The Journal of Animal and Plant Sciences, 23(1 Suppl.): 2013, Page: 62-74
ISSN: 1018-7081

COMPOSITION AND PHYSICO-CHEMICAL CHARACTERISTICS OF BUFFALO MILK WITH PARTICULAR EMPHASIS ON LIPIDS, PROTEINS, MINERALS, ENZYMES AND VITAMINS

Ahmad, S.*, F. M. Anjum, N. Huma, A. Sameen and T. Zahoor

National Institute of Food Science and Technology, University of Agriculture Faisalabad
*Corresponding author Email: sarfraz.ahmad@uaf.edu.pk
National Institute of Food Science and Technology, University of Agriculture, Faisalabad-Pakistan

ABSTRACT

The businesses community of different sectors affected by current energy crisis in Pakistan tends towards dairy business. They are highly interested by the information on milk composition particularly of buffalo milk due to its major contribution in national's milk production i.e. 63% according to the FAO's published data of 2010. It is necessary to know for maximum value addition in dairy food chain as the nutrients not only determine the dietary value of milk for human consumption but also help to define market strategies for various classes of consumers like growing children, nursing mothers, young persons involved in hard jobs or elderly people. Buffaloes are most important sources of milk for human consumption in several parts of the world including Pakistan. It is characterized by higher solids contents for being richer source of lipids, protein, lactose and minerals. Buffalo milk has long been valued by its important chemical composition determining nutritive properties and suitability in the manufacture of traditional as well as industrial dairy products. Recently buffalo milk's constituents, their nutritional importance and bioactive properties have received much attention. In this paper, the composition and physico-chemical properties of major constituents of buffalo milk with particular emphasis on lipids, protein, minerals, enzymes and vitamins have been presented. The concentration and partition of major elements between different phases of buffalo milk are also given. The enzymic profiles as well as the nutrient molecules have been presented for the said milk which determines its suitability for various processes and end products. The available technologies need some modifications even from milking machines to industrial processing. It is a golden opportunity for the investors to come into buffalo milk business to get advantage from the government initiatives in the current period. In this way, we will be able to improve the genetic potential of buffaloes in getting more milk of higher quality and experimenting diversity of products particularly cheeses and other fermented dairy products for the local market and export by better exploiting the uniqueness of buffalo milk.

Key words: Buffalo milk, chemical composition, physico-chemical properties.

INTRODUCTION

Pakistan is among top ten milk producer of the world with 35.5 billion liters annual milk production (FAOSTAT, 2010). It is the 2nd largest buffalo and goat milk producer in the world with 22.3 and 0.7 billion liters production after India and Bangladesh, respectively. Buffalo milk's share is highest (62.8%) followed by cow (34.9%), goat (2.0%), camel (0.2%) and sheep (0.1%). Pakistan is blessed with a fairly large population of buffaloes, cows, sheeps, camels and goats (30.8, 34.3, 27.8, 1.0 and 59.9 million, respectively) (FAOSTAT, 2010) with vast tracts of pastoral lands where a large number of rural population engaged in cattle-raising. In Pakistan, dairy is majorly considered as an unorganized sector where the share of formal sector for industrial processing is only 3-4% of the total national milk production processed by very few multi-national and national industries like Nestle, Haleeb, Engro, Millac, Halla, Shakargaj, Noon, Premier, Gourmet, Prime etc.

Dairy sector remained a much neglected and under-developed sector of agriculture for several decades but now is considered as one of the priority area due to increased internal mobility of investors with great interest. Creation of a separate ministry of livestock and dairy development is a clear recognition of the importance of this sector. The businesses community of different sectors of Pakistan has been badly affected by current energy crisis (electricity and gas) particularly textiles industries and started to shift towards dairy business. They imported quite a large number of high producing dairy animals and installed well-managed large dairy farms with modern facilities. This class of businessmen is highly interested by the information on milk composition and their nutritional importance to exploit maximum marketing worth.

The major aim of this manuscript is to provide valuable information on chemical composition and physico-chemical characteristics of buffalo milk. Considerable amount of segregated published data is available in recent years with respect to the chemical composition, properties and processing of buffalo milk. A need is left for a critical review and updating of knowledge on the detailed composition and properties of buffalo milk as a base for better processing and production of innovative dairy products. The major constituents of buffalo milk for being higher in concentration than that of human, cow, goat and camel milk have been discussed in detail with their nutritional values. In addition to its advantage as a rich source of nutrients, a recent study by Sheehan *et al.* (2009) indicated that subjects with cow milk allergy are able to tolerate buffalo milk. Buffalo milk may contain almost all the beneficial compounds found in other milks, e.g., proteins, peptides, fatty acids, vitamins, and other bioactive compounds. Buffalo milk has higher levels of total protein, medium chain fatty acids, CLA, and contents of retinol and tocopherols than those of cow milk. Some components may only be present in buffalo milk such as specific classes of gangliosides (Berger *et al.,* 2005). Interest in buffaloes is growing globally as the first import of buffaloes to France took place in 1998 (Coop de Bufflonnes, 2010) and to Sweden in the beginning of 2009 (Larsson *et al.,* 2009) after observing successes in other countries of Europe like Italy and Bulgaria in order to obtain high quality milk and meat with less cholesterol level in both products.

Because of the increasing of people awareness for food safety, knowing the chemical composition of buffalo milk has a great significance for further development of hygienic processing into quality products for the consumer (Mihaiu *et al.,* 2010). The information in this manuscript will definitely provide a roadmap to create awareness about this unique milk for which we can get international fame by achieving large increases in productivity, value added, marketed milk, develop new products and improve quality of the existing products. Because of the differences in compositional and physiochemical properties between buffalo and other milks, processing technology and equipment designed for other milks need sensible modification for buffalo milk processing. Buffalo milk can be utilized for manufacture of a wide variety of dairy products with limited modifications in processing technology.

COMPOSITION OF BUFFALO MILK

According to the definition of USDA (2011), water buffalo milk is the normal lacteal secretion practically free of colostrum, obtained by the complete milking of one or more healthy water buffalo. Water buffalo milk shall be produced according to the sanitary standards of this ordinance. Quite a number of studies focused on cow milk, even if the milk produced by other animals such as buffaloes are essential in human diet in different parts of the world. Buffalo milk is a totally natural product that can be consumed like any other milk. It is one of the richest products from a compositional point of view and characterized by higher fat, total solids, proteins, caseins, lactose and ash contents than cow, goat, camel and human milk. Monitoring changes in composition of buffalo milk over years is important as an overall index for the combined effects of environmental and genetic factors. Zicarelli (2004a) recorded an increase in fat content of Italian buffalo milk from 7.3 to 8.3% and its protein content from 4.4 to 4.8% respectively from 1967 to 2000. Differences in the composition of buffalo milk in different localities reflect differences in breeds, management, feeding and environmental conditions. General composition of buffalo is given in the table 1.

The high milk solids of buffalo milk not only make it ideal for processing into superb dairy products but also contribute to significant energy savings in conducting that process. Buffalo milk yogurts and cheeses are natural thick set without recourse to adding addition milk proteins or gelling agents as with lesser milks. Dairies love to work with buffalo milk, which we all know makes the best mozzarella. The smooth texture and richness converts into a truly wonderful range of multiple award winning products.

Proteins: The protein content of buffalo milk is higher than in cow (Ragab *et al.,* 1958; Ganguli, 1973, Ahmad *et al.,* 2008).Of the total proteins of buffalo milk, ~80% are caseins and ~20% are whey proteins with traces of minor proteins (Laxminarayana and Dastur, 1968; Sirry *et al.,* 1984; Sahai, 1996). Whey proteins and minor proteins are even higher in colostrum than mature buffalo milk.

Caseins: Almost all casein of buffalo milk is present in the micellarform (Ganguli, 1973; Sabarwal and Ganguli, 1970a). Buffalo milk contains a negligible proportion of soluble casein (0.03 $g.100mL^{-1}$) about 1 % of the total casein unlike cow milk (0.11 $g.100mL^{-1}$) about 5 % of the total casein (Sabarwal and Ganguli, 1971). The caseins in buffalo milk are sub-classified into α_{s1}-, αs_2-, β- and κ-casein and the concentrations are given in the table 2.

Physico-chemical characteristics of caseins micelles: Casein micelles of buffalo have average diameter of 190 nm (Ahmad, 2010) which found about 10-20 nm bigger in size than that of cow agree with the findings of other authors (Ganguli, 1973; Sood *et al*., 1976;Sirry *et al.*1984; Sarswat, 1985).Overall, micelles of buffalo milk found globally spherical and individual (Fig. 1).

