ^2	4.952	0.049	0.096
	RMS%	0.179	0.008	0.108
	P	37.72	6.983	20.154
Lewicki-I	A	8.743	3.842	1.504
(a_w=0.10-0.92)	B	0.553	0.366	0.089
	R^2	0.958	0.985	0.973
	χ^2	6.375	1.439	2.023
	RMS%	0.126	0.048	0.067
	P	17.52	9.821	12.79
Mizrahi	A	4.952	0.0904	0.179
(a_w=0.10-0.92)	B	-3.034	-1.4591	1.063
	R^2	0.959	0.9343	0.917
	χ^2	3.809	4.095	4.167
	RMS%	0.039	0.125	0.298
	P	7.83	16.615	21.722
Kuhn	A	-1.523	-1.189	-0.9403
(a_w=0.10-0.85)	B	5.477	1.937	0.6428
	R^2	0.994	0.994	0.989
	χ^2	2.14	2.345	3.671
	RMS%	0.065	0.523	8.367
	P	11.177	25.206	90.765
Caurie	M_0	6.258	3.48	1.817
(a_w=0.10-0.85)	Cc	1.398	1.104	0.828
	R^2	0.9581	0.985	0.973
	χ^2	6.358	1.44	2.026
	RMS%	0.126	0.048	0.067
	P	17.522	11.835	12.801
Oswin	A	0.4467	0.634	0.911
(a_w=0.10-0.92)	B	8.743	3.842	1.504
	R^2	0.9579	0.985	0.973
	χ^2	6.375	1.439	2.026
	RMS%	0.126	0.048	0.067
	P	17.52	11.837	12.798
Halsey	A	25.455	3.171	0.797
(a_w=0.10-0.92)	Mr	3.236	1.154	-0.226
	R^2	0.987	0.997	0.925
	χ^2	1.911	0.611	5.676
	RMS%	0.044	0.027	0.324
	P	10.76	7.396	27.52
GAB	K	0.948	0.981	0.973
(a_w=0.10-0.85)	C	-25.425	11.705	0.919
	M_0	3.457	1.893	1.763
	R^2	0.971	0.995	0.989
	χ^2	3.476	0.673	0.938
	RMS%	0.037	0.021	0.037
	P	9.113	7.158	9.915
Modified Mizrahi	A	4.448	-0.738	0.208
(a_w=0.10-0.85)	B	0.531	-3.670	-2.992
	C	2.228	3.188	1.718
	R^2	0.986	0.993	0.973
	χ^2	1.777	0.901	2.029
	RMS%	0.021	0.027	0.108
	P	6.069	8.307	16.200

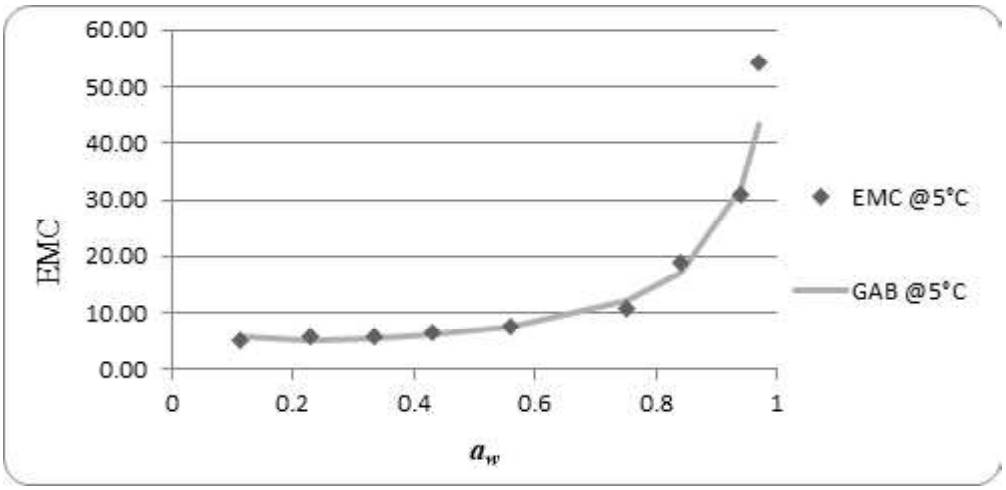

Fig. 2(a) Fitting of GAB model at 5°C temperature

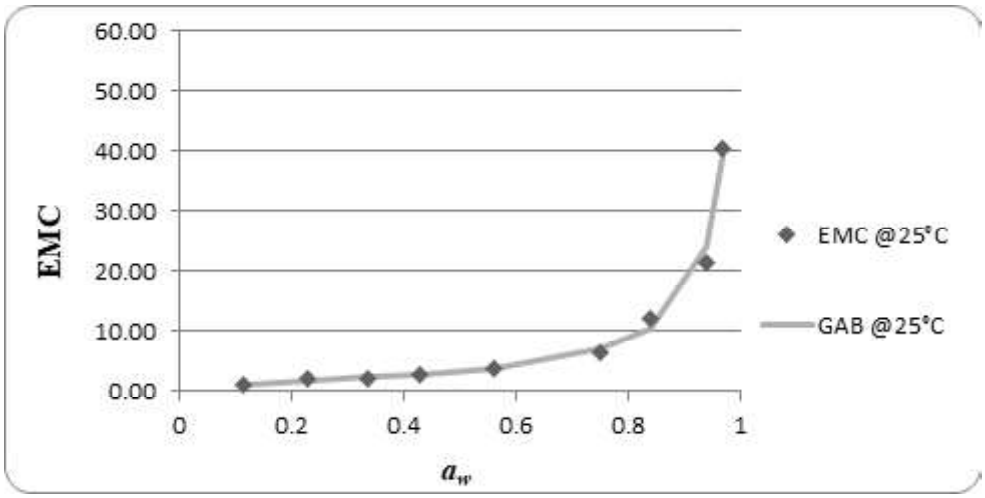

Fig. 2(b) Fitting of GAB model at 25°C temperature

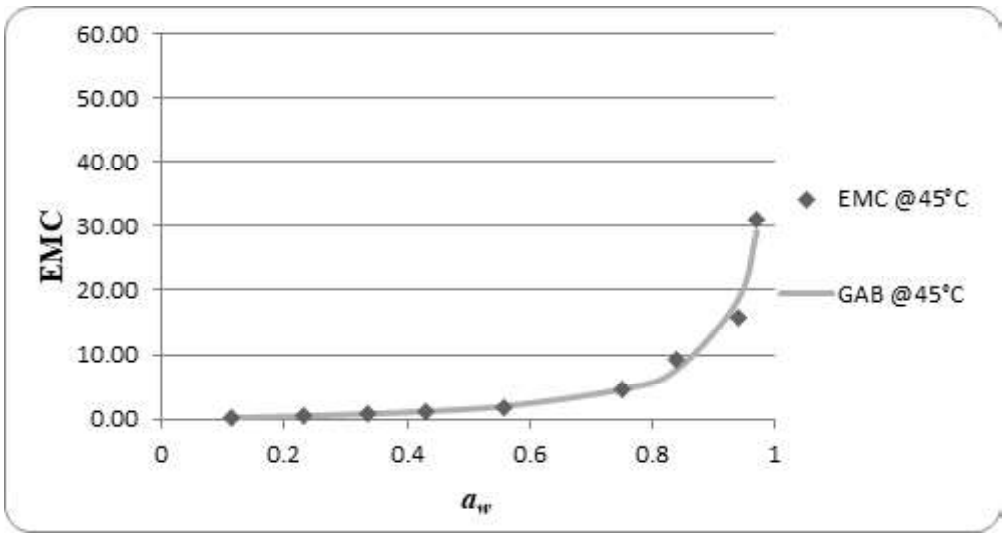

Fig. 2(c) Fitting of GAB model at 45°C temperature

1973). Rao et al. (2006) reported reduction in EMC of *chhana podo* when the temperature was increased from 5 °C to 35 °C. The EMC values at 25 °C were however low as compared to that at 40 °C, but the difference gradually declined as a_w increased above 0.7. This behavior is typical of sugar rich food systems such as raisins, fig, prunes, kheer etc. as sugar absorb more water at higher water activities thus overcoming the negative temperature effect due to increase in solubility of sugars in water (Sharma et al. 2009).

Table 5 Properties of Sorbed water in *Mohanthal*

Temp (ºC)	Number of adsorbed monolayer	Monolayer moisture content (g/100g solids)	Density of sorbed water (g/ml)	Surface area of adsorption (m^2/g)	Bound water (%)
5	4.48	6.25	1.39	121.87	28.01
25	3.15	3.48	1.10	85.82	10.97
45	2.19	1.82	0.83	59.75	3.98

Properties of Sorbed water in *Mohanthal*

Properties of the water sorbed by *Mohanthal* were calculated according to Caurie's model (Table 5). The monolayer moisture content (M_0), number of adsorbed monolayers, bound water content, density of sorbed water and surface area of adsorption decreased with increase in temperature. The M_0 signifies the amount of water strongly binding to the specific sorption sites. It is at these values the food is most stable. The M_0 values for *Mohanthal* decreased from 6.25 g/100g solids at 5 °C to 3.48 and 1.82 g/100g solids at 25 and 45 °C, respectively. This may be due to the loss of some moisture sorption sites as a result of some physical and chemical changes on temperature rise (Mazza, 1982). In region of lower a_w, proteins and carbohydrates in *Mohanthal* are the preferred sorption sites at lower temperatures than at higher temperatures because the components present in food have hydrophilic polar groups with high water binding capacity. Hydrophobic hydration is a result of water forming hydrogen bonds with certain specific groups (Kinsella and Fox, 1987) but as the temperature increases the number of hydrophilic group decreases and the biopolymers hydrophobic hydration also start to break when on attaining higher temperature. This explains reduced mono layers at higher temperatures. They feature a critical moisture content called the monolayer value at which they show maximum physical and chemical stability (Iglesias and Chirife, 1976). When moisture content in the food crosses this monolayer value, it leads to food spoilage (Labuza, 1968). In the present study, the safe storage moisture content values (M_C) predicted using Caurie's model was found to be between 1.82 and 6.25 g/100g solids. The initial moisture level of *Mohanthal* was 10.41 g/100g solids which is higher than the calculated monolayer values. This explains why *Mohanthal* is shelf-stable only to a certain extent.

Surface area of the globular proteins also shows a direct relation with the monolayer moisture content. A larger surface area means greater number of polar groups that are exposed, resulting in an increase in water sorption. With an increase in temperature the surface area decreases (Zettlemoyer, 1968) as reported in some products, like wadi (Rakshit et al. 2014) and dudh churpi (Hossain et al. 2002), where this value decreased from 203.8 to 151.1 m^2/g in the former and from 239.7 to 214.1 m^2/g in the later as the temperature increased from 15 to 45 °C.

Bound water content of *Mohanthal*, which is directly related to monolayer moisture content, was found to decrease from 28.01 % to 3.98 % with the rise in temperature from 5 °C to 45 °C. Density of sorbed water decreased from 1.39 g/ml at 5 °C to 1.10 and 0.83 g/ml at 25 °C and 45 °C, respectively.

Conclusions

The present study was conducted with an intention to understand the sorption characteristics of *Mohanthal* at 5–45 °C and describe the various properties of sorbed water such as monolayer moisture content, number of adsorbed monolayers, density of sorbed water, bound water content and surface area of adsorption. Moisture sorption isotherm exhibited type II sigmoid shape which is typical for most food products. This study also can serve as the possible explanation for the onset of various microbiological events in *Mohanthal* that may lead to its spoilage. The present study revealed that the amount of bound water was more at lower temperatures and it decreased with an increase in temperature. This water is not available for microorganisms; so the shelf-life of *Mohanthal* at lower temperatures will be more than at higher temperatures.

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RESEARCH ARTICLE

Bile salt hydrolase and cholesterol assimilation potential of lactobacilli from Nigerian fermented foods and human sources

Rachael T. Duche[1,2,4], Tochukwu N. Nwagu[4], Anamika Singh[1], Arundhati Ganesh Wandhare[1], Manvesh Kumar Sihag[3], Harsh Panwar*[1], Lewis I. Ezeogu[4]

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Abstract: Bile salt hydrolases (BSH) results in microbes releasing enzymes conferring physiological functions for both the producing organisms and the host and subsequently lowering serum cholesterol. In recent years, these attributes have generated interest towards screening of lactic acid bacteria (LAB) for functional food production. In this study, lactobacilli (n=34) (13 *L. casei*, 6 *L. paracasei*, 11 *L. brevis*, 3 *L. pentosus* and 1 *L. plantarum*) from various sources *viz*. fermented sorghum products (Kunu, Burukutu and Pito), fermented cassava products (Garri and Akpu), Human milk, Infants' feces and fermented cow milk (Fura da Nono). These samples were screened qualitatively and quantitatively for BSH activity using the direct plate assay and cell free extracts (CFE) from overnight cultures. To establish a correlation between BSH and cholesterol reduction, 12 isolates displaying high precipitation over bile salt agar plates, and high BSH enzyme activity in CFE were selected for *in vitro* cholesterol reduction assay. Results showed that 58% of the isolates from humans, and 36% from fermented food origin showed larger zones of precipitation. The highest BSH activity was exhibited by *Lactobacillus casei* GR4 (76mM) after 10 min of incubation, an isolate from fermented food. Isolates from human specimens expressed higher enzyme activity compared to those from fermented foods with significant increase ($P<0.05$) after 30 min incubation. Cholesterol assimilation levels varied as *L. pentosus* 8ST5 and 8ST7 had 2 and 4% cholesterol levels in the medium; while *L. casei* and *paracasei* group (*Lactobacillus casei* group, LCG) 3MB4, 8BM6, BK4 and *L. brevis* GR29 had 7%/ml each proving their application in food formulation, reduction of hypercholesterolemia, and as prophylaxis for controlling serum cholesterol levels.

Keyword: Bile salt hydrolase, Cholesterol; Probiotics; Lactic acid bacteria; *Lactobacillus*

Introduction

Bile acids are synthesized in the liver from cholesterol; before leaving the liver, the unconjugated bile acids get converted into bile salts *via* the replacement of the hydrogen ion at the end of the carboxyl group with either the amino acid glycine or the amino acid taurine (Fig. 1). The process leads to the formation of four primary bile salts *viz*. glycocholic acid (glycine + cholic acid), glycochenodeoxycholic acid (glycine + chenodeoxycholic acid); taurocholic acid (taurine + cholic acid), and taurochenodeoxycholic acid (taurine + chenodeoxycholic acid) (Chand et al. 2016). Primarily, bile acids on their own, if not converted to bile salts retain a hydrogen ion that binds to the carboxyl group which is more soluble when the hydrogen ion or amino acid is separated from it. Therefore, bile salts which have amino acids that can completely separate from the carboxyl group, are more water-soluble than bile acids, which have a hydrogen ion that easily sticks to the carboxyl group. This makes bile salts more effective than bile acids at mixing fats with water and water-soluble enzymes that digest them. Glycine forms of bile salts

[1]Department of Dairy Microbiology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana – 141004, Punjab, India

[2]Department of Microbiology, College of Biological Sciences, Federal University of Agriculture, Makurdi-Nigeria

[3]Department of Dairy Chemistry, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana – 141004, Punjab, India

[4]Department of Microbiology, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria
Harsh Panwar (✉)
Department of Dairy Microbiology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana – 141004, Punjab, India. Email: drhpanwar@gmail.com, Contact No. +91-9501260540

outnumber taurine forms of the same by 3 to 1 ratio. After bile salts are produced in the liver, they either flow through the bile duct into the duodenum, to be used immediately for digestion, or they are stored in the gall bladder, where they are saved for future digestive requirements.

BSH also called choloylglycine are intracellularly located enzymes that are highly sensitive to oxygen but specialize in the hydrolysis of the bile salt steroid nucleus and its amino acid moieties (Horackova et al. 2020). The exact function of this BSH in human metabolism is still under investigation and yet to be fully understood, although it has always been included among the criteria for selecting probiotic bacteria (Reis et al. 2017). Deconjugation of bile salts is being considered as a mechanism of removing cholesterol from the human system thereby reducing the risk of cardiovascular disease. Bile acids are synthesized from cholesterol in the liver, usually conjugated with either taurine or glycine, and lactobacilli with BSH activity can endure and colonize the lower small digestive system where the enterohepatic cycle happens (Tsai et al. 2014). Numerous probiotic microorganisms capable of producing the enzyme BSH, catalyze the deconjugation of bile salts connected with glycine or taurine, as displayed in Fig. 1. The process involves hydrolysis of the C_{24} N-acyl amide bond linking bile acids to their amino acid conjugates thus liberating unconjugated bile salts and their amino acid moieties. Literature suggests that this reaction confers some advantages to both producing organisms and the host. To the organisms, the glycine/taurine moiety released serves as different sources of energy, CO_2, NH_3, etc. and as such adds to their pool of nutrients. Deconjugated bile acids are more inhibitory to pathogens compared to their conjugated counterparts (Begley et al. 2006). As such, this could be a desirable trait for probiotic bacteria because it will maximize their survival in the gut epithelium. Although, the possibility of conjugated bile acid precipitation at low pH in the gut (a phenomenon usually observed in the qualitative determination of BSH using agar plate assay) cannot be ruled out, (Fig. 2). However, to the host, it is said that conjugated bile acids are easily assimilated, unlike their unconjugated counterparts. When deconjugation of bile salts occurs, only a little amount of it is absorbed by the bloodstream while the majority of bile salts get excreted in feces as free bile acids. To compensate for the loss, there is an increase in *de novo* bile salt biosynthesis in the liver, leading to high demand for cholesterol, which subsequently reduces it at serum level (Adebola et al. 2020). During the 1970s, a wild *Lactobacillus* strain was isolated from fermented milk which exhibited some cholesterol-lowering effect in humans (Mann and Spoerry, 1974). There onward, numerous tests have been directed *in vitro* or *in vivo* to explore this attribute of LAB for the production of probiotic products.

The intake of food rich in cholesterol often leads to cholesterolemia, a high risk for coronary heart disease (Nichols et al. 2016). Consumption of foods such as saturated fats and trans fats is likely to increase cholesterol levels in serum. However, studies have also shown that the consumption of probiotic foods in adequate amounts is a promising remedy that can reduce cholesterol in serum up to 3% (Liong and Shah, 2005). High serum cholesterol is the leading risk of cardiovascular diseases (WHO, 2013) and how BSH activity is ascribed to a reduction in hypercholesterolemia is somehow complicated. Among the mechanisms used by probiotics to remove cholesterol is the ability to assimilate cholesterol in their cell membrane, and also bile salt deconjugation by BSH activity (Tsai et al. 2014). Some research findings postulate that BSH-positive bacteria are not present in fermented foods because the habitat is devoid of bile salts (Tanaka et al. 1999; Begley et al. 2006). In this study, we screened isolates from both fermented foods and human sources for their ability to deconjugate bile salts through the amount of enzyme produced and the potential to reduce cholesterol from blood serum. Previous studies have also attempted to find a relationship between BSH and bile tolerance but their claims have not been substantiated.

The present study aimed to assess the ability of both intestinal (human origin) and non-intestinal LAB to exhibit bile salt hydrolase activity leading to cholesterol reduction in serum. Our result is expected to present the best performing isolates as potential probiotic candidates which can be incorporated into food for the control of hypercholesterolemia and the management of the cardiovascular disease.

Materials and Methods

Bacterial cultures

Thirty-four (34) LAB used for the study were isolated from a variety of sources including Nigerian traditional fermented foods and beverages (garri, akpu, kunu, burukutu and pito, and fura da nono) as well as human milk and infant feces. These isolates had been screened previously for acid and bile tolerance. Isolates identification was done by matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectrometry (MS) (Table 1).

Screening for bile salt deconjugation

The differential medium of Dashkevicz and Feighner (1989), for bile salt hydrolase-active *Lactobacillus* species was employed. Bile salts, sodium glycodeoxycholate and sodium taurodeoxycholate (Sigma, Taufkirchen, Germany), at 0.2% (w/v) each, were incorporated into MRS agar containing L-cysteine-HCl. An aliquot (10µl) of bacterial overnight growth was streaked across the medium, before incubation at 37°C, for 72h. Formation of opaque halos (bile acid precipitation) around colonies served as evidence of bile salts deconjugation. Differences in individual abilities of isolates to deconjugate bile salts were assessed semi-quantitatively by measuring precipitation halo diameters. Isolates

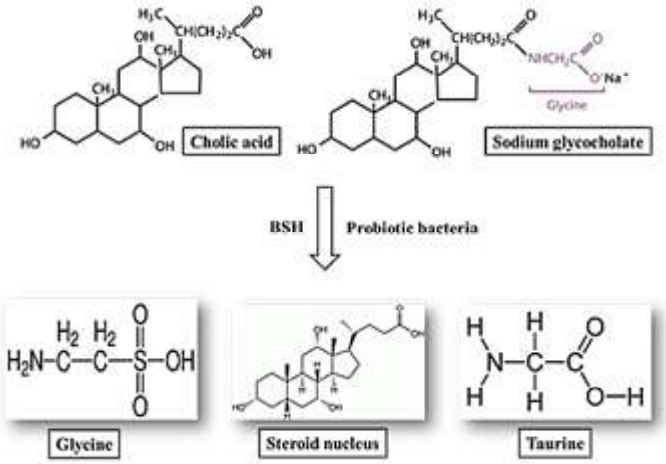

Fig. 1: Chemical structure of conjugated bile acid with glycine and taurine, deconjugated core and the amino acids released

grown on MRS agar without bile salts served as negative controls. Results were presented as mean values of three different replicates.

Quantitative determination of bile salts hydrolase (BSH) activity

The method described by Tanaka et al. (1999) was used for the quantitative determination of bile salt hydrolase activity with little modifications. Briefly, overnight cultures were centrifuged and washed twice in 0.1M phosphate buffer (pH 6.8), containing 10 mM dithiothreitol (DTT), Cell precipitates were resuspended in the same buffer (A_{600} value of 3.0) and sonicated (Misonix S-4000 Sonicator), for 60s on ice, for two cycles, before centrifugation (14,000 × g, 10 min, 4ÚC). The supernatant was recovered and used as crude enzyme for BSH activity determination. Enzyme (BSH) activity assay was by a two-step procedure. In brief, cell-free enzyme extract (10µl) was added to a reaction vessel containing 180µl phosphate buffer (0.1 M, pH 6.0) and bile salt solution (Ox gall, 200 mM, 10µl). The mixture was incubated at 37°C. Samples (35µl) were withdrawn after 10 and 30 min during incubation, mixed immediately with trichloroacetic acid (TCA) (15% (w/v) 50µl), before centrifugation (14,000×g, 4°C, 10 min) to remove the precipitate. In the second step of the assay, aliquots of the supernatant (50µl) from above were mixed with deionized water (50µl). 1.9ml Ninhydrin reagent [0.5ml of 1% (w/v) ninhydrin in 0.5 M sodium-citrate buffer, pH 5.5; 1.2ml glycerol and 0.2ml sodium citrate 0.5M, pH 5.5] was then added and the mixture boiled (100°C, 14 min) in a water bath. Tubes were allowed to cool to room temperature (25°C). Absorbance was then read at 570 nm in a spectrophotometer. The amount of amino acid released by the reaction was read off a standard curve generated (R^2 = 0.9958) for each assay with glycine concentrations (10, 20, 30, 40 50, and 60mg/mL in ddH_2O). A unit of BSH activity was defined as any amount of the enzyme that released 1 mM of amino acid per min.

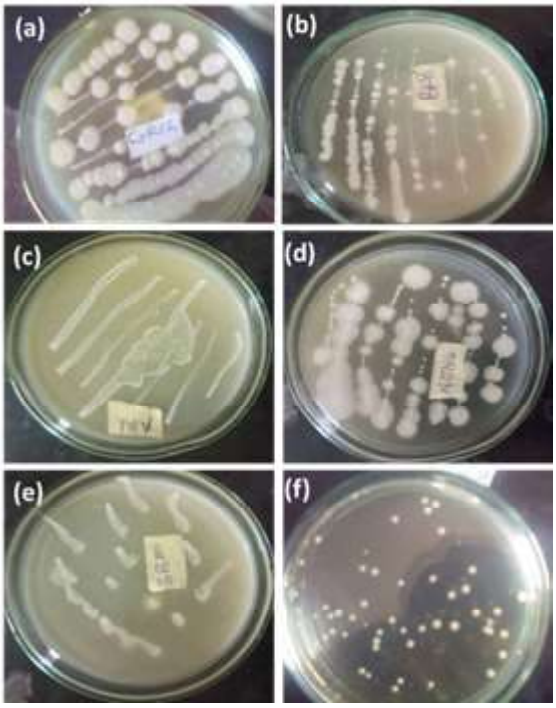

Fig. 2: Precipitation of unconjugated bile salts on agar plates by *Lactobacilli* strains: (a) GR12 (b) BK8 (c) AK1 (d) NON4 (e) 3BM1 (f) Negative control

Cholesterol reduction assay

The protocol of Mishra and Prasad (2004), which is based on 0.2% sodium thioglycolate and 0.3% ox-gall supplementation of MRS broth was employed. Ethanol-soluble cholesterol and o-phthlaldehyde stock standards were prepared according to the method of Zlatkis and Zak (1969). Cholesterol was added into sterile MRS broth (40ml) in test tubes to a final concentration of 100µg/ml. The supplemented medium was then inoculated with 2% of active test cultures, while a tube with uninoculated cholesterol-supplemented MRS was kept as control. Tubes were incubated at 37°C for 24h. Subsequently, cells were harvested by centrifugation (10,000×g, 15min). Aliquots of cell-free culture supernatant (0.5ml) were then placed in fresh test tubes and combined vigorously with 3ml 95% ethanol (3ml), followed by 2ml of 50% KOH (Sigma Aldrich). The mixture was heated (60°C, 10min) in water bath and then allowed to cool. N-hexane (5ml) was added and followed by 3ml of distilled water. Tubes were shaken for 1min to ensure complete mixing and then allowed to stand for 15 min at room temperature (25°C) for phase separation. Aliquots (2.5ml) of the upper n-hexane fractions were collected in clean dry test tubes and the solvent evaporated at 60°C, under a flow of nitrogen gas. O-phthalaldehyde reagent (4ml) was added to the dry tubes. The mixture was allowed to stand for 10min. Conc. sulphuric acid (2ml) was added, slowly, along the sides of the tubes, before mixing and standing for 10min. Absorbance was then read off a spectrophotometer at A_{550nm}. Residual

cholesterol was determined by reading off a standard curve (R^2=0.9876) prepared using cholesterol, o-phthalaldehyde and concentrated H_2SO_4 at suitable concentrations (0, 2, 4, 6, 8, 10µg/ml). Percentage reduction of cholesterol was calculated as follows:

$$A = (B - C/B) \times 100$$

Where; A = % removed cholesterol, B = absorbance of blank, C = absorbance of spent broth

Statistical Analysis

Data were analyzed based on IBM SPSS statistics, 25 (IBM Corporation, USA) using one-way analysis of variance (ANOVA). Student t-tests were used for mean separation and comparison. A level of $P < 0.05$ was considered statistically different. Results presented are mean values of triplicates for each assay expressed as mean ±SD.

Results and Discussion

Bacterial cultures: All 34 isolates were identified as members of *Lactobacillaceae*. Out of the 34 isolates, the most abundant isolates were *L. casei* (13 isolates, or 38.2% of total isolation), while the least isolated species was *L. plantarum*, occurring at 2.9% of all isolations (or just one isolate). For the other species, rates of isolation were: *L. paracasei* (6 isolates, 17.6%); *L. brevis* (11 isolates, 32.4%), and; *Lb. pentosus* (3 isolates, 8.8%). *L. casei* was also the most isolated strain from human sources (5 isolates out of 12 (or 41.7%).

Table 1: Identity of isolates used in the study with their respective origins

S. No.	Origin	Isolate Code	Identification	Source
1	Infant feces at 3	3ST2	*Lactobacillus casei*	Human Source
2	Infant feces at 3	3ST3	*L. paracasei*	
3	Infant feces at 3	3ST5	*L. casei*	
4	Infant feces at 3	3ST7	*L. casei*	
5	Human milk at 3	3BM1	*L. brevis*	
6	Human milk at 3	3BM3	*L. casei*	
7	Human milk at 3	3BM4	*L. paracasei*	
8	Human milk at 8	8BM6	*L. casei*	
9	Human milk at 8	8BM9	*L. brevis*	
10	Infant feces at 8	8ST5	*L. pentosus*	
11	Infant feces at 8	8ST7	*L. pentosus*	
12	Infant feces at 15	15ST2	*L. pentosus*	
13	*Fura da nono*	NON4	*L. brevis*	Fermented Nigerian Foods
14	*Kunu*	KN3	*L. casei*	
15	*Kunu*	KN5	*L. brevis*	
16	*Kunu*	KN6	*L. casei*	
17	*Kunu*	KN9	*L. brevis*	
18	*Kunu*	KN10	*L. brevis*	
19	*Garri*	GR5	*L. brevis*	
20	*Garri*	GR4	*L. casei*	
21	*Garri*	GR11	*L. paracasei*	
22	*Garri*	GR8	*L. casei*	
23	*Garri*	GR12	*L. plantarum*	
24	*Garri*	GR13	*L. brevis*	
25	*Garri*	GR2	*L. brevis*	
26	*Garri*	GR27	*L. casei*	
27	*Garri*	GR29	*L. brevis*	
28	*Garri*	GR32	*L. casei*	
29	*Burukutu*	BK4	*L. paracasei*	
30	*Burukutu*	BK5	*L. paracasei*	
31	*Burukutu*	BK8	*L. brevis*	
32	*Pito*	PT1	*L. paracasei*	
33	Fermenting *Akpu*	AK1	*L. casei*	
34	Fermenting *Akpu*	AK5	*L. casei*	

Bile salt deconjugation ability of lactic acid bacteria

All the isolates grew on MRS containing bile salts and showed some BSH activity. A minority (10) of the isolates were however, active against only one of the two bile salt substrates (sodium deoxycholate or deoxycholic acid) tested (Table 1). For the *Lactobacillus* strains which were active against both substrates, fourteen isolates, representing 7 each from the human and fermented food sources, formed large (3.0mm - > 6.0mm) precipitation zones, although with varying diameters on plates (Fig. 2a – 2f). These isolates, 8BM6, 8ST7, 8ST5, 3ST5, 3ST2, 3BM3 and 3BM4 (from human sources, Table 2), and GR29, BK4, BK5, KN6, NON4, GR12 and AK1 (from fermented food sources, Table 2) showed activity against both bile salts, as well as displayed the largest zones of precipitation on both sodium deoxycholate and taurocholic acid. Only one (or 8.33%) of the human isolates (isolate 15ST2) did not show capacity to deconjugate one of the bile salts (taurodeoxycholic acid) tested, the rest indicated active deconjugation of both bile salts. Conversely, a highly significant ($p < 0.001$) number (9 or 40.1%) of isolates from fermented food sources lacked BSH activity against one of either sodium deoxycholate or taurodeoxycholic acid. Moreover, a higher proportion (58.3%) of the human isolates, compared to fermented food isolates (36.4%), produced

Table 2 Bile salts deconjugation

	Source	Isolate	Activity score on bile salt substrates	
			SDA*	TDA**
Fermented Food Sources	*Kunu*	*KN3*	-	+
		KN10	+	-
		KN6	+++	++
		KN5	+++	+
		KN9	++	-
	Burukutu	*BK8*	+	+
		BK5	+++	+++
		BK4	+++	+++
	Fura da nono	*NON4*	++	+++
	Garri	*GR2*	-	+
		GR4	-	+
		GR5	++	+
		GR8	+	-
		GR11	+	++
		GR12	+++	++
		GR13	-	+
		GR27	-	+
		GR29	+++	+++
		GR32	+	-
		AK1	+++	++
	Akpu	*AK5*	+	+
	Pito	*PT1*	++	+
Human sources	*Infant Feaces*	*3ST2*	+++	++
		3ST3	+	++
		3ST5	+++	+++
		3ST7	+	++
		8ST5	+++	+++
		8ST7	+++	+++
		15ST2	+	-
	Human milk	*3BM1*	+	+
		3BM3	++	+++
		3BM4	+++	+++
		8BM6	+++	+++
		8BM9	+	+

* Sodium deoxycholate; ** taurodeoxycholic acid; +=1 – 3mm halo, ++ = 4 – 6mm halo, +++ = >6mm

deconjugation zones with diameters of 3 to > 6.0 mm, while growing on either of the salts or both.

Bile acid biotransformation is of critical dietary, hormonal, and physiological significance to the wellbeing of the human organism, as its products (secondary bile acids) are crucial participants in important health-defining physiological processes of the body, like dietary lipid absorption regulation, molecular signaling and cholesterol and triglyceride metabolism modulation, as well as glucose and energy homeostasis. The implication is that BSH-catalyzed primary bile acid deconjugation, as gateway reaction in BA metabolism, is of central importance, as it makes downstream processing of secondary BA possible. While all the tested isolates showed evidence of BSH production (on bile salts-containing MRSA), enzyme, in ten, was active against only one of either of the two bile salt substrates (sodium glycodeoxycholate or taurodeoxycholic acid). Previous studies (Corzo and Gilliland, 1999; Tanaka et al. 2000; Kim et al. 2004; Begley et al. 2006; Marius et al. 2018; Foley et al. 2021 and Hernandez-Gomez et al. 2021) have reported similar findings, whereby potential probiotic isolates showed varying/differential activity towards bile salt substrates, depending on whether these salts are glycoconjugated or tauroconjugated. As in those reports, the differences noted in the current study, on the inability of the BSH enzymes of ten of the isolates to deconjugate one of either conjugated bile salts, appear to be dependent on whether the amino acid substituent at the C_{24} position was glycine or taurine. Previous reports have also shown that most BSHs are more efficient at hydrolyzing glycoconjugated bile salts than they are on tauroconjugated bile salts (Taranto et al. 1999; Tanaka et al. 2000; Kim et al. 2004; Begley et al. 2006 and Dang and Lee, 2018). Although, our result partially agreed with the above assertion as some of the isolates (8) exhibited substrate specificity for either sodium glycodeoxycholate or taurodeoxycholic acids. Twenty-four of the isolates however produced BSHs which were active against both substrates. No detailed investigation was carried out to establish the reasons for the 'broad' activities observed against the bile salts, it is conceivable that the isolates' activities may be due to: (1) possible synthesis by isolates of multiple BSH types, or the (2) production, by isolates, of BSHs with broad substrate specificities (i.e., acting against both glyco- and tauro-conjugated substrates). Multiple BSHs have been observed in some bacteria, with up to four homologues in *L. plantarum* ST-111 (Ren et al. 2011) and *L. johnsonii* 100-100 (Elkin et al. 2001), three in *Bifidobacterium bifidum* (Kim et al. 2004) and two in *L. salivarius* (Bi et al. 2013), with each homologue varying from the others in substrate specificity, as well as other important kinetic properties. On the other hand, BSH homologues characterized from many bacteria (e.g. *L. acidophilus* (Carzo and Gilliland, 1999), *Bifidobacterium longum* (Tanaka et al. 2000), *Clostridium. Perfringens* (Rossocha et al. 2005) and *E. faecalis* (Chand et al. 2016) have been reported as having broad substrate specificity, being active on both glycoconjugated and tauroconjugated substrates, although, each may show greater preferential activity towards either of the tauroconjugated or the glycoconjugate substrate (Rohawi et al. 2018). In their study of bile salts deconjugation by *L. plantarum* LAB12 and *Pediococcus pentosaceus* LAB6, Rohawi et al. (2018) observed that both microbes had broad BSH activity against both tauro- and glycoconjugates, though preferentially for the latter. This however varied from the findings made by Bi et al. (2013) who established that whereas BSH1 and BSH2 of *L. salivarius* had broad substrate specificity, against 6 bile acids (3 tauroconjugates and 3 glycoconjugates), one of the BSH homologues (BSH2) exhibited greater preference for tauroconjugated bile salts, while the other (BSH1) preferred glycoconjugates more. The observations by Rohawi et al. (2018) and Bi et al. (2013) probably explain the situation observed in the current study, whereby isolates deconjugating both bile salts appeared to be more active on either glycodeoxycholate (isolates KN6, KN5, GR5, PT1AK1, and 3ST2) or taurodeoxycholic acid (isolates NON4, GR11, 3ST3 and 3ST7) than the other, respectively. Phylogenetic analysis of BSH isotypes from various bacteria (and with available substrate binding profile) by Dong and Lee (2018) has divided BSH isotypes into four clusters, with, most interestingly, each clusters containing some isotypes with broad substrate specificity as well as those having narrow substrate specificity. Results for the same are documented in Table 1 and 2. Only one (or 8.33%) of the human isolates (isolate 15ST2) did not show capacity to deconjugate one of the bile salts (taurodeoxycholic acid) tested, with the rest indicating active deconjugation of both bile salts. Conversely, a highly significant ($p < 0.001$) higher number (9 or 40.1%) of isolates from fermented food sources lacked BSH activity against one of either sodium deoxycholate or taurodeoxycholic acid. Also, a higher proportion (58.3%) of the human isolates, compared to fermented food isolates (36.4%), produced deconjugation zones with diameters of 3 to > 6.0 mm, while growing on either of the salts or both.

