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The First Step to Standardization of Iranian Buffalo Milk: Physicochemical Characterization

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Abstract. Nowadays, buffalo's milk due to have highly nutritional properties, has a special place among consumers and its application for the production of dairy products due to the high technological properties is increasing day by day. In the present study, the physicochemical characteristics of Iranian buffalo's milk were compared with cow's milk. According to chemical analysis, the amount of fat, protein, and total solid was higher in buffalo milk than cow's milk (respectively, 8.2%, 4.73% and 15.92% compared with 3.5%, 3.25% and 12.5%). Also, the percentage of cholesterol buffalo's milk was less than in cow's milk. In contrast, no significant difference between the pH, acidity, and specific gravity was observed. The size of buffalo milk fat globules was larger than cow's milk. In addition, the profile of buffalo free fatty acids milk showed the relatively high distribution of long chain saturated fatty acids. The presence of four major bands related to αs casein, β casein, β-lactoglobulin, and α-lactalbumin with quite higher intensity than cow's milk was also observed. The results obtained will provide a reference investigation to improve the developing of buffalo milk standard.

Keywords: buffalo milk, physicochemical characterization, standardization

1. Introduction

Buffalo (*Bubalus bubalis*) milk with 12.5% of milk produced each year after cow's milk (84% with 551 billion liters) has occupied the second place in the world [1], [2]. In terms of components, fat comprises the main portion of buffalo milk which is responsible for its high nutritional value and in terms of technological properties (high total solids, fat, protein, colloidal calcium, caseine micelles, and larger fat globules) it can used for providing various dairy products such as: butter, butter oil (ghee), soft and hard cheeses, condensed or evaporated milks, ice cream, yogurt, and buttermilk [1], [3]-[5]. However, great deals of studies were focused on cow's milk and there is no comprehensive information about the physicochemical characteristics of buffalo's milk. Some studies conducted in the case of buffalo's milk are summarized as following. In the investigation of 112 raw buffalo milk samples at four locations in China by Han et al [6], the average chemical composition was reported as 7.59% (v/v) fat, 4.86% (w/w) crude protein, 4.74% (w/w) lactose, 18.44% (w/w) total solids, 0.85% (w/w) ash, and pH 6.65. In another study performed by Bonfatti et al [7], the analysis of total casein content of water buffalo milk by RP-HPLC method showed 32.2% α_{S1}, 15.8% α_{S2}, 36.5% β_{γ} and 15.5% κ-casein, whereas the content of α-lactalbumin was less than β-lactoglobulin. Mahmood and Usman with comparison the physicochemical parameters of buffalo, cow, goat and sheep milk samples from the diverse regions of Gujrat (Pakistan), indicated that the content of fat, protein, and lactose were higher in buffalo milk than the other samples [8]. Such differences in composition increased the buffering capacity of buffalo milk which affecting on quantitative molecular changes such as: caseins aggregation, calcium and inorganic phosphate solubilization, and decrease in casein hydration during acidification [1]. In

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addition, changes in the rheological and micellar of mozzarella-type curd made from buffalo and cow's milk were occurred due to the different milk compositions [4]. Although some of the physicochemical characteristics of buffalo milk have been investigated by a number of researchers, there is no study on Iranian buffalo milk. For this purpose and to perform a comparative analysis between Iranian buffalo and cow milk, characterization of some physicochemical properties and determination of fat globules diameter, protein and free fatty acid profiles were conducted.

2. Materials and Methods

2.1. Materials

Reagents and chemicals of analytical grade were obtained from Merck Chemical Ltd. (Darmstadt, Germany). Unstained protein molecular weight marker 14.4 to 116.0 kDa (#SM0431) was obtained from Fermentas (USA).

2.2. Sampling

The fresh natural milk from buffalo and cow animals were obtained from local markets in East Azerbaijan (north-west of Iran). A total of 60 milk samples corresponded to a mixture of the milks produced by 30 buffaloes and 30 cows were collected at the end of August. The samples of milks were stored in properly washed and cleaned polyethylene bottles and brought to the laboratory for performing various physicochemical analyses.

2.3. Physicochemical Analysis

The buffalo and cow's milk fat and protein content were determined by the Gerber (BSI Standard 696, part 2) and Kjeldahl (FIL-IDF Standard 20A) methods, respectively. The milk total solid contents were obtained by oven drying (FIL-IDF Standard 4A). The pH of buffalo and cow's milk was measured using a digital pH-meter and total titratable acidity was measured by titrimetric method (ISO 6092:1980). Also, specific gravity was determined by the standard method (AOAC). All the analyses were run in triplicate, and SAS statistical software (version 8.2, SAS Institute Inc., Cary, NC) was applied for the statistical analysis of the experimental data. Multifactor analysis of variance (ANOVA) with the least significant difference (LSD) test ($p<0.05$) was used.

