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A comprehensive review on the composition and properties of buffalo milk

Mohamed H. Abd El-Salam · Safinaz El-Shibiny

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Abstract The aim of this review is to update the knowledge regarding the composition and properties of buffalo milk (BM). Buffalo milk has higher levels of fat, lactose, protein, ash and Ca, and vitamins A and C and lower levels of vitamin E riboflavin and cholesterol; an absence of carotene; and the presence of the blue-green pigment (biliverdin) as well as a bioactive pentasaccharide and gangliosides not present in cow milk (CM). The fat globules of BM are larger but are less stable and contained less membrane materials than that of CM. Buffalo milk fat has slightly higher levels of saturated fatty acids and has quantitative differences in the distribution of triglycerides and physical properties in comparison to CM. The casein micelles of BM are larger and richer in minerals and can be disrupted by alkali at higher pH values than that of CM. The primary structures of all BM proteins have been established. High homologies have been found between the proteins of BM and CM, but BM α_{s1} -casein and α_{s2} -casein have lower levels of phosphorylation. The activities of several enzymes in BM are presented and discussed. The viscosity and curd tension of BM are higher; rennet coagulation is faster, and heat stability is lower than that of CM. The available published data gives a better understanding of BM, but more studies are required on some aspects to give a clearer picture on the detailed composition and properties of BM.

水牛乳组成和特性的综合评述

摘要 该综述旨在提供水牛乳(BM)的组成和特性的最新知识。与牛乳(CM)相比,水牛乳含有较高含量的脂肪、乳糖、蛋白质、灰分、钙、维生素A和C以及较低含量的维生素E、核黄素和胆固醇。水牛乳不含有胡萝卜素,但是含有一些牛乳中没有的生物活性物质,如胆绿素、五糖和神经节苷脂。水牛乳脂肪球比牛乳要大,但是不稳定且脂肪球膜物质含量较低。水牛乳脂肪的饱和脂肪酸比牛乳要稍高,其三酰甘油的分布以及脂肪的物理特性与牛乳也有一定的差异。水牛乳的酪蛋白胶束比牛乳要大且富含矿物质,但水牛乳酪蛋白胶束在较高pH条件下比牛乳酪蛋白胶束易解聚。水牛乳蛋白质的一级结构已经被确定,结果发现水牛乳蛋白和牛乳蛋白有很高的同源性,但是水牛乳的 α_{s1} -酪蛋白和 α_{s2} -酪蛋白磷酸化作用较低。该综述也讨论了水牛乳中一些酶的活性,水牛乳的粘度和凝乳张力比牛乳要高,凝乳的速度更快、

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热稳定性要低。本文提供的数据可以对水牛乳有较全面的了解,但是要在水牛乳的组成和特性有更清晰和更具体的描述,还有大量的研究需要去做。

Keywords Buffalo milk · Major constituents · Minor constituents · Physical properties · Technological properties

关键词 水牛乳 · 主要组分 · 次要组分 · 物理特性 · 工艺特性

1 Introduction

Bubalus bubalis is the name of the domesticated buffaloes. The farming of buffaloes has long been favored due to their efficient utilization of low-quality high-roughage diet (Larsson 2009), resistance to parasites, quick and easy calf growth, good quality meat, and rich milk and milk products.

Buffaloes are widely distributed throughout Asia, the Middle East, Europe, China, South America, the former Soviet Union Countries, and the Caribbean. However, the largest number of buffaloes and buffalo breeds are found in India and Pakistan producing 68% and 28% of the world buffalo milk production (IDF 2010). Originally, buffaloes were considered as a multipurpose animal, but breeds of buffalo for milk production have been selected and improved considerably. Twenty-two lactating buffalo breeds are found all over the world, and their characteristics and milk production have been described (Mohran 1991). Of the buffalo breeds, the Nili Ravi, Kundi, Egyptian, and Murrah represent the largest buffalo populations. Buffalo is the second global milk-producing animal all over the world. About 90 million tons were produced in 2009 representing 13% of the total world milk production with an annual growth rate of ~3.1% as compared with 1.3% annual increase in cow milk (CM) production (IDF 2010).

Buffalo milk (BM) plays an important role in human nutrition particularly in the developing countries. Compared with CM, buffalo milk is richer in almost all the main milk nutrients. Also, some milk products such as Mozzarella cheese and ghee are the specialties of buffalo milk. In addition, a recent study (Sheehan and Phipatanakul 2009) indicated that subjects with CM allergies are capable of tolerating BM, thus adding to the nutritional benefits of buffalo milk. The composition, properties and processing of buffalo milk and milk products has been the subject of several reviews (Laxminaryana and Dastur 1968; Abd El-Salam 1990; Gokhale et al. 2001; Pandya et al. 2004). However, considerable literature has been accumulated in recent years on the composition, properties and processing of BM. The focus of the present review is to highlight the recent knowledge on the detailed composition and properties of buffalo milk.

2 General composition and properties

2.1 General composition and factors of variation

Buffalo milk is characterized by high levels of fat, lactose, protein, casein, and ash contents as described in an early review (Laxminaryana and Dastur 1968). Published

data from more recent studies (Table 1) are in line with that of the earlier studies. Milk fat represents the major constituent of buffalo milk with a minimum and maximum of 6.6 and 8.8 g·100 g⁻¹, respectively (Table 1). Lactose is the second major constituent of buffalo milk with a minimum and maximum of 4.5 and 5.2 g·100 g⁻¹, respectively. Wide variations were found in the reported protein contents of BM (Table 1), but most figures fall within the range of 3.8–4.5 g·100 g⁻¹. The reported minimum and maximum ash content of BM were 0.71 and 0.90 g·100 g⁻¹ (Table 1). These data indicated that BM is richer than CM in all major constituents.

Monitoring changes in the composition of BM over the years is an important index for the combined effects of environmental and genetic factors. Zicarelli (2004) recorded an increase in fat and protein contents of Italian BM from 7.3% to 8.3% and 4.4% to 4.8%, respectively, between 1967 and 2000.

Differences in the composition of BM in different locations (Table 1) reflect differences in breeds, management, feeding, and environmental conditions. The gross composition of buffalo milk was affected by several factors as follows:

1. Breed. The breed of buffalo has a significant ($P \leq 0.05$) effect on the milk composition and yield traits (Misra et al. 2008). A comparison of the composition of milk from four different breeds of Indian buffaloes (Misra et al. 2008) revealed that Murrah was the best performing breed for fat, total protein, and casein contents, Mehsana for solids not fat, and Bhadawari for total solids (Table 2). Several studies did not mention the breed of BM analyzed which may be a factor for differences in milk composition in these studies. Apart from the wide differences in fat content of buffalo breeds (Moioli and Borghese 2007), differences in other milk constituents were not reported.
2. Lactation number. The total solids, solids not fat, lactose, and ash content increased with the increase in the number of lactation while the fat and total protein contents were not affected (Kholif 1997; Sodi et al. 2008).
3. Stage of lactation and seasonality. The content of fat and total solid content increased; lactose decreased, and protein and ash initially decreased before re-increasing with advanced stage of lactation (Shah et al. 1983; Kholif 1997; Dubey et al. 1997; Bhonsie et al. 2003). Also, seasonal variations in the major milk constituents have been reported (Bhonsie et al. 2003; Ranjupt et al. 1982). The percentages of fat, solids, and solids not fat (SNF) were the highest during summer (Bhonsie et al. 2003). Also, the percentage of Ca, P, K, Na, Cu, Mn, and Fe were the highest in summer and the lowest in winter (Ranjupt et al. 1982).
4. Feeding. The composition of the diet and the composition and particularly the quantity and quality of protein have been reported to affect the composition of BM (Tufarelli et al. 2008; Khattab et al. 1998). Feeding buffaloes on a diet containing added fats increased milk yield and fat content and, in particular, with dietary tallow (Nawaz et al. 2009). A positive correlation has been found between the energy content of the diet and fat, milk protein and lactose contents of BM (Tufarelli et al. 2008).
5. Incidence of subclinical mastitis. Buffaloes are less susceptible to subclinical mastitis and with somatic cell lower than those of cows under similar conditions

Table 1 Gross chemical composition of buffalo milk in different localities (grams per 100 grams)

Fat	TS	SNF	Protein	Lactose	Ash	Locality/reference
7.6±1.3	18.4±1.6	ND	4.74±0.2	ND	0.85±0.05	China (Han et al. 2007)
7.1±1.0	ND	ND	3.63±0.34	4.99±0.37	ND	Egypt (Kholif 1997)
7.0–7.2	16.9–17.4	9.8–10.1	3.60–3.85	4.99–5.24	0.79±0.03	Egypt (Abd El-Salam and El-Shibiny 1966)
8.0±0.6	16.3±0.8	8.3±0.3	2.70±0.08	ND	ND	India (hills; Meena et al. 2007)
7.7±0.1	17.0±0.1	9.4±0.1	3.81±0.02	4.83±0.01	ND	India (Sodi et al. 2008)
6.80	–	9.61	3.91	ND	ND	India (Dubey et al. 1997)
8.1±1.9	ND	ND	4.65±0.48	4.85±0.78	ND	Italy (Tufarelli et al. 2008)
7.6±0.1	–	9.8±0.1	4.11±0.02	4.67±0.02	ND	Pakistan (Imran et al. 2008)
7.0±0.6	–	–	4.35±0.34	5.21±0.11	0.84±0.02	Pakistan (Arian et al. 2008)
8.4±0.3	17.7±0.4	9.5±0.2	3.97±0.06	4.80±0.1	0.71±0.02	Bangladesh (Khan et al. 2007) swamp buffalo
7.3±0.5	16.7±0.1	9.2±0.2	3.77±0.26	4.76±0.18	0.71±0.02	Bangladesh (Khan et al. 2007) water buffalo
8.4–9.0	ND	ND	4.24–4.45	4.90–5.05	0.78–0.80	Azerbaijan (Akhundov and Farzalieva 1979)
6.6	17.0	10.4	4.13	ND	ND	Brazil (Macado et al. 2001)
7.0±1.3	16.6±2.6	–	3.73±0.82	4.57±0.23	0.85±0.05	Argentina (Patino et al. 2003; Patino and Stefani 2005) Murrah
7.6±1.8	16.8±2.6	–	3.73±0.88	4.51±0.21	0.80±0.05	Argentina (Patino et al. 2003) cross-bred ^a
8.8±0.3	18.4±0.2	–	5.20±0.14	4.55±0.0	0.78±0.03	Argentina (Patino and Stefani 2005) Jafarabadi
7.9	17.7	9.9	4.49	4.86	ND	Bulgaria (Peeva 2001)
7.1±1.4	16.6±1.6	9.6±0.8	4.40±0.51	ND	0.76±0.07	Turkey (Sekerden et al. 1999)
7.5–9.6	17.2–21.3	–	4.90	ND	ND	Germany (Braun and Preuss 2008)
7.0±0.6	ND	ND	4.35±0.34	5.21±0.11	0.84±0.02	France (Ahmad et al. 2008)

Values to the computed to the first decimal

TS total solids, SNF solids not fat, ND not determined

^a Murrah × Mediterranean

- (Ghosh et al. 1971). However, the content of fat, SNF, casein, and lactose decreased in subclinical mastitic BM, with an increase in the chloride content (Ghosh et al. 1971; Haggag et al. 1991).
- Genetic polymorphism of milk proteins. The genotypes of α -s₁ and α -s₂ caseins had no significant correlation with milk composition except for a weak ($P \leq 0.1$) correlation being reported between α -s₂ casein and total solids (Misra et al. 2008).