Quantitative assessment of bile salt hydrolases

BSH activity was assayed spectrophotometrically by determination of taurine and glycine release. The 34 Lactobacillus isolates and 4 reference strains, *L. acidophilus, L. fermentum*, *L. rhamnosus*, and *L. casei*, when subjected to quantitative BSH assay, gave mean BSH activities of 528 to 76.5 units (Fig. 3). Overall, mean average BSH activity was always higher in value with the 30 min-incubated assay, compared to 10min incubation, irrespective of whether isolates were from a human or fermented food origin. For most of the other isolates (15 isolates), and on an individual basis, BSH activity varied substantially ($p < 0.05$), depending on whether enzyme activity was measured after 10 or 30 min. Four (4) isolates (3BM4, 3BM3, GR4, and AK5) exhibited significantly ($p < 0.05$) higher BSH activity at 10 min than at 30 min, however for fifteen (15) of the other isolates, increasing assay time to 30 min resulted in markedly ($p < 0.05$) more BSH activity than was observed after 10 min of incubation. The highest value for BSH activity was exhibited by *L. casei* 3ST7 (76.45U),

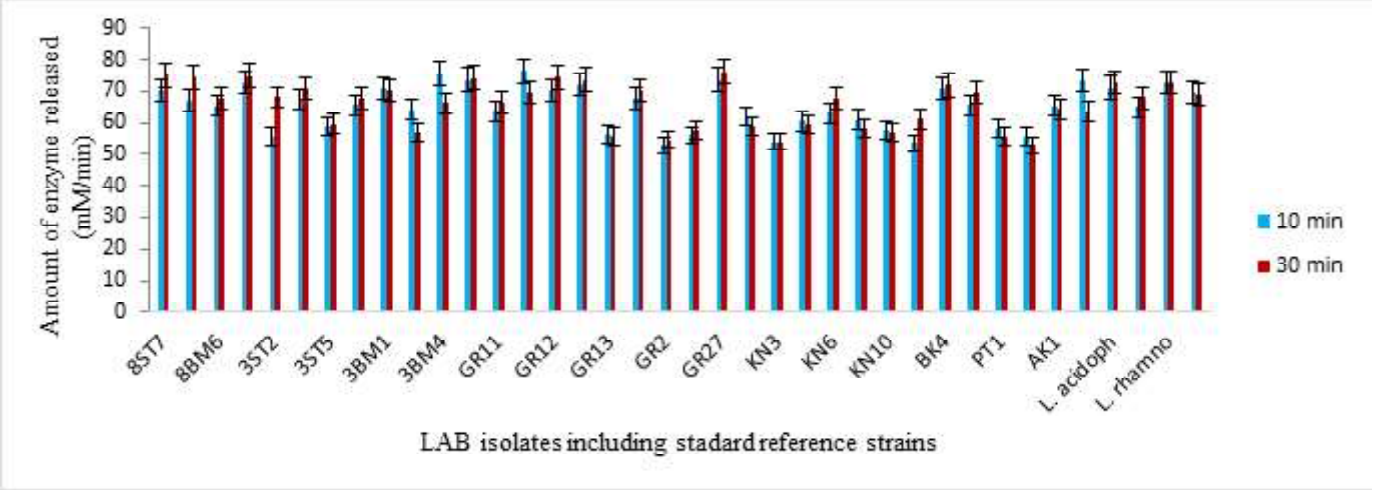

Fig. 3 Intracellular bile salt hydrolase activity of LAB at 10- and 30-mins incubation. Amounts of enzyme were expressed in mM/min Results are shown as mean ± SD (n=3)

after 30 min of assay), followed immediately in sequence by *L. casei* GR4 (76.41U), with the 10 min assay protocol. This isolate however showed less activity with the 30 min protocol. On the other hand, BSH activity when assayed at both 10 and 30 min was statistically always similar for 15 isolates (NON4, PT1, KN3, KN10, KN5, AK1, GR5, GR13, GR2, GR32, GR27, 8BM6, 3BM1, 8BM9 and GR8). BSH activity, for the four reference strains, *L. acidophilus, L. fermentum, L. rhamnosus,* and *L. casei,* remained also always similar, irrespective of assay time. To establish whether results of bile salt precipitation assay could be used directly as indicator of the actual (quantitative) BSH production by isolates, a correlation analysis was undertaken. The correlation coefficient (R^2) was 0.0624. On average, isolates from human sources exhibited markedly ($p < 0.05$) higher BSH activity (68.04U), compared to their counterparts (Fig. 4a and 4b) isolated from fermented foods (62.38U). A total of 12 isolates (including 7 and 5 from human and fermented food sources, respectively) exhibited BSH activities which were similar or higher than the highest BSH value (72.69U) given by the highest BSH-producing reference strain (*L. rhamnosus*). Based on the results from qualitative plate assay and quantitative CFE determination, 12 cultures were selected for cholesterol assimilation assay.

Several factors, including microbial strain and growth condition, affect BSH production by probiotic organisms. In the current work, BSH activity ranged from 52.9U – 76.4U and 52.8 – 76.5U, depending on whether enzyme assay was carried out using the 10 or 30min assay protocol, respectively. Also, the highest BSH activity by the 10 min assay protocol was given by isolate GR4, from garri fermentation, in contrast to that of the 30 min assay protocol (76.5U) obtained with 8ST7, an isolate from infant stool. Variations in fixed-time BSH activity for the 10 and 30min assays are possibly due chiefly to differences in enzyme reaction rates occasioned by variations in enzyme: substrate ratios, over the duration of assay. In a previous study, Corzo and Gilliland (1999), who had made similar observations as above, had resolved the discrepancy in fixed-time assay BSH values by diluting enzyme samples and, subsequently, correcting for dilution factors. If the above explanation is accepted as valid, then the implications for the 15 isolates, for which BSH activities were similar during the 10 and 30min assays, is that their enzyme reaction rates remained constant, probably because their enzymes did not operate under conditions of complete substrate limitation throughout the assay period (Corzo and Gilliland, 1999). On the other hand, for the 4 isolates (3BM4, 3BM3, GR4, and AK5), exhibiting significantly higher ($p < 0.05$) BSH values when assayed by the 10min protocol, the marked reduction in enzyme activity at 30min could be ascribed to possible substrate limitations, probably due to initial faster reaction rates (rapid substrate consumption), by their BSH enzymes. It is quite notable that, with the 10min protocol, three of these isolates showed, sequentially (GR4, 76.41U; 3BM4, 75.34U, and: AK5, 73.22U), the three highest BSH enzyme activities of all the isolates tested. BSHs and the bacterial penicillin V acylases (PVAs), with their quite striking sequence and structural homology, as well as distinct significant similarities in active site architecture and catalytic mechanisms, are, together, members of the N-terminal nucleophile hydrolase structural superfamily (Kumar et al. 2006; Ru et al. 2018; Daly et al. 2021). This implies that, like PVAs, BSHs may be liable to inhibition of their activity by their products. It is, thus, additionally possible that the lower BSHs values for isolates 3BM4, 3BM3, GR4, and AK5, when assayed by the 30-min fixed-time protocol, be partly due to inhibition of their BSH activities by products of these enzymes' actions on oxgall, the assay substrate. Indeed, Kumar et al. (2006) showed evidence of the competitive inhibition by cholic acid (CA) and deoxycholic acid (DCA) of the *B. longum* BSH activity. For the remaining 15 isolates, the BSH activity was always markedly ($p < 0.05$) higher with the 30min protocol. This could be attributed due to the complex processes involved in the processing of the complex bile substrate by the BSH enzyme

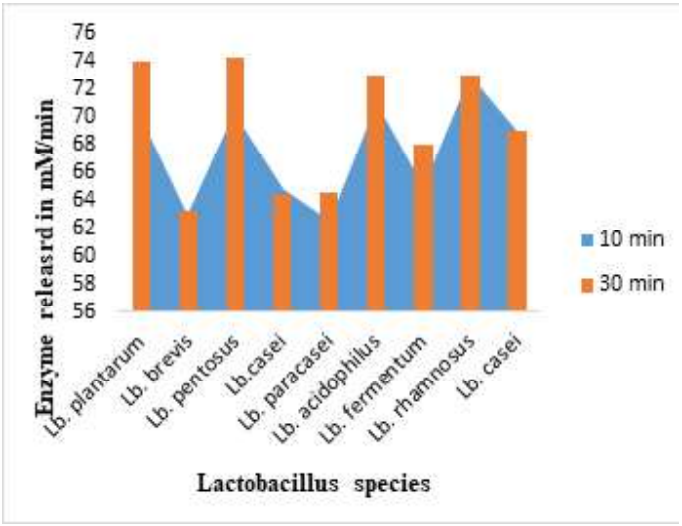

Fig. 4a Amount of enzyme released as compared by each of the species expressed in mM/min based on one way ANOVA. Results are shown as mean ± SD (n=3) (P<0.05 by t-test).

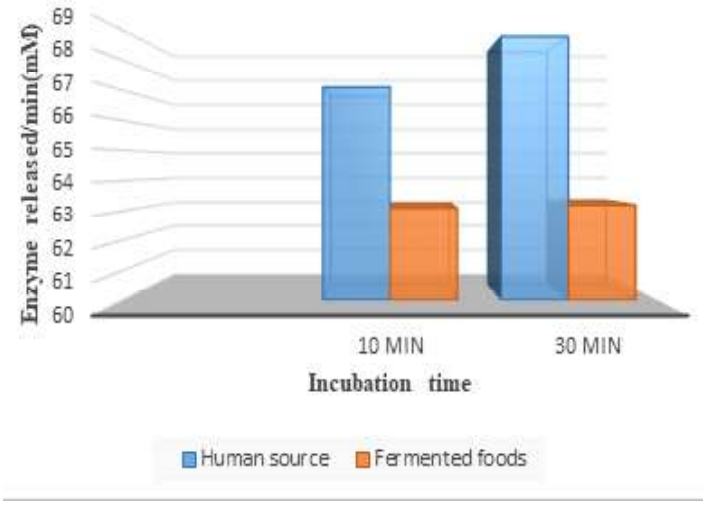

Fig. 4b. Amounts of enzyme released as compared by human source (n=12) and fermented foods (n=22) expressed in mM/min. Results are shown as mean ± SD (n=3)(P<0.05 by t-test)

activity of these microbes. In the current study, unlike that reported by Corzo and Gilliland (1999), oxgall, a mixture of taurocholic acid and glycocholic acid (in addition to cholesterol and lecithin), instead of sodium glycolate or sodium taurocholate, was the substrate for BSH assay. In a previous study investigating the activities of different microbial BSHs towards human bile salts, Jiang et al. (2010) had observed a great drop in BSH activity when oxgall was included in the growth medium of some strains of Lactobacillus (*L. gasseri* and *L. acidophilus*), but not in others, suggesting the possibility that components of bile (oxgall, in the current instance) may exert some complex as yet unknown regulatory process on the manner in which different BSHs process their bile acid components. In fact, Patel et al. (2010) have reported that exposure to bile engendered a 6-fold increase in *L. plantarum* BSH1 activity, while simultaneously inhibiting the organism's BSH3 ability to deconjugate bile acids by 5 times.

Our isolates, alongside *L. acidophilus*, *L. fermentum*, *L. rhamnosus*, and *L. casei* (reference strains), gave a mean range of 52.9 to 76.5U in BSH activity (Fig. 3). These results show that BSH production by our isolates generally conformed with the range of BSH production *(*4.78 to 93.0U and 2.31 to 42.62U of BSH activity, on oxgall and taurodeoxycholate (TDC), respectively) reported by Marius et al. (2018) from their fermenting cassava Lactobacillus isolates, although the lowest values of BSH given by our isolates far outstripped the lowest recorded by those authors on oxgall and the highest value obtained by the same authors for their isolates on TDC. On average the BSH activity of our isolates was also higher than that the 22.18 – 57.63U as reported by Bhat and Bajaj (2020) for their isolates. On the other hand, the highest BSH activity of our isolates fell short of values of 93.0U and 99.0U reported for their highest BSH producers by Marius et al. (2018) and Kumar et al. (2011), respectively. Parvez et al. (2006) had used precipitation zone diameters on BSH qualitative assay plates as measures of isolates' capacities to produce BSH, as well as an index of their bile salt deconjugation efficiencies. An attempt, in this work, to investigate the correlation between diameters of bile salt deconjugation zones on qualitative plate assays and actual BSH values, obtained via quantitative analysis, shows a complete lack of relationship, suggesting the ineffectuality of using deconjugation zone diameter as effective predictor of isolates' actual capacities to deconjugate bile salts. This lack of effective relationship between qualitative plate assessment results and actual BSH activity is, in this work, further highlighted by findings that isolates, like GR4, which showed quite low zones of precipitation on agar plates gave one of the two highest values (76.4U) for actual BSH activity obtained during the course of the current study . Essentially, BSH is a cytoplasmic enzyme. It may thus be that differences in bile acid precipitation zone diameters observed during qualitative plate assays be related more to the readiness with individual microbial strains autolyze to release intracellular contents than to the actual amounts of the enzymes synthesized by isolates. Most importantly, BSH activity was present in strains isolated from fermented foods, ecosystems naturally deficient in bile salts. This suggests that, contrary to previously held opinion (Tanaka et al. 1999; Begley et al. 2006), presence of bile acids may be no sine qua non for the successful isolation of BSH-active Lactobacillaceae from a given ecosystem. This assertion is supported by reports of Singhal et al. (2021) who, most unexpectedly, reported isolating highly BSH-active, hypocholestorelemic, probiotic-capable Lactobacillaceae from rhizospheric soils in India. Free-living bacteria isolated from marine sediments (*Methanosarcina acetorum) and Antarctica lakes (Planococcus antarticus*) have, most unexpectedly, been reported to also synthesize BSH (Jones et al. 2008). Prete et al. (2020) had proposed a complete reconsideration of the idea that

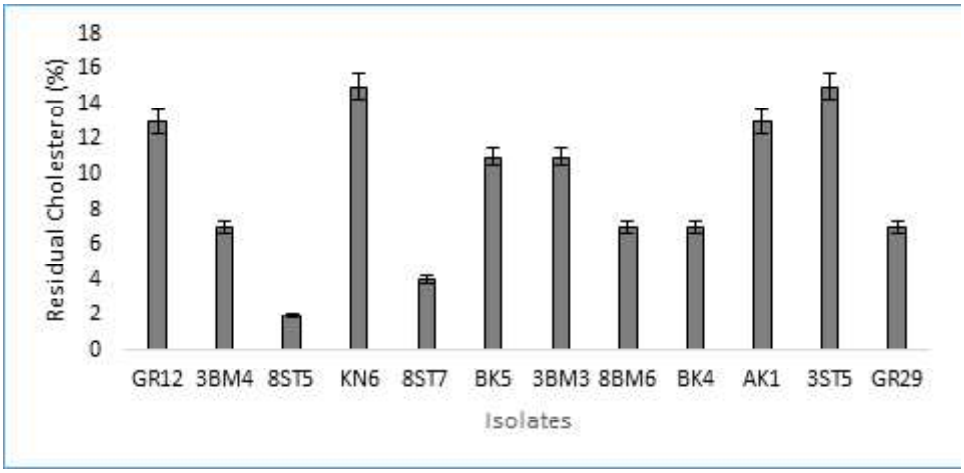

Fig: 5. Cholesterol removal by selected isolates after 24 h incubation in MRS-THIO containing 0.3g oxgall. Results are shown as mean ± SD (n=3)

BSH activity is strictly limited to intestinal residents or to tourist microbial strains. We strongly support that proposal. As in their study (Singh et al. (2021), Choi et al. (2015), Ding et al. (2017), the Lactobacillus isolates obtained from fermenting food ecosystems in the present work showed high BSH activity (sometimes, higher than was obtained for traditional enteric strains), despite not coming from enteric sources, or not having any known history of prior contact with bile salts-containing ecosystems. On average, *Lact obacillus* isolates from human sources appeared to show more ($p < 0.05$) BSH activity, compared to those of fermented food source origins, thus, somewhat, partially agreeing with the hypothesis that BSH activity may be more pronounced in *Lact obacillus* strains from bile salts-impacted ecosystems (Tanaka et al. 1999).

BSH expression has been proposed as an indirect indicator of the ability of potential probiotic strains to reduce serum cholesterol levels (Begley et al. 2006). Additionally, the application of highly BSH-active probiotics has been proposed as possible vital strategy for the management of serum cholesterol levels (Tsai et al. 2014; Bhat and Bajaj, 2020). BSH is believed to reduce serum hypercholesterolemia by creating increased demand for its (cholesterol's) *de novo* synthesis, itself, engendered by the gateway-catalyzed BSH deconjugation of primary bile salts and the products' subsequent modification to less soluble and less readily absorbed derivatives (like coprostanol) excreted with fecal matter (Patel et al. 2010; Chen et al. 2011 and Kumar et al. 2012).

Ability to assimilate cholesterol

The ability of LAB to remove cholesterol *in vitro* is shown in Fig. 5. All 12 tested isolates successfully removed cholesterol from the culture medium, although, individually, isolates varied substantially ($p < 0.05$) in their ability to remove the steroidal substrate. Residual cholesterol level, after 24h of cultivation, was reduced to ≤ 15%, in the following order: 8ST5 (2.0%) < 8ST7 (4.0%) < 8BM6, BK4, 3BM4, and GR29 (7.0%, each) < BK5 and 3BM3 (11.0%, each) < AK1 and GR12 (13.0% each) < 3ST5 and KN6 (15% each).

All the isolates tested in this study, assimilated above 50% cholesterol from the culture medium, with some removing up to 98% of initial medium cholesterol content. Although there are several reports of high-level cholesterol reduction by members of the Lact obacillus genus (Albano et al. 2018), only very few report cholesterol removal of up to 90% (Singhal et al. 2021), especially for isolates originating from sources other than those not naturally impacted by bile salts. For Lact obacillus strains coming from non-enteric sources, reduction of cholesterol to the levels observed for some of the isolates. In this study, residual cholesterol, after 24 h, ranged from 2.0 to 15.0%, indicating extensive (85–98%) removal by tested isolates of the steroidal substrate from the culture medium. There was difference statistically ($P<0.05$) between specific strains and percentage of cholesterol assimilation. Residual cholesterol levels were least with *L. pentosus* 8ST5 and *L. pentosus* 8ST7, while the strains *L. casei* KN6 and *L. casei* 3ST5 showed the highest value (H" 15%) of cholesterol remaining after 24h. These results for cholesterol removal, obtained in this work for our isolates, are quite high, comparing favorably to the 3.51 – 80.86%, 3.8 – 55%, and 48.84–60.52% reported by Choi et al. (2015), Ma et al. (2019), and Singhal et al. (2021), respectively, for cholesterol reduction in media not previously supplemented with bile salts. Ding et al. (2017) had also observed maximum cholesterol removal rates of up to 75.9% for a *L. plantarum* strain isolated from yak, a Tibetan fermented milk product. The fact that the values for cholesterol reduction by our isolates were achieved in media not previously supplemented with any form of bile salt, makes the results obtained in this study much more meaningful. From previous reports (Singhal et al. 2021 and Xiong et al. 2017), such high values for cholesterol reduction by members of Lact obacillus have been achieved only when the medium contained bile salts. For example, Singhal et al. (2021) markedly improved cholesterol reduction by *L. plantarum* strain NS14 to 92.20%, from 60.52%, just by including bile salts in the growth medium. In another study by Singhal et al. (2019), cholesterol assimilation by a rhizospheric *Enterococcus faecium* was markedly increased to 98% (from 75%), also by incorporation into the cholesterol

assimilation medium of bile salts. At 85%, the lowest value for cholesterol removal by our isolates (given by *L. casei* 3ST5 and *L. casei* KN6) surpasses in magnitude the highest values of cholesterol removal, obtained for their isolates by others (Choi et al. 2015; Ma et al. 2019 and Singhal et al. 2019; 2021). Such high values for cholesterol removal suggest, for present study, potential future roles in hypercholesterolemia management.

Our attempt to correlate BSH activity by the isolates to their cholesterol removal ability showed a weak relationship (R^2= 0.39), indicating the possibility that factors (or mechanisms) other than BSH activity may additionally be contributing to cholesterol removal by isolates. Indeed, other mechanisms, such as co-precipitation, adhesion to probiotic cell wall, micellar sequestration and assimilation have been shown to contribute to the hypocholesterolemic functions of probiotics (Brashears et al. 1998; Kimoto et al. 2002; Kumar et al. 2012; Tsai et al. 2014; Ding et al. 2017 and Ma et al. 2019). It may, therefore, just be that, unlike what has been reported by some others (Iranmanesh et al. 2014; Tsai et al. 2014), the BSH-engendered cholesterol precipitation is not sole (or principal mechanism) underlining cholesterol reduction by our isolates. Indeed Xiong et al. (2017) reported on an isolate of *L. casei*, which, despite not showing evidence of BSH production, displayed good ability to remove cholesterol from the culture medium.

Conclusions

Deconjugated bile salts are more inhibitory to pathogens than their conjugated counterparts, therefore isolates screened here for BSH activity will serve as good probiotics candidates when administered in food to colonize the gastrointestinal tract. Some strains were positive for one salt and not the other is because sodium salts have either of the amino acid groups and also differ in their steroid moieties at the alpha positions, which leads to the organisms being selective in substrate utilization and activity. Another reason may be that although, BSH is present, mechanisms that contribute to its expression may not be present under the conditions in which the organism is been cultivated; hence the need for further investigation for *bsh* genes is warranted. The presence of BSH activity in LAB from human sources particularly from the intestines is well documented but only a few literatures report it from fermented foods. This study documents the presence of high BSH activity of LAB of non-intestinal origin from fermented foods from Nigeria, Africa.

Many factors contribute in the production of BSH by organisms which makes the study a complex one. Among these are, sonication efficiency, oxidation which is not part of our study for now but can be investigated in future research. It is suggested that each study should clearly state the mechanism used by their cultures in cholesterol removal to give a clear picture to some extent on the results presented. Mechanism of cholesterol removal was not part of our study and so, we recommend further study to investigate same isolates for this. Our in vitro data shows that members of lactobacilli screened here are capable of assimilating cholesterol in their cell membrane, but we are not very sure as to whether the same effect will be exhibited in in vivo experiments. We therefore recommend in vivo evaluations to substantiate our result.

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RESEARCH ARTICLE

Coliform bacteria screening and evaluating chemical composition of raw milk from dairy farms of Sylhet Sadar, Bangladesh

Tonmoy Chowdhury[1], Shamsul Alam Roky[2], Md. Ashik-Uz-Zaman*[3], Md. Tanvir Islam[4] and Bikash Mohonto[5]

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Abstract: The study aims screening of coliform bacteria and evaluates chemical constituents of farm supplied raw milk at Sylhet sadar, Bangladesh. A total of 50 milk samples (5 samples from each farm) were collected from randomly selected 10 different farms. Samples were diluted (1:$10^{6)}$ and bacteria cultures were done in EMB (Eosin methylene blue) agar. Further, Indole test, Methyl red test and Voges-Proskauer test were done to differentiate environmental and fecal coliform (*Escherichia coli*). Chemical analysis of milk was done using automatic milk-analyzer. Total coliform count (TCC) was found to be ranged between 0.6 × 10^6 to 7.8 × 10^6 colony forming unit/ml (cfu/ml) and the biochemical tests showed all isolated coliform bacteria from milk samples were negative for Indole and Methyl red test, and positive for Voges-Proskauer test, respectively confirming the absence of fecal coliform (*Escherichia coli*) and presence of environmental coliforms. Mean values of Chemical constituents were found to be fat% (4.48%), Solid-Not-Fat% (8.55%), Protein% (3.20%), Lactose% (4.66%) and Mineral% (0.68%). Adulteration with added water in milk was found with a mean of 0.89%. This study will help to generate an idea about coliform contamination scenario and quality of farm milk found at the particular study area.

Keywords: Bangladesh; Coliform; Escherichia coli; Milk; Milk Composition,

Introduction

According to U.S. Public Health Services (1995), milk is the lacteal secretion obtained by full milking of one or more healthy cows, 5 days after and 15 days before parturition, containing not less than 8.5% milk solids-not-fat and not less than 3.5% milk fat (Paul et al. 2018). The amount of milk produced in Bangladesh is reported 10.47 million tons (Uddin et al. 2021) . Milk from dairy cows roughly comprises 90% of total milk production in Bangladesh and the rest comes from buffalos, sheep, and goats (Alam et al. 2018). With a population of 890,180 people, Sylhet city requires 78.59 tons (considering 250 ml/person) of milk per day and also with a 4.5% population increase rate milk consumption is increasing parallelly (World Population Review 2021). To cope up with increasing milk demand new farms are being introduced simultaneously- raising concern regarding the safety of public health due to the consumption of unsafe and poor-quality milk (Uddin et al. 2021). Moreover, milk adulteration not only alters the chemical quality of milk but also increases the risk of introducing germs into milk.

Milk itself acts as an excellent culture medium for the growth and multiplication of a wide variety of microbes. Microorganisms can enter milk in various ways, including the udder, cow's body, floor, flies, insects, rodents, milker, milk utensils, and the environment to contaminate and affect the quality of milk (Cousin 1982). In Sylhet, milk that is collected by the middleman and supplied to the consumer is often found to be adulterated with water and other things, and poor in quality (Paul et al. 2018). Among various microorganisms, coliforms are almost always present in raw milk which indicates unhygienic processing, inappropriate handling of either milk or milk utensils, fecal and environmental contamination (Kagkli et al. 2007; Mhone, Matope, and Saidi 2011; Salman and Hamad 2011). However, coliform bacteria not only portray intestinal (fecal in origin) bacteria but also other free-living (non-fecal in origin) coliforms (Paruch and Mæhlum

[1,2,3] Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[4] Department of Physiology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[5] Executive, Veterinary services, Agro-vet division, Square Pharmaceuticals Ltd., Sylhet-3100, Bangladesh

Md. Ashik-Uz-Zaman (✉)
Dept. of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh
E-mail: ashik.ds@sau.ac.bd

2012). Among the coliforms, the most prevalent contaminant in raw and processed milk is *Escherichia coli*, which is the most accurate indicator of fecal contamination (Mhone et al. 2011). Although most of the *Escherichia coli* strains are not harmful, but the existence of enteropathogenic and/or toxigenic strains in food may result in public health risk. Infants and young children are susceptible to enteropathogenic *Escherichia coli* strains, which can cause extreme diarrhea and vomiting, while toxigenic strains such as *Escherichia coli* O157:H7, produce hemolytic uremic syndrome (Mhone et al. 2011; Paruch and Mæhlum 2012).

In the Sylhet sadar region, different studies were conducted to analyze microbiological and physio-chemical properties of farm milk (Alam et al. 2018; Kader et al. 2015; Paul et al. 2018). Those studies revealed total coliform count without differentiating environmental and fecal coliforms, and physio-chemical properties may also alter with time, based on the introduction of new farms. Considering those facts, the sole purposes of this study were to do screening of coliform bacteria (environmental and fecal origin) and to evaluate the major chemical compositions of farm supplied raw milk at Sylhet Sadar, Bangladesh.

Methods and Materials

Study Period and Study Sites

The study was conducted from 1st December 2020 to 31st January 2021. A total of 50 samples (5 samples from each farm) were collected from randomly selected 10 dairy farms situated at Sylhet Sadar, Sylhet-3100, Bangladesh. Microbiological and chemical analyses (Fat%, SNF%, Protein%, Lactose%, Mineral%, Added Water%) were performed in Dairy Technology Laboratory under the Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University-3100, Sylhet, Bangladesh.

Sampling

Before collection of the milk from the bulk milk container, the container was stirred thoroughly for the uniform mixing of milk. From each farm, each day approximately 50 ml of raw milk were collected for consecutive 5 days. Samples were collected on aseptic clean plastic tubes and stored in an airtight icebox and transported to the laboratory in aseptic condition. The samples were examined quickly after arriving at the laboratory.

Microbiological tests

Colony count of coliform bacteria was done using Eosin Methylene Blue (EMB) agar (Oxoid media, UK) as growth media. 1ml of diluted (10^{-6} dilution factor) raw milk was taken from each raw milk sample and was poured on the petri dish followed by autoclaved EMB agar. Petri dishes were incubated for 24 hours at 37° Celsius temperature. After incubation, Petri-dishes were observed for pink colonies to be identified as coliform and green metallic sheen with a dark circle in the center were identified es *Escherichia coli* colony (Antony et al. 2016). In case of biochemical tests, Indole test, Methyl red test, Voges-Proskauer were done to differentiate environmental coliform from fecal coliform (*Escherichia Coli*) (Islam et al. 2021; Paruch and Mæhlum 2012).

Chemical analysis

Ultrasonic Milk analyzer (Lactoscan) manufactured by WINCOM was used for the analyses of chemical properties (Fat%, Solid-

Table 1 Microbiological test results of collected raw milk samples from different farms

Farm ID	Total Samples (n)	Colony positive samples in EMB agar	Mean TCC (cfu/ml)	Indole Test Positive Samples	Methyl-Red Positive Samples	Voges-Proskauer Positive Samples
Farm1	5	3	3.4×10^6	0	0	3
Farm2	5	5	7.8×10^6	0	0	5
Farm3	5	3	3.6×10^6	0	0	3
Farm4	5	3	4.6×10^6	0	0	3
Farm5	5	1	0.6×10^6	0	0	1
Farm6	5	2	2.8×10^6	0	0	2
Farm7	5	3	1.8×10^6	0	0	3
Farm8	5	4	3.2×10^6	0	0	4
Farm9	5	2	1.0×10^6	0	0	2
Farm10	5	2	1.8×10^6	0	0	2
Total	50	28	-	0	0	28
p-value		-	0.155	-	-	-

In the table, TCC = Total coliform count which is showed as cfu/ml meaning Colony forming unit per milliliter. Statistical significance was considered as ($p < 0.05$).

not-fat% (SNF%), Protein%, Lactose%, Mineral% and Added Water%).

Statistical analysis

Data were recorded in Microsoft Excel 2019 and the descriptive analyses was done using both IBM SPSS Statistics v26 and Microsoft Excel 2019. We conducted one-way ANOVA to compare the means.

Results and Discussion

Microbiological analysis

The result of microbiological test with EMB (Eosin Methylene Blue) agar showed a total of 28 samples out of 50 raw milk samples grew several colonies including green metallic sheen with a dark circle in the center (Table 1), which had been stated as the characteristic of coliform bacteria colony in previous study (Antony et al. 2016). Hence, raw milk samples supplied from farms had presence of coliform bacteria. Microbiological test results demonstrate total coliform count (TCC) ranges from 0.6×10^6 to 7.8×10^6 cfu/ml (Table 1). The total coliform count (TCC) showed no significant ($p>0.05$) difference among samples (Table 1). In a previous study it was found that total coliform count (TCC) ranged between 1.0×10^4 and 2.0×10^5 cfu/ml in raw milk which is lower than the obtained results of current study (Banik, Das, and Uddin 2014). On the other hand, (Sraïri et al. 2006) found total coliform count ranged between less than 30 to 20.8×10^6 in raw milk. Another study found presence of coliform bacteria at a higher number ranging between 6.34×10^{10} to 7.50×10^{10} cfu/ml in raw milk (Kakati et al. 2021) which supports the current fundings (Table 1).

Further in (Table 1), all the samples (n=28) showed negative results in case of the Indole test and the Methyl-Red test, but in the case of Voges-Proskauer test all the samples (n=28) gave positive results. *Escherichia coli* gives positive results in Indole test and Methyl-Red test among Indole test, Methyl red test and Voges-Proskauer test; other coliforms except *Escherichia coli* give positive results in case of Voges-Proskauer and/or Citrate utilization test and shows negative results in Indole test and Methyl Red test (Zhang et al. 2017). Hence, the results depicts that all the milk samples were free from *Escherichia coli* which is considered as a fecal coliform (Devane et al. 2020). But the positive result of Voges-Proskauer depicts that samples contained other non-fecal origin or environmental origin coliform. So, the contamination of milk may be occurred from milkers and/or uncleaned utensils. (Banik et al. 2014) found presence of both

Table 2 Comparison among chemical constituents of collected raw milk samples from different farms

Farm ID (Total samples)	Fat % (Mean ± SD)	SNF % (Mean ± SD)	Protein % (Mean ± SD)	Lactose % (Mean ± SD)	Mineral% (Mean ± SD)	Added Water % (Mean ± SD)
Farm1 (n=5)	2.52 ± 0.03	8.83 ± 0.12	3.24 ± 0.09	4.92 ± 0.01	0.74 ± 0.10	0 ±.00
Farm2 (n=5)	4.78 ± 0.05	7.43 ± 0.13	2.71 ± 0.06	4.08 ± 0.02	0.59 ± 0.07	8.91 ± 0.53
Farm3 (n=5)	4.70 ± 0.07	9.51 ± 0.10	3.44 ± 0.12	5.18 ± 0.05	0.76 ± 0.12	0 ± .00
Farm4 (n=5)	4.86 ± 0.04	8.53 ± 0.24	3.23 ± 0.05	4.80 ± 0.03	0.70 ± 0.12	0 ± .00
Farm5 (n=5)	6.40 ± 0.08	8.69 ± 0.13	3.17 ± 0.01	4.61 ± 0.14	0.69 ± 0.12	0 ± .00
Farm6 (n=5)	8.05 ± 0.06	8.13 ± 0.04	2.82 ± 0.07	4.55 ± 0.09	0.63 ± 0.12	0 ± .00
Farm7 (n=5)	3.36 ± 0.03	9.12 ± 0.01	3.37 ± 0.33	5.61 ± 0.01	0.72 ± 0.12	0 ± .00
Farm8 (n=5)	3.47 ± 0.13	9.04 ± 0.02	3.28 ± 0.03	4.92 ± 0.03	0.71 ± 0.12	0 ± .00
Farm9 (n=5)	3.15 ± 0.04	8.18 ± 0.05	3.31 ± 0.16	4.14 ± 0.03	0.65 ± 0.14	0 ± .00
Farm10(n=5)	3.52 ± 0.10	8.05 ± 0.04	3.43 ± 0.18	3.76 ± 0.18	0.60 ± 0.15	0 ± .00
p value	0.000	0.000	0.000	0.000	0.343	0.000

In the table, SD = Standard deviation and SNF = Solid-Not-Fat. Statistical significance was considered as ($p < 0.05$).

Table 3 Average value of chemical constituents of collected raw milk

Properties	Total Number of Samples(N)	Mean±SD	95 percent confidence interval	
			Lower range	Upper range
Fat (%)	50	4.48 ± 1.61	4.02	4.94
SNF[b] (%)	50	8.55 ± 0.59	8.38	8.72
Protein (%)	50	3.20 ± 0.27	3.12	3.28
Lactose (%)	50	4.66 ± 0.53	4.50	4.81
Mineral (%)	50	0.68 ± 0.12	0.64	0.72
Added Water (%)	50	0.89 ± 2.70	0.12	1.66

In the table, SD = Standard deviation and SNF = Solid-Not-Fat

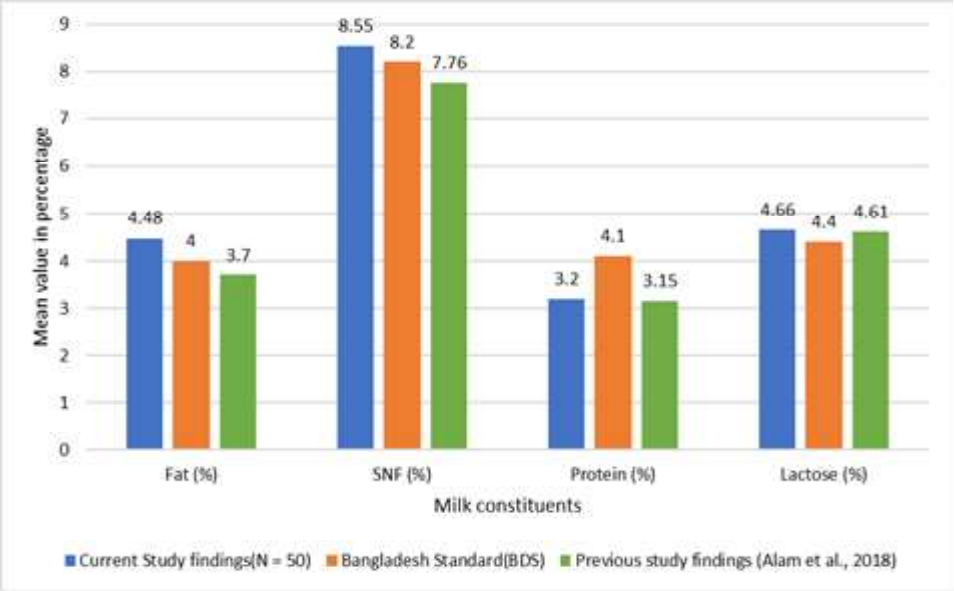

Fig 1. Comparison of mean value of major milk constituents among current study findings, Bangladesh standard (BDS) and previous study findings

environment and fecal coliforms and stated these contaminations as a result of faulty hygiene management. On the other hand, based on (Paul et al. 2018) study findings, it was assumed that the degradation of milk quality (chemical and microbiological) might be a result of adulteration done by middleman (who collects the milk from farms and supplies to consumers). However, we collected the milk samples directly from farm without the involvement of middleman. So, the current study results demonstrate that environmental contamination may start from the events of milk collection and storing procedure.

Chemical analysis

The results of chemical analysis and comparison of different chemical constituents of milk have been shown in Table 2, Table 3 and Figure 1.

Fat

According to (Table 2), it can be depicted that fat% was the highest in Farm6 (8.05%) and the lowest in Farm1 (2.52%). Average fat% was found 4.48% in the collected milk samples with a standard deviation of 1.61 (Table 3). Also, significant difference ($p < 0.05$) was found among fat% of farm samples which supports the findings of (Alam et al. 2018) (Table 2).

Solid-Not-Fat (SNF)

We found the highest solid-not-fat (SNF%) in Farm3 (9.51%) and the lowest in Farm2 (7.43%) (Table 3). Average SNF% was found 8.55% with a standard deviation of 0.59 in the collected milk samples (Table 3). (Alam et al. 2018) found significant differences ($p < 0.05$) among SNF% which is similar to the current study findings ($p < 0.05$) (Table 2).

Protein

In case of protein% the lowest value was in Farm2 (2.71%) and the highest was in Farm3 (3.44%) (Table 2). The mean protein% we found in the collected raw milk samples was 3.20% (Table 3). However, (Alam et al. 2018) found no significant difference ($p > 0.05$) among protein% which contradicts with the findings of current study findings ($p < 0.05$) (Table 2).

Lactose

Farm7 contained the highest lactose% of 5.61% and Farm10 had the lowest lactose% (3.76%) (Table 2). Mean lactose% was found 4.66% and standard deviation was 0.53 (Table 3). The differences among mean lactose% of farms samples were significant ($p < 0.05$) which supports the study of (Alam et al. 2018) (Table 2).

Mineral

Mineral% was the highest in Farm3 (0.76%) and the lowest in Farm2 (0.59%) (Table 2). The average mineral% was found to be 0.68% (Table 3). The average mineral% was slightly lower than the findings of (Paul et al. 2018) who reported mineral% range between 0.73% and 0.81%. However, difference found among the farm milk samples in current study were not significant ($p > 0.05$) which supports the findings of (Paul et al. 2018) (Table 2).

Added water

Added water was found only in Farm2 milk samples among all the farm samples (Table 2). The average added water% was 0.89% (Table 3) and the difference among mean added water% of milk samples were significant ($p < 0.05$) (Table 2). This indicates the adulteration of raw milks which can degrade the nutritional quality of milk. (Paul et al. 2018) also reported 25% farms from which

consumers collect milk directly, seems to adulterate milk with water. Moreover, (Paul et al. 2018) also reported, when a middleman supplies milk from farm to consumer,75% of the farm milk was found adulterated with water.