2.4. Fat Globule Size Measurements

The fat globule size distributions in the buffalo milk was measured by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK), equipped with a He/Ne laser (k = 633 nm) and an electroluminescent diode (k = 466 nm). The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm [9].

2.5. Analysis of Free Fatty Acids and Cholesterol

Gas chromatography according to Turkoglu method [10] was used for analysis the free fatty acids of buffalo milk. As described, the milk fat was extracted by a mixture of methanol and methylene chloride (1:9), followed by evaporation of the solvent at 40 ℃ under vacuum [11]. After methylation of extracted fats by Sukigi method [12], injection to the gas chromatograph (Shimadzu GC-17 AAF, V3, 230V series; Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID), and fitted with a fused silica capillary column (SP-2380, 100 m × 0.25 mm; Supelco Inc., Bellefonte, PA) was done. Determination of total cholesterol content in buffalo milk was also performed by the standard method (ISO 12228: 1999).

2.6. Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)

Buffalo's milk (5 g) with 20 mL water was mixed for 3 min at 20°C. The pH of the mixture was adjusted at 4.6 and kept at 40 ℃ for 1 hour. Then, centrifugation of prepared samples was performed at 3000 ×g for 20 min at 5°C. At last, the pellets were vortexed with 5 mL of urea (7 M) and stored in freezer (−18°C) [13]. The profile of buffalo milk casein was evaluated electrophoretically with SDS-PAGE according to the Laemmli method [14], using a vertical slab gel apparatus with 5% stacking and 15% running gel. Before the loading of samples into the gel, proteins and peptides were extracted using Vannini et al. method [15].

Electrophoresis was done at a constant 125 V (8.0 mA) for 4 h in tris-glycine-SDS buffer pH 8.3. After electrophoresis, proteins in the separating gel were made visible by staining with coomassie brilliant blue.

3. Results and Discussion

3.1. Physicochemical Parameters

The pH, acidity, fat, protein, total solid content, and specific gravity of buffalo and cow's milk were shown in Table 1. No significant differences between the pH and acidity of buffalo milk and cow's milk were observed ($p<0.05$). It was reported that the pH of buffalo milk can vary 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 [2]. Also, buffalo milk titratable acidity which is due to the presence of lactic acid, citric acid and phosphoric acid [8] varies from 0.05% to 0.20% and even higher in colostrums than mature buffalo milk [16].

Table 1: Physicochemical Characteristics.

Characteristics	Buffalo Milk	Cow's Milk
pH	6.73 ± 0.12^a	6.50 ± 0.20^a
Titratable Acidity (%, W/W)	1.70 ± 0.10^a	2.00 ± 0.10^a
Fat (%, V/V)	8.20 ± 0.10^a	3.50 ± 0.26^b
Protein (%, W/W)	4.73 ± 0.01^a	3.25 ± 0.01^b
Total Solid (%, W/W)	15.92 ± 0.09^a	12.50 ± 0.29^b
Specific Gravity (g/cm^3)	1.034 ± 0.001^a	1.032 ± 0.001^a

Different superscripts (a and b) in each raw show significant difference ($p<0.05$).

As indicated, the fat, protein, and total solid percentage were higher in buffalo milk compared with cow's milk. Similar results related to chemical properties of buffalo and cow's milk have been reported by Ahmed et al. [2], Mahmood and Usman [8], and Menard et al [17]. According to the obtained values for specific gravity of buffalo (1.034 ± 0.001) and cow's milk (1.032 ± 0.001), no significant differences were observed ($p<0.05$). The specific gravity of buffalo milk was similar to the findings of Mahmood and Usman [8]. As it clears, the specific gravity of milk is influenced by the proportion of its constituents. Among various compounds, fat affect the density and due to its low mass and high volume, the fat amount is inversely related to density. Besides, changing nutritional requirements and additives adding to milk can also cause changes in the specific gravity [8].

3.2. Fat Globules Size

The size of buffalo milk fat globules was calculated and shown the highest percentage of buffalo fat globule milk had a diameter of 0.1-0.2 and 4.5-7.5 μm. Various studies have shown that the fat globule in buffalo milk has a size between 3.5 to 7.5 μm and its average size (5 μm) is higher than cow, goat and sheep fat globules milk [2]. The larger size of buffalo milk fat globules (8.7 μm) as compared to 3.8, 3.8, 3.2 and 3.0 μm for cow, sheep, goats and camel milks was also reported by El-Zeini [18]. It is clear that milk fat globules contribute to some characteristics such as color and creaming of dairy products, and are essential to processing and manufacturing properties of many dairy products. Besides, the size of milk fat globules is a key factor affecting the physical properties of the emulsion and influences on characteristics of the cheese manufacturing process (like storage potential, gel structure, firmness) and yogurt texture.

