Studies on the Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis* ssp. *Cremoris* and *Lactococcus lactis* ssp. *lactis*

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SANJAY SINGH

B.V.Sc. & A.H.

THESIS

Master of Veterinary Science

(Livestock Products Technology)

2017

Department of Livestock Products Technology

College of Veterinary and Animal Science

Rajasthan University of Veterinary and Animal Sciences

Bikaner – 334001 (Rajasthan)

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THESIS

Submitted to the

Rajasthan University of Veterinary and Animal Sciences, Bikaner
In partial fulfillment of the requirements for

The degree of

Master of Veterinary Science
(Livestock Products Technology)

FACULTY OF VETERINARY & ANIMAL SCIENCE

By

SANJAY SINGH

B.V.Sc. & A.H.

2017

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – I

Date……..

This is to certify that **Mr. SANJAY SINGH** has successfully completed the **comprehensive examination** held on …… as required under the regulation for **M.V.Sc. degree.**

(Prof. Basant Bais)

HEAD

Department of Livestock Products Technology

College of Veterinary and Animal Science,

Bikaner

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – II

Date……..…

This is to certify that the thesis entitled **"Studies on The Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* "** submitted for the degree of Master of Veterinary Science in the subject of **Livestock Products Technology** embodies bonafide research work carried out by **Mr. Sanjay Singh** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the Advisory Committee on …….

(Prof. Basant Bais)
HEAD
Department of Livestock Products Technology
College of Veterinary and Animal Science,

(Prof. Basant Bais)
Major Advisor

Bikaner

DEAN

College of Veterinary and Animal Science,

Bikaner

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE - III

Date……

This is to certify that the thesis entitled **"Studies on The Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* "** submitted by **Mr. Sanjay Singh** to Rajasthan University of Veterinary and Animal Sciences, Bikaner, in partial fulfillment of requirements for the degree of **Master of Veterinary Science** in the subject of **Livestock Products Technology** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory, we, therefore recommend that the thesis be approved.

(Prof. Basant Bais) *(Dr. Raghvendar Singh)*

Major Advisor Co Advisor

(Dr.Rajkumar Berwal) (Dr. Rajni Joshi)

Advisor Advisor

(Dr. Anju Chahar)

Dean PGS Nominee (Prof. Basant Bais)

Head

Department of Livestock Products Technology

College of Veterinary and Animal Science, Bikaner

DEAN

Post Graduate Studies

RAJUVAS, Bikaner

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE - IV

Date……

This is to certify that **Mr. Sanjay Singh** of the **Department of Livestock Products Technology, College of Veterinary and Animal Science, Bikaner** has made all corrections/modifications in the thesis entitled "**Studies on The Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis***" which were suggested by the external examiner and the advisory committee in the oral examination held on…….. The final copies of the thesis duly bound and corrected were submitted on ……, are enclosed herewith for approval.

(Prof. Basant Bais)

Major Advisor

Enclosed one original and two copies of bound thesis. Forwarded to the Dean, Post Graduate Studies, RAJUVAS, Bikaner through the Dean, College of Veterinary and Animal Science, Bikaner

(Prof. Basant Bais)
Head

Department of Livestock Products Technology
College of Veterinary and Animal Science

Bikaner

Dean

College of Veterinary and Animal Science

Dean, PGS Bikaner

RAJUVAS, Bikaner

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1. INTRODUCTION

The Camel (state animal of Rajasthan) is an important component of Indian fragile desert ecosystem. The genus Camelus have two important species first one is Dromedary camel (*Camelus dromedarius,* single humped) that mainly live in the desert areas (arid) and second one is Bactrian camel (*Camelus bactrianus*, double humped) which prefer living in the cooler areas.

World Camel population is estimated to be around 25.89 million spread across 47 countries. Somalia has the highest population of 7.00 million followed by Sudan 4.25 million, Ethiopia 2.40 million, Niger 1.65 million, Mauritania 1.49 million, Chad 1.39 million, Mali 1.15, Pakistan 0.95 million and Kenya 0.94 million. India stands tenth (10^{th}) in the world ranking with 0.40 million camels (FAOSTAT, 2013).

The states of India, Rajasthan 0.498 million, Haryana 0.128 million, Punjab 0.043 million and Gujarat 0.058 million contribute 93.12% in Indian camel population. Eleven arid districts of Rajasthan contribute 78.86% to the total Rajasthan camel population and 55.70% to the Indian camel population (19^{th} Livestock Census -2012).

Camel milk is unique in terms of low fat (1.5-3%), low protein (2.5%) and longer shelf life, higher ratio of ß-casein to k-casein, absence of Lysozyme-C and ß-lactoglobulin and presence of Whey Acidic Protein (WHP) and Peptidoglycan Recognition Protein. There are reports on its antibacterial and other therapeutic properties. Fresh and fermented camel milk is an important nutritional and functional source.

The milk composition of dromedary camel is excellent in nutritional point of view. Camel milk also contains a high proportion of antibacterial substances and higher concentration of vitamin C in comparison with cow milk.

Grigoryants NN (1954), Rao *et al.,* (1970), Shalash MR (1979) reported that fresh camel milk has a high pH and the pH of milk is between 6.5-6.7 and this is similar to the pH of sheep milk. When camel milk is left to stand, the acidity rapidly increases. The lactic acid content increases from 0.03 percent after standing 2 hours to 0.14 percent after 6 hours.

Normally camel milk has a very white colour and is foamy (El – Agamy, 1983). The taste of camel milk is usually sweet, when camels are fed on green fodder, but sometimes salty, due to feeding on certain shrubs and herbs in the arid regions (El–Agamy, (1983 and 1994), Indra and Erdenebaatar, 1998).

The *Camelus dromedarius* is good producer of milk which differs from bovine milk in the composition and structure of protein content and thus has different functional and medicinal properties. Caseins (CNs) are the major proteins in camel milk, and α, β and κ-CN constitutes about 65, 21 and 3.47% respectively, of total caseins present in milk (Kappeler *et al.,* 2003).

Camel milk demonstrate to alike with human milk as it contains a high amount of β-CN; due to high amount of β-CN, camel milk have higher digestibility and lower incidence of allergy in infants, as β-CN is more sensitive to peptic hydrolysis than α-CN (El-Agamy *et al.,* 2009). α-lactalbumin of camel milk was reported to have a molecular weight of 14.6 kDa having 123 residues, which is similar to that of bovine, human and goat milk (Beg *et al.,* 1985).

According to 19th Livestock Census -2012, the female buffalo population has increased by 7.99% over the previous census and the total number of female buffalo is 92.5 million numbers in 2012. Buffalo account 21.23% to India's livestock population. The milch buffaloes increased from 48.64 million to 51.05 million with increased by 4.95% over previous census. Rajasthan has about 12.97 million population of buffaloes.

Buffalo milk contains all the nutrients in higher proportions than cow milk as per the nutrient components. The compositional differences between buffalo and cow milk are reflected on their physico-chemical properties. Milk from buffalo preferred for preparing dairy products of western and traditional (indigenous) type and nutritionally superior. Buffalo milk contains less cholesterol (total cholesterol 275 mg and free cholesterol 212 mg per 100 g of fat) in compared to cow milk (total cholesterol 330 mg and free cholesterol 280 mg per 100 g of fat) and more tocopherol (334.21 µg per kg for buffalo and 312.3µg per kg of cow milk). Due to high peroxidase activity, buffalo milk can be preserved naturally for a longer period. Buffalo milk contains more calcium, better calcium: phosphorous ratio and less sodium and potassium than cow milk which makes it a better nutritional supplement for infants.

Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health (Kitts and Wailer, 2003).

Bioactive peptides are synthesized in the cell in the form of large prepropeptides, which are further cleaved and modified to give active products. Peptides are inactive within the sequence of the parent protein and can be released in three ways: (1) enzymatic hydrolysis by digestive enzymes, (2) food processing and (3) proteolysis by enzymes derived from microorganisms or plants.

Many food proteins have peptide sequences with potential multifunctional activities, and there are many reports on antioxidant, antimicrobial, ACE-inhibitory, opioid, antithrombotic, anticancer activities of peptides derived from milk proteins and various food proteins (Rossini *et al.,* 2009; Salami *et al.,* 2011; Umuhumuza *et al.,* 2011; Kumar *et al*., 2016).

Potent biologically active peptides have been isolated from a number of fermented dairy products such as cheese, fermented milk

and yogurt (Korhonen and Pihlanto, 2006).

As indicating molecules, the bioactive peptides play important roles in physiological functions and pathogenesis. According to Haque *et al.,* (2008), Milk derived bioactive peptides play important roles in human health and nutrition. Milk derived bioactive peptides have been identified as potential ingredients for health-promoting functional foods. These bioactive peptides are targeted at diet-related chronic diseases especially the non-communicable diseases viz., obesity, cardiovascular diseases and diabetes. Peptides derived from the milk of cow, goat, sheep, buffalo and camel exert multifunctional properties, including antimicrobial, immune modulatory, antioxidant, inhibitory effect on enzymes, antithrombotic, and antagonistic activities against various toxic agents. Majority of those regulate immunological, gastrointestinal, hormonal and neurological responses, there by playing a vital role in the prevention of cancer, osteoporosis, hypertension and other disorders. Recently, milk proteins have been recognized as one of the most significant sources of bioactive peptides.

A number of natural antioxidative agents have been produced from plants, and some dietary proteins have also been reported to have antioxidant activity (Okada and Okada, 1998). Several food protein hydrolysates have been found to exhibit antioxidant activity (Gutteridge and Halliwell, 2000; Suetsuna *et al.,* 2000; Abuja and Albertini, 2001; Kudoh *et al.,* 2001; Saiga *et al.,* 2003; Wong and Kitts, 2003; Davalos *et al.,* 2004; Halliwell and Whiteman, 2004; Liu *et al.,* 2005; Pihlanto, 2006).

Limited studies have been done on camel and buffalo milk proteins and their milk products as sources of bioactive peptides. The main buffalo milk proteins show high homology to their cow counterparts (D'Ambrosio *et al.,* 2008). Therefore, buffalo milk proteins are potential precursors for bioactive peptides of diversified functionalities.

There are various traditional fermented camel milk products that are produced in different parts of the world by camel herders. Suusac and garris are fermented camel milk products in Kenya, Somalia and Sudan. Ititu is produced in the Kereyu area of the Oromia region in the eastern part of Ethiopia. These products are Ethiopian indigenous traditionally fermented camel milk food products (Zahran and Al-Saleh 1997; Abdelgadir WS *et al*., 1998; Lore TA *et al*., 2005; Hassan RA *et al*., 2008; Abdel Rahman IE *et al*., 2009).

Due to growth requirements, dairy starter cultures have developed highly sophisticated proteolytic system that capable of break down milk proteins, mainly α1 and β-caseins. The proteolytic structure of lactic acid bacteria (LAB) and their activities in dairy products including yogurt and cheese have been studied extensively (Nakajima *et al.,* 1995; Lankaputhra and Shah, 1996; Rybka and Fleet, 1997; Dave and Shah, 1998; Christensen *et al.,* 1999; Gomes and Malcata, 1999; Shah and Ravula, 2000; Lourens-Hattingh and Viljoen, 2001; Hernandez *et al.,* 2005; Pan *et al.,* 2005).

Lactococcus lactis has two subspecies with few phenotype and genotype differences, *Lactococcus lactis* ssp. *lactis* and ssp*. cremoris*, where ssp. *lactis* is preferred for making soft cheese while ssp. *Cremoris* is for hard cheese.

Lactococcus lactis is a gram-positive bacterium used extensively in the production of buttermilk and cheese. It has become famous as the first genetically modified organism to be used alive for the treatment of human disease. *Lactococcus lactis* cells are cocci that group in pairs and short chains, and depending on growth conditions, appear ovoid with a typical length of 0.5 - 1.5 µm. *Lactococcus lactis* are non sporulating and not motile. They have a homo fermentative metabolism and have been reported to produce exclusive L(+)lactic acid. However, reported D(-)lactic acid can be produced when cultured at low pH. The capability to produce lactic acid is one of the reasons

why *Lactococcus lactis* is one of the most important microorganisms in the dairy industry.

Lactococcus lactis has crucial importance for manufacturing dairy products, such as buttermilk and cheeses. When *Lactococcus lactis* ssp. *lactis* is added to milk, the bacterium uses enzymes to produce energy molecules (ATP), from lactose. The by-product of ATP energy production is lactic acid. The lactic acid produced by the bacterium that curdles the milk and separates from curd, which is used to produce cheese. Other uses that have been reported for this bacterium include the production of pickled vegetables, beer or wine, some bread, and other fermented foodstuffs, such as soymilk kefir, buttermilk etc. *Lactococcus lactis* is one of the best characterized low GC gram positive bacteria with detailed knowledge on genetics, metabolism and biodiversity.

Industrial research on *Lactococcus lactis* deals with the production of L-alanine, which is used as sweetener in dairy products. Many microorganism produce L-alanine, but the maximum conversion rate from carbohydrates remain only between 50–60%. The increase in performance of *Lactococcus lactis* reveals industrial advantage for producing amino acid and genetic engineering.

The dried fruit of the jujube (Ber) tree, scientifically known as *Ziziphus jujuba*, is a pectoral fruit similar to dates and figs. Once confined largely to China, the fruit now grows in many areas of the world, including southern Europe and the southern United States. It is a small deciduous tree or shrub. The fruit is an edible oval drupe 1.5–3 centimeters (0.59–1.18 in) deep; when immature it is smooth-green, with the consistency and taste of an apple, maturing brown to purplish-black and eventually wrinkled, looking like a small date. Jujube is rich in biological components and various secondary metabolites, which are related to both nutritional values and biological activities.

Jujube fruit are rich in carbohydrates; dried jujube fruits contain a broad array of vitamins and minerals. Calories in 100 g of dried

jujube total are 350; the dried jujubes contain 84 g of carbohydrates, 7.3 g of protein, 4 g of dietary fibre and 1.2 g of fat. The fruit also contains roughly 300 mg of vitamin C, 125 mg of vitamin A, 2.8 mg of niacin, 0.2 mg of riboflavin, 0.1 mg of thiamine, 1050 mg of potassium, 168 mg of phosphorus, 130 mg of calcium, 12 mg of sodium and 3.5 mg of iron. Dried jujube also contains a wide variety of antioxidant rich phytochemicals including saponins and flavonoids.

Saponins and triterpenoids that are present in jujube fruit, play an important role in digestion by enhancing the uptake of nutrients and encourage healthy movement of food through the bowel, prevent constipation, cramping, bloating, and excess flatulence, as well as more serious gastrointestinal conditions, like colorectal cancer.

Jujube is one of the good sources of antioxidant content, like vitamin C, vitamin A, and numerous organic compounds and acids that help to neutralize free radicals, the dangerous by-products of cellular respiration, which are liable for several chronic diseases and illness within the body.

Limited studies have been done on camel and buffalo milk proteins and their milk products as sources of bioactive peptides. Keeping in view the aforesaid facts, the present investigation has been planned to find out the fermentative potential (antioxidant nature) of camel and buffalo milk during fermentation by lactic acid bacteria (LAB); *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* with the following objectives:-

1. To estimate the physico-chemical properties of Camel and Buffalo milk.
2. To produce bioactive peptides from Camel and Buffalo milk by bacterial fermentation.
3. To determine antioxidative property of fermented Camel and Buffalo milk.

4. To produce and evaluate fermented Camel and Buffalo Milk Product.

2. REVIEW OF LITERATURE

The available literature over viewing the composition, physicochemical properties and nutritive value of camel and buffalo milk, lactic acid bacteria (LAB) and their fermentative potential, bioactive peptides and their effect on health and enriched fermented milk products study have been presented here under the following sub headings:

2.1 Camel and Buffalo Milk and its properties

2.2 Bioactive peptides

2.3 Lactococcus and its bioactive properties

2.4 Fermented milk product study

2.1 Camel and Buffalo Milk and its properties:

The most important factor in camel milk is water content. Young camels and especially the humans living in drought areas are in need of fluid to maintain homeostasis and thermo neutrality. The water content of camel milk fluctuates from 84 percent (Bremaud, 1969) to 90 percent (Yagil and Etzion , 1980).

Kunji *et al.,* (1996) reported that amino acids which are required for the growth of lactic acid bacteria are present in milk casein protein. Nevertheless, only less than 1% of the total casein constituents are actually required. Milk proteins during fermentation are subjected to slight proteolytic degradation resulting in a number of potentially bioactive peptides which may vary between 2-20 amino acid residues. Many of them are known to express multi-functional physiological properties. Proteolysis is a cascade process involving a number of steps including (a) an extracellular proteinase initiating degradation of casein into oligopeptides, (b) transport systems that translocate

peptides and amino acids across the cell wall, (c) various intracellular peptidases for further degradation of peptides into amino acids, and (d) different enzymes that convert liberated amino acids into various components.

Rogelj, (2000) reported that increasing amount of scientific evidences confirm that many chronic diseases such as cancer, osteoporosis, coronary heart disease and hypertension are connected to consumption of unbalanced milk diet.

Milk and other dairy products have long been recognized as a significant component of a balanced diet. Milk is a natural source which contains various essential nutrients and biologically active compounds with potential health benefits (Lourens-Hattingh and Viljoen, 2001).

Agrawal *et al.,* (2003) suggested that camel milk show significant therapeutic properties such as anticancer and antidiabetic properties.

Camel's milk has a bacteriostatic effect against *E. coli* and *L. monocytogenes*, while the colostrum is bactericidal to *E.coli* and bacteriostatic to *L. monocytogenes*, suggesting that different antimicrobial systems occurring in camel's milk may be responsible for the inhibition of each of the pathogens tested (Benkerroum *et al.,* 2004).