The higher casein concentration in buffalo milk and almost 100% in colloidal form seems to have primary impact to increase the numbers of casein micelles and secondary impact on size.More casein concentration is possibly translated into more number of casein micelles in buffalo $milk.mL^{-1}$ as compared to number of casein $micelles.mL^{-1}$ of cow milk.

Charges could be due to glycosylated parts present on κ-CN, the protein being present at the periphery of casein micelles which is similar as for cow milk (Ahmad, 2010). The lower hydration of buffalo milks' casein micelles as compared to cow milk could be due to their larger size and high concentration of colloidal calcium so it leaves less space for water molecules to inculcate. Some other authors (Kuchroo and Malik, 1976; Sabarwal and Ganguli, 1970a) also found the similar results. Further investigations are needed to observe the structural differences and compactness of casein micelles as compared to the casein micelles of other milks to better exploit the functional and nutritional characteristics of individual caseins.

Opacity of the buffalo casein micelle is greater than that of the cow casein micelle (Sabarwal and Ganguli, 1970b). Buffalo casein contains lower proportions of sialic acid (2.0mg.g^{-1} casein), hexose (2.5mg) and hexosamine (1.8mg), but higher proportions of calcium (Sabarwal *et al.,* 1972), while heating of milk reduces sialic acid, hexose and hexosamine contents (Sabarwal and Ganguli, 1973). Electrophoretic separation of casein components showed 44, 53 and 3% for buffalo αs-, β-, and κ-casein vs. 55, 39 and 6% for the cow milk casein fractions(Ganguli and Bhalerao, 1964). All three fractions of buffalo milk casein have slower mobility than cow milk casein. The proportions of αs_1-, αs_2-, β- and κ-caseins were 40, 6–9, 35 and 12%, respectively (Yamauchi *et al*., 1983). The N and P contents of buffalo αs_1-casein are about 15 and 0.1%, respectively. Amino acid composition of buffalo and cow αs_1-casein is similar. Buffalo κ-casein is heterogeneous with eight sub fractions, which are similar in P but different in carbohydrate contents. Amino acid composition of buffalo κ-casein is comparable to that of cow milk, but poorer in sialic acid. Overall 95% homology exists in the amino acids sequences of all casein classes between buffalo and cow milk.

Whey Protein: The proportions of whey proteins in buffalo milk are similar to those in cow milk, and the amino acid composition of buffalo β-lactoglobulin (β-Lg) is identical to that of cow milk(Mawal *et al.,* 1965) except that it does not exhibit genetic polymorphisms (Senand Sinha, 1961). The molecular weight of buffalo β-Lg is 38.5 kD. Buffalo and cow α-lactalbumin (α-LA) have the same crystalline form and similar nitrogen content. The molecular weight of buffalo α-LA is 16.2 kD, and no genetic polymorphisms have been observed (Malik and Bhatia, 1977). Buffaloα-LA has one major and three minor fractions, but all are active in modifying the activity of glactosyltransferase in the synthesis of lactose (Sindhu and Singhal, 1988). The concentrations of immunoglobulins (Ig) are very high in buffalo colostrum (Kulkarni, 1981),and four classes have been identified (IgGa, IgA1, IgA2 and IgM). Lactoferrin content of buffalo milk is much higher than in cow milk (Sahai, 1996). Its content in buffalo colostrums is still higher (0.75 mg.mL^{-1}). The molecular weight is 73.7–74.0 kD.

Fat: Buffalo milk is nearly twice as rich in fat as compared to cow milkand the most important fraction responsible for its high energetic and nutritive value. Varrichio *et al.* (2007) reported the fact that the fat content has an average value of 8.3% but can also reach upto 15% under normal conditions. Tonhati *et al.,* (2011) found the fat yield means 90.1±24·6 g.kg^{-1}. Medhammar *et al.* (2011) also found the interbreed differences of in total fat in buffalo, yak, mare and dromedary camel milks and as well in the mineral contents.

Fat related constituents: Fatty acid composition, however, in buffalo milkfat is different from that of cow milk fat (Ramamurthy and Narayanan, 1971;Joshi and Vyas, 1976;Arora *et al.,* 1986;Zicarelli, 2004b,Menard *et al.,* 2010).Some authors reported changes in the fatty acids composition of buffalo milk as a function ofbreed (Talpur *et al.,* 2007), lactatingstage (Arumughan and Narayanan, 1981), season (Talpur *et al.,* 2008;Asker *et al.,* 1978) and diet (Patiño*et al.,* 2008).The differences are present not only among species but also within species i.e. among breeds of buffaloes regarding fat and fatty acids concentration. Talpur *et al.* (2007) studied two major buffalo breeds of Pakistan i.e. Nili-Ravi and Kundi for fat. The milk fat of Kundi buffalo was found to contain significantly lower amount of saturated fatty acid contents than Nili-Ravi buffaloes (66.96 and 69.09 g.100g^{-1}),higher monounsaturated fatty acid contents (27.62 and 25.20 g.100g^{-1}) and total *trans* fatty acids (3.48 vs. 2.48). In another study, Qureshi *et al.* (2010) found that Nili-Ravi dairy buffaloes produce milk almost similar to dairy cows regarding availability of cardio protective fatty acids, with the highest concentration of oleic acid (C18:1cis-9, 29.47 g/100 g). Buffaloes with moderate body condition yielded greater concentrations of these fatty acids followed by poorand highest ones. Two hypercholestolemic fatty acids (C12:0 and C14:0) were associated with higher body condition.Proportions of C4, C16,C17, and C18 fatty acids (FA) are higher, but C6,C8, C10, C12, C14, and C14:1fatty acids are lower in buffalothan in cow milk fat. Soliman *et al.* (1979) gave an average total saturated and unsaturated fatty acid content of 71.7% and 28.3% respectively in Egyptian buffalo milk fat. The intra-molecular fattyacid distribution is similar to that of other species (Freeman *et al.,* 1965).Buffalo milk fat has a greater proportion of high melting triglycerides than that of cow milk fat(9–12% and 5–6%, respectively) (Ramamurthy and Narayanan, 1974).The high melting triglycerides fraction contains less short-chain and unsaturated fatty acids. High, medium and low molecular weight triglycerides in buffalo milk are 42%, 17%, and 41% of total, respectively (Arumughan and Narayanan, 1982). Colostrum and late lactation milk are rich in unsaturated but poor in saturated fatty acids (Anantakrishnan *et al.,* 1946). Buffalo milk fat contains more tetraenoic and pentaenoic but less dienoic and trienoic fatty acids than cow milk fat (Ramamurthy and Narayanan, 1971). Buffalo milk fat has a higher melting point, density, specific gravity and saponification value, but lower refractive index, acid and iodine values than cow milk fat, although they are affected by stage of lactation, season, feed and thermal oxidation (Angelo and Jain, 1982). Buffalo milk and ghee contain less free fatty acids than milk and ghee from cows (Pantulu and Ramamurthy, 1982; Lal and Narayanan, 1983).

Cholesterol levels (total and free) in buffalo milk fat appear to be lower than in that of cow milk as shown in the table 5 (Zicarelli, 2004b). Colostrum and mastitic milk contained more cholesterol than normal milk. Cholesterol content in fore-milk is higher than in stripping; also, it is higher in milk during the spring season. Esterified cholesterol, however, was higher in buffalo than in cow milk fat (64 and 48 $mg.100g^{-1}$, respectively) (Bindal and Jain, 1973; Prasad and Pandita, 1987).The phospholipids content of milk is a function of fat content and size of fat globules. A significant correlation has been found between PL and fat content of buffalo milk. The phospholipid contents of buffalo milk is slightly higher (29.6$mg.100mL^{-1}$) in summer time than in winter (24.7 $mg.100mL^{-1}$).The phospholipid content of buffalo milk, butter and ghee per unit weight of fat is much lower than in cow milk fat (Baliga and Basu, 1956). Colostrum has more phospholipids, which becomes normal in 15 days. The phospholipid contents are maximum in January and minimum in July. The ratio of lecithin: cephalin: sphingomyelin is 48:40: 12 in cow milk and 40:48:12 in buffalo milk (Rawat, 1963).Buffalo milk contains gangliosides which are not present in cowmilk (Berger *et al.,* 2005). A gangliosides fraction in buffalo milk show a GM1- specific binding to cholera a toxin subunit B. Also the lipholipic gangliosides of buffalo milk have anti-inflamatry activity (Colarow *et al.,* 2003).Milk Fat contains wide range of carbonyl compounds and their precursor keto-glycerides a part of the delicate flavor system of milk fat. Monocarbonyl content of buffalo milk fat is higher than that of cow milk fat. Colostral fat contained 60-70% of total carbonyls in normal milk fat, increased rapidly during early lactation and then gradually therefore (Bhatand Rao, 1983).