Table 2 Average composition of milk from four breeds of Indian buffaloes (Misra et al. 2008)

Breed	Fat	Total protein	Casein	Solids not fat	Total solids
Bhadawari	7.43±0.26 a	3.92±0.07	3.16±0.06	8.99±0.10 ab	17.70±0.28 a
Mehsana	6.46±0.17 b	3.87±0.05	3.07±0.04	9.13±0.06 a	15.59±0.18 c
Murrah	7.53±0.19 a	4.03±0.05	3.20±0.04	9.00±0.07 a	16.53±0.20 b
Surti	6.17±0.20 b	3.93±0.05	3.11±0.04	8.80±0.07 b	14.96±0.21 c

Values in grams per 100 milliliters

Similar letters in the same column indicates no significant differences

2.2 Physical properties

2.2.1 Viscosity

The viscosity of BM is generally higher than that of CM (Ismail and El-Deeb 1973; Tambet and Sirinivasan 1979). However, the viscosity of milk from both species is largely dependent on fat content. Skim, standardized (3% fat), and whole BM (6.1% fat) showed viscosities of 1.33, 1.70, and 2.02 cP, while skim, standardized (3% fat) and whole cow milk had viscosities of 1.17, 1.44, and 1.66 cP, respectively (Ismail and El-Deeb 1973). This may explain the variations in viscosity of BM cited in different studies. Tambat and Srinivasan (1979) and Haggag et al. (1991) reported a similar value of 2.19 cP for buffalo bulk milk samples, while Singh et al. (1987) reported an average value of 2.06 ± 0.034 cP for viscosity of whole BM. Arain et al. (2008) recorded a rapid decrease in the viscosity of postpartum BM from 6.80 cP for the first milking to reach the 1.64 cP on the sixth day (normal milk). Incidence of mastitis increased the viscosity of BM to 2.79 and 2.43 cP for milk from animals with clinical and subclinical mastitis, respectively (Haggag et al. 1991). At pH 8.6 and 10.8 the viscosities of BM were twice as high as CM (Ahmad et al. 2009) which was attributed to induced changes in the interactions between water and casein micelles.

2.2.2 Specific gravity

Buffalo milk is characterized by higher specific gravity than CM (Laxminaryana and Dastur 1968). Colostrum had higher specific gravity (1.061) than normal BM (1.037; Arian et al. 2008). Haggag et al. (1991) reported values of 1.036 and 1.032 for the specific gravity of colostrum and normal BM and that mastitic BM had a lower specific gravity of 1.014 and 1.028 in clinical and subclinical cases, respectively.

2.2.3 Freezing Point

The FP or cryoscopic index of milk is related to its soluble constituents (i.e., lactose and soluble salts) and is usually used to detect water added to milk. Hofi et al. (1966a) showed that the FP of BM (-0.518 °C to -0.590 °C) was less than that of CM. The FP of Italian BM was affected by season (-0.528 °C and -0.531 °C in warm and cold

weather, respectively) and farm size ($-0.532\text{ }^{\circ}\text{C}$ and $-0.519\text{ }^{\circ}\text{C}$ in small and large farms, respectively; Terramoccia et al. 1999) and between organic and conventional farming methods (-0.526 ± 0.01 and -0.537 ± 0.01 , respectively; Di Francia et al. 2007). The FP of BM in Germany ranged from $-0.5509\text{ }^{\circ}\text{C}$ to $-0.5146\text{ }^{\circ}\text{C}$ (Braun and Preuss 2008).

2.2.4 Buffering Capacity (BC)

The pH of BM decreased more slowly than did the pH of CM during acidification due to the higher buffering capacity (BC) of BM (Imam et al. 1974; Ahmad et al. 2008) resulting from the high casein and inorganic phosphate contents of BM. The pK_a of BM (pH 5.32) was higher than that of CM (pH 4.90; Imam et al. 1974). However, both milks showed a higher BC at the acid side than the alkali side of the titration curve (Imam et al. 1974).

2.2.5 Thermal Conductivity

Knowledge of the thermal properties of milk is essential to the design of heat exchangers, condensers, and evaporators commonly used in dairy plants. The average thermal conductivity (TC) of whole BM has been reported to be 0.5689 ± 0.00734 at $42\text{--}43\text{ }^{\circ}\text{C}$ (Sharma and Roy 1983). Normal variations in the ranges of fat and SNF had no significant effect on the TC of BM. However, the TC of BM was higher than that reported for CM (Sharma and Roy 1983) which was attributed to the differences in the fat and fat/SNF ratio of the two milks.

2.2.6 Electrical Conductivity

Electrical conductivity (EC), like other milk properties, is related to the milk composition, particularly the ionized constituents. Buffalo milk has a lower EC (average 9.17 ± 1.51 mmhos) in comparison to CM (11.12 ± 1.56 mmhos; El-Shibiny and Abd El-Salam 1973).

2.2.7 Other physical properties

The only cited reference for oxidation reduction potential (Hofi et al. 1966c) gave average values of 0.5391 ± 0.0069 V and 0.5367 ± 0.0069 V for BM and CM, respectively. CM and BM had similar surface tension values of 42.75 and 42.57 dynes/in, respectively (Tambet and Sirinivasan 1979). Hofi et al. (1966b) reported a higher average refractive index (1.3454) for BM in comparison to CM (1.3441).

2.3 Technological properties

2.3.1 Acid gelation

The acid induced gelation of BM using glucono-delta-lactone (GDL) was monitored using thromboelastography which can separate gelation into two phases, the onset gelation time (GT) and the time to the development of firm

curd (K_{20} ; Abd El-Salam et al. 1996). The pH at GT ranged from 5.5 to 5.9 which was higher than that reported for CM (pH 5.1–5.2; Kim and Kinsella 1989). The pH at GT of BM increased with increasing protein content (Abd El-Salam et al. 1996) which may explain the higher pH at GT of BM as compared with CM. Also, the pH of BM at K_{20} was 5.40 to 5.65 which was higher than that of CM. Linear relations were found between GT, K_{20} of BM, and GDL concentration and gelation temperature (Abd El-Salam et al. 1996).

2.3.2 Rennet coagulation

Buffalo milk is characterized by a faster coagulation than CM (Ibrahim et al. 1973a; El-Shibiny and Abd El-Salam 1980). Also, the Rennet Coagulation Time (RCT) of BM was almost unchanged when the milk was diluted with an equal volume of water (Ibrahim et al. 1973a; El-Shibiny and Abd El-Salam 1980) while a similar treatment markedly increased the RCT of CM. A close correlation was found between the RCT and the levels of colloidal calcium in diluted milk from different species. RCT increased sharply at a colloidal calcium level of $<50 \text{ mg} \cdot 100 \text{ mL}^{-1}$ (El-Shibiny and Abd El-Salam 1980). The RCT of BM was affected differently than the RCT of CM by the following factors:

1. The type of milk clotting enzyme. Using *Endothia parasitica* protease, both buffalo and cow's milk showed similar RCT, while BM coagulated faster with the use of calf rennet (Wahba 1979).
2. The RCT of BM was more sensitive to the addition of NaCl, H_2O_2 , and NaCO_3 than CM (Abd El-Hamid et al. 1981).
3. Storing of BM at 7°C for up to 24 h had a slight effect on its RCT while the RCT of CM increased under the same conditions (Amer et al. 1974).
4. The increase in the RCT of BM by heat treatments was less pronounced than that of CM (Amer et al. 1974).

These differences can be attributed to the differences in the colloidal phase of the two milks and explain the differences in the behavior of buffalo and cow's milks during cheese-making.

The enzymatic and non-enzymatic phases of rennet coagulation of BM were determined as clotting time (r) and curd firming time (K_{20}). Both parameters were found to be markedly affected by pH. The calculated Q_{10} s (rate of increase with 10°C increase) were 1.69, 1.75, and 4.00 for r and 2.28, 3.0, and 2.5 for K_{20} at pH 6.5, 6.0, and 5.5, respectively (El-Dein 1994).

2.3.3 Curd Tension (CT)

Early studies have shown that the CT of rennet-coagulated BM is nearly 1.5-fold that of CM mainly due to its high casein and calcium contents (Laxminaryana and Dastur 1968). Tambat and Srinivasan (1979) reported a CT of 27.90 and 32.25 g for CM and BM, respectively, but a higher CT value of 55.70 g has been reported for BM (Abd El-Salam et al. 1974) which may be due to differences in animal breeds and methods used for the determination of CT. However, the CT of BM was greatly reduced by heating to 85°C , boiling and homogenization. The addition of phosphate and citrate

to heated milk further decreased the curd tension of BM (Abd El-Salam et al. 1974). Lowering the pH increased the CT of rennet-coagulated BM. However, for milk containing NaCl, the CT increased when the pH was lowered to 6.0, but further lowering of the pH caused the CT to decrease (Asker et al. 1981). Buffaloes infected with clinical mastitis and subclinical mastitis reduced the CT, giving CT values of 6.2, and 21.5 g, respectively, compared with a value of 39.3 g for milk from normal animals (Haggag et al. 1991). Also, buffalo colostrum had a lower (33.3 g) CT than normal milk (Haggag et al. 1991). The CT of acid coagulated BM (Patel and Chakraborty 1985) increased with an increase in the inoculum level and incubation temperature giving values of 83.56, 90.82, and 91.7 g for 1%, 2%, and 3% inocula, respectively, at 37 °C and 92.80, 98.30, and 99.35 g, respectively at 42 °C.

2.3.4 Heat stability

Wide variations have been reported in values for the heat stability of BM due to the heating temperature as well as in the methods used to measure the heat stability (Pandya et al. 2004). However, most studies (Ghatak et al. 1980; Pandya et al. 2004) agreed that BM was less heat-stable than CM due to the following factors:

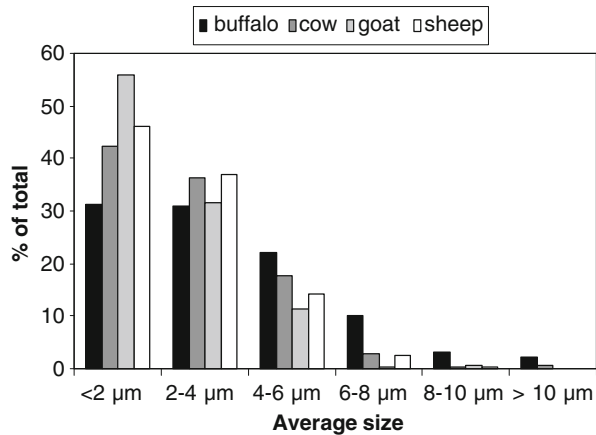
1. The high fat and Ca contents. High negative correlations (R , -0.65 and -0.75) have been reported between fat and Ca contents, respectively, and the heat stability of BM (Puri et al. 1977).
2. pH. Alteration in pH caused considerable changes in the heat stability of buffalo milk and exhibited type A milk with a maximum (\sim pH 6.7) and minimum (\sim pH 6.9) stability (Ranjupt et al. 1982).
3. The urea level. The low level of urea in BM ($17.5 \text{ mg} \cdot 100 \text{ mL}^{-1}$) as compared with CM ($40 \text{ mg} \cdot 100 \text{ mL}^{-1}$; Ganguli and Bhavadasan 1980) was considered to be a factor responsible for the low heat stability of buffalo milk. Addition of small amounts of urea has been reported to improve the heat stability of BM (Ranjupt et al. 1982).