Camel and buffalo produces nutritious milk for human consumption. If camels are reared under same environment as buffalo, there is no doubt it will produce milk of high quality. A wide variation was observed in the quality of raw camel milk. Specific gravity ranged between 1.014 to 1.017 (1.015±0.001), pH 6.57 to 6.97 (6.77 ± 0.07), and acidity 0.12 to 2.00 (0.18 ±0.01 g per 100 g). Total solids, solids not fat, fat, protein, casein, lactose, ash and chlorides contents ranged between 7.76 to 12.13, 5.56 to 8.29, 1.8 to 5.0, 1.8 to 3.2, 0.78 to 2.76, 2.9 to 4.12, 0.85 to 1.00 ,0.20 to 0.28 g per 100 g, respectively. While

the mean values (g per 100 g) were 9.74 ± 0.49 for TS, 7.12 ± 0.35 for SNF, 2.63 ± 0.40 for fat, 2.54 ± 0.19 for protein, 2.21 ± 0.02 for casein, 3.65 ± 0.16 for lactose, 0.94 ± 0.02 for ash and 0.26± 0.01 for chlorides (Khaskheli *et al.,* 2005).

Rachid, (2006) Observed that a diet rich in cultured dairy products may inhibit the proliferation of many cancerogenous cells. The epidemiological studies had suggested that the oral intake of LAB dairy products may minimize the incidence of colon cancer. It has also been reported that there was a relationship between low fat dairy products consumption and the possibility of reducing the overweight syndrome. Furthermore, oral administration of milk and milk products has been linked with the reduction of hypertension. All these health beneficial effects may be due to the biological compounds derived from milk proteins hydrolysis and other effects, such as the weight control effects of milk calcium.

Buffalo milk had higher concentrations of protein, fat and ash than cow milk. The casein micelles from buffalo milk were more mineralized and less hydrated than their counterpart's cow milk. During acidification, some molecular changes, such as precipitation/aggregation of casein, solubilizations of calcium and inorganic phosphate and decrease in hydration of casein occurred. These molecular changes were qualitatively similar for both species. The buffering capacity was higher for buffalo milk than cow milk (Ahmad *et al.,* 2008).

The antibacterial activity of lactophorin (PP3 fraction) isolated from camel milk show highest antibacterial activity in respect of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) from milk of other species animals (Conesa *et al.,* 2008).

Sharma *et al.,* (2011) found that most of the recognized peptides are derived from α-casein and have been shown to possess free

radical scavenging activities and to hamper enzymatic and non enzymatic lipid per oxidation. ACE inhibitory peptides have been found in processed dairy products (cheese, milk etc.) without any intended functional role.

The bioactivity of peptides obtained from camel milk casein upon fermentation has not been extensively studied so far (Salami *et al.,* 2008 and 2011), however, enzymatic hydrolysis of camel caseins and improvement of biological activities have been reported (Jrad *et al.,* 2014).

2.2 Bioactive peptides:

Chen *et al.,* (1998) studied that the properties of 22 synthetic peptides containing histidine, which were designed on the basis of the antioxidative peptide (Leu-Leu-Pro-His-His) derived from proteolytic digestion of a soybean protein, were examined with regard to their antioxidative activity against the peroxidation of linoleic acid and the scavenging effects on active oxygen and free radical species. The antioxidative activities of these peptides in an emulsion oxidation system determined by using 2,2‘-azobis(2-amidinopropane) dihydrochloride as a radical initiator correlated well within an aqueous system. Although the histidine-containing peptides had a quenching activity on singlet oxygen, they did not show antioxidative activity in an 2,2‘-azobis(2,4-dimethylvaleronitrile)-induced oxidation system or scavenging effects on 1,1-diphenyl-2-picrylhydrazyl radical and superoxide. The metal-ion chelating activities and the hydrophobicities of these peptides showed no direct correlation with their antioxidative activities. Leu-Leu-Pro-His-His was modified with a hydroxyl radical in an aqueous ethanol system during the peroxidation of linoleic acid.

Antioxidant activity of fermented milk generated using LAB oxidative metabolism is crucial for the survival of human cells. However, the risk associated with this activity is that the production of free radicals may cause oxidative changes. For example, in some of

the age specific diseases, oxidative stress has been reported to play an important role in the genesis of these diseases. Free radicals have also been linked with many other pathological conditions such as atherosclerosis, diabetes, rheumatoid arthritis. Inhibition of the free radicals formed in the living body and foodstuff is an important way to protect body from these serious diseases (Cervato *et al.,* 1999).

Christensen *et al.,* (1999) have been isolated potent biologically active peptides from a number of fermented dairy products such as cheese, fermented milk and yogurt. Due to growth requirements, dairy starter cultures have developed highly sophisticated proteolytic system that capable of breaking down milk proteins, mainly α1 and β-caseins.

A food can be said to be functional if it meets one of the following criteria: (a) It includes a food component (being nutrient or not) which have positive effects on one or a limited number of function(s) in the body (b) It has physiological implication as a result of the traditional nutritional effect. Collectively, functional foods should have a positive impact on well-being and health or lead to a reduction of risk of many diseases. The biological active substances in functional foods can be either an essential macronutrient if it has specific physiological effects or an essential micronutrient if its intake is over and above the daily recommendations (Roberfroid, 1999).

Clare and Swaisgood, (2000) reported that bioactive peptides are synthesized in the cell in the form of large prepropeptides and they remain inactive within the sequence of native milk proteins, but they can be released invivo by digestive proteases or invitro by enzymatic hydrolysis either by digestive, microbial, plant proteases or by fermentation using different Lactic Acid Bacteria with proteolytic activities. The peptides derived from camel milk proteins have been shown to exert various functionalities such as antioxidant activities, anticancer activities, reduction of blood pressure (ACE), opioid activities, mineral binding, growth stimulation and antimicrobial

activities. It is reported that casein derived bioactive peptides decrease the risk of heart disease, diabetes and cancer.

Numerous casein and whey protein-derived angiotensin-I-converting enzyme (ACE) inhibitory peptides/hydrolysates have been identified. Clinical trials in hypertensive animals and humans show that these peptides/hydrolysates can bring about a significant reduction in hypertension. These peptides/hydrolysates may be classified as functional food ingredients and nutraceuticals due to their ability to provide health benefits i.e. as functional food ingredients in reducing the risk of developing a disease and as nutraceuticals in the prevention/treatment of disease (Fitzgerald and Meisel, 2000).

Shah, (2000) studied that milk contains various components with physiological functionality. Peptides derived from caseins and whey proteins including opioid peptides, antihypertensive peptides, casein phosphopeptides, α and β-lactorphins and albutensin have been shown to possess various bioactive properties.

Rival *et al.,* (2001) found that most of the identified peptides are derived from α-casein and have been shown to possess free radical scavenging activities and to inhibit enzymatic and non enzymatic lipid peroxidation, most likely by being a preferred target over fatty acid free radicals.

Bioactive peptides have been isolated from many protein sources such as soy proteins, gelatin, fish proteins and maize but milk proteins appear to be the most important sources of bioactive peptides identified thus far (Farnworth, 2003).

Bioactive peptides are defined as specific protein fragments that have a positive impact on body functions or conditions and may finally influence health of consumer (Kitts and Weiller, 2003).

Bioactive peptides usually contain 3 to 20 amino acid residues per molecule. They have been found to have specific activities, such as

antihypertensive, antioxidative, antimicrobial, immunomodulatory, opiod or mineral-binding activities. Many milk-derived bioactive peptides reveal multifunctional properties, i.e. specific peptide sequences may exert two or more different biological activities. Due to their physiological and physicochemical versatility, milk-borne bioactive peptides are regarded as important ingredients for health-promoting functional foods (Korhonen and Pihlanto-Leppala, 2004).

The main sources of biologically active peptides or bioactive peptides are caseins and whey proteins. Bioactive peptides are described as 'food derived components that in addition to their nutritional value exert a physiological effect in the body (Vermeirssen *et al.,* 2004).

Magjeed, (2005) reported that milk-based bioactive peptides have been focused until now mainly on bovine and to smaller extent on ovine and caprine milk proteins.

The consumption of fermented goat milk improved antiatherogenicity in healthy subjects by prolonging the resistance of the lipoprotein fraction to oxidation, lowering the levels of peroxidized lipoproteins, oxidized LDL, 8-isoprostanes and the glutathione redox reaction, and enhancing total antioxidative activity (Kullisaar *et al.,* 2006*).*

Bioactive peptides are protein sequences that remain inactive in the native protein primary structure, but when released, for example by proteolytic enzymes, may regulate the most body's physiological functions (Dziuba and Darewicz, 2007).

The preparation of an ovine αs2 and bovine κ-casein hydrolysate show antibacterial, ACE-inhibitory and antioxidant activities. In addition, the presence of multifunctional peptides in these hydrolysates was also demonstrated (Lopez-Exposito *et al.,* 2007).

Hayes *et al.,* (2007) found that a variety of milk-derived biologically active peptides have been shown to exert both functional and physiological roles invitro and invivo, and because of this are of particular interest for food science and nutrition applications. Biological activities associated with such peptides include immunomodulatory, antibacterial, anti-hypertensive and opioid-like properties. Milk proteins are recognized as a primary source of bioactive peptides, which can be encrypted within the amino acid sequence of dairy proteins, requiring proteolysis for release and activation. Fermentation of milk proteins using the proteolytic systems of lactic acid bacteria is an attractive approach for generation of functional foods enriched in bioactive peptides given the low cost and positive nutritional image associated with fermented milk drinks and yogurt.

A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus*, *Bifidobacterium* ssp*.*, and *L. casei*. These benefits include antimutagenic effects, anticarcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol, and immune system stimulation. Because of the potential health benefits, these organisms are increasingly being incorporated into dairy foods, particularly yogurt. In addition to yogurt, fermented functional foods with health benefits based on bioactive peptides are released by probiotic organisms (Shah, 2007).

Li *et al.,* (2008) analyzed that antioxidant properties of the peptides are related to their composition, structure and hydrophobicity, position of amino acid residue and molecular weight.

Bioactive peptides are substances that can affect the biological processes of the body functions with beneficial effects. Milk proteins have been recognized as potential sources of biological active peptides that are latent and encrypted in their native form. Protein-derived compounds known as bioactive peptides may exert a number of activities affecting the digestive, endocrine, cardiovascular, immune

and nervous systems under invitro and invivo conditions (Moller *et al.,* 2008).

Hogan *et al.,* (2009) reported three microbial proteases, validase (Val) from *Aspergillus oryze*, alkaline protease (AP) from *Bacillus licheniformis*, and neutral protease (NP) from *Bacillus subtilis*, were investigated for producing antioxidant hydrolysates/peptides from milk protein isolate. The hydrolysates were fractionated by sequential ultra-filtration and they show antioxidant properties.

Production of bioactive peptides derived from fermented milk with starter cultures have been studied extensively (Gobbetti *et al.,* 2002; Fitzgerald and Murray, 2006). Such peptides are inactive in their parent protein sequences, and these biologically active peptides can be released by three ways including digestive enzymes during passage through the gastrointestinal tract, during processing of foods and by fermentation. Upon consumption of microbial fermented milk, bioactive peptides may affect many human physiological systems including digestive, nervous, endocrine, immune and cardiovascular (Korhonen, 2009).

According to Korhonen, (2009) bioactive peptides were first reported in 1950 when casein-derived phosphorylated peptides enhanced vitamin D-independent calcification in rachitic infants upon ingestion. In recent years, a number of in vitro studies have been provided evidence for the existence of biological active peptides and proteins derived from foods that might have beneficial effects on human health. These primary studies have opened a new scientific field to examine the production of bioactive peptides from many types of dietary proteins. Proteins in the diet have been increasingly acknowledged and confirmed by new scientific findings as a great value of vital source of amino acids and biologically active substances. These biologically active peptides are hidden in their parent protein sequence and can be released by gastrointestinal tract (GIT) enzymes,

food processing and fermentation. Various health benefits including anticarcinogenic, weight management, antithrombotic, antioxidative, immunomodulatory and antihypertensive properties, have been reported. Sources of bioactive peptides in addition to milk proteins, as an important source of bioactive peptides, plants such as wheat, maize, soy, rice, mushroom, pumpkin and sorghum, as well as meat, fish, eggs from animals have been identified as other sources of bioactive peptides.

Bioactive peptide fragments can be obtained through hydrolysis of whole milk or precursor protein by digestive enzymes. This powerfully hypothesizes the existence of such peptides in the GIT after consumption of milk. The quantity and composition of milk, presence of additional food, instance, pH and enzymatic action utter the type and destiny of peptides released in digestive system. These factors are influenced by age, genetic makeup, nutritional patterns and health status of the consumer (Kamau *et al.,* 2010).

Many artificial antioxidative agents are prohibited in some countries because of the risk associated with their consumption. Only a few details are available about antioxidant peptides derived from fermented dairy products. Milk proteins have been suggested to have possible free radical scavenging by amino acids such as tyrosine and cysteine. Peptides derived from casein hydrolysis have been reported to have antioxidant activity. Peptide produced from β-casein f (177–183), for example, which known as ACE-I, it has been reported to have antioxidant activity. Furthermore, a potent antioxidant activity was found in the peptide Tyr–Phe–Tyr–Glu-Pro–Leu. Casein hydrolysates were reported to have higher concentration of histidine, lysine, proline and tyrosine, which are able to react with free radical and serve as scavengers. However, few antioxidant peptides have been observed in microbial fermented milk. A κ-casein derived 61 peptide with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity has been

found in fermented milk. The antioxidative activity of milk-kefir and soymilk- kefir and found that the DPPH radical-scavenging activity of milk-kefir and soymilk-kefir was significantly higher than that of milk and soymilk. These results suggest that this activity may be, in part, attributed to the peptides derived from degradation of milk and soybean proteins. These results of scavenging properties of free radicals by dairy cultures might be useful in food manufacturing and can present additional sources of health enhancing antioxidants (Osuntoki and Korie, 2010).

Salami *et al.,* (2010) Studied on antimicrobial properties of whey protein before and after limited proteolysis and size-based fractionation done. Both wps and their hydrolysates were assessed for their antibacterial effects against *E.coli. E.coli* was cultured in luria broth medium with the addition of different Wps/Wp hydrolysates. The specific growth rates were compared. Both bovine and camel wps, and their hydrolysates, inhibit the growth of *E.Coli.* Camel wps revealed markedly greater antimicrobial activities than bovine wps before hydrolysis. This finding can be explained by the higher content of antimicrobial factors such as lysozyme, lactoferrin, and immunoglobulin's in camel milk. There is a great interest in camel milk immunoglobulin's (IGgs), which are quite unique in the animal world and could be used to neutralize bacterial and viral enzymes.

It is studied that when camel milk hydrolyzed with pepsin and pancreatin then it shows the antimicrobial, radical-scavenging and ACE (angiotensin l converting enzyme) inhibitory activities (Jrad *et al.,* 2014).

Kumar *et al.,* (2016) determined the antioxidant activity of camel milk by enzymatic hydrolysis using 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) method. They also reported that Many food proteins have peptide sequences with potential

multifunctional activities, and there are many reports on antioxidant, antimicrobial, ACE-inhibitory, opioid, antithrombotic, anticancer activities of peptides derived from milk proteins and various food proteins.

2.3 Lactococcus lactis and its bioactive properties:

A study on peptide importers in *Lactococcus lactis* has shown that relatively hydrophilic di and tripeptides are transported by a proton motive force-driven transport system (Smid *et al.,* 1989).

It has been shown that *Lactococcus lactis* possesses a transporter that is specific for oligopeptides (Opp) (Kunji *et al.,* 1993).

Juillard *et al.,* (1995) confirmed that the quantity of free amino acids and short peptides in milk are very low. Therefore, LAB use developed proteolytic system allowing for degradation of milk proteins for their growth.

Gobbetti *et al.,* (2000) reported that fermented milk containing angiotensin-I-converting-enzyme (ACE)-inhibitory peptides were produced by using *Lactococcus lactis* ssp*. cremoris* FT4.

Korhonen and Pihlanto, (2006) reported the proteolytic structure of lactic acid bacteria (LAB) and their activities in dairy products including yogurt and cheese.

Amino acids and peptides produced by enzymatic hydrolysis of milk proteins by LAB proteolytic system and utilization of these amino acids are a central and integral part of their metabolic activity. During fermentation, milk, as stated above cannot supply all essential amino acids required for LAB growth in free form; therefore, LAB have developed ability to degrade milk proteins, mainly caseins, by their proteolytic system producing initially peptides, and then amino acids needed for their growth (Savijoki *et al.,* 2006).

Milk fermentation by proteolytic lactic acid bacteria (LAB) is one of the economical and practical methods for the production of fermented dairy products enriched in bioactive peptides (Hayes *et al.,* 2007).

Tamime *et al.,* (2007) reported that viability of LAB and culture performances (growth rate, acid production, proteolytic activity) is important detriments of the culture selection in industrial applications. It has been suggested that the concentration of dairy lactic acid bacteria should be at least 107 colony forming units (CFU) per gram of a medium to obtain desired health benefits. A consumer should consume about 108 cfu/g of product daily to recompense the possible losses during the transit in the gastro intestinal tract (GIT) as well as regular washouts due to poor adhesion of these bacteria. Factors affecting viability of LAB many factors, such as species specificity, interactions between strains, acidity and hydrogen peroxide production as a result of bacterial metabolism, have been reported to exert their effect on the culture activity and viability. These factors can affect the viability of fermented lactic acid bacteria even during fermentation. Moreover, storage temperature, concentration of produced organic acids in the medium, growth promoters and inhibitors, inoculation level, fermentation time and post-acidification and oxygen content can also affect the viability and growth of LAB during fermentation and storage in dairy products adding enhancers with appropriate LAB strains can improve the viability and survival of LAB in fermented dairy products. Physiological functions of dairy derived bioactive peptides fermented dairy foods, in addition to providing energy and nutrients, also are a source of physiologically important peptides that have a positive impact on body’s functions. These potential health benefits may be due to the production of microbial metabolites, such as cell wall components, bacteriocins and the hydrolysis of cell-free extracts containing proteinase and peptidase activities on milk proteins substrates.

Lactic acid bacteria (LAB) are a group of bacteria which may convert up to 50% of carbohydrate to lactic acid via fermentation process, and have been studied for their possible health beneficial physiological effects on human and animals (Yamamoto Y, 2009).

Lactobacilli are normally found in the mouth, intestinal tract and vagina, where they play major part in preventing from mouth sores and vaginal infections caused by bacteria and yeast infection (Admin, 2010).

