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Comparative Assessment of Physicochemical and Structural Properties of Buffalo and Bovine Casein

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Abstract – **This research was carried out to evaluate and camper the physiochemical and characteristics of buffalo casein with bovine casein. The casein powder was characterized in terms of particle size distribution, zeta-potential, solubility, and Scanning electron microscopy. The bovine casein contained highest protein content (93.96%). Buffalo casein had the largest particle size and more white color than bovine casein (173.7 and 94.7 nm) respectively. Zeta-potential for buffalo and bovine casein showed negative charge and mobility suitable to solubility index (99 and 95) in aqueous. Scanning electron microscopy images of buffalo casein was showed large size, as well as surface of smooth of these structural characteristics which was similar bovine casein. Hence, buffalo casein could be considered as a natural source for coffee whiteners and ingredient food in supplement food products to use in functional foods and pharmaceutical applications.**

Keywords – **Buffalo Casein, Bovine Casein, Physicochemical, Particle Size.**

I. INTRODUCTION

Casein are extensively used in food industry because of their physicochemical, nutritional and functional properties, such as enzymatic hydrolysis, emulsifying and gelation capacities, thus contributing to food texture, However, the micellar structure of casein is destroyed during the manufacture of sodium caseinate [1]. Casein is account 80% of milk proteins and plays an important role in the development of new food products due to a wide range of uses as a functional food ingredient. However, the dominant physiological feature of the casein micelle system has more recently been proven to be an excellent source of bioactive peptides and related ingredients such as its enzymatic hydrolysates and peptides [2]. Additionally, various sources of casein were previously produced from bovine milk [3], yak milk [4], goat milk [5], and buffalo milk [6].

Buffalo casein is one of the most casein products of our diet with regard to its physical and physicochemical features, because of its very high protein content. Buffalo milk is ranked at second after cow milk, based on worldwide milk production and distribution with leading countries in Asia (India, Pakistan and China) and the Middle East (Egypt) and Europe. Notably, about 103 million tons of buffalo milk was produced in 2013, representing 13% of the total world milk production with an annual growth rate of ~3.3% which is higher than cow milk (annual growth rate 0.9%)[7]. In addition, if compared with bovine milk, buffalo milk has a protein content in the range of 3.8-4.5% with a high casein ratio [8] and [9]. However, there could be some limitations owing to inherent differences in the compositional and physiochemical properties between cow and buffalo milk [10].Among the various factors, structural characteristics and particle size distribution of protein and its hydrolysates are two major attributes controlling their solubility. The physicochemical of casein behavior might impede the use of proteins in products, for example, particle size or limited solubility restricts the protein concentration, which can be used by enzymatic hydrolysis to produce bioactive peptides.

According to buffalo casein is less studied than the bovine casein. The information on characterize and compare buffalo and bovine casein which naturally occurring bioactive peptides, that information about buffalo casein needs to be improved. The objective of this study was to compare of the physicochemical characteristics of buffalo casein and bovine casein include microstructure, particle size, solubility and chemical composition.

II. MATERIAL AND METHOD

A. Materials

Sodium caseinate bovine milk (CN) was purchased from Tokyo chemical industry Co., LTD. Preparation of sodium caseinate from buffalo milk (CB), Skim milk was prepared from fresh whole buffalo milk obtained from the Farm of Faculty of Agriculture, (Cairo University, Cairo, Egypt) by centrifugation (Milk centrifuge Elecrem, Helmut Rink GmbH, Amtzell, Germany) at 2000 ×g for 30 min at 4 °C. The average composition of skim milk in dry weight was 10.62, 0.1, 4.23, 4.92, and 0.97% for total solids, fat, protein, lactose and ash respectively.

Sodium caseinate from buffalo milk was prepared according to the method of Mulvihill [11] with some modifications. Skim milk was acidified to pH 4.6 with hydrochloric acid (1 M) under continuous stirring by stirrer (IKA@-works guangzhou Co., Guangzhou, China) at 25 °C. After leaving of curd deposition for 20 min, the mixture was filtered (Whatman no. 40). The precipitated casein was washed with distilled water, then dissolved with the addition of NaOH (1 M) at pH 7.0, and again left for precipitation. Precipitation and washing steps were repeated four times. The final precipitates were dissolved in NaOH (1 M) to pH 7.0, thereafter heated at 80 °C for 30 min to inactivate plasmin, dialysed against distilled water and lyophilized.