3 Lipids

3.1 Physico-chemical properties of fat globules

Several studies have demonstrated that the size of the fat globule (FG) of BM is larger in comparison to milk from other ruminants (Abo-Elnaga et al. 1966; Abd El-Hamid and Khader 1982; Menard et al. 2010). The average FG size in BM as measured by the microscopic method ($4.07 \mu\text{m}$) was higher than that of cow's ($3.16 \mu\text{m}$), goat's ($2.57 \mu\text{m}$), and sheep ($3.02 \mu\text{m}$) milks (Abd El-Hamid and Khader 1982). A larger average size of $5.0 \mu\text{m}$ has been reported (Menard et al. 2010) for FG in BM using the particle size measurement. The size of the native milk FG affects the physico-chemical and functional properties of cheeses (Michalski et al. 2004) and the larger size of BM fat globules may be a factor affecting the properties of dairy products made from BM. Also, the frequency distribution of FG showed a higher percentage of large FG in BM than in CM (Fig. 1). The fat globule sizes

Fig. 1 Size distribution of fat globules in buffalo, cow, goat, and sheep milks (Abd El-Hamid and Khader 1982)



(FGS) in German buffalo breeds have been reported to have a more balanced distribution, with the main FGS between 3 and 6 μm (Schafberg et al. 2007). Seasonal and lactational variations were found in the FGS with lower sizes reported during late lactation (Schafberg et al. 2007). The FGS of BM showed a positive correlation ($R^2=0.85$) with milk fat content (Menard et al. 2010) and negative correlation (Schafberg et al. 2007) with diurnal fat yield ($R^2=0.24$). It has been suggested that the increase in the FGS of buffalo milk may be due to the enhanced cell metabolism of the buffalo mammary gland and ability to produce milk FG (Schafberg et al. 2007). The creaming of BM was poorer and affected more by temperature than CM due to the weak clustering ability of buffalo FG (Abo-Elnaga et al. 1966). The BM fat globule differed markedly from FG of other ruminants (El-Zeini 2006) in its rheological parameters. The respective parameters of buffalo and cow milk FG were: compactness 0.71 and 0.81; sphericity 0.59 and 0.91; surface roughness 0.91 and 0.99; surface area 58.34 and 12.26 μm^2 ; length 9.85 and 4.67 μm ; width 4.15 and 2.02 μm , and orientation 107.46 and 90.79, respectively (Ganguli and Bhavadasan 1980). The apparent zeta-potential of buffalo FG was reported to be -11.0 ± 0.7 mV as compared with -9.4 ± 0.6 mV for cow FG (Menard et al. 2010) which can be attributed to differences in the composition of the milk FG membrane in the two milks. Fat globules of BM are less stable and can be churned faster than that of CM (Hammad 1993). The sensitivity of buffalo milk FG was attributed to its lower Laplace pressure (0.8–1.6 kPa) in comparison to cow milk FG (1.1–2.2 kPa; Menard et al. 2010).

3.1.1 Fatty acids composition

Soliman et al. (1979) reported an average total saturated (SFA) and unsaturated fatty acids (USFA) contents of 71.7% and 28.3%, respectively, in Egyptian buffalo milk fat (BMF), which coincided with that reported for Indian buffaloes (Ramamurthy and Narayana 1971) and Italian buffaloes (Secchiari et al. 2004). A recent study (Talpur 2007) showed that BMF contained higher total SFA, C16:0 and C12:0, and lower monounsaturated fatty acids (MUFA) than cow's milk fat (CMF) under similar conditions (Table 3). The fatty acid composition of BMF was

Table 3 Fatty acid composition of buffalo and cow milk (percent of total methyl esters, nearest decimal)

Fatty acid	Cow (Talpur 2007)	Buffalo (Talpur 2007)	Cow (Menard et al. 2010)	Buffalo (Menard et al. 2010)	Buffalo		
					(Varricchio et al. 2007)	(Bergamo et al. 2003)	(Bergamo et al. 2003)
4:0	3.2–4.2	3.6–4.3	2.5±0.5	2.8±0.5	3.3–3.5	5.1	5.2
6:0	1.4–2.9	1.4–3.0	2.1±0.4	1.9±0.3	1.7–1.9	2.5	2.6
8:0	1.7–2.9	1.6–3.9	1.4±0.4	1.1±0.2	0.9–1.1	1.4	1.4
10:0	2.3–4.6	1.5–3.8	2.5±0.3	1.8±0.3	1.7–2.4	1.5	1.6
12:0	1.0–2.6	2.2–4.1	2.9±0.2	2.3±0.2	2.3–2.9	2.1	2.1
14:0	9.1–13.0	9.4–11.4	11.1±0.4	11.8±0.2	10.0–11.5	9.1	10.2
14:1c 9	0.7–1.4	0.6–0.9	1.1±0.2	0.7±0.01	0.9–1.2	0.5	0.6
15:0	–	–	1.2±0.03	1.74±0.08	0.9–1.2	1.1	1.2
16:0	24.7–28.8	25.7–32.3	33.8±0.9	36.0±1.2	29.4–32.4	26.8	29.6
16:1c 9	1.0–2.8	1.0–2.8	1.6±0.05	1.9±0.02	1.4–1.9	1.7	1.9
17:0	0.4–0.7	0.4–0.7	0.6±0.05	0.8±0.02	–	0.5	0.5
18:0	8.9–13.9	8.6–14.5	11.1±0.9	9.9±0.2	10.3–14.0	10.7	10.0
18:1c 9	23.3–26.4	21.9–26.2	22.1±1.7	20.3±0.7	20.2–33.5	17.8	17.4
16:1t 9	0.2–0.6	0.2–0.6	–	–	–	–	–
18:1t 11	1.7–3.1	1.7–2.7	0.2±0.1	0.1±0.03	1.8–2.1	–	–
18:2t 9 t 12	0.2–0.6	0.2–0.7	–	–	–	–	–
18:2c 9 t 11	0.4–1.0	0.3–0.6	0.7±0.02	0.9±0.1	–	0.7	0.5
18:2t10c12	0.03–0.05	0.02–0.03	–	–	–	–	–
18:2c9 c 12	1.0–2.1	1.0–2.2	1.34±0.1	0.9±0.1	2.0–2.9	1.6	1.3
18:3 n-3	0.5–1.1	0.5–1.2	0.6±0.03	0.7±0.2	1.0–1.4	0.3	0.2
20:4 n-6	0.1–0.5	0.1–0.2	–	–	–	–	–
20:5 n-3	0.1–0.4	0.1–0.3	–	–	–	–	–
22:6 n-6	0.1	0.1–0.2	–	–	–	–	–
20:0	–	–	0.1±0.06	0.2±0.03	–	–	–
SCFA	8.6–14.5	8.0–14.9	–	–	–	–	–
SFA	55.7–67.5	62.1–70.2	69.6±1.7	70.8±0.8	–	–	–
MUFA	25.0–30.3	24.0–29.4	–	–	–	–	–
PUFA	2.7–3.0	2.3–3.9	–	–	–	–	–
CLA	0.5–1.1	0.4–0.6	0.7±0.02	0.9±0.1	–	–	–

SCFA short-chain fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *CLA* conjugated linoleic acid

affected by breed and stage of lactation (Talpur 2007), organic and conventional farming (Bergamo et al. 2003), as well as feed (Varricchio et al. 2007). Despite of the differences in the fatty acid composition between buffalo and cow milk, the atherogenic index of the two fats was nearly the same at 1.92–2.56 and 1.77–2.55 for BM and CM fats, respectively (Talpur 2007), in Pakistan and 2.15–2.61 (Varricchio et al. 2007) for Italian BMF. Special interest has been given to the conjugated linoleic acid (CLA) content of milk due to its potential health effects. The CLA content of BMF ranged widely from a minimum of 0.39% for total CLA and 0.29% *cis*-9, *trans*-11 isomer in Bulgaria (Mihaylova and Peeva 2007), 0.48 g·100 g⁻¹ fat for river buffalo in Argentina (Gonzalez et al. 2004) to 0.77 g·100 g⁻¹ fat for

Murrah buffaloes (Tyagi et al. 2007) fed on green fodder, to $0.46\text{--}0.66\text{ g}\cdot 100\text{ g}^{-1}$ fat (Sindhu and Roy 1982) and $0.51\text{--}1.06\text{ g}\cdot 100\text{ g}^{-1}$ fat (Varricchio et al. 2007) for Italian buffaloes, 0.90 ± 0.06 and $0.70\pm 0.02\text{ g}\cdot 100\text{ g}^{-1}$ fat for buffaloes and cows, respectively (Menard et al. 2010) in France and 0.8 and $0.71\text{ g}\cdot 100\text{ g}^{-1}$ fat for Kundi and Nili Ravi buffaloes in Pakistan (Talpur 2007). Under similar conditions of milk production, BMF contained less CLA than cow's (Talpur 2007), while Kumar and Kansal (2005) found buffalo (Murrah) milk fat to contain a greater ($15.5\text{ mg}\cdot\text{g}^{-1}$ fat) CLA content than CMF ($9.2\text{--}13.1\text{ mg}\cdot\text{g}^{-1}$). These variations reflected the differences in the genetic and environmental factors of the lactating animals and milk production, respectively. The Δ^9 desaturase enzyme activity is an index for the CLA level in milk. In buffalo mammary gland, Δ^9 desaturase enzyme activity was greater on pasture grazing which can explain the differences in CLA of BM on different rations (Fernandes et al. 2007).

3.2 Triglycerides

Buffalo milk fat was separated into high (HMT), medium (MMT), and low (LMT) molecular weight triacylglycerol by thin-layer chromatography (Arumughan and Narayanan 1982). The LMT was unique in containing almost completely the C4:0 present in whole BMF. Using low temperature crystallization and lipase hydrolysis, it has been revealed that oleic acid, non-conjugated dienes (linoleic), and trienes (linolenic acid) were preferentially esterified in the 1- and 3-position of BMF (El-Sadek et al. 1972). However, FA distribution was non-random in buffalo milk triglycerides with SFA prevalently esterified in third position while MUFA in the second position (Blasi et al. 2008). Using GLC methods, triglycerides (TGs) of BMF consist of carbon chains lengths of C24 and C54 (Kuzdzal and Savoie 1980; Fatouh et al. 2005; El-Shibiny et al. 2005). The TG pattern of BMF shows two maximums at C38 and C50, respectively, similar to the TG pattern of CMF. Grouping the TGs into short (C26–C34), medium (C36–C44), and long (C46–C54) chains revealed a higher percentage of medium-chain TGs in BMF while CMF contained more long-chain TGs (El-Shibiny et al. 2005).