Pan and Mei, (2010) reported that a novel exopolysaccharide (EPS) was obtained by ultra-filtration, ion exchange and sizing chromatography from a culture of *Lactococcus lactis subsp. lactis* 12. The EPS was mainly composed of fructose and rhamnose with a mean molecular weight of 6.9×10^5 Da. The EPS and its antioxidant properties were evaluated invitro and invivo. The EPS displayed strong antioxidant effects, exhibited the ability to scavenge hydroxyl and superoxide anion radicals, and significantly decreased the level of malondialdehyde (MDA), even while increasing the activity of catalase (CAT) and superoxide dismutase (SOD) in mice in a dose-dependent manner. The results suggest that the EPS has direct and potent antioxidant properties.

Milk and fermented dairy products represent a good source of natural folate and folate binding proteins, which improve the bioavailability and stability of folate. Effective manipulation of medium additives and cultivation conditions enhanced the folate level in skim milk by *Lactococcus lactis* ssp *cremoris*, an isolate from raw cow's milk. *Lactococcus lactis* ssp. *cremoris* also proved to be an excellent source for the enrichment of the folate content in cucumber and water melon juice (Gangadharan and Nampoothiri, 2011).

Nguyen and Nguyen, (2014) studied that the water-soluble polysaccharides (EPS) of *Lactococcus lactis* NCR 112 express the inhibition of human cancer cells as well as the antioxidant activity. The

water-soluble polysaccharide of *Lactococcus lactis* NCR 112 was studied on the antioxidant activity by using DPPH radical scavenging assay and sulforhodamine B (SRB) assay for anticancer activity tests on two HeLa and Hep G2 tumor cell lines.

2.4 Fermented milk product study:

Karleskind *et al.,* (1993) studied that chemical and microbiological properties of plain nonfat yogurt were determined after 2, 6 and 12 days refrigerated storage. Sensory properties were determined after 6 days storage. Viable culture bacteria concentrations ranged from 140 to 8,000 x 106/g and ratios of lactobacillus to streptococcus ranged from 0.18 to 15.4. Chemical criteria used to characterize products included: pH, titratable acidity, lactic and four other organic acids and lactose by HPLC, and 23 major volatile organic compounds by dynamic headspace analysis. Lactic acid concentrations ranged from 7.5 to 9.9 mg/g. Major flavor volatiles in all yogurts included: acetaldehyde, heptane, acetone, diacetyl, and benzothiazole. Untrained sensory panels showed differences for flavor, aroma and acceptability.

There was an observed sharp decline in coliform counts from 5.2 $\log_{10}$ cfu/ml to almost undetectable levels in the later stages of the fermentation. This reduction in pH as a result of the production of organic acids (e.g. lactic acid) is likely to have caused the suppression of coliform population in *suusac* (Garotte *et al.,* 2000).

Attia *et al.,* (2001) reported that camel milk fermented with lactic acid starter culture show a great decline in pH (near pH 4.4) to form a heterogeneous and fragile structure, which is not true curd but can be define as coagulum.

Fitzgerald and Meisel, (2003) reported that risks of acquiring some of chronic diseases and metabolic disorders that are associated with unbalanced diet may be reduced by consumption of fermented

dairy products. Some of the explored risk lowering effects involved cancer, osteoporosis, coronary heart diseases, hypertension and obesity.

The inhibition of *E. coli* and other coliforms by low pH caused by the production of organic acids in fermented milk products (Gran *et al.,* 2003).

Narvhus and Gadaga, (2003) analyzed that the proteolytic and lipolytic activity of yeast strains in fermented milk is likely to contribute towards development of flavor compounds and, in the case of kefir and koumiss, the desirable properties of carbon dioxide and ethanol production.

Diet is important in the prevention and treatment of the metabolic syndrome, a cluster of metabolic risk markers, leading to type 2-diabetes and coronary heart disease (CHD). Epidemiological studies suggest a beneficial relationship between the consumption of low-fat dairy products and type 2-diabetes. For CHD, relationships are less consistent. These associations, however, do not prove causality, which can only be demonstrated by intervention studies. Several intervention trials have indeed shown positive effects on metabolic risk markers, of which dairy products were a part. Milk products contain a bioactive component that can explain these findings (Mensink, 2006).

Sulieman *et al.,* (2006) studied that some of the chemical and microbiological characteristics of garris, a Sudanese traditionally fermented camel's milk product, were investigated. The chemical analyses included pH, titratable acidity and ethanol contents. A total of 100 strains of lactic acid bacteria (LAB) were isolated from twenty samples of traditionally fermented household garris. The selected isolates were phenotypically characterized by their ability to ferment carbohydrates using API 50 CHL kits and additional biochemical tests. LAB dominated the microflora of garris samples, and the major genera were Lactobacillus (74%), followed by Lactococcus (12%),

Enterococcus (10%) and Leuconostocs (4%). The most predominant Lactobacillus species were identified as Lactobacillus paracasei ssp. paracasei (64 strains), *L. fermentum* (seven strains) and only three strains as *L. plantarum*. Most strains produced the enzymes that are relevant to cultured dairy product processing. The Lactococcus species were identified as *Lactococcus lactis*. The average pH value of the samples was 4.42 ± 0.21. The pH values were accompanied with increasing of titratable acidity which averaged 1.72 ± 0.04%. The relatively high amounts of ethanol detected in all samples (average 1.40 ± 0.03%) together with the high yeasts counts (6.0 ± 0.53 log10 cfu/ml), indicated that the fermentation process of garris is a yeast lactic fermentation.

Parvez *et al.,* (2006) studied that functional foods in modern era migrations and industrialization have introduced new eating habits followed by innovative production and processing of foods, consumption of which had substantial social and health impacts. Metabolic syndrome has been related to high energy dense foods, and thus an unbalanced diet has become a major health related challenge in most developed countries around the world. Recently, a great deal of attention has been paid by food scientists, nutritionists and health professionals to functional foods and biological active compounds that can potentially reduce the risk of chronic diseases beyond their basic nutritional functions.

Huang *et al.,* (2007) demonstrated that induction of apoptosis is one of the mechanisms for anticancer activities of jujube extracts in different cell lines investigated the anticancer activity of *Z. jujuba* Mill and its underlying mechanisms of action in human hepatoma cells (HepG2) and found that the extract of jujubes decreased the viability of the cells.

Brennan *et al.,* (2008) reported that barley beta-glucan, partially hydrolysed guar gum and inulin were used in the processing of low-fat

yogurts. The possible beneficial effects of carbohydrate, fat replacers on the rheological, textural and sensory quality of low fat yogurt based products were determined. Comparisons were made between the sample, yogurts made from a low-fat milk base, and full-fat and low-fat yogurt controls. The inclusion of the carbohydrate components reduced product syneresis and improved the texture and rheological properties of the low fat based products so that their quality characteristics were similar to yogurt made with full-fat milk. Both the type and also the amount of carbohydrate component altered product characteristics. Beta-glucan addition at low level (0.5%) was effective in improving serum retention of the yogurt and its viscoelastic nature. In contrast, higher levels (above 2%) of inulin and guar gum were needed to exert significant improvements in the textural characteristics of yogurt. Sensory analysis conducted on the samples illustrated that the inclusion of carbohydrate based fat replacers could be successfully utilized to mimic full fat products.

Vahedi *et al.,* (2008) reported that water extract of dried fruit of *Ziziphus Jujuba* was tested for its possible anticancer effect and induction of apoptosis on human tumour cell lines, HEp-2, HeLa and Jurkat cell lines. The inhibitory effect of water extract of this fruit on cell proliferation was assessed by MTT colorimetric assay. The induction of apoptosis of this extract was analyzed by DNA fragmentation analysis. *Ziziphus Jujuba* extract showed inhibitory effects on mentioned cell lines. Jurkat leukemic line was found the most sensitive cells with IC 50 of 0.1 µg ml^{-1}. The present study showed cytotoxic activity of *Zizihus Jujuba* on tumor cells. Although *Ziziphus Jujuba* has useful compounds for medical applications.

There are various traditional fermented camel milk products that are produced in different parts of the world by camel herders. Suusac and garris are fermented camel milk products are produced in Kenya,

Somalia and Sudan and ititu is produced in the Kereyu area of the Oromia region in the eastern part of Ethiopia.

These two products are Ethiopian indigenous traditionally fermented camel milk food products (Abdel Rahman IE *et al*., 2009).

Physico-chemical, microbiological and sensory attributes before and after storage for different periods of time (5, 10 and 15 days) of yogurt produced from camel milk with reference to cow milk were analyzed. Yogurt processing significantly ($P \leq 0.01$) decreased the initial pH of fresh camel and cow milk. Cow yogurt was found to be more viscous than camel yogurt. Storage resulted in significant changes in gross composition of both yogurt types. Nutrients content of cow yogurt was more affected than that of camel yogurt during storage. *Lactobacillus* spp. and *Streptococcus* spp. were main fermentative organisms in both yogurt types. Yeast and moulds were detected and increased with storage time up to day 10 and thereafter decreased for both yogurt types. *Staphylococcus aureus* and *Salmonella* were absent in both yogurt types. Coliforms and fecal coliforms were only detected in cow yogurt and then disappeared after day 5 of storage. Organoleptic tests indicate that camel yogurt had significantly lower consumer preferences. In terms of sensory tests, camel yogurt can withstand storage for longer period than cow yogurt (Eissa *et al.,* 2010).

Wang *et al.,* (2010) found that the content of phenolic acids in jujube fruit ranged from 751.39 μg/g dry weight (DW) in jujube peel to 143.59 μg/g DW in pulp, and the phenolic acids are mainly found in the insoluble bound form in both jujube seed and peel, whereas in the glycosided form in the pulp. Free phenolic acids constitute from 5.2% in peel to 20.7% in seed of the total phenolic acids present in jujubes, whereas the phenolic acids released from soluble esters make up 6.2% in seed and 27.5% in pulp. Glycosides account for 44.7%, 11.6%, and 22.3% of the total phenolic acids present in jujube pulp, seed, and peel,

respectively. P-Hydroxybenzoic acid is the dominant phenolic acid in both pulp and seed of jujubes, even in the whole jujubes, with 51.7%, 47.7%, and 25.0% of the total contents in the pulp, seed, and whole jujubes, respectively, whereas p-coumaric, cinnamic, and chlorogenic acids are present in high amount in peel. Hydroxybenzoic acids include protocatechuic and gallic acid in jujubes. Caffeic, p-coumaric, cinnamic acid, and ferulic acid are hydroxycinnamic acids found in jujubes.

The proteolytic activities of traditional and commercial yogurt culture and evaluated the antioxidant activities by ABTS and DPPH assay. They further reported that the water soluble extracts of commercial yogurt had significantly higher antioxidant activities than of traditional cultures (Aloglu and Oner, 2011).

The anti-proliferative and apoptosis mechanisms of betulinic acid isolated from sour jujube fruits, on human breast cancer MCF-7 cells. They found that the complexation model inhibited the growth of MCF-7 cells and arrested cell cycle in the G2/M phase and induced apoptosis via the mitochondria transduction pathway. Gene and protein analyses showed that the complexation model significantly inhibited Bcl-2 expression and promoted Bax expression, causing caspase-3 and caspase-9 cascade activation (Sun *et al.,* 2011).

Giampieri *et al.,* (2012) confirmed the antioxidant capacity of jujube fruit is closely correlated with the presence of efficient oxygen radical scavengers, such as phenolic compounds.

Jujube extracts inhibited the growth of selected cancer cell lines. This indicated that triterpenic acids resulted from bioactive compounds present in the most effective extracts and showed that they inhibited the growth and induced apoptosis in MCF-7 and SKBR3 breast cancer cell lines (Plastina *et al.,* 2012).

3. MATERIAL AND METHODS

To study on the fermentative potential of camel and buffalo milk by Using *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp*. lactis*, an experiment was designed and conducted at NRCC Bikaner to estimate physico-chemical properties and to produce bioactive peptides from camel and buffalo milk. A pre experimental trial was done by using different starter cultures of lactic acid bacteria procured from NCDC, NDRI karnal. On the basis of antioxidant activity the two cultures viz. *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* were choosen for the present investigation.

3.1 Location:

Experiment was conducted at ICAR-National Research Centre on Camel, Bikaner (Rajasthan), Department of Livestock Products Technology and Department of Animal Nutrition, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan).

3.2 Chemical and Reagents:

Fine chemicals such as ABTS (2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH (2,2′-diphenyl-1-picrylhydrazyl), potassium persulphate ($K_2S_2O_8$), Tris-HCl were obtained from Sisco Research Laboratories (SRL) Pvt. Ltd., India and TBA (2-Thiobarbituric acid) was obtained from Merck Life Science Private Limited, India and other chemicals were of analytical grade from reputed companies and used without further purification. All solutions, prepared with double-distilled water, were kept at 4°C before further use.

3.3 Collection of samples:

About 2 liter of fresh camel milk was collected from camel dairy maintained at ICAR-NRC on Camel, Bikaner and about 2 liter of fresh buffalo milk was collected from buffaloes maintained under the project "Establishment of live demonstration models of diversified livestock production systems for motivating adaption to enhancing agricultural income (RKVY-15)" College of Veterinary and Animal Science, RAJUVAS, Bikaner at weekly interval for period of 2 months to perform the different experiments as mentioned below under the study.

3.4 Experimental Details:

Following parameters were estimated during this phase:

3.4.1: Determination of Physico-chemical Properties of Camel and Buffalo Milk

3.4.2: Periodical Evaluation of Fermented Camel and Buffalo Milk

3.4.3: Characterization of Bioactive Potential (Antioxidant property) of Camel and Buffalo Milk during fermentation process

3.4.4: Production, Evaluation and Accessibility of Fermentative Camel and buffalo milk product

3.4.1: Determination of Physico-chemical Properties of Camel and Buffalo Milk

To determine the physico-chemical properties of camel and buffalo milk (about 500 ml) fresh camel milk and fresh buffalo milk was collected and analyzed for pH, SNF, fat, specific gravity, water content, protein, lactose, freezing point depression, salts and conductivity by using Milkoscan (Lactoscan Milk Analysar sr. no. 0564, made in Bulgaria) at camel milk research laboratory, ICAR-NRC on Camel, Bikaner.

3.4.2: Periodical Evaluation of Fermented Camel and Buffalo Milk

Fresh camel and buffalo milk were skimmed to bring the fat contents to below 0.5% using cream separator. The samples were heated to boil at least for 5 min to inactivate/kill the inherent microbial population present in milk. Then *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* cultures were inoculated @ 1% and after proper mixing the samples were inoculated at 30^0C and samples were drawn at 0, 2, 4, 6, 8, 10, 12 hours and were subjected to analysis for change in pH, TA (Titratable Acidity) and soluble protein concentration etc.

3.4.2.1: Bacterial cultures and their propagation:

Glass ampoules containing Lyophilized powder of *Lactococcus lactis* ssp. *cremoris* NCDC 81 and *Lactococcus lactis* ssp*. lactis* NCDC 88 were obtained from the NCDC (National Collection of Dairy Cultures) Dairy Microbiology Division ICAR-National Dairy Research Institute, Karnal (INDIA). The organisms were stored at 4°C. The propagation for each strain was performed according to Donker *et el.,* (2007) with slight modification. Sterile 5 ml aliquots of reconstituted sterile skim milk (RSM) (Himedia Laboratories) were inoculated with each strain individually and incubated at 30°C for 24h in BOD incubator. After incubation, the pre-inoculated cultures were prepared by transferring loop full of activated culture to 10 ml aliquots of litmus milk (Himedia Laboratories) to determine the activation of culture activity by observing change in colour of litmus milk after 24 hour of inoculation. The skim milk and litmus milk were autoclaved following the standard procedure (121°C for 15 min @15 lbs).

3.4.2.2: Culture performance during cultivation in milk:

Formation of serial dilution of the culture was done and appropriate dilution was selected for enumeration by the pour plate technique. All samples were enumerated on MRS agar at 30°C for 24 hours. Plates containing 30–300 colonies were enumerated and the colony forming units (CFU) per gram of the product was calculated. These cultures were then used for fermentation of fresh pasteurized milk samples for 12 hour at 30°C corresponding to cell count 10^7–10^8 CFU/ml as Per suggested by Ramesh V. *et al.*, (2012).

3.4.2.3: PH measurement of milk samples:

The pH of samples was measured by using combined glass electrode of Milkoscan at camel milk research laboratory, ICAR-NRC on Camel, Bikaner.

3.4.2.4: Titratable acidity (TA) measurement:

Titratable acidity expressed as percentage of lactic acid, was determined by 10 ml of each sample titrating with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink colour.

Calculation:

$$\% \text{ Lactic acid} = \frac{\text{Number of ml. of 0.1 N NaOH solutions required for neutralization} \times 0.009}{\text{Weight of sample}} \times 100$$

(Weight of sample = Volume of milk x specific gravity)

NaOH = sodium hydroxide

3.4.3: Characterization of Bioactive Potential (Antioxidant property) of Camel and Buffalo Milk during fermentation process

The supernatants collected by centrifugation of Camel and Buffalo Milk during fermentation and then utilized for antioxidant assay (ABTS, DPPH, etc.).

3.4.3.1: DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical-scavenging activity:

The ability to scavenge DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical by added antioxidants in samples was estimated by following the method of Brand-Williams *et al.,* (1995) with slight modification. About 2 ml of DPPH reagent (100 µM) was mixed with 0.50 ml of 0.1 M Tris-HCl buffer (pH 7.4) and 50 µl of hydrolysate sample in test tubes and the content was gently mixed and immediately absorbance was measured at 517 nm (nanometre) by using a spectrophotometer and then the sample tubes were incubated at room temperature under dark for 20 minutes and then again measured the absorbance. Ethanol was used as blank. The free radical-scavenging activity was calculated from the following equation:

DPPH radical Scavenging activity (% inhibition) = 100−[(At20/At0)x100]
Where At20= absorbance at 20 minute

At0= absorbance at zero minute

3.4.3.2: ABTS+ (2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity:

The spectrophotometric analysis of ABTS+ radical-scavenging activity was determined according to method described by Salami *et al.* (2009). ABTS radical cation (ABTS+) was produced by reacting ABTS+ stock solution with equal volume of 2.45mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hours before use. For making working solution of ABTS, the stock solution of ABTS was diluted with distilled water to make its absorbance 0.70 and equilibrated at 30°C exactly 6 min after initial mixing. About 4 ml of ABTS+ working standard solution was mixed with 40µl of hydrolysate/standard and absorbance was measured after 20minuts @ 734 nm by using spectrophotometer.