Comparison of milk constituents with Bangladesh standard (BDS) and previous findings

The mean fat% (4.48%) is higher than the findings of (Alam et al. 2018) (3.7%) and the Bangladesh standard (BDS) (4%) (Hossain and Dev 2013) (Figure 1). The mean SNF% (8.55%) of current study is higher than the findings of (Alam et al. 2018) (7.76%) and Bangladesh Standard (8.2%) (Hossain and Dev 2013) (Figure 1). Average protein% (3.20%) of current study seems to be lower than the protein% values of Bangladesh Standard (4.1%) (Hossain and Dev 2013), but slightly higher than the findings of (Alam et al. 2018) study (3.15%) (Figure 1). The average lactose% (4.66%) of current study is somewhat higher to the standard of BDS (4.4%) (Hossain and Dev 2013) and findings of (Alam et al. 2018) (4.61%) (Figure 1).These differences may occur due to several factors like study area, feeding, farming condition, season, parity etc.

Conclusions

The result of the present study depicted that the raw milk supplied from the dairy farms of Sylhet Sadar had no presence of fecal coliform (*Escherichia coli*) but there was presence of environmental origin coliform bacteria. Among chemical properties of milk, we found fat%, SNF% and lactose% higher than the requirement of Bangladesh Standard (BDS) but the protein% were lower than the Bangladesh Standard (BDS). However, the presence of added water was found only in one farm sample. Further studies with larger samples are needed to assess the scenario of milk adulteration.

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RESEARCH ARTICLE

Optimization of biofunctional jaggery yogurt: It's physicochemical and antioxidant properties

Pankaj[1], Abhishek Dutt Tripathi and Aparna Agarwal[2]

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Abstract: In addition to the standard healthy diet needs, balance food is taught to be a healthier diet that will promote human health. It's also known as functional food, which is food that clearly claims to have health advantages as well as the ability to enhance the immune system. Yoghurt is a fermented dairy product that is in great demand for its nutrient content, bacterial activity and wide product range in terms of flavours and textures. In this study, physicochemical, microbiological and sensory differences of probiotic set yoghurt incorporated with different concentration of Jaggery were evaluated, as well as the changes taking place during storage at 4°C for 21 days. During the storage, the addition of jaggery improved survival of *Lactobacillus delbrueckii* subspecies *bulgaricus* (ABT-7) and *Streptococcus thermophiles* (YoFlex Express 1.0) from CHR HANSEN, Denmark were used as starter culture for yogurt preparation. All yogurts exhibited a decrease in pH accompanied by an increase in titratable acidity during storage. Therefore, this study revealed that jaggery could be used to produce probiotic set yoghurt with improved physicochemical, microbiological and sensorial attributes.

Keywords: Jaggery; Milk; Microbial Analysis; Physicochemical; Sensorial; Yoghurt

[1]Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005

[2]Department of Food Science and Nutrition, Lady Irwin College, University of Delhi, New Delhi

Abhishek Dutt Tripathi (✉)
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005
Email: abhishek.tripathi@bhu.ac.in; Mob: +91-9450533651

Introduction

Sugarcane (*Saccharum spp*.) is a major cash crop grown in more than hundred nations, mostly for sugar and bioethanol. Despite the fact that India is the world's second largest producer of sugarcane, after Brazil, its output must be increased to meet the growing need for renewable energy and green energy **(**Aguiar et al. 2016). Sugarcane is extremely important to the country's economy. In 2018, 79.9% of India's total sugarcane production went into the manufacturing of white sugar, 11.29 percent went into the production of jaggery, and 8.80 percent went into seed and feed materials. Jaggery, also known as Gur, is an unrefined, unpurified sugar that has been consumed throughout the Asia, Africa, Latin America, and the Caribbean for thousands of years (Pandraju et al. 2021; Revathy et al. 2021; Rao and Singh, 2021). According to FSSAI (2018), "Gur or Jaggery means the product obtained by boiling or processing juice pressed out of sugarcane or extracted from palmyra palm, date palm or coconut palm". It is natural sweetener with win fragrance and flavour. A quality jaggery is golden yellow in colour, hard in texture, crystalline in structure, sweet in taste and low in moisture. A good quality Jaggery/Gur contains over 70% sucrose, below 10% glucose and fructose (invert sugars), less than 5% minerals and under 3% moisture (Ghosh et al. 1998). Most of traditional sweets use jaggery as a sweetener. Jaggery has number of advantages in comparison to conventional table sugar as it is, high in minerals like calcium, potassium, and magnesium (Chandrakanth et al. 2019). Magnesium, which is contained in jaggery, has been shown to improve our neurological system, help with muscular relaxation, reduce fatigue, and protect our blood vessels. Magnesium, when combined with selenium, acts as an antioxidant, scavenging free radicals from our body. Jaggery contains potassium and a trace of sodium, which aid in the maintenance of the acid–base balance in our cells, as well as the regulation of acids, acetone levels, and blood pressure. It is a good source of iron and helps to prevent anaemia (Jaffe, 2012). Thus, jiggery contains variety of nutrients that are essential for growth and maintenance of human body. (Shori et al. 2021). However, in the majority of sweet items such as bread, confectionery, dairy, and beverages, the processed food industries mainly relies on refined sugar as a sweetener and bulking agent. Milk and Milk products have been an important part of Indian culture because of their health-promoting properties

and organoleptic features. In India, 50% of total milk produced is utilized for traditional milk products manufacture (Bandyopadhyay and Khamrui, 2007). Among the various traditional dairy products, fermented milk products provides significant nutritional value as well as the health benefits (Baba et al. 2014). According to FSSAI, 2016 "Fermented Milk is a milk product obtained by fermentation of milk, which may have been manufactured using other permitted raw material, by the action of suitable microorganisms and resulting in lowering of pH with or without coagulation (iso-electric precipitation). Fermented milk may be heat treated after fermentation. The raw material used shall be subjected to a heat treatment as defined in the General Standard for Milk and Milk Products." Yogurt is one of the categories of the fermented milk in which symbiotic cultures of S*treptococcus thermophilus* and *Lactobacillus delbrueckii sub sp. bulgaricus* should be used. Microorganisms present in the yoghurt provides therapeutic benefits such as immune system modification, cholesterol reduction, relief in lactose intolerance, improved gastrointestinal microbial balance, and antioxidant activity (Oak and Jha, 2019; Shori et al. 2021). Due to sulfur-containing amino acids, bioactive peptides, vitamins, and minerals created during fermentation, fermented dairy products such as yoghurt have been recognised to have strong antioxidant activity (Alenisan et al. 2017). Consumers are demanding yoghurt due to its bioavailability of essential nutrients resulting from yoghurt bacterial activity (Hill et al. 2017).

Therefore, the present study was planned to study the effects of addition of jaggery on changes in post-acidification (pH and titratable acidity), viability of lactic acid bacteria of yoghurt, physicochemical analysis and antioxidant and total phenolic activity during storage . In addition, sensory evaluation of all yoghurt samples was conducted the first day of storage.

Materials and Methods

Lactobacillus delbrueckii Subsp. *bulgaricus* and *Streptococcus thermophilus* (YoFlex Express 1.0) procured from Chr Hansen, Denmark were used as starter culture for yogurt preparation. Raw milk used for the research work was procured from the Dairy Farm, Department of the Dairy Science and Food Technology, Banaras Hindu University, Varanasi. Fresh good quality Jaggery was procured from Sundarpur market Varanasi, India. All chemicals used were of analytical grade and were obtained from Sigma Chemical Company (St Louis, MO, USA).

Preparation of Yoghurt

As per FSSAI, yogurt should contain not less than 3.0 % fat, 8.0 % Milk solids-not-fat , 2.9% milk protein and minimum 0.6% acidity expressed as lactic acid. . Raw milk was standardized to 3.5 % fat and yoghurt was prepared from the standardized milk using two starter culture namely; *Lactobacillus delbrueckii* Subsp. *bulgaricus* and *Streptococcus thermophilus.* Jaggery was added as an additive at different levels (5%, 10% and 15%). Control yoghurt sample was prepared with the standard method using *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* as starter culture without any additive. All yoghurt samples were prepared using the method described by (Tamine and Robinson, 1999) with slight modification.

Control yoghurt

Milk was standardized using Pearson square method to 3.5 % fat. It was pasteurized at 80-85 °C for 30 mins and homogenized at 17 MPa at 60-70 °C. It was cooled to 42 °C and inoculated with 1% starter culture. The obtained final mixture was poured in pre-sterilized LDPE cups and incubated at a temperature of 40 – 42 °C for 3 to 4 hours. After 4 hours, pH was measured and fermentation process was stopped by cooling and storing it at a temperature of 4-6 °C for further analysis

Jaggery yoghurt

Milk was standardized using Pearson square method to 3.5 % fat. It was pasteurized at 80-85 °C for 30 mins and homogenized at 17 MPa at 60-70 °C. Temperature was decreased to 60-62 °C and jaggery was added at different levels vis-a-vis 5, 10 and 15 % and stirred well to mix the ingredients . The mixture was cooled to 42 °C and inoculated with 1% starter culture. It was poured in pre-sterilized LDPE cups and incubated at a temperature of 40 – 42 °C until pH of 4.5 was achieved. After 4 hours of incubation, pH was measured and fermentation process was stopped by cooling and storing it at a temperature of 4-6 °C for further analysis (Figure 1a).

Total Solids content

The total solids content of the raw milk and yoghurt samples was determined according to the gravimetric method described in (AOAC, 2007). The moisture was calculated as 100 -% total solids content.

pH measurement

The pH value of the raw milk and yoghurt samples was determined by a digital pH-meter (Lab Man Scientific Instruments, Chennai, Tamilnadu, India.) equipped with a glass electrode without dilution of samples.

Acidity

Percentage titratable acidity of the samples was measured by titration with 0.1 N NaOH (AOAC, 2005) and expressed as % lactic acid.

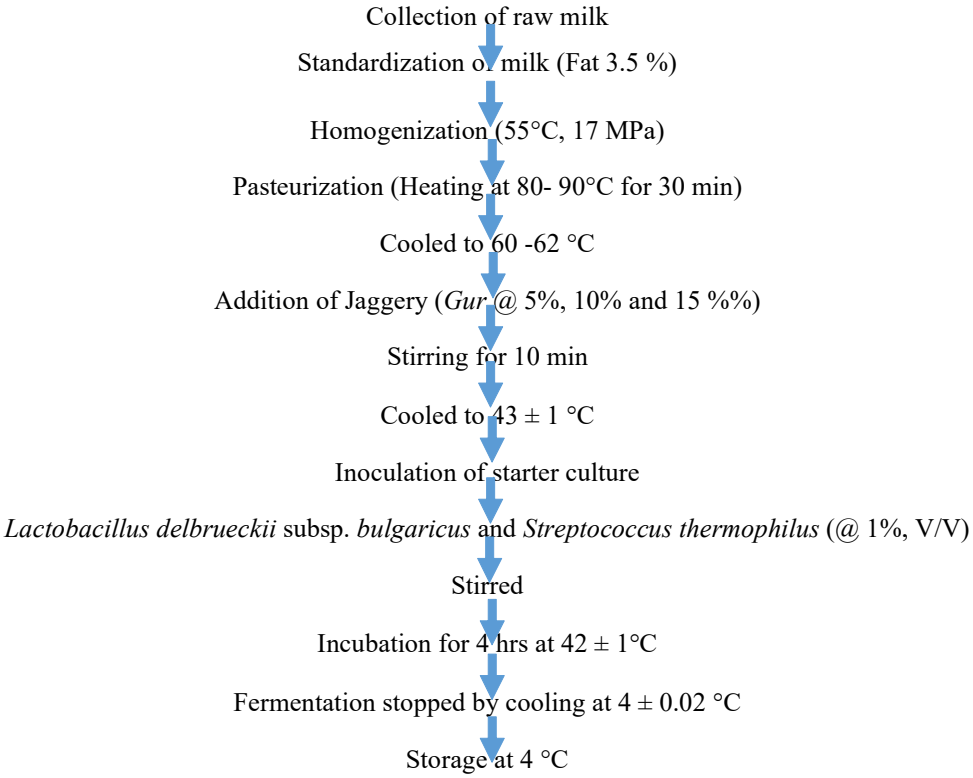

Fig.1a Flow diagram for preparation of Jaggery yoghurt

Ash content

Total mineral content by dry ashing procedure using muffle furnace (Maurice and Marshall, 2010).

Fat content

Fat content in the yogurt samples was estimated by standard method of (AOAC, 2005, Kleyn et al. 2001).

Protein content

Protein content in the samples was determined by Kjeldahl method considering factor as 6.25 (Lynch and Barbano, 1999).

Colour

The colour parameters L″ lightness (100) to darkness (0), a″ redness (+) to greenness (“), and b″ yellowness (+) to blueness (“) values were measured in a container using a colourimeter (Colour Flex EZ, (Model-CFEZ1048, Hunter Association Laboratory Inc., Reston, VA, USA). Standard white tile was used for calibration and measurements were conducted at room temperature. All measurements were taken in triplicate.

Determination of syneresis

The syneresis index of the yoghurt was determined according to Keogh and O’Kennedy (1998). Twenty grams of yoghurt sample was centrifuged (Sigma, 3-30k , Germany) at 1500 RPM for 10 min at 4 °C by using test tube. The clear supernatant was poured off, the weight of supernatant was measured, weighed, and recorded as syneresis (%), which was calculated by using equation-

$$\text{Syneresis (\%)} = \frac{Wf}{Wi} \times 100$$

Where W_i = weight of initial sample and W_f = weight of supernatant.

Texture profile analysis

Flow properties of the yoghurt samples were tested with viscosity and texture assessments. Viscosity values were measured with a viscometer (Brookfield Engineering Laboratories, model DV-II+, Inc., Stoughton, MA, USA) with spindle TE, and speed at 100 rpm after 24 h at 4 °C according to the slightly modified method of (Ghasempour et al. 2012). For textural analysis (TA. XT. Plus Texture Profile Analyser Stable Micro Systems, UK) was used with a solid rod probe back extrusion (35 mm diameter) into a cup holding the test sample using a 5-kg load cell (Marshall and

Rawson, 1999). The textural properties namely hardness and springiness were assessed. Each test was done in triplicate.

Antioxidant activity

The antioxidant activities of yoghurt samples were analysed following the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay (Dudonné et al. 2009).

Total phenolic content

Folin-Ciocalteau method was used to measure total phenolic content of the sample (Dudonné et al. 2009).

Sensory evaluation

Sensory evaluation of the final product was done by 30 trained panellists of the Department of the Dairy Science and Food Technology, Banaras Hindu University, Varanasi, India as described by the procedures in Folkenberg et al. (2006). The panellists analysed 5 sensory attributes as colour, odour, flavour, texture and overall acceptability according to the 9-point hedonic scale (9 –Liked extremely and 0 – Disliked Extremely). Yoghurt samples were coded and presented in transparent plastic cups under daylight and presented in random order.

Total Plate count, Coliform counts and LAB Count

Total Plate count, coliform counts and LAB count were performed as per the standard method described in FSSAI, 2011. Standard plate count agar was used for TPC and for ***Streptococcus*** *thermophilus* M17 agar, MRS agar for *Lactobacillus delbrueckii* subsp. *Lactis were being used for enumeration.* All plates were incubated at 37 ± 1 °C for 48 h and the viable cell count, coliform count and LAB count were calculated as CFU per gm.

Statistical analysis

All analytical experiments were repeated in triplicates and the results reported as a mean of the values along with standard deviation. The obtained data were statistically analysed by Software Minitab 17 is used to carry out statistical analysis and MS Excel data were evaluated by one-way ANOVA. The level of significance was set at $P < 0.05$.

Results and Discussion

Physicochemical analysis

The objective of this study was to optimize the process for preparation of the jaggery enriched yoghurt. Physicochemical properties of the control as well as jiggery yoghurt samples are depicted inTable 1. Moisture percentage of jaggery yoghurt ranged from 74 ± 0.24 % whereas the moisture values for control were 80.33 ± 0.17 %. Significant ($p<0.05$) difference in moisture with different treatments was observed. This may be due the fact that added jaggery contributes to the total solids owing to decrease in moisture content. This attributed to the increased storage stability of the developed product. Increase in total solids may also have a positive impact on the texture of the resultant product reported (Abd El-Tawab, 2009). Physicochemical analysis of yoghurt samples is depicted in Table 1.

For colour (Table 2), lightness (L*) decreased significantly ($p< 0.05$), whereas red/green (a*) increased due to addition of jaggery. Variation in the a* and b* value was significant ($p < 0.05$), as jaggery enriched yoghurt has high redness compared to control yoghurt. Jaggery contributes to the colour of the product. Fermentation process may also have affected the colour results from the absorption of water by mineral content, vitamins, antioxidants and phenolic contents in jaggery. The fermentation process might have favoured the release of some pigments from the jaggery, mainly carotenoids, making the product more yellow. Therefore, jaggery enriched yoghurt samples showed a higher b* value than the control yoghurt samples.

Addition of jaggery in yoghurt, substantially reduced syneresis. Syneresis is the separation of liquid that occurs in weak gel-like structures in yoghurt, and it is a visible defect with a negative influence on consumer acceptability. It is mainly related to rearrangement of protein molecules (or aggregation) caused by the difference in density between phases where whey proteins accumulate on the surface of yoghurt and expel the serum out of the food matrix. Syneresis was reduced with increased concentration of jaggery which may be attributed the complex

Table 1 Physicochemical analysis of Yogurt and Jaggery Yogurt

Parameters	Control Yoghurt	Jaggery Yoghurt 15 %
Total solids (%)	13.60 ± 0.22	20.60 ± 0.22
Total protein (%)	3.4 ± 0.14	3.6 ± 0.14
Fat (%)	3.52 ± 0.12	3.82 ± 0.12
Ash (%)	0.57 ± 0.196	0.97 ± 0.196
Titratable acidity (%)	0.73 ± 0.55	0.79 ± 0.55
pH	4.56 ± 0.13	4.46 ± 0.13

Table 2: Effect on colour and texture of control yoghurt and optimized jaggery enriched yoghurt

Parameters	Control yoghurt	Jaggery enriched yoghurt
Lightness (L*)	91.61 ± 0.86	67 ± 0.16
Redness (a*)	-0.70 ± 0.11	19.56 ± 0.24
Yellowness (b*)	13.62 ± 0.42	14.01 ± 0.31
Firmness (g)	41.22 ± 0.01	43.75 ± 0.02
Consistency (g.s)	811.42± 0.04	863.03 ± 0.03
Cohesiveness (g)	34.48 ± 0.03	36.33 ± 0.21

Table 3 Effect of storage period on sensory scores of control yoghurt

Parameters	Storage Days			
	0	7	14	21
Colour and Appearance	7 ± 1.01	6 ± 1.03	6.5± 0.92	5 ± 0.84
Flavour	8 ± 0.74	7 ± 0.61	6 ± 0.81	6.5 ± 0.83
Mouth feel	7 ± 0.57	6.5 ± 0.67	6 ± 0.71	5.5 ± 0.73
Texture	8 ± 1.07	7 ± 1.02	6.5 ± 0.84	6 ± 0.66
Whey Syneresis	8 ± 1.01	7 ± 1.03	6.5 ± 1.01	6 ± 1.01
Overall Acceptability	8.5 ± 1.01	7.5 ± 1.01	7 ± 1.01	6 ± 1.01

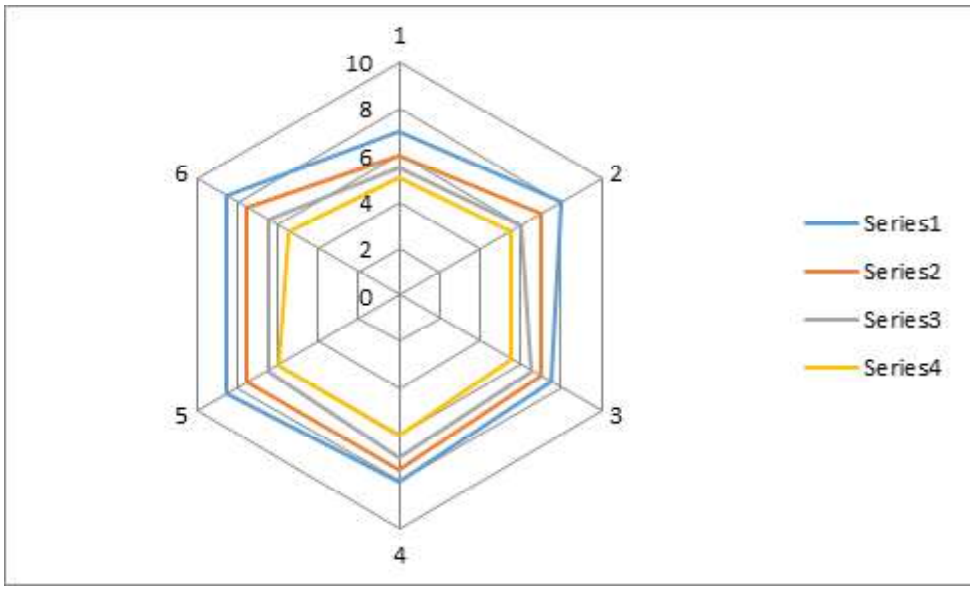

Fig. 1 (b) Effect of storage period on sensory scores of jaggery yoghurt(Series 1: Day 0, Series 2: Day 7.,Series 3: Day 14 and Series 4: Day 21)

formation of the milk proteins with the jaggery and also due to increase in the total solids content.

The fat percentages of jaggery yoghurt is higher than the control yogurt. Jaggery yogurt has 3.85 ± 0.22 % as compared to the control which has 3.82 ± 0.12 % fat. There is insignificant difference observed between the fat contents of both the samples. There is significant increase in % fat content due to decrease in moisture content, also with storage there will be changes in the fat with respect to oxidation. It has been reported that fat % has a positive impact on the sensory and physical attributes of yogurt as it adds mouthfeel and improves the texture of the product (Farinde et al. 2009, Shori, 2012). Ash content was significantly higher in the Jaggery yogurt as compared to the control yogurt owing to the fact that jaggery powder accounts for higher total inorganic matter contributing towards high ash content. Titratable acidity was comparable for both the products and ranged from 0.73% to 0.79%. pH of Jaggery yogurt was found to be 4.54 ± 0.01 and for control yoghurt pH value was 4.56 ± 0.13. Sample of jaggery enriched yoghurt had less pH value than the control yoghurt.

The optimization of yoghurt was done at three levels of Jaggery *viz*., 5, 10 and 15%. Physico-chemical and sensory analysis revealed that jaggery yogurt with 15% jaggery was found most acceptable by the trained panellists.

Storage Study

The sensory scores of the control and jaggery yogurt during the storage period are shown in Table 3. There is a significant increase in total solids and decrease in the moisture content of the jaggery yogurt as well as control yoghurt, because of loss of moisture during the storage period. The findings are in agreement with Sonwane and Sonkamble (2020) and Gavali et al. (2021).

The % fat increased significantly owing to the decrease in the moisture content as well as the oxidation of fats during storage. The similar finding of was also reported by Gaikwad and Hembade (2013) and Gavali et al. 2021. pH of both the products decreased significantly and acidity increased significantly due the microbial growth during the storage time. The % ash increased significantly which might be due to increase in minerals by enzymatic and microbial activities and addition of jaggery also contributed to the inorganic ash content, observations of present study agreed with those of Gavali et al. (2021). There was no significant difference observed in the protein percentages of both the samples during the storage. (Table 4 and Figure 1b)

Table 4 Physicochemical analysis of Control and Jaggery yoghurt during storage

Parameters	Control yoghurt Days of storage				Jaggery yoghurt Days of storage			
	0	7	14	21	0	7	14	21
pH	4.56 ± 0.03	4.49 ± 0.05	4.41 ± 0.02	3.98 ± 0.06	4.54 ± 0.01	4.50 ± 0.03	4.48 ± 0.02	4.01 ± 0.06
Titratable acidity (%)	0.71 ± 0.55	0.89 ± 0.41	0.91 ± 0.53	1.10 ± 0.41	0.71 ± 0.33	0.76 ± 0.41	0.87 ± 0.53	0.98 ± 0.41
Ash (%)	0.72 ± 0.19	0.70 ± 0.11	0.68 ± 0.16	0.68 ± 0.14	0.98 ± 0.13	1.2 ± 0.11	1.4 ± 0.16	1.6 ± 0.14
Total solids (%)	13.6 ± 0.22	13.85 ± 0.13	14.1 ± 0.08	15.5 ± 0.82	21 ± 0.81	21.5 ± 0.13	21.5 ± 0.83	22 ± 0.82
Fat (%)	3.52 ± 0.12	3.62 ± 0.02	3.76 ± 0.04	3.79 ± 0.01	3.85 ± 0.22	3.89 ± 0.22	4.0 ± 0.22	4.1 ± 0.22
Total protein (%)	3.4 ± 0.14	3.4 ± 0.31	3.55 ± 0.29	3.65 ± 0.11	3.7 ± 0.27	3.7 ± 0.31	3.65 ± 0.29	3.44 ± 0.11
Yoghurt Viscosity(mPa·s)	2879 ± 535	2750 ± 231	2036 ± 100	2022 ± 110	2922 ± 535	2750 ± 231	2036 ± 100	2022 ± 110
Yoghurt Syneresis (%)	31.59 ± 0.06	32.89 ± 0.01	31.63 ± 0.02	31.17 ± 0.02	32.28± 0.07	32.89 ± 0.01	31.63 ± 0.02	31.17 ± 0.02

Table 5 LAB, Coliform, Yeast and mould, counts of jaggery enriched yoghurt and control yoghurt during 21 days of storage

Treatment	Microorganisms	1st	7th	14th	21st
Control Yoghurt	*LAB*	$1.5x\ 10^8$	$2x\ 10^7$	$1\ x\ 10^7$	$2x\ 10^6$
	Coliform	Nil	Nil	Nil	Nil
	Yeast and mould	10 ± 14.16	30 ± 14.16	Â20	Â20
Optimized Yoghurt	*LAB*	$1.5x\ 10^9$	$2x\ 10^8$	$3\ x\ 10^7$	$2x\ 10^7$
	Coliform	Nil	Nil	Nil	Nil
	Yeast and mould	<20	10 ± 14.15	20	10 ± 14.15

Antioxidant activity and Total Phenolic content:

Jaggery is considered to be rich in phytochemicals such as flavonoids, polyphenols, phytosterols. These compounds contribute to the antioxidant activity of the jaggery. Antoxidant activity and total phenolic activity of Jaggery yogurt is significantly higher than the control yoghurt. The results are in agreement with the findings by Nayaka et al. (2009) and Srivastava et al. 2015.

Conclusions

Jaggery has been popularly used for manufacture of various food products such as jaggery basundi, jaggery kulfi, jaggery chocolates. Jaggery is said to provide various health benefits such as improvement in digestion, anti-cancer, relieves constipation, boosts energy and antioxidant activity.

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RESEARCH ARTICLE

Process optimization for the development of quinoa based sugar free ice-cream

Vasundhara and Amrita Poonia

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Abstract: Quinoa has high nutritional value and rich source of lipids, proteins, vitamins, fiber, minerals and excellent balance of amino acids. The major objective of this study was to develop and optimize a functional quinoa based ice cream with no sugar. The standard method of ice cream preparation was followed with incorporation of 10, 15 and 20 per cent malted quinoa flour with proper homogenization. Incorporation of more than 20 per cent malted quinoa flour in ice-cream mix imparted increased mild nutty flavor, hardness and increase in viscosity. Based on sensory evaluation, ice cream which was prepared with the incorporation of 15 per cent malted quinoa flour was accepted as final product and was subjected to further testing due to higher values of acceptance for color, flavor, texture and overall acceptability. The physico-chemical composition of optimized ice cream observed were 10.50 ± 0.08 % fat, 4.21 ± 0.08 % protein, 14.01 ± 0.05 % carbohydrates, 37.92 ± 0.39 % total solids, 1.84 ± 0.25 % crude fibre, 16.91 ± 0.02 mg/g GAE total phenolic content and 66.25 ± 2.53 % antioxidants. During storage period some of the sensory attributes, physico-chemical, bio-active compounds and microbiological count of the quinoa ice-cream and the control sample were analysed after every 7th day for 35 days. At the end of the storage period, anti-oxidant, overrun, melting resistance and pH decreased but acidity increased slightly.

Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Amrita Poonia (✉)
Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India
Email: amrita12@bhu.ac.in

Keywords: Antioxidants; Bioactive compounds; Overrun; Quinoa; Sensory attributes; Texture

Introduction

Recently quinoa (*Chenopodium quinoa*) has gained worldwide popularity due to its nutritional profile. It is a pseudo cereal with high nutritional value. Quinoa is a rich source of proteins, essential fatty acids and dietary fibers, lipids, vitamins, minerals and essential amino acids (Abugoch, 2009). Quinoa also contains phytochemicals including phytosterols, saponins and phytoecdysteroids which are beneficial for human health. Quinoa seeds also contains high amount of naturally occuring antioxidants such as, tocopherols, flavonoids, phenolic acids and betalains (Abugoch, 2009; Tang et al. 2015). Quinoa provides perfect amino acid balance and also rich source of thionic amino acids and lysines (Food and Agriculture Organization (FAO). Maradini et al. (2015) reported that quinoa is one of the few plants which provide almost all amino acids which are necessary for human life, and has a perfect amino acid balance and high quality proteins. Hubner and Arendt, (2013); Singh et al. (2015) reported that germination of quinoa seeds improve its nutritional quality, functional properties and also reduce the anti- nutritional factors. Germination is a popular method of grain processing due to its low cost, acceptability, low energy requirements and assorted flavor for human consumption. Malting of quinoa increases the effect of amylose by four folds (Atwell et al. 1988). Two to three folds of iron solubility is increased upon malting and the anti-nutritional factors is reduced to about 35-39% (Valencia et al. 1999). Quinoa seeds are known for their considerable positive effects on cardiovascular, metabolic and gastrointestinal, health in humans. Quinoa being gluten free has known health benefits on the peoples suffering from celiac disease.

Ice cream is a very popular dairy product and it is also very popular among the consumers of all age group. This growing popularity and acceptability of ice cream among consumers leads the manufacturers to endeavor for development through innovations. Now- a -days consumers are very health conscious and ready to pay for innovative and new products which will add on to their good health. Ice cream and other frozen desserts can

easily be fortified with compounds which can make them more nutritious and increase their acceptability. Addition of functional compounds will not only attract the attention of consumers but also provide them with nutritious and healthy options to choose from. Commercially available ice cream is not so functional due to lack of antioxidants and phenolic compounds. Waterhouse et al. (2013) reported that the increased demand and liking of the consumers for nutritious products has drawn the attention of ice cream manufactures to find out some innovative options in nutritious and functional ingredients. Addition of malted quinoa flour in ice cream could improve the nutritional aspect of the functional product as the germination process reduces the anti-nutritional factors i.e. phytic acid and saponins content to about 35-39% in quinoa. Along with this, the germination process also increases the iron content to about 2-4 folds (Valencia et al. 1999). So, the main aim of this study was to develop an ice cream with the incorporation of malted quinoa flour in three variations of 10%, 15% and 20% and to evaluate its effect on quality parameters of ice cream.

Materials and Methods

Raw materials

The ingredients for ice-cream formulations (milk, cream, SMP, stevia, butter and quinoa) were procured from local market of Varanasi. Chemicals used in the estimation process were procured from different sources (Hi Media Laboratories Pvt. Ltd., Mumbai, India; Sigma Chemicals Co. St. Louise, M.O., USA; Fisher scientific, Mumbai, India; Merck Specialists Pvt. Ltd., Mumbai, India

Preparation of malted quinoa flour

Quinoa seeds were cleaned and washed with running tap water and were soaked in water (1:3 w/v) a period of 5-6 hrs. Water was drained off and the wet seeds were transferred to a moist muslin cloth and the seeds were covered entirely with the muslin cloth for proper germination. The germination was done in the incubator at a temperature of 27±2°C for a period of 24 hrs (Patel et al. 2015). The sprouted seeds were dried in tray drier at 55°C. for 2hrs. The dried sprouts were milled to a fine flour and sieved on a 100 µm sieve (Valencia et al. 1999). The dried quinoa flour were vacuum packed in low density polyethylene (LDPE) packages of size 12 cm x 9 cm.

Preparation of quinoa ice -cream

The process of preparation of quinoa ice-cream using malted quinoa flour is depicted in Fig 1. (Clarke, 2015). The list of ingredients used for preparation of ice cream has given in Table1. Different combinations were prepared by varying the amount of malted quinoa flour. The proportions of ingredients which was produced by the varying the level of malted quinoa flour were considered as treatments and are given below.

Treatment details:

C_0 (Control) = 0% quinoa flour
C_1 = 10% quinoa seed flour
C_2 =15% quinoa seed flour
C_3 =20% quinoa seed flour

Sensory evaluation

The developed product was organoleptically evaluated by semi-trained panel of 15 judges from Department of Dairy Science and Technology, Banaras Hindu University. Control sample was prepared in the laboratory itself considering commercial process by using the basic ingredients, excluding the flavoring agents and any additional ingredients. Optimized ice cream was prepared using malted quinoa flour, milk, cream, skim milk powder, stevia and sodium alginate at different levels for different combinations. The samples were coded to avoid any biased judgement. The attributes were evaluated on the basis of 9-point hedonic scale. The product was judged for color & appearance, body & texture, flavor and overall acceptability using a score card of 9 point Hedonic Rating Scale.

Physico-chemical analysis

The chemical analysis namely moisture (%), ash content (%), crude fibre (%), fat (%) taking 5 g moisture free sample and using Soxhlet apparatus, total solids (% dry matter) and protein (%) was estimated by the macro-Kjeldahl method in which the percentage total nitrogen present in the sample was calculated and then it was multiplied by a factor of 6.38 in order to get the final protein content. Carbohydrate (%) and energy was calculated by the AOAC, 2000 methods of analysis. The physical analysis namely acidity (% citric acid) was determined by taking 1.0 g of sample and dissolved in 100 ml distilled water and taking 10 ml from the solution for estimation purpose, melting rate (g of ice-

Table 1 List of ingredients used for preparation of quinoa ice-cream

Ingredients	Milk (ml)	SMP (gm)	GMS (%)	Stevia (gm)	Sodium Alginate(%)	Quinoa Flour(%)	Sugar (%)
Control(C)	400	20	0.5	Nil	0.5	Nil	15
Sample 1(C_1)	400	20	0.5	5	0.5	10	Nil
Sample 2(C_2)	400	20	0.5	5	0.5	15	Nil
Sample 3(C_3)	400	20	0.5	5	0.5	20	Nil

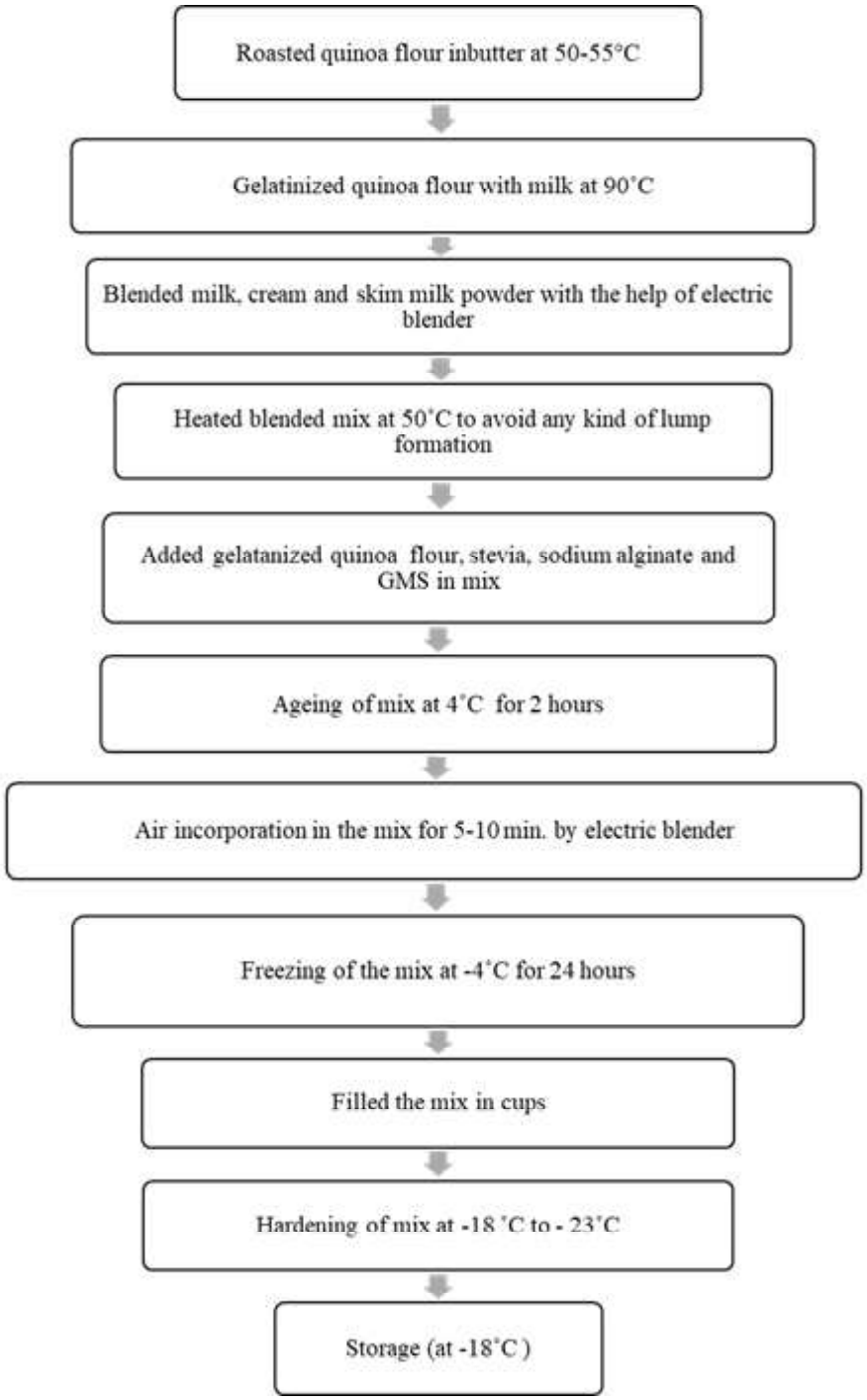

Fig. 1 Flow diagram for preparation of quinoa ice cream

cream melted at room temperature for 30 min.) by using 5.0 g of sample, pH using 5.0 g of sample dissolved in 100 ml of the distilled water was estimated by using the methods of AOAC, 2000. Viscosity (mPa.s) was determined by the method of Lowenstein and Haddad (1972) by using Brookfield viscometer: Model-DV-II + Pro (Brookfield Engineering Laboratories, USA). The overrun (%) was determined by using the method of FSSAI, 2015. Minerals were determined using Atomic Absorption Spectroscopy (Thermo Fisher Scientist-IN) (Mowuta, and Mayangsari, 2022).