3.3 Cholesterol

The cholesterol content of BM in Pakistan ranged from $8.89\text{--}10.24\text{ mg}\cdot 100\text{ mL}^{-1}$ (Talpur 2007), $9.18\text{--}12.7\text{ mg}\cdot 100\text{ mL}^{-1}$ (Zicarelli 2004) and $14.2\text{--}16.0\text{ mg}\cdot 100\text{ g}^{-1}$ (Mattera et al. 2007) in Italian buffaloes and $9.18\text{--}12.7\text{ mg}\cdot 100\text{ g}^{-1}$ in lactating buffaloes in Nepal (Hayashi et al. 2005), and $8.06\text{--}17.96\text{ mg}\cdot 100\text{ mL}^{-1}$ in Indian breeds of buffalo (Sharma et al. 1996). Although BM contains higher fat contents, it had slightly less cholesterol than bovine milk which can be attributed mainly to differences in the size of the FGS in the two species. Levels of 2.62, 3.47, and $3.54\text{ mg}\cdot\text{g}^{-1}$ fat have been reported (Prasad and Pandita 1990) for cholesterol in Murrah buffalo, Haryana and Sahiwal cows milk respectively. Also, Fatouh et al. (2005) reported an average cholesterol of $2.80\pm 0.02\text{ mg}\cdot\text{g}^{-1}$ fat in Egyptian butter oil.

3.4 Milk Fat Globule Membrane

The phospholipid (PL) content of milk is a function of fat content and FGS. A significant correlation ($R\ 0.65$) was found between the PL and fat content of buffalo

milk (Hofi et al. 1973). The PL content of BM was slightly higher ($0.42 \text{ g} \cdot 100 \text{ g}^{-1}$ fat) in summer time than in winter time ($0.38 \text{ g} \cdot 100 \text{ g}^{-1}$ fat; Hofi et al. 1973) due to differences in the FGS in the two seasons. Also, the PL content of BM was affected by the individual animal and lactation period (Mahran et al. 1973). The reported amount and composition of buffalo milk fat globule membrane (MFGM) differed in the literature (Table 4) which may be due to the season, stage of lactation, and breed of the animal. Seasonal variations in the amount but not the composition of MFGM (Asker et al. 1978) and by the stage of lactation (Hofi et al. 1977b) were reported mainly due to changes in the FGS. The total SFA content of buffalo MFGM from different breeds varied from 66.20 to 78.33 and the USFA from 21.65% to 33.47% (Beri et al. 1984; Sharma et al. 1994). The presence of membranes in buffalo skim milk was ascribed to fragments, microvilli, and membrane-bound vesicles (Sharma et al. 1996). The protein/lipids ratios of the isolated milk membranes varied from 3.17:1 to 4.70:1, but the total neutral and polar lipids were very similar in the different seasons (Sharma et al. 2001).

Buffalo milk contained bioactive gangliosides not present in cow milk which showed a GM1-specific binding to cholera toxin subunit B and anti-inflammatory activity (Colarow et al. 2003).

Table 4 Amount and composition of MFGM of buffalo and cow milk (values to the nearest decimal)

Constituent	Buffalo (Beri et al. 1984)	Cow (Beri et al. 1984)	Buffalo (Hofi et al. 1977a)	Cow (Hofi et al. 1977a)	Buffalo (Menard et al. 2010) ^a	Cow (Menard et al. 2010) ^a
MFGM ($\text{g} \cdot 100 \text{ g}^{-1}$ fat)	1.40	1.47	1.57	1.97	ND	ND
Protein ^b	58.2	57.8	33.1	32.7	ND	ND
Lipids ^b	38.5	36.7	66.9	67.3	ND	ND
Neutral lipids ^c	74.4	65.7	69.5	57.3	ND	ND
Polar lipids ^c	19.7	18.6	30.5	29.3	$2,627 \pm 496$	$3,641 \pm 748$
Posphatidylinositol	0.8	0.7	0.7	0.7	276 ± 46	377 ± 68
Phosphatidylserine	3.1	2.4	0.9	1.0	298 ± 73	442 ± 101
Sphingomyelin	4.3	3.2	7.8	7.9	652 ± 132	970 ± 151
Phosphatidylcholine	5.3	5.4	10.3	9.5	624 ± 84	784 ± 100
Phosphatidylethanolamine	6.3	6.7	10.7	9.8	777 ± 175	$1,069 \pm 269$
Cerebrosides	—	—	0.1	0.2	ND	ND
Monoglycerides	4.1	3.6	2.2	2.2	ND	ND
Cholesterol	3.6	3.0	3.7	3.3	ND	ND
1,2 Diglycerides	7.4	5.5	3.3	3.7	ND	ND
1,3 Diglycerides			2.0	2.8	ND	ND
Free fatty acids	4.8	4.4	4.2	4.3	ND	ND
Short-chain triglycerides	25.4	23.4	12.6	13.3	ND	ND
Long-chain triglycerides	27.3	24.6	39.7	39.2	ND	ND
Cholesterol esters	1.8	1.2	1.1	1.5	ND	ND
Squalene	—	—	0.7	0.8	ND	ND

^a Values in micrograms per gram lipids

^b Percent of total membrane materials

^c Percent of total membrane lipids

3.5 Carbonyl and lactones

Milk fat contains a wide range of carbonyl compounds and their precursor ketoglycerides as part of the delicate flavor system of milk fat. The monocarbonyl content of buffalo milk fat has been reported to be higher than that of cow milk fat (Bhat et al. 1981; Ahmed et al. 1984) while cow milk fat contained higher quantities of β -ketoglycerides (nearly twice) and methyl ketones than buffalo milk fat (Bhat et al. 1981). Colostral fat contained 60–70% of carbonyls in normal milk fat which increased rapidly during early lactation (Bhat et al. 1981). A total of $16.8 \mu\text{g}\cdot\text{g}^{-1}$ (3.0 of δ -10, 6.3 of δ -12, and 7.3 of δ -14, respectively) of lactones has been reported in BMF (Fatouh et al. 2005).

3.6 Physical properties and crystallization of milk fat

Buffalo milk fat has been reported to melt over a higher but narrower temperature range (11 – 38 °C) than CMF (5 – 35 °C) due to the considerably higher proportion of high melting components in BMF as compared with CMF (Patel and Frede 1991). No differences were found in the microscopic crystal structure of BMF and CMF (El-Ghandour et al. 1976), although BMF developed larger-size grains (0.20 – 0.41 mm) than CMF (0.098 – 0.190 mm) during the manufacture of ghee (Gokhale et al. 2001). BMF and CMF exhibit similar polymorphism when affected by the cooling treatment (Helal et al. 1977). Three phases namely; α , β , and β' similar to that of CMF were identified in the high melting fraction of BMF in addition to a new phase, termed δ (Rifaat et al. 1973) characterized by a strong X-ray reflection at 14.88 Å spacing. The rheological properties of BMF are affected by season (El-Nimr et al. 1979). Plastic viscosity and yield values of 5.5×10 dyne $\cdot\text{cm}^{-2}$ and 0.25×10 poise were recorded in summer BMF samples and 8×10 dyne $\cdot\text{cm}^{-2}$ and 0.55×10 poise in winter BMF. Also, the solid fat (T%) and melting coefficient (D) determined during stepwise heating from 0 °C to 50 °C revealed that the polymorphic transformations were weaker and occurred over a narrower range of temperature in CMF than in BMF (El-Nimr and El-Ghandour 1980). The differential scanning calorimetry (DSC) curve of BMF and CMF can be divided into low, medium, and high temperature melting regions. In BMF, the area of low melting region was larger than the area of the medium temperature melting region (≥ 1.6 -fold), but the two areas were nearly equal in CMF (Tunick and Malin 1997).

4 Proteins

4.1 Major milk proteins

The major proteins of BM are: α_{s1} -casein (α_{s1} -CN), α_{s2} -casein (α_{s2} -CN), β -casein (β -CN), κ -casein (κ -CN), β -lactoglobulin (β -LG), and α -lactalbumin (α -LA; Table 5). Compared with CM, BM contained higher α_{s2} -CN and κ -casein (Table 5). The larger quantities of κ -CN in BM can be considered as a factor to speed up the enzymatic phase of rennet coagulation and the requirement of smaller quantity of chymosin in cheese manufacture. Also, the optimal curd elasticity for Mozzarella

Table 5 Concentration and some properties of the main proteins of buffalo milk

Protein (abbreviation)	Content	No. of P atoms	Genetic variants	Ip	MW	Ref.
α -s1-casein (α -s1-CN)	8.89 \pm 2.15 ^a	6, 7, 8	A, B	4.24–4.63	22,773	(Felgini et al. 2009)
	24.8% ^b					(D'Ambrosio et al. 2008)
	31.0 \pm 2.0 ^c					(Bramanti et al. 2003)
	30.2 ^c					(Addeo et al. 1977)
α -s2-casein (α -s2-CN)	5.08 \pm 1.56 ^a	10, 11, 12, 13	–	4.84–5.13	24,700	(Felgini et al. 2009)
	11.9% ^b					(D'Ambrosio et al. 2008)
	13.0 \pm 2.0 ^c					(Bramanti et al. 2003)
	17.6 ^c					(Addeo et al. 1977)
α -s1-CN + α -s2-CN	44.1 ^c	5	A, B	4.55–5.09	23,582	(Mehanna et al. 1982)
β -casein (β -CN)	47.8 ^c					(Addeo et al. 1977)
	20.91 \pm 0.75 ^a					(Felgini et al. 2009)
	24.9 ^b					(D'Ambrosio et al. 2008)
κ -casein (κ -CN)	28.0 \pm 2.0 ^c	1, 2	–	4.80–6.85	19,247	(Bramanti et al. 2003)
	30.3 ^c					(Mehanna et al. 1982)
	33.9 ^c					(Addeo et al. 1977)
	4.13 \pm 1.53 ^a					(Felgini et al. 2009)
β -Lactoglobulin (β -LG)	10.8 ^b	–	A, B, C	4.71–6.77	18,287	(D'Ambrosio et al. 2008)
	7.0 \pm 1.0 ^c					(Bramanti et al. 2003)
	14.0 ^c					(Mehanna et al. 1982)
	15.4 ^c					(Addeo et al. 1977)
α -Lactalbumin (α -LA)	7.7 ^b	–	A, B	4.55–4.66	14,244	(D'Ambrosio et al. 2008)
	50.3 ^d					(Mohran 1991)
Serum albumin (SA)	4.8 ^b	–	A, B	4.44–4.74	69,293	(D'Ambrosio et al. 2008)
	16.2 ^d					(Mohran 1991)
Immunoglobulin (Ig)	10.0 ^d					(Mohran 1991)
	8.7 ^d					(Mohran 1991)

Ip isoionic point, MW molecular weight (theoretical) (D'Ambrosio et al. 2008)

^a In grams per liter of skim milk

^b Percent of total gel absorbance

^c Percent of total casein

^d Percent of total whey proteins

cheese manufacture was obtained for BM at pH 4.9 and for CM at pH 5.0–5.2 (Addeo et al. 2007). The relative concentration of whey proteins in BM was similar to that of CM, i.e., B-LG is the major protein (>50% of total whey (Mohran 1991). The relative distribution (in percent) of casein and whey proteins in BM was affected by its somatic cell count (Pasquini et al. 2003). Buffalo milk containing high (>1,500,000), medium (\pm 211,000), and low (13,000) somatic cell per milligrams had

the immunoglobulins, serum bovine albumin, casein, β -lactoglobulin, and α -lactalbumin contents shown in Table 6 (Pasquini et al. 2003). In addition to the quantitative differences in the content of the main protein fractions between buffalo and cow milk, qualitative differences have been found in the amino acid sequence of the main proteins of BM and other ruminants. However, high percentages of homology were found between buffalo and cow main milk proteins; both have similar number of amino acid residues and variable amino acid substitutions in their polypeptide chains. Table 7 summarizes the substitutions in the amino acid residues of the main proteins of buffalo and cow milks. Based on the amino acid composition, buffalo α_s -CN and β -CN were more hydrophobic than their bovine counterpart. Woodward (Yousef et al. 1983) calculated the hydrophobicity of buffalo α_s -CN and β -CN as 1,190 and 1,360, respectively and for cow α_s -CN and β -CN hydrophobicities as 1,170 and 1,330, respectively. On the other hand, buffalo κ -CN was less hydrophobic (1,200) than cow κ -CN (1,220) (Woodward 1976).