Physico-chemical characteristics of fat globules: The fat globule in buffalo milk is coarse and bigger than in cow milk (1 ml buffalo milk contains about 2.7 million fat globules), with 60% having a size between 3.5 to 7.5 µm(Akhundov, 1958; Akhundov, 1959; Abd El-Hamid and Khader, 1989; Ahmad *et al.,* 2008).The average size of fat golubles in buffalo milk (5µm) is higher than cow, goat and sheep milk being 3.2, 2.6 and 3.0 µm respectively. El-Zeini *et al.* (2006) reported much average globule sizes (8.7µm) in buffalo milk as compared to 3.8, 3.8, 3.2 and 3.0 µm for cow, sheep, goats and camel milks. Higher percentage (20.34%) of large fat globules (16-18µm) has been found in buffalo milk but not in the milk of other ruminants. The buffalo milk fat globule has spherical shapes (Fig. 2) as that of other milk but differs markedly from fat globules of other ruminants in its rheological characteristic. The respective parameters of buffalo milk fat globules are compactness, sphericity, surface roughness, length, width, orientation are 0.71, 0.59, 0.91, 58.34, 9.85, 4.67, 9.85, 4.15 and 107.46 respectively. The total concentration of saturated fatty acid of buffalo milk fat globule membrane varied from 66.2 to 78.3 and unsaturated fatty acids from 21.7 to 33.5% in agreement with result of milk fat globule membrane of buffalo milk from different breeds. The proteins:lipids ratios of isolated membranes vary from 3.2 to 4.7 but the total neutral and polar lipids are almost similar in different seasons (Sharma *et al.,* 1994; Sharma *et al.,* 1996).

Lactose: Lactose is a disaccharide made up of glucose and galactose bonded together in buffalo milk like other milks. Buffalo milk is richer source of lactose than cow, goat, sheep and camel milk so agood of source of energy for body activities particularly of brain and hormonal regulation. Before it can be used by the body, the bond must be broken by the enzyme lactase in the small intestine. People that have decreased activity of lactase in the small intestine may have problem of lactose digesting and this is referred to as lactose intolerance or malabsorption. Due to higher concentration, the chances of such problems are more by using buffalo milk but cases have not been noticed as for cow milk, may be due different repartition of lactose in the buffalo milk.

Complex oligosaccharides constitute a large portion of lactose of milk and perform biological functions that are closely related to their structural conformation. They contribute to the growth of beneficial intestinal flora in the colon, postnatal stimulation of the immune system and provide defense against bacterial and viral infections by acting as competitive inhibitors for binding sites on the intestinal epithelial surface (Kunz *et al.,* 2000; Kunz and Rudloff, 2002). Varman and Sutherland (2001) have explained that lactose makes a major contribution to the colligative properties of milk, such as osmotic pressure, freezing point depression and boiling point elevation. Oligosaccharide distributions in human milk and colostrum and milk of domestic animals (cows, goats, sheep, and buffaloes) have also been studied by Mehra and Kelly (2006). The levels of oligosaccharides in cow, sheep and goat milk are much lower (Urashima*et al.,* 1997; Martinez-Ferez*et al.,* 2006), whereas comparable in buffalo milk. The low concentration of oligosaccharides in cow milk and colostrum has stalled their utilization as biologically active ingredients in the health care and food sector but it opens the door for milk and colostrum like of buffaloes having comparable oligosaccharides levels as in human milk. Much research interest is being shown in recent period on the potential of milk oligosaccharides in infant nutrition. A processed oligosaccharide mixture of buffalo milk induced significant stimulation of antibody, delayed type hypersensitivity response to sheep red blood cells in BALB/c mice and also stimulated nonspecific immune response of the animals in terms of macrophage migration index. Saksena *et al.* (1999) isolated a novel pentasaccharide from buffalo milk oligosaccharides containing a fraction with immune stimulant activity. Currenly, there is only limited data and research findings on oligosaccharides in buffalo milk. Abd El-Fattah *et al.* (2012) observed that at calving, all components decreased gradually as the transition period advanced except lactose which conversely increased. Milk oligosaccharides are divided into neutral (don't contain any charged monosaccharides residues) and acidic (contain one or more residues of sialic acid that are negative charged) classes (Gopal and Gill, 2000). The galactose, *N*-acetylgalactosamine and sialic acid contents of buffalo κ-CN fractions ranged from 0 to 4.3, 5.5 and 8.5 moles.mole^{-1} protein, respectively (Addeo *et al.,* 1977). Aparna and Salimath (1995) reported the composition of oligosaccharides, and isolation and structural elucidation of disialyl lactose, from the colostrum of buffalo as three fractions with different concentration of glycopeptides (i) 0.2-0.8%, (ii) 0.3-1.5%) and (iii) 2.2-2.8%. A sialoglycopeptide was isolated from buffalo colostrum in pure form which consists of fucose, galactose, mannose, *N*-acetyl glucosamine and *N*-acetyl neuraminic acid in the ratio 1:2:3:4:1, and aspartic acid, serine, threonine, proline and glutamic acid as the major amino acids. Glycine was identified as the *N*-terminal amino acid residue.

Minerals: Buffalo milk has been found to contain more minerals than cow milk. Contents of macro minerals and selected trace elements in dairy products have been published by Cashman (2002a, b). The chemical form in which a macro mineral and trace element is found in milk or in other foods and supplements is important, because it will influence the degree of intestinal absorption and utilization, transport, cellular assimilation, and conversion into biologically active forms, and thus bioavailability. Buffalo milk is characterized by high calcium content than in cow, goat and camel milk). Most of calcium is found in insoluble form mainly due to the high casein contents of buffalo milk which plays an important role in determining the properties of buffalo milk. Based on the available data the insoluble calcium represents 67.6-82.6% of the total calcium. It has been estimatedthat micellarcalcium in buffalo milk to be 1.12 mM.g^{-1} casein as compared to 0.84 mM.g^{-1} casein in cow milk (Ahmad *et al.,* 2008). The ionizablecalcium of buffalo milk represents 34.6% of the soluble calcium. Buffalo milk is also rich in phosphorous contents. The phosphorous is disturbed between colloidal inorganic phosphate (42.4% of total), soluble inorganic phosphate (30.0%of total) and esters phosphorous (9.2% of total) (Abd El-Salam and El-Shibiny, 1966). The soluble magnesium represents 50% of total magnesium and soluble citrate represents 85% of the total citrate while sodium, potassium and chloride are almost completely present as soluble salts.

The presence of 15 elements presents as traces in buffalo milk has been reported in the table 7. Wide variations are found in reported levels of traces elements in buffalo milk which reflect the difference variable on the composition of milk in addition to differences in the used methods of analysis. Zinc, iron, and copper content of buffalo milk have received special attention. Trace elements are distributed variably between different phases of buffalo milk. Boron is found in buffalo milk as 44.8% soluble, 37.6% associated with fat and 17.6% associated to casein. It contains 18%, 72%, and 10% of zinc as soluble associated with casein and lipid phase, While, 36.5, 42.5 and 21% of iron are found in cream, rennet whey and rennet curd. The secretion of some trace elements seems to be affected by hormonal like oxytocin administration which increases copper and manganese contents and decreases magnesium, iron and zinc contents without altering the calcium concentration of buffalo milk (Sheehan *et al.,* 2009).