Heat treatment

Heat treatment was done by the method of (Arbuckle, 1986) by taking 50 g of sample. It was hardened at -23° C for 24 hrs and then transferred to -18±2°C for 1.5 hr, same samples were again taken to the same temperature for 0.5 hr for the shock and then returned back to the freezing temperature. This treatment was done daily for 6 days. After that the samples were kept at freezing temperature and checked for sensory attributes.

Texture analysis

Texture profile analysis of ice cream was performed by using the texture profile analyzer TA.XT plus, Exponent Lite (Stable Micro Systems, Surrey, UK) using probe P-32 up-to 80 mm distance at 2.0 mm/s test period. The samples were kept at freezing temperature i.e. -18 to -23 ºC and at the time of the texture analysis the cups were taken out and brought straight for analysis in order to avoid any textural changes. The probe used was automatically inserted into the cups (one cup at a time). Graphs and values were obtained by using a software on the computer which was attached to the Texture profile analyzer (Sert et al. 2021).

Bioactive compounds

Total phenolic contents were determined using the Folin-Ciocalteau reagent and Gallic acid as a standard (Slinkard and Singleton, 1997). The free radical scavenging activity was determined by using DPPH assay with modified method of Brand-Williams et al. (1995). A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%) was added to 0.1 mL of methanolic ice cream sample extract and shaken. The samples were kept in the dark room for 30 minutes after which absorbance was recorded at 575nm. The results of total phenolic content were expressed in mg/g GAE.

Anti-nutritional compounds

$$\text{Saponins \%} = \frac{\text{Dried sample (g)}}{\text{Wt. of sample taken (g)}} \times 100$$

Saponins were determined using methods of Obadoni and Ochuko (2002). Twenty grams of sample was taken into a conical flask and then added with 100 ml of 20% aqueous ethanol. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred to a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated.

Statistical analysis

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

Results and Discussion

Incorporation of malted quinoa flour in ice cream

The malted quinoa flour was mixed in different proportion i.e. 10%, 15% and 20% (w/w) with the milk for optimization of ice cream on the basis of initial trials in the laboratory. Incorporation of 20% quinoa flour resulted in high viscous and sticky mix for ice cream preparation. Similar observations have been reported by Patel et al. (2014). The best results were obtained on mixing 100 parts of milk with 15 parts of malted quinoa flour. The optimization of ice cream was done on the basis of sensory evaluation. The optimized ice cream was then compared with control ice cream.

Sensory attributes

The sensory mean score for color and appearance of ice cream varied from 7.22 ± 0.21 to 7.65± 0.22, body and texture from 6.89± 0.19 to 7.89± 0.10, flavor from 6.56± 0.22 to 7.78± 0.21 and overall acceptability from 6.89± 0.14 to7.70± 0.09. It is evident from the data presented in (Table 2) that most of the samples prepared are

Table 2 Mean sensory score for ice-cream prepared by using different levels of malted quinoa flour

Level of Incorporation (%)	Color and Appearance	Body and Texture	Flavor	Overall Acceptability
0	7.44* ± 0.22	7.56* ± 0.27	7.44* ± 0.16	7.58* ± 0.15
10	7.65*± 0.22	6.89 ± 0.19	6.78 ± 0.13	7.11*± 0.13
15	7.44* ± 0.16	7.89* ± 0.10	7.78* ± 0.21	7.70* ± 0.09
20	7.22* ± 0.21	6.89 ± 0.29	6.56 ± 0.22	6.89 ± 0.14

Values are mean ± standard deviation (n = 3)
*Means in the same column are not significantly different (P<0.05)

significantly different (P<0.05) from the control sample. The overall acceptability score for the quinoa flour ice cream prepared with 15 % quinoa was found to be most satisfactory by the panelist. The sample containing 20% quinoa flour had a mild nutty flavor. Though it is the characteristic flavor of the quinoa flour it was not much liked by the sensory panel when compared to other combinations. The formulation containing 15% quinoa flour had an acceptable flavor and mouthfeel because it balanced the taste of a typical ice-cream and quinoa flour. The optimized ice cream sample with 15% malted quinoa flour (C_2) was finally selected for further analysis in the study.

Physico-chemical analysis

Physical analysis

The acidity of the optimized quinoa ice cream containing 15% malted quinoa flour was more than that of the control i.e. 0.22 ± 0.00 and 0.14± 0.02 per cent, respectively. The pH of the optimized ice cream was 6.09±0.01 which is less than that of the control sample i.e. 6.44± 0.00. The pH of the optimized ice cream was less due to increase in acidity. Similar results were reported by (Patel et al. 2014). According to their research, incorporation of malted ragi flour at higher levels showed an increase in the acidity and decrease in the pH of mixes significantly at (P<0.05). Somewhat similar results were also observed by Desai et al. (2010) while investigating the performance of malted ragi flour in bakery product such as cake. This may be due to the production of the ascorbic acid during the germination process or due to the hydrolysis of fat which produces fatty acids. The viscosity of the mix increased after the addition of the malted quinoa flour. The viscosity of the control sample was found to be 72.48± 0.39 (mPa.s) which is much lower than that of optimized ice cream i.e. 82.55±0.45 (mPa.s). Viscosity may be increased due to several factors such as processing, kind of ingredients used, temperature, concentration and holding of the mix. Cottrell et al. (1980); Schmidt et al. (1993) reported that the ice-cream mixes which contains carbohydrate-based fat replacers exhibit a very thick and viscous behavior because of its capability to imbibe water. This type of behavior would tend to increase the viscosity of the mixes. This fact was accepted because the carbohydrate has good water binding capacity and sometimes even better than proteins as reported by (Clark (1994); Akoh, 1998). Arbuckle (1986) reported that the acidity of the ice cream also affects the viscosity of the mixes.

The melting resistance is an important factor for ice cream. Ice-cream should melt down to a smooth texture. It is very important to consider that the ice cream does not melt down fast and should not be too hard. Marshall et al. (2003) reported that the melting down rate of the ice-cream can be affected because of many factors such as nature of the ice crystals, amount of air incorporated, network of fat globules formed during freezing etc. The melting rate of the quinoa based ice- cream 6.61±0.01 g/min was more than that of the control sample 6.20±0.01 g/min. High melting rate of the quinoa based ice- cream may be due to the high value of total solids with further contribution of the sodium alginate which help in stabilizing the mix in optimized ice cream.

The overrun of the ice-cream increases its palatability and the profit. It is the amount of air incorporated into the mix. The overrun of the ice-cream is affected by many factors such as type of ingredients used in the mix, type and amount of emulsifiers used and the type of processing of the mix. The overrun of quinoa ice cream was found to be 75.65 ± 0.09 % which is less than that of the control sample which is 78.69 ± 0.32%. Marshall et al. (2003) observed that the premium ice cream commercially marketed have a significantly low amount of overrun that is 25% - 45 %. This may be due to the high value of the viscosity in the optimized sample. Das et al. (1989) found that high value of viscosity has an adverse effect on the whipping quality or the overrun of the mix.

Chemical analysis

The optimized ice cream contains less moisture content that is 65.61± 0.31% than the control sample 68.92± 0.14%. From the result shown in (Table 3) it is seen that all the factors other than ash are significantly affected. The optimized quinoa flour ice cream shows total solids more than in control. Similar findings are reported by (Cody et al. 2007). They reported an increase in the amount of total solids with the incorporation of rice flour in ice cream. It may be due to incorporation of SMP and level of quinoa flour in the ice cream. Quinoa flour contains 7.14 g/100g of fat. Due to the incorporation of quinoa flour the fat of the optimized ice cream increased slightly from 10.03± 0.10 % to 10.50± 0.08 %. The protein content in the quinoa flour is 14.29g/100g. The protein increased from 3.99± 0.16 % in control sample to 4.21± 0.05% in the optimized ice cream, respectively. Patel et al. (2014) reported that addition of malted gelatinized ragi flour increased the fat and protein of the ice cream. So, the rise in the fat and protein content in this study may be due to the incorporation of the malted quinoa flour. The carbohydrate content of the control sample was less than that of the quinoa ice cream (Table 3). The total energy value of the quinoa ice cream was less than that of the control sample. Control sample contained total energy of 166.17±1.64 Kcal/100 g. and optimized sample contained 183.63±0.07 Kcal/100g, respectively. This may be due to the less amount of the carbohydrate in the quinoa ice cream. Vilche et al. (2003) reported that the proximate composition of the pseudo cereal quinoa is found to be 54.1% -64.2% carbohydrate, 10% to 18% protein, 2.4% to 3.65% ash, 2.1% to 4.9% crude fibre and 4.5% to 8.75% crude fat.

The optimized quinoa ice cream contains high value of crude fiber that is 1.84± 0.02% than in the control sample which is 0.24± 0.01 %, respectively. The crude fiber content is more in optimized

ice cream due to incorporation the high fiber quinoa flour which itself contains about 2.18% crude fiber.

Textural attributes

Textural attributes such as cohesiveness, consistency, index of viscosity, firmness and gumminess was measured for the control as well as the optimized ice cream (Table 4). Texture analysis of ice cream was done in the frozen state to avoid the error that may cause due to increase in temperature. The firmness of the control sample was found to be 0.148±0.04 (N) and the optimized sample was 0.250±0.05 (N). Index of viscosity in the control was -0.005±0.06 (N.s) and the optimized sample was -0.167±0.05 (N.s). The cohesiveness of the control sample was found to be -0.105±0.04 (N) and optimized sample was -0.218±0.03 (N). Consistency in the control sample was 2.15±0.02 (N.s) and the optimized sample was 3.27±0.04 (N.s).

Mineral content

Among all the minerals it was found that the level of calcium was highest (Table 5) i.e. 238.21±4.68 mg/100g. This increase in Ca content can may be due to incorporation of malted quinoa flour as quinoa is rich in calcium content. The magnesium and zinc content were 41.03 mg/100g and 1.55 which were higher than the control sample which contained 13.88 mg/100g and 0.21 mg/100g, respectively. The manganese content of the control sample was 0.71mg/100g which is very much less than quinoa ice cream which has a Mn value of 1.26 mg/100g. Ascheri et al. (2002) reported that quinoa flour is rich in minerals such as Fe (11.77 mg/100 g), Ca (38.26 mg/ 100 g), Mg (160 mg/100 g), K (546 mg/100 g) and P (357 mg/100 g). It has been thus concluded that quinoa flour has high levels of some nutrients and does not contain gluten, so it may find application in foods e.g. for people suffering from celiac disease.

Bioactive compounds

The optimized quinoa ice cream (C_2 with 15 % of quinoa flour) contained high amount of antioxidant which is 66.25± 2.53 (%) and it may be due to incorporation of the quinoa flour which contains total phenol content and antioxidants (Table 6). It has á-tocopherol, ã-tocopherol and phytoestrogens are present in the quinoa seeds as antioxidant component (Bkowska et al. 2003).

Table 3 Physico-chemical analysis of optimized quinoa ice-cream

Parameters	Control (C)	Optimized ice -cream (C_2)
Acidity (%)	0.14* ± 0.02	0.22* ± 0.00
Melting resistance**(g/min)	6.20* ± 0.01	6.61* ± 0.01
Overrun (%)	78.69 ± 0.32	75.65 ± 0.09
pH	6.45 ± 0.00	6.49 ± 0.01
Viscosity (mPa.s)	72.48 ± 0.39	82.55 ± 0.45
Moisture (%)	68.92 ± 0.14	65.61 ± 0.31
Ash (%)	0.79* ± 0.03	0.77* ± 0.01
Fat (%)	10.03* ± 0.10	10.50* ± 0.08
Protein (%)	3.99* ± 0.16	4.21* ± 0.05
Carbohydrates (g)	14.01 ± 0.19	19.37 ± 0.39
Total solids (%)	36.13* ± 0.29	37.92* ± 0.25
Crude fiber (%)	0.24* ± 0.01	1.84* ± 0.02
Energy (Kcal)	183.63 ± 0.07	166.17± 1.64

Values are mean ± standard deviation (n = 3)
*Means in the same row are not significantly different (P<0.05)
**g/min. of ice-cream melted at room temperature (25±2°C) for 30 min.

Table 4 Instrumental texture profile analysis of quinoa ice-cream

Attributes	Control (C)	Optimized quinoa ice-cream (C_2)
Cohesiveness (N)	-0.105 ± 0.04	-0.218 ± 0.03
Index of Viscosity(N.s)	-0.005 ± 0.06	-0.167 ± 0.05
Firmness (N)	0.148*± 0.04	0.250 ± 0.02
Gumminess (g)	0.86* ± 0.03	0.90* ± 0.04
Consistency (N.s)	2.15* ± 0.02	3.27*± 0.04

Values are mean ± standard deviation (n = 3)
*Means in the same row are not significantly different (P<0.05)

Anti-nutritional factors

The saponins found in optimized quinoa ice cream were very less i.e. 0.18± 0.03%. (Table 6). This may be due to reduction of anti -nutritional factors during soaking, heating and germination process (Liu, 2019). Koziol (1992) reported that for different quinoa variety the saponin content varied in the range of 0.001% to 4.65%.

Effect of storage on antioxidants and some physical properties of quinoa ice cream

The optimized ice-cream was stored at -18°C and analyzed for, antioxidant %, overrun %, pH, acidity % and the melting rate (g/min) at an interval of 7 days for 35 days (Table 7). During the storage period the antioxidant content was seen to be decreasing from 68.60 ± 0.10% to 66.12 ± 0.01% and this may be due to the decrease in the amount of the total phenolic content. Patthamakanokporn et al. (2008) reported that the decrease in the amount of the antioxidant is due to the reaction of the polyphenol oxidase endogenously with the phenols as the time passes and also reported the significant reduction in the amount of the antioxidant and TPC (Total phenolic compounds) when a guava fruit was homogenized and stored at -2° C for a period of 3 months. Goraya and Bajwa, (2015) also observed a decrease in the amount of the antioxidants in amla incorporated ice-cream and also in bakery products.

The melting resistance as per the analysis was found to be more in quinoa ice cream that is 6.61± 0.00 g/min. when the ice cream was allowed to melt for 30 minutes than the control sample which is 6.20± 0.00g/min. The melting resistance may have increased due to the incorporation of the quinoa flour and sodium alginate which is used as a stabilizer. Moenfard and Tehani (2008) reported that the melting rate ice-cream of is an important factor and is at a large extend influenced by its additives that is being used at the time of the manufacturing, composition, overrun, network of fat globules and formation of ice crystal. The pH of the control sample was 6.45± 0.00 and optimized product was 6.49± 0.02. The acidity of optimized product was found to be 0.22± 0.00% and the control sample was found to be 0.14 ± 0.00 %. The overrun per cent of

Table 5 Mineral analysis of the quinoa ice-cream

Minerals (mg/100 g)	Control (C)	Optimized quinoa ice-cream (C_2)
Calcium	127.62 ± 0.23	238.21± 4.68
Magnesium	13.88* ± 0.01	41.03* ± 0.28
Iron	0.10 ± 0.00	1.77 ± 0.07
Zinc	0.21* ± 0.14	1.55* ± 0.13
Manganese	0.71± 0.00	1.26 ± 0.06

Values are mean ± standard deviation (n = 3)
*Means in the same row are not significantly different (P<0.05)

Table 6 Bioactive compounds and saponins in quinoa ice-cream

Parameters	Control (C)	Optimized quinoa ice-cream (C_2)
Total phenolic content (mg/g GAE)	Nil	16.91 ± 0.26
Anti-oxidants (%)	Nil	66.25 ± 2.53
Saponins (%)	Nil	0.18± 0.03

Values are mean ± standard deviation (n = 3)

Table 7 Effect of storage period on some properties of the quinoa ice-cream

Control (C)	Antioxidants (%)	Acidity (%)	pH	Over-run (%)	Melting Rate(g/min.)
0 day	Nil	0.14* ± 0.00	6.49* ± 0.00	78.69* ± 0.00	6.20* ± 0.00
7th day	Nil	0.13* ± 0.00	6.47* ± 0.00	78.52* ± 0.00	6.18* ± 0.00
14th day	Nil	0.14* ± 0.00	6.45* ± 0.00	78.10* ± 0.00	6.15* ±0.00
21st day	Nil	0.14* ± 0.00	6.46* ± 0.00	77.82* ± 0.00	6.13* ± 0.00
28th day	Nil	0.15* ± 0.00	6.45* ± 0.00	77.10* ± 0.00	6.10* ± 0.00
35th day	Nil	0.14* ± 0.00	6.47* ± 0.02	76.21* ± 0.00	6.05* ± 0.00
Optimized quinoa ice-cream (C_2)	Antioxidants (%)	Acidity (%)	pH	Over-run (%)	Melting Rate(g/min.)
0 day	68.60* ± 0.10	0.22* ± 0.00	6.10* ± 0.00	75.65* ± 0.00	6.61* ± 0.00
7th day	67.40* ± 0.03	0.21* ± 0.00	6.10* ± 0.00	75.61* ± 0.00	6.58* ± 0.00
14th day	67.23* ± 0.01	0.20* ± 0.00	6.09* ± 0.00	75.60*± 0.00	6.57* ± 0.00
21st day	67.11* ± 0.00	0.20* ± 0.00	6.10* ± 0.00	75.58* ± 0.00	6.54* ± 0.00
28th day	66.51* ± 0.00	0.20* ± 0.00	6.10* ± 0.00	75.56* ± 0.00	6.49* ± 0.00
35th day	66.12* ± 0.01	0.20* ± 0.00	6.06* ± 0.02	75.54* ± 0.00	6.48* ± 0.00

Values are mean ± standard deviation (n = 3)
*Means in the same column are not significantly different (P<0.05)

the quinoa ice cream was reduced from 78.69± 0.32 to 75.65 ± 0.09 and this decrease may be due to the shrinkage of ice-cream and formation of the ice crystals during storage.

Effect of storage on microbial count

During the storage period of quinoa ice-cream *E-coli* and yeast & mould growth has not been reported. The Standard Plate Count (SPC) of developed ice cream was seems to be decreasing from 5.41 cfu/ml to 4.31 cfu/ml. The decrease SPC may be due to the storage of quinoa ice-cream at low temperature and because of very low temperature the microbes could not survive in the product and thus lowering the count. The results obtained for standard plate count were below the range of FSSAI standards i.e. (1 x10^5g to 2 x10^5 g). Lee and White (1991) also reported a decline in SPC of ice cream samples during storage period. Davidson et al. (2000) also reported that the decline in SPC or the microbial count during storage of the ice cream could mainly be due to ice crystals formation that often damages the cell wall of the microbes and leads to the lysis of the micro- organisms.

Conclusions

Quinoa incorporated ice-cream can be made commercially and can be stored at freezing temperature. Addition of 15 % malted quinoa flour to ice cream positively affected its sensory attributes, physico-chemical properties and bioactive compounds. Quinoa flour contains about 7.1 g/100g of protein, 29.0g/100g of carbohydrates and 184 Kcal of energy. Incorporation of quinoa in a product like ice-cream which lacks certain nutrients will enhance its nutritional qualities. The polyphenols in the form of antioxidants present in quinoa will help to enhance the biological value of ice-cream. The developed product has a very unique mild nutty flavor due to incorporation of malted quinoa flour. So, the optimized product does not require any additional artificial flavoring agent. Quinoa can be a core ingredient of great importance in many other value added products such as bread, cake, cookies and many more products. Gluten free property of quinoa makes it valuable product for those having celiac disease and digestive problems. Quinoa incorporated ice-cream containing stevia can be a promising product for the diabetic patients as well because, quinoa has a low glycemic index. Use of stevia as a replacement of sugar may not cause any negative effect on diabetic patients.

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RESEARCH ARTICLE

Non genetic factors affecting udder type traits in Sahiwal cattle

Oshin[1], S M Deb[1], Sagar Kadyan[2], PB Nandhini[1] and Sushil Kumar[3]

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Abstract: The present study was conducted to find the magnitude of environmental factors affecting udder type traits. 100 lactating Sahiwal cattle were selected to measure the fourteen types of udder traits. These animals were maintained at Livestock Research Centre, NDRI, Karnal, India. The data was analysed by least squares method using the general linear model procedures including parity, stage of lactation and season of calving as fixed effects and it was observed that traits viz. Udder balance, Rear udder Height, Teat diameter, Teat length, Central ligament, Udder circumference, Udder width and Teat circumference were significantly ($P<0.05$) affected by parity. The Stage of lactation was significant ($P<0.05$) source of variation for shortest distance from teat ends to floor, rear udder height, central ligament and udder circumference while season of calving significantly ($p<0.05$) affected rear udder height, teat length and rear udder width. Therefore, it is suggested that to keep these environmental factors in consideration for genetic improvement of cattle on the basis of udder confirmation traits.

Keywords: Parity; Stage of lactation; Sahiwal; Season of calving; Udder type traits

[1]Animal Genetics & Breeding Division, ICAR-National Dairy Research Institute, Karnal-132001, (Haryana), India

[2]Department of Livestock Production Management, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar- 125004, (Haryana), India
E-mail: sagar.kadyan21@gmail.com

[3]Krishi Vigyan Kendra, Jaora, Ratlam- 457340, India.
E-mail: vetsushil09@yahoo.com

Oshin (✉)
Animal Genetics & Breeding Division, ICAR-National Dairy Research Institute, Karnal-132 001, (Haryana), India.
E-mail: toglaoshin2121@gmail.com

Introduction

Dairy industry has always shown a remarkable interest in the relationships between body conformation measurements and production performance of dairy cows. Several studies have shown that the emphasis of selection for increasing milk production may result in a decrease in the merit of some type traits which negatively influence health and fitness. Recent emphasis has, therefore, been directed to improve functional traits like reproductive traits, type traits, disease tolerance etc. improvement of which contribute to the economics of cattle rearing substantially. Linear type traits are very significant while making reproduction and selection decisions in dairy cows (Schneider et al. 2003). Udder traits are reported to have an impact on cow health, production longevity (Stefani et al. 2018) and survival (Sewalem et al. 2004). Since the udder traits are known to have moderate genetic correlations with milk yield, they might be used as indirect predictors of the production traits. They are known to have direct and indirect effect on milk production, longevity and culling decisions (Zavadilova and Stipkova, 2012) which further is utilized to define the dairyness of a cow (Dubey et al. 2014). A well-balanced and strongly attached udder with fine texture will support high and persistent production over the cow's lifetime. The cattle genetic resource base of India includes Sahiwal breed that stands on the top of indigenous cattle breeds in terms of production aspects (Ahmad et al. 2019). This breed has been used for various breed improvement programmes and development of synthetic crosses in India (Singh et al. 2017). Since it is the heaviest milkers of all zebu breeds, it displays a well-developed udder (Mason,1996). It has been reported in the various studies that udder type traits are affected by various genetic and non- genetic factors (Khan et al. 2009, Mazza et al. 2013) etc. Genetic factors are inherited individually from the parents and possessed from birth, whereas the environment is the influence of non-genetic factors (Santosa et al. 2019). These are the factors with measurable effects for example: age of the parent when giving birth, stage of lactation, age of cow, season, calving year, parity, body weight, lactation period, etc. Control of non-genetic factors is expected to provide ideal conditions to the animal to perform its genetic potential at its best. If the environment and breeding practices are improved, favourable phenotypic and genetic trends can be achieved.

Measurable effects are to be standardized for the non-genetic sources of variation to decrease known environmental differences among animals to imply in livestock improvement programs. There is a need to specify these factors for unbiased genetic evaluation while applying the breeding technology to the dairy cows. Season of calving, Parity, stage of lactation and age of the cow at time of classification are considered as the most significant environmental factors, although several non-genetic factors affecting linear type traits were pointed out by Dubey et al. (2014), Mazza et al. (2013). There is less information on the effects of parity, stage of lactation and season of calving on udder type traits in Sahiwal cow which can be achieved by a thorough investigation. Therefore, the magnitude of these non-genetic factors is required for more precise assessment of the udder type traits. In this context, the study of udder characteristics and their main variation factors in Sahiwal cow, could permit to know its dairy potential in depth, and to recognise those mammary traits more desirable for specific breeding programs.

Materials and Methods

Subject and site of experimentation

The study was carried out at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, India which is located between 29° 42'N latitude and 72° 54'E longitude. It has subtropical climate and temperature remains between 45°C during summer to 2°C during winter. The annual rainfall is 760 to 960 mm and relative humidity ranges from 40 to 85%. The feeding practices were same for all the animals and animals were free from mastitis.

Source of information and data

100 lactating Sahiwal cattle were selected for measurement of udder type traits as per guidelines of the International Committee of Animal Recording ICAR (2012) and World Holstein Friesian Federation, WHFF (2012), from August 2018 to July 2019 where udder type traits viz. Fore udder attachment (FUA), Rear udder height (RUH), Udder depth (UD), Udder balance (UB), Rear udder width (RUW), Central ligament (CL), Udder length (UL), Udder width (UW), Udder circumference (UC) and teat type traits viz. Teat diameter (TD), Teat length (TL), Teat circumference (TC), Average distance between teats (DBT) and shortest distance from teat ends to floor (DFF) were measured 2 hours before evening milking, and vernier callipers was used for teat measurement.

Statistical analysis

The data was analysed by using general linear model (SAS, 2002) including parity, season of calving and stage of lactation as fixed effects. The dependent variable were udder morphology traits. The year was classified into four major seasons, viz. (1) winter (December - March), (2) summer (April - June), (3) rainy (July - September) and (4) autumn (October - November). All animals were divided into five groups based on parity i.e., Group 1 (1st parity), Group 2 (2nd parity), Group 3 (3rd parity), Group 4 (4th parity) and Group 5 (5th and above). The stage of lactation was divided into Class 1 (0-90 days), Class 2 (90-180 days), Class 3 (180-270 days) and Class 4 (more than 270 days) and following statistical model use to analyse the data.

$$Y_{ijkl} = \mu + L_i + P_j + S_k + e_{ijkl},$$

Where,

Y_{ijkl} = L^{th} Observation of cow in i^{th} stage of lactation, j^{th} parity and k^{th} season of calving

μ = Overall mean

L_i = Fixed effect of i^{th} stage of lactation (i= 1 to 4)

P_j = Fixed effect of j^{th} parity (j =1 to 5)

S_k = Effect of k^{th} season of calving (K = 1 to 4)

e_{ijkl} = Random error NID $(0, \sigma^2_e)$

For all parameters, model effects were declared significant at $p<0.05$, unless otherwise stated and difference of means between subclasses was tested for significance using Duncan's Multiple Range Test (DMRT) modified by Kramer (1957).

Results and Discussion

The overall least squares mean for FUA, RUH, UD, UL, UW, TC, TL, DFF, TD, UB, DBT, RUW, CL and UC were 119.79± 2.40 degree, 21.88 ± 0.28, 48.01± 0.17, 45.61 ± 0.61, 63.93 ± 0.52, 7.58 ± 0.10, 5.08 ± 0.107, 46.76 ± 0.53 , 1.91 ± 0.705, 0.75 ± 0.114, 5.27 ± 0.12, 8.74 ± 0.106, 3.93 ± 0.14 and 149.25 ± 0 .63 cm, respectively. According to the descriptive structure of data depicted in Table 1, udder of the population is well developed.

Parity effect

Traits viz. UB, RUH, TD, TL, CL, UC, UW and TC were significantly ($p<0.05$) affected by parity and results were presented in Table 2. The UB values showed an increasing trend among cows of parity 1st to 3rd and thereafter, decline which shows that cows of 3rd parity have most well-balanced udder. In case RUH, cows in the 1st parity had the lowest RUH and highest in 4th parity and decline thereafter. Similarly, cows in 1st parity had the smallest TD and cows in 3rd parity had the largest declining thereafter. An increasing trend from 1st to 4th parity in cows was seen for UW, CL, UC and TL with a small decline in 5th parity showing that cows of 4th parity had the large, widest udder with deepest cleft and longer teats than cows in other parities. Inconsistent trend was observed in TC where cows in 3rd parity had largest TC and smallest in 2nd parity. It was observed in the

present investigation that overall udder measurements increased with the advancement in the parity where maximum values are attained up to 3rd or 4th parity and then exhibiting a decline. This might be due to the continuous development of udder tissues up to certain parity, after which the tissues start to regress as the age advances. The results resemble the findings of Khan and Khan, (2015) who stated that cows with more parities had higher scores for RUH, RUW, CL and fore TL. Similarly, Lavania et al. (2011) found gradual increment of udder measurement occurred with parity and declined from 5th parity onwards in Surti buffaloes. Kuczaj (2003) reported that parity significantly affects almost all udder traits, and lowest values were for primiparous. Mingoas et al. (2017) reported an increment in Udder Height in 2nd and 3rd parities because of progressive udder hypertrophy concerning cow's age and parity. Modh et al. (2017), Patel et al. (2016) and Prasad et al. (2010) reported that parity was a significant source of variation for UW (P<0.05). Similar results were established by Modh et al. (2017) who concluded that there was a gradual increase in length of fore (7.56 %) and rear (8.28%) teat with the advancement of parity. The findings of Patel et al. (2016), Modh et al. (2017) and Prasad et al. (2010) reported that parity was a significant source of variation for UW (P<0.05). They observed increase in udder characteristics with parity could be due to proportion of alveoli developed in previous lactations which did not regress completely but was added to those which developed in the subsequent lactations, thus increasing the size of udder (Peris et al. 1999).

Stage of lactation effect

The stage of lactation was significant (P<0.05) source of variation for DFF, RUH, CL and UC (Table 3). The declining trend in DFF

Table 1 Descriptive statistics of udder and teat type traits

S No.	Traits (cm)	Mean Value ± SE	Range	Minimum value	Maximum value	Sample variance	Standard deviation
1	RUW	7.00 ± 0.21	8.12	3.30	11.43	3.30	1.81
2	RUH	21.39±0.53	25.95	4.34	30.30	135.70	11.64
3	FUA (^{0})	119.32±2.07	85	60	145	248.03	15.74
4	UL	52.89 ± 1.19	47.75	23.36	71.12	107.15	10.35
5	UW	63.76±1.006	43.43	43.68	87.12	75.95	8.71
6	UC	125.71±1.21	51.81	94.18	145.99	110.31	10.50
7	UD	30.68 ± 0.32	12.73	24.13	36.83	7.98	2.82
8	UB	-1.73 ± 0.41	25.43	-20.32	5.08	12.93	3.59
9	TL	7.82 ± 0.19	8.22	3.73	11.96	2.82	1.67
10	CL	7.50± 0.21	7.95	2.94	10.89	1.83	3.37
11	TC	8.84 ± 0.21	9.144	5.52	14.66	3.39	1.84
12	TD	1.84± 0.06	2.47	1.045	3.51	0.53	0.29
13	DBT	5.80 ± 0.23	8.76	0.95	9.71	4.01	2.00
14	DFF	42.20 ± 0.98	51.47	25.61	77.08	72.4133	8.50

Table 2 Influence of parity on udder type traits in Sahiwal cows

S.No.	Traits	P1	P2	P3	P4	P5
1	UD	50.31±0.42	48.79±0.21	49.074±0.31	49.10±0.41	48.76±0.38
2	UB	-2.54±0.27^{a}	1.24±0.13^{b}	1.73±0.20^{c}	-3.03±0.27^{cd}	-3.29±0.25^{d}
3	FUA	118.58±5.87	115.95±2.94	116.52±4.38	127.52±5.74	120.37±5.29
4	RUH	17.36±0.68^{a}	18.78±0.34^{bc}	19.50±0.51^{cd}	19.77±0.67^{d}	18.01±0.61^{ab}
5	DBT	5.20±0.30	5.37±0.15	5.37±0.22	5.10±0.29	5.31±0.27
6	RUW	8.55±0.25	8.67±0.13	9.09±0.19	8.71±0.25	8.70±0.23
7	TD	1.65±2.61^{a}	1.76±1.31^{a}	2.27±1.94^{c}	2.14±2.55^{b}	2.14±2.35^{b}
8	UW	62.30±1.27^{a}	68.13±0.64^{b}	68.82±0.95^{b}	69.64±1.24^{b}	62.73±1.15^{a}
9	UL	46.02±1.50	45.16±0.75	43.80±1.12	47.39±1.47	43.67±1.35
10	DFF	47.96±1.31	46.58±0.66	46.11±0.98	45.81±1.28	47.33±1.18
11	TL	4.6±0.26^{a}	4.87±0.13^{ab}	5.1±0.19^{bc}	5.61±0.25^{d}	5.23±0.23^{c}
12	CL	2.01±0.34^{a}	2.94±0.17^{b}	3.17±0.26^{b}	3.7±0.34^{c}	2.82±0.31^{b}
13	TC	7.29±0.25^{a}	7.27±0.12^{a}	8.2±0.18^{b}	7.86±0.24^{b}	8.1±0.22^{b}
14	UC	147.94±1.55^{a}	149.72±0.78^{b}	149.45±1.16^{b}	151.1±1.52^{c}	147.9±1.4^{a}

^{a,b,c}Means within the same row with different superscripts are significantly different (*p <0.05

was observed upto 3rd stage of lactation, but increased during 4th stage of lactation. The UC values consistently increased from 1st to 3rd stage of lactation but cows in 4th stage of lactation, udder with smaller circumference were noticed showing that teat ends distance to floor became shorter or in other words teat size increased in 3rd stage of lactation achieving the highest value of udder circumference. The rear udder were higher with deeper clefts in cows in 2nd stage of lactation as compared to cows in other stage of lactation. It is inferred from the results of present investigation that udder measurements were in accordance to the milk production in each stage of lactation. This might be due to nutrient requirement varies with the stage of lactation and mammary tissue function declines after peak lactation due to decrease in mammary cell quantity. Krastanov (2006), Petkov and Stoyanova (2006) also found significant effect of the lactation stage on CL. Krastanov (1995), reported a significant effect of the stage of lactation on RUH. Khan and Khan (2015) and Mazza et al. (2013) observed a consistent reduction in values for RUH from early to later stages of lactation. As lactation continue the UH increased ($p<0.05$) from 15 d (110.8 mm) to 75 d (125.3 mm) postpartum, but it diminished ($p<0.05$) after 105 d postpartum (115.0 mm) coinciding with the involution of mammary gland at end of lactation. The udder bottom height and udder upper height increased throughout lactation while UC and UW diminished throughout lactation, but reduction evident at 100th day of postpartum. These values of UC and UW were similar to those reported by Unal et al. (2008).

Season of calving effect

Table 3 Influence of stage of lactation on udder type traits in Sahiwal cows

S.No.	Traits	L1	L2	L3	L4
1	UD	48.90±0.30	49.37±0.2	48.05±0.28	48.70±0.35
2	UB	1.39±0.19	1.62±0.16	1.49±0.18	1.78±0.23
3	FUA	118.76±4.19	118.82±3.52	118.6±3.97	122.95±4.91
4	RUH	20.34^{a}±0.49	22.58^{c}±0.571	19.57^{b}±0.46	19.25^{b}±0.41
5	DBT	5.21±0.21	5.22±0.18	4.97±0.20	5.67±0.25
6	RUW	8.93±0.18	7.88±0.15	8.76±0.17	8.41±0.21
7	TD	1.81±1.86	2.15±1.56	2.13±1.76	1.87±2.18
8	UW	61.85±0.91	61.73±0.76	61.75±0.86	63.37±1.06
9	UL	45.58±1.07	43.52±0.90	43.47±1.02	47.86±1.26
10	DFF	46.45^{a}±0.94	45.74^{a}±1.10	45.99^{a}±0.89	48.85^{b}±0.79
11	TL	5.00±0.18	5.03±0.15	5.13±0.17	5.17±0.21
12	CL	3.09^{a}±0.24	3.98^{a}±0.2	2.19^{b}±0.23	2.03^{b}±0.29
13	TC	7.76±0.17	7.78±0.15	7.56±0.16	7.23±0.21
14	UC	148.9^{b}±1.11	149.77^{b}±0.93	150.91^{b}±1.05	140.3^{a}±1.30

[a,b,c]Means within the same row with different superscripts are significantly different (*p <0.05)

Table 4 Influence of season of calving on udder type traits and overall mean in Sahiwal cows

S.no.	Traits	S1(w)	S2(s)	S3®	S4(a)
1	UD	50.26±0.24	48.97±0.37	48.69±0.38	49.11±0.22
2	UB	1.56±0.16	1.84±0.24	1.48±0.25	1.39±0.14
3	FUA	125.05±3.41	120.61±5.20	111.75±5.37	121.74±3.08
4	RUH	$20.58±0.39^{a}$	$18.92±0.60^{cd}$	$18.05±0.62^{c}$	$19.19±0.36^{ab}$
5	DBT	5.19±0.17	5.10±0.26	5.23±0.27	5.36±0.15
6	RUW	$9.50±0.15^{a}$	$8.04±0.23^{d}$	$8.59±0.23^{bc}$	$8.84±0.13^{b}$
7	TD	1.90±1.51	1.90±2.31	2.09±2.39	2.08±1.37
8	UW	62.17±0.74	68.13±1.13	62.68±1.17	68.73±0.67
9	UL	47.84±0.87	45.12±1.33	44.11±1.38	44.36±0.79
10	DFF	46.79±0.76	46.27±1.16	47.33±1.20	46.65±0.69
11	TL	$5.03±0.15^{a}$	$4.23±0.23^{c}$	$4.95±0.23^{b}$	$5.02±0.13^{ab}$
12	CL	2.76±0.2	2.89±0.3	2.86±0.31	3.21±0.18
13	TC	8.58±0.14	7.85±0.22	7.49±0.22	7.42±0.13
14	UC	150.2±0.9	148.73±1.37	147.8±1.42	150.26±0.81

[a,b,c]Means within the same row with different superscripts are significantly different (*p <0.05)

The results of least square analysis (Table 4) revealed that season of calving significantly ($p<0.05$) affect the RUH, TL and RUW, however, winter calvers exhibit higher and wider udders with longer teats followed by autumn calvers, but summer calvers had the higher udder but less wide and smaller teats than rainy calvers.

It can be surmised from the results that winter calvers had higher values of udder morphometric traits than the rest of calvers, which could be attributed to the ample nutrition and favourable temperature range during this season due to which milk production is higher resulting into higher udder capacity and measurements. According to (M'hamdi et al. 2012), effects of calving season on milk yield and lactation length was significant and milk yield was high (5827±69.23) in cows calving during winter season. Marai et al. (2001) stated that season of calving showed significant ($p <0.01$) effects on TL, TD and distances between front teats. He further added that highest values of TL and diameter were found during winter season, probably due to increase in the perpendicular position of teats due to increase in milk, but report of Rahman et al. (1989) is in agreement with the results that all udder measurements were the highest during winter and the lowest during summer, in lndian buffaloes.