4.1.1 α_{s1} -CN

α_{s1} -CN of BM consists of a single 199 residue polypeptide chain of high homology to variant B of α_{s1} -CN of bovine milk (D'Ambrosio et al. 2008). Only 9 substitutions were found between buffalo and cow α_{s1} -CNs (Table 7) which correspond to 97.2 % homology (Sukla et al. 2006; D'Ambrosio et al. 2008). However, the buffalo α_{s1} -CN exhibited lower mobility in alkaline PAGE than its bovine counterpart but with isoelectric focusing, they had similar pI (Chianese et al. 2009). The primary structure of water buffalo α_{s1} -CN (Ferranti et al. 1998; D'Ambrosio et al. 2008) was different to that resulting from nucleotide sequencing of Indian river buffalo α_{s1} -CN cDNA (Genbank Accession No. 062823) for the amino acid substitution Leu¹⁷⁸ (Italian Buffalo) \rightarrow Ser¹⁷⁸ (Indian buffalo). Three isoforms were found in buffalo α_{s1} -CN containing 8, 7, and 6 phosphate groups, respectively (Ferranti et al. 1998). Phosphorylation occurs in similar sites as in cow α_{s1} -CN, i.e., at Ser 41, 46, 48, 64, 66, 67, 68, and 75 respectively but not at Ser 115 as in cow α_{s1} -CN being surrounded with hydrophobic amino acids. The absence of phosphorylation at Ser 115 strengthens the hydrophobic nature of buffalo α_{s1} -CN (Ferranti et al. 1998). This may explain the higher sensitivity of buffalo α_s -CN to Ca^{++} in comparison to that of cow milk (Abd El-Salam 1975). Generally, buffalo α_{s1} -CN showed reduced phosphorylation in comparison to α_{s1} -CN of other ruminants (Ferranti et al. 1998; Chianese et al. 2009; D'Ambrosio et al. 2008). Buffalo α_{s1} -CN has long been regarded as monomorphic, but a new genetic variant

Table 6 Effect of somatic cell count on the contents of different proteins in buffalo milk (mean \pm SE; Pasquini et al. 2003)

Somatic cell count (cell \cdot mL ⁻¹)	Ig	BSA	Casein	β -LG	α -LA
>1,500,000	0.44 \pm 0.20	0.74 \pm 0.15	69.51 \pm 5.29	16.31 \pm 2.47	8.65 \pm 2.33
\pm 211,000	0.47 \pm 0.36	1.35 \pm 0.85	70.16 \pm 9.01	14.88 \pm 4.42	9.56 \pm 3.79
13,000	0.04 \pm 0.06	0.34 \pm 0.09	76.48 \pm 2.13	12.41 \pm 1.10	7.05 \pm 1.91

Ig immunoglobulins, BSA blood serum albumin, β -LG β -lactoglobulin, α -LA α -lactalbumin

Table 7 Differences in the amino acid sequence of the main proteins of buffalo and cow milk (D'Ambrosio et al. 2008)

Amino acid sequences															
α_{s1} -CN		4	14	42	74	115	119	148	174	192					
	Buffalo	Gln	Gly	Thr	Ile	Leu	Gln	Gln	Pro	Gly					
	Cow	His	Glu	Lys	Asn	Ser	Arg	Glu	Thr	Glu					
α_{s2} -CN		2	29	44	147	157	170	175	176	182	199				
	Buffalo	His	His	Ile	Ile	Asp	His	Thr	Try	Tyr	Asn				
	Cow	Asn	Asn	Val	Phe	Glu	Arg	Ala	Leu	His	Lys				
β -CN		25	41	57	84	108	164								
	Buffalo	His	Met	Met	Lys	Ile	Pro								
	Cow	Arg	Thr	Thr	Asn	Val	His								
κ -CN		14	19	80	96	126	128	138	140	147	149	156	162	168	
	Buffalo	Glu	Asn	Pro	Thr	Val	Val	Ile	Asn	Ala	Ser	Val	Ala	Val	
	Cow	Asp	Ser	Ser	Ala	Ala	Gly	Val	Ser	Asp	Pro	Pro	Val	Ala	
β -LG		1	163												
	Buffalo	Ile	Val												
	Cow	Leu	Ile												
α -LA		17													
	Buffalo	Asp													
	Cow	Gly													

has been discovered in the Italian buffaloes (Chianese et al. 2009). The two genetic variants were termed buffalo α_{s1} -CN A and B having a single silent amino acid substitution Leu¹⁷⁸(A)→Ser¹⁷⁸(B) with allelic frequencies of 0.31 and 0.69, respectively (Chianese et al. 2009).

4.1.2 α_{s2} -CN

Buffalo α_{s2} -CN is a single polypeptide chain of 207 residues in length and has very high homology (97.9%) compared with cow α_{s2} -CN (Sukla et al. 2007; D'Ambrosio et al. 2008). Also, the genetic sequence of Indian river buffalo α_{s2} -CN has been deposited (Genbank Acc No 062825). Only ten substitutions were found between buffalo and cow α_{s2} -CN (Table 7). Chianese et al. (1996) reported the presence of three phenotypes of buffalo α_{s2} -CN that differ only in their phosphorus content, while D'Ambrosio et al. (2008) found buffalo α_{s2} -CN isoforms containing 13, 12, 11, and 10 phosphate group and that phosphorylation occurred at similar sites as in cow α_{s2} -CN, i.e., Ser 8, 9, 10, 16, 31, 53, 56, 57, 58, and 61, respectively.

4.1.3 β -CN

β -CN of BM is a single polypeptide chain of 209 residues. Several isoforms have been identified (D'Ambrosio et al. 2008) in β -CN of BM which contained five, four, three, two, and one phosphate group respectively (at Ser 15, 17, 18, 19, and 35). The

lack of putative phosphorylation sites within the buffalo β -CN amino acid sequence resulted in a reduced degree of phosphorylation of this protein with respect to that observed in β -CN of other ruminants (Ferranti et al. 1998). The complete sequence of buffalo β -CN has been determined by a combination of mass spectrometry and Edman degradation (Ferranti et al. 1998) and by DNA sequencing in Indian and German water buffalo (Klotz et al. 2000). Both cow and buffalo β -CN have a very similar amino acid composition and sequence. Only six single substitutions were found in the two β -CNs (Table 7) indicating high homology (95%) between the two proteins. Two genetic variants (A and B) were identified in buffalo β -CN (Ferranti et al. 1998). These two variants differed in two amino acid substitutions PThr⁴¹(A)→Met⁴¹(B) and Asn⁶⁸(A)→Lys⁶⁸(B). Buffalo β -CN B variant showed similar mobility in PAGE and a pI similar to that of cow β -CN A variant. Peptides released from buffalo and cow β -CN by trypsin were not identical (Abd El-Salam and El-Shibiny 1975) and buffalo β -CN was hydrolyzed by both chymosin and *Mucor miehei* protease at a slower rate than cow β -CN, and the resultant peptides were found to be unidentical (El-Shibiny and Abd El-Salam 1976). Plasmin hydrolyzed a less sensitive bond at position 68–69 on buffalo β -CN and released a peptide (f 69–209) which had no counterpart in cow β -CN (Di Luccia et al. 2009).

4.1.4 κ -CN

κ -CN is the casein fraction characterized by having a carbohydrate moiety and all the *N*-acetylneuramenic acid present in casein micelles. An early study (Abd El-Salam and Manson 1966) showed differences in the C-terminal amino acid sequence of buffalo and cow κ -CN. κ -CN consists of a single polypeptide chain of 169 amino acid residues in both buffalo and cow κ -CNs. Buffalo/cow κ -CNs showed substitutions in 13 sites (Table 7) and 92.6% homology. Six to seven components were separated from buffalo κ -CN (Addeo et al. 1977; Mehanna et al. 1983). Buffalo κ -CN had less sialic acid content than cow κ -CN (Addeo et al. 1977; Mehanna et al. 1983). The carbohydrate free fraction of κ -casein represents 40% of κ -CN in buffaloes where it accounted for only 25% of total κ -CN in cow's milk (Addeo et al. 1977) which explains the low sialic acid content of buffalo κ -CN. Based on sequence analysis and coding region, Mukesh et al. (2006) concluded that the κ -CN of Indian riverine buffalo seems to be an intermediate between A and B variants of cow κ -CN. On the other hand, Abdel Dayem et al. (2009) demonstrated that Egyptian buffalo bulls were monomorphic for the κ -CN gene and that they possessed the only B allele. Del Lama and Zago (1996) found that Brazilian buffaloes had only κ -CN B allele.

4.1.5 β -LG

The amino acid composition and sequence of buffalo β -LG were similar to that of cow β -LG B (Braunitzer et al. 1979) except in its C- and N-terminal acids (Table 7). Also, buffalo and cow β -LG had striking similarities in structure and solution conformation (Ghosh et al. 2004; Shazly et al. 1973b). The heat denaturation of buffalo and cow β -LG followed a second-order reaction with comparable heat of activation, free energy, and entropy for heat denaturation (Shazly et al. 1973a). Also,

a strong cross-reactivity between buffalo and cow β -LG was apparent from indirect ELISA, Western blotting, and inhibition ELISA indicating that β -LG from both species share similar epitopes (Li et al. 2008). The presence of three alleles (A, B, and C) were reported in Murrah, Bhadawari, and Mehsana breeds from the nucleotide sequence (Vohra et al. 2006). Del Lama and Zago (1996) found that Brazilian buffaloes had only β -LG B allele.

4.1.6 α -LA

Buffalo α -LA differs from cow α -LA variant B in a single amino acid residue, i.e., Asp¹⁷→Gly¹⁷ (Table 7) as revealed from amino acid sequence analysis (Chianese et al. 2004). This substitution may explain the slower mobility on PAGE of buffalo α -LA compared with cow α -LA. Also, buffalo α -LA retained the compact ellipsoidal structure with a small hydrophobic box as bovine α -LA since one bound Ca⁺⁺ and four disulphide bridges were conserved (Calderone et al. 1996). It has long been believed for buffalo α -LA to be monomorphic, but a new variant (buffalo α -LA A) was discovered in a low allelic frequency (0.5%) in Italian buffaloes which differed from the B variant in a single amino acid substitution, Asn⁴⁵(B)→Asp⁴⁵(A) (Chianese et al. 2004). As this amino acid substitution altered the N-glycosylation sequence consensus Asn⁴⁵-x-Ser⁴⁶, the protein glycosylation level of α -LA A would decrease. Similar to cow α -LA, a non-glycosylated and a glycosylated form were found in buffalo α -LA (Chianese et al. 2004). Dayal et al. (2005) found two fragments of α -LA gene to be polymorphic in Bhadwari, Mehsana, Surti, and Murrah riverine buffalo breeds and that genotypes and alleles varied between breeds.