Enzymes: Milk contains numerous minor proteins having physiological effects. These minor proteins include enzymes, metal-binding proteins, enzyme inhibitors, vitamin binding proteins, and numerous growth factors (Fox, 2001). The enzymes concentrations in buffalo milk are given in the table 8.Lysozyme (LZ) is a basic protein enzyme with a low-molecular weight and important component of the antibacterial system in milk. Priyadarshini and Kansal (2002b) found the molecular weight of buffalo milk LZ to be 16 kDa, and determined its antibacterial activity. Buffalo colostrum contains five-times more LZ activity than mature milk (Priyadarshini and Kansal 2002a) and higher specific activity than that of cow milk LZ. Buffalo milk LZ is active over a wide range of pH and its activity is strongly influenced by the molarity of the medium. LZ activity in buffalo milk was not influenced by parity and stage of lactation; however, it increased during extreme weather conditions in winter and summer. The higher LZ activity in buffalo milk possibly is one of the factors responsible for lesser incidences of udder infections in buffaloes. Buffalo calf receives greater amounts of LZ during first few days after birth in colostrum which shows five times greater LZ activity than mature milk, one of the main reasons of prevention of enteric infections (Priyadarshini and

Kansal, 2003). LZ has found application in food preservation like egg-while lysozyme is already being used successfully as an antimicrobial in many foods, especially in cheese (Benkerroum, 2008). LZ in buffalo milk is more stable than in cow milk during storage and heat treatment (Priyadarshini and Kansal, 2002b).Buffalo milk LZ found fully stable (El-Dakhakhny 1995; Priyadarshini and Kansal 2002a), whereas cow milk LZ was partly inactivated by pasteurization. Buffalo milk lactoperoxidase (LP) has been studied extensively (Kumar and Bhatia, 1994; Kumar *et al.,* 1995; Kumar and Bhatia, 1998, 1999; Van Nieuwenhove *et al.,* 2004). Kumar and Bhatia (1999) observed that LP is more stable in whey prepared from buffalo milk. Buffalo LP is pH sensitive undergoing denaturation at low pH, while relatively stable in the range of pH 5-10 (Kumar and Bhatia, 1994). Kumar and Bhatia (1999) reported that at 72°C buffalo milk LP alone in acetate buffer (0.1 M, pH 6.0) was completely inactivated at zero time, while the presence of salts induced thermal protection to LP structure. LP content found the most abundant enzyme in buffalo milk. LP has antimicrobial properties and because of its broad biocidal and biostatic activity, LP has found many commercial applications, especially targeting oral pathogen (Tenovuo, 2002).In buffaloes the xanthine oxidase (XO) activity gradually increases in colostrum and then decreases with the changeover to the secretion of milk (Gandhi and Ahuja, 1979).XO is negatively correlated with the fat content of the milk. The activity of partially purified XO from buffalo milk fat globules was optimal at pH 7.6. The Km and Vmax values were 48-55 μM xanthine and 92-125 mμ.mg^{-1} protein, respectively. In the presence of phospholipids, especially phosphatidyl serine and phosphatidyl inositol, the temperature-dependent inactivation of XO is decreased, indicating a protective effect of phospholipids on XO.

Vitamins: Buffalo milk contains only traces of carotene, but higher vitamin A than cow milk (Narayanan *et al.,* 1952). Carotene and vitamin A in different seasons are 3.0 and 67.1, 2.9 and 73.3, 1.8 and 48.1, 2.2 and 48.4µg.$100mL^{-1}$ in winter, spring, summer and autumn, respectively (Narayanan *et al.,* 1956; Ibrahim *et al.,* 1983). However, due to the absence of carotenoids and high fat content, its total vitamin A potency per unit weight of fat is lower than in cow milk fat (Sampath *et al.,* 1955). The feeding of cotton seed to buffaloes leads to an increase in vitamin A content in its milk fat (Pandya and Patel, 1972). Heating of milk causes a decrease in its vitamin A content (El-Abd *et al.,*1986). Several studies reported that buffalo milk contains higher ascorbic acid (vitamin C) than cow milk (Singh and Gupta, 1986; Mohammad *et al.,* 1990). The thiamine content of buffalo milk varies from 38.7to 53.0µg.$100mL^{-1}$.Buffalo milk contains less riboflavin than cow milk and riboflavin of milk from the two species decrease markedly with exposure to sunlight whilst exposure to fluorescent light has much less effect. Buffalo milk contains average riboflavin contents of 158.8µg.$100mL^{-1}$(146.4 and 183 µg.$100mL^{-1}$on berseem and oats diets, respectively) (Sikka*et al.,* 1993).Buffalo milk from Indian breeds contains less folic acid than cow milk and goat milk. The bound and total folate decreased from 22.7 and 54.7 ng.mL^{-1} in the first day after calving to 18.2 and 37.8 ng.mL^{-1}, respectively after 55 days of parturition. The folate binding capacity of buffalo milk is less than that for cow and goat milk. Folic acid content in Egyptian buffalo milk was reported to be 1.368 µg.$100g^{-1}$ dry matters similar to that of Indian buffalo milk (Sharaf, 1989).The average niacin, biotin and B12 contents in buffalo milk are 1.3mg.$100g^{-1}$, 6.7µg.$100g^{-1}$ and 1.9 µg.$100g^{-1}$dry matters content. Buffalo milk contains 4 folds higher vitamin B12 (21.7 ppb) compared to cow milk (4.9ppb) (Sharma *et al.,* 2007).The concentrations of B vitamins in buffalo milk are: thiamin, 0.5; riboflavin, 1.0; nicotinic acid, 2.6; biotin, 26.8; folic acid, 0.1; pantothenic acid, 1.5; pyridoxine, 3.8; vitamin B12, 3.4; and p-aminobenzoic acid, 26.8 µg.ml^{-1} (Pasricha, 1969). Raw buffalo milk contained more riboflavin, B6, and folic acid and less thiamin than raw cow milk. Heat treatment of the milk caused the loss of 7 – 37% of thiamin, 8 – 35% of B6 , 8 – 45% of folic acid, and 0.4 – 4% of riboflavin. Losses of all vitamins were higher in cow milk than in buffalo milk. Losses were lower for pasteurization than by microwave or conventional boiling and in - bottle sterilization (Sharma and Darshan, 1998).Buffalo Milk also contains high levels of the natural antioxidant tocopherol. Peroxidate activity is normally 2-4 times that of cows' milk.

PHYSICO-CHEMICAL PROPERTIES OF BUFFALO MILK

Buffalo milk is very white and beautifully smooth. The pH of buffalo milk ranges from 6.57 to 6.84 and is not influenced by month, lactation number, or season of calving, but correlated with solid-not-fat and lactose contents (Minieri *et al*., 1965). Acidity varies from 0.05% to 0.20% (Dharmarajan *et al.,* 1950.), and its colostrum has greater acidity than mature milk. In fresh milk, lactic acid accounted for 25% of total acidity. Acidity was correlated with fat and solid-not-fat percentage in buffalo milk but not in cow milk (Hofi *et al.,* 1966a). The freezing point of buffalo milk is in the range of – 0.552 to – 0.558° C (Hofi *et al.,*1966b), but boiling and souring decrease the freezing point, and vacuum treatment, cold storage, and the addition of water increase the freezing point. The maximum buffering index was 0.042 at pH 4.9–5.1 for buffalo milk and 0.035 at pH 5.1–5.2 for cow milk (Rao *et al.,*1955; Rao *et al.,* 1956). The refractive index of buffalo milk (at 40°C) varies from 1.346 to 1.353 compared to cow milk, which is 1.345 to 1.348, with proteins and lactose contributing most (Rangappa, 1947).Buffalo milk with 6.4% fat and 10.2% solid-not-fat had mean density of1.034 g.mL^{-1} at its freezing point (Roy and Chandra, 1978) with little difference between cow and buffalo milk, but separation of cream increased the density of buffalo milk (Abo-Elanga, 1966).Curd tension in buffalo milk (32–85 g) is nearly 1.5 times that of cow milk (28–54 g) and increases at the end of lactation (Rao *et al.,*1964), but heat treatment from pasteurization decreases it by 10–28%, boiling by 58%, sterilization by 87%, homogenization by 24–73%, and addition of sodium citrate or sodium hexa-meta-phosphate by up to 97% (Tambat and Srinivasan, 1979).