Conclusions

The results of present investigation showed that cows of 3rd parity had the most ideal udder balance with larger teat diameter as compare to cows in other parities, while cows of 4th parity had the higher, large, widest udder with deeper cleft and longer teats. Cows in 3rd stage of lactation had the shortest distance of teat ends to floor and a big udder with large circumference, while rear udder were higher with deeper clefts in cows of 2nd stage of lactation as compare to cows in other stage of lactation. The winter calvers exhibited higher and wider udders with longer teats followed by autumn calvers. The variability of udder dimensions suggested that udder traits have adequate genetic variation to allow selection. Also, that udder confirmation of Sahiwal cows at Livestock Research Centre, NDRI, Karnal, India is affected by the seasonal, parity and stage of lactation. Therefore, it becomes necessary to standardize the data for same should be considered for genetic evaluation of udder traits in Sahiwal cows and these results can be used as a management tool to improve selection criteria for dairy animals.

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RESEARCH ARTICLE

Non - genetic factors affecting milk composition traits and association of milk quality parameters with udder and teat type traits of Sahiwal and Karan Fries cows

Nisha Sharma[1], Revanasiddu D[1], Sushil Kumar[2], Beena Sinha[3], Ragini Kumari[4], Rebeka Sinha[1], ID Gupta[1] and Archana Verma[1]

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Abstract: The study was carried out to determine the effect of season of calving, stage of lactation and parity on 207 cattle compromising of Sahiwal (107) and Karan Fries cattle (100) of different parities and lactation stages.In Karan Fries cows, Season had significant effect on all milk composition traits viz., average test day milk yield (ATDMY), average test day fat yield (ATDFY), average test day solid not fat yield (ATDSNFY), average test day protein percentage (ATDP%) and average test day lactose yield (ATDLY). In Sahiwal cows, parity had non-significant effect on all milk composition traits whereas, in Karan Fries cows, parity had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP%, ATDLY and average test day protein yield (ATDPY). In Sahiwal cows, Season had non-significant effect on all milk composition traits whereas, in Karan Fries cows (KF) had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP% and ATDLY in Karan Fries cows. In Sahiwal cows, parity had non-significant effect on all milk composition traits whereas in Karan Fries cows, parity had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP%, ATDLY and ATDPY. The phenotypic correlation between udder and teat type traits and composition were low in magnitude. It can be concluded that changing patterns of milk composition with different seasons and parities provide scientific evidence to improve the nutrition management.

Keywords: Karan Fries; Milk composition traits; Pearson Correlation; Sahiwal cows

Introduction

Animal husbandry sector is emerging as an important growth engine of the Indian economy. The share of livestock sector in GDP has gradually risen because of the growing demand for livestock products impelled by urbanization and population growth. Livestock sector contributes 4.11% to GDP and 25.6% of total Agriculture GDP in India. About 20.5 million people depend upon livestock for their livelihood and it provides employment to about 8.8 % of the population in India (20th Livestock Census, 2019). The economics of any dairy enterprise is influenced by the production, reproduction and health status of livestock. Selection pressure on production traits increase milk yield in dairy animals but leads to an increased incidence of diseases and reduced fertility. Milk composition traits are the factors which drives the economic profitability of dairy farms. Efforts to improve the traits that are greatly influenced by the environment should primarily focus on the managerial inputs that modify the conditions under which the genotypes are expected to perform is the important key to improve the profitability to the dairy farmers.

Comprehensive research works on major constituents in cow milk and the factors affecting milk yield and composition is the need of the hour, as many farmers have complaints regarding low fat and SNF in their cow's milk. A strong positive genetic correlation has been found between linear type traits and production traits i.e. udder width and fat yield (Zink etal. 2014).Wagay et al. (2017) investigated that Fat% and SNF% showed a negative and highly significant (P<0.01) correlation with teat diameter, udder length and fore udder depth. Selection within the best environment allowed better gene expression and selection response; when the genetic effect on a trait is weak then, the environment has a greatest influence on the trait. Keeping the fore going in consideration, the present study was

[1] Animal Genetics and Breeding Division, ICAR-National Dairy Research Institute, Karnal – 132 001, Haryana India

[2]Department of Animal Genetics & Breeding, College of Veterinary Science and A.H. (NDVSU), Rewa -486 001 (Madhya Pradesh) India
Email: vetsushil09@yahoo.com

[3]DAHO Campus, Nawada-805 110, Bihar, India
Email: bkumarvet@gmail.com

[4]Block Animal husbandry officer,Ekangarsarai-801 301, Bihar, India
Email: raginindri@gmail.com

Nisha Sharma (✉)
Animal Genetics and Breeding Division, ICAR-National Dairy Research Institute, Karnal – 132001, Haryana, India
E-mail: nishasharma1777@gmail.com

conducted to analyze the association of udder and teat characteristics with milk quality parameters in Sahiwal and Karan Fries cows.

Materials and Methods

The milk composition data of lactating animals (2017-2019) were collected from milk composition register maintained in the Livestock Record Unit of Animal Breeding & Genetics Division of ICAR- NDRI on 207 cattle compromising of Sahiwal (107) and Karan Fries (100). Standardization of the data for milk composition traits was done by selecting only those animals which were having Average test day milk yield (kg) ATDMY more 2.5 kg and >5 kg in Sahiwal and Karan Fries cows, respectively.

Udder and teat type traits

Data on udder and teat type traits were measured and documented from the herd of all lactating Sahiwal and Karan Fries cows maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal

Udder and teat measurements were done by Metal tape and verniercalliper before afternoon milking. The udder and teat measurements recorded were: fore udder attachment, rear udder width, rear udder height, udder balance (The level of the rear udder was assessed in relation to the depth of the front udder), udder depth (It is the distance from the hock to the lowest part of the udder floor by a line drawn imaginary), udder length, udder width, udder circumference (round the udder), central ligament) and 8 measurements described the teat conformation (teat circumference, fore teat length, rear teat length, distance between fore and rear teat, distance between left and right teat, shortest distance of floor from fore teat, shortest distance of floor from rear teat and teat diameter). The 16 udder and teat measurements were in centimeters except for udder attachment (The soundness of attachment of fore udder to abdominal wall) (degrees). RUW, RUH and CL were measured as guidelines by (NDDB 2017). The udder length was measured from the rear attachment of the udder, close by the escutcheon, to the front of the udder where it combines evenly with the body. The udder width was measured as a distance linking two lateral lines of attachment of the udder to abdominal wall, below the flank. Front and rear teat length was measured from the higher part of the teat, where it dangle perpendicularly from the quarter to the tip of teat. DFR and DLR was measured as the distance linking teats at midpoint of the teat length. Mean of distance from pair of the front teat ends to floor was taken as SDF and from rear teats was SDR. Teat diameter was measured at the mid-point length by Vernier Calliper and Teat circumference was measured with a measuring tape at the midpoint of teat length.

To study the influence of season, stage of lactation and parity, these were divided into different groups. Season was divided into four groups, viz. Summer (April to June), Rainy (July to August), Autumn (September to November), Winter (December to March) while, Stage of lactation-was divided in three groups, viz. early (0–3 months), mid (3–6 months), and late (above 6 months) stage of lactation in Karan Fries cows and four stages of lactation in Sahiwal cows; (0-90 days), (91 days – 180 days), (181 days– 270 days) and (> 270 days)while, Parity-wise animals divided in five groups, viz. first, second, third, fourth, and fifth and above. Current lactation length was calculated from the difference of date of calving to the date of type scoring. Composition traits was recorded from the date of type scoring. The different traits were estimated as below:

$$\text{Average test day milk yield (kg)} = \frac{TD_1MY + TD_2MY + TD_NMY}{\text{No. of Test days}}$$

$$\text{Average test day fat \%} = \frac{TD_1F\% + TD_2F\% + TD_NF\%}{\text{No. of Test days}}$$

$$\text{Average test day Protein\%} = \frac{TD_1P\% + TD_2P\% + TD_NP\%}{\text{No. of Test days}}$$

$$\text{Average test day Lactose\%} = \frac{TD_1L\% + TD_2L\% + TD_NL\%}{\text{No. of Test days}}$$

$$\text{Average test day SNF\%} = \frac{TD_1SNF\% + TD_2SNF\% + TD_NSNF\%}{\text{No. of Test days}}$$

Average test day fat yield (grams) = ATDMY ×ATDF% × 10

Average test day Protein yield (grams) = ATDMY ×ATDP% × 10

Average test day Lactose yield (grams) = ATDMY ×ATDL% × 10

Average test day SNF yield (grams) = ATDMY ×ATDSNF% × 10

Statistical analysis

Least squares analysis of variance for unequal and non-orthogonal data using the technique described by Harvey (1990) was used to study effect of non-genetic factors. The model was used with the assumptions that, different components being fitted into the model are linear, independent and additive.

Results and Discussion

The mean and standard error of different milk composition traits viz., average test day milk yield (ATDMY), average test day fat percentage (ATDF%),average test day solid not fat percentage (ATDSNF%), average test day protein percentage (ATDP%), average test day lactose percentage (ATDL%), average test day fat yield (ATDFY),average test day solid not fat yield (ATDSNFY), average test day protein yield (ATDPY), average test day lactose yield (ATDLY) in Sahiwal and Karan Fries cows were presented in Table 1.

In Sahiwal cows, the mean of ATDMY, ATDF%, ATDSNF%, ATDP%, ATDL%, ATDFY, ATDSNFY, ATDPY and ATDLY were 7.21±0.24 kg, 4.30±0.02%, 8.64±0.03%, 3.31±0.01%, 4.6±0.03%, 310.8±10.73gm, 623.95±21.01gm, 239.67±8.09gm, 331.34±11.65gm, respectively. In Karan Fries cows, the mean of ATDMY, ATDF%, ATDSNF%, ATDP%, ATDL%, ATDFY, ATDSNFY, ATDPY and ATDLY were 11.26±0.34 kg, 4.21±0.03%, 8.71±0.01%, 3.36±0.01%, 4.48±0.02%, 474.83±15.85 gm, 981.25±30.67gm, 378.44±11gm, 502.85±15.86 gm, respectively. Lal et al. (1984) found protein, SNF percentage as 4.9 ± 0.01% and 9.1 ±0.01% in Tharparkar cows. Sarkar et al. (2006) reported that Least square means for TDMY, fat, SNF, protein and Lactose, were 8.45±0.93%, 4.23±0.18%, 9.77±0.11%, 3.60±0.05%, 5.38±0.07%, respectively in Sahiwal cows whereas in Karan Fries cows, 11.19±0.70%, 3.91±0.14%, 9.78±0.09%, 3.58±0.04%, 5.39±0.05%. Painkra (2007) reported that the least square means for fat, SNF, protein and Lactose, were 4.28±0.04%, 11.07±0.05%, 4.25±0.02%, 6.08±0.03%, respectively in Sahiwal cows. Khan et al. (2007) reported the range for Fat% as 3.33 to 4.88 percent in different stages of lactation and 8.90 percent SNF in Sahiwal cows. Kayastha et al. (2008) reported that least square means for fat, SNF and protein percentages were 5.341±0.067%, 8.544±0.035%, 3.047±0.036, respectively in native cattle of Assam. Suman et al. (2009) found that the milk solid-not-fat (SNF) averaged to 8.54±0.01, 8.50±0.01 and 8.65±0.02 per cent respectively in Holstein Friesian x Hariana (FH), Brown Swiss x Hariana (BH) and Jersey x Hariana (JH) crosses of cattle. Mishra and Joshi (2009) reported fat, protein percentage as 4.02 ± 0.02% and 3.35 ± 0.03% in Karan Fries cows. Verma et al. (2016) found least squares means for lactational average fat percentage (LFA), lactational average solid not fat percentage (LSA) were 4.71±0.01%, 8.81±0.01%, respectively in Sahiwal cattle. Sudhakar et al. (2013) found that the fat, SNF, protein and lactose content were 4.50±0.35, 8.92±0.17, 3.25±0.06 and 4.88±0.089 per cent respectively in Jersey crossbreds whereas in Holstein crossbreds, were 3.81±0.34, 9.13±0.16, 3.33±0.06 and 5.06±0.09.

Season, Parity and stage of lactation showed non-significant effect on all milk composition traits in Sahiwal cows. Season had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP% and ATDLY in Karan Fries cows and non-significant effect on ATDF%, ATDSNF%, ATDPY and ATDL%.

Table 1 Estimates of different milk composition traits (Mean±SE) in Sahiwal and Karan Fries cows

Traits/Genetic group	Sahiwal	Karan Fries
Average test day milk yield (ATDMY) kg	7.21±0.24	11.26 ±0.34 kg
Average test day fat percentage (ATDF) %	4.30±0.02	4.21±0.03
Average test day solid not fat percentage (ATDSNF) %	8.64±0.03	8.71±0.01
Average test day protein percentage (ATDP) %	3.31±0.01	3.36±0.01
Average test day lactose percentage (ATDL)%	4.6±0.03	4.48±0.02
Average test day fat yield (ATDFY)g	310.8±10.73	474.83±15.85
Average test day solid not fat yield (ATDSNFY) g	623.95±21.01	981.25±30.67
Average test day protein yield (ATDPY) g	239.67±8.09	378.44±11
Average test day lactose yield (ATDLY) g	331.34±11.65	502.85±15.86

Table 2 Effect of non genetic factors on milk composition traits in Sahiwal cattle

Effects	ATDMY	ATDF%	ATDFY	ATDSNFY	ATDP%	ATDPY	ATDL%	ATDLY
SEASON								
Summer (39)	7.34±0.56	4.33±0.04	317.59±24.66	629.23±49.35	3.33±0.0 (37)	239.31±16.55	4.66±0.08	334.95±23.7
Rainy (23)	7.05±0.61	4.43±0.04	311.13±27.14	615.12±54.30	3.33±0.02(21)	234.13±18.37	4.57±0.09	322.53±26.3
Autumn (4)	6.66±1.66	4.60±0.12	302.78±73.03	577.87±146.1	3.43±0.07 (3)	224.86±52.91	4.55±0.26	301.96±75.8
Winter (41)	7.22±0.52	4.23±0.03	306.79±22.97	613.85±45.9	3.34±0.02(41)	243.16±15.03	4.70±0.07	343.61±21.5
PARITY								
1 (21)	6.42±0.75	4.39±0.05	280.32±33.12	553.26±66.26	3.34±0.03(20)	214.71±22.62	4.62±0.11	299.54±32.4
2 (37)	7.17±0.61	4.39±0.04	313.55±26.98	623.48±53.99	3.34±0.02(37)	241.00±18.17	4.52±0.08	328.71±26.0
3(19)	8.45±0.77	4.42±0.05	370.77±33.95	729.85±67.94	3.38±0.03(17)	271.13±23.78	4.66±0.11	375.62±34.1
4(12)	6.74±0.92	4.41±0.07	298.85±40.41	571.26±80.86	3.40±0.03(11)	231.03±27.57	4.78±0.13	325.86±39.5
>5(18)	6.55±0.69	4.37±0.05	284.38±30.50	567.23±61.02	3.32±0.02(17)	218.95±21.08	4.51±0.10	299.10±30.2
STAGE OF LACTATION								
1(53)	6.88±0.62	4.34±0.04	298.32±27.51	601.94±55.05	3.32±0.02(53)	228.23±18.92	4.49±0.09	308.45±27.1
2(26)	7.88±0.79	4.49±0.06	351.74±34.71	682.34±69.45	3.36±0.03(24)	253.04±23.93	4.63±0.11	351.89±34.3
3(15)	5.52±0.77	4.41±0.05	243.79±34.15	475.59±68.33	3.35±0.03 (12)	189.30±24.49	4.61±0.12	263.93±35.1
4(13)	7.99±0.90	4.34±0.06	344.44±39.74	676.19±79.51	3.38±0.03 (13)	270.89±26.53	4.75±0.11	378.79±38.0

Table 3 Effect of non-genetic factors on milk composition traits in Karan Fries cattle

Effects	ATDMY	ATDF%	ATDFY	ATDSNF%	ATDSNFY	ATDP%	ATDPY	ATDL%	ATDLY
				SEASON					
Summer (18)	10.69^{b}±0.69	4.16±0.07	446.87^{a}±31.40	8.69±0.03	926.51^{b}±60.75	3.42^{b}±0.03	367.02±23.3	4.49±0.04	480.08^{a}±31.41
Rainy (16)	9.62^{a}±0.82	4.20±0.08	405.93^{a}±37.57	8.69±0.04	839.97^{a}±72.69	3.42^{b}±0.04	330.49±27.9	4.57±0.05	438.90^{a}±37.59
Autumn (22)	12.72^{c}±0.62	4.21±0.06	536.54^{b}±28.52	8.75±0.03	1112.89^{c}±55.19	3.30^{a}±0.03	419.67±21.2	4.42±0.04	561.66^{b}±28.54
Winter(44)	11.99^{c}±0.47	4.27±0.04	509.96^{b}±21.48	8.72±0.02	1045.63^{c}±41.57	3.30^{a}±0.02	396.56±15.9	4.43±0.03	530.75^{b}±21.50
				PARITY					
1(33)	13.66^{c}±0.54	4.14±0.05	568.43^{c}±24.97	8.70±0.02	1190.13^{c}±48.32	3.35^{ab}±0.02	457.59^{ab}±18.5	4.46±0.03	607.85^{c}±24.99
2(24)	11.27^{b}±0.62	4.28±0.06	486.03^{b}±28.29	8.73±0.03	985.98^{b}±54.74	3.45^{c}±0.03	387.12^{a}±21.0	4.55±0.04	512.28^{b}±28.31
3(16)	10.53^{ab}±0.66	4.21±0.06	444.08^{ab}±30.11	8.67±0.03	917.19^{ab}±58.27	3.33^{ab}±0.03	351.39^{ab}±22.3	4.46±0.04	467.85^{ab}±30.13
4(15)	10.99^{ab}±0.72	4.22±0.07	467.53^{ab}±33.03	8.75±0.03	961.44^{ab}±63.91	3.31^{ab}±0.03	365.04^{bc}±24.5	4.40±0.04	482.24^{ab}±33.05
e"5(12)	9.83^{a}±0.77	4.20±0.07	408.05^{ab}±35.20	8.72±0.04	851.51^{ab}±68.11	3.36^{b}±0.03	331.03^{a}±26.1	4.50±0.05	444.01^{ab}±35.22
				STAGE OF LACTATION					
1(20)	10.89±0.66	4.15±0.06	454.14±30.27	8.70±0.03	948.40±58.58	3.36±0.03	365.13±22.5	4.45±0.04	482.83±30.29
2(32)	10.89±0.63	4.21±0.06	457.88±28.76	8.75±0.03	952.48±55.65	3.40±0.03	372.00±21.3	4.52±0.04	492.06±28.78
3(48)	11.99±0.41	4.27±0.04	512.46±18.98	8.69±0.02	1042.87±36.72	3.32±0.02	398.18±14.1	4.46±0.02	533.65±18.99

Means with different superscripts (a, b, c) indicates significant difference (P<0.05).

Parity had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP%, ATDLY and ATDPY in Karan Fries cows. Stage of lactation had non-significant effect on all milk composition traits in Karan Fries cows. The results have been depicted in Table 2 and 3. The results of the present study indicated that season of calving showed non-significant effect on all milk composition traits in Sahiwal cows. Mishra (2001) and Kumar et al (2020) reported non-significant effect of season of calving on 305 days fat yield in Sahiwal cows as observed in present study. Season had significant effect on milk composition traits viz., Average test day milk yield (ATDMY), Average test day fat yield (ATDFY), Average test day Solids- Not - Fat yield (ATDSNFY), Average test day protein percentage (ATDP%) and Average test day Lactose yield (ATDLY) in Karan Fries cows. Total solid content was higher in autumn period than in summer period in Karan Fries cows, but there was no significant variation in the autumn and summer periods. This is in line with study reported by Parmar et al. (2020). However non-significant effect on ATDF%, ATDSNF%, ATDPY and ATDL%. On the contrary, Painkra (2007) reported that Fat, protein, solid not fat and lactose percentage were significantly (P<0.01) affected by season in Sahiwal cows. Sarkar et al. (2006) found significant effect of season of calving on protein percentage, lactose percentage and non significant on fat and SNF percentages in dairy animals. Radhika et al. (2012) reported that season of calving had non-significant effect on milk fat% and SNF% in crossbred cows. In contrary to the present finding, Suman et al. (2009) found that season of calving had significant effect on SNF percentage in two breed crosses of cattle. Verma et al. (2016) found that the effect of season was found to be statistically non-significant on lactational average fat percentage (LFA), lactational average solid not fat percentage (LSA) in Sahiwal cattle. Highest value of ATDF% in Karan Fries cows was recorded in winter and lowest in summer season because in summer season (fresh grass) was fed to the animals as compared to silage feeding in winter. Linolenic acid content found to be associated with production of specific long chain unsaturated fatty acids that inhibit the denovo fatty acid synthesis in the mammary gland and reduce the milk fat content (Baumgard et al. 2000, Heck et al. 2009). In Sahiwal cows, Parity had non-significant effect on all milk composition traits. Parity had significant effect on milk composition traits viz., ATDMY, ATDFY, ATDSNFY, ATDP%, ATDLY and ATDPY and non-significant effect on ATDF%, ATDSNF%, ATDL% in Karan Fries cows. Painkra (2007) reported that Fat, protein and lactose percentage, respectively were found to be significantly (P<0.01) affected by parity in Sahiwal cows. Sarkar et al. (2006) reported that effect of order of parities was not significant for TDMY, Fat%, Protein %, SNF % and Lactose% in dairy animals. Similar results have been reported by Radhika et al. (2012) that parity had non-significant effect on milk fat% and SNF% in crossbred cows as observed in present study. Sudhakar et al. (2013) found the non- significant effect of parity on fat%, fat yield, SNF yield, Protein percentage, Protein yield, Lactose percentage and Lactose yield in crossbred cattle. Verma et al.

Table 4 Phenotypic correlation coefficients between milk quality parameters and udder traits in Sahiwal and Karan Fries cows

Breed	Traits	ATDMY	ATDF%	ATDFY	ATDSNF%	ATDSNFY	ATDP%	ATDPY	ATDL%	ATDLY
Sahiwal	UW	0.327**	-0.037	0.318**	0.174	0.349**	-0.042	0.319**	-0.134	0.272**
	UD	-0.114	-0.044	-0.118	-0.506**	-0.168	0.071	-0.098	0.386	-0.007
Karan	UW	-0.234*	-0.042	-0.228*	-0.245*	-0.243*	0.057	-0.236*	-0.064	-0.248*
Fries	UD	-0.179	-0.073	-0.199*	0.212*	-0.169	0.021	-0.175	-0.159	-0.201*

* Significant at 5% level of significance; ** Significant at 1% level of significance

(2016) found that the effect of order of parity were found to be statistically non-significant on lactational average fat percentage (LFA), lactational average solid not fat percentage (LSA) in Sahiwal cattle. Duncan's multiple comparison of parities showed that ATDF% in the first parity was lower than the other parities in both the breeds. Yang et al. (2013) reported similar findings in Chinese Holstein cows that milk fat percentage in the first parity was lower than the other parities. This can be supported by the reason that heifers need more amino acids and fat for their body growth. Stage of lactation had non-significant effect on all milk composition traits in both the breeds. Painkra (2007) reported that Fat, SNF, protein and lactose percentage, respectively were found to be significantly ($P<0.01$) affected by stage of lactation in Sahiwal cows. Sarkar et al. (2006) reported that effect of stage of lactation was significant on TDMY, Protein, SNF and lactose & non significant on fat percentages in dairy animals. Kayastha et al. (2008) found that stage of lactation showed highly significant effect on fat percentage, solids-not fat percentage and non significant on protein percentage in native cattle of Assam. Sudhakar et al. (2013) found the non- significant effect of stage of lactation on fat%, fat yield, SNF yield, Protein percentage, Protein yield, Lactose percentage and Lactose yield in crossbred cattle as observed in present study. Stage of lactation lead to variation in milk fat and protein percentages and highest percentages are usually found in colostrum, followed by a decline during the first 2 months of lactation, followed by slow increase as lactation progresses as observed in present study.

Pearson correlation between milk composition traits and udder & teat type traits presented in Table 4. The strongest positive phenotypic correlation was estimated between udder width, ATDMY (0.32) and ATDPY (0.31) in Sahiwal cows. Similar correlationwere estimated for the same traits in Czech Holstein cows by Zink et al. (2014). In KF cows, Udder depth had negative correlation with all milk composition traits in both the breeds. Zink et al. (2014) also reported negative correlation between Udder depth, milk yield and protein yield. Literature on correlation between milk composition traits and udder & teat type traits is scanty.

Conclusions

Non genetic factors play a significant role in variation of milk yield and their components. Nowadays, milk pricing system is based on percentage of milk composition. The present study highlights the usefulness of changing patterns of milk composition with different seasons and parities as well as provide scientific evidence to improve the nutrition management of the herd to obtain nutritive milk. There is need to focus attention on milk constituents as a selection criteria in breeding programme to obtain healthy milk products. Animal breeders while paying attention to udder depth trait as a predictor of useful life in the dairy herd can also lead to sacrifice yield.

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RESEARCH ARTICLE

A study on the trends of antibiotic usage in dairy animals of Jammu division

Rakhshan Jeelani, Asma Khan, Dipanjali Konwar and Biswajit Brahma

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Abstract: Antibiotics are becoming increasingly ineffective as drug-resistance spreads globally. This study documents the trend of usage of antibiotics in dairy animals of Jammu. Data regarding the usage pattern of veterinary antibiotics was collected from the records of government veterinary hospitals and veterinary pharmaceutical companies from 2016-2021.The data were analysed to check the widely used antibiotics and there trend of usage. The study revealed that beta-lactums were the most used antibiotics, followed by floroquinolones. The aminoglycosides were also used in noticeable amount with maximum usage. In cattle and buffalo beta-lactums and floroquinolones were the most used antibiotics, respectively. Beta-lactums were preferred in case of cattle calf while as in case of buffalo calf tetracyclines were the drug of choice. In case of clinical mastitis beta lactum class of antibiotics was the most used drug for treatment. In case of fever, diarrhoea, metritis and other diseases such as retention of placenta, abscess, or wound, floroquinolones were most widely used antibiotics.

Keywords: Antimicrobial resistance (AMR); Beta-lactum; Drug resistance; Jammu region

Introduction

The term antibiotics includes a broad range of chemical substances that are produced naturally, semi-synthetically and synthetically used to inhibit bacterial growth (bacteriostatic) or kill them (bactericidal). Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites transform with time to render the action of drugs as ineffective, thus increasing the hazard of disease spread, morbidity and mortality. Antibiotics are used in livestock production as therapeutics, growth promoters, and prophylactics. Antibiotics are becoming increasingly ineffective as drug-resistance spreads globally. Usage of antibiotics is an important element in the management of udder hygiene to treat clinical or sub-clinical mastitis (Steeneveld et al. 2011; van den Borne et al. 2010), and at dry-off to prevent mastitis cases (Halasa et al. 2009) in dairy herds. Despite large scale usage of antibiotics in livestock production, comparatively less attention has been given to its contribution to the overall problem of antibiotic resistance. In September 2016, the United Nations (UN) General Assembly recognized the inappropriate use of antimicrobials in animals as a leading cause of rising AMR.

The dairy industry is a major consumer of antibiotics globally and treatment of infected udder is the most common reason of use of antibiotics in cows (Pol and Ruegg, 2007). The occurrence of Staphylococcus aureus and methicillin resistant Staphylococcus aureus (MRSA) in foods of animal origin, pose a serious threat to the well-being of humans due to innumerable clinical implications. There is a potential risk of transmission of S. aureus and MRSA to humans through raw milk if consumed without maintaining adequate hygienic standards. The new WHO recommendations (WHO, 2017) urges prudent usage of antibiotics to reduce the risk of antimicrobial resistance.

To optimize usage of antibiotics, it is important to understand antimicrobial treatment patterns for udder health in dairy cattle herds and investigate factors that influence or enhance the treatments, and also other factors, such as spread of pathogens (Halasa et al. 2010) and antimicrobial resistance pattern. Unlike for human medicine, there is only limited information (public or private) on veterinary antimicrobial usage accessible to the public health community. The emergence of antibiotic resistance is threatening the public health in industrialized as well as developing countries, resulting in therapy failure and increased health-care expenditures. Recent reviews have identified a lack of data on the pattern of antimicrobial use as an impediment to the design of measures to tackle this growing problem (Jones et al. 2015). Antibiotic resistance is a dynamic process and needs

Division of Livestock Production & Management, University of Agricultural Sciences & Technology of Jammu, R. S. Pura, Jammu 181 102, India

Asma Khan (✉)
Division of Livestock Production and Management, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India 181104
E-mail: asmakhan70m@gmail.com; Phone: +91-9419112132

continuous monitoring to follow its proper pattern. The data for antibiotic usage is not available publicly thus it is difficult to track the trend of antibiotic usage. The present study was therefore, aimed to analyze pattern and extent of usage of veterinary antibiotics in dairy sector.

Material and Methods

Information on the usage of veterinary antibiotics was collected from documented records of government hospitals, and the records of wholesale distributors for the period 2015-2021. The clinical treatment registers of veterinary hospitals were the primary source of data and information on livestock species, disease by which animal was suffering, the antibiotic used and duration of treatment were collected. Different antibiotics were categorised into six major class of commercially available antibiotics (Beta-lactums, floroquinolones, tetracycline, aminoglycosides, sulphonamide, and nitroimidazole).

Data on wholesale distribution of antibiotics for seven districts (Jammu, Samba, Kathua, Doda, Bhaderwah, Kishtwar, Pounch, Rajori) of Jammu region was collected from office of Deputy Drug controller, Drug and Food control organisation, Jammu. The data included volume of sales of different types of antibiotic and units sale price of each type. The antibiotics were categorised in six major categories of antibiotics as described earlier. The total sale price was deducted by multiplying sale volume with unit price. The data accounted for approximately 70 % of sale volume of antibiotic for the region. All the statistical analysis were performed in SYSTAT 12 software package.

Results and Discussion

Two dataset that included sale data of antibiotics of wholesale drug dealers in the region and treatment history of clinical cases in veterinary hospitals were analysed.

Trends in sale of antibiotics in the region

Trends in overall sale volume of antibiotics in the region

Total sale quantity of all classes of antibiotics by major wholesale veterinary drug dealers during previous six years has been presented in figure 1 (A). The sale of antibiotics from these wholesale dealers covers seven districts and approximately 60 percent of total consumption of antibiotics in the Jammu region. Overall, a whopping 519.45 percent increase in sale volume of antibiotics was observed during the period of 2015-16 to 2020-21. It was evident that the sale volume was minimal during 2015-16 but experienced a major increase (261.67%) in the next year (2016-17). An increasing trend in sale volume was observed since then, however, another major increase (73.13%) in sale volume of antibiotics was during the period 2018-19 to 2019-20. The trend indicated there was a major increase in usage of antibiotics in dairy animals over last six years. This usage is still expected to rise as population is increasing and more livestock will be required to meet the needs of people. Thus, will cause an increase in the usage of antibiotics as growth promoters. Similarly Organisation for Economic Cooperation and Deveopment (OECD) assess that the quantity of antimicrobials used in food animals will rise globally by 67% that is from 63,151 tons in 2010 to 105,596 tons by 2030 (Laximinarayan et al. 2015). Overuse and misuse of antimicrobials are the main driving forces for expansion of high rates of resistance. However, there are also other reasons for increase in antibiotic consumption which include driven by rising incomes, health insurance, and load of infectious diseases (Singh et al. 2019).

Trends in sale volume of different categories of antibiotics in the region

The sale volume of different antibiotics categorized under major six classes has been depicted in figure 1 (B) for the period. The data suggest beta-lactums (4029 kg) as major antibiotics type used in the region followed by floroquinolones (1069 kg) and tetracycline (576 kg) for the period 2015-16 to 2020-21. It was concluded that Beta lactums were the most widely used class of antibiotics. Sawant et al. (2005) found similar pattern in his study and concluded that beta-lactums are the most widely used antibiotics. Consumption of sulphur group of antibiotics was less (3.76 kg) as compared to other groups for the period. The trend indicated beta-lactum group was the major type and maintained a steady sale volume over the period of study. Sale volume of tetracycline and sulphur groups showed a flip-flop pattern. Starting at a negligible quantity during 2015-16, sale of sulphur group reached the peak in 2017-18 with an increase over 266 percent and again declining to minimal during 2019-20. Major increase (4828.57%) in the sale volume was observed in 2019-20 (517.5 kg) over the previous year. A remarkable increase in sale volume of floroquinolones was observed for the study period. First major increase (3775%) for the group was in 2016-17 and the second sharp rise (1818%) in sale volume was in 2020-21 indicating a progressive usage of the antibiotics in the region.

Trends in sale values of different categories of antibiotics in the region

The sale values of different categories of antibiotics were deduced by multiplying quantity of sale with unit price. The overall trend suggested that beta-lactums registered maximum sale values (INR 405.75 lacs) followed by tetracyclines (INR 28.81 lacs), floroquinolones (INR 20.34 lacs) and sulphur drugs (INR 6.89 lacs). The disparity in trends of sale volume and sale values of different categories of antibiotics was attributed to different unit prices for different categories and formulations. The total sale values of all categories showed an increase (135.40%) during the period 2015-16 (INR 44.86 Lacs) to 2020-21 (INR 105.61 lacs). Barring a transitory drop in sale value of sulphur group in 2020-

21, all the categories of antibiotics showed a steady increase in sale values during the period.

Share of distributors in sale values of different categories of antibiotics

The share of different wholesale distributors in total sale values of antibiotics over the study period has been presented in figure 1 (C). It was evident that while sale of beta lactum and floroquinolones was shared by almost all the dealers, the sale of tetracycline was limited to two distributors (Dealer B and C). Barring a drop in sale values in 2019-20, three major wholesale dealers (Dealer A, B and C) experienced increase in sale values of beta lactum. The major rise in sale value of floroquinolones in the years 2019-20 and 2020-21 was contributed two new firms (Dealer D and F) for which data of previous years was not available.

The share of beta-lactum and tetracycline sale quantity by different wholesale distributors during the period of study has been presented in figure 2 (C) and 2 (D). Two farms (Dealer B and C) shared the major sale quantity of both the antibiotics group. The sale of beta-lactum group showed a steady rate barring the year 2015-16, where the quantity was minimal. A major increase in sale quantity of beta lactum was contributed by Dealer A and Dealer C. The disparity in sale quantity and sale value of beta-lactum for the year 2019-20 was attributed to the change in formulation and type of beta-lactum that were different from previous years. Tetracycline sale showed a major drop for the period 2016-2018, but increased again for the year 2019-20 for both the distributors.

Share of distributors in sale volume of different categories of antibiotics

The share of beta-lactum and tetracycline sale quantity by different wholesale distributors during the period of study has been presented in figure 2(A) and 2(B). Two forms (Dealer B and C) shared the major sale quantity of both the antibiotics group. The sale of beta-lactum group showed a steady rate barring the year 2015-16, where the quantity was minimal. A major increase in sale quantity of beta lactum was contributed by Dealer A and Dealer C. The disparity in sale quantity and sale value of beta-lactum for the year 2019-20 was attributed to the change in formulation and type of beta-lactum that were different from previous years. Tetracycline sale showed a major drop for the period 2016-2018, but increased again for the year 2019-20 for both the distributors.