B. Chemical Properties Analysis

B.A. Determination of Chemical Composition

Moisture contents of samples were determined according to weight loss (powder 2g) at 105°C in an oven. Protein (N × 6.38) contents were determined using the macro-Kjeldahl method. Ash contents were determined at 550 °C for 6 h. Fat contents were determined using the Gerber method, and carbohydrate contents were determined by subtracting the percentages of other components (protein, moisture, fat and ash) from 100. Subsequently, pH values were measured using a digital pH meter (HANNA instrument, Italy) with a glass electrode. Chemical analyses of powders are presented on a wet basis, and were performed in triplicate (**Table 1**).

C. Physicochemical Properties Analysis

C. A. Measurement of Solubility and Water Activity

To determine buffalo and bovine casein solubility, 1 g of sample was dispersed in 100mL of distillated water and pH of the mixture was adjusted to 7 with HCl 1 N and NaOH 1 N. The mixture was stirred at room temperature for 15 min and centrifuged at 6000×g for 15 min. Protein contents in the supernatant were determined. Casein solubility was calculated according to the equation 3:

$$Solubility\ (\%) = \frac{SP}{TP} \times 100$$

where SP is the protein content in supernatant and TP is the total protein content in sample.

Water activity at 25°C was measured using the AquaLab Water Activity Meter (Series 4 TEV, Decagon Devices Inc., Pullman, WA, USA).

C. B. Scanning Electron Microscopy (SEM)

Buffalo and bovine casein powder were fixed on metal stubs with double-sided tape and coated with gold by a gold sputter coater (Hitachi, E-1010) in a high-vacuum evaporator and examined on a scanning electron microscope (SU-1510, Hitachi, Japan) at a magnification of 10009.

C. C. Color Measurements

The color values of buffalo and bovine casein were evaluated using a Colorimeter (Minolta Company Ltd., Osaka, Japan), which was standardized using a white reference tile. The results were expressed as L*(lightness), a*(redness to green), and b*(yellow to blue) tri stimulus values. The analysis was repeated three times.

C. D. Practical Size of Casein Analysis

Casein powder was measured using a NanoBrook Omni particle size analyzer (Brookhaven Instruments, USA)

C. E. Zeta-potential (ζ potential) Measurement

An aliquot sample from the top layer of the supernatant was collected using a pipette. To obtain an optimal concentration for particle size measurement, the sample was diluted with deionized (DI) water. The samples were placed in a quartz cuvette, and particle size measurements via dynamic light scattering were conducted utilizing a A Zetasizer Nano ZS® (Malvern Instruments, 179 Ltd., UK). Three repetitions of three measurements were performed and the average of the three repetitions was calculated.

D. Statistical Analysis

Data for each treatment condition are presented as mean ± standard deviation. One-way ANOVA was conducted using SPSS 19 (SPSS Inc., Chicago, USA) with significance defined as the 95% confidence limit ($P<0.05$).

III. Results and Discussion

A. Chemical Composition

Proximate analysis was conducted to examine the chemical composition and pH value of buffalo and bovine casein and the results are summarized in Table 1. From the table, it is clear that on dry basis, buffalo casein contains is higher total fat, carbohydrate and ash contents than bovine casein, the major differences between buffalo and bovine casein were those concerning protein content, which was significantly higher for bovine. These values of protein contents (CN 93.96% and CB 86.68%) are similar to those obtained by Hassan & Heikal (2010) [12] from the same animal of buffalo and bovine. Fat contents of buffalo casein (1.15%) were higher than those of bovine casein (0.01%). Percentage fat contents depended on the amount of fat in raw milk, and high fat contents of CB reflected high fat contents of buffalo milk. Also, ash content of buffalo casein (6.12%) had higher than bovine casein (1.04%). The high ash content was indicative of the presence of large amounts of minerals reflecting adjustments of pH with NaOH, pH control during the hydrolytic process, and final pH adjustments of casein before drying [13]. Buffalo milk casein micelles are larger than bovine casein micelles, and contain more calcium, phosphorous, magnesium, and citric acid than cow casein micelles [8].