Table-1: General composition of buffalo milk (g.kg^{-1})

Protein	Fat	Lactose	Ash	Total solids	References
43	77	47	8	175	Altman and Dittmer (1961)
40	70	51	8	167	Sindhu and Singhal (1988)
40	80	49	8	175	Jan (1999)
44	71	52	8	175	Ahmad *et al.* (2008)
46	73	56	-	176	Menard *et al.* (2010)
50	71	46	9	177	Han *et al.* (2012)

Table-2:Proteins and nitrogenous fractions in buffalo milk

Nitrogenous fractions	Concentrations	Nitrogenous fractions	Concentrations
Total casein	**37.8[b]**	Proteose peptone (g.kg^{-1})	3.3[b]
αS1-casein (g.kg^{-1})	16.2[b]	Serum albumin (g.kg^{-1})	0.3[b]
αS2-casein (g.kg^{-1})	2.5[b]	Lactoferrin (g.kg^{-1})	0.3[b]
β-casein (g.kg^{-1})	14.2[b]	**Non protein nitrogen (g.kg^{-1})**	**1.7[c]**
κ-casein (g.kg^{-1})	4.9[b]	Amino acid (mg N.100g^{-1})	5.13[a]
Non casein nitrogen (g.kg^{-1})	**8.9[c]**	Creatinine (mg N.100g^{-1})	0.37[a]
Whey protein (g.kg^{-1})	**6.2[c]**	Creatine (mg N.100g^{-1})	0.92[a]
β-lactoglobulin (g.kg^{-1})	3.9[b]	Uric acid (mg N.100g^{-1})	0.24[a]
α-lactalbumin (g.kg^{-1})	1.4[b]	Ammonia (mg N.100g^{-1})	0.26[a]
Immunoglobulins (A, M & G)	10.7[b]	Undetermined (mg N.100g^{-1})	9.30[a]

[a]Sahai (1996); [b](Pandya and Khan, 2006); [c]Ahmad*et al.* (2008)

Table-3: Physico-chemical characteristics of casein micelles of buffalo milk

Parameters	Buffalo milk
Size of micelles (nm)	190[b]
Charge of micelles (mV)	-20[b]
Water content (g H_2O.g^{-1} dry pellet)	1.90[b]
Voluminosity (mL.g^{-1})	3.20[a]

[a]Sood*et al.* (1976); [b]Ahmad (2010)

Table-4: Amino acids contents of buffalo milk (g.kg^{-1})

Amino acids	Concentrations	Amino acids	Concentrations
Lysine	3.51	Glycine	0.81
Histidine	1.66	Alanine	1.57
Arginine	1.17	Valine	2.52
Aspartic acid	2.94	Methionine	0.62
Threonine	1.22	Isoleucine	2.48
Serine	0.72	Leucine	4.24
Glutamic acid	9.96	Tyrosine	0.48
Proline	4.44	Phenylalanine	2.31

(Aliyev, 2005)

Table-5: Fatty acids and other fat related constituents of buffalo milk (% wt)

Fat related constituents	Concentrations	Fat related constituents	Concentratios
C4:0 butyric acid	2.8[d]	C18:2 c9, t11 (Main CLA)	0.9[d]
C6:0 caproic acid	1.9[d]	Saturated fatty acids	70.8[d]
C8:0 caprylic acid	1.1[d]	Unsaturated fatty acids	29.2[d]
C10:0 capric acid	1.8[d]	ω6/ω3	1.3[d]
C10:1 caproleic acid	Trace[a]	C18:1 *trans* octadecenoic acid	2.70[d]
C12:0 lauric acid	2.3[d]	Total *trans* (C18:1 *trans + CLA)*	3.61[d]
C14:0 myristic acid	11.8[d]	Monounsaturated fatty acids	29.1[a]
C14:1 c9 myritoleic acid	0.7[d]	**Dienoic**	
C15:0 pentadecanoic acid	1.7[d]	Conjugated	0.7[a]
C15:1 c10 pentadecenoic acid	0.4[d]	Non-conjugated	0.8[a]
C16:0 palimitic acid	36.0[d]	Total	1.5[a]
C16:0 branched	0.2[a]	**Trienoic**	
C16:1 c9 palmitoleic acid	1.9[d]	Conjugated	0.04[a]
C17:0 heptadecanoic acid	0.8[d]	Non-conjugated	0.4[a]
C17:1 c10 heptadecenoic acid	0.3[d]	Total	0.5[a]
C18:0 branched	0.2[a]	**Tetraenoic**	
C18:0 stearic acid	9.85[d]	Conjugated	0.01[a]
C18:1 t6+t7+t8+t9 octadecenoic acid	0.4[d]	Non-conjugated	0.2[a]
C18:1 t10 octadecenoic acid	0.2[d]	Total	0.2[a]
C18:1 t11 octadecenoic acid	2.0[d]	**Pentaenoic**	
C18:1 t12 octadecenoic acid	0.1[d]	Conjugated	0.003[a]
C18:1 c9 oleic acid	20.3[d]	Non-conjugated	0.09[a]
C18:2 c9,c12 (ω6) linoleic acid	0.9[d]	Total	0.09[a]
C18:3 c9, c12, c15 (ω3) linolenic acid	0.7[d]	Total cholesterol (mg.100g^{-1})	275[c]
C20:0 arachidic acid	0.2[d]	Free cholesterol (mg.100g^{-1})	212[c]
C20:4 arachidonic acid	0.2[a]		

[a]Ramamurthy and Narayanan (1971);[b]Arora *et al.* (1986);[c]Zicarelli (2004a),[d]Menard *et al.*(2010); t:trans; c:cis

Table-6: Physico-chemical characteristics of fat globules of buffalo milk

Parameters	Values
Size (μm)	5[b,c]
Charge (mV)	-14[b]
d_{32}(μm)	3.7[c]
d_{43} (μm)	5.2[c]
Span	1.4[c]
Specific surface area (m^2.g^{-1}fat)	1.8[c]
Number of fat globules (million.mm^3)	3.2[a]

[a]Laxminarayana and Dastur, (1968); [b]Ahmad (2010); [c]Menard *et al.* (2010); d:diameter

Table-7: Average concentrations of major minerals and trace elements in buffalo milk

Minerlas	Concentrations
Total Calcium (mM)	47.1[a]
Colloidal (mM)	38.9[a]
Soluble (mM)	8.2[a]
Total Phosphate (mM)	27.7[a]
Colloidal (mM)	18.5[a]
Soluble (mM)	9.2[a]
Calcium:Phosphate	1.8[a]
Total Magnesium (mM)	7.3[a]
Colloidal (mM)	3.8[a]
Soluble (mM)	3.5[a]
Total Citrate (mM)	8.3[a]
Colloidal	1.2[a]
Soluble	7.1[a]
Sodium (soluble) (mM)	20.3[a]
Potassium (soluble) (mM)	28.7[a]
Chloride (soluble) (mM)	16.6[a]
Boron (μg.100mL^{-1})	52-145[b]
Cobalt (μg.100mL^{-1})	0.69-1.6[b]
Copper (μg.100mL^{-1})	7-2[b]
Iron (μg.100mL^{-1})	42-152[b]
Manganese (μg.100mL^{-1})	38.2-65.8[b]
Sulphur (μg.100mL^{-1})	15700-31400[b]
Zinc (μg.100mL^{-1})	147-728[b]

[a]Ahmad *et al.* (2008); [b]Sahai (1996)

Table-8: Concentrations of major enzymes in buffalo milk

Enzymes	Concentration
Lysozyme (μg.mL^{-1})	0.2
Lactoperoxidase (Units.mL^{-1})	5.2-9.8
Xanthine Oxidase (Units.mL^{-1})	0.1
Lipase (Units.mL^{-1})	0.2-1.1
Alkaline phosphatase (Units.mL^{-1})	0.1-0.2
Ribonuclease (μg.mL^{-1})	9.8
Protease (Units.mL^{-1})	0.8

Sahai (1996)

Table-9: Vitamins concentrations of buffalo milk

Vitamins	Concentrations
Vitamin A (IU.mL^{-1})	340
Vitamin C (ascorbic acid) (mg.L^{-1})	0.67
Riboflavin (mg.L^{-1})	1.59
Pryridoxine (mg. L^{-1})	3.25
Thiamine (mg.L^{-1})	0.2-0.5
Tocopherol (μg.g^{-1})	334.2

Sahai (1996)

Table-10: Physico-chemical characteristics of buffalo milk

Characteristics	Buffalo milk
pH	6.81[b]
Acidity (D°)	16.2[b]
Freezing point (°C)	-0.526[b]
Urea (mg.L^{-1})	237[b]
Viscosity (cp)	2.04[a]
Refractive index	1.345[a]
Surface tension (dynes.cm^{-1})	55.4[a]
Heat coagulation time at 140V	8 min 48 sec[b]
Phosphate test at 100°C (mL)	0.8[a]
Energy (kcal)	412[a]
White (L) (a.u.)	74[b]
Green (-a) (a.u.)	-1.6[b]
Yellow (b) (a.u.)	5.6[b]

[a]Laxminarayana and Dastur, (1968); [b]Ahmad (2010);