Trends in usage of antibiotics for treatment of clinical cases

Trends in overall usage of antibiotics for treatment of clinical cases

To study the trends of actual usage of different types of antibiotics, the data of treatment history of two veterinary

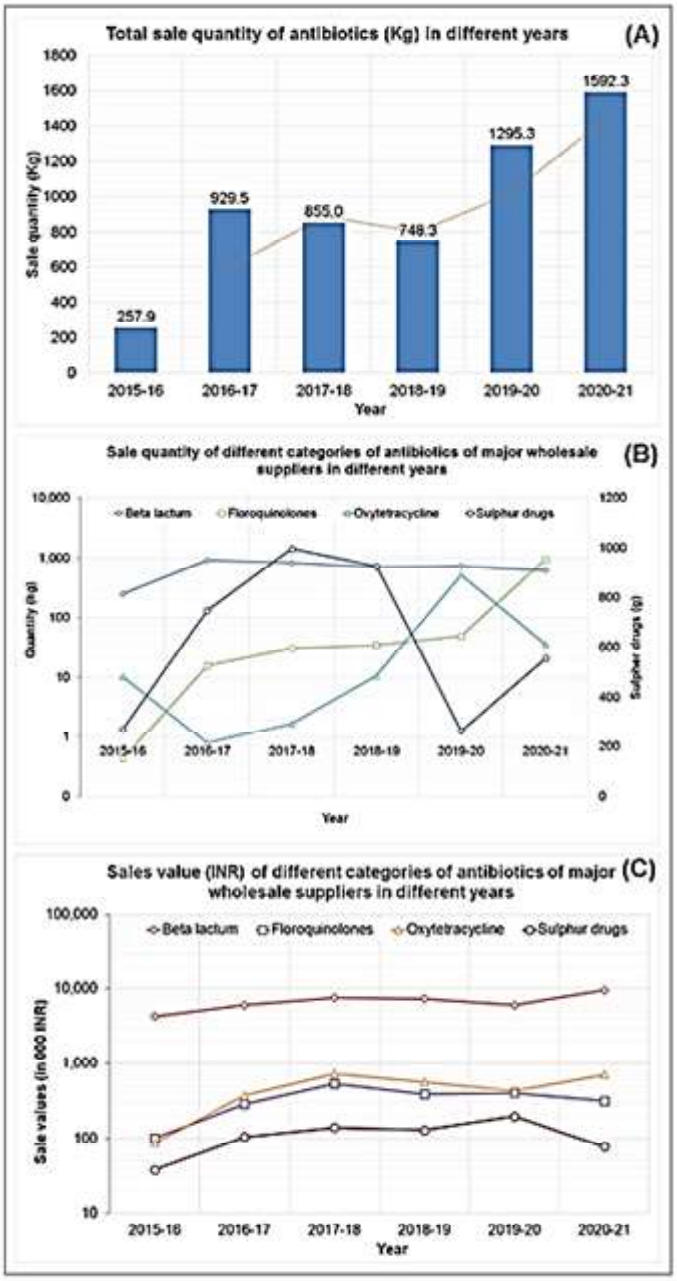

Fig. 1 Sales value and sales quantity of different antibiotics. (A): Total sale volume of antibiotics (Kg) over the period 2015-16 to 2020-21 in the region. (B): Sale volume of different categories of antibiotics of major wholesale suppliers in different years. (C): Sales value (INR) of different categories of antibiotics of major wholesale suppliers in different years.

hospitals of Jammu region over the period 2015-2019 were analysed. The overall share of different types of antibiotics used for treatment of clinical cases has been presented in figure 3. Coinciding with sale data, the usage of beta lactum was highest in both the hospitals, followed by floroquinolones and

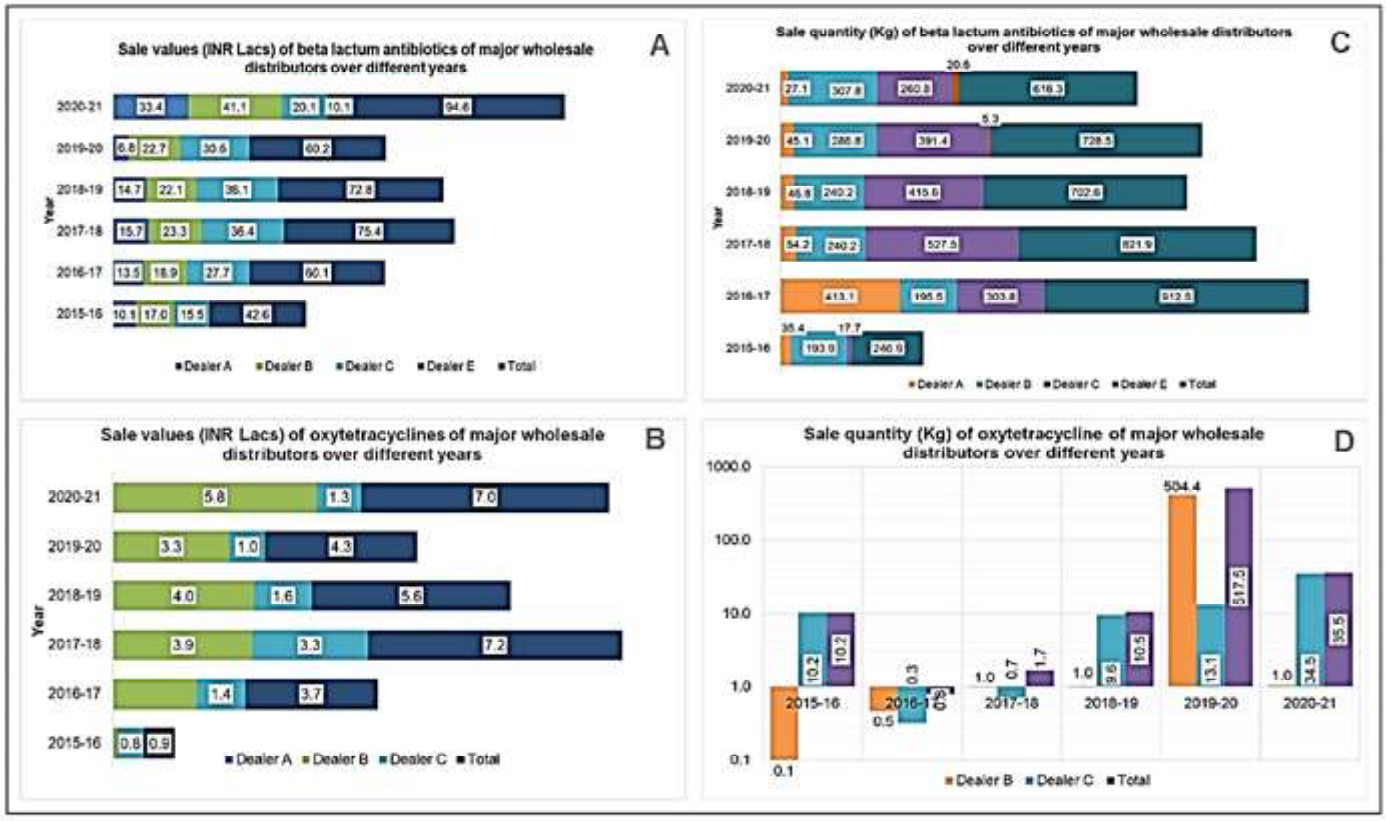

Fig. 2 Share of sale quantity and sale values of different categories of antibiotics by different wholesale distributors for the period 2015-2021. (A) Sale value of Beta lactum, (B) Sale value of Oxytetracycline, (C): Sale quantity of Beta lactum, (D): Sale quantity of oxytetracycline**.**

aminoglycosides. Contrary to sale data, the use of tetracycline was very low in these government hospitals. Use of sulphonamide that shared a fair 10 percent of treated cases, was exclusive to one hospital. Use of nitromidazole was negligible and was limited to one hospital.

Year wise usage of different categories of antibiotics for clinical treatments

The year wise usage of different categories of antibiotics for treatment of clinical cases has been presented in Figure 3 (A) and 3 (B). The usage of beta-lactum was highest ranging 6.67 to 49.43 percent of treated cases in hospital 1 and 39.50 to 53.45 percent of treated cases in hospital 2. Use of aminoglycosides for treatment of clinical cases varied for the hospitals. For hospital 2, the usage was between 9.05 to 21.37 percent, whereas, for hospital 1 it had a wide range (1.15-33.89%). Use of fluoroquinolones was second to beta-lactum usage that varied between 31.31 to 36.78 percent for hospital1 and 21.85 to 49.47 percent for hospital 2. The trend indicated a steady use of beta-lactum and fluoroquinolones over the period, whereas, there was varied usage of aminoglycosides. Usage of tetracycline also varied between the hospitals and ranged from as low as 0.63 percent to 9.44 percent of the treated cases. Use of sulphonamides in hospital 1 showed a consistent rate of usage ranging between 8.04 to 12.78 percent. No major changes was also observed in usage of nitroimidazole (<1%) over the study period.

Species wise usage of different categories of antibiotics for clinical treatments

The usage of different classes of antibiotics in different species over the study period is presented in figure 3 (C) and 3 (D). Although this data is not a true representative of species wise usage of antibiotics as the data takes account of only clinical cases that visited veterinary hospitals and also the data of other livestock species was not included in the analysis. Nevertheless, this analysis still provides an impression that the usage of antibiotics for therapeutic purpose was maximal in cattle followed by buffalo. The overall data suggest beta-lactum (42.68 - 46.46%) followed by floroquinolones (36.03-38.53%) as major antibiotics used for treatments of dairy animals. For dairy cattle, 47.01 to 49.89 percent clinical cases received treatments with beta-lactum antibiotics, followed by floroquinolones (35.61-36.21%) group of antibiotics. Use of aminoglycosides (12.67%) and sulphur group (11%) were specific to hospitals. Usage of tetracycline (1.82-5.34 %) and nitroimidazole (0.42%) were limited for dairy cattle. The treatment regime of buffalo followed an almost similar

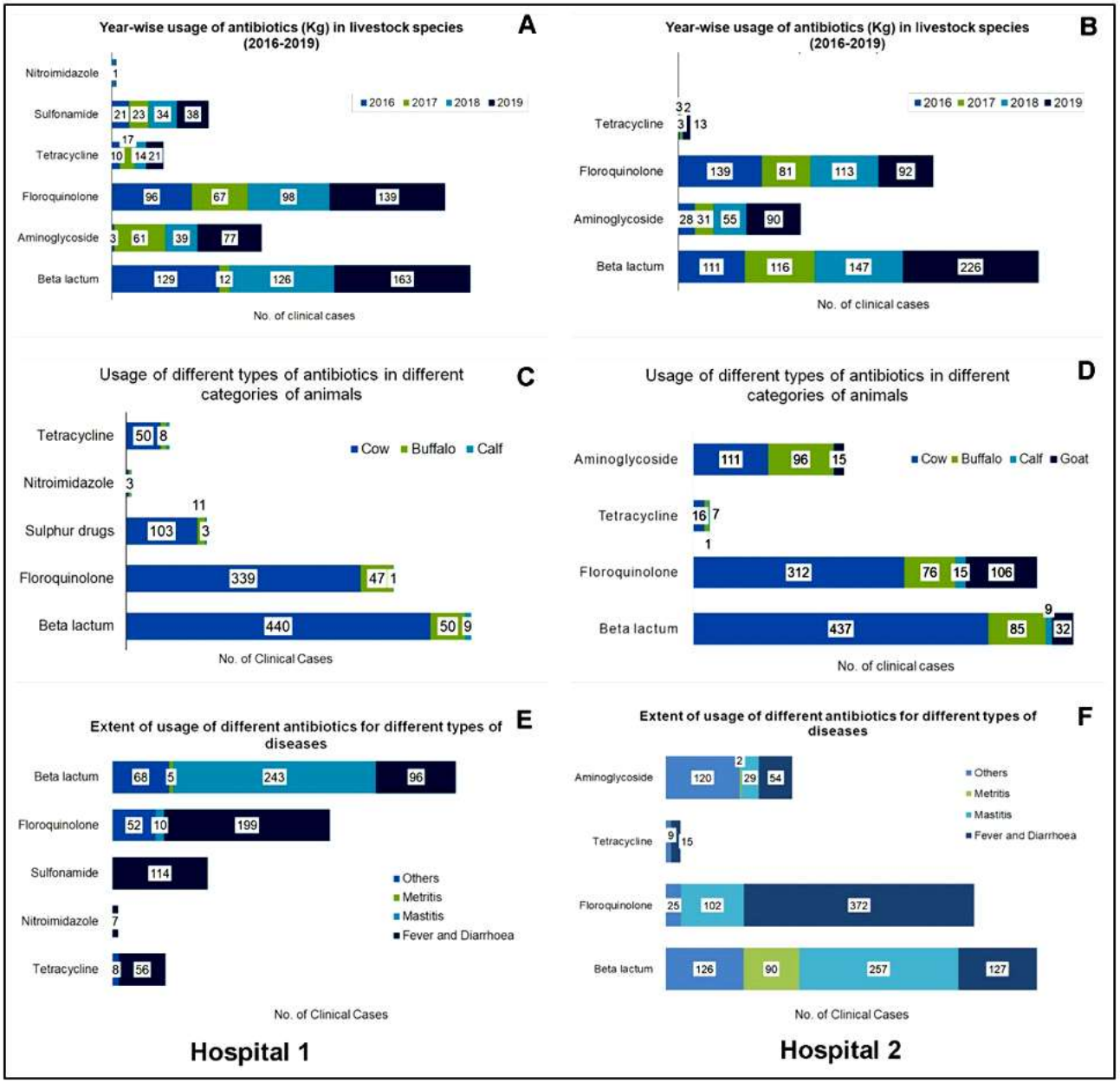

Fig. 3 Trends in usage of antibiotics for treatment of clinical cases in hospitals. (A): Year wise usage of antibiotics in livestock species (2016-2019) in Hospital 1. (B): Year wise usage of antibiotics in livestock species (2016-2019) in Hospital 2. (C): Usage of different types of antibiotics in different categories of animals in Hospital 1. (D): Usage of different types of antibiotics in different categories of animals in Hospital 2.(E): Extent of usage of different antibiotics for different types of diseases in Hospital 1. (F): Extent of usage of different antibiotics for different types of diseases in Hospital 1.

pattern with maximal use of beta-lactum (32.20-42.01%), floroquinolones (28.78-39.50%). However, the data of hospital 2 suggested that aminoglycosides (36.36%) were also used fairly for treatment of clinical cases of buffalo. A significant observation on usage of floroquinolone was that this antibiotic was predominantly used in treatments of calf (57.69%) and goats (69.28%) in the hospital 2. Alike other categories of animals, use of tetracycline and nitroimidazole was limited in calf and goats.

Usage of different categories of antibiotics for clinical treatments of different diseases

The usage pattern of different types of antibiotics to treat different types of disease has been presented in figure 3 (E) and 3 (F). The data indicated beta-lactum was used to treat nearly half (45.18-48.02%) of all clinical cases. Maximum use of beta-lactum was in treatment of mastitis (66.23-96.04%), metritis (97.82-100%), cases such as enteritis, wound, retention of placenta, FMD, bloat,

abscess etc. (45.00-53.12%). Overall use of floroquinolones (30.42-37.57) was next to beta lactum and with maximum usage for treatment of fever and diarrhoea (42.16-65.49%) and cases such as enteritis, wound, abscess etc. (8.92-40.62%). A significant observation was that none of the hospitals used tetracycline for treatment of clinical cases of mastitis or metritis. The data suggested that sulfonamides were exclusively used for treatment of fever and diarrhoea. Use of floroquinolones were less often for treatment of mastitis (3.95-26.28%), whereas no usage of this group was recorded for treatment of metritis.

Conclusion

Beta lactums and floroquinolones are the most extensively used antibiotics in dairy farms which makes it important that public health issues related to antibiotic usage and emerging antimicrobial resistance should take into consideration such antibiotics. The increasing trends in consumption of antibiotics for veterinary usage also warrants creating awareness among people how resistance to antibiotics can affect dairy herds and farmer in long run.

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RESEARCH ARTICLE

Impact of COVID-19 lockdown on various stakeholders associated with dairy food supply chain in Karnataka, India- An evidence based study

Gurrappanaidu Govindaraj[1], Shanabhoga MB[1], Swamy HM[1], Nagalingam M[1], Shome BR[1], Habibar Rahman[2]

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Abstract: The present study assessed the disruptions in the dairy food supply chain and associated stakeholders due to COVID-19 in Karnataka, India. A cross-sectional survey using a multistage random sampling procedure was conducted and data was collected from the dairy food supply chain associated stakeholders in Karnataka, India. The results revealed that milk providers to the co-operatives increased by 4.67 % and the quantity of milk collection increased by 25.15 % during the lockdown. On the service front, 41.3% of the dairy co-operative societies faced animal feed shortage. The average per day milk consumption increased by 42.7 % during lockdown mainly driven by the return of family members from cities to villages. However, the gross income realized through milk sales by the dairy farmers decreased during the lockdown period mainly due to reduced milk prices offered by the co-operatives. The gross income realized through the sale of milk products by the vendors declined by 5.11% due to a fall in demand for various milk products. Despite many problems faced by the co-operative sector during the lockdown, it acted as a buffer and protected the dairy food supply chain from the free market and capitalist breakdown in Karnataka.

Keywords: COVID-19 Impact Evaluation; Dairy Cooperatives; Dairy Food Supply Chain; Stakeholders; Food Security

Introduction

The dairy food supply chain in India has grown substantially over the years. India ranks first among the world's milk producing nations, achieving an annual output of 187.7 million tonnes during the year 2019 which is approximately 2 times that of the USA and over 5 times of China (DAHD&F, 2019a). Also, the per capita availability of milk in the country registered 394 grams per day in 2019 (DAHD&F, 2019a) which is more than the world average of around 294.2 grams per day in 2017 (Food Outlook, 2018). The bovine population in India is 303.76 million, of which, 193.46 million is cattle and 109.85 million is buffaloes encompassing 125.75 million milch animals (DAHD&F, 2019a). According to National Account Statistics (2019), the dairy food supply chain in India has the highest value of the output of milk Rs.44,850 crore, which was more than the combined output value of major agricultural crops such as wheat (Rs. 1,660 crore) and paddy (Rs. 2,400 crore). Out of the total value of output from the agriculture and allied sector, the dairy food supply chain alone contributes more than 25 percent (DAHD&F, 2019a). Dairying is an important secondary source of income for millions of rural families in India (DAHD&F, 2019b) and contribute significantly in alleviating poverty and unemployment especially in the rain-fed and drought-prone areas (Karmakar and Banerjee, 2006). Around 69 percent of India's farming community is engaged in dairy activities and contributes one third of the gross income of rural households and nearly half of gross income of the landless farmers (Jaiswal et al. 2018).

The Indian dairy food supply chain comprise organized and unorganized segments. The organized segment includes cooperatives and private dairies whereas the unorganized segment includes traditional milkmen, vendors, and self-consumption at home. Despite existence of wide network of milk co-operatives & private dairies, they procure only about 20% of the milk produced in the country, while 32% is sold in the unorganized market and 48% is consumed locally (DAHD&F, 2019b). The Indian dairy & dairy products industry comprises milk and a large variety of milk products like flavored milk, ghee,

[1]ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)
Ramagondanahalli, Yalahanka, Bangalore-560064, Karnataka, India

[2]Regional Representative
International Livestock Research Institute (ILRI), South Asia Region, New Delhi

Gurrappanaidu Govindaraj (✉)
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)
Ramagondanahalli, Yalahanka, Bangalore-560 064, Karnataka, India
Email: govindaraj.naidu@icar.gov.in; mggraj74@gmail.com
ORCID: 0000-0003-3461-2446

butter, curd, buttermilk, cheese, paneer, ice cream, etc. The cooperatives were set up by respective state milk federation to collect the raw milk from farmers and process them into various milk products and sell to the consumers in urban areas. Indian dairy food supply chain is an interconnected linkage of different stakeholders like farmer producer, dairy cooperatives, feed sellers, veterinarians, milk vendors, and consumers. Hence, any alteration in one segment of the supply chain affects other segments in the dairy food supply chain considerably (Bhandari and Ravi Shankar, 2020).

The COVID-19 pandemic has created a serious impact, not only for human health sector in the country but also all aspects of living conditions of various stakeholders associated with dairy food supply chain. To mitigate the COVID-19 spread, Government of India announced nationwide lockdown from 25th March 2020 to 14th April of 2020 (Lockdown 1.0) allowing only essential commodities. The lockdown was extended from 15th April to 3rd May (Lockdown 2.0) with a provisional relaxation on agriculture, livestock, plantation, aquaculture including essential goods. Since, there was no decline in the number of COVID 19 active cases, further lockdown was extended from 4th May to 17th May (Lockdown 3.0) with more easing on the farm activities and business. Finally, lockdown 4.0 was announced from 18th May to 31st May with some more easing on the previous guidelines (Chand, 2020). In the first phase of the lockdown, several services in the livestock sector like animal health and supply of inputs were not considered as essential services. Hence, there was disruption in the dairy production and supply including animal health services. Realizing the consequences of lockdown on the livestock and dairy food supply chain, the government included dairy food supply chain activities in the essential services category. The Indian economy was already facing challenges from demonetization and Good and Services Tax (GST) implementation and the nationwide lockdown has hampered the economy further. The sudden nationwide lockdown affected many businesses including the key sectors that contribute to the nation's food security, nutrition, and livelihood like the livestock and dairy food supply chain. Though dairy activities like production, collection, processing, marketing, and distribution were permitted as essential services during lockdown, the dampening demand of various dairy products impacted the stakeholders in the whole value chain significantly. Though there are few studies on the impact of COVID-19 on different sectors in India using secondary data, the empirical studies on the impact on dairy food supply chain and its associated stakeholders is lacking. Therefore, to address the gap, the present study assessed the disruptions in dairy food supply chain and impact on dairy associated stakeholders in Karnataka, the second highest milk producing state in India.

Methodology

Study Area

Karnataka is the seventh largest state in India with a total geographical area of 191,791 km^2. As per the 20th Livestock census, Karnataka comprise 11.45 million bovine population of which 8.46 million are cattle and 2.98 million are buffaloes (DAH&VS, 2017). The in-milk cattle population in the state is 2.98 million (includes crossbred and indigenous/non-descriptive animals) and in-milk buffalo's population is 1.6 million with a total milk production of 5.93 million liters/year (DAHD&F, 2019a). Ballari district comprise 0.161 million in-milk bovine population and produces 0.158 million tonnes per year whereas Chitradurga comprises 0.137 million in-milk bovine population and produces 0.114 million tonnes of milk per year (NDDB, 2015).

Milk Cooperative System in Karnataka

The Karnataka Cooperative Milk Producers' Federation Limited (KMF), is a state-level cooperative in Karnataka and it was setup during 1974. It was India's first World Bank funded dairy development program modelled on the *AMUL* pattern. The KMF today is the second largest milk cooperative in India and the largest in South India in terms of procurement and sale (Gopal and Mathew, 2020). The main function of the federation is milk procurement, processing and marketing of hygienic milk and milk products at affordable prices to customers across India under the brand name *Nandini*.

The structure of the KMF supply chain is based on the three tier model, which comprises of the milk producer and the village cooperative at the village level, the district dairy and the district unions at the district level and the state marketing federation representing all dairies at the state level. KMF has 14 Milk Unions (the jurisdiction of each union will be 3-4 districts) covering all the districts of the State which procure milk through Primary Dairy Cooperative Societies (DCS) and distribute milk to the consumers in various Towns/Cities/Rural markets in Karnataka.

The products of KMF include pasteurized and homogenized milk, Ultra-High Temperature processed (UHT) milk, flexi pack milk, curd, ghee and butter, milk powder, ice cream, milk sweets, chocolates, paneer (It is made by curdling heated milk with acids like lemon juice, vinegar etc.), cheese, processed biscuits etc. The total milk procurement by the KMF accounts to more than 8.4 million liters per day (KMF, 2021) which accounts 18.43 per cent of the total milk procurement in the country (FICCI, 2020). Besides KMF, other organized private player's viz., *Doldla, Thirumala, Arokya* and *Heritage dairies* also involved in milk and milk products business in Karnataka. The study district, Ballari is under the jurisdictions of Raichur, Ballari and Koppal Milk Union Limited (RBKMUL) and Chitradurga district is under

Shivamogga, Davanagere and Chitradurga Co-operative Milk Union Limited (SHIMUL), respectively.

Sampling size and procedure

The sample size for the study was calculated using the Cochran (1963) formula as below

$$n = z^2 \frac{p(1-p)}{e^2}$$

Where

n is the required sample size

p is the (estimated) proportion of the population

Z value found in z-table (for 95% confidence interval the z- value is 1.96)

e is the desired level of precision

To calculate the dairy farmers sample size, p=0.95 (proportion of livestock rearing households to total households in Karnataka) and e=0.025 was considered and for rural consumers sample size, p= 0.36 (proportion of rural households not having livestock to total households in Karnataka) and e=0.053 was considered. Accordingly, the calculated sample size for dairy farmers and rural consumers was 256 and 316, respectively. Of the planned samples, only 236 dairy farmers and 300 rural consumers were surveyed. The multistage sampling procedure was adopted for the selection of the study units and the details are presented in Table.1. Besides, dairy farmers and consumers, the other stakeholders associated with dairy viz., dairy cooperative societies (n=109), private feed sellers (n=12) and milk vendors (n=31) were also considered for survey.

Data Collection

A cross-sectional survey was undertaken in the study districts of Karnataka for three months (October-November 2020) after removal of restrictions that were imposed during the lockdown. The before, during and after lock down period refers to January 2020 to March 2020, April 2020 to July 2020 and August 2020 to October 2020, respectively. The data on dairy asset pattern, milk sale pattern, consumption pattern were collected from dairy farmers and number of milk pourers, milk collected, feed sale pattern and animal health care services were collected from dairy co-operative societies. Further, the quantity of feed sold by the private feed sellers during different periods of lockdown; milk sale pattern from the milk vendors and consumption pattern of the consumers were also collected to assess the COVID 19 impact on dairy food supply chain.

Description of different stakeholders in dairy food supply chain and eestimation of change in income and expenditure patterns

Dairy Cooperative Societies (DCS)

The DCS's are the lowest tier in the dairy co-operative structure in Karnataka and acts as pivotal point in milk collection and providing various dairy related services to the farmers at village level. The DCS's on an average have about 200 members each from whom milk is collected every day. Each member of the DCS has a commitment to supply a certain amount of milk to the society. Payments towards milk procurement are made based on the test results obtained by the quality of milk supplied. The procured milk is then sent to the district dairy for processing (Gopal and Mathew, 2020). The quantity of milk collected by dairy societies and the price per litre during different periods of lockdown was collected to calculate the expenditure towards milk purchase by the DCS's during different periods of lockdown.

Dairy Farmers

The households rearing dairy animals were considered as dairy farmer and they earn income by selling milk to co-operatives, local consumers and commercial establishments. Some farmers are exclusive dairy farmers and some practices dairying along with agriculture. The information on the quantity of milk provided per day by the dairy farmer and the corresponding farm-gate milk prices offered by the milk unions or the consumer were collected to calculate the income earnings during different periods of lockdown. The dairy farmers were post-classified small, medium and large depending on the number of in-milk dairy animals reared (small farm (1-2 animal), medium (2-3 animals), and large (three and above)) (Govindaraj et al. 2015; Ganesh Kumar et al. 2006).

Consumers

The households rearing dairy animals were considered as livestock farmer, whereas other households in the same village who are not rearing any dairy animals were considered as consumers of dairy products. The expenditure pattern of consumers on major and regularly consumed milk products like milk and curd was collected from consumers to assess the expenditure pattern during different periods of lock down.

Private feed sellers

Private feed sellers are those who sell dairy animal feeds and mainly located at block level and not in every village. The quantity of animal feed sold and their respective prices for different periods of the study was collected to calculate the gross income of private feed sellers during different periods of lockdown.

Local milk vendors

The milk vendors include exclusive milk shops (set up cooperatives viz *Nandini Milk Parlours*), Super Markets, Bakeries and Provision Stores. All the milk vendors who have participated in the survey were selling milk and curd followed by other dairy products. The exclusive milk shops and super markets were selling all milk products whereas the bakeries sold only milk and curd. The weekly gross income of local milk Vendors was estimated by considering their procurement quantity of different milk products and their respective prices at different

Table 1. Sampling procedure for farmers and consumers for primary survey in Karnataka

Stages	Selection	Unit Name/Number	Criteria/procedure
First Stage	Two districts in Karnataka	Ballari and Chitradurga districts	The data on livestock population density, number of COVID-19 cases per lack human population[2] and number of veterinary institutions[3] were normalized and Z scores were calculated and grouped into high and low corona risk districts using Z median scores. Ballari from high-risk group and Chitradurga from low-risk group were randomly selected.
Second Stage	Two taluks[1] (Out of Seven) in Ballari and two taluks (Out of Six) in Chitradurga	Huvina Hadagali and Kudligi taluks in Ballari district and Hiriyuru and Challakere taluks in Chitradurga district	The taluks were selected randomly in the district.
Third stage	Two blocks each (out of four) from Hiriyur and Chellakere taluks in Chitradurga district.	Dharmapura and Aimangala blocks (Hiriyur taluk) and Parushurampura and Nayakanahatti blocks (Chellakere taluk)	The blocks were selected randomly in each of the selected the taluks.
	Two blocks (out of three) in Huvina Hadagali and two blocks (out of four) in Kudligi taluks in Ballari district.	Itigi and Hirehadagali blocks (Huvinahadagali taluk) and Gudekote and Hoskerehalli blocks (Kudligi taluks)	
Fourth Stage	Dairy Cooperative Societies (DCS) at block level	In each block three DCS were selected (out of ten to twelve)	The DCS were selected randomly at block level for primary survey. However, secondary data were collected from all the DCS in the selected taluks.
	Villages under the jurisdiction of DCS	One village each from three selected DCS	For primary survey the villages under the jurisdiction of DCS were listed and three villages were selected randomly
Fifth Stage	Households in the village	Livestock farmers and rural consumers in the village	The households rearing dairy animals were considered as livestock farmer, whereas other households in the same village were considered as consumers

[1]Taluks are administrative sub-units of the district and it may vary from 2 to 10 or more depending on the geographical area of the district.

[2] As on September 12, 2020

[3] Department of Animal Husbandry & Veterinary Services, as on September 2020).

periods of lockdown. The milk products were classified into two categories such as, regularly consuming milk products (milk and curd) and intermittently consuming milk products (butter, paneer, ice cream and ghee).

Statistical Analysis

Descriptive statistics like frequencies, percentages, means, standard deviations, etc. were performed in Microsoft Excel, 2019.

Results and Discussion

Dairy food supply chain plays an important role in the overall livestock sector growth in Karnataka. The COVID-19 and subsequent lockdown had affected the income generation and expenditure pattern of the various stakeholders associated in dairy food supply chain and also disrupted the regular activities considerably. The details of COVID 19 impact to various stakeholders associated in the dairy food supply chain in Karnataka is discussed and presented below.

Karnataka Milk Federation (KMF), Dairy co-operative societies (DCS) and Farmer's milk selling pattern

The KMF is an apex tier in the three-tier co-operative structure in Karnataka. The number of milk pourers under the Federation declined before the lockdown 1.0 (January to March) from 862,000 to 844,000, whereas after lock down 1.0 and before the complete restrictions were eased (April 2020 to August 2020), the increasing trend was observed (Fig 1 &2). The quantity of milk collection also increased 6,944,000 kg's/day to 8,691,000 kg's/day (25.15%) between lock down period (March 2020) and removal of all restrictions (July 2020) period. During the same period, at federation level, the milk providers were increased from 0.83 million

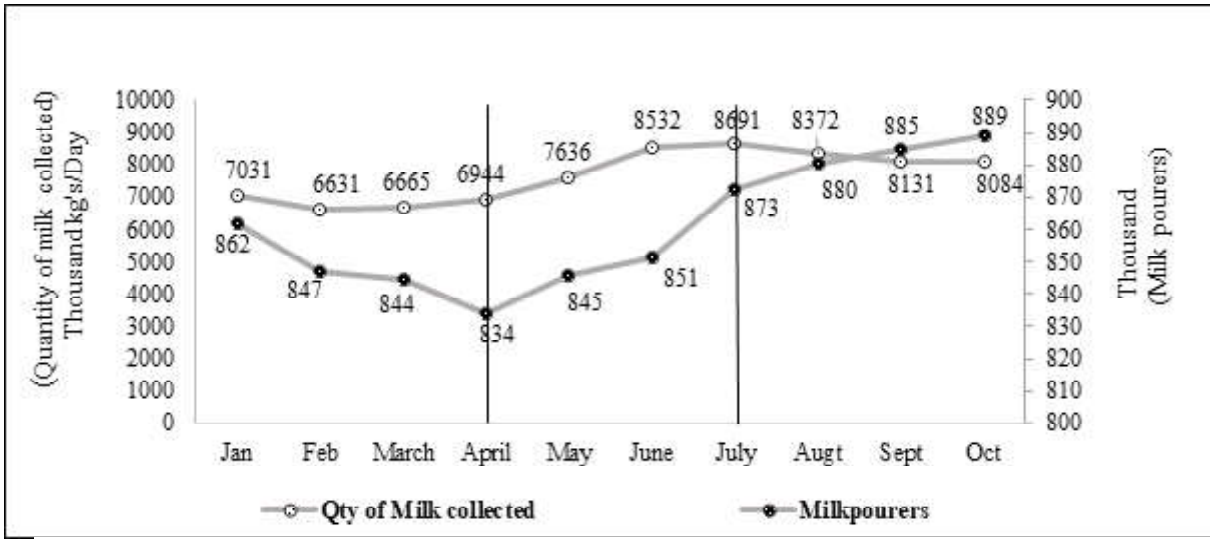

Fig 1. Relationship between quantities of milk collected per day and number of milk pourers in the KMF

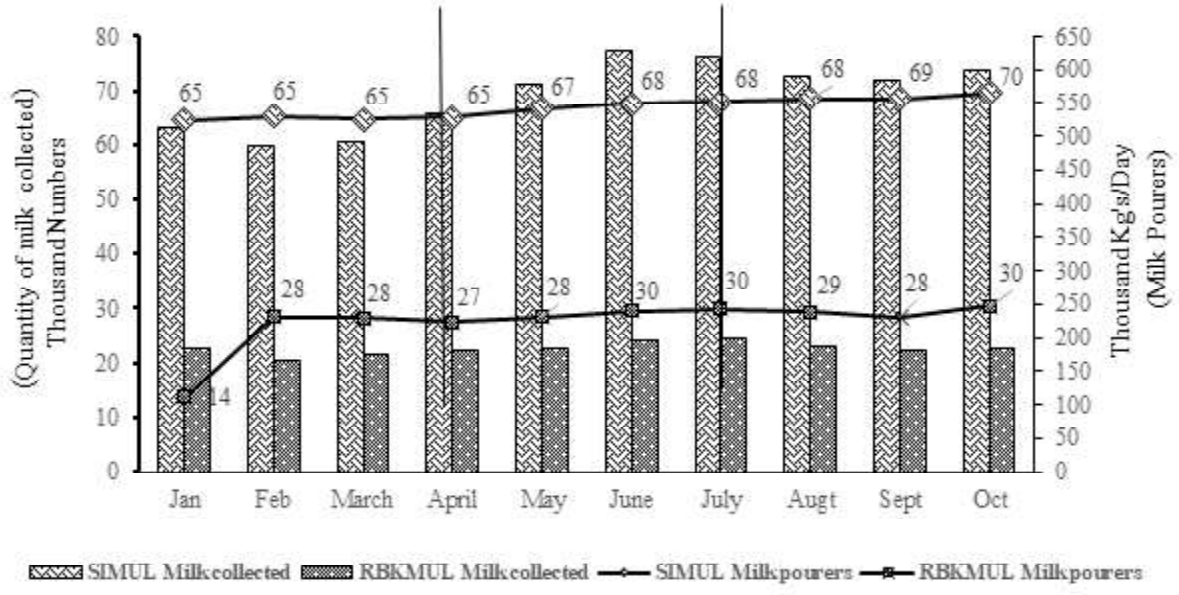

Fig 2. Relationship between quantity of milk collected and number of milk pourers in the milk unions

to 0.87 million (4.67%). Similarly, at the milk unions level (the second tier in the cooperative dairy food supply chain), the increase in the number of milk pourers (4.61 % and 11.11 %) and quantity of milk collected per day (15.91 % and 8.79 %) was observed in the Shivamogga and Ballari milk unions, respectively. The DCS are the lowest tier in the dairy co-operative structure and acts as pivotal point in milk collection and providing various dairy related services to the farmers. It was observed that in 50% of the surveyed DCS were having addition of new members ranging from 1-3 persons. But, after lockdown restriction was eased, 36.7% of the societies observed reduction in the new milk pourers. On the service front, the 41.3% of the dairy co-operative societies faced animal feed shortage, 52.3% of the societies reported delay in payment of milk bills by four to six days and in 53.2% of the societies, planned animal health camps could not be organized during the lockdown. The details are presented in Table 2. Among the dairy farmers, before lockdown, 51.2% of the surveyed dairy farmers sold milk to dairy cooperative societies, 21.2% to local consumers (households) in the village and 27.5% to local commercial ventures like, hotels, tea stalls, sweet shops etc., whereas during lockdown the share of sale to dairy co-operatives and local consumers (households) increased to 70.8% and 24.6%, respectively. However, the share of sale to commercial ventures declined significantly due to closure of various activities during lockdown. After lockdown restrictions were eased, the milk sale to dairy cooperatives declined whereas the sale to other ventures increased to pre-lockdown levels. Though there is variation of average milk sold by the farmers before, during and after lock down in different survey districts, the pooled results revealed no difference in average milk sold by the farmers. However, the price offered by the dairy co-operative declined from Rs 28.5 (pre lockdown) to Rs. 24 (after lockdown). The details of selling pattern and the milk prices prevailed in the survey districts are presented in Table 3.

The number of milk pourers to the co-operative sector (KMF/ DC's) increased during the lockdown due to dairy enterprises started by the unemployed urban people who reverse-migrated to their native villages. Further, avenues for milk sale declined for the existing rural dairy farmers due to closure of major commercial ventures viz., tea stalls and sweet shops and sudden closure of the milk procurement operations by the private milk traders/ dairies. Furthermore, the restriction of social gatherings like marriages, functions, parties also affected the avenues of private sale resulting in more milk supply to co-operative sector. Similar finding has been reported in Canada (Weersink et al. 2020). The milk procured by KMF on a single day is either consumed within the state, converted into skimmed milk powder or into milk that has a long shelf life. The KMF burdened with the excess milk due to closure of schools and abrupt halt of milk supply of 0.80 million

Table 2 Lockdown and its effect on dairy co-operative societies in the study area

Sl. No.	Questions	Response	Ballari (n=57) No (%)	Chitradurga (n=52) No (%)	Pooled (N=109) No (%)
1	Was there any newly added member who came from city and started selling milk to dairy co-operatives?	Yes	28 (49.12)	26 (50.00)	54 (49.54)
		No	29 (50.87)	26 (50.00)	55 (50.45)
	Newley added members in each DCS	Range	1 to 2	1 to 3	1 to 3
2	Were there any farmers sold their animals who come from city after lockdown?	Yes	19 (33.33)	21 (40.38)	40 (36.69)
		No	38 (66.66)	31 (59.61)	69 (63.30)
3	Was there any shortage in feed availability/supply during lockdown?	Yes	21 (36.84)	24 (46.15)	45 (41.28)
		No	34 (59.64)	28 (53.84)	62 (56.88)
4	Was there any delay in payment of milk bills of the farmers due to lockdown?	Yes	30 (52.63)	27 (51.92)	57 (52.29)
		No	27 (47.36)	25 (48.07)	52 (47.70)
	Average number of days delayed	Number	6	4	5
5	Was there any disruptions in organizing planned animal health camp?	Yes	19 (33.33)	32 (61.53)	51 (46.78)
		No	38 (66.66)	20 (38.46)	58 (53.21)

Table 3 Milk selling pattern of livestock farmers in the study area

Particulars	Ballari (n=118)			Chitradurga (n=118)			Pooled (n=236)		
	BL	DL	AL	BL	DL	AL	BL	DL	AL
Farmers milk selling pattern (Frequency)									
Dairy Cooperative societies (DCS)	56 (47.46)	84 (71.19)	69 (58.47)	65 (55.08)	83 (70.34)	68 (57.63)	121 (51.27)	167 (70.76)	137 (58.05)
Local Consumers	25 (21.19)	29 (24.58)	26 (22.03)	25 (21.19)	29 (24.58)	24 (20.34)	50 (21.19)	58 (24.58)	50 (21.19)
Commercial Ventures (Hotels, Tea Stalls etc.,)	37 (31.36)	05 (4.24)	23 (19.49)	28 (23.73)	06 (5.08)	26 (22.03)	65 (27.54)	11 (4.66)	49 (20.76)
Total	118 (100.00)	118 (100.00)	118 (100.00)	118 (100.00)	118 (100.00)	118 (100.00)	236 (100.00)	236 (100.00)	236 (100.00)
Average quantity of milk sold (l/day)									
Small farm	7.81	8.24 (5.51)	7.35 (-10.80)	8.02	8.12 (1.25)	7.12 (-12.32)	15.83	16.36 (3.35)	14.47 (-11.55)
Medium farm	8.65	8.51 (-1.62)	7.58 (-10.93)	8.22	8.51 (3.53)	7.81 (-8.23)	16.87	17.02 (0.89)	15.39 (-9.58)
Large farm	6.59	7.54 (14.42)	6.87 (-8.89)	7.85	6.24 (-20.51)	7.32 (17.31)	14.44	13.78 (-4.57)	14.19 (2.98)
Average milk sold	7.68	8.10 (5.38)	7.27 (-10.25)	8.03	7.62 (-5.06)	7.42 (-2.71)	15.71	15.72 (0.04)	14.68 (-6.59)
Price of milk (Rs/L)	28.50	26.25	24.75	28.50	24.75	22.5	28.50	25.50	24.00

Note: The figures in the parenthesis indicates the percentage change
BL- Before lockdown (January to March), DL- During lockdown (April to July), AL- After lockdown (August to October)

liters/day to primary and high school students under *Ksheera Bhagya* scheme of Karnataka government. Further, transport restrictions disrupted the supply of 0.45 to 0.61 million litres/day to neighboring states viz., Maharashtra, Telangana, Kerala, Andhra Pradesh. Since, excess milk production has gone up in the country, people prefer drinking fresh milk to converted form or stored form (Nayantra, 2015). Hence, there may be less demand for the converted milk products like milk powder and Ultra Heat Treatment (UHT) milk. Despite the burgeoning demand and supply gap during the lockdown, the KMF procured the milk unabated with a 21% reduction in milk prices in two tranche. The excess procurement and processing added cost of milk and its products processed in co-operative sector, which is already operating under least margins (Akshata, 2020).

