Pak. J. Agri. Sci., Vol. 52(1), 191-197; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

TECHNOLOGICAL CHARACTERIZATION OF PHOSPHOCASEINATE POWDER OF BUFFALO MILK

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Globally, milk is extensively used for the production of value added dairy products owing to its rich nutritional profile. Similarly, innovative dairy products can be formulated by changing the functional characteristics of milk caseins. Current research was planned to assess the compositional, functional and physicochemical characteristics of phosphocaseinate powder prepared from buffalo milk. The size of casein was determined through master sizer. Chemical profiling of the tested powder revealed that it contains total protein, non casein nitrogen, non protein nitrogen, moisture, ash and total solids as 87.82±0.28, 0.79±0.02, 0.48±0.01, 4.22±0.10, 8.57±0.11 and 95.76±5.10%, respectively. Furthermore, phosphocaseinate powder contained sodium (6.79±0.39 mg/100g), potassium (2.98±0.75 mg/100g), calcium (9.68±0.08 mg/100g), magnesium (10.44±0.03 mg/100g) and phosphorus (12.25±0.05 mg/100g). Different suspensions of phosphocaseinate powder *i.e.* 1, 2, 3, 4 and 5% were used to optimize the functional properties. The results revealed that 5%suspension had the highest foaming stability (89.17±3.79%), foaming capacity (49.15±2.82%), emulsion capacity (39.57±1.97%), emulsion stability (74.49±0.98%) and protein solubility (37.09±1.48%). However, the particle size of phosphocaseinate suspensions was increased by lowering the pH. Conclusively, phosphocaseinate of buffalo milk has exceptional protein features which can be exploited in numerous food products to expand their functional properties.
Keywords: Dairy products, Casein, Non casein nitrogen, Non protein nitrogen, Functional properties.

INTRODUCTION

In the food system, the utilization of buffalo milk proteins is limited due to variability in their functional properties. Generally, protein characteristics are affected by their structure, configuration and molecular weight. Information about functional and thermal properties of protein is critically important for food processing strategies as well as heat treatment design. The behavior and functionality of protein is affected during processing, storage and consumption ultimately associated with quality and organoleptic characteristics of food (Singh, 2009). In this context, numerous test procedures are carried out to evaluate the functionality of food proteins such as solubility, water & oil binding, emulsifying, foaming and buffering capacity. Viscosity is also considered as an important parameter as it stabilizes the emulsions by maintaining the native structure between casein micelles through ionic, hydrogen, disulfide and hydrophobic interactions. Several surface-active components in milk also maintain surface tension, bulk properties and globular formation (Singh 2009; Jayasena *et al.,* 2010).

The emulsions and foams are immiscible liquids in which small droplets dispersed in continuous state. The proteins have ability to stabilize emulsion and foam by decreasing surface tension, adsorption on the interface and formation of cohesive film around oil droplets. The solubility plays a key role in emulsification whilst, it has been recorded that less soluble protein possesses poor emulsifying properties (Marinova *et al.* 2009). The stability of protein at oil/water interface is dependent on equilibrium between protein molecules and both phases. The emulsifying properties may improve upon limited protein denaturation which does not decrease the solubility drastically but increase the surface hydrophobicity (Wong and Kitts, 2003).

In contrast to low molecular weight emulsifiers, the protein structure may change due to adsorption. Hydrophobic regions of protein domains orient towards oil phase whereas hydrophilic attract water molecules. Newly formed interfacial surface of protein can be stabilized in two ways, either by electrostatic or steric effects (Darewicz *et al.*, 2000).

The calcium ions have ability to manipulate hydrophilic properties of caseins. The functional properties of casein alter the molecular configuration of protein such as size, shape, charge and particle size distribution. Furthermore, proteins and their peptides make complex systems with other food components. In food product development, technological characteristics are important to improve the functional properties (Anema, 2000; Morel and Harper, 2002). Likewise, different researchers; Sodini *et al.* (2006) and Sevilla *et al.* (2013) explored that the increase in emulsion activity and stability result in reduced proteolysis of acid casein. Moreover, casein hydrolysis can improve its

digestibility in various food formulations. Thus, the objectives of this study were to determine the compositional, physicochemical and functional characteristics of phosphocaseinate powder of buffalo milk.

MATERIALS AND METHODS

Procurement of raw materials: The phosphocaseinate powder of buffalo milk was procured from Lab UMR-STLO (Science and Technology of Milk and Egg), Rennes-France. The compositional and functional analyses were done in Dairy lab, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The physicochemical characteristics were assessed at School of Agriculture and Food Science, University of Queensland, Australia.