The ABTS+ activity was calculated by using the following formula:

ABTS activity (% inhibition) = [(0.70−At20)/ 0.70] x 100

Where $At_{20=}$ absorbance of mixture at 20 minute

0.70=absorbance of working solution of ABTS

3.4.4: Production, Evaluation and Accessibility of Fermentative Camel and buffalo milk product

The most effective bacteria in terms of properties was utilized for production of fermented milk product. The developed products were packed in LDPE (Low Density Poly Ethylene) bags and stored under refrigeration condition for 7 days. The samples were evaluated for sensory evaluation at day 0 and drawn for 0, 3, 5 and 7 days for physico-chemical changes including change in pH, titratable acidity, proximate analysis, TBA value, change in ABTS %, DPPH %, microbial count Including standard plate count (SPC), Coliform count, Yeast-Molds count and MRS count measured as per method described earlier.

3.4.4.1: Production of fermented camel and buffalo milk product:

3.4.4.1.1: Preparation of jujube extraction:

About 500g of dry jujube cleaned and the seeds were removed, then soaked with 1500 ml of lukewarm distilled water overnight, then were good blended with the electrically laboratory blender, then filtered through very fine sieve (0.5mm), and the extract stored in refrigerated temperature at 4°C.

3.4.4.1.2: Preparation of jujube syrup dilutions:

Jujube syrup was diluted with distilled water until the total solid been 13-14%, then were taken 2, 5, 8 and 10 % W/W of yogurt and placed in the plastic cups for making yogurt.

3.4.4.1.3: Yogurt Making:

The fermentated camel and buffalo milk were taken at the time of fermentation where highest antioxidant property was observed and mixing of sugar and jujube syrup at a level of 2, 5, 8 and 10% (W/W) of yogurt take place in a hygienic way. Then the mixture was blended with laboratory blender until all ingredients were dissolved in the fermentated camel and buffalo milk and further use for sensory evaluation.

3.4.4.2: Sensory evaluation of Camel and buffalo milk product:

The investigated samples were evaluated using a panel test at day 1st of storage at room temperature. Ten panelists consisting of worker and staff. Yogurt samples were presented in transparent plastic cups under fluorescent light. All samples were marked with digital code, and the order of presentation of samples was randomized for each panelist. The panelists rated as per given performa:-

Table 3.4.4.2 Hedonic scale for sensory evaluation of buffalo and camel milk product

Attributes	Scale of descriptive attributes of product							
	8	7	6	5	4	3	2	1
Appearance/colour	Excellent	Very good	Good	Fair	Slightly poor	Moderately poor	Very poor	Extermely poor
Flavour	Extremely Desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extermely undesirable
Texture/ Viscosity	Extremely Thick/viscous	Very Thick/viscous	Moderately Thick/viscous	Slightly Thick/viscous	Slightly thin	Moderately thin	Very thin	Extermely thin
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly unacceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Sample no.	Appearance/colour	Flavour	Texture/ Viscosity	Overall Acceptability
1				
2				
3				
4				

Remarks

Signature

3.4.4.3: Accessibility of Fermentative Camel and buffalo milk product

3.4.4.3.1: TBA (2-Thio Barbituric Acid) value:

The TBA values were determined according to the extraction method described by Witte *et al.,* (1970). About 10 gram of the sample was blended with 25 ml of cold solution containing 20% trichloroacetic acid in 2M orthophosphoric acid. The resulting slurry was homogenized by shaking and filtered through whatman no.1 filter paper. After that 3 ml of the filtrate was pipette into a test tube while another 3 ml of fresh chilled 2-thiobarbituric acid (0.005 M in 0.05 M NaOH) was added. The test tube was shaken well and placed in the dark at room temperature (25°C) for 15-17 hours to develop the color reaction. The blank was formed by mixing 3 ml of fresh chilled 2-thiobarbituric acid (0.005 M in 0.05 M NaOH) with 1.5 ml of cold solution containing 20% trichloroacetic acid in 2M orthophosphoric acid and 1.5 ml of distilled water. After 15-17 hours the resulting color was measured in spectrophotometer at 532 nm to calculate the TBA values. The TBA value was expressed as mg MDA/kg of sample, which was calculated by multiplying the absorbance by 5.2 factors as follows:

TBA (mg (MDA)/kg) = A532 × 5.2

Where A= absorbance of mixture, 532=frequency of spectrophotometer and 5.2=factor

3.4.4.3.2: Microbial count:

Microbial count was determined by using pour plate method. Standard plate count (SPC) was determined on plate count agar medium and the plates of different dilutions were incubated at 30°C for 24 hour. The total number of colonies per gram (CFU/g) was determined. Violet Red Bile (VRB) Agar medium was used for determination of coli form bacteria and the plates of different dilutions were incubated at 30°C for 24 hour by pour plate method, and the number of dark red colonies was calculated. Man, Rogosa and Sharpe (MRS) agar medium used for enumeration and cultivation of Lactococcus species and by pour plate method, the and the plates of different dilutions were incubated at 30°C for 24 hour, Lactococci colonies appear slightly opalescent, and medium amber in color. Yeast molds (Y-M) count take

place by Potato dextrose agar (PDA) medium by pour plate method and the plates of different dilutions were incubated at 30°C for 24 hour, for yeast and mold count.

3.5: Statistical analysis:

All the experiments of fermentation study were repeated three times and samples were drawn in duplicate. Data collected during the Present investigation were subjected to statistical analysis by using F- test and adopting appropriate methods of analysis of variance as described by Snedecor and Chochran (1994). Wherever, the variance ratio were found significant at 5 percent and highly significant at 1 percent levels of probability, the significance of mean differences were tested by Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1957).

4. RESULTS AND DISCUSSION

The studies on the fermentative potential of camel and buffalo milk by using *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* were carried out in terms of antioxidant potential of fermentated camel and buffalo milk by using ABTS and DPPH method. The other parameters included in to study were physico-chemical properties of camel and buffalo milk, pH and TA of fresh milk as well as during fermentation, proximate analysis, sensory evaluation and storage study of the products.

The present chapter describes the results obtained from different experiments carried out in accordance with the objectives as per materials and methods. The results are presented through the narration and supported with tables, figures and photographs of products. The results have been critically analyzed in the present chapter.

The results obtained in present investigation along with their discussions are presented under following headings:

4.1 Physico-chemical properties of camel and buffalo milk

4.2 pH of camel and buffalo milk during fermentation

4.3 Titratable Acidity (TA) of camel and buffalo milk during fermentation

4.4 ABTS activity of camel and buffalo milk during fermentation

4.5 DPPH activity of camel and buffalo milk during fermentation

4.6 Production, Evaluation and Accessibility of fermented camel and buffalo milk product

4.7 Sensory evaluation of fermented camel and buffalo milk product

4.8 Proximate analysis of fermented camel and buffalo milk product

4.9 Storage study of fermented camel and buffalo milk product

4.1 PHYSICO-CHEMICAL PROPERTIES OF CAMEL AND BUFFALO MILK

Fresh buffalo milk was obtained from buffaloes maintained under the project “Establishment of live demonstration models of diversified livestock production systems for motivating adaption to enhancing agricultural income (RKVY-15)” C.V.A.S., RAJUVAS, Bikaner and was kept in chilled condition till further use. Fresh camel milk was collected from camel dairy maintained at ICAR-NRC on Camel, Bikaner. All samples were collected manually in sterile bottles once per day (usually in the morning), milk samples were analyzed for pH, SNF, fat, specific gravity, water content, protein, lactose etc. using Milkoscan at camel milk research laboratory, ICAR-NRC on Camel, Bikaner.

The mean values of physico-chemical properties of fresh buffalo and camel milk (from 8 buffalos) and camel milk (from 10 camels) has been presented in Table 4.1.

Table 4.1 Physico-chemical properties of buffalo and camel milk

Physico-chemical Property	Camel milk	Buffalo milk
Fat	3.63 ± 0.08	8.53 ± 0.16
SNF	7.16 ± 0.12	7.73 ± 0.04
Density	1.025 ± 0.23	1.031 ± 0.41
Protein	2.43 ± 0.05	3.09 ± 0.02
Lactose	3.92 ± 0.05	3.85 ± 0.03
Water	13.22 ± 1.25	6.05 ± 0.65
Salt	0.80 ± 0.01	0.76 ± 0.01
Freezing Point	-0.47 ± 0.01	-0.49 ± 0.01
pH	6.44 ± 0.01	6.71 ± 0.01
Conductivity	5.28 ± 0.07	2.56 ± 0.03

The overall compositions of buffalo and camel milk showed that the buffalo milk had higher concentrations of protein, fat and solid not fat (SNF) than camel milk. The casein micelles from buffalo milk were more mineralized and less hydrated than their counterparts camel milk. The results related to physico-chemical properties of buffalo milk were in conformity with Ahmad *et al.,* (2008).

In general the present study showed a wide variation in the gross composition of camel milk. The results obtained for camel milk were in agreement with studies of Ahmed, (1990), Lapsson, (1990) and Khaskheli *et al.,* (2005). This variation was concluded to be partly due to the inherited

capabilities of the animals and/or attributed due to various seasonal and environmental factors as well as stage of lactation, age and number of calving. In addition, the feed and water quality and quantity available to the animals also play an important role (FAO, 1982).

4.2 pH OF CAMEL AND BUFFALO MILK DURING FERMENTATION

Change in pH during hydrolysis:

The data related to pH of camel milk has been presented in Table 4.2(a) and depicted in figure 1. The pH of fresh milk was found to be 6.50±0.006 for *Lactococcus lactis* ssp. *cremoris* and 6.50±0.005 for *Lactococcus lactis* ssp. *lactis* before inoculation. The value of pH was dropped significantly as the fermentation hour were increased, and at 12 hour of fermentation it was observed to be 4.82±0.005 and 4.93±0.008 for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* respectively whereas the overall pH was 5.66±0.088 and 5.71±0.083 was observed for *Lactococcus lactis* ssp*. cremoris and Lactococcus lactis* ssp. *lactis*.

The statistical analysis of data shown in Table 4.2(b), revealed that there was a highly significant ($P<0.01$) decrease in the pH value of camel milk samples with advancement of fermentation hours as well as with treated bacteria. The results were similar with the findings of Minieri *et al.,* (1965).

Process of fermentation is affected by several factors including the structure of the protein, temperature, enzyme/protein ratio, enzyme concentration and pH. In the present study, almost linear drop in pH was observed during the fermentation process in both camel and buffalo milk samples. The release of protons (H+ ion) and/or production of acidic amino acids into the surrounding medium results in reduction in the pH of the reaction mixture.

Table 4.2(a) pH (Mean ± SE) of camel milk during fermentation

Treatment	***Lactococcus lactis* ssp. *cremoris***	***Lactococcus lactis* ssp. *lactis***	**Overall**
Fresh	6.50± 0.006	6.50±0.005	6.50^{g}± 0.004
Hour2	6.06± 0.008	6.06± 0.004	6.06^{f}± 0.004
Hour4	6.04± 0.005	6.04± 0.006	6.05^{e}±0.006

Hour6	5.77± 0.005	5.95± 0.010	5.86^{d}±0.027
Hour8	5.36±0.004	5.47± 0.005	5.41^{c}±0.017
Hour10	5.05±0.010	5.06± 0.011	5.06^{b}±0.007
Hour12	4.82± 0.005	4.93±0.008	4.88^{a}±0.018
Overall	**5.66^{a}±0.088**	**5.71^{b}±0.083**	**5.69±0.060**

Note – Means bearing different superscripts differ significantly.

Table 4.2(b) Analysis of variance for pH of camel milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	0.062	**
Hour	6	4.190	**
Reminder	76	0.002	

** = Significant at 1% (P<0.01)

Fig. 1 pH of camel milk during fermentation

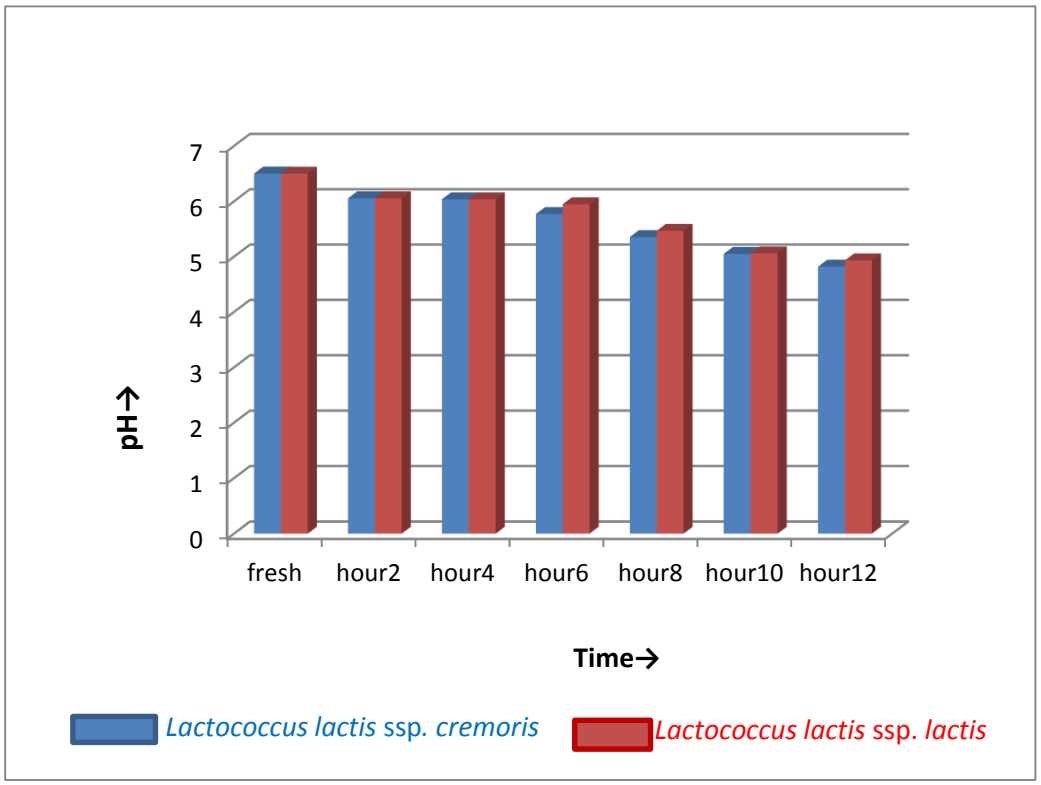


Fig. 2 pH of buffalo milk during fermentation

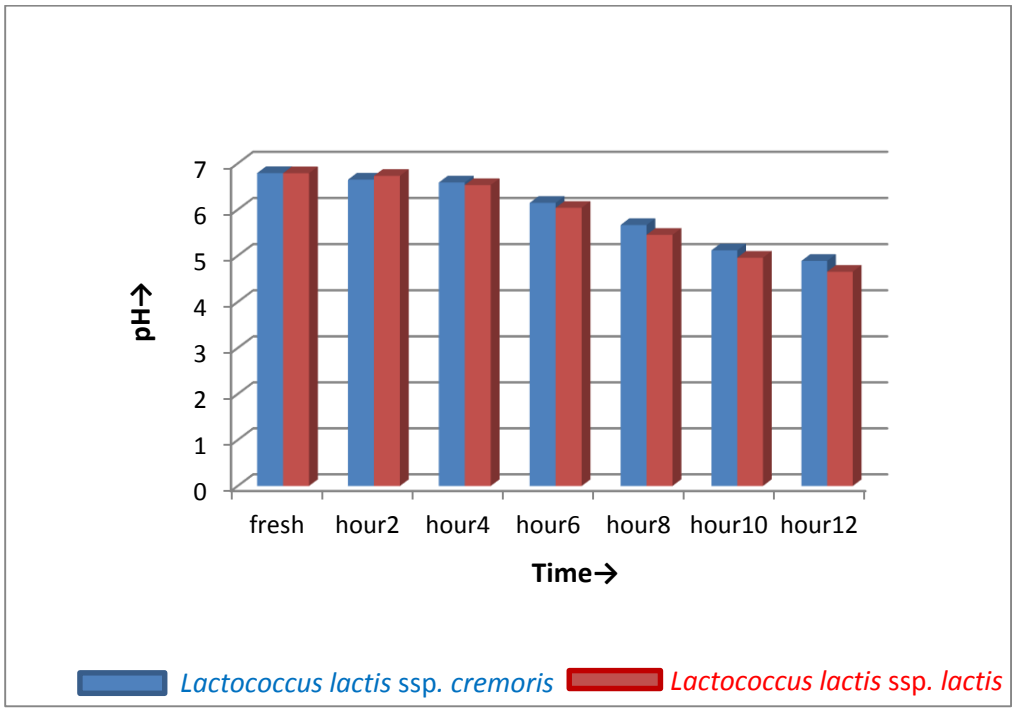


Table 4.2(c) pH (Mean ± SE) of buffalo milk during fermentation

Treatment	*Lactococcus lactis* ssp. *cremoris*	*Lactococcus lactis* ssp. *lactis*	Over all
Fresh	6.79±0.004	6.79±0.007	6.79^{g}±0.004
Hour2	6.65±0.005	6.73±0.003	6.69^{f}±0.012
Hour4	6.59±0.006	6.53±0.003	6.56^{e}±0.009
Hour6	6.15±0.005	6.04±0.009	6.09^{d}±0.017
Hour8	5.67±0.006	5.45±0.010	5.56^{c}±0.033
Hour10	5.12±0.005	4.96±0.007	5.04^{b}±0.025
Hour12	4.89±0.004	4.66±0.011	4.77^{a}±0.036
Overall	**5.98^{b}±0.111**	**5.88^{a}±0.126**	**5.93±0.084**

Note – Means bearing different superscripts differ significantly.