Dairy feed sales

1. The *Ksheera Bhagya* Scheme was launched by the State Government of Karnataka in association with KMF for school and *anganwadis* children's in the state

Anganawadis are the primary education centres for children's below 6 years

The supply and demand for animal feed by the dairy farmers through milk cooperatives and the selling pattern of the private feed sellers in the study area was quantified and among which 11.29% and 14.13% decline in quantity of feed with no change in feed price supplied through the milk cooperatives was observed during lockdown, respectively in Ballari and Chitradurga districts. However, in the same period, the feed sale from private feed sellers increased by 58.75% and 92.62% in the surveyed districts (Ballari and Chitradurga), with the marginal increase in price per bag (Rs. 63.75/50 kg).

Table 4 Change in income and expenditure pattern due to COVID-19 among the different stakeholders in the study area

Particulars	Ballari			Chitradurga			Pooled		
	BL	DL	AL	BL	DL	AL	BL	DL	AL
A. Milk cooperatives* (million)		n=126			n=80			N=206	
Payment of milk bills by the milk union (Rs)	89.02	141.75 (59.22)	104.25 (17.10)	89.7	127.5 (42.14)	99.75 (11.20)	178.5	269.25 (50.84)	204.75 (14.71)
B. Gross income of dairy farmers (Rs/farm)		n=118			n=118			N=236	
Small farm	13046	11866 (-9.05)	10161 (-14.37)	13397	12161 (-9.23)	9022 (-25.81)	26442	23558 (-10.91)	18336 (-22.17)
Medium farm	14449	12254 (-15.19)	10479 (-14.49)	13731	12745 (-7.18)	9897 (-22.34)	28180	24509 (-13.03)	19502 (-20.43)
Large farm	11008	10858 (-1.37)	9497 (-12.53)	13113	9345 (-28.73)	9276 (-0.74)	24121	19843 (-17.73)	17982 (-9.38)
Average gross income	12834	11659 (-9.16)	10045 (-13.84)	13413	11417 (-14.89)	9398 (-17.68)	26248	22637 (-13.76)	18607 (-17.80)
C. Consumers (Rs)		n=138			n=163			N=300	
Monthly Expenditure on milk and curd	675	975 (44.44)	750 (11.11)	675	1125 (66.67)	675 (0.00)	675	1050 (55.56)	750 (11.11)
D. Weekly gross income of local milk Vendors (Rs/vender)		n=16			n=15			N=31	
Da. Regular consuming milk products (Milk & Curd)									
Milk	63975	60000 (-6.21)	66000 (3.17)	60825	58425 (-3.95)	66000 (8.51)	124800	118425 (-5.11)	132000 (5.77)
Curd	12750	14850 (16.47)	13575 (6.47)	14250	15975 (12.11)	12750 (-10.53)	27225	32025 (17.63)	27225 (0.00)
Db. Intermittent consuming milk products (Butter, Paneer, Ghee)									
Ice Cream	21000	6600 (-68.57)	10575 (-49.64)	18975	5625 (-70.36)	8625 (-54.55)	41475	12225 (-69.42)	19200 (-51.97)
Butter	19425	12375 (-36.29)	19425 (0.00)	18375	11250 (-38.78)	18150 (-1.22)	38775	24225 (-37.52)	38775 (0.00)
Paneer	13125	17250 (31.43)	12150 (-7.43)	12150	16125 (32.72)	12150 (0.00)	24300	35175 (44.75)	24300 (0.00)
Ghee	26925	31875 (18.38)	31125 (15.60)	28500	32625 (14.47)	30750 (7.89)	53925	63825 (18.36)	53925 (0.00)
Total	157200	142950 (-9.06)	152850 (-2.77)	153075	140025 (-8.53)	148425 (-3.04)	309000	285900 (-7.48)	295425 (-4.39)
E. Private feed sellers (Rs/seller)		n=6			n=6			N=12	
Gross income earned	86325	144375 (67.25)	96300 (11.56)	131625	267225 (103.02)	150075 (14.02)	217950	411600 (88.85)	246375 (13.04)

Note: *Secondary Data of DCS collected from milk union of the respective district; the figures in the parenthesis indicate the percentage change; BL- Before lockdown (January to March), **DL**- During lockdown (April to July), **AL**- After lockdown (August to October)

The animal feed supply from 41.3% of the DC's were less than the required quantities due to shutdown of feed plants during lockdown and threated more dependency of farmers on private feed sellers. This has paved way for increased supply through the private feed traders. The private feed sellers made significant profit by increasing their sale with marginal increase (Rs. 65.25/ 50Kg) in feed prices during lockdown. Some farmers resorted to feed rationing by cutting the external feed and increasing the fresh green fodder (Biswal J et al. 2020), as sufficient rains was received in the study areas during lockdown.

Consumption and expenditure pattern of milk products (milk and curd)

The number of family members among the surveyed households in rural areas had increased by 46% during lockdown. In consonance with increased family members, the average per day milk and curd consumption increased by 33.3% and 70% in Ballari district and 52.6% and 76.5% in Chitradurga districts, respectively. The purchase of fresh milk directly from farmers and from the local milk Vendors had increased during the lockdown period whereas the fresh milk purchased from the dairy co-operatives decreased during the lockdown period.

The reverse-migration to rural areas during the lockdown had increased the family members in rural areas leading to increased milk and curd consumption. The 43% increase in milk consumption in the study area could be attributed to increased household members and also increased consumption of milk, tea and coffee during lockdown. However, the increase in curd consumption could be partially attributed to extreme hot weather during summer season along with lockdown effect. Though there was marginal increase in fresh milk consumption in rural areas, only 25-30% of milk produced in India is meant for household consumption and such marginal increase in consumption was insufficient to compensate for its decline in use in other sectors (Jha, 2020). Some consumers sourced their daily milk requirement directly from dairy farmers instead of regular purchase from the dairy co-operatives, due to fear of spread of virus during the purchase in the co-operative societies. However, purchase behavior of consumer changed after the lockdown restrictions were eased out.

Local milk Vendors

The milk procured by the local milk vendors decreased by 5.12% during the lockdown period whereas during the same period, the curd purchased by them had increased by 17.6% implying variation in procurement pattern of milk and curd in the study area. The procurement of other processed milk products like paneer (44.73%) and ghee (18.36%) had increased and butter procurement by the vendors had decreased by 37.5% during lockdown. The Ice cream procurement decreased significantly (69.5%) during lockdown period.

The local milk vendors play the role of a "gatekeeper" within the dairy supply chain. Some of the vendors sell exclusively the milk and milk products and some vendors sell milk and milk products along with other groceries. The milk vendors procured less milk and butter during lockdown mainly due to fall in demand from commercial establishments like hotel, bakeries, tea stall etc., whereas curd, paneer and ghee procurement had increased due to increased demand from the households. The ice-cream procurement had fallen considerably due to dampened consumer demand, mainly due to fear of flu infection. This finding is consistent with Thinking Hats Consumer Insights LLP (2020) study that found in-store purchase from local dairy/vendors has sustained at 15% as compared to 17% before lockdown.

Change in income and expenditure pattern of different stakeholders

The effect of COVID 19 on income and expenditure pattern of different stockholders associated with dairy food supply chain is presented in the Table 4. An increased payment of milk bills was observed in Ballari (59.2%) and Chitradurga (42.1%) milk unions, despite decrease in price of milk by the union twice during the lockdown period. Even after lockdown is eased, the payment of milk bills was higher by 17.1% and 11.2% in Ballari and Chitradurga districts, respectively. The gross income realized through milk sale by the dairy farmers decreased during the lockdown period. Among the small farmers, the decrease in income was 17.7% whereas among the medium and large farmers, 13.0% and 10.91%, respectively.

The monthly expenditure of consumers on milk and curd showed upward surge of 44.4% and 66.7%, respectively during lockdown period in Ballari and Chitradurga districts, respectively. However, considerable reduction was observed after the lockdown. For the milk vendors, the pooled results revealed that the gross income realized through sale of milk declined by 5.11% whereas in the same period 17.6% increase in income through curd sale was observed. Among the intermittent consuming milk products, 69.4% decline in the income was observed in ice cream and 37.5% decline was observed through butter sales whereas increased gross income was observed through paneer (44.8%) and ghee (18.4%) sales. After lockdown restrictions were eased, except ice cream, the gross income realized through butter, paneer and ghee was restored to the pre-lockdown level revenues. The gross income earned by private feed sellers increased considerable during lockdown (67.25%) and it reduced after the lockdown.

Despite the fall in demand, milk procurement has not decreased through the dairy cooperatives. The co-operatives played a significant role in protecting the livelihood of dairy of farmers, though procurement was at a reduced price in tranche (from Rs. 28.50/l to Rs. 21.75/l). Due to increased milk procurement, the milk bills payment through the co-operatives increased and profit margins squeezed (Sharath, 2020). The individual dairy farmer's

gross income through milk sale decreased due to price reduction by the co-operatives. Though the expenditure surge in households for milk and curd was observed, the overall dampened demand from other commercial enterprises affected the incomes of all the stakeholders in the dairy supply chain except the private feed sellers who nearly doubled their income during lockdown.

Conclusion

KMF, despite facing many problems during lockdown viz., fall in demand for milk and milk products, excess milk procurement and dip in supply of milk to neighboring states, provided the helping hand to nearly two million dairy farm families in Karnataka by ensuring timely procurement of milk, as private dairies and other commercial establishments stopped buying milk. It also provided the employment opportunity especially for the families who migrated from cities and also ensured continuous and timely supply of milk to consumers across the state during lockdown. Hence, the milk co-operative system acted as buffer and protected the dairy food supply chain from free market and capitalist breakdown during the unprecedented COVID-19 lockdown crisis. The financials of the cooperative system was badly affected and hence for long-term sustainability of the dairy co-operatives, the immediate need is to increase the processing capacity of the plants and also to ensure product diversification. The consumers prefer fresh milk in Karnataka and to tap its complete potential, the cooperatives instead of converting milk into milk powder, can develop and promote new products like immunity booster milk, *ayurvedic* milk, herbal milk, A2 milk, organic milk etc., To overcome wastage of excess milk collected, government needs to continue and extend proposing free milk to poor, children and pregnant women. This ensures nutritional security of poor, as well as utilization of excess milk procured through the co-operatives effectively.

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RESEARCH ARTICLE

Forecasting of milk production of crossbred dairy cattle by Autoregressive Integrated Moving Average (ARIMA) model

Rohit Sharma[1]*, JK Chaudhary[2], Sanjeev Kumar[3], Ranjit Rewar[4] and Sandeep Kumar[5]

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Abstract: The objective of this study was to forecast the milk production in crossbred dairy cattle. In this study secondary data collected from Livestock Farm of CVSc. & A.H., CAU, Aizawl, Mizoram, from year 2010 to 2019 was used. The main focus of our study was on forecasting the milk production through the ARIMA model. To perform exploratory information examination, box-plot was used while the stationarity of data was checked with Augmented Dicker-fuller test, Autocorrelation Function (ACF) and Partial autocorrelation function (PACF). Model fit checking and forecasting of milk was done through software package R. The results indicated that ARIMA (1, 0, 0) was the most suitable model for forecasting of milk for our dataset. Milk production is expected to be 1910.20 litres by 2022 with 95% confidence interval.

Keywords: ARIMA; ACF; Forecasting; Milk production; PACF

Introduction

Dairy cattle contribute significantly in Indian economy as each house & family requires milk for daily consumption. India ranks 1st in cattle population of the world and it shares 35.94 % of India's total livestock population. The total cattle population has increased by 0.8 % in 2019 census over 2012 census. According to livestock census (2019), there are 192.49 million total cattle count in India that contributed 187.7 million tonnes of milk. Due to daily increasing demand of milk, milk yield's exact prediction is an important aspect of any dairy industry. Dairy producers need exact prediction of milk yield for individual cow as lactation length is the main factor affecting milk yield in cross-bred cows (Sharma et al. 2020) and milk yield prediction can help to improve their yield by maintaining their lactation length and at group level, change in milk yield decreases with time. One-year increase in age of animal corresponds to 0.0012 units less in milk yield change rate (Chaudhary et al. 2020). Therefore, accuracy in the forecast of milk production in advance provides benefits for management & farmers at farm level as the total milk production influences optimum farm configurations and cash flow/income. For genetic analysis, milk yield forecasting models have been proven useful by Ptak and Schaeffer (1993). Simultaneously, for farm management support, herd management analysis and economic prediction; correct milk production forecasts are helpful (Shalloo et al. 2004; Murphy et al. 2013; Upton et al. 2015). Milk yield forecasting system's usefulness depends mainly on 2 things; first how accurately it predicts daily milking patterns, second its capacity to change in accordance with factors influencing flexibly.

Time series forecasting is a vital area of forecasting where past observations of the same variable are collected and analyzed to develop a model to describe the relationship between past and future data. Various models are used to extrapolate the time series data into the future data. This modeling approach is particularly useful when some knowledge is available on the underlying data and when no satisfactory explanatory model is available to relate the prediction variable to other explanatory variables. Much effort has been devoted over the past several decades toward the development and improvement of time series forecasting models. One of the most important and widely used time series models is the Autoregressive Integrated Moving Average (ARIMA) model.

[1]Department of AGB, CVSc. & A.H.,CAU, Selesih, Aizawl, Mizoram, India -796014
Email: r20rohitsharma.r@gmail.com

[2] Department of AGB, CVSc. & A.H.,CAU, Selesih, Aizawl, Mizoram , India -796014
Email: vetjitu@gmail.com

[3] Department of Veterinary Microbiology, CVSc. & A.H., CAU, Selesih, Aizawl, Mizoram, India -796014
Email: sk62270@gmail.com

[4]Department of LPM, CVSc. & A.H.,CAU, Selesih, Aizawl, Mizoram, India -796014
Email: Ranjitrewar5772@gmail.com

[5]Department of LPT, CVSc. & A.H.,CAU, Selesih, Aizawl, Mizoram, India -796014
Email: dr.saharans123@gmail.com

Rohit Sharma (✉)
Department of Animal Genetics & Breeding, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl-796014, Mizoram, India
E-mail: r20rohitsharma.r@gmail.com

ARIMA models are improbable and flexible class of forecasting models that use historical information to make predictions.

Materials and Methods

The main focus of our study was based on forecasting through ARIMA model i.e. Auto_Regressive Integrated Moving Average model. ARIMA model, presented by Box and Jenkins (1976), has been habitually used for foreseeing the future qualities and example of the measurement information. In an auto-regression model, we figured out the variable of interest by using a direct blend of variable's past information. The term auto-regression indicates that it's a regression of the variable against itself. Instead of using past estimations of the figure variable in an extreme regression, a MA (moving average) model uses past forecast errors in regression like model. Time series when differentiated follows both AR and MA models and thus is known as autoregressive integrated moving average. There are 3 pieces of ARIMA model: 1. 'AR' segment represents designs between any one timeframe and past periods, 2. 'MA' part gauges the variety of most recent estimates to past figure blunders and 3. 'I' part imply diverse coordinated technique inside the information. In ARIMA (p, d, q) time series, p denotes the number of autoregressive terms (AR), d the number of times the series has to be differenced before it becomes stationary (I), and q the number of moving average terms (MA). In general an ARIMA (p,d,q) model can be explained as:

$$Autoregressive\ process\ of\ order\ (p)\ is,$$
$$Y_t = \mu + \phi_1 Y_{t-1} + \phi_2 Y_{t-2} + \ldots\ldots\ldots + \phi_p Y_{t-p} + \varepsilon_t;$$
$$Moving\ Average\ process\ of\ order\ (q)\ is,$$
$$Y_t = \mu - \theta_1 \varepsilon_{t-1} - \theta_2 \varepsilon_{t-2} - \ldots\ldots\ldots - \theta_q \varepsilon_{t-q} + \varepsilon_t;$$
$$and\ the\ general\ form\ of\ ARIMA\ model\ of\ order\ (p, d, q)\ is,$$
$$Y_t = \mu + \phi_1 Y_{t-1} + \phi_2 Y_{t-2} + \ldots + \phi_p Y_{t-p} + \mu - \theta_1 \varepsilon_{t-1} - \theta_2 \varepsilon_{t-2} - \ldots - \theta_q \varepsilon_{t-q} + \varepsilon_t$$

where, Y_t is milk production, $\int_t$'s were distributed independently and normally distributed with zero mean and constant variance for t = 1, 2 ...n; d is the fraction differenced whereas interpreting AR and MA and φs and θs are the coefficients to be estimated.

ARIMA modeling system of the Milk Yield dataset was followed as follows:

Perform exploratory data examination

To perform exploratory investigation, the boxplot work was utilized with 5 numbers outline (which are namely minimum, first quartile, median, third quartile & maximum) of a bunch of data. A box was plotted from 1st quartile to the third quartile. The worth goes from every quartile to the minimum or maximum.

Decomposition of data

We decomposed the information (time series) for the evaluation/ estimates of trend, seasonal, and arbitrary segments utilizing moving average (MA) strategy.

Testing the stationarity

For testing the stationarity of time series, Augmented Dickey-Fuller test was utilized and was additionally checked with the assistance of ACF and PACF.

Fit a model

For a given time series, the "forecast" package in R automatically chose the best fit ARIMA model with the auto.arima () function.

Calculate forecasts

We utilized the forecast function, from the forecast R package to calculate forecast.

Statistical Analysis

To analyze the data and computation of results Software Package (R -3.6.2) was used. For our objective software packages used were; *t-series* and *forecast*.

Results and Discussion

The results of forecasting of milk production through ARIMA model at various step of analysis are shown in subsequent headings.

Step 1: Performing exploratory data analysis

The milk yield increase over time with each year which may be indicative of an increasing linear trend, perhaps due to increasing demand for that time period. In the boxplot there is more milk yield in 7 to 11 months than the other months, indicating seasonality with an apparent cycle of 12 months. The rationale for this could be cows are more productive in those months. Milk Yield appears to be multiplicative time series as the MY increase, it appears so does the pattern of seasonality.

Step 2: During time series decomposition

We decomposed the time series arrangement for appraisals of pattern/trend, seasonal, and irregular segments utilizing moving average (MA) technique and got outcome as appeared in figure 1.

After decomposition of data the outcome appeared to be like this, in these disintegrated plots we considered trend and irregularity, however under the "remainder" noticed the assessment of the arbitrary segment.

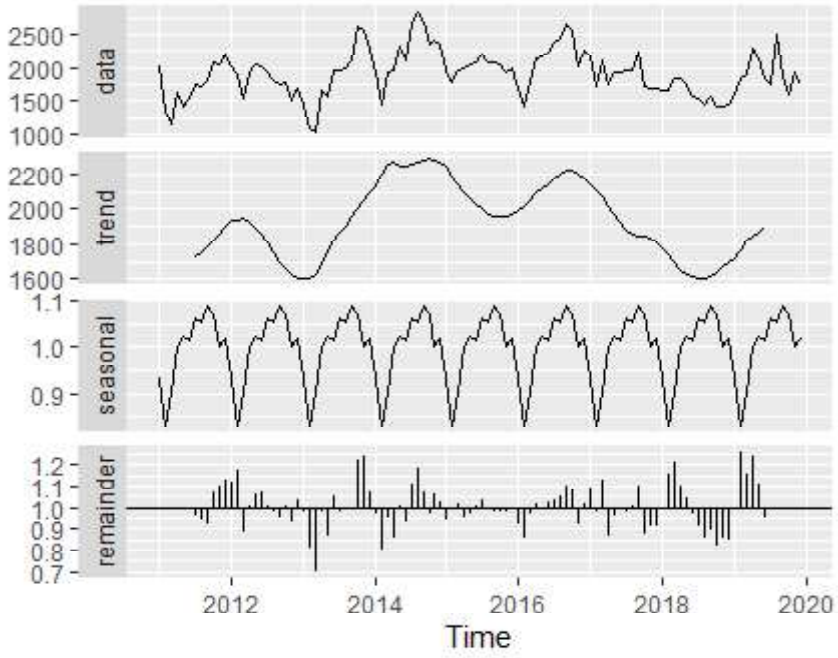

Fig. 1 Decomposition of multiplicative time series

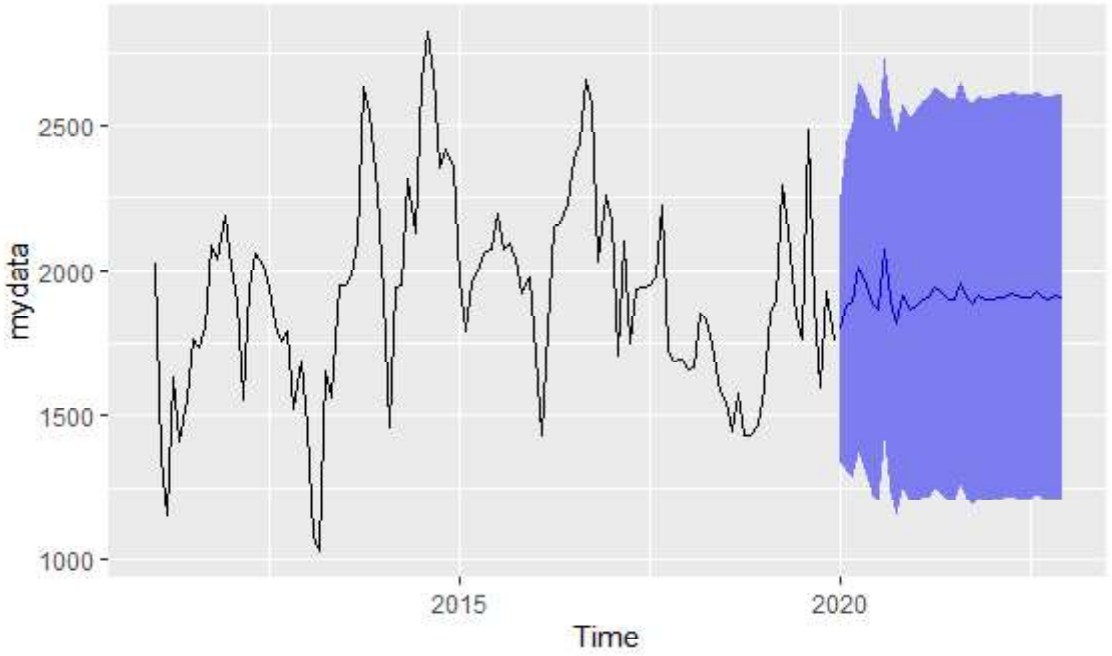

Fig. 2 Forecast from ARIMA (1,0,0)

Step 3: During stationarity test of the time series

We utilized Augmented Dickey-Fuller test to check the stationarity of time series. So we originally fixed testing theory (null and alternative speculation). The null hypothesis, H_0: that the time series is non-stationary; alternative hypothesis, H_A: that the time series is stationary. At p-value <0.05, we reject the null hypothesis for the alternative hypothesis that the time series is stationary. The stationarity of information was additionally checked with the assistance of ACF and PACF. As the estimated values of ACF and PACF lied between -0.5 to +0.5, it indicated that the data is stationary.

Step 4: Fit a time series model

The ARIMA (1,0,0) (1,0,0) [12] model boundaries are slack 1 differencing (d), an autoregressive term of second lag(p) and a moving average model of order 1 (q). At that point the occasional model has an autoregressive term of first slack (D) at model period 12 units, for our situation months. The leftover residual plots

Table 1 Forecast for the period of 2020 to 2022

		2020	2021	2022
January	Point Forecast	1797.592	1877.488	1901.011
	Lo 95	1338.236	1198.822	1205.149
	Hi 95	2256.948	2556.154	2596.873
February	Point Forecast	1877.695	1900.772	1907.747
	Lo 95	1311.162	1214.405	1211.248
	Hi 95	2444.228	2587.139	2604.246
March	Point Forecast	1896.431	1906.276	1909.341
	Lo 95	1281.409	1215.93	1212.51
	Hi 95	2511.454	2596.622	2606.171
April	Point Forecast	2013.461	1940.182	1919.147
	Lo 95	1374.629	1247.772	1222.144
	Hi 95	2652.294	2632.593	2616.151
May	Point Forecast	1968.84	1927.323	1915.429
	Lo 95	1317.946	1233.839	1218.336
	Hi 95	2619.734	2620.806	2612.523
June	Point Forecast	1885.57	1903.273	1908.475
	Lo 95	1228.478	1209.231	1211.335
	Hi 95	2542.661	2597.316	2605.616
July	Point Forecast	1864.752	1897.276	1906.741
	Lo 95	1204.454	1202.943	1209.576
	Hi 95	2525.05	2591.61	2603.906
August	Point Forecast	2075.459	1958.229	1924.369
	Lo 95	1413.497	1263.744	1227.191
	Hi 95	2737.422	2652.713	2621.546
September	Point Forecast	1900.899	1907.759	1909.773
	Lo 95	1238.071	1213.195	1212.589
	Hi 95	2563.727	2602.322	2606.958
October	Point Forecast	1817.729	1883.715	1902.82
	Lo 95	1154.451	1189.11	1205.633
	Hi 95	2481.008	2578.32	2600.008
November	Point Forecast	1914.231	1911.629	1910.893
	Lo 95	1250.718	1217.003	1213.703
	Hi 95	2577.744	2606.256	2608.082
December	Point Forecast	1863.994	1897.106	1906.693
	Lo 95	1200.358	1202.468	1209.502
	Hi 95	2527.63	2591.743	2603.883

give off an impression of being based on 0 as clamor, with no pattern. The ARIMA model was a decently good fit.

Step 5: Calculate forecasts

Our findings revealed that ARIMA (1, 0, 0) was the most suitable model for our dataset. Finally, a forecast graph of our time series was plotted by using the *forecast* function from the forecast R package with a 95% confidence interval (Figure 2). Month wise point forecasts for the period of 2020-22 with 95% confidence interval are shown in table 1. This measure additionally showed that the estimating incorrectness was low.

In view of the model fitted, average forecasted milk yield (in liters) for 2020, 2021, and 2022 were 1906.38L, 1909.25L and 1910.20L, respectively. The determining capacity of fitted ARIMA model was evaluated by the proportions of the sample time frame estimates' exactness processing.

A forecast graph of time series was plotted which showed the genuine and forecasted estimation of milk yield (with 95% certainty limit). The outcomes in the past investigations by Sankar and Prabakaran (2012) and Chaudhari and Tingre (2013) varied from our current examination in terms of best fit model as the most fit model proposed by their examination was ARIMA (1, 1, 0). The most fitting ARIMA model for fish item trade determining

was discovered to be ARIMA (0, 1, 2) by Sankar (2011). Deshmukh and Paramasivam (2016) while determining milk production in India discovered ARIMA (1,0,0) with GRETL as appropriate for their information while when SPSS programming is utilized, ARIMA (1, 1, 1) found as best reasonable. The models can be distinctive relying upon information of the researcher however different authors have suggested ARIMA as the decision of technique for time-arrangement investigation over different strategies.

Conclusions

On the basis of our current investigation regarding forecasting of milk production in cross-bred cows, it may be concluded that ARIMA model using R software '*forecast'* package is much useful for time series analysis. For our dataset ARIMA (1, 0, 0) was best fit with software R. The models can be distinctive relying upon information however, various researchers have suggested ARIMA as the decision of technique for time-arrangement investigation over different strategies. Using time series data, this study provides evidence on future milk production, which can be considered for future policy making. Although, GOI is implementing various programs like Intensive Dairy Development Programme, Strengthening Infrastructure for Quality and Clean Milk Production, Assistance to Cooperatives and Dairy Entrepreneurship Development Scheme, deeper and wider penetration and implementation is required.

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RESEARCH ARTICLE

Does livestock help in compensating land inequalities in rural India: Evidences from agricultural census?

Manpreet Kaur[1] and Naresh Singla[2]

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Abstract: Using data of various rounds of Agricultural Census, the study has revealed that rural inequalities are largely attributed to disparities in ownership of land leading to unequal distribution of income. Land inequalities during various rounds among various farmer categories have remained almost constant with minor fluctuations, whereas inequalities in ownership of various livestock such as cattle, buffalo, horse and pony have reduced drastically. The extent of land inequalties calculated with the help of Gini coefficient and Lorenz curve is also found to be much larger than livestock inequalties. Small size of operated area among marginal and small cultivators is compensated by ownership of cattle and buffalo, which helps to mitigate inequalities caused by land. The study concludes that government policies should be targeted at strengthening sustained development of livestock activities, which can go a long way in mitigating land inequalities and removing agrarian distress.

Keywords: Gini Coefficient; Inequality; Livestock; Rural Change; Rural Assets

Introduction

The continous persistence of inequalities in the distribution of income and wealth acts as one of the main impediments for causing economic slowdown in developing economies of the world.As a result, various problems of poverty, malnutrition, corruption, unemployment, mortality risk, mental illness or depression, and other social problemstend to perpetuate in such economies (Larrea and Kawachi, 2005; Rowlingson, 2011; Vries et al. 2011; Zheng, 2012; Norris et al. 2015; Brian, 2015; Ribeiro et al. 2017). Consequently, eradication of unequal distribution of income, asset and wealth has emerged as a pre-requisite for sustainable economic development and inclusive growth in less developed and devloping countries (Norris et al. 2015). Further, it has also been argued that the inequalties tend to be much dominant in the faster growing developing economies of the world such as India, where majority of the population lives in rural areas (OECD, 2011). The introduction of various structural reforms in India particularly after globalisation has widened the inequalties in terms of health, education and basic amenities in rural as well as urban areas (Deb, 2018).

The rural economy of India continues to be agrarian in character. The path of economic development in India has remained highly skewed and largely unparalleled with rest of the economies of the world. The relative contribution of agriculture and allied sectors in the Gross Value Added (GVA) has continously declined to 17.4 %, while as per population census 2011, around 54.6% of the population continue to drive their livelihoods from agriculture and its various sub-sectors (GoI, 2015-2016; GoI, 2015). After independence, one of the major structural reforms introduced was implemenation of land reforms to abolish intermediaries, ensuring rights of tenants through security of tenure, providing ownership rights, regulating rent etc., put ceiling on land holdings and consolidate land holdings in order to provide land rights to landless. However, the implementation of land reforms has remained patchy due to bureaucratic interference, poor maintenance of land records, existence of absentee landlords etc. (Devi and Parisad, 2011). The introduction of green revolution has also accounted for increase in land and wealth inequalties as the benefits of capital intensive mode of agricultural production have largely accrued to the large land cultivators (Dhanagare, 1987). The inequalities among land cultivators have further accentuated due to dwinding profit margins among small scale cultivators due to increase in cost of production, stagnation in productivity and production of major crops, inadequacy of agricultural price policy, low marketable surplus etc. As a result, the farming is no longer a profitable enterprise for small and marginal land cultivators. As a result, they tend to lease out their land to large cultivators.Thus, the distribution of land in rural

[1]Baba Farid Law College, Faridkot, Punjab, 151203, India
E-mail: manpreetkaur.mk1989@gmail.com

[2]Department of Economic Studies,Central University of Punjab, Ghudda (Bathinda), Punjab, India
Email: naresh.singla@cup.edu.in

Manpreet Kaur(✉)
Baba Farid Law College, Faridkot, Punjab, 151203, India
Email: manpreetkaur.mk1989@gmail.com

economy of India continues to be skewed without showing any signs of reduction in land inequality (Singhla et al. 2016).

Therefore, the policy makers have been looking for more viable and equitable enterprises within the wider spectrum of agriculture sector in India, which are not only inclusive, but also pro-poor in nature. In this regard, livestock sector has been emerging as one of the pioneer sub-sectors of agriculture sector, which provides income and employment oppurtunities; and leads to asset creation among rural poor. In India, out of the total agricultural GDP, livestock sector contributes around 25.7 % during 2016-17 (GoI, 2017; Kaur & Singla, 2018). Within livestock sector, around 70 % of the milk is largely produced by landless, marginal and small farmers (Malliga et al. 2012; Kumar et al. 2013), thereby help to augument their income from crop farming. Such producers with own tiny pieces of land and large unskilled family size find it difficult to get employment oppurtunities in other enterprises (Padhi, 2014; Bharti et al. 2015). Thus, livestock sector acts as a significant tool for poverty reduction as the incidence of poverty has been found less in the states having higher share of livestock in agricultural output (Ali, 2007; Kumar et al. 2008; Kumar and Singh, 2008; Birthal andNegi, 2012; Birthal, 2014; Birthal, 2016). In this context, it has been argued that livestock sector may act as boon for reducing the land inequalities among various categories of the farmers in rural India.

In India, significant number of studies have investigated the asset inequalties in terms of income and wealth distribution (Subramanian and Jayaraj, 2014; Jayaraj and Subramanian, 2018; Mandela, 2018; Shetty, 2018; Suryanaryana and Mamgain, 2018; Sengupta and Mukherjee, 2018; Sen, 2018).In rural as well as urban India, the inequalities have increased during the post-reform period (Pal and Ghosh, 2007). In Tamil Nadu, rural inequalities during 1993-1994 to 2004-2005 have increased due to increase in inequalities between classes, whereas within classes, inequalities have largely remained constant (Mandela, 2018). However, thereafter, during 2004-2005 to 2011-2012, due to the revival of growth in agriculture and allied sector activities, increase in agricultural wages, increase in non-farm employment opportunities and effective implementation of state welfare programmes such as Mahatma Gandhi National Rural Employment Guarantee Scheme (MGNREGS) and Public Distribution System (PDS), the extent of inequalities between classes has declined (Mandela, 2018).Jayaraj and Subramanian (2018) worked out the rural as well as urban inequalities at India level during 1991-1992 to 2012-2013 and found that rural as well as urban inequalities in India have increased during the period of two decades. Further, the absolute Gini Coefficient has witnessed a sharp increase in inequality, especially in the urban sector (Jayaraj and Subramanian, 2018). The exisiting literature on inequalities in India largely pertains to income inequalities (Chaudhuri and Ghosh, 2021; Ghatak, 2021; Rao, 2003; Pal and Ghosh, 2007; Kumar, 2017; Deb, 2020; Sarkar and Mehta, 2010). In this paper, we largely focus on inequalities caused by the ownership of land in rural areas. Since land is considered as main determinant of rural wealth and source of income distribution, its unequal distribution leads to rural inequalities. We built this paper on the hypothesis that ownership of livestock as an asset may lead to reduction in poverty and rural inequalities caused by the unequal distribution of land. It acts as a significant tool for poverty reduction as the incidence of poverty has been found less in the states having higher livestock share in total agricultural output (Ali, 2007; Kumar et al. 2008; Kumar and Singh, 2008; Birthal and Negi, 2012; Birthal, 2014; Birthal, 2016). Livestock is a labour intensive activity; therefore, it creates gainful employment for marginal and small farmers who, in general, are characterised as having abundant family labour, but tiny pieces of land (George, 1996; Pandit and Dhaka, 2004; Ali, 2007; Bardhan, 2007; Kumar et al. 2007; Baba et al. 2011; Kumar et al. 2011; Birthal, 2016).

Materials and Methods

The entire study is based on land and livestock asset inequalities among different farm size categories. The data on land and livestock is extracted from various rounds of Agricultural Census, Ministry of Agriculture, Government of India from 1981-1982 to 2011-2012. The agricultural census provides quinquennial data on various structural aspects of operational holdings among different farm-size categories for different states of India. This data has been extracted and analysed to work out land inequalities. The agricultural census also provides input survey data, which provides data on consumption of various agricultural inputs including ownership of various types of livestock according to major size group of landholdings.The same has also been extracted to work out the inequality in ownership of livestock assets.All census rounds of input surveydata except 2011-2012, provide data on ownershipof land and area operated by each farm size category; and ownership of livestocks such as cattle, buffalo, sheep, goat, horse, pony, mule, donkey, pig and camel.The agricultural census for 2011-2012 provide data on ownership of land and area operated by each farm size category; and ownership of cattle and buffalo only. Further, the data for 2015-2016 round of agricultural census on input survey has not been published yet. Therefore, the study period is only confined to 2011-2012.The land and livestock inequalities are calculated and compared for each farm size category as well at state level using coefficient of variation (CV) and Gini Coefficient as outlined below:

Coefficient of Variation (CV)

This measure of asset inequality is worked out by dividing the standard deviation of the land and livestock asset distribution by its mean. More equal asset distributions will have smaller standard deviations; therefore, the CV will also be smaller in more equal ownership of assets. The Coefficient of Variation is calculated with the help of formula given below;

$$Coefficient\ of\ Variation = \frac{Standard\ Deviation}{Mean} * 100$$

Lorenz Curve

This method is one of the most convenient methods for graphical representation of the distribution of income/wealth, which was developed by Max Lorenz in 1906. In the present study, this method has been used for analysing the graphical distribution of landand livestock ownership (cattle and buffalo) among the various operational landholdings of the farmers. The line at the 45º angle shows perfectly equal ownership of land and livestock asset distribution among all the operational landholdings,while the other line shows the actual distribution of land and livestockasset among all the operational landholdings. The farther away the curve from the diagonal or line of perfect equality, the more unequal is size of the distribution of land and livestock asset ownership. Therefore, this Lorenz Curve relates the cumulative proportion of operational landholding units to cumulative proportion of land as well as livestock asset ownership, when these units are arranged in ascending order.