Compositional Analyses of phosphocaseinate powder: The moisture content of buffalo milk phosphocaseinate was determined by drying the samples in an Air Forced Draft Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5°C till constant weight by following the Method No. 44-15A of AOAC (2006). The ash was calculated by direct incineration of sample; igniting in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) at 550°C till grayish white residue (AOAC, 2006 Method No. 942.05).The nitrogen content in powder was measured by using Kjeltech Apparatus (Technik GmbH D-40599, Behr Labor, Germany) based on Kjeldahl's Method No 991.20 (AOAC, 2006). Following is the mathematical expression for the % total protein.

$$\text{Total protein (\%)} = \text{Nitrogen} \times 6.38$$

The NCN of phosphocaseinate powder was estimated according to the protocol of Heino *et al.* (2007). The NCN content in phosphocaseinate powder was calculated following equation;

$$\text{NCN (\%)} = \text{N (\%)} \times 6.25$$

The NPN in powder was measured according to IDF Method No 20-4 (International Dairy Federation, 2002). The NPN content was determined through following equation;

$$\text{NPN (\%)} = \text{N (\%)} \times 6.19$$

Total solids were determined by adopting the Method No. 990.20 as described in AOAC (2006).

Mineral profile of phosphocaseinate powder: The phosphocaseinate powder of buffalo milk was assessed for its mineral contents according to the guidelines of AOAC (2006). Purposely, calcium and magnesium were determined through Atomic Absorption Spectrophotometer (Varian AA240, Australia). Nevertheless, sodium and potassium were measured by Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge.

Rehydrated casein solutions: Different concentrations of buffalo caseins powder were rehydrated in deionized water at 40ºC. The solution was kept under continuous agitation at 4ºC overnight followed by centrifugation @ 160*g* for 5 min to separate the non-dissolved caseins. In this context, only homogenized casein solutions were used for further studies (Mimouni *et al.,* 2009).

Functional properties

Foaming properties: The phosphocaseinate foaming capacity (FC) or foam expansion (%) was determined by the method of Mao and Hua (2012). The volume of suspensions of different protein concentrations were adjusted followed by agitation at 980 rpm in an orbital shaker at 25ºC for 5 min. The developed foam was immediately transferred to a graduated cylinder and the initial volume of foam (V_0) was measured. The retention of foam volume after 60 min (V_{60}) was indicative of foam stability (FS). The expression for foaming stability and foaming capacity are given below;

$$\text{FS (\%)} = \frac{\text{Volume of foam after 60 min}}{\text{Volume of foam at initial level}} \times 100$$

$$\text{FC (\%)} = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

Emulsifying properties: The emulsifying capacity of phosphocaseinate powder suspension was determined according to the procedure of Wong and Kitts (2003). The dispersions of various concentrations of protein and corn oil were prepared in Ultra Torex T-25 homogenizer (IrkaWerk, Germeny) at 9500 rpm for 3 min to form emulsion. Purposely, 40 mL (V_T) of emulsions were poured into a 50 mL centrifuge tube followed by centrifugation (model Sorvall RC-5B, DuPont Instruments) at 1475*g* for 5 min and the volume of emulsified fraction (V_{F1}) was recorded. The tubes containing oil in water emulsified fraction were heated in a water bath at 80 °C for 30 min and cooled to room temperature (25°C). Afterwards, tubes were centrifuged at 1475*g* for 5 min, and the volume of the remaining emulsified fraction (V_{F2}) was recorded. Emulsion capacity and emulsion stability were reported as

$$\text{Emulsion capacity (\%)} = V_{F1} / V_T \text{ X } 100$$

$$\text{Emulsion stability (\%)} = V_{F2} / V_T \text{ X } 100$$

***Protein solubility (PS)*:** The protein solubility was determined by the standard method of Kaur and Singh (2007). The beaker was placed on a magnetic stirrer. For the purpose, 2.5 cm long smooth, plastic-coated stir bar was used to stir up the dispersions for 1 hr. Afterwards, the dispersion was transferred into a 50 mL volumetric flask and mixing was done. An aliquot of the dispersion was centrifuged for 30 min at 20,000*g* and the resulting supernatant fraction was filtered through whatman no. 1 filter paper. The protein content of the filtrate was determined through micro-Kjeldahl apparatus.

Modifications of pH: Different concentrations (3, 4 and 5%) of phosphocaseinate powder were rehydrated in deionized water at room temperature. The stirring was performed at constant speed (700 rpm) using an electric overhead mixer (RW20, IKA, Staufen, Germany) and four blade propeller stirrer of 50 mm diameter (R 1342, IKA, Staufen, Germany). The solution was kept under continuous agitation for 3 hr followed by centrifugation at 1000*g* for 10 min to separate

non-dissolved suspension. The suspension was filtered through glass fiber filter paper. The only filtered solution was used for further analysis. The solutions were studied under various pH ranging from 4 to 11 to observe the effects of acidification and alkalization on casein micelles of phosphocaseinate suspension solution. The phosphocaseinate solutions were acidified with 1 mol/L HNO_3 under vigorous stirring at 25ºC. The solutions were alkalinized at pH 7, 8, 9, 10, 11 by the addition of 1 mol/L NaOH under vigorous stirring at 25ºC in addition to the control sample that was unmodified (Mimouni *et al.,* 2009).