4.2 Minor proteins

D'Ambrosio et al. (2008), in their proteomic study, identified the presence of several minor proteins in whey which they classified according to their function into:

1. Defense/immunity components which include lactoferrin, IgM heavy chain, Ig λ chains, polymeric immunoglobulin receptor, complement C3, cluster of differentiation CD14, Zn- α 2 glycoprotein, factor XIIa inhibitor, inter α -trypsin inhibitor, and nucleobindins 1 and 2.
2. Transport components which include albumin, folate-binding protein, and mammary-derived growth inhibitor.
3. Metabolic components which include lactoperoxidase and 1,4 galactosyl transferase.

The presence of a free secretory component in buffalo milk whey was reported (Kaur and Bhatia 1989) with an average level of 7.09 mg·L⁻¹ whey. However, limited numbers of minor whey components were studied in detail. The following were the subject of more detailed studies:

4.2.1 Immunoglobulins

Immunoglobulins of BM consisted of IgG as the major Igs, IgM, and IgA with respective concentrations of 8.71, 1.91, and 0.04 mg·mL⁻¹, respectively (El-Loly et

al. 2007). Determination of IgG by immunosensors (Campanella et al. 2009) revealed averages of IgG at 0.67 and 0.77 mg·mL⁻¹ in buffalo and cow milks, respectively. Interestingly, it was reported (El-Loly et al. 2007) that IgG and IgM were not completely denatured in BM upon heating up to 88 °C which may be due to the protective effect of the high protein content of BM.

4.2.2 Lactoferrin

The lactoferrin (LF) content of buffalo and cow colostrum were extremely high but reached the normal level in mature milk after 15–30 days of parturition (Abd El-Gawad et al. 1996; Campanella et al. 2009). The LF of buffalo milk varies widely from 0.05 mg·mL⁻¹ (Abd El-Gawad et al. 1996) in Egyptian buffaloes to 3.40 mg·mL⁻¹ in Indian Murrah buffaloes (Maheshwari and Bhatia 1990) and in Italian buffaloes, 0.23 mg·mL⁻¹ (Campanella et al. 2009). In addition to differences in breeds, the method of analysis affected greatly the measured LF content of milk (Campanella et al. 2009). The iron and iron saturation of LF from colostrum were much lower than that of mature milk with levels of 0.12–0.20 mg·g⁻¹ and 8–14% in colostrums being reported which reached 1.10–1.50 mg·g⁻¹ and 77–104%, respectively, in normal milk (Mahfouz et al. 1997). Also, the composition of carbohydrate moieties of buffalo and cow LF were nearly the same (Mahfouz et al. 1997). The peptic hydrolyzates of lactoferrin at pH 2.5 had an antibacterial effect on *Bacillus subtilis*. However, cow lactoferrin hydrolyzates were more effective than that of buffalo's (Sangwan et al. 2007).

4.3 Casein micelles

The caseins of BM were almost completely (90–95%) present in the micellar form (Rifaat et al. 1969a). Buffalo milk had larger micelles than CM (102, 152, 17). The average micellar size of BM, as measured by a simple turbidimetric method, was 86.5 nm (Ibrahim et al. 1973b) as compared with 71.4 nm for CM (Ibrahim et al. 1973b). The size of buffalo casein micelles increased from 55 nm in the colostrum to 85 nm after 1 month of lactation and then decreased to 75 nm at the end of lactation period (Salama et al. 1978). Using particle size analyzer, casein micelles from buffalo and cow milk showed normal distribution curve ranging from 30 to 400 nm with similar average of about 180 nm (Ahmad et al. 2008, 2009). El-Zeini (2001) reported that buffalo casein micelles were roughly spherical of large sizes (320–400 nm) as measured by scan electron microscopy and image analysis. The heat treatments of casein micelles increased the micellar size (480–560 nm) and their surface roughness, intensity, and compactness (El-Zeini 2001). The increase in casein micelle size of buffalo and cow's milk by heat treatment was demonstrated by gel filtration (Abd El-Salam et al. 1978b). Also, BM had a greater percentage of larger micelles than CM (Ooman and Ganguli 1973; Abd El-Salam et al. 1978a). The casein micelles of BM had more Ca⁺⁺ (2.85–3.58 g·100 g⁻¹), inorganic P (1.42–1.85 g·100 g⁻¹), Mg⁺⁺ (0.12–0.19 g·100 g⁻¹), and citric acid (0.61–0.77 g·100 g⁻¹) than cow casein micelles with values of 2.52–2.98, 1.11–1.48, 0.10–0.15, and 0.52–0.67 g·100 g⁻¹, respectively (Abd El-Salam et al. 1978a).

Buffalo milk casein micelles were less hydrated than bovine casein micelles (Ahmad et al. 2008, 2009). The amounts of water associated to the ultracentrifuged casein pellet from buffalo and cow milk were 1.9–2.0 and 2.24–2.3 g·g⁻¹ of dry pellet, respectively (Ahmad et al. 2008, 2009). The dissociation/aggregation changes of buffalo and cow casein micelles as a function of added alkali or acid were qualitatively similar but quantitatively different (Ahmad et al. 2009). The dissociation took place at pH 9.7 and 8.6 for buffalo and cow milks, respectively (Ahmad et al. 2009). It was suggested that buffalo casein micelles are assembled in a similar way to cow casein micelles (Abd El-Salam et al. 1978a).

4.4 Bioactive peptides

Cow milk proteins are good sources of bioactive peptides which have been reported to have various physiological effects (Clare and Swaisgood 2000). However, studies on buffalo milk proteins as sources of bioactive peptides have received much less attention. Based on the similarity of the amino sequences of β -LG and α -LA of buffalo and cow milks, it is expected that they would yield similar bioactive peptides. However, no literature on bioactive peptides from buffalo whey proteins has been cited.

On the other hand, reports on bioactive peptides from buffalo milk caseins have been cited but are very limited. Treatment of a beta-casomorphine-containing fragment from buffalo β -casein (residues 49–68) with pancreatic proteases was not able to release β -casomorphine (Petrilli et al. 1987). An angiotensin I-converting enzyme inhibitory peptide corresponding to β -CN 58–66 was produced by the action of *Lactobacillus helveticus* PR4 proteinase on buffalo milk casein (Minervini et al. 2003). Cationic peptides were separated from the peptic digest of α_{s1} - and α_{s2} -caseins of buffalo milk (Bajaj et al. 2005) which had antibacterial activity. However, the antibacterial activity of α_{s1} - derived cationic peptide was greater than that of α_{s2} - against both Gram-positive and Gram-negative organisms. De Simone et al. (2009) reported the presence of β -CN f57-68 and f60-68 which are precursors of the agonist opioid β -casomorphines 7 and 5, respectively, in buffalo whey of Mozzarella (BWW) cheese. Also, they (De Simone et al. 2009) found that the peptides in BWW exerted a significant antiproliferative effect on CaCo2 cell line. More research along these lines is needed in order to fully explore buffalo milk proteins as potential sources of bioactive peptides.

4.5 Milk Fat Globule Membrane Proteins

The MFGM contains a large number of heterogeneous proteins with diversified functions. The polypeptide products found in the MFGM fraction of BM were investigated using a proteomic approach (D'Ambrosio et al. 2008). Out of the 50 proteins identified, 65% were membrane-associated proteins, with the remainder being cytoplasmic (25%) and secreted (10%) polypeptides (D'Ambrosio et al. 2008). Several of these proteins were found in multiple species that arose due to limited proteolysis of the native protein and/or type and level of glycosylation and phosphorylation (D'Ambrosio et al. 2008). Similar to the MFGM of other mammals, the most abundant protein species identified in buffalo MFGM were xanthine dehydrogenase/oxidase, butyrophilin, adipophilin, lactadherin, platelet glycoprotein 4, and mammary-derived growth inhibitor (D'Ambrosio et al. 2008). However, the

homologies of buffalo MFGM proteins with that of other ruminants remain to be explored. A single study on butyrophilin gene revealed close homology between the MFGM proteins of cow and buffalo milk (Bhattacharya et al. 2004).

5 Enzymes

The milk of different mammals contain a large number of enzymes, some of which are of technological significance, e.g., markers of heat treatments and milk preservation (anti-microbials) and can result in undesirable changes during storage. Limited studies have been performed on the enzymes of BM. However, many enzyme activities have been detected in BM, and some were isolated and partially characterized. Generally, it was difficult to compare the obtained results obtained from different studies due to the use of different methodologies.

5.1 Lipases

The lipase activity of bulk and individual buffalo's milk were 1.39 ± 1.01 and 1.43 ± 1.81 unit·mL⁻¹, respectively (Hofi et al. 1976) and 3.73 ± 0.03 unit·mL⁻¹ in bulk samples from Murrah buffaloes (Srivastava et al. 1989). The lipase activity of BM increased gradually during the lactation period from 0.86 unit·mL⁻¹ in the first month to a maximum of 2.48 unit·mL⁻¹ at the sixth month (Hofi et al. 1976). Also, lipase activity of BM was affected by feeding system (high in dry compared with green feeding), decreased with increased number of lactations, but no differences have been reported between morning and afternoon milking (Abd El-Hamid et al. 1977). However, feeding-protected fat (Srivastava et al. 1989) had no significant effect on lipase activity of BM.

5.2 Lysozyme

The reported concentration and properties of lysozyme in BM are controversial. Lysozyme was present at lower concentration in BM (average 15.21 µg·100 mL⁻¹) in comparison to CM (average 18.00 µg·100 mL⁻¹; Kumari and Mathur 1981), while a more recent study (Kansal and Priyadarshini 2002) reported a twofold lysozyme activity in BM ($60 \pm 3.9 \times 10^{-3}$ units·mL⁻¹) than in CM ($29.1 \pm 1.5 \times 10^{-3}$ units·mL⁻¹) and that buffalo colostrum showed lysozyme activity five times that of mature milk. The lysozyme activity of BM in Argentina was 2.49 ± 0.86 U·mL⁻¹ (Nieuwenhove et al. 2004). The lysozyme activity of BM increased with advanced lactation (Kumari and Mathur 1981) and in milk of buffaloes reared under extreme weather conditions (Kansal and Priyadarshini 2002). The lysozyme activity of BM decreased markedly on storage particularly at high temperature. Buffalo lysozyme had an optimum pH and temperature of 6.65 °C and 37 °C, respectively, and appears as a homogeneous protein on polyacrylamide gel electrophoresis (Kumari and Mathur 1981). The specific activity of BM lysozyme was ten times that of CM and retained full activity after 1 min at 74 °C or 30 min at 63 °C (Priyadarshini and Kansal 2003). The sequence of 23 amino acid residues at the N-terminal end of buffalo lysozyme showed a 56.5% homology with CM lysozyme (Priyadarshini and Kansal 2002). Buffalo milk lysozyme inhibited four out of seven Gram-positive

bacteria, but Gram-negative bacteria were resistant (Priyadarshini and Kansal 2002). Buffalo milk lysozyme was greatly influenced by the ionic strength and the concentration of metal ions in milk and in buffers (Priyadarshini and Kansal 2003).

5.3 Lactoperoxidase

Lactoperoxidase is the most abundant enzyme in cow and buffalo milks. The lactoperoxidase (LP) activity of BM varies widely, mainly due to the assay method used, from $0.9 \text{ U}\cdot\text{mL}^{-1}$ (Hamulv and Kandasamy 1982) to $16.84 \text{ U}\cdot\text{mL}^{-1}$ (Tayefi-Nasrabadi and Asdpour 2008). Sharma et al. (2009) found that LP was largely found free in BM and exhibited 1.76-fold higher activity ($12.02\pm0.83 \text{ U}\cdot\text{mL}^{-1}$) than LP of CM ($6.83 \text{ U}\cdot\text{mL}^{-1}$).