3.3. Free Fatty Acid Composition and Cholesterol Content

In Fig. 1., the percentage of different free fatty acids in buffalo and cow's milk was compared. As shown, fatty acid composition in buffalo milk fat is different from that of cow milk fat. However, both milks contained the higher amounts of saturated fatty acids and the major fatty acids were myristic acid, palmitic acid, and stearic acid. These results are in accordance with Ménard et al studies [17]. Among various medium chain saturated fatty acids, the presence of caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0) in buffalo milk were dominant. Long chain fatty acids including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (18:1 cis-9) had also relatively high distribution in buffalo milk.

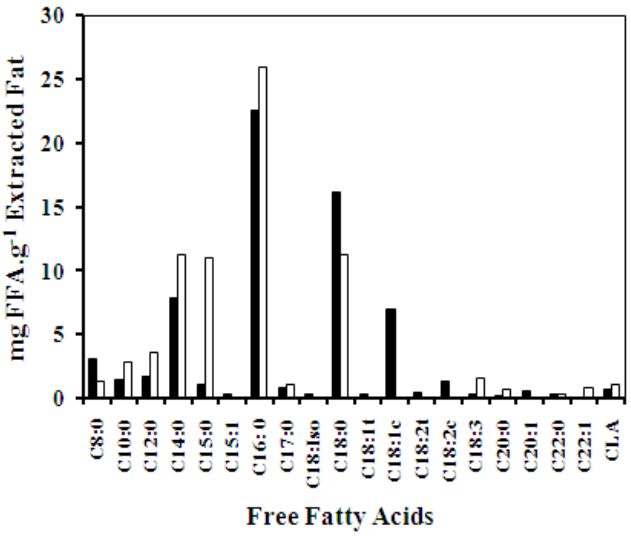

Fig. 1: The profile of free fatty acids of Iranian buffalo (■) and cow's (□) milk.

Regarding the medium and long chain fatty acids, buffalo milks contained significantly higher contents of caprylic acid and stearic acid, respectively and lower content of lauric acid, myristic acid, and palmitic acid than cow milks. Considering the long chain unsaturated fatty acids, oleic acid was significantly higher in buffalo milk than cow's milk. Since, medium chain saturated and long chain unsaturated fatty acids include beneficial effects on human health [16] such considerable results are important for buffalo milk utilization. Besides, the presence of conjugated linoleic fatty acid (CLA; C18:2 cis-9 tr-11) increases the importance of buffalo milk consumption due to various roles of CLA isomers as anticarcinogenic, antidiabetic, antiobesity and antiatherogenic components [16]. The measurement of total cholesterol in buffalo and cow's milk fat has shown that the buffalo milk has lower cholesterol content (85.20 ± 0.01 %) than cow's milk (90.00 ± 0.01 %). This result and similar observations [1]–[3] confirms the better nutritional value of buffalo milk than cow's milk. The higher cholesterol content in buffalo milk was also reported in colostrums, mastitis, fore-milk, and in the spring season [16].

3.4. Protein Profile

The electrophoretic band pattern of buffalo and cow's milk proteins was identified by the SDS-polyacrilamide gel electrophoresis (SDS-PAGE) and shown in Fig. 2. As indicated, the presence of four major bands related to α_s casein (~ 29 kDa), β casein (~ 25 kDa), β-lactoglobulin (~ 18 kDa), and α-lactalbumin (~ 14 kDa) was observed. Although the same values of buffalo and cow's milk (30 µl) were loaded to the gel, the intensity of casein and whey protein bands in buffalo milk was stronger than cow's milk. The result obtained from buffalo milk total protein measurement was also indicated the higher protein content than cow. In addition, all fractions of buffalo milk protein showed a slightly slower mobility than cow milk proteins. Ganguli and Bhalerao by paper disk electrophoresis of casein components showed 44, 53 and 3% for buffalo αs-, β-, and κ-casein versus 55, 39 and 6% for the cow milk casein fractions [19].

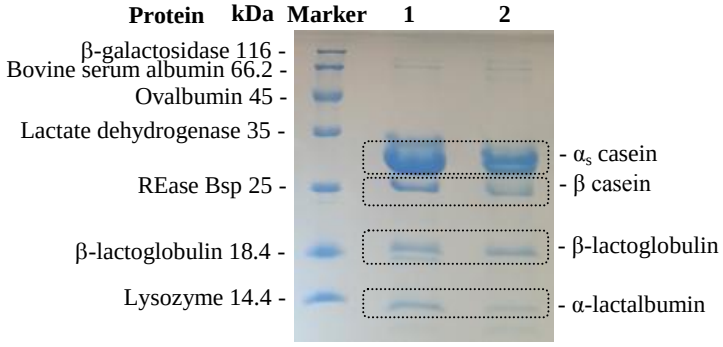

Fig. 2: SDS-PAGE of buffalo (lane 1) and cow's (lane 2) milk, Mw of protein electrophoresis standards are shown on the left.

4. Acknowledgements

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5. References

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