Table 1. Chemical composition and pH of bovine casein (CN) and buffalo casein (CB)

Parameters	CB	CN
pH	6.86 ± 0.01	7.10 ± 0.01
Ash (%)	6.12 ± 0.20	1.04 ± 0.06
Fat (%)	1.15±0.01	0.01±0.03
Protein (%)	86.68 ± 0.38	93.96 ± 0.41
Carbohydrate (%)	1.55 ± 0.2	0.15 ± 0.2
Moisture (%)	4.5 ± 0.11	4.84 ± 0.14

Means of three determinations ± SD

B. Physical Properties of Buffalo and Bovine Casein.

The functional properties include solubility and water activity which effect of food ingredient and pharmaceutical applications. Casein powder particle is formed as soon as the surface reaches the critical concentration which is a function of the solubility of constituents at the wet-bulb temperature. The bovine casein (99%)show higher solubility properties than buffalo casein powder (95%) Table 2. Based on differences in solubility in aqueous systems, suggested that isoelectric casein is heterogeneous, which was confirmed by the electrophoretic studies of [14].

Casein products can absorb substantial amounts of water, so they can modify the texture of dough or baked products, serve as the matrix former in cheese-type products, produce specialized plastic materials, or increase the consistency of solutions such as soups. The water activity of buffalo casein

(0.2141) record value lower than bovine casein (0.3164) Table 2. The low water activity was similar to that previously reported for spray-dried whole milk powder [15]. Ahmed et al., (2013) [16] observed that the hydration (solvation) of buffalo casein is lower as compared to casein of cow milk.

Table 2. Physical properties of bovine casein (CN) and buffalo casein (CB)

Parameters	CN	CB
aW	0.3164 ± 0.001	0.2141 ± 0.001
Solubility (%)	99	95

Means of three determinations ± SD

C. Scanning Electron Microscopy (SEM)

The structure, density and particle size play important role in reconstitution of powder in to milk. In order to understand the morphology of casein powder, SEM analysis was performed and the results are presented in Fig. 1a and b at size resolution 5 and 20 µm. These images provide visual information regarding presence of casein powder in the gel condition.

Scan Electron Microscopy has proven to be a useful tool for studying rheological properties of the casein mass are thus conditioned by interfacial properties and microstructure of the dispersed phase. Fig. 1 (A and B). It can be observed from the figure that B. at the same size resolution (5 - 20 µm), buffalo casein displayed larger size than bovine casein. The surface of all particles was smooth and most of these structural characteristics were similar to those of UF milk from cows [17]. These characteristics suggest that the differences in composition between skim milks of buffalo and cows might not be large enough to affect greatly the structural properties of the powder.

D. Particle Size Distribution of the Buffalo and Bovine Casein.

Particle size distribution of the material is an important factor influencing the efficiency of value-added processing and a valuable indicator of quality and performance [18]. The structure, density and particle size play important role in reconstitution of powder in to milk. Particle size of buffalo and bovine casein include diameter (nm), volume (%), and width Table 3 and Fig 2. particle sizes of casein dried influence the flowability of powders, while large particle sizes of dried products are generally associated with good dispersibility [18]. Using particle size analyzer, buffalo casein (173.7nm) showed higher particle size than and bovine casein (94.68 nm). Whole of the caseins in buffalo milk is present in miceller form while in cow milk only 90-95% is the miceller casein and rest is present in the serum phase. The particle size of the buffalo miceller casein is larger at (110- 160 nm) than that of cow miceller casein (70-110 nm) [8].While volume of buffalo casein distribution curve ranging from 12 to 87 % with similar average of [19].