Table 4.2(d) Analysis of variance for pH of buffalo milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	0.207	**
Hour	6	8.034	**
Reminder	76	0.003	

** = Significant at 1% (P<0.01)

The data related to pH of buffalo milk has been shown in Table 4.2(c) and depicted in figure 2. The pH of fresh milk was found to be 6.79±0.004 for *Lactococcus lactis* ssp. *cremoris* and 6.79±0.007 for *Lactococcus lactis* ssp*. lactis* before inoculation of treated bacteria.

The value of pH was dropped significantly as the fermentation hour were increased and at 12 hour of fermentation and it was observed to be 4.89±0.004 and 4.66±0.011 for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* respectively whereas the overall pH was 5.98±0.111 and 5.88±0.126 for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* respectively.

The statistical analysis of data as shown in Table 4.2(d), revealed that there was a highly significant ($P<0.01$) decrease in the pH value of buffalo milk samples with advancement of fermentation hours as well as between the treated bacteria that is *Lactococcus lactis* ssp*. cremoris* and *Lactococcus lactis* ssp. *lactis*.

4.3 TITRABLE ACIDITY (TA) OF CAMEL AND BUFFALO MILK DURING FERMENTATION

The data related to TA of camel milk has been presented in Table 4.3(a) and depicted in figure 3. The TA value of fresh camel milk was found to be 0.14 ± 0.003 for *Lactococcus lactis* ssp. *cremoris* and 0.14 ± 0.002 for *Lactococcus lactis* ssp. *lactis* before inoculation. These results were in line with those reported by Ahmed (1990), Elamin and Wilcox (1992) which is *0.13* and 0.15%, respectively.

Table 4.3(a) TA (Mean ± SE) of camel milk during fermentation

Treatment	*Lactococcus lactis* ssp. *cremoris*	*Lactococcus lactis* ssp. *lactis*	Over all
Fresh	0.14±0.003	0.14±0.002	0.14^{a}± 0.003
Hour2	0.25±0.004	0.24±0.006	0.25^{b}±0.004
Hour4	0.27±0.004	0.27±0.003	0.27^{c}±0.003
Hour6	0.35±0.004	0.32±0.005	0.33^{d}±0.006
Hour8	0.48±0.005	0.46±0.006	0.47^{e}±0.005
Hour10	0.68±0.004	0.64±0.005	0.66^{f}±0.006
Hour12	0.83±0.005	0.72±0.006	0.77^{g}±0.017
Overall	**0.44^{b}±0.034**	**0.34^{a}±0.031**	**0.50±0.022**

Note – Means bearing different superscripts differ significantly.

Table 4.3(b) Analysis of variance for TA of camel milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	0.034	**
Hour	6	0.601	**
Reminder	76	0.001	

** = Significant at 1% (P<0.01)

Fig. 3 TA of camel milk during fermentation

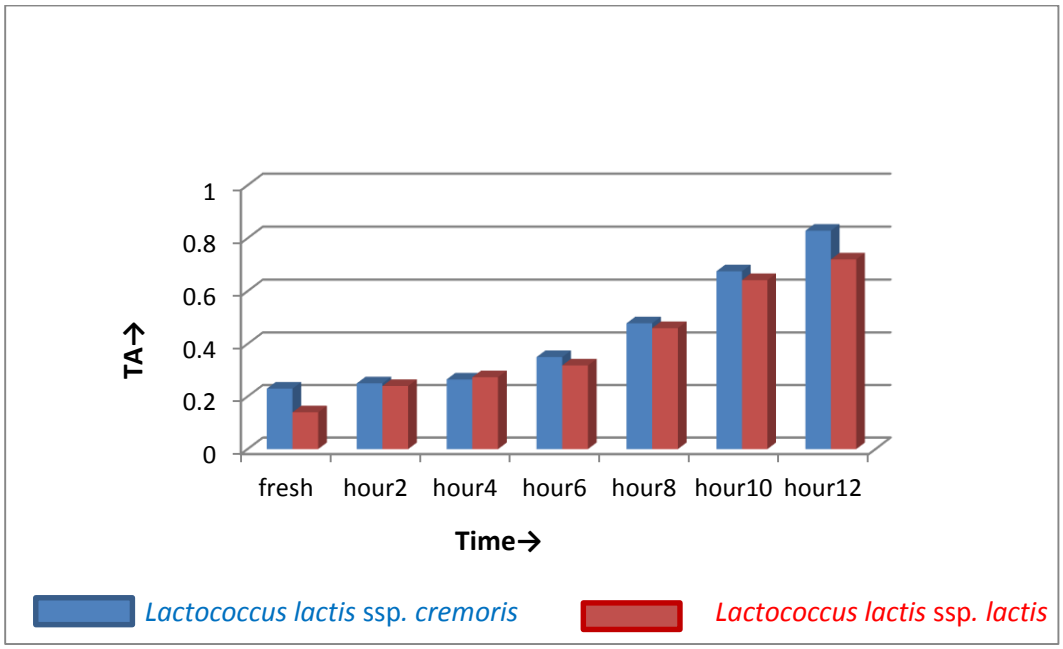


Fig. 4 TA of buffalo milk during fermentation

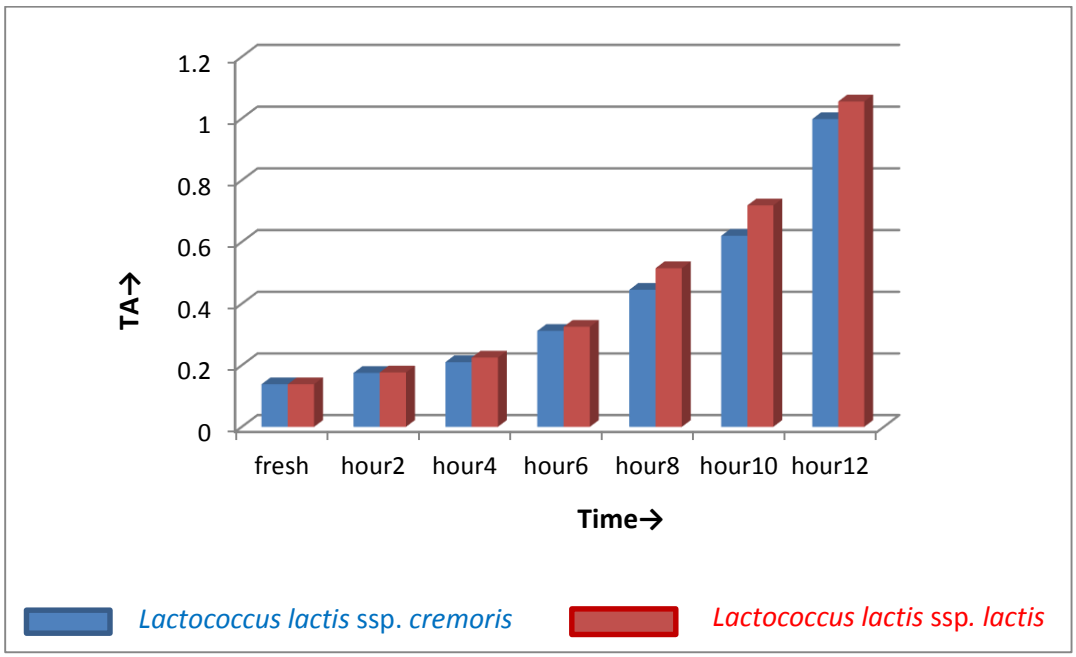


Table 4.3(c) TA (Mean ± SE) of buffalo milk during fermentation

Treatment	*Lactococcus lactis ssp. cremoris*	*Lactococcus lactis* ssp. *lactis*	Overall
Fresh	0.138±0.003	0.14±0.005	0.139^{a}±0.004
Hour2	0.175±0.003	0.18±0.003	0.177^{b}±0.003
Hour4	0.217±0.003	0.23±0.004	0.223^{c}±0.004
Hour6	0.318±0.003	0.33±0.004	0.324^{d}±0.004
Hour8	0.48±0.011	0.52±0.005	0.50^{e}±0.008
Hour10	0.66±0.015	0.72±0.005	0.69^{f}±0.010
Hour12	1.00±0.018	1.06±0.008	1.029^{g}±0.013
Overall	**0.43^{a}±0.008**	**0.49^{b}±0.005**	**0.440±0.006**

Note – Means bearing different superscripts differ significantly.

Table 4.3(d) Analysis of variance for TA of buffalo milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	0.042	**
Hour	6	1.180	**
Reminder	76	0.008	

** = Significant at 1% (P<0.01)

The value of TA was increased significantly as the fermentation hour were increased, and at 12 hour of fermentation it was observed to be 0.83 ± 0.005 and 0.72 ± 0.006 for *Lactococcus lactis* ssp*. cremoris* and *Lactococcus lactis* ssp*. lactis* respectively whereas the overall TA was 0.44 ± 0.034 and 0.34± 0.031 was observed for *Lactococcus lactis* ssp*. cremoris* and *Lactococcus lactis* ssp*. Lactis*.

The statistical analysis of data shown in table 4.3(b), revealed that there was a highly significant (P<0.01) increases in the TA value of camel milk samples with advancement of fermentation hours.

According to Hofi *et al.,* (1966) in fresh buffalo milk, lactic acid accounted for 25% of total acidity. Acidity was correlated with fat and solid-not-fat percentage in buffalo milk. The values of the titratable acidity in fresh buffalo milk were in accordance with Mahmood *et al.,* (2010).

The data related to TA of buffalo milk has been presented in Table 4.3(c) and depicted in figure 4. The TA value of fresh buffalo milk was found to be 0.14 ± 0.003 for *Lactococcus lactis* ssp*. cremoris* and 0.14 ± 0.004 for *Lactococcus lactis* ssp. *lactis* before inoculation. increased significantly to mean value of 1.00 ± 0.018 for *Lactococcus lactis* ssp*. cremoris* and 1.06 ± 0.008 for *Lactococcus lactis* ssp*. lactis* respectively after 12 hour of fermentation.

The statistical analysis of data shown in table 4.3(d), revealed that there was a highly significant (P<0.01) increases in the TA value of buffalo milk samples with advancement of fermentation hours.

During 12 hour of fermentation process, the TA value of both type of milk samples increased continuously, due to conversion of lactose into lactic acid. Results were showing similar trend with Mahmood *et al.,* (2010).

4.4 ABTS ACTIVITY (% INHIBITION) OF CAMEL AND BUFFALO MILK DURING FERMENTATION

The data related to ABTS activity (% inhibition) of camel and buffalo milk has been shown in Table 4.4(a) and 4.4(c), whereas it is depicted in figure 5 and 6 respectively.

The ABTS radical-scavenging activity increased significantly (P<0.01) with the advancement fermentation time up to 8 hour for buffalo milk whereas, for camel milk, it increased significantly (P<0.01) up to 10 hour, after that, decrease in activity was observed.

Milk inoculated with *Lactococcus lactis* ssp. *cremoris* had the highest antioxidant capacity which increased from mean value of 1.57 ± 0.001% at zero hour (fresh milk) in camel milk to 18.46 ± 0.013% at 10 hour, and for buffalo milk, it was 1.14 ± 0.001% at zero hour which increased to 14.50 ± 0.142% at 8 hour of fermentation respectively, after that it decreased significantly in both cases. Similar trends were observed with *Lactococcus lactis* ssp*. lactis* during the same incubation time and similar free radical scavenging activity at zero hour in both camel and buffalo milk samples, which reached to 13.64 ± 0.031% and 10.25 ± 0.054% in camel and buffalo milk samples, at 10 hour and 8 hour of fermentation, respectively. After that a significant fall in ABTS free radical scavenging activity takes place in both types of milk samples. Results were showing similar trend with Ramesh *et al.,* (2012).

A more apparent increase in the ABTS antioxidant activity was observed in the milk inoculated with *Lactococcus lactis* ssp. *cremoris* strains and incubated for 12 hour at 30°C for both camel and buffalo milk.

According to table 4.4(a), the overall ABTS activity (% inhibition) for camel milk was observed 9.94 ± 0.897 and 8.19 ± 0.688, for *Lactococcus lactis* ssp. *cremoris and Lactococcus lactis* ssp*. lactis* respectively.

Table 4.4(a) ABTS (Mean ± SE) activity (% inhibition) of camel milk during fermentation

Treatment	***Lactococcus lactis* ssp. *cremoris***	***Lactococcus lactis* ssp. *lactis***	**Over all**
Fresh	1.57±0.001	1.57±0.001	1.57[a]±0.001

Hour2	4.74±0.054	3.24±0.015	3.99^{b}±0.227
Hour4	5.31±0.070	5.85±0.016	5.58^{c}±0.089
Hour6	10.82±0.022	8.73±0.020	9.78^{d}±0.316
Hour8	14.80±0.022	12.74±0.018	13.77^{f}±0.311
Hour10	18.46±0.013	13.64±0.031	16.05^{g}±0.726
Hour12	13.84±0.010	11.53±0.019	12.69^{e}±0.349
Overall	**9.94^{b}±0.897**	**8.19^{a}±0.688**	**9.061±0.570**

Note – Means bearing different superscripts differ significantly.

Table 4.4(b) Analysis of variance for ABTS activity (% inhibition) of camel milk during fermentation

Source	D.F.	Mean square	Level of sig.
Treated bacteria	1	64.108	**
Hour	6	357.387	**
Reminder	76	0.727	

** = Significant at 1% (P<0.01)

Fig. 5 ABTS activity (% inhibition) of camel milk during fermentation

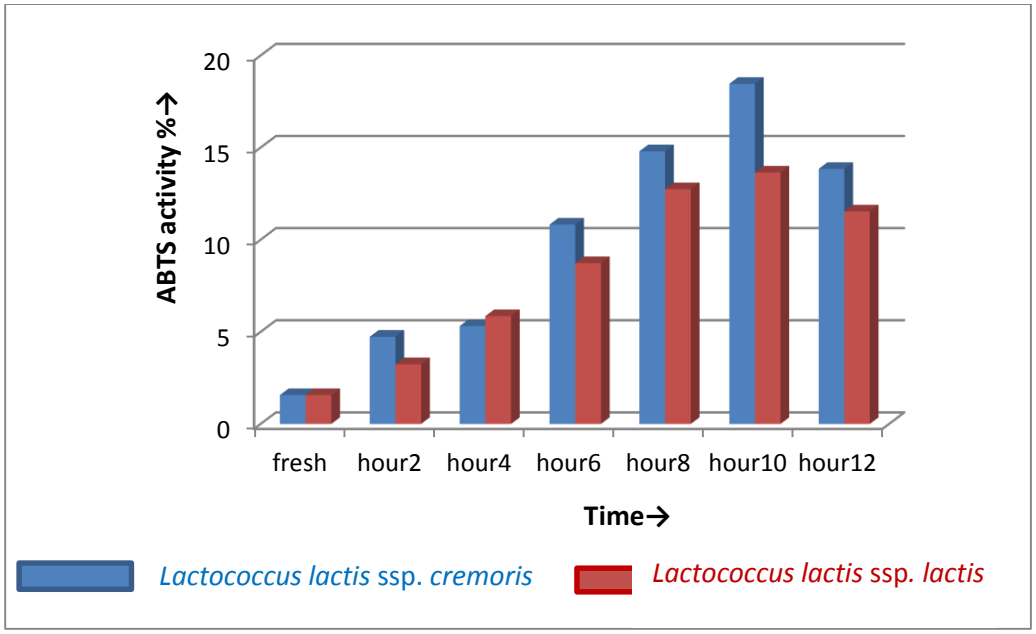


Fig. 6 ABTS activity (% inhibition) of buffalo milk during fermentation

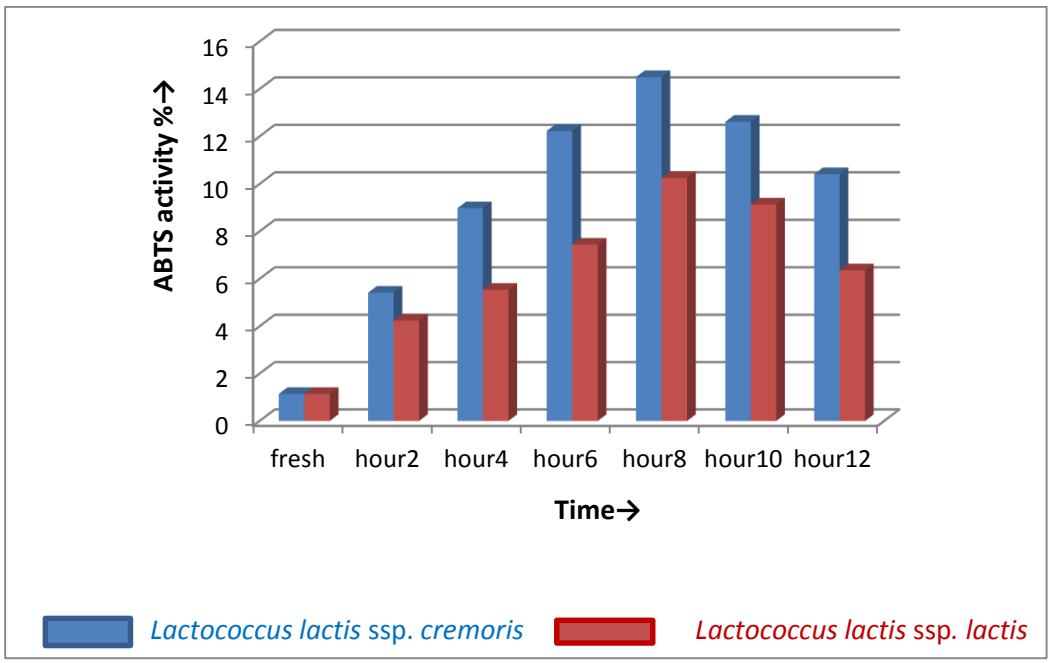


Table 4.4(c) ABTS (Mean ± SE) activity (% inhibition) of buffalo milk during fermentation

Treatmet	*Lactococcus lactis* ssp. *cremoris*	*Lactococcus lactis* ssp. *lactis*	Over all
Fresh	1.14±0.001	1.14±0.001	1.14^{a}±0.001
Hour2	5.42±0.004	4.23±0.060	4.82^{b}±0.180
Hour4	8.98±0.004	5.54±0.025	7.26^{c}±0.518
Hour6	12.23±0.047	7.44±0.025	9.84^{e}±0.723
Hour8	14.50±0.142	10.25±0.054	12.38^{g}±0.645
Hour10	12.62±0.144	9.13±0.033	10.88^{f}±0.530
Hour12	10.41±0.123	6.36±0.020	8.38^{d}±0.613
Overall	**9.33^{b}±0.673**	**6.30^{a}±0.443**	**7.81±0.434**

Note – Means bearing different superscripts differ significantly.