5.4 Alkaline Phosphatase

Contrary to early reports (Laxminaryana and Dastur 1968), more recent studies reported that BM contained higher alkaline phosphatase (ALP; $79.2\pm15.16 \text{ U}\cdot\text{mL}^{-1}$) than CM ($61.39\pm6.89 \text{ U}\cdot\text{mL}^{-1}$), but the differences were not significant (Sharma et al. 2009). Srivastava et al. (1989) reported an average value of $34.00\pm10.94 \text{ U}\cdot\text{mL}^{-1}$ for ALP of milk from Murrah buffaloes. Lombardi et al. (2000) gave a value of $295\pm164 \text{ IU}\cdot\text{mL}^{-1}$ for ALP in BM. Both studies (Lombardi et al. 2000; Sharma et al. 2009) indicated that the thermal inactivation of ALP in BM was similar to that in other milks.

5.5 Xanthine oxidase/dehydrogenase

The activity of xanthine oxidase/dehydrogenase (XO) in BM was reported to be less than in CM. Buffalo milk from Indian breeds contained slightly less (average $0.075\pm0.027 \text{ U}\cdot\text{mL}^{-1}$) XO activity than in CM (average $0.093\pm0.029 \text{ U}\cdot\text{mL}^{-1}$; Sanhotra and Dutta 1986). However, XO activity of BM from an Egyptian breed ($0.045\pm0.016 \text{ U}\cdot\text{mL}^{-1}$) was much less than that of CM ($0.070\pm0.007 \text{ U}\cdot\text{mL}^{-1}$; El-Gazzar et al. 1999). The major part (66.74%) of XO activity in BM was found in skim milk, and only 24.49% activity were found in cream (El-Gazzar et al. 1999). Heating at $55^\circ\text{C}/1 \text{ min}$ increased the XO activity of BM by 9.29%, but it decreased by 75.35% and 89.43% at $70^\circ\text{C}/1 \text{ min}$ and $80^\circ\text{C}/1 \text{ min}$, respectively (El-Gazzar et al. 1999).

5.6 γ -Glutamyltranspeptidase

Buffalo milk has been reported to contain an average γ -glutamyltranspeptidase (GGTP) activity of $8.14\pm0.51 \text{ U}\cdot\text{mL}^{-1}$ (Sharma et al. 2009) and $7.12\pm6.01 \text{ IU}\cdot\text{mL}^{-1}$ (Lombardi et al. 2000). GGTP lost most of its activity after heating to 70°C for 10 min. GGTP has been suggested as a potential marker for heat treatments of BM due to its high concentration and sensitivity to heat treatments (Lombardi et al. 2000).

5.7 *N*-acetylglucosamineidase

Buffalo milk contained slightly higher, but not significant, *N*-acetylglucosamineidase activity ($22.49\pm6 \text{ IU}\cdot\text{mL}^{-1}$) than CM ($19.86\pm4.75 \text{ IU}\cdot\text{mL}^{-1}$; Sharma et al. 2009).

5.8 Aspartate Amino Transferase

Minor aspartate amino transferase (AST) activity ($18 \pm 4 \text{ IU.L}^{-1}$) was found in BM, which maintained 50% of its activity even after 30 min at 70°C and some of its activity after heating to 80°C for 30 min (Lombardi et al. 2000).

5.9 Proteinases

A limited number of studies have measured the proteinase activity (micrograms Newton per milliliter) of BM in comparison to CM, and the results are controversial at 149.9 ± 66.9 and 103.9 ± 72.9 , respectively (Rifaat et al. 1969b), and 55 and 70.2, respectively (Manjunath and Bhat 1992). Many factors can be responsible for this discrepancy, and more controlled studies are needed to verify the proteinases activity of BM due to their importance to the ripening changes in cheeses. The presence of plasmin (Madkor and Fox 1991) and plasminogen and activators (Fantuz et al. 1998) in BM have been reported. Plasmin activity of BM was associated primarily with the casein micelles, but completely dissociated at $\text{pH} < 4.6$ (Madkor and Fox 1991). Addition of urokinase, which converts plasminogen to plasmin to buffalo milk or its micellar casein suspension increased the proteolytic activity almost 3.5- and fivefold, respectively (Madkor and Fox 1991). Addition of NaCl (3–15%) decreased the plasmin activity, and this decrease was more pronounced at high NaCl concentrations (Madkor and Fox 1991). Heating to 70°C for 10 min increased the plasmin activity of buffalo milk but lost 15% of its activity at $80^\circ\text{C}/10 \text{ min}$. Most of the plasminogen activators (PA) were associated with casein micelles (Fantuz et al. 1998). Several PAs were identified in buffalo milk: two major fractions with molecular masses of 75 and 120 kDa and two minor fractions with molecular masses of 48 and 38 kDa (Fantuz et al. 1998). The 38, 75, and 120 kDa PAs were tissue PA, and the 48 kDa was urokinase PA (Fantuz et al. 1998). It is likely that BM contains the complete plasmin system: plasmin, plasminogen, plasminogen activators (PA) and inhibitors of PA, and plasmin similar to milk of other ruminants.

5.10 Other enzymes

1. The lactic dehydrogenase activity of BM was estimated to be $386 \pm 183 \text{ IU.L}^{-1}$ and lost most activity after 10 min at 70°C (Lombardi et al. 2000).
2. Buffalo milk contained much less acid phosphatase (AP; $57.5 \pm 8.7 \mu\text{g phenol}\cdot\text{mL}^{-1}$) than cow milk ($175.8 \pm 60.5 \mu\text{g phenol}\cdot\text{mL}^{-1}$; Rifaat et al. 1969a). Acid whey of BM contained higher AP than rennet whey (Ismail et al. 1976), suggesting that the AP associated with casein was set free by acidity.
3. Both buffalo's and cow's milk showed comparable cytochrome C reductase activity being $1.5 \pm 2.7 \text{ U}\cdot\text{mL}^{-1}$ in the former and $1.16 \pm 0.76 \text{ U}\cdot\text{mL}^{-1}$ in the latter (Rifaat et al. 1969a).
4. Buffalo milk contained $64.9 \pm 5.6 \text{ U}\cdot 100 \text{ mL}^{-1}$ of amylase in comparison to $57.7 \pm 4.6 \text{ U}\cdot 100 \text{ mL}^{-1}$ in CM (Rifaat et al. 1969b).
5. The average catalase activity of BM was $10.9 \pm 4.8 \text{ U}\cdot\text{mL}^{-1}$ while that of CM was 12.1 ± 3.0 (Rifaat et al. 1969a).

6. Carbonic anhydrase activity was detected in both buffalo's and cow's milk with averages of 0.82 ± 0.62 and 0.77 ± 0.63 mL $\text{CO}_2 \cdot 100 \text{ mL}^{-1}$, respectively (Rifaat et al. 1969a).
7. The ribonuclease (RNase) activity of BM ($158.3 \pm 34.6 \mu\text{g} \cdot \text{mL}^{-1}$) was less than that found in CM, namely $225.6 \pm 35.3 \mu\text{g} \cdot \text{mL}^{-1}$ (Rifaat et al. 1969a). The optimum pH of RNase in buffalo rennet whey was 6.8 and showed high stability up to 90°C (Ismail et al. 1976).

6 Minerals

6.1 Major elements

Buffalo milk is characterized by high calcium content (about 1.5-fold Ca than in cow's milk) as was apparent from several studies, except in BM from Argentina (Patino et al. 2007) which had Ca content comparable to that of CM. It seems that the Ca content of BM was considerably affected by breed, environmental factors, and methods used for analysis. Most of the Ca was in the insoluble form (67.6–82.6% of total Ca) mainly due to the high casein content of BM (Table 8). The soluble Ca content of BM was similar to that found in CM where the aqueous phase of milk is considered to be saturated with respect to Ca. The ionizable Ca of BM represented 34.56% of the soluble Ca (Abd El-Salam and El-Shibiny 1966). The wide range of total P in BM (Table 8) reflected the various factors affecting the composition of milk and the sensitivity of the methods of analysis. The P was distributed between colloidal inorganic P (42.4% of total), soluble inorganic P (29.9% of total), casein P (17.3% of total), and organic P (9.2%) of total (Abd El-Salam and El-Shibiny 1966). The soluble Mg represents ~50% of total Mg, while most of the citrate was present in the soluble form. The Na, K, and Cl were almost completely present as soluble salts.

6.2 Trace elements

The presence of 15 elements (Zn, Fe, Cu, B, Mn, Co, Cd, Cr, Ni, Sn, Pb, As, Br, Se, and Rb) in BM as traces has been reported in different studies (Abd El-Salam 1968; Al-Jobori et al. 1990; Mathur and Roy 1981a, 1981b, 1982; Singh et al. 1981; Yousef et al. 1983). However, Zn, Fe, and Cu contents of BM have received special attention (Table 9), while the other trace elements were reported only in one or two studies. Wide variations have been reported in the levels of these elements in BM which reflected the effects of different variables on the composition of milk. Also, different methods of which had different levels of sensitivity were used in their determinations, i.e., colorimetric, atomic absorption, and spectrographic methods which add to the variability of the reported results. Variations in the distribution of trace elements have been reported between the different phases of BM. The distribution of boron ($0.5\text{--}1.4 \mu\text{g} \cdot \text{kg}^{-1}$) found in BM was found as 44.8% soluble, 37.6% associated with fat, and 17.6% associated to casein (Mathur and Roy 1981a). Buffalo milk was reported to contain 18%, 72%,

Table 8 Major mineral element contents of buffalo milk (in milligrams per 100 grams)

Ca	P		Mg		Citrate		Na	K	Cl	Ref
	Soluble	Total	Soluble	Total	Soluble	Total				
159.6–166.2	ND	118.8–120.6	ND	17.7–18.8	ND	ND	39.0–42.3	102.3–108.8	ND	(Kapoor and Ludri 1984)
159.8–190.5	ND	ND	ND	18.3–39.0	ND	158.9–321.6	52.9–95.5	112.0–136.0	ND	(Kumar et al. 1985)
182.0–195.0	ND	93.0–103.0	ND	13.0–17.0	ND	ND	56.0–59.0	155.0–178.0	ND	(Kholif 1997)
188.4±4.8	32.8±8.0	85.1±4.3	28.5±1.3	16.5±1.2	8.4±0.2	159.4±7.7	52.3±1.2	114.8±2.8	58.1±2.8	(Ahmad et al. 2008)
165.9	37.8	109.9	35.8	17.7	8.4	161.3	43.4	92.9	61.3	(Sindhu and Roy 1982),
170.6±14.4	55.2±10.9	145.3±26.9	56.9±12.9	ND	ND	ND	ND	ND	ND	(Ariota et al. 2007)
153.6±16.0	34.2±9.4	112.4±15.3	33.7±5.1	35.5±9.1	17.7±6.3	143.9±28.3	52.8±11.6	181.5±21.2	74.9±6.0	(Abd El-Salam and El-Shibiny 1966)
112.0±40.0	ND	99.0±32.0	ND	8.0±2.0	ND	ND	35.0±11.0	92.0±25.0	ND	(Patino et al. 2007)

Na, K, and Cl are completely soluble

ND not determined

Table 9 Zinc, iron, copper, and manganese contents (in micrograms per kilogram) of buffalo milk