Table 3. Particle size characteristics of bovine casein (CN) and buffalo casein (CB)

	Dia(um)	Vol%	Width
SCC	94.68	80.4	131.2
	8.28	19.6	8.01
SCB	173.7	87.2	224.6
	6.2	12.8	6.55

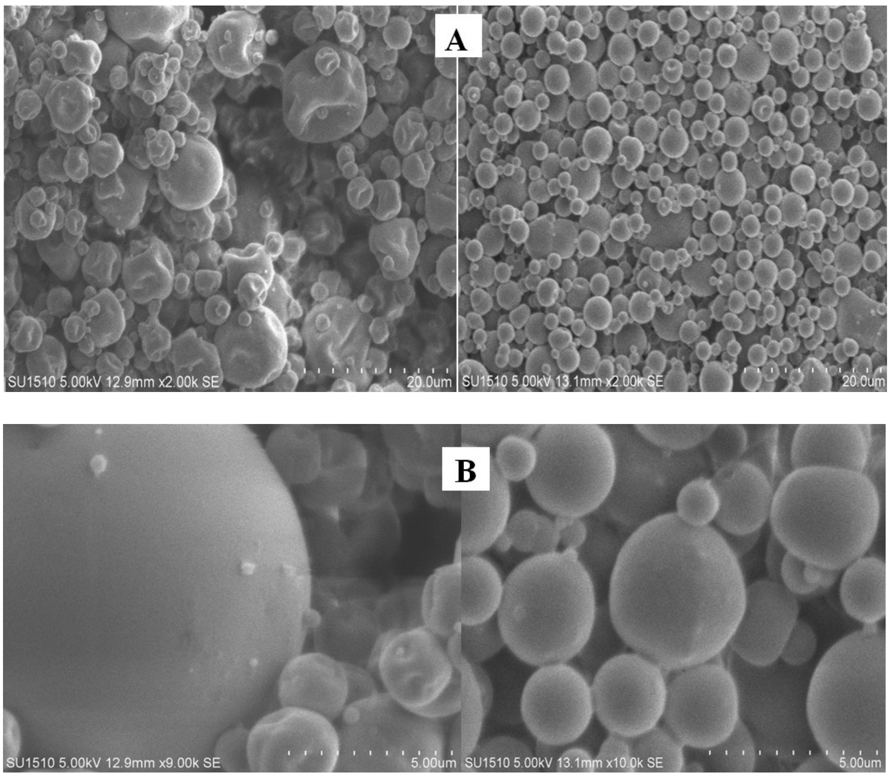
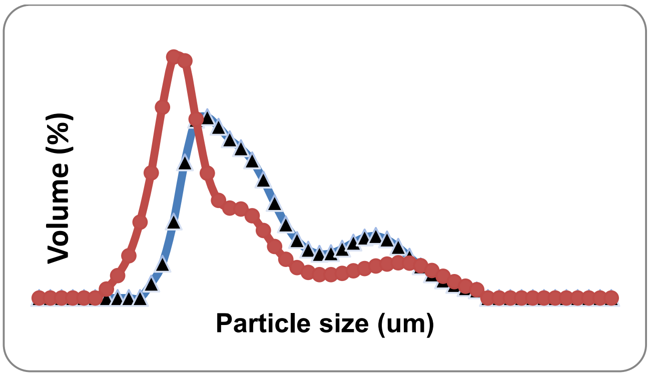


Fig. 1. Scanning electron micrographs of Sodium casein Buffalos(A) and Sodium Casein Bovine (B)



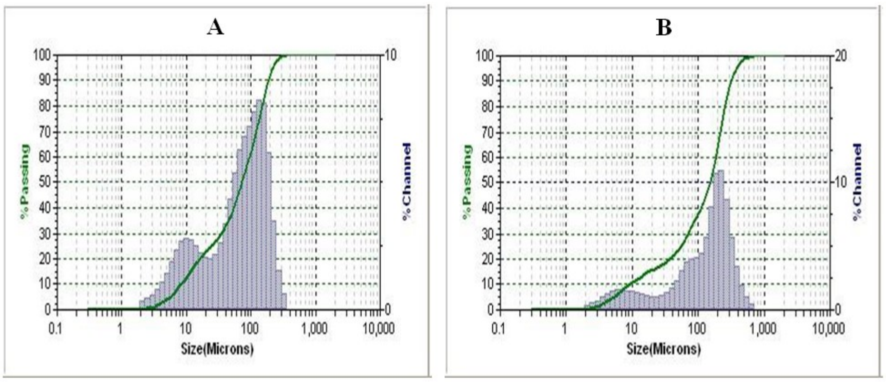


Fig. 2. Particle Size Distribution of the buffalo (red line)(A) and bovine casein(blue line)(B)