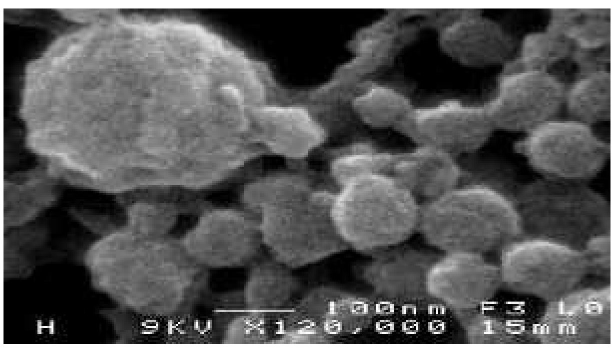

Figure-1: Image of caseins micelles of buffalo milk through scanning electron microscope (Ahmad, 2010)

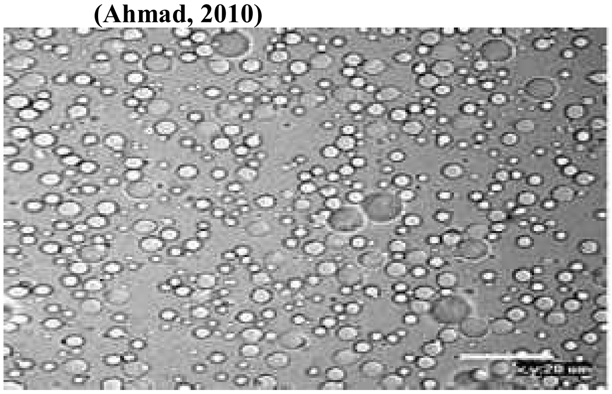

Figure-2: Fat globules of buffalo milk through optical microscope (Menard *et al.*, 2010)

Conclusions: Buffalo milk is a richer source of major and minor components which are essential to provide the nutritional requirements to human body. The compositional and characteristics differences from milk of other mammalian species depict that exiting technological or processing effects must be different. These available technologies need some modifications even from milking machines to industrial processing. It is a golden opportunity for the investors to come into buffalo milk business to get advantage from the government initiatives in the current period. In this way, we will be able to improve the genetic potential of buffaloes in getting more milk of higher quality and experimenting diversity of products particularly cheeses and other fermented dairy products for the local market and export by better exploiting the uniqueness of buffalo milk.

REFERENCES

Abd El-Fattah, A. M., F. H. R. Abd Rabo, S. M. EL-Dieb, and H. A. El-Kashef. (2012). Changes in composition of colostrum of Egyptian buffaloes and Holstein cows. B. M. C. Vet. Res.8(1):19.

Abd El-Hamid, L. B. and A. E. Khader. (1989). Size distribution of fat globules in buffalo, cow, goat and sheep milk. Egyp. J. Dairy Sci. 10: 43-46.

Abd El-Salam, M. H., and S. El-Shibiny. (1966). The chemical composition of buffalo milk. I. General composition. Indian J. Dairy Sci. 19: 151-154.

Abo-Elanga, I. G. (1966). Factors affecting creaming of cows and buffalo milk. Milchwissenschaft. 21: 210-213.

Addeo, F., J. C. Mercier, and B. Ribadeau-Dumas. (1976). Eléments de structure primaires de l'α-lactalbuminet de la β-lactoglobulin de buffle. F.E.B.S. Letters. 63(2):255-259.

Ahmad, S., I. Gaucher, F. Rousseau, E. Beaucher, M. Piot, J. F. Grongnet and F. Gaucheron (2008). Effects of acidification on physicochemical characteristics of buffalo milk: A comparison with cow's milk. Food Chem. 106:11-17.

Ahmad S. (2010). Understanding of the molecular changes in casein micelles of buffalo milk as a function of physico-chemical conditions: a comparison with cow milk. PhD Thesis, Agrocampus Ouest-INRA, France.

Akhundov, D. (1958). Chemical composition of milk of Azerbaijan buffaloes. Dairy Sci. Abstr.22:1708.

Akhundov, D. (1959). Some data on Buffaloes' milk. Dairy Sci. Abstr.22:211.

Aliyev, M. M., T. B. Iskenderov, and O. V. Aliev (2005). Amino acid ingredients of milk Azeri buffalo. YYÜ Vet. Fak. Derg.16:103-104.

Altman, P. L., and D. K. Dittmer (1961). Blood and other body fluids. Fed. Am. Soc. Exp. Biol. Washington, D.C.

Anantakrishnan, C. P., V. R. Bhalerao, T. M. Paul, and M. C. Rangaswami (1946). The component of cow colostrum fat. J. Biol. Chem. 116:31-37.

Angelo, I. A., and M. K. Jain (1982). Physico-chemical properties of ghee prepared from the milk of cows and buffaloes fed with cottonseed. Indian J. Dairy Sci. 35:519-525.

Aparna, H. S., and P. V. Salimath (1995). Disialyl-lactose from buffalo colostrum. Carb. Res. 268:313-318.

Arumughan, C. and K. M. Narayanan (1981). Influence of stage of lactation on the triacylglycerol composition of buffalo milk fat. Food Chem. 16:155-164.

Arumughan, C. and K. M. Narayanan (1982). Triacylglycerol composition of buffalo milk fat. J. Dairy Res. 49(1):81-85.

Arora, S. P., K. K. Singhal and R. C. Chopra (1986). Fatty acids composition of fat in milk and milk replacer diets. Indian J. Dairy Sci 39(4):495-497.

Asker, A. A., L. F. Hamzawi, A. E. Hagrass and L. B. Abd El-Hamid (1978). Studies on buffaloe's milk fat globule membrane: II-Seasonal variations. Egyp. J. Dairy Sci. 6:63-67.

Baliga, B. R. and K. P. Basu (1956). Phospholipids in milk and milk products. Indian J. Dairy Sci. 9:95-104.

Benkerroum, N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. African J. Biotechnol. 7(25):4856-4867.

Berger, A., M.E.Turini, and L. Colarow.(2005). Buffalo milk gangliosides. US Patent No. 20, 050, 107, 311.

Bhat, G. S., and M. B. Rao (1983). Glyceryl ethers in cow and buffalo milk. Indian J. Animal Sci. 53:321-323.

Bindal, M. P. and M. K. Jain (1973). A note on the unsaponifiable matter of ghee and its quantitative relationship with cholesterol content. Indian J. Animal Sci. 43:900-902.

Cashman, K. D. (2002a). Trace elements in milk and dairy products, nutritional significance. In: Roginski, H., Fox PF, Fuquay JW. (Eds.), Encyclopedia of Dairy Sciences. London, UK: Academic Pres:2058-2065.

Cashman, K. D. (2002b). Macrominerals in milk and dairy products, nutritional significance. In: Roginski, H., Fox PF, Fuquay, J. W. (Eds.), Encyclopedia of Dairy Sciences. London, UK: Academic Pres:2051-2058.

Colarow, L., M. Turini, S. Teneberg and A. Berger (2003). Characterization and biological activity of gangliosides in buffalo milk.Biochemistry and Biophysics Act. 163: 94-106.

Coop de bufflonnes (2010). Les bufflonnes: historique. http://coopdebufflonnes.free.fr/page2.html.

Dharmarajan, C. S., M. N. Menon, R. Venkatrao and N. N. Dastur (1950). Composition of milk of Indian animals. I. Freezing point, Lactose, Chloride and Acidity in the milk of different breeds of animals. Indian J. Vet. Sci. 20:35-46.

El-Abd, M. M., F. H. Ragab, I. A. Abd El Gawad, M. A. El Aasar, I. A. El Gawad, M. A. El. Abd Aasar, and I. A. Abd El. Gawad (1986). Study on vitamin A in milk and some milk products. Annals. Agr. Sci. 24:2129-2147.

El-Dakhakhny, E. A. (1995). The phenomena of lysozyme activity in cow and buffalo milk as affected by heat treatment, presence of sodium chloride or antibiotics. Alexandria J. Agric. Res. 40 (3):201-208.

El-Zeini, H. M. (2006).Microstructure, rheological and geometrical properties of fat globules of milk from different animal species. Polished J. Food and Nutr. Sci. 15: 147-154.

FAOSTAT (2010).Food and Agriculture Organization of the United Nations.http://faostat. fao.org /site /569/default.aspx#ancor.

Fox, P. F. (2001). Milk proteins as foodingredients. Int. J. Dairy Technol. 54:41-55.

Freeman, C. P., E. L. Jack, and L. M. Smith (1965). Intramolecular fattyacid distribution in the milk fat triglycerides of severalspecies. J. Dairy Sci. 48:853-858.

Gandhi, M. P. and S. P. Ahuja (1979). Partial characterization of xanthine oxidase (XO) from buffalo milk fat globules and variation in the XO content of milk. ZentralblVeterinarmed A.26(9): 765-769.