Zn	Fe	Cu	Mn	Reference
5.8 (1.4–10.0)	2.1 (0.6–4.8)	0.29 (0.12–0.41)	ND	(Abd El-Salam 1968)
(4.5–5.9)	(1.1–1.9)	ND	ND	(Mathur and Roy 1981b)
(3.3–4.0)	(0.7–1.0)	(0.17–0.21)	ND	(Mebta and Gangwar 1984)
6.1 (2.8–9.6)	1.5 (0.6–1.9)	0.25 (0.15–0.34)	0.05 (0.02–0.08)	(El-Shabrawy and Hagrass 1980)
(4.8–5.9)	(1.2–1.4)	(0.30–0.43)	(0.25–0.28)	(Kholif 1997)
ND	4.0 ±0.1	0.05 ±0.01	0.29 ±0.05	(Yousef et al. 1983)
6.3 ±0.6	3.2±0.8	0.29±0.04	ND	(Singh et al. 1981)
6.3 ±0.3	3.1±0.6	0.33±0.06		
5.3 ±0.2	1.8±0.2	0.35±0.06		
4.4 ±0.8	1.0 ±0.4	0.21 ±0.10	0.08 ±0.04	(Enb et al. 2009)
4.1 ±1.4	1.6 ±0.6	0.35 ±0.16	0.27 ±0.01	(Patino et al. 2007)
47.6 ±157 ^a	ND	ND	ND	(Al-Jobori et al. 1990)

Figures in bold indicate mean values; figures between parentheses indicate the range of values

ND not determined

^a In micrograms per gram milk powder

and 10% of zinc distributed as soluble, associated with casein, and with lipid phases, respectively (Mathur and Roy 1981b), while 36.5%, 42.5%, and 21.0% of iron were found in cream, rennet whey, and rennet curd, respectively (Mathur and Roy 1982).

7 Vitamins

Cow's milk is a good source of vitamin A potency due to its content of carotene and vitamin A. However, BM contained only vitamin A (Table 10). Variations in the reported data of the vitamin A content of BM were due to physiological and environmental factors and sensitivity of the methods of analysis. However, BM generally contained higher levels of vitamin A than CM (Table 10). The 13 *cis* isomer of retinol, which has the maximum vitamin A potency, was present in very small amounts in fresh raw BM (2.4–5.2 $\mu\text{g}\cdot 100\text{ g}^{-1}$) but increased through heat treatment (Mattera et al. 2007). The concentration of vitamin E in buffalo milk and milk fat was less than that of cow milk which supports the relation between the tocopherol content and the polyunsaturated content of milk fat (Fatouh et al. 2005). The only cited report on vitamin D content of buffalo milk fat (Fatouh et al. 2005) reported a value of 0.03 $\mu\text{g}\cdot\text{g}^{-1}$ indicating that BM was not a good source of vitamin D. Buffalo milk contained higher vitamin C than CM with levels of 20.3 ± 0.07 and $8.6\pm 0.07\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ being reported, respectively (Mohamed et al. 1990). The thiamin content of BM vary from an average of $38.7\text{ }\mu\text{g}\cdot 100\text{ mL}^{-1}$ (El-Abd et al. 1986) to $53.0\pm 0.7\text{ }\mu\text{g}\cdot 100\text{ mL}^{-1}$ (Mohamed et al. 1990). Buffalo milk contained less

Table 10 Fat-soluble vitamins and carotene contents of buffalo and cow milk

Species	Carotene	Vitamin A	Vitamin E	Vitamin D	Remarks	Ref
Cow ^a	6.8 ±0.1	27.8 ±0.4	30.2 ±0.7	ND	1–3 lactation	(Lal and Narayanan 1984)
	7.0 ±0.1	28.9 ±0.8	32.6 ±0.5	ND	4–6 lactation	
	7.2 ±0.1	30.7 ±0.6	34.6 ±0.8	ND	>7 lactation	
Buffalo ^a	–	28.7 ±0.7	23.8 ±0.5	ND	1–3 lactation	(Lal and Narayanan 1984)
	–	29.8 ±0.8	25.4 ±0.5	ND	4–6 lactation	
	–	30.7 ±0.6	26.6 ±0.7	ND	>7 lactation	
Cow ^b	3.2 ±0.7	7.2 ±1.8	21.9 ±2.5	ND	Organic	(Bergamo et al. 2003)
	2.0 ±0.7	7.8 ±1.8	15.0 ±2.0	ND	Conventional	
Buffalo ^b	–	7.2 ±0.5	18.3 ±2.3	ND	Organic	(Bergamo et al. 2003)
	–	12.1 ±1.2	12.1 ±1.6	ND	Conventional	
Buffalo ^c	ND	35.5–117.6	38.0–136.3	ND	Farm samples	(Mattera et al. 2007)
	ND	127.0–190.1	66.3–84.2	ND	Lactation period	
Buffalo ^b	–	7.8	10.1	0.03		(Fatouh et al. 2005)

Figures in bold are mean values

Endash not present, ND not determined

^a In micrograms per 100 milliliters

^b In micrograms per gram of fat

^c In micrograms per 100 grams

riboflavin than CM (Sikka et al. 1993). However, the riboflavin content of milk from the two species was reported to decrease markedly with on exposure to sunlight while exposure to fluorescent light had much less of an effect (Sikka et al. 1990). Buffalo milk contained average riboflavin contents of 158.8 $\mu\text{g}\cdot 100\text{ mL}^{-1}$ (Sikka et al. 1993), $132\pm 1.3\text{ }\mu\text{g}\cdot 100\text{ mL}^{-1}$ (Petrilli et al. 1987), $138.3\text{ }\mu\text{g}\cdot 100\text{ mL}^{-1}$ (Abd El-Gawad et al. 1988), and 183 and $146.4\text{ }\mu\text{g}\cdot 100\text{ mL}^{-1}$ on oats and barseem diets, respectively (Sikka et al. 1993).

Buffalo milk from Indian breeds contained less folic acid (both bound and free) than cow and goat milks. The bound and total folate decreased from 22.67 ± 5.3 and $54.68\pm 6.58\text{ ng}\cdot\text{mL}^{-1}$ on the first day after calving to 18.16 ± 4.8 and $37.75\pm 4.46\text{ ng}\cdot\text{mL}^{-1}$, respectively, after 55 days of parturition (Singh and Sharma 1984). Also, the folate-binding capacity of BM ($72\text{ ng}\cdot\text{mL}^{-1}$) was less than that for cow's ($200\text{ ng}\cdot\text{mL}^{-1}$) and goat's ($532\text{ ng}\cdot\text{mL}^{-1}$) milk after 16 days of parturition (Singh and Sharma 1984). The folic acid content of Egyptian BM was almost similar to that of Indian BM (Sharaf 1988).

The average niacin, biotin, and B12 contents in BM were $1.304\text{ (}0.877\text{--}1.668\text{) mg}\cdot 100\text{ g}^{-1}\text{ DM}$, $6.73\text{ (}0.87\text{--}12.39\text{) }\mu\text{g}\cdot 100\text{ g}^{-1}\text{ DM}$, and $1.86\text{ (}0.67\text{--}3.17\text{) }\mu\text{g}\cdot 100\text{ g}^{-1}\text{ DM}$ (Sharaf 1988). Buffalo milk contained approximately fourfold higher levels of vitamin B12 ($21.68\pm 2.69\text{ }\mu\text{g}\cdot\text{kg}^{-1}$) compared with CM ($4.91\pm 0.4\text{ }\mu\text{g}\cdot\text{kg}^{-1}$; Sharma et al. 2007).

8 Other minor constituents

8.1 Oligosaccharides

Buffalo milk has higher oligosaccharide content in comparison to cow milk. The average total oligosaccharides in cow and buffalo colostrums were reported to be 20.3 and 26.0 mg·100 mL⁻¹, respectively, which decreased to 14.7 and 17.2 mg·100 mL⁻¹, respectively, in mid-lactation and to 8.7 and 10.2 mg·100 mL⁻¹ in late lactation (Balasubramanta et al. 1993). An interesting finding (Saksena et al. 1999) was the immuno-stimulant activity of pooled oligosaccharides obtained from BM which induced a two- and sixfold increases in antigen-specific immune response to sheep red blood cells and a twofold increase in non-specific immune response. Saksena et al. (1999) characterized a pentasaccharide which was isolated from BM which has the following structure:



Further studies are needed in this area to explore the significance of oligosaccharides in BM.

8.2 Pigments

Buffalo milk contained 23–155 mg·100 mL⁻¹ of dormant blue-green pigment (Chandradavdana and Dastur 1976) which was associated with the casein micelles and was identified as an isomer of biliverdin which arises from the oxidation of bilirubin in the mammary gland by biliverdin reductase. The biliverdin content was influenced by breed (51.8 and 65.3 µg·100 mL⁻¹ for Murrah and Serti breeds, respectively), stage of lactation (low in colostrums, 30.0–34.8 µg·100 mL⁻¹) and number of lactations (third to sixth lactation had significantly higher concentration than first lactation; Kumar et al. 1987).

8.3 Orotic acid

Orotic acid is found in the non-protein fraction of milk from ruminants. It has a number of physiological functions, e.g., intermediate in pyrimidine synthesis, growth factor for yogurt starters. Buffalo milk has been reported to contain an average orotic acid content of 28.26 µg·mL⁻¹ (Hewedy 1990), 29.0 µg·mL⁻¹ (Larson and Hegarty 1979), and 19.55 µg·mL⁻¹ (Parkash and Sharma 1985). The orotic acid content increased with advanced lactation (Hewedy 1990).

8.4 Volatile compounds

Buffalo milk contained a complex mixture of 75 volatile compounds 50% of which were identified as esters, 14% as aldehydes, 13% as nitrogen compounds, 9% as ketones, 5% as aliphatic alcohols, 2.5% aromatic, and 4% sulfur compounds (Moio et al. 1993).

8.5 Thiocyanate

The natural thiocyanate content of milk is an important constituent for the activity of the lactoperoxidase/H₂O₂ system in the preservation of milk. The average natural thiocyanate content of raw BM varies from 5.90 ± 2.17 and 8.94 ± 4.54 mg.L⁻¹ in the morning and evening milk from individual buffaloes (Abd El-Ghani and Sayed 1997). Also, differences have been reported in raw BM delivered to collecting centers in different localities in Egypt but were generally lower than that found in the milk of individual buffaloes (Abd El-Ghani and Sayed 1997). The average thiocyanate content of BM in Argentina has been reported to be 8.64 ± 2.08 mg.L⁻¹ (Nieuwenhove et al. 2004).

9 Conclusion

Progress has been achieved in understanding the composition and properties of BM particularly on BMF and proteins. However, several points should be taken into consideration for further studies on BM:

1. Most of cited studies were performed on a limited number of breeds of buffalo milk, and in several studies, the breed was not mentioned. Detailed studies on buffalo milk from the different breeds would give better insight on the genetic diversity of buffaloes.
2. The minor constituents of BM (e.g., enzymes and vitamins) have not been studied thoroughly. Controversial data have been reported in the limited studies performed on these constituents, and the methodology used should be updated.
3. Recent studies have covered most aspects of the chemistry of BM proteins. However, more investigation on BM proteins as a source of bioactive peptides, their functional properties, and nutritional aspects need to be performed. Also, studies on the presence of genetic variants in BM proteins which cover a large number of animals from different breeds need to be performed.

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