Table 4.4 (d) Analysis of variance for ABTS activity (% inhibition) of buffalo milk during fermentation

Source	D.F.	Mean Square	Level of sig.
Treated bacteria	1	192.580	**
Hour	6	176.721	**
Reminder	76	0.760	

** = Significant at 1% (P<0.01)

According to table 4.4(c), the overall ABTS activity (% inhibition) for buffalo milk was observed 9.33 ± 0.673 and 6.30 ± 0.443, for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* respectively.

According to Table 4.4(b) and 4.4(d), the free radical scavenging activity in all samples changed significantly ($P< 0.01$) from zero to 12 hour. On the data basis shown in figure 5 and 6, the ABTS anti oxidant activity (% inhibition) of *Lactococcus lactis* ssp. *cremoris* was significantly higher, when compared with *Lactococcus lactis* ssp. *lactis* in both camel and buffalo milk samples during fermentation process.

According to Donkor *et al.,* (2007) the variations of biological activities may be attributed to the production of different bioactive peptides, which may or may not have antioxidant properties and it is likely to be strain dependent.

These findings of proteolytic activity were in accordance with the findings of Salami *et al.,* (2011) and Jrad *et al.,* (2014) but the method of production of bioactive peptides from milk sample were different (digestive enzymes v/s LAB fermentation) .

4.5 DPPH ACTIVITY (% INHIBITION) OF CAMEL AND BUFFALO MILK DURING FERMENTATION

The mean data related to DPPH activity of camel milk has been presented in Table 4.5(a) and depicted in figure 7 whereas the mean data related to DPPH activity of buffalo milk has been presented in Table 4.5(c) and depicted in figure 8

Table 4.5(a) DPPH (Mean ± SE) activity (% inhibition) of camel milk during fermentation

Treatment	*Lactococcus lactis* ssp. *cremoris*	*Lactococcus lactis* ssp. *Lactis*	Over all
Fresh	0.67±0.001	0.68±0.005	0.67^{a}±0.003
Hour2	1.95±0.004	1.76±0.007	1.85^{b}±0.028
Hour4	3.78±0.004	2.32±0.005	3.05^{c}±0.220
Hour6	4.84±0.047	3.07±0.006	3.95^{d}±0.267
Hour8	6.86±0.012	5.87±0.004	6.36^{f}±0.151
Hour10	9.29±0.005	6.22±0.004	7.75^{g}±0.464
Hour12	5.25±0.062	4.56±0.004	4.90^{e}±0.109
Overall	**4.66±0.420**	**3.50±0.305**	**4.08±0.266**

Note – Means bearing different superscripts differ significantly.

Table 4.5(b) Analysis of variance for DPPH activity (% inhibition) of camel milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	28.618	**
Hour	6	74.048	**
Reminder	76	0.267	

** = Significant at 1% (P<0.01)

The DPPH activity of fermented camel milk increased significantly (P<0.01) with the progress in fermentation time, and a positive relationship

between fermentation time and DPPH activity could be established; however, the higher DPPH-scavenging activity was decreased after 10 hour of fermentation. Data show in Table 4.5(a) reveals that, at 10 hour of fermentation, the DPPH activity of camel milk samples were highest (9.29±0.005 and 6.22±0.004 respectively for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis*). Similarly DPPH-scavenging activity of buffalo milk samples increased significantly (P<0.01) with the progress in fermentation time as per data show in Table 4.5(c), but after 8 hour of fermentation, a significant fall in DPPH activity was seen in buffalo milk samples. At 8 hour of fermentation, DPPH activity (% inhibition) in buffalo milk samples was highest (5.58±0.007 and 5.15±0.011 respectively for *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis*). Results demonstrated a similar pattern of fermentative potantial with Ramesh *et al.,* (2012).

A more apparent increase in the DPPH antioxidant activity was observed in the milk inoculated with *Lactococcus lactis* ssp*. cremoris* strains and incubated for 12 hour at 30°C for both camel and buffalo milk.

According to Table 4.5 (b) and 4.5 (d) the free radical scavenging activity in all samples changed significantly (*P*< 0.01) from zero to 12 hour. Milk inoculated with *Lactococcus lactis* ssp. *cremoris* had the highest antioxidant capacity which increased from mean value of 0.67±0.001 at zero hour (fresh milk) in camel milk to 9.25±0.005 at 10 hour and for buffalo milk, it was 0.54±0.005 at zero hour which increased to 5.58±0.007 at 8 hour of fermentation after that it decreased significantly in both cases.

Similar trends were observed with *Lactococcus lactis* ssp. *lactis* during the same incubation time and similar free radical scavenging activity at zero hour in both camel and buffalo milk samples, which reached to 6.22±0.004 and 5.15±0.011 in camel and buffalo milk samples, at 10 hour and 8 hour of fermentation, respectively. Subsequently a significant fall in DPPH radical scavenging activity takes place in both types of milk samples.

On the data basis shown in figure 7 and 8, the DPPH antioxidant activity (% inhibition) of *Lactococcus lactis* ssp. *cremoris* was significantly

higher, when compared with *Lactococcus lactis* ssp*. lactis* in both camel and buffalo milk samples during fermentation process.

According to Ramesh *et al.,* (2012) ABTS and DPPH radical scavenging antioxidant activity of both camel and buffalo milk samples shown in table 4.4(a), 4.4(b), 4.4(c) and 4.5 (d). The fermentative potential of *Lactococcus lactis* ssp. *cremoris* was found more, when it was compared with *Lactococcus lactis* ssp. *lactis*, thus milk samples fermented with *Lactococcus lactis* ssp. *cremoris* of both camel and buffalo milk were used for production of fermented camel and buffalo milk products at the time period of fermentation, where it show highest antioxidant activity (both ABTS and DPPH basis) (*i.e.* 10 hours of fermentation for camel milk and 8 hours of fermentation for buffalo milk).

Table 4.5(c) DPPH (Mean ± SE) activity (% inhibition) of buffalo milk during fermentation

Treatment	***Lactococcus lactis* ssp. *cremoris***	***Lactococcus lactis* ssp*. lactis***	**Over all**
Fresh	0.54±0.005	0.54±0.004	0.54^{a}±0.003
Hour2	1.84±0.013	1.34±0.011	1.59^{b}±0.076

Hour4	3.08±0.012	2.36±0.009	2.72^{c}±0.110
Hour6	4.55±0.013	4.23±0.009	4.39^{f}±0.048
Hour8	5.58±0.007	5.15±0.011	5.36^{g}±0.065
Hour10	3.06±0.016	3.28±0.008	3.17^{e}±0.035
Hour12	2.45±0.013	3.05±0.013	2.75^{d}±0.091
Overall	**3.01^{b}±0.241**	**2.85^{a}±0.230**	**2.93±0.166**

Note – Means bearing different superscripts differ significantly.

Table 4.5(d) Analysis of variance for DPPH activity (% inhibition) of buffalo milk during fermentation

Source of variation	D.F.	Mean Square	Level of sig.
Treated bacteria	1	0.561	**
Hour	6	31.362	**
Reminder	76	0.052	

** = Significant at 1% (P<0.01)

Fig. 7 DPPH activity (% inhibition) of camel milk during fermentation

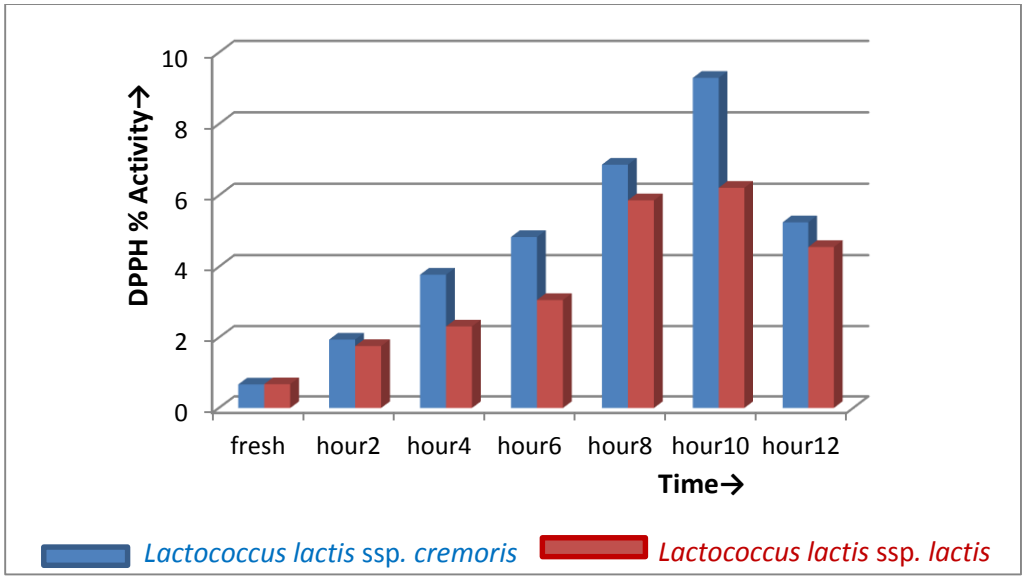
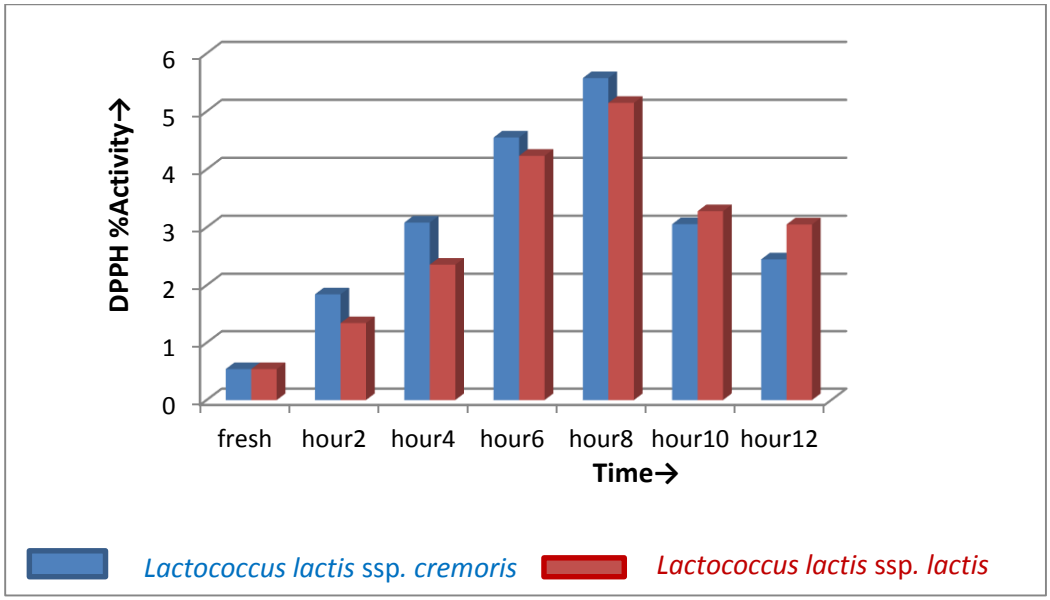


Fig. 8 DPPH activity (% inhibition) of buffalo milk during fermentation



4.6 PRODUCTION, EVALUATION AND ACCESSIBILITY OF FERMENTATIVE CAMEL AND BUFFALO MILK PRODUCT

Lactic acid bacteria *Lactococcus lactis* ssp. *cremoris* was utilized for production of fermented camel and buffalo milk product that is yogurt. Both milk samples were used to produce milk product at the time of fermentation, where highest fermentative potential (antioxidant property in term of ABTS and DPPH radical scavenging activity) was obtained (*i.e*. 10 hours of fermentation for camel milk and 8 hours of fermentation for buffalo milk). The samples were drawn for product formation by adding of 5% jujube syrup (W/W) and 5% sugar (W/W) and were used sensory evaluation and packed in LDPE bags and were stored under refrigeration condition for 7 days for estimation of physico-chemical and microbial changes.

4.7 SENSORY EVALUATION OF FERMENTATED CAMEL AND BUFFALO MILK PRODUCT

The mean values of sensory evaluation scores of fresh camel and buffalo milk yogurt (day 1 of storage) fortified with jujube syrup are summarized in table 4.7(a) and 4.7(b). It was shown that, yogurt made up of fermented buffalo milk had more acceptable flavor, texture, colour and overall acceptability compared to camel milk yogurt.

Table 4.7(a) Sensory evaluation (Mean ± SE) of fermented camel milk product

Attribute	Score
Appearance/colour	7.00 ± .0.258
Flavor	7.02 ± 0.197
Texture/ Viscosity	7.05 ± 0.189
Overall Acceptability	7.08 ± 0.227

Table 4.7(b) sensory evaluation (Mean ± SE) of fermented buffalo milk product

Attribute	Score
Appearance/ Colour	7.35 ± 0.167
Flavor	7.23 ± 0.185
Texture/ Viscosity	7.16 ± 0.248
Overall Acceptability	7.22 ± 0.170

Texture properties of buffalo milk yogurt and camel milk yogurt had the heights 7.16 ± 0.248 and 7.05 ± 0.189 score respectively. The body and texture of all fermented camel milk products was weak and showed a brittle structure. Similar result reported by Brennan *et al.,* (2008) and Peng *et al.,*(2009) who stated that the texture improved by increasing the total solids content of milk. so It could conclude that the excellent effect of adding jujube syrup on texture properties, may be related to the dietary fibre in the jujube extraction absorbing more moisture because of its higher water-holding capacity as it presented as the sucrose, fructose etc. in the jujube syrup increase the total solids of the mix and strengthens the gel network which described by Hashim *et al.,* (2009).

The overall acceptability scores of the sensory evaluation revealed that the buffalo milk fermented by culture *Lactococcus lactis* ssp. *cremoris* and fortified by jujube syrup of level 5% was the most acceptable. While fermented camel with fortification was the least.

Similar results were obtained by Attia *et al., (*2001) who observed that the fermentation of camel milk by starter culture did not reveal a good curd formation but indicated a fragile and heterogeneous structure. Also, the camel milk failed to reach a gel-like structure after sensory attributes.

It could be concluded that, camel and buffalo milk products fortified with jujube syrup can be considered as a good source of minerals, vitamins,

and dietary fibres resulted in a good properties and higher acceptable sensory values in fresh condition.

4.8 PROXIMATE ANALYSIS OF FERMENTATED CAMEL AND BUFFALO MILK PRODUCT

Proximate analysis of buffalo and camel milk product was done according to method described by A.O.A.C. (2000) (Official methods of analysis), including estimation of Moisture content, Dry Matter (DM), Crude Protein(CP), Ether Extract(EE), Crude Fibre (CF) and Total ash.

Table 4.8 Proximate analysis (Mean ± SE) of fermented camel and buffalo milk product

Constituent (%)	Buffalo milk product	Camel milk product
Moisture	78.08±0.001	88.47±0.002
Dry matter	21.92±0.004	11.53±0.004
Crude protein	9.92±0.001	4.63±0.001
Crude fibre	0.72±0.003	0.75±0.003
Ether extract	8.53±0.004	5.06±0.002
Total ash	0.72±0.002	0.75±0.004

Moisture content in camel milk product was higher (average 88.47±0.002%) than buffalo milk product (average 78.08±0.001%). Due to less dry matter content and higher water percentage in camel milk, the camel milk product also had higher moisture content comparing to buffalo milk product.

The dry matter percentage (mean value) of camel and buffalo milk products was 11.53±0.004 and 21.92±0.004 respectively. Crude protein and ether extract of buffalo milk product were higher than camel milk product due to higher content availability in raw milk respectively. Total ash of camel and buffalo milk products was almost similar.

The crude fibre percentage (mean value) in camel and buffalo milk was 0.75±0.003 and 0.72±0.003 respectively. These findings were found in line with result demonstrated by Sulieman *et al*., (2006), Eissa *et al.,* (2010) and Ahmad *et al.,* (2013).

4.9 STORAGE STUDY OF FERMENTATED CAMEL AND BUFFALO MILK PRODUCT

Yogurt of fermented camel and buffalo milk were analyzed for nutrients content (proximate analysis), pH, TA, antioxidant activity (ABTS and DPPH activity), lipid per oxidation (TBA value) and microbiological study (Plate count, Coliform count, Yeast-Mold count and MRS count for Lactococcus) as shown in Table 4.9(a) and 4.9(b) Nutrients composition of camel and buffalo milk was affected by processing into yogurt. The pH of buffalo milk products decreased significantly after storage of 7 days. Results were found by Guler, (2007) who studied that nutrients constituents in yogurt are influenced by the fermentation process, draining of yogurt, cooking and processing relatively in decreasing of Ph.