Particle size distribution: The size of casein micelles of powder solution was determined using protocol of Khalid *et al.* (2000). Accordingly, 3, 4 and 5% casein solution was rehydrated for 3 hr. The rehydrated casein solution was centrifuged at 10, 000*g* for 5 min and filtrated through glass fiber filter paper of pore size 50 mm. The size distribution of filtrated casein solution was assessed with master size 2000 laser particle sizer. The principle of respective technique is based on the diffraction of a laser beam particles of the analyzed product. For measurement, the sample was directly injected into the dispersion cell containing 700 mL distilled water under agitation of 2000 rpm. The refractive index of dispersion medium was set at 1.33 and particle size 1.57. Further, 12 mL sample was dispersed into apparatus circulating cell containing 700 mL of distilled water at 25°C. The particle size distribution was calculated by master sizer software and the result of model diameter was expressed as µm.

Statistical analysis: The effect of treatments on tested parameters was determined by analysis of variance (ANOVA) using Statistical 8.1 soft ware version. One way ANOVA at 5% significant level ($\alpha = 0.05$) was carried out to assess various treatments resulted in statistically significant differences in the variables evaluated.

RESULTS

Compositional analysis of phosphocaseinate powder: The phosphocaseinate powder was assessed for various quality attributes and results depicted that total protein, non-casein nitrogen, non-protein nitrogen, moisture, ash and total solids were 87.82±4.28, 0.79±0.02, 0.48±0.01, 4.22±0.10, 8.57±0.11 and 95.76±5.10%, respectively.

Table 1. Compositional analysis (%) of phosphocaseinate powder

Constituents	Concentrations (%)
Total protein	87.82± 4.28
Non casein nitrogen	0.79±0.02
Non protein nitrogen	0.48±0.01
Moisture	4.22±0.10
Ash	8.57±0.11
Total solid	95.76±5.10

Mineral profile of phosphocaseinate powder: The results regarding the mineral contents; sodium, potassium, calcium, magnesium and phosphorus were 6.79±0.39, 2.98±0.75, 9.68±0.08 and 10.44±0.03, 12.25±0.05 mg/100g concentration, respectively.

Table 2. Mineral profiles of phosphocaseinate powder of buffalo milk

Minerals	(mg/100g)
Sodium (Na)	6.79±0.39
Potassium (K)	2.98±0.75
Calcium (Ca)	9.68±0.08
Magnesium (Mg)	10.44±0.03
Phosphorous (P)	12.25±0.05

Functional properties of native phosphocaseinate powder

Foaming capacity: The means pertaining to foaming capacity showed momentous effect of treatments (1, 2, 3, 4 and 5%) on this trait. The mean values for foaming capacity were 35.33±1.52 (1%), 38.78±1.72 (2%), 42.45±2.60 (3%), 45.58±2.98 (4%k2) and 49.15±2.82% (5%) (Figure 1)

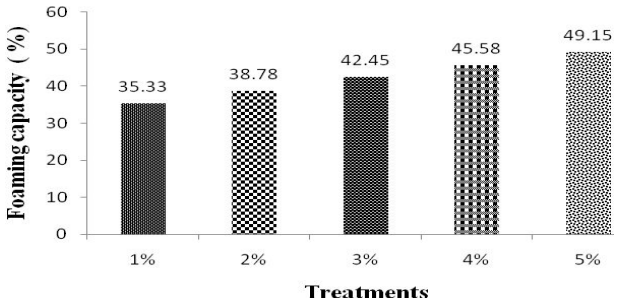

Figure 1. Effect of treatments on foaming capacity (%) of phosphocaseinate powder

Foaming stability: The results for foaming stability showed highly significant effects within the suspensions. The means related to foaming stability of phosphocaseinate powder were 72.75±3.65,75.78±3.86, 81.78±4.13, 84.87±2.89 and 89.17±3.79% for 1%, 2%, 3%, 4% and 5%, respectively (Figure 2).

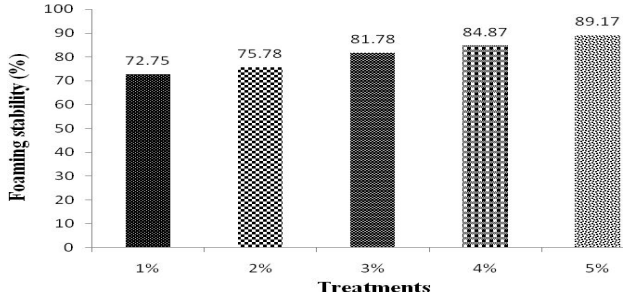

Figure 2. Effect of treatments on foaming stability (%) of phosphocaseinate powder

Emulsifying capacity: The mean values showed that emulsion capacity and emulsion stability have significantly affected due to suspensions. The mean values regarding emulsion capacity of phosphocaseinate treatments; 1%, 2%, 3%, 4% and 5% were 30.09±1.50, 33.37±1.66, 37.25±1.86, 38.39±1.78 and 39.57±1.97%,respectively as shown in Figure 3.