Gini Coefficient

Gini Coefficient is derived from Lorenz Curve. The Lorenz Curve depicts the distribution of income in an economy, while the Gini Coefficient is used to measure the degree of income inequality. However, in this study, Gini Coeffient is calculated for finding the land and livestock inequalities among the farmers. The value of Gini Coefficient varies between 0 to 1. A Gini Coefficient of zero means perfect equality that means each landholding has the same land and livestock ownerhip, while a coefficient of one represents one category of operational landholding of farmers is receiving all the land as well as livestock ownership (Kashish et al.2017). This Gini Coefficient is calculated with the help of the formula given below;

$$G = \left[1 - \sum_{k=1}^{n} (X_k - X_{k-1})(Y_k + Y_{k+1})\right]$$

G= Gini Coefficient; X_k = Cumulative proportion of the operational landholdings variable for k=0,….,n, with X_0= 0, X_n= 1; Y_n= Cumulative proportion of land, cattle and buffaloes each, for k=0,….,n; with Y_0=0, Y_n=1.

In other words, value of Gini coefficient is graphically represented through Lorenz curve. The Gini coefficient is computed by subtracting area below the line of perfect equality (0.5 by definition) from the area below the Lorenz curve, divided by the area below the line of perfect equality.

Results and Discussion

In India, inequalities in ownership of land are prevalent among all the states (Datta et al. 2015) From Table 1, it can be inferred that in India, around 85 % of the operational holdings belong to marginal and small farmers, who collectively cultivate only about 46% of the operated land. The semi-medium, medium and large farmers combining together are 15 %, which cultivate about 54% of the operated area in India. This also reflects poor implementation of land reforms since independence. However, ownership in possession of livestock assets, particularly in cattle and buffalo is found to be more equally distributed than operated land(.On the other hand, the marginal and small farmers jointly own 77 % of cattle and 72 % of buffalo. Thus, the possession of cattle and buffalo in India is helpful in compensating rural asset inequalities, which are largely caused by land.

Consequently, livestock sector in India has been emerged as a breakthrough for the sustainable development of agriculture and allied sectors. In India, around 65.07% of the population lives in villages and agriculture and allied sectors is one of their primary occupations (World Bank, 2021). However, there exist large variations and inequalities in the ownership of landholdings. Among all the assets owned by the farmers, large inequalities still exist in ownership of operated area as the value of coefficient of variation (CV) is found to be the highest in case of operated area during the entire period under study. However, along with operational landholdings, inequalities are also higher in the ownership of camels due to high value of CV. However, the value of CV is high as camels are mostly found in the desert spots of Rajasthan and their price is very high. Moreover, camel is acquired by medium and large farmers only. However, among other species of livestock, the magnitude of inequalities is found to be very small as comaped to operated area and camel. Further, by taking into consideration, the period-wise analysis, the study also shows

Table 1 Category-Wise Distribution of Land and Livestock in India during 2011-2012 (in %)

Category	Operational Landholding	Operated Area	Cattle	Buffalo
Marginal	67.11	23.63	54.66	49.5
Small	17.92	22.13	22.3	22.17
Semi-medium	10.04	23.71	14.29	16.73
Medium	4.24	21.18	7.23	9.48
Large	0.69	9.35	1.52	2.12

Source: Based on authors' own calculations.

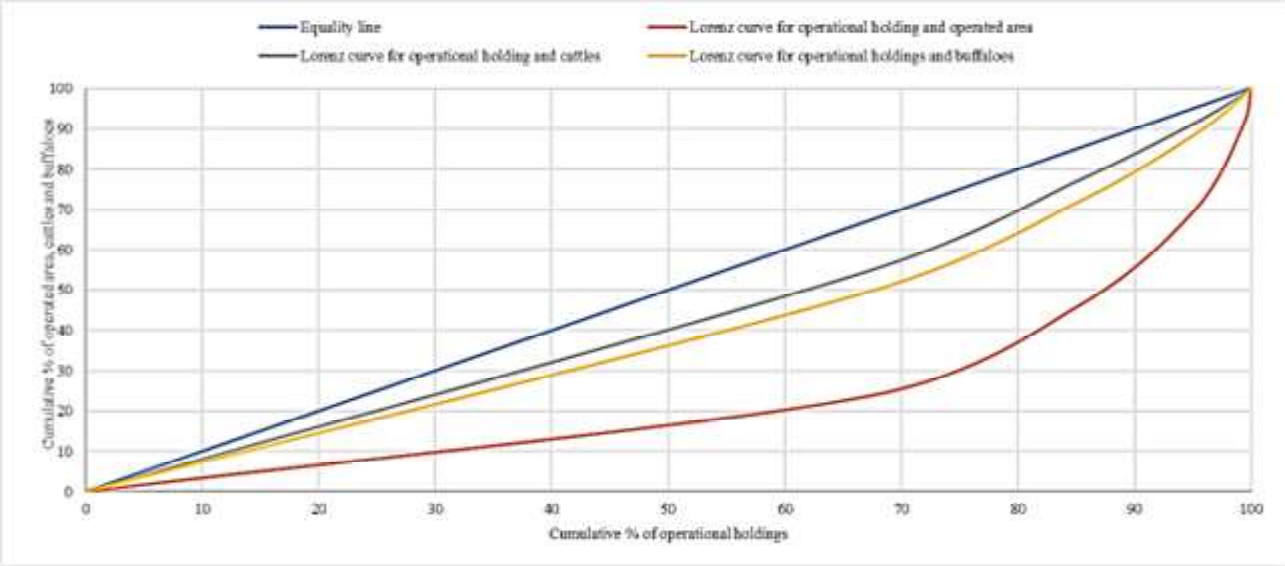

Fig 1. Lorenz Curve for Operational Holdings and Operated Area, Cattles and Buffaloes during 2011-2012
Source: Based on authors' own calculations

Table 2 Coefficient of Variation (CV) in Operational Holdings and Ownership of Livestock among Farmer Categories.

Particulars	1981-1982	1986-1987	1991-1992	1996-1997	2001-2002	2006-2007	2011-2012
Operated area	120.12	112.00	120.31	122.09	121.10	121.65	122.03
Cattle	49.87	44.71	51.39	37.50	38.56	44.02	44.33
Buffalo	59.13	49.52	57.89	49.65	52.61	57.69	50.41
Sheep	70.76	85.29	74.93	93.10	75.73	77.30	-
Goat	31.94	42.62	55.63	65.67	51.67	63.31	-
Horses and pony	54.15	50.03	44.28	51.41	97.47	29.30	-
Mule	45.15	38.18	27.89	14.14	16.74	54.84	-
Donkey and ass	50.03	112.05	77.36	79.13	131.25	139.93	-
Pig	17.96	35.37	33.27	25.15	52.49	30.69	-
Camel	112.10	144.11	122.87	167.45	146.44	149.26	-

Source: Based on authors' own calculations.

that, with the passage of time, inequalities between operational landholdings among various farmer categories have remained almost constant with slight fluctuations. On the other hand, inequalities in ownership of various livestock such as cattle, buffalo and horse and pony among farmer categories have reduced drastically. Though, inequalities in case of some other species such as sheep, goat, mule, donkey and ass and pig have increased but, the extent of these inequalities is found to be much lesser than inequalities in operated area among the farmers except camel.

The magnitude of inequalities in the ownership of land and livestock among different farmer categories is also worked out with the help of Gini Coefficient (Table 3). During 2011-2012, the value of Gini Coefficient is found to be the highest in operational landholdings (0.53) followed by buffalo (0.20) and cattle (0.13). Further, agricultural census-wise analysis shows that Gini Coefficient has decreased across all the livestock among the farmers except in case of camel and donkey and ass. Hence, the value of Gini Coefficient for livestock is turned out to be far less than the value of the same in case of operational landholding except camels. Therefore, it can be concluded that possession of livestock assets among the farmers leads to reduction of inequalities among them.

The state-wise in-depth of inequalities during 2011-2012 is also analysed with the help of coefficient of variation (CV) in Table 5. The value of CV in operated land among various farmer categories is found to be the highest in Himachal Pradesh (144.6%) followed by Assam (140%), Jammu and Kashmir (139%), Kerala (134.6%), whereas, value of CV in ownership of cattle among various farmer categories is turned out to be only 18.26%, 7.17%, 36.42% and 36.62% respectively. Similarly, the value of CV in ownership of buffalo is worked out to be 35.2%, 64.98%, 54.78% and 108.26% respectively. This reveals that extent of inequalities is much less in ownership of cattle and buffalo than ownership of land among the farmers. The value of CV in the distribution of operated area is least in Manipur (101.51%) followed by West Bengal (102.31%)

and Uttar Pradesh (106.97%). Further, the value of CV in per operational landholding distribution of cattle is found to be highest in Bihar (51.45%) and least in Assam (7.17%). Similarly, in case of per operational landholding distribution of buffalo, the value of CV is highest in Mizoram (142.59%) and least in Rajasthan (10.3%). Therefore, the above analysis reveals that inequalities in ownership of land are much higher than ownership of cattle and buffalo as the value of coefficient of variation among all the states is worked out to be more than 100%.

State-wise inequalities between operational landholdings and operated area are found to be much prevalent in the states such as Rajasthan, Haryana, Jharkhand, Sikkim, Himachal Pradesh, Chhattisgarh as Gini Coefficient is worked out to be equal to or more than 0.50 (Table 5). However, in these states, the Gini Coefficient between operational landholdings and cattle as well as operational landholding and buffalo is much lower than the Gini Coefficient between operational landholding and operated area. In all the states of India, the Gini Coefficient in case of

Table 3 Gini Coefficient in Operational Holding and Ownership of Livestock among Farmer Categories

Particulars	1981-1982	1986-1987	1991-1992	1996-1997	2001-2002	2006-2007	2011-2012
Operated area	0.58	0.57	0.56	0.56	0.55	0.54	0.53
Cattle	0.24	0.24	0.26	0.13	0.17	0.16	0.13
Buffalo	0.30	0.26	0.28	0.22	0.24	0.23	0.20
Sheep	0.31	0.29	0.25	0.24	0.25	0.21	-
Goat	0.13	0.15	0.16	0.10	0.13	0.10	-
Horses and pony	0.28	0.09	0.20	0.05	0.13	0.13	-
Mule	0.15	0.02	0.05	0.00	0.03	0.08	-
Donkey and ass	0.18	0.25	0.15	0.04	0.26	0.36	-
Pig	0.09	0.16	0.12	0.10	0.13	0.09	-
Camel	0.57	0.60	0.60	0.68	0.48	0.70	-

Source: Based on authors' own calculations.

Table 4 State-Wise Coefficient of Variation (CV) in Distribution of Operated Area, Ownership of Cattle and Buffalo in 2011-2012

State	Operated Area	Cattle	Buffalo
Andhra Pradesh	111.76	32.60	39.01
Assam	140.12	7.17	64.98
Bihar	117.17	51.45	58.51
Chhattisgarh	117.81	41.37	47.00
Gujarat	109.85	37.26	17.23
Haryana	116.71	25.58	32.75
Himachal Pradesh	144.58	18.26	35.20
Jammu & Kashmir	139.25	36.42	54.78
Jharkhand	119.33	47.86	37.79
Karnataka	110.54	19.00	33.48
Kerala	134.58	36.62	108.26
Madhya Pradesh	118.13	12.81	57.33
Maharashtra	115.76	32.00	26.32
Manipur	101.51	14.59	65.68
Meghalaya	129.59	34.74	83.86
Mizoram	114.88	18.08	142.59
Odisha	121.37	21.13	72.62
Punjab	114.51	33.65	42.74
Rajasthan	118.35	31.43	10.30
Sikkim	120.74	41.51	92.73
Tamil Nadu	118.27	32.16	38.74
Tripura	118.27	9.67	87.04
Uttar Pradesh	106.97	26.59	38.78
Uttarakhand	124.79	16.15	40.15
West Bengal	102.31	34.36	98.31

Source: Based on authors' calculations

Table 5 State-Wise Gini Coefficient of Land and Bovine during 2011-2012

State	Operated Area	Cattle	Buffalo
Andhra Pradesh	0.44	0.10	0.15
Assam	0.38	0.03	0.23
Bihar	0.32	0.18	0.19
Chhattisgarh	0.50	0.19	0.21
Gujarat	0.45	0.12	0.07
Haryana	0.53	0.08	0.15
Himachal Pradesh	0.50	0.08	0.14
Jammu & Kashmir	0.38	0.05	0.17
Jharkhand	0.53	0.05	0.15
Karnataka	0.47	0.07	0.15
Kerala	0.30	0.08	0.08
Madhya Pradesh	0.48	0.05	0.24
Maharashtra	0.44	0.12	0.09
Manipur	0.36	0.06	0.14
Meghalaya	0.38	0.13	0.26
Mizoram	0.33	0.05	0.45
Odisha	0.36	0.09	0.29
Punjab	0.43	0.13	0.17
Rajasthan	0.56	0.13	0.06
Sikkim	0.51	0.27	0.18
Tamil Nadu	0.43	0.09	0.17
Tripura	0.39	0.03	0.53
Uttar Pradesh	0.41	0.07	0.11
Uttarakhand	0.40	0.06	0.11
West Bengal	0.28	0.09	0.25

Source: Based on author's calculations

operational landholdings and operated area is much higher than the Gini Coefficient of operational landholdings and cattle and the operational landholding and buffalo except Mizoram and Tripura, where the Gini Coefficient between the operational landholding and buffalo is higher (Gini value= 0.45 in Mizoram and Gini value= 0.53 in Tripura) as compared to the Gini value between operational landholding and operated area (Gini value= 0.33 in Mizoram and Gini value= 0.39 in Tripura). Thus, it can be concluded that land inequalities are much higher in India as compared to bovine inequalities (Datta et al. 2015).

Conclusion

It has been argued that the moderate success of land reforms and implementation of green revolution in few selected regions of India have a greater role in causing land inequalities (Sebby, 2010). The policy makers are looking at viable and equitable enterprises such as livestock within wider spectrum of agriculture sector in India, which are not only inclusive, but also pro-poor in nature. The findings of the study revealed that inequalities as measured by coefficeint of variation and Gini Coefficient between operational landholdings in India among various land size categories have remained almost constant with minor fluctuations, whereas inequalities in ownership of various livestock such as cattle, buffalo and horse and pony among farmer categories have reduced drastically. The extent of land inequalties is also worked out to be much larger than livestock inequalties. Further, state-wise analysis revealed that small possession of operated area among marginal and small cultivators is compensated by ownership of cattle and buffalo, which helps to mitigate inequalities caused by land. Therefore, it can be concluded that policies should be targeted at the strengthening sustained development of livestock enterprises in wider parts of India, which can go a long way in mitigating land inequalities and removing agrarian distress at large.

Notes

1. Under agriculture census, data is collected under two heads viz. agricultural data and input survey data. There is the time lag of one year between the agricultural data and input survey data. The agricultural data (publishes data on operational landholdings, average size, operated area etc.), therefore, confined to 1980-1981 to 2010-2011 whereas the input survey data (publishes data on the usage of various agricultural inputs and possession of agricultural assets) is related to the time period of 1981-1982 to 2011-2012. However, the input survey data also includes the data related to agricultural data (which is used in the study) and it remains same for both the years.

2. Along with providing data on operational landholdings and operated area, the input survey data round of 2011-2012 provides data on the possession of cattles and buffaloes only and it did not publish data on the ownership of other species of livestock such as sheep and goat, mules, horses and pony, donkey and ass, camels etc.

3. The census rounds are conducted after every five years. The reports on agricultural census data for the year 2015-2016 have been published which discusses data related to operational landholdings and operated area only but the data related to input survey for the year 2016-2017 on the possession of livestock species has not been published anywhere either on reports or on website.

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SHORT COMMUNICATION

Tick control management practices of dairy farmers in Kangra district of Himachal Pradesh, India

Devesh Thakur [1], Krishnender Dinesh [2], Manoj Sharma[1], RK Ravikumar [3], Rupali Masand [4] and Devina Sharma[5]

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Abstract: Tick infestation is a major problem affecting farm animal welfare and productivity. The study was conducted to assess the nature of tick infestation, awareness and methods of tick control among eight purposively selected village panchayats of district Kangra, Himachal Pradesh. Further out of these 8 panchayats, 200 dairy farmers were randomly selected during 2018-2019.The study indicated that tick infestation is acute in hilly regions of Himachal, India and farmers rely mainly on animal husbandry institutions for seeking scientific inputs/information. Synthetic acaricides were widely in practice (100.0%), though 40 percent of farmers relied on both synthetic and indigenous herbal medications. The high use of acaricides was due to the presence of veterinary institutions in every village panchayat in the region. The majority of dairy farmers (79.5%) reported that these synthetic acaricides were effective up to 30 days only and recurrent infestation on animals was noticed. Suitable extension interventions for improved housing management and proper tick disposal must be applied to overcome the problem in the region.

Keywords: Acaricides; Dairy; Herbal Medications; Indigenous Knowledge; Ticks, Veterinary Institutions

Himachal Pradesh is a hilly state situated in western Himalayan region of India and bestowed with rich biodiversity (Tewari et al. 2017). Inspite of availability of plant based resources, in hill farming system, the medicinal usage of herbs have become limited and chemical-based application is increasingly practised. In Himachal Pradesh, the ectoparasitic infestation had emerged as a major constraint to livestock health and productivity (Thakur et al. 2012). Ticks have an adverse effect on livestock in terms of blood loss, decrease in body weight, damage to hides, udder and reduction in milk yield. Major losses caused by ticks are due to their ability to transmit protozoan, rickettsial and viral diseases in livestock such as babesiosis, theileriosis, anaplasmosis, dermatophytosis and myiasis (Scholtz et al. 1991).

Ticks also decrease fertility rate and causes hindrance in introducing improved breeds of cattle (FAO, 2004). In India, tick control by synthetic acaricides is widespread (Ghosh et al. 2007). However, absolute reliance on any one method of controlling ticks fails to ensure efficient, sustainable and long-term control measure (Nolan, 1990). Indigenous knowledge systems are widely prevalent and delivery of health service, by complementing such knowledge, needs to be identified for developing suitable implementation strategies. Accordingly, ectoparasitic infestation may be controlled with the prevalent knowledge (local/ indigenous) by practicing and sharing within livestock health care system.

(✉)[1]Department of Veterinary and AH Extension Education, DGCN, COVAS, Palampur, Himachal Pradesh, India
E-mail: drdth4@gmail.com

[2]Department of Animal Genetics and Breeding, DGCN, COVAS, Palampur, Himachal Pradesh, India
E-mail: krishanender25@gmail.com

[1]Department of Veterinary and AH Extension Education, DGCN, COVAS, Palampur, Himachal Pradesh, India
E-mail: manojsharma2808@gmail.com

[3]National Innovation Foundation-India, Amrapur, Gandhinagar
Email:drrkravikumar@gmail.com

[4]Department of Veterinary and AH Extension Education, DGCN COVAS, Palampur, Himachal Pradesh INDIA
E-mail: r.masand93@gmail.com

[5]Department of Parasitology, DGCN COVAS Palampur, Himachal Pradesh, India
E-mail: devinasharma23@yahoo.co.in

Thus, the study was proposed and conducted during 2018-2020 to provide insights into nature of tick infestation, level of knowledge among farmers on ticks and traditional tick management practices in Himalayan regions. Such measures will enhance scope of technological intervention, which are suitable to dairy farmers more specifically in hilly regions.

The present study was conducted in purposively selected district Kangra, Himachal Pradesh. This district was selected to assess tick management practices adopted by farmers under state funded project by Himachal Pradesh Council of Science, Environment & Technology, Shimla. Data were collected from June 2018 to December 2019 through a semi-structured interview schedule which was prepared in consultation with experts from different departments of Dr GC Negi College of Veterinary & Animal Sciences, Palampur. The schedule was pretested and later used to collect data from farmers apart from conducting focus group discussion (FGD). The FGDs was done to initially pretest the interview schedule. Eight village panchayats viz., Gunehar, Chowgan, Bharmat, Saliyana, Banuri, Menjha, Kandwari & Jandpur were selected purposively from Kangra district. The village panchayats selected were at convenient locations(within 40 km) to DGCN COVAS CSKHPKV Palampur to implement HIMCOSTE project activities. Twenty-five individual farmers were selected randomly from each these 8 village panchayats thereby making a total sample size of 200 respondents. (Focused Group Discussions)FGD was conducted among these respondents from each of these 8 villages to identify farmers' practices in the control of tick infestation.

The majority (81.50%) of dairy owners reported that tick problem infestation is more during summer and rainy season followed by 13.50 percent who had observed throughout the year. Five percent farmers reported tick infestation in their animals in summer, rainy & autumn season (Table 1). Acaricides were sought either from veterinary institutions (62.50%) or procured directly by dairy

Table 1: Data collected from farmers on awareness & control methods for ticks (N=200)

1. Prevalence of ticks	Frequency (%)
i. Summer & rainy Season (April-September)	163 (81.50)
ii. Summer, rainy & autumn season	10 (5.00)
iii. Year around	27 (13.50)
2. Methods to control ticks	
i. Experience of using synthetic acaricides	200 (100.00)
ii. Experience of using synthetic acaricides & local medications	81 (40.50)
3. Source of procurement of medicine	
i. Veterinary institutions	125 (62.50)
ii. Medical shop	75 (37.50)
4 Time duration of effect of chemical acaricides	
i. Up to 7 days	21 (10.50)
ii. Up to 15 days	79 (39.50)
iii. 16-30 days	59 (29.50)
iv. 31-45 days	16 (8.00)
v. 46-60 days	25 (12.50)
5. Problems in medications	
i. Need for frequent use	126 (63.00)
ii. Costly	83 (41.50)
iii. Unavailable	73 (36.50)
iv. Dangerous/Not appropriate for surrounding	35 (17.50)
v. Less effective	23 (11.50)
6. Manual remove of ticks	
i. Yes	149 (74.50)
ii. No	51 (25.50)
7 Cracks & crevices in walls of animal sheds	
i. Yes	161 (80.50)
ii.No	39 (19.50)
8 Awareness about harmful effects	
i. Direct effects (blood loss/ weakness/ milk production)	193(96.50)
iiDisease transmission among animals	7 (3.50)
iii. Disease transmission among humans	0

Table 2 Indigenous management practices against Tick Control adopted by farmers

S. No.	Local methods used in the region	Possible explanation of the practice	Reported similar findings in different parts of world
1	Salt is applied over the body	Coarseness of salt causes tearing at ticks' bodies until they fall apart from infested site of animal	Farmers in Sri Lanka, Phillipines and Thailand regularly bathe their animal in sea or rub the entire body of animal with a mixture of 200 g of salt and 4 liters of water
2	Soap solution is applied over the body	Soap removes waxy cuticle that protects insects and mites from drying out.	Soap detergent is widely used to control Ticks in Malawi (Chikomola and Phoya 2019).
3	Cloth is dipped in kerosene & then applied over the animal body	Insects and mites need oxygen just like any other animal. Oil kill them by clogging pores that deliver oxygen (Pedretti, 2014).	Ponnusamy and Devi (2017a) and Ponnusamy et al.(2017b) reported similar findings in northern parts of India. Masai tribes a well known tribe known for their knowledge on tick & tick borne diseases do apply kerosene oil to control ticks (Kioko et al. 2015).
4	Mango leaves are grinded and mixed with ash. This preparation to be applied over the body	Herbal indigenous knowledge	The biological compounds of *Magnifera indica* have reported acaricidal properties (Parte et al.2014)

owners from local chemists' shops (37.50%) on the prescription of veterinary officer. The advice of veterinarians & para-veterinarians of veterinary institutions was relied upon by majority (87.00%) of respondents to apply acaricides. The observations reflect that knowledge of veterinarian and para-veterinarian are pertinent factors in the control of tick infestation. The farmers rely on the knowledge of veterinarians to control ectoparasites (Jadav et al. 2021). The effect of synthetic acaricides was short-lived as the majority (79.5 %) of the farmers reported a re-occurrence of infestation within 30 days of application. This meant that the re-appearance of tick infestation is a common factor and respondents have to use medications frequently to overcome the problem. The efficacy of maximum duration of upto 60 days was reported by only 12.50 percent of respondents. This reflects nature of difficulties faced by respondents in addressing farm animal welfare and productivity.

Dairy owners expressed that these synthetic medications required frequent application (63.00%), were too costly (41.50%), lesser availability(36.50%) ,inimical to surroundings (17.50 %) and were less effective (11.50 %) . Kunz and Kemp (1994) reported that chemical acaricides do not provide long term protection against ticks & tick control as they are expensive, environmentally damaging and ticks develop resistance to such chemicals.

This necessitates development and sharing of environmental friendly technologies. In such situation, technological inputs derived from societal/indigenous wisdom are pertinent and need to be shared with farmers as an alternative. Efforts made to popularize indigenous formulation developed and standardized by National Innovation Foundation-India, Gandhinagar were found effective. Dr. GC Negi, College of Veterinary and Animal Sciences, Palampur, Himachal Pradesh undertook implementation activities to popularize the formulation with the support of state veterinary institutions (Ravikumar et al. 2017). Such alternative models to scientifically valorize and diffuse cost effective, herb based indigenous acaricides are relevant. Manual removal of ticks from animal body for at least once was practiced by 74.50% farmers in the region. Generally it was observed that livestock keeper remove the ticks through bare hands without any proper precautions to prevent infections to humans. Only 25.50 per cent of dairy owners did not use manual removal and there is a need for advisory role among these dairy owners who were practicing manual removal of ticks.High percentage of farmers did not practice proper disposal of ticks as they threw them in cow dung (23.50%), fields (21.50%), water sources (19.00%) and kill ticks with stone (19.00%). Only 8.50 percent of dairy owners informed that they destroy them by putting these ticks in fire. Ticks should never be thrown away or squeezed as it may release eggs and in no case crushed between fingers. They should be preferably removed with forceps, collected in leaves or plastic bags and should be burnt (OIE, 2004). It was observed that majority (80.50%) of sheds had cracks & crevices which are the major areas/hideouts for tick breeding. Only 19.50 percent of dairy owners had proper animal sheds. This reflects the nature of livestock rearing practices among study population and to propose an intervention strategy suitable to such dairy owners. Proper disposal of urine and cow-dung provision was absent among majority (91.50%) of the cowsheds. This can be a major reason for the severity and recurrence of tick problem in the region. Higher percentage (96.50%) of livestock owners had knowledge about direct effects of ticks in terms of blood loss, emaciation and loss in milk production. It was also found that

about three and half percent of dairy owners were aware that disease transmitted by ticks to animal populations

The study found that the majority of farmers (100.00%) depend on chemical acaricides to control ticks in their animals and about 40.50 percent of dairy owners adopted both synthetic & local/ indigenous medicinal practices. Thus, difficulties in control of tick infestation through medication available in veterinary institutions/ chemist shops led dairy owners to resort to their own management practices (Table 2). There is a need to reinforce scientifically proven, cost-effective herbal medications for livestock health services by popularizing indigenous technical knowledge with the support of animal husbandry institutions.

Conclusions

The problem of tick infestation is severe and farmers mostly observed recurrence of ticks within 30 days of using acaricide. Dairy farmers could not find alternative methods to overcome this situation and relied primarily on measures recommended by formal veterinary institutions. The absence of proper tick management practices such as improved housing and proper disposal of ticks were associated risk factors in smallholder livestock systems. The study noted that indigenous knowledge of livestock farmers can complement the design of tick disease control programme and add value to the animal health care system. Education and training program for farmers and veterinary professionals on aspects of utilizing an established indigenous system needs attention.

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SHORT COMMUNICATION

Effect of processing by batch type scraped surface heat exchanger (SSHE) on sensory attributes of bottle gourd (*Lagenaria siceraria*) *halwa*

Sapna Jain[1], S Kartikeyan[2] and AG Bhadania[3]

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Abstract: The awareness among consumers for healthier and functional food has led to increase in the market size for valued added products. The present study was undertaken to manufacture valued added Indian indigenous dairy product, bottle gourd *halwa* in a multipurpose batch type SSHE. The performance of the developed SSHE was evaluated at three different steam pressures (1, 1.5 and 2 kg/cm^2), scraper speeds (10, 20 and 30 rpm) by keeping batch size constant (5kg) during manufacturing of bottle gourd *halwa*. The product was evaluated for sensory characteristics with a 9-point Hedonic scale. The overall acceptability of the product was considered on the basis of sensory attributes. The bottle gourd *halwa* prepared in SSHE at 1kg/cm^2 steam pressure and 30 rpm scraper speed (P1S3) had the score of 7.9 for flavour, 7.85 for body and texture and 7.4 for colour & appearance and the highest overall acceptability score (7.72) as compared to the product prepared at other operating conditions. However, it was not equivalent to control for these attributes.

Keywords: Bottle gourd *halwa*, Scraped surface heat exchanger, Scraper speed, Sensory and Steam pressure

[1]Dairy Engineering Department, College of Dairy Science & Food Technology, Raipur, DSVCKV, CG., India

[2]Dairy Technology Department, College of Dairy Science & Food Technology, Raipur, DSVCKV, CG., India

[3]Dairy Engineering Department, SMC College of Dairy Science, AAU, Anand, Gujarat, India

Sapna Jain (✉)
Dairy Engineering Department, College of Dairy Science & Food Technology, Raipur, DSVCKV, CG.
Email id.: er.sapnajain@gmail.com

The increased availability of milk and inadequate facilities to keep liquid milk fresh during transit from rural production areas to urban market have led to the conversion of milk into traditional dairy products. The mechanized production of traditional dairy products helps the milk producers to fetch better price for milk and provides an opportunity for employment generation in rural and semi-rural areas. Industrial production of many ethnic products presents a unique opportunity, as profit margin is higher in Traditional Indian Dairy Products (TIDP). The production and marketing of TIDP by organized sector can bring about remarkable value addition to the extent of 200%, as compared to only 50% in Western products. The consumption of traditional dairy products is likely to grow at an annual growth rate of more than 20% (Bandyopadhyay, 2006).

Suitably designed Scraped Surface Heat Exchanger (SSHE) has a huge potential in manufacture of TIDP in which multiphase fluid systems are treated very effectively. Many small scale dairy entrepreneurs are interested to adopt mechanization for the production of many TIDP in order to get uniform and improved product quality. The efforts were being made to develop a SSHE for the manufacture of indigenous products like bottle gourd *halwa, kheer* etc at small scale (Velpula et al. 2019, Jain et al. 2021).

Bottle gourd (*Lagenaria siceraria*) *halwa* also called as *Lauki halwa*, is a popular dessert of North India, specially favoured during the winter season. Bottle gourd *halwa* is light greenish, with glossy fragments of grated bottle gourd cooked in milk or *khoa*. It has a lumpy body and crunchy texture but with typical mellowness. The characteristic aroma is a blend of cooked bottle gourd, heat concentrated milk and caramelized sugar. Bottle gourd is a low-priced vegetable with good amount of nutrients, low in fat and cholesterol yet, high in dietary fibre. The white pulp of bottle gourd is emetic, purgative, diuretic and antibilious. Bottle gourd juice is very effective in reducing high cholesterol levels in the blood (Rahman et al. 2008).

Upadhyay et al. (1993) made an attempt to study the feasibility of manufacturing bottle gourd *halwa* in a batch type SSHE which was designed for the manufacture of *khoa* and reported that the product was acceptable in terms of rheological attributes.

Baladhiya et al. (2018) standardized process technology for manufacture of bottle gourd *halwa* using *khoa*, which was prepared in *karahi*. They reported that there was no adverse effect found on sensory attributes of the product during storage. Velpula et al. (2018) prepared bottle gourd *halwa* using skim milk powder and *khoa* using SSHE and studied the shelf-life of the product. They observed that the product prepared using 20% SMP (w/w) of shredded bottle gourd with batch size of 4 kg, steam pressure of 1.0 kg/cm^2 and scraper speed of 30 rpm yielded *halwa* with highest overall acceptability score.

A multipurpose batch type SSHE was designed and developed for preparation of *kheer* and *halwas* (Jain et al. 2021) at Dairy Engineering lab of the SMC College of Dairy Science, Anand Agricultural University, Anand. The standardised pasteurized milk (4.5% fat and 8.5% SNF) procured from Amul was used for preparation of *lauki halwa*. The bottle gourd, fine crystalline sugar (sucrose), cardamom, saffron and green colours of food grade quality were obtained from the local market of Anand for *halwa* making. The fresh bottle gourds were washed, peeled and shredded in the food processor (Philips food processor, model no. 59 HR7625/70).

The preparation of the *halwa* mainly involved two stages, *viz*. steaming of bottle gourd for its cooking and evaporation of water from the blend of bottle gourd and milk. The product lots were prepared in the clean and dry SSHE. Ghee at the rate of 3.5 % of the shredded bottle gourd was taken in the SSHE, steam was admitted in the jacket. On heating, 5 kg shredded bottle gourd was fed in the SSHE. As heating continued, steam cooking of shredded bottle gourd took place. The cooking of shredded bottle gourd was judged by crushing between thumb and forefingers. When shredded bottle gourd was cooked properly, 7.5 kg standardized milk (1.5kg milk/1kg shredded bottle gourd) with 4.5% fat was added in the equipment. The process of evaporation and concentration were continued and during this operation sugar was added in the SSHE. The permitted green food grade colour (Bush natural green) was added at this stage to impart little greenish appearance of the product. On achieving desired lump formation, the steam supply was stopped and the

Table 1: ANOVA table for Sensory scores of control and experimental samples of Halwa

Source	Flavour			Body & Texture			Colour & Apperance			Overall acceptability		
	SEm	C.D.	Result	SEm	C.D.	Result	SEm	C.D.	Result	SEm	C.D.	Result
Control	0.246	0.692	*	0.112	0.315	*	0.104	0.293	*	0.105	0.296	*
P	0.149	0.420	*	0.066	0.187	*	0.063	-	NS	0.064	0.179	*
S	0.149	-	NS	0.066	-	NS	0.063	-	NS	0.064	-	NS
PxS	0.149	-	NS	0.115	-	NS	0.109	0.307	*	0.110	-	NS

*Significant at p≤ 0.05, NS-Non significant

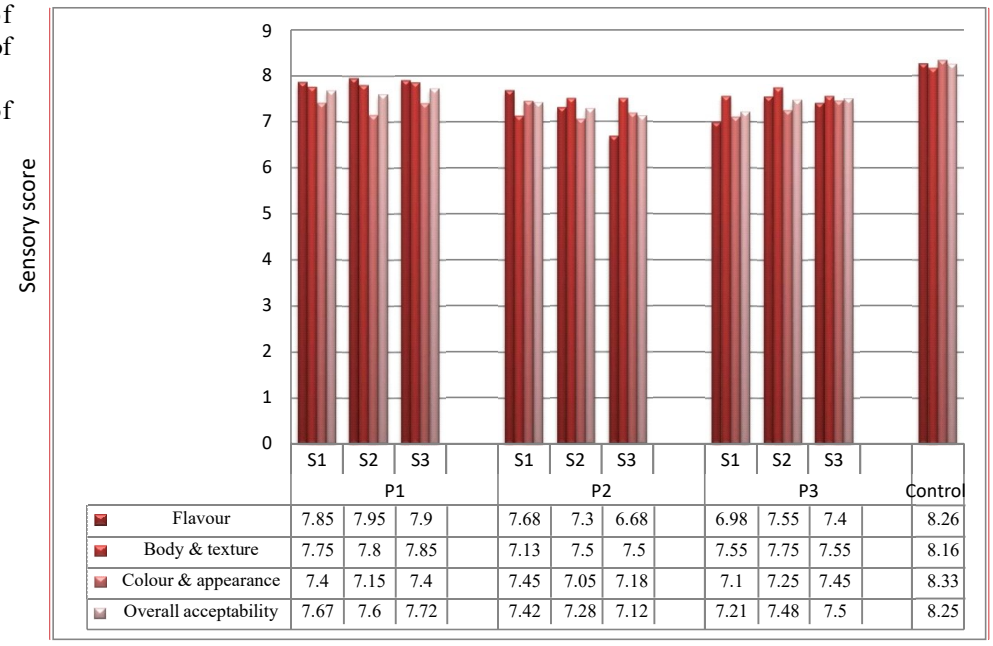

Fig.1 Average sensory score of Experimental and Control samples of Bottle gourd *halwa* at different operating conditions of batch SSHE#

Scores are average of 3 replications

product was removed from the SSHE. The product was allowed to set for 4-5 hours on a clean tray before sensory evaluation of the product.

The experimental trials were carried out at different operating parameters viz., scarper speeds (S1=10 rpm, S2=20 rpm, S3=30 rpm) and operating steam pressures (P1=1.0 kg/cm^2, P2=1.5 kg/cm^2, P3=2.0 kg/cm^2) by keeping batch size of 5 kg as constant during manufacture of bottle gourd *halwa.* The control sample of *lauki halwa* was prepared in the lab by conventional method (Aneja et al. 2002). The experiment was replicated three times. The sensory attributes of product was evaluated as a 9-point Hedonic scale by a panel of 8 judges. Factorial Completely Randomized Block Design described by Snedecor and Cochran (1994) was adopted to analyze the data.

The statistical analysis revealed that there was a significant ($p<0.05$) difference between scores of control and the experimental samples of *halwa* for various sensory attributes studied. The graphical representation and statistical analysis of average values of sensory of *lauki halwa* manufactured at different operating conditions and control sample are shown in Fig 1 and Table 1 respectively. The steam pressure had significant ($p<0.05$) effect on the scores of *halwa* for flavour, body and texture and overall acceptability. The average sensory scores of control sample of *halwa* were 8.26, 8.16, 8.33 and 8.25 for flavour, body & texture, colour & appearance and overall acceptability, respectively. Among experimental sample, the *halwa* prepared using P1S2 combination had the highest score of 7.95 for flavour and the lowest score of 7.15 for colour & appearance attributes. The interaction effect of steam pressure and scraper speed had significant effect only on colour and appearance scores of *halwa*. This might be due to Maillard browning, which improved the flavour characteristics but decreased the colour and appearance of the product. While, the product made using the P1S3 combination had higher score for body and texture (7.85) and colour & appearance (7.4), however, the flavour score (7.9) was lower as compared to P1S2. The *lauki halwa* prepared using P1S3 combination had the highest overall acceptability (7.72)

Among experimental samples; however, it was inferior to control (8.25). Almost similar results have been reported by Velpula et al. (2018). Statistical analysis reveals that the steam pressure and scraper speed interaction effect was found to be non significant (ANOVA Table 1). However, out of 9 combinations of Pressure and Scraper speed, the product prepared by P1S3 combination had the highest score (7.72) and nearer to the control (8.25).

Conclusion

From the present study, it can be inferred that good quality *lauki halwa* could be prepared using low steam pressure with high scrapper speed (P1S3) in a batch type multi-purpose SSHE. Though the experimental sample was not sensorily at par with control, yet it had added advantages like more uniform and cohesive characteristics that could be adopted hygienically (enclosed system) for larger batch size with less labour requirement for the manufacture of *lauki halwa*.

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