Ganguli, N. C. and V. R. Bhalerao (1964). A comparative study on the caseins of buffalo and cow milks by paper disk electrophoresis. Milchwissenschaft. 19: 535-538.

Ganguli, N. C. (1973). State of the casein micelle in buffalo milk. Netherlands Milk Dairy J. 27:258-272.

Gopal, P. K., and H. S. Gill (2000). Oligpsaccharides and glycoconjugates in bovine milk and colostrum. Brit. J. Nutr. 84(Suppl.1):S69-S74.

Han, X., F. L. Lee, L. Zhang and M. R. Guo (2012). Chemical composition of water buffalo milk and its low-fat symbiotic yogurt development. Functional Food in Health and Disease 2(4):86-106.

Hofi, A. A., I. D. Rifaat and M. A. Khorshid (1966a). Studies on some physico-chemical properties of Egyptian buffaloes and cows' milk. I. Freezing point. Indian J. Dairy Sci. 19:113-117.

Hofi, A. A., I. D. Rifaat and M. A. Khorshid (1966b). Studies on some physico-chemical properties of Egyptian buffaloes and cows' milk. V. Acidity and hydrogen ion concentration. Indian J. Dairy Sci. 19:158-161.

Ibrahim, E. M., M. A. Mohran and M. R. Said (1983). Seasonal variation in fat, carotene and vitamin A contents of milk from buffaloes and cows herds. J.Agri. Sci. 14: 195-206.

Jan (1999). Water buffalo in Brazil. http://ww2.netnitco.net/ users/ djligda/ wbbraz.htm

Joshi, C. H. and S. H. Vyas (1976). Studies on buffalo ghee. I. Seasonal variation in fatty acid composition and other properties of buffalo ghee. Indian J. Dairy Sci. 29:7-12.

Kuchroo, C. N. and R. C. Malik (1976). Voluminosity and hydration of buffalo milk casein micelles. Milchwissenschaft. 31:28-31.

Kulkarni, B. A. (1981). Immunoglobulins of the Indian buffalo. Part V: Changes in colostrum milk and neonatal calf serum immunoglobulins in early lactation. Indian J. Biochem. Biophys. 18(1):28-31.

Kumar, R. and K. L. Bhatia (1994). Lactoperoxidase activity in buffalo milk and whey. 1:57. In: Proceedings of IV World Buffalo Congress, Sao Paulo, Brazil, 27-30 June, 1994. Vale WG, Barnabe VH, de Mattos JCA (Eds.). 2:168-170.

Kumar, R., K. L. Bhatia, Z. Dauter, C. Betzel and T. P. Singh (1995). Purification, crystallization and preliminary X-ray crystallographic analysis of lactoperoxidase from buffalo milk. Acta Crystallographica Sec-D Biol. Crystallogr. 51(6):1094-1096.

Kumar, R. and K. L. Bhatia (1998). Kinetics of heat inactivation of buffalo lactoperoxidase. Indian J. Dairy Sci.51(5):323-327.

Kumar, R. and K. L. Bhatia (1999). Thermostability of lactoperoxidase in buffalo milk and whey. Indian J. Dairy Sci.52(3):142-148.

Kunz, C., S. Rudloff, W. Baier, N. Klein and S. Strobel (2000). Oligosaccharides in human milk. Structural, functional and metabolic aspects. Ann. Rev. Nutr. 20:699-722.

Kunz, C. and S. Rudloff (2002). Health benefits of milk-derived carbohydrates. Bull. Int. Dairy Fed. 375:72-79.

Lal, D. and K. M. Narayanan (1983). Effect of lactation number on fatty acid and physico-chemical constants of milk fats. Asian J. Dairy Res. 2:191–195.

Larsson, M. (2009). Water buffalo identifying and possibilities from Swedish perspective. Alaval Publications. 30.

Laxminarayana, H., and N. N. Dastur (1968). Buffaloe's milk and milk products – Part I. Dairy Sci. Abst. 30: 177-186.

Malik, R. C., and K. L. Bhatia (1977). Annual report, CIRD, Karnal, India.

Martinez-Ferez, A., S. Rudloff, A. Guadix, C. A. Henkelm, G. Pohlentz and J. J. Boza (2006). Goat's milk as a natural source of lactose-derived oligosaccharides: Isolation by membrane technology. Int. Dairy J. 16(2):173-181.

Mawal, R. B., T. Barnbas, and J. Barnbas (1965). Identification of cow β-lactoglobulin 'B' and buffalo β-lactoglobulin. Nature. 205: 175-176.

Medhammar, E., R. Wijesinha-Bettoni, B. Stadlmayr, E. Nilsson, U. R. Charrondiere, and B. Burlingame (2011). Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. J. Sci. Food Agric.92(3):445-474.

Mehra, R. and P. Kelly (2006). Milk oligosaccharides: structural and technological aspects. Int. Dairy J. 16(11):1334-1340.

Menard, O., S. Ahmad, F. Rousseau, V. Briard-Bion, F. Gaucheron and C. Lopez (2010). Buffalo vs. cow milk fat globules: Size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. Food Chem. 120:544-551.

Mihaiu, M., A. Lapusan, C. Jecan, R. Mihaiu, S. D. Dan, and C. T. Carsai (2010). Researches regarding leptin's influence on the fat and protein percent in buffalo milk", e.q. Scientific papers in veterinary medicine XLIII, Timişoara. http://m-business.ro/ro/e-articole/researches-regarding-leptin%E2%80%99s-influence-on-the-fat-and-protein-percent-in-buffalo-milk/

Minieri, L., G. de. Franciscis and F. Intrieri (1965). Relationship between pH, acidity and composition of buffalo milk. Dairy Sci. Abstr. 28: 1676.

Mohammad, K. S., W. A. Talib and L. A. Kashab (1990). Some water vitamins in different types of milk and their stabilities towards light and oxygen. Egyp. J. Dairy Sci. 18: 43-56.

Narayanan, K. M., T. M. Paul, C. P. Anantakrishnan, and K. C. Sen (1952). Studies on vitamin A in milk. V. The vitamin A content of buffalo colostrum. Indian J. Dairy Sci. 5:45-50.

Narayanan, K. M., C. P. Anantakrishnan and K. C. Sen (1956). Co-vitamin studies. I. Variations in tocopherol, carotene and vitamin A contents in milk and butterfat of cows and buffaloes. Indian J. Dairy Sci. 9:44-51.

Pandya, A. J. and M. M. H. Khan (2006). Buffalo milk. In: Handbook of milk of non-bovine mammals.

Park YW, Haenlein GFW (Eds.) Ames, Iowa: Blackwell Publishing Professional:195-273.

Pandya, M. P. and B. M. Patel (1972). Effect of prolonged feeding of concentrates on vitamin A and physico-chemical properties of butterfat. Indian J. Dairy Sci. 25:215-221.

Pantulu, P. C. and M. K. Ramamurthy (1982). Lipid composition of skimmed milk and whey. Asian J. Dairy Res. 1:17-20.

Patiño, E. M., M. A. Judis, C. S. Guanziroli, D. O. Pochon, J. F. Cedres and M. M. Doval (2008). Determination of milk fatty acids in buffaloes (Bubalus bubalis) from Corientes, Argentina. Revista Veterinaria. 19:28-32.

Prasad, R. and N. N. Pandita (1987). Variations in the cholesterol content of butterfat. Indian J. Dairy Sci. 40:55-57.

Priyadarshini, S. and V. K. Kansal (2002a). Lysozyme activity in buffalo milk: effect of lactation period, parity, mastitis, season in India, pH and milk processing heat treatment. Asian-Aust J. Animal Sci.15(6):895-899.

Priyadarshini, S. and V. K. Kansal (2002b). Purification, characterization, antibacterial activity and *N*-terminal sequencing of buffalo-milk lysozyme. J. Dairy Res. 69(3):419-431.

Priyadarshini, S. and V. K. Kansal (2003). Biochemical characterization of buffalo (*Bubalusbubalis*) milk lysozyme. J. Dairy Res. 70:467-472.

Qurshi M. S., A. Mushtaq, S. Khan, G. Habib and Z. A. Swati (2010). Variation in milk fatty acid composition with body condition in dairy buffaloes (*Bubalusbubalis*). Asian-Aust J. Animal Sci. 23(3):340-346.