Table 4.9(a) and 4.9(b) illustrates changes in physico-chemical and microbial changes of camel and buffalo milk yogurt during storage at 4°C for different periods of time (1, 3, 5 and 7 days). The results obtained revealed that the stability of camel and buffalo yogurt decreased day by day with the storage period. Moreover, the good microbial load (MRS count) of camel and buffalo yogurt decreased significantly during the whole storage period. However initially coliform count and yeast-mold (Y-M) count remains nil which show hygienic production of the products. Changes in pH and acidity of the products during storage, suggests effect of yogurt microflora on its nutrient composition. Similar changes were observed by Guler, (2007), Vargas *et al*., (2008) and Eissa *et al.,* (2010), in some yogurt products. Camel and buffalo milk yogurt had mean pH values of 5.07±0.025 and 5.59±0.010 respectively, coupled with acidity of 0.68±0.005 and 0.46±0.005, respectively. After 7 days of storage an increase in acidity by mean value of 1.17±0.015 in camel yogurt and by 0.86±0.015 in buffalo yogurt was observed. However, the trend of increase in acidity continued from day 1 to day 7 of storage. According to Valli and Traill, (2005) the activity and growth rate of the starter cultures are strain

dependent. Hence, the acidification rate of lactic acid bacteria varied with the type of milk, being some yogurt starters more active than others.

Table 4.9(a) and 4.9(b) Showed significant changes in total viable count (plate count) of camel and buffalo yogurt during storage. The results revealed that camel and buffalo milk yogurt contained a mean value of 9.42±0.95 and 9.46±0.300 log cfu/ml total viable cells of bacteria initially. The initial plate count of camel and buffalo milk yogurt decreased during storage for 7 days viz. 9.05±0.09 and 8.78±0.390 log cfu/ml. Increase in acidity of the growth media in camel and buffalo milk yogurt may retard bacterial growth. The results obtained in the study are in agreement with the findings of Masud *et al*., (1991). The MRS count (*Lactococcus* count) in camel and buffalo milk yogurt was 9.38±0.54 and 9.46±0.54 log cfu/ml initially which were significantly decreased during storage (8.96±0.920 and 9.07±0.920 log cfu/ml at day 7 of storage respectively).

The load of lactic acid bacteria found significant in both yogurt types, which stated as both yogurts have probiotic nature. Tamime and Robinson, (1999) also reported similar findings as in our study.

On the other hand, yeasts and molds were significantly increased in camel milk yogurt with storage period of day 7 but not found at day 1 to day 5 of storage period. An increase in acidity and/or reduction in potential oxygen during fermentation process may provide suitable state for growth of yeasts and molds. Contamination by yeasts and molds during processing of yogurt was reported (Karleskind *et al.,* 1993).

Table 4.9(a) Storage study (Mean ± SE) of fermented camel milk product

Parameter	Day 1	Day 3	Day 5	Day 7
PH^{NS}	5.07±0.025	4.97±0.015	4.69±0.010	4.44±0.015
TA^{NS}	0.68±0.005	0.77±0.015	0.92±0.005	1.17±0.015
TBA**	0.01^{a}±0.005	0.14^{b}±0.003	0.24^{c}±0.003	0.43^{d}±0.030
ABTS**	19.54^{d}±0.037	18.26^{c}±0.111	16.80^{b}±0.120	11.31^{a}±0.056
DPPH**	10.45^{d}±0.346	8.41^{c}±0.044	6.31^{b}±0.056	4.31^{a}±0.056

Coliform count** (log CFU/ml)	Nil	Nil	1.25^{a}±0.300	1.75^{b}±0.300
Plate count* (log CFU/ml)	9.42^{b}±0.950	9.24^{a}±0.970	9.13^{a}±0.920	9.05^{a}±0.900
Yeast mould count* (logCFU/ml)	Nil	Nil	Nil	0.97^{a}±0.170
MRS count* (log CFU/ml)	9.38^{c}±0.540	9.27^{b}±0.690	9.14^{b}±0.770	8.96^{a}±0.920

Note – Means bearing different superscripts differ significantly.

NS= Non-significant * = Significant at 5% (P<0.05) ** = Significant at 1% (P<0.01)

Table 4.9(b) Storage study (Mean ± SE) of fermented buffalo milk product

Parameter	Day 1	Day3	Day 5	Day 7
PH**	5.59^{d}±0.010	5.27^{c}±0.015	4.97^{b}±0.015	4.54^{a}±0.010
TA**	0.46^{a}±0.005	0.53^{b}±0.010	0.69^{c}±0.010	0.86^{d}±0.015
TBA**	0.03^{a}±0.001	0.16^{b}±0.003	0.26^{c}±0.003	0.49^{d}±0.026
ABTS**	15.28^{c}±0.018	14.98^{c}±0.012	12.26^{b}±0.099	9.60^{a}±0.054
DPPH**	6.71^{d}±0.018	5.50^{c}±0.045	4.096^{b}±0.028	3.34^{a}±0.016
Coliform count* log (CFU/ml)	Nil	Nil	1.06^{a}±0.390	1.60^{b}±0.840

Plate count** log (CFU/ml)	9.46^{d}±0.300	9.23^{c}±0.650	9.08^{b}±0.390	8.78^{a}±0.390
Yeast mould count^{NS} log (CFU/ml)	Nil	Nil	Nil	0.39±0.170
MRS count* log (CFU/ml)	9.46^{c}±0.540	9.32^{b}±0.900	9.17^{a}±0.600	9.07^{a}±0.920

Note – Means bearing different superscripts differ significantly. **NS= Non-significant * = Significant at 5% (P<0.05) ** = Significant at 1% (P<0.01)**

Fig 9 Sensory evaluation of camel and buffalo milk products

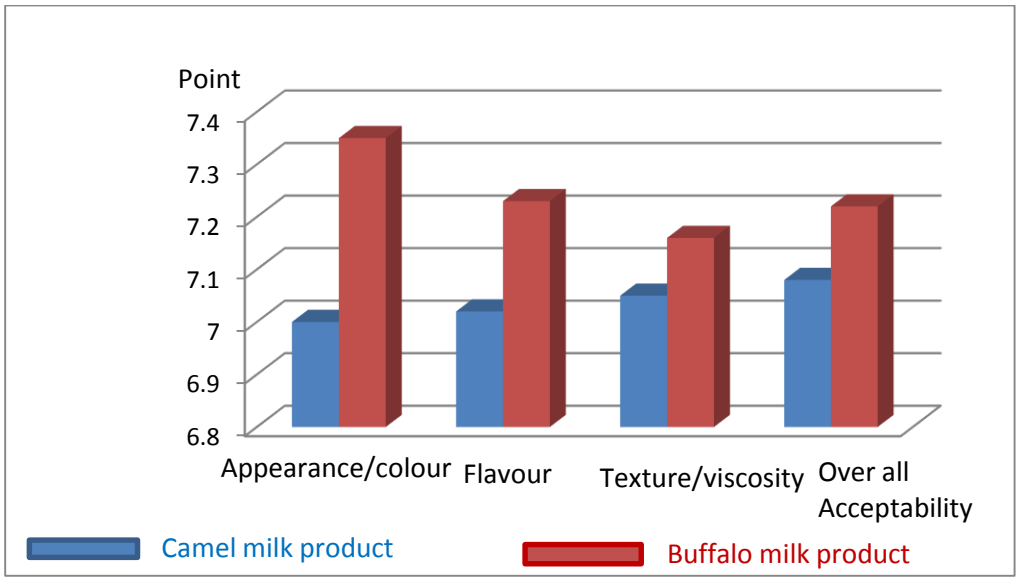


Fig 10 TBA value of camel and buffalo milk products during storage period

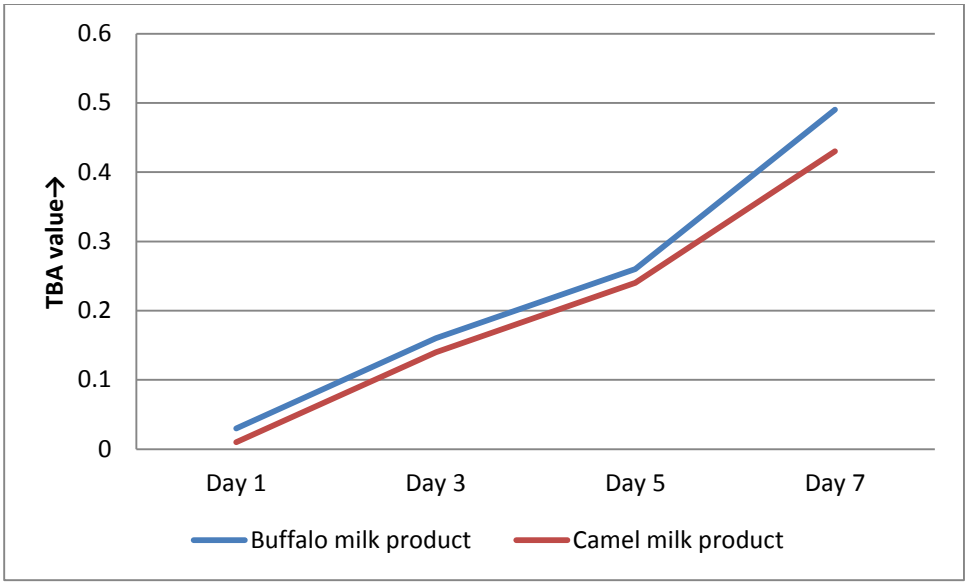


Fig 11 Change in ABTS activity (% inhibition) in camel and buffalo milk products during storage

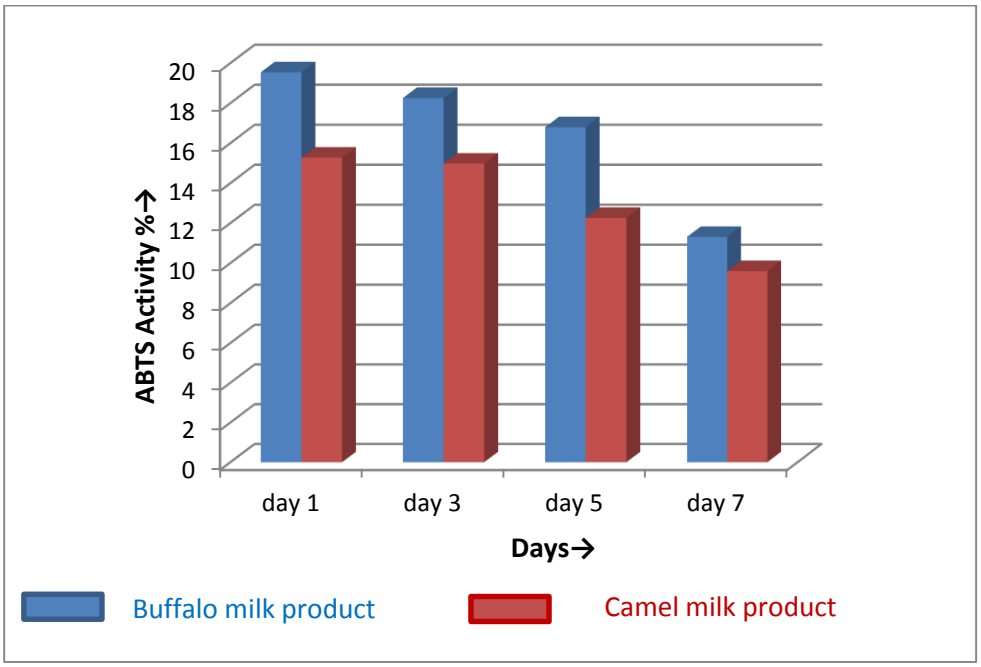
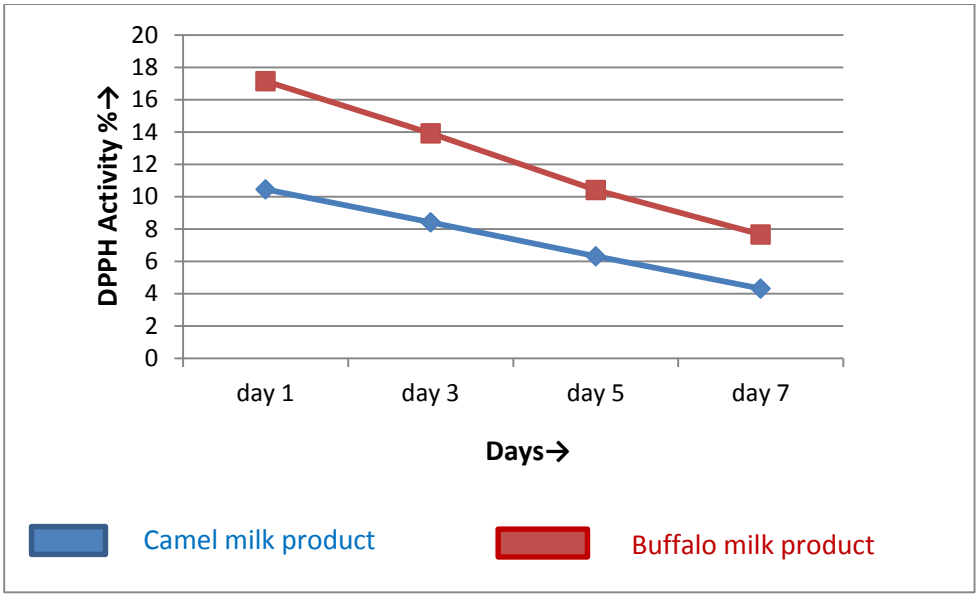


Fig 12 Change in DPPH activity (% inhibition) in camel and buffalo milk products during storage



Coliforms were absent in fresh camel and buffalo yogurt and also at day 3 of storage period, but found at day 5 and day 7 of storage and increased significantly from 5 days of storage to 7 day of storage, which may be due to contamination or any other reason related to hygiene. However, many workers reported on the survival of coliforms, if present, in yogurt to a maximum of 3 days (Karleskind *et al.,* (1993) and Farida *et al.,* 2000). According to El-Agamy *et al.,* (1996) and Mainfreni *et al.,* (2002) *E. coli* was observed to survive the low pH domestic yogurt developed during cold storage and could tolerate lower acidity up to 6 days.

Usually, lipid per oxidation is not a major problem in yogurt due to the low pH, the low storage temperature and low permeability to oxygen of packaging materials. Furthermore when Lactococcus are used as starter culture, it helps to reduce the rate of lipid per oxidation by its natural anti oxidant potential.

Lipid per oxidation of camel and buffalo milk products was determined by TBA (Thio Barbituric acid) value, which gave the idea about rancidity of both milk products. In both products the initial TBA value at day 1 was very low and it significantly increased at day 3 and day 5 of storage which show slow elevation till day 7 of storage, but it was lower than 1 which can gave an idea about no foul smell till 7 day of storage of both camel and buffalo milk product. The results were define similar conclusion as provided by Saide and

Gilliland, (2005) that Lactococcus as a starter culture was helpful to prevent lipid per oxidation due to its anti oxidant potential, however elevation of TBA value at day 7 of storage was due to change in its physico-chemical as well as microbial properties.

5. SUMMARY AND CONCLUSION

The components of milk provide critical nutritive elements, immunological protection, and biologically active substances to both neonates and adults. It is not surprising, therefore, that the nutritional value of milk is high. Milk is not only consumed as a raw material but it is transformed in a variety of products to preserve its nutrients.

As signaling molecules, the bioactive peptides play important roles in physiological functions and pathogenesis. Milk derived bioactive peptides play vital roles in human health and nutrition. Milk-derived bioactive peptides have been identified as potential ingredients of health-promoting functional foods. These bioactive peptides are targeted at diet-related chronic diseases especially the non-communicable diseases viz., obesity, cardiovascular diseases and diabetes. The main factors influencing the bioavailability of peptides are the resistance to digestion enzymes of and the absorption by the intestinal epithelium.

The present investigation designed to elucidate the function and the bioavailability of bioactive peptides present in camel and buffalo milk-derived products, with the purpose to identify the crucial aspects that have to be taken into consideration for an efficient production of bioactive peptides from milk proteins. In this context, special attention has been given to the antioxidant activity and to specific milk-derived peptides associated to this bioactivity.

Considering the vitality of above facts in mind, the present study entitled **"Studies on The Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis*"** was undertaken to estimate the physico-chemical properties, to produce bioactive peptides, to determine antioxidative property of fermented milk and also to produce and evaluate fermented camel and buffalo milk product that is yogurt.

Present study takes place on:-

The overall compositions of buffalo and camel milk showed that the buffalo milk had higher concentrations of protein, fat and solid not fat (SNF)

than camel milk. In general the present study showed a wide variation in the gross composition of camel milk.

The statistical analysis of data revealed that there was a highly significant (P<0.01) decrease in the pH value of camel milk samples with advancement of fermentation hours as well as with treated bacteria.

The statistical analysis of data revealed that there was a highly significant (P<0.01) decrease in the pH value of buffalo milk samples with advancement of fermentation hours as well as between the treated bacteria that is *Lactococcus lactis* ssp*. cremoris* and *Lactococcus lactis* ssp*. lactis*.

The statistical analysis of data revealed that there was a highly significant (P<0.01) increases in the TA value of camel milk samples with advancement of fermentation hours.

The statistical analysis of data revealed that there was a highly significant (P<0.01) increases in the TA value of buffalo milk samples with advancement of fermentation hours.

ABTS activity in all samples changed significantly (P< 0.01) from zero to 12 hour. On the data basis the ABTS antioxidant activity (% inhibition) of *Lactococcus lactis* ssp. *cremoris* was significantly higher, when compared with *Lactococcus lactis* ssp. *lactis* in both camel and buffalo milk samples during fermentation process.

DPPH activity in all samples changed significantly (*P*< 0.01) from zero to 12 hour. On the data basis the DPPH antioxidant activity (% inhibition) of *Lactococcus lactis* ssp. *cremoris* was significantly higher, when compared with *Lactococcus lactis* ssp*. lactis* in both camel and buffalo milk samples during fermentation process.

Lactic acid bacteria *Lactococcus lactis* ssp. *cremoris* was utilized for production of fermented camel and buffalo milk product that was yogurt and highest fermentative potential (antioxidant property in term of ABTS and DPPH radical scavenging activity) was obtained (*i.e*. 10 hours of fermentation for camel milk and 8 hours of fermentation for buffalo milk). ABTS and DPPH activity of both camel and buffalo milk products decreased significantly (*P*< 0.01) from day 1 to day 7 of storage.

The mean values of sensory evaluation scores of fresh camel and buffalo milk yogurt fortified with jujube syrup was shown that, yogurt made up

of fermented buffalo milk had more acceptable flavor, texture, colour and overall acceptability compared to camel milk yogurt.