E. Color Parameters

Casein micelles are just large enough to deflect light, contribute to the opaque white color of milk, which it is combined with calcium and phosphorus as clusters of casein molecules. Casein with more white can be used in in coffee whiteners, infant formulas, processed cheese, and for use in pharmaceutical products [20].

The color parameters of buffalo and bovine casein are given in Table 4. L and a values of bovine casein showed lower than buffalo casein values, while B and b values of bovine casein showed higher than buffalo casein values. That is indicating the white color of buffalo casein better than bovine casein. The buffalo casein micelle is more opaque, about three times, when suspended in a different medium, than bovine milk micelle. Buffalo casein has superior whitening as compared to bovine casein due to a higher proportion of calcium present in it [9]. Also, it indicated to bovine casein more yellow than buffalo casein (Fig. 3)

Table 4. Color characteristics of bovine casein (CN) and buffalo casein (CB)

.Color attributes	CN	CN
L*	94.66 ±0.21	98.36 ±0.16
a*	-1.63 ± 0.03	-0.19 ± 0.02
b*	9.63 ± 0.12	1.55 ±0.11
B*	16.75±0.17	2.57±0.24
Whiteness	86.66 ± 0.12	97.04 ±0.21

Means of three determinations ± SE.

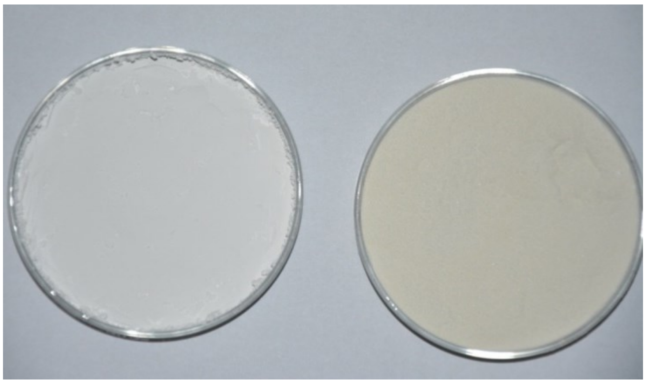


Fig. 3. Powder pictures of Sodium casein Buffalos (A) and Sodium Casein Bovine (B)

F. ζ -Potential

ζ -Potential (surface charge) is a very good index of the magnitude of the interaction among colloidal particles and is used widely to assess the stability of colloidal systems [21]. Both of two type casein have negative ζ –potentials Table 5, as well as bovine have more charge and mobility than buffalo casein -77.74 and -23.30 mV respectively. These results suggest that bovine casein might dispersion in aqueous solution. All case in types had z-potential values greater. Particles with ζ -potentials - 20 mV are normally considered stable for buffalo casein [9]. Negatively charged surface groups provide electrostatic repulsive forces between emulsion droplets and stabilize them in suspension. Charges could be due to glycosylated parts present on k-CN, the protein being present at the periphery of casein micelles which is similar as for cow milk [9].

Table 5. Particle size characteristics of bovine casein (CN) and buffalo casein (CB)

	Zeta Potential (mV)	Mobility (μ/s)/(V/cm)	Droplet size (nm)
CN	-77.74	- 4.049	657.81
CB	-23.30	-1.21	706.57

IV. Conclusions

Buffalo casein powder is providing physiochemical and characteristics differences from bovine casine such as white color and larger particle size which can be used in coffee whiteners. As well as, it is a golden opportunity for its suitability for use in patented food, products particularly cheeses, other fermented dairy products, infant formulas, processed cheese, and pharmaceutical products.

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