Ragab, M. T., A. A., Asker and T. H. Kamal (1958). The effect of age and seasonal calving on the composition of Egyptian buffalo milk. Indian J. Dairy Sci. 11(1):18-28.

Ramamurthy, M. K., and K. M. Narayanan (1971). Fatty acid composition of buffalo and cow milk fats by gas-liquid chromatography (GLC). Milchwissenschaft. 26:693.

Ramamurthy, M. K. and K. M. Narayanan (1974). Partial glycerides of buffalo and cow milk fats. Milchwissenschaft. 29:151-154.

Rangappa, K. S. (1947). Studies on the refractive index of milk. II. Some factor affecting refractive index and refractive constant of milk. Proc. Indian Academy Sci. 25:125–135.

Rao, Bhimsen, and N. N. Dastur (1955). Hydrogen ion concentration of milk. I. pH of milk of animals of different breeds and individuals. Indian J. Dairy Sci. 8:158-172.

Rao, Bhimsen, and N. N. Dastur (1956). Hydrogen ion concentration of milk. II. Effect of some factors on the pH of milk. Indian J. Dairy Sci. 9:114-123.

Rao, R. V., V. C. Chopra, J. Stephen and V. R. Bhalerao (1964). Studies on curd tension of milk. J. Food Sci. Tech. India. 1:19-22.

Rawat, R. S. (1963). Phospholipids content of milk of different species. Agra Univ. J. Res. 12:245-249.

Roy, N. K., and S. Chandra (1978). Variation in physicochemical properties of buffalo milk with temperature and milk constituents. III. Density. Milchwissenschaft. 33:690-692.

Saksena, R., D. Deepak, A. Khare, R. Sahai, L. M. Tripathi and V. M. Srivastava (1999). A novelpentasaccharide from immune stimulantoligosaccharide fraction of buffalo milk. Biochimicaet Biophysica Acta. 1428(2-3):433-445.

Sampath, S. R., C. P. Anantakrishnan and K. C. Sen (1955). Studies on vitamin A in milk. VIII. vitamin A in market milk. Indian J. Dairy Sci. 8:129-134.

Sharaf, E. S. (1989). Studies on the levels of certain members of B complex vitamins in some Egyptian dairy products. MSC Thesis, Alexandra University Egyptian.

Sharma, K. C. A. P. Kaur and S. A. Singh (1994). A comparative lipid composition of milk fat globule membrane isolated from milk of murrah and niliravi breeds of buffalo. Archive Animal Breeding. 3: 527-533.

Sharma, K. C., C. Varindra and V. S. Singh (1996). Day to day variations in total cholesterol and some other components of milk from cow and buffalo during summer. Indian J. Dairy Sci. 49: 472-476.

Sharma, R. and L. Darshan (1998). Influence of various heat processing treatments on some B-vitamins in buffalo and cow’s milk. J. Food Sci. Technol. Mysore. 35(6):524-526.

Sharma, R., Y. S. Rajputt, G. Dogra and S. K. Tomar (2007). Estimation of vitamin B12 by ELISA and its status in milk. Milchwissenschaft. 62: 127-131.

Sheehan, W. J. and W. Phipatanakul (2009). Tolerance to water buffalo milk in a child with cow allergy. Allergy Astma and Immunolog.102: 349.

Sikka, P. R. Narayan and U. K. Atheya (1993). Effect of feed and foder on milk riboflavin of sahwial, crossbred cows and Murrah buffaloes. Indian Vet. J. 70: 79-80.

Singh, S. P., and M. P. Gupta (1986). Influence of certain treatments on the ascorbic acid content of buffaloes’ and cows’ milk. Indian Dairyman. 38:379-381.

Sirry, I., F. A. Salama, A. E. Salam, A. A. Mohamed, and H. A. Ahmed (1984). Studies on the physico-chemical properties of skim and standardized cow and buffalo milk. I. Effect of heating. Deutsche Lebensmittel-Rundschau.8: 242-245.

Soliman, M. A. A. A. Mohamed. A. E. A. Hagrass and S. A. El-Shabrawy (1979). Fatty acid composition of buffalo milk fat. Egyp. J. Dairy Sci. 7: 177-182.

Sabarwal, P. K., and N. C. Ganguli (1970a). The status of casein micelles in buffalo milk. Proc. 18th Int. Dairy Congress. 1E:24.

Sabarwal, P. K. and N. C. Ganguli (1970b). Studies on casein micelle of buffalo milk. I. Opacity of casein micelle as affected by buffer constituents. Indian J. Dairy Sci. 23:24– 32.

Sabarwal, P. K. and N. C. Ganguli (1971). Studies on the casein micelle of buffalo milk. III. Distribution pattern of micellar and soluble caseins as revealed by differential ultracentrifugation. Indian J. Dairy Sci. 24:16-24.

Sabarwal, P. K., S. Oommen and N. C. Ganguli (1972). Inorganic constituents of casein micelles from cow and buffalo milk. Indian J. Food Sci. Technol. 9:144–146.

Sabarwal, P. K. And N. C. Ganguli (1977a). A note on certain characteristics of casein micelles isolated from dialyzed milk. J. Anim. Sci. 47: 422-424.

Sabarwal, P. K. And N. C. Ganguli (1977b). Studies on micellar kappa-casein of cow and buffalo milk in relation to sialic acid and rennet action. Milchwissenchaft. 32: 202-206.

Sahai, D. (1996). Buffalo milk: Chemistry and processing technology. Karnal: Shalini International (SI) Publications, India, 132001.

Sarswat, B. L. (1985). Effect of season on the nitrogen distribution of cow and buffalo milk. *Asian* J. Dairy Res. 4: 108-110.

Sen, A. and N. K. Sinha (1961). Comparison of the beta-lactoglobulins of buffalo's milk and cow's milk. Nature.190:343-344.

Sindhu, J. S. and O. P. Singhal (1988). Qualitative aspects of buffalo milk constituents for products technology. In: "Buffalo Production and Health – A compendium of latest research information based on Indian studies", Indian Council of Agricultural Research, New Delhi.

Sood, S. M., K. S. Sidhu and R. K. Dewan (1976). Voluminosity of different-sized casein micelles from the buffalo and the cow. New Zealand J. Dairy Sci. Techn. 11: 79-82.

Talpur, F. N., N. N. Memon and M. I. Bhanger (2007). Comparison of fatty acid and cholesterol content of Pakistani water buffalo breeds. Pak. J. Analytical and Environ. Chem. 8:15–20.

Talpur, F. N., M. I. Bhanger, A. A. Khooharo and M .G .Zuhra (2008). Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan. Livestock Sci. 118:166-172.

Tambat, R. V. and M. R. Srinivasan (1979). Changes in surface tension, viscosity and curd tension of buffalo and cow milk during Cheddar cheese manufacture. Indian J. Dairy Sci. 32:173-176.

Tenovuo, J. (2002). Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: efficacy and safety. Oral Dis.8(1):23-29.

Tonhati, H, A. L. Lima, D. P. Lanna, G. M. de Camargo, F. Baldi, L. G. de Albuquerque and J. M. Montrezor (2011). Milk fatty acid characterization and genetic parameter estimates for milk conjugated linoleic acid in buffaloes. J. Dairy Res. 4:1-6.

Urashima, T., S. Murata, and T. Nakamura (1997). Structural determination of momosialyltrisaccharides obtained from caprineclostrum. Comp. Biochem. Physiol. 116B(4): 431-435.

USDA (United States Department of Agriculture). (2011). Milk for manufacturing purposes and its production and processing: recommended requirements. http://www.ams.usda.gov/ AMSv 1.0/getfile?dDocName=STELDEV3004791

Van Nieuwenhove, C. P., C. V. Gutierrez, M. S. Nunez and S. N. Gonzalez (2004). Lactoperoxidase and lysozyme activity in buffalo milk from Argentine. J. Ani. Vet. Adv.3:431-433.

Varman, H. A., and J. P. Sutherland (2001). Milk and milk products: technology, chemistry and microbiology. ASPEN Publishers Inc. USA.

Varrichio, M. L., A. Di Francia, F. Masucci, R. Romano and V. Proto (2007). Fatty acid composition of Mediterranean buffalo milk fat. Italian J. Animal Sci. 6:509-511.

Zicarelli, L. (2004a). Water buffalo nutrition. Zootech, 28-31 May, 2004-Brazil, DF:1-22. http://www.scribd.com/ doc/41329997/ W Buffalo -Nutrition.

Zicarelli, L. (2004b). Buffalo milk: its properties, dairy yield and mozzarella production. Vet. Res. Communications 28:127-135.