The overall acceptability scores of the sensory evaluation revealed that the buffalo milk fermented by culture *Lactococcus lactis* ssp. *cremoris* and fortified by jujube syrup of level 5% was the most acceptable while fermented camel with fortification was the least.

The mean values of moisture content in camel milk product was higher (average 88.47±0.002%) than buffalo milk product (average 78.08±0.001%). The mean values of dry matter percentage of camel and buffalo milk products were 11.53±0.004 and 21.92±0.004 respectively. The mean values of crude fibre percentage in camel and buffalo milk were 0.75 and 0.72 respectively. Crude protein and ether extract of buffalo milk product were higher than camel milk product.

The pH of buffalo milk products decreased significantly after storage of 7 days. Camel and buffalo milk yogurt had mean pH values of 5.07±0.025 and 5.59±0.010 respectively, coupled with acidity of 0.68±0.005 and 0.46±0.005, respectively. After 7 days of storage an increase in acidity by mean value of 1.17±0.015 in camel yogurt and by 0.86±0.015 in buffalo yogurt was observed.

Total viable count (plate count) of camel and buffalo milk yogurt Showed significant changes during storage. The results revealed that camel and buffalo milk yogurt contained a mean value of 9.42±0.95 and 9.46±0.300 log cfu/ml total viable cells of bacteria initially. The initial plate count of camel and buffalo milk yogurt decreased during storage for 7 days viz. 9.05±0.09 and 8.78±0.390 log cfu/ml.

Coliforms were absent in fresh camel and buffalo yogurt and also at day 3 of storage period, but found at day 5 and day 7 of storage and increased significantly from 5 days of storage to 7 day of storage.

Yeasts and molds were significantly increased in camel milk yogurt with storage period of day 7 but not found at day 1 to day 5 of storage period.

In both products the initial TBA value at day 1 was very low and it significantly increased at day 3 and day 5 of storage which show slow elevation till day 7 of storage, but it was lower than 1 which can gave an idea about no foul smell till 7 day of storage of both camel and buffalo milk product.

The results obtained revealed that the stability of camel and buffalo yogurt decreased day by day with the storage period.

In conclusion, the main contribution of this study was to provide new knowledge about milk-derived products with bioactivities. In particular, original contributions are in relation to the mechanisms by which milk-derived bioactive peptides are generated and express their bioactivities. The mechanisms of generation of the bioactivities from the raw milk, notably the effect of the bacterial strain on the digestive phenomena intervening in the production of fermented milks rich in antioxidant activity, have been studied.

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Studies on the Fermentative Potential of Camel and Buffalo Milk by Using *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis*

Department of Livestock Products Technology
College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner

Submitted by : Sanjay Singh
Advisor : Prof. Basant Bais

ABSTRACT

The present study was carried out with objectives to estimate the physicochemical properties of camel and buffalo milk and to determine the antioxidant property of fermented camel and buffalo milk. The overall compositions of buffalo and camel milk showed that the buffalo milk had higher concentrations of protein, fat and solid not fat (SNF) than camel milk. The statistical analysis of data revealed that there was a highly significant ($P<0.01$) decrease in the pH value of camel and buffalo milk samples with advancement of fermentation hours as well as between the treated bacteria that is *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis*. There was a highly significant ($P<0.01$) increases in the TA value of camel and buffalo milk samples with advancement of fermentation hours. ABTS and DPPH activity in all samples changed significantly ($P< 0.01$) from zero to 12 hour. The ABTS antioxidant activity (% inhibition) of *Lactococcus lactis ssp. cremoris* was significantly higher, when compared with *Lactococcus lactis ssp. lactis* in both camel and buffalo milk samples during fermentation process. Lactic acid bacteria *Lactococcus lactis ssp. cremoris* was utilized for production of fermented camel and buffalo milk product that was yogurt, ABTS and DPPH activity of both camel and buffalo milk products decreased significantly ($P< 0.01$) from day 1 to day 7 of storage. The overall acceptability scores of the sensory evaluation revealed that the buffalo milk fermented using culture *Lactococcus lactis ssp. cremoris* and fortified by jujube syrup of level 5% was the most acceptable while fermented camel with fortification was the least. The mean values of moisture content and crude fibre percentage in camel milk product was higher than buffalo milk product. The pH of camel and buffalo milk products decreased and acidity increased significantly after storage of 7 days. The initial plate count of camel and buffalo milk yogurt decreased significantly during storage for 7 days and coliforms were absent in fresh camel and buffalo yogurt and also at day 3 of storage period, but found at day 5 and day 7 of storage and increased significantly from 5 days of storage to 7 day of storage. Yeasts and molds were significantly increased in camel and buffalo milk yogurt with storage period of day 7 but not found at day 1 to day 5 of storage period. In both products the initial TBA value at day 1 was very low and it significantly increased at day 3 and day 5 of storage which shows slow elevation till day 7 of storage. Hence it may be concluded that fermented camel and buffalo milk showed significant antioxidant property and fermented buffalo milk fortified with jujube syrup more acceptable with regards to sensory attributes. The results revealed that the stability of camel and buffalo yogurt decreased day by day with the storage period.

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Appendix-I Score given by panelist to camel milk product according to 8 point hedonic scale

SN.	Appearance/ Colour	Flavour	Texture/ Viscosity	Overall Acceptability
1.	8	8	7	7
2.	7	7	8	8
3.	6.5	6.7	7	7
4.	8	6.5	6.5	8
5.	6.5	6.2	8	8
6.	6.5	7	7.5	6.5
7.	5.5	8	7	6
8.	7	7.5	6.5	6.3
9.	7	6.5	6.5	7
10.	8	6.8	6.5	7

Appendix-II Score given by panelist to buffalo milk product according to 8 point hedonic scale

SN.	Appearance/ Colour	Flavour	Texture/ Viscosity	Overall Acceptability
1.	8	7	8	8
2.	7.5	7.5	6	7.2
3.	7	6	7.5	6.8
4.	8	7	7.7	7.7
5.	7	8	8	8
6.	7.5	7.1	7.9	7.5
7.	8	7.5	6.5	7
8.	7	8	7	6.5
9.	6.5	7.3	6	7
10.	7	6.9	7	6.5

Plate 1: Activation of culture into reconstituted skim milk (RSM) media

Plate 2: Subculturing of cultures into litmus milk media

Plate 3: Inoculation of cultures into milk samples

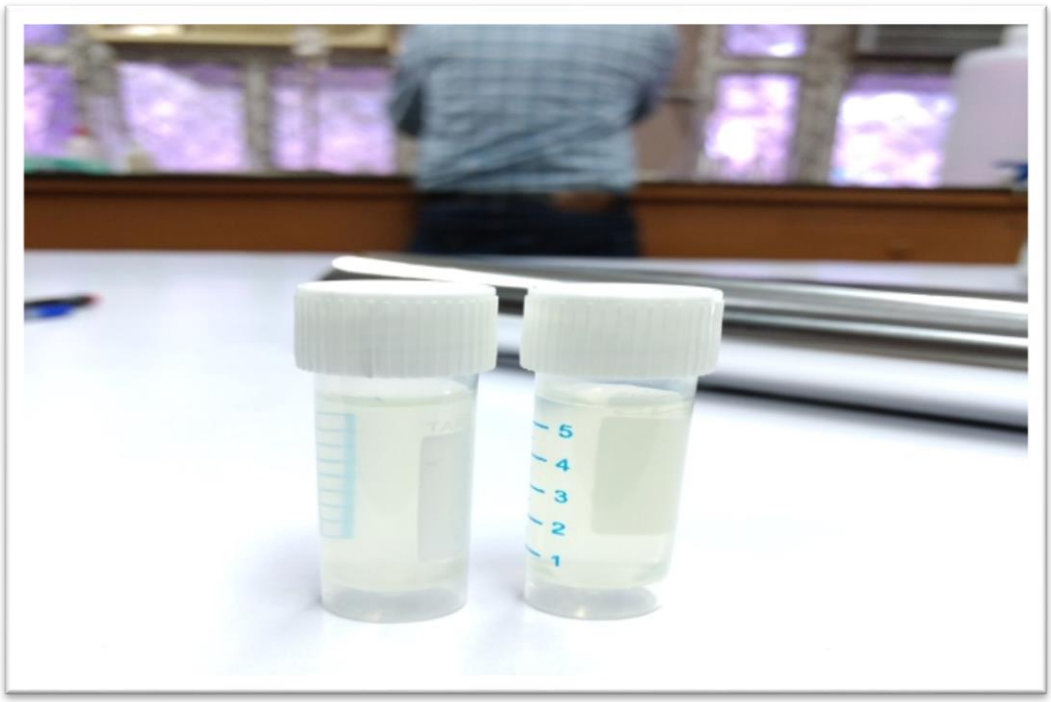

Plate 4: Supernatents/Hydrolysates of milk samples obtained by centrifugation of milk samples during fermentation

Plate 5: Proximate analysis of fermented milk samples

Plate 6: Fermented milk product samples displayed for sensory evaluation

Plate 7: Coliform count of fermented milk product on VRB media

Plate 8: Viable plate count of fermented milk product on plate count media

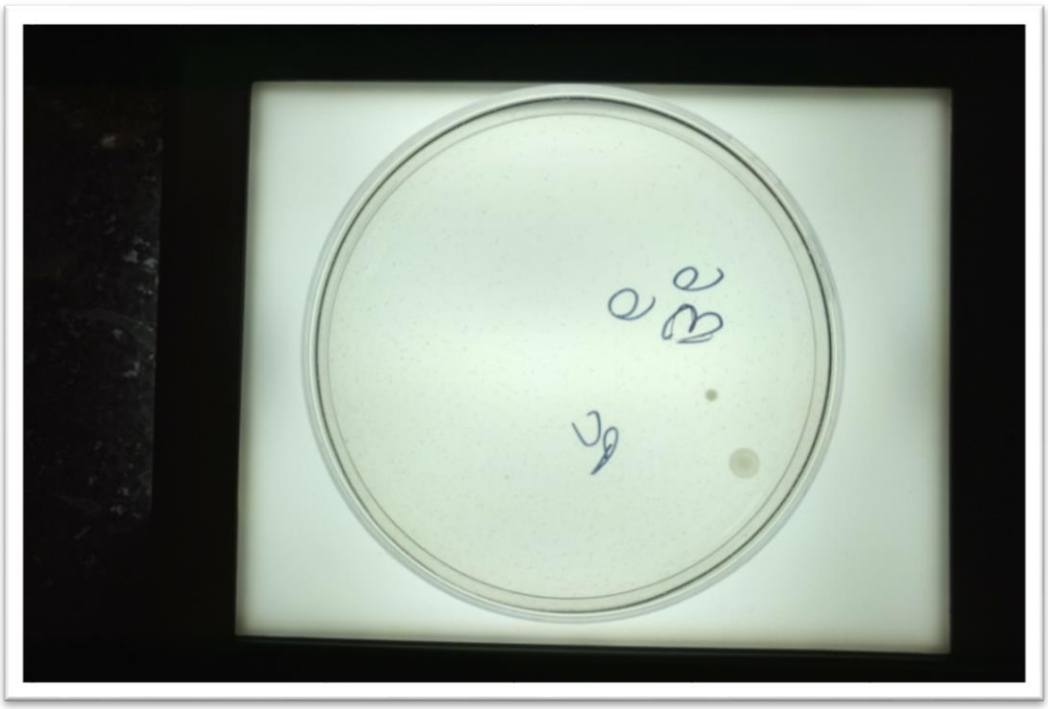

Plate 9: Yeast-Mold count of fermented milk product on PDA media

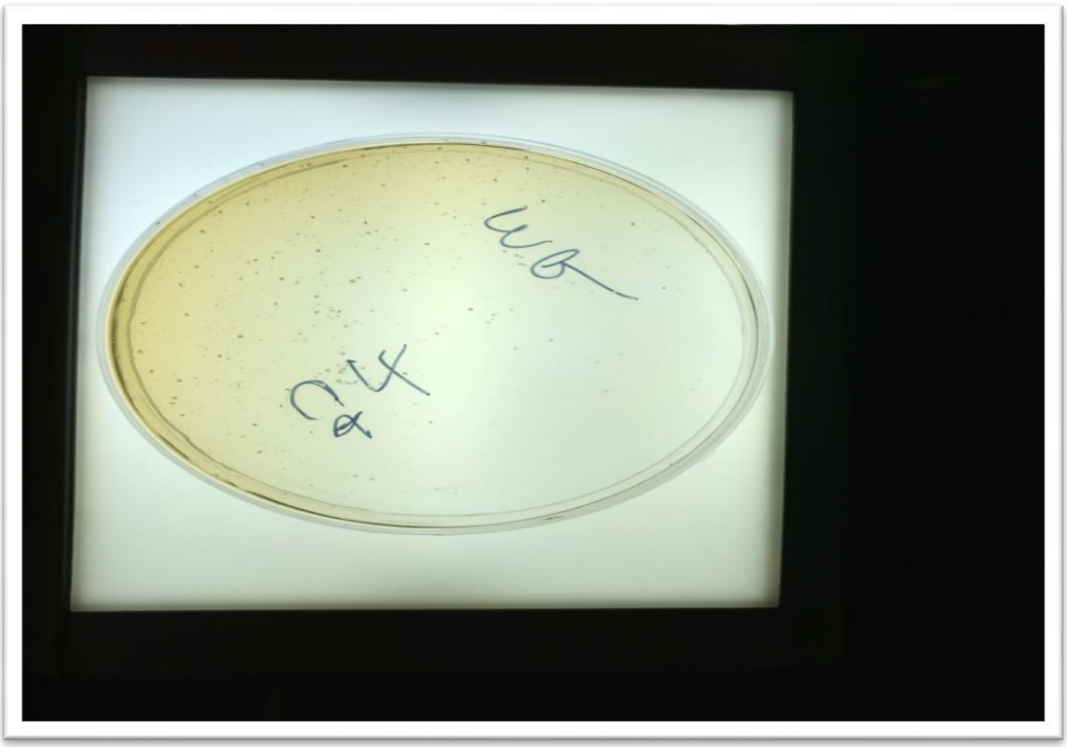

Plate 10:Lactococcus count of fermented milk product on MRS media

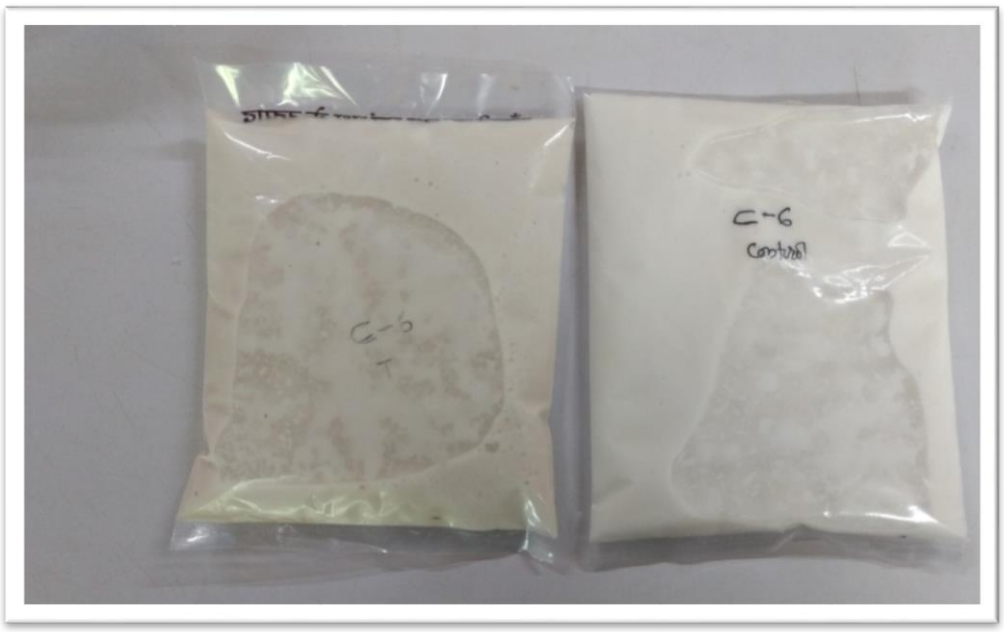

Plate 11: Fermented camel milk product samples

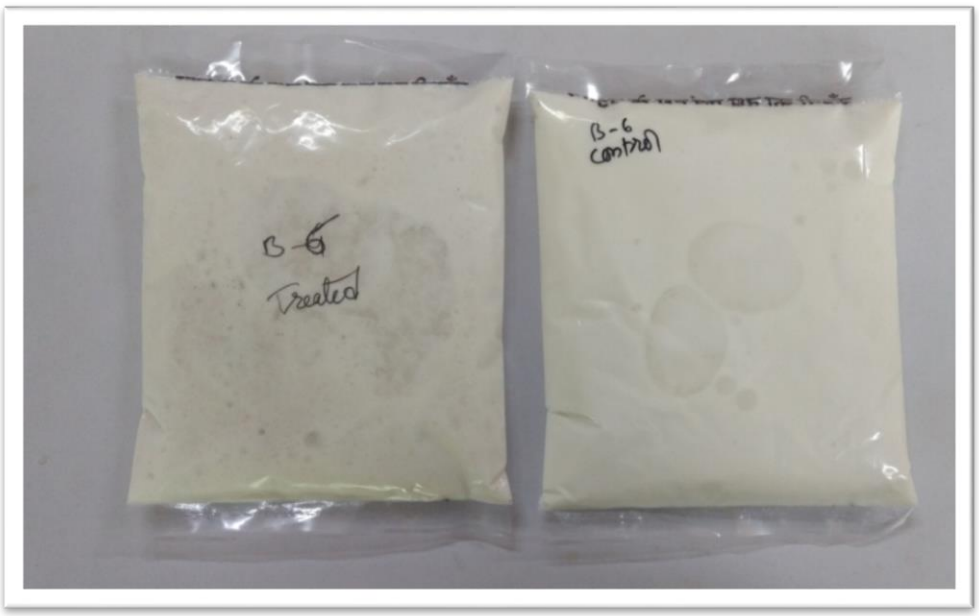

Plate 12: Fermented buffalo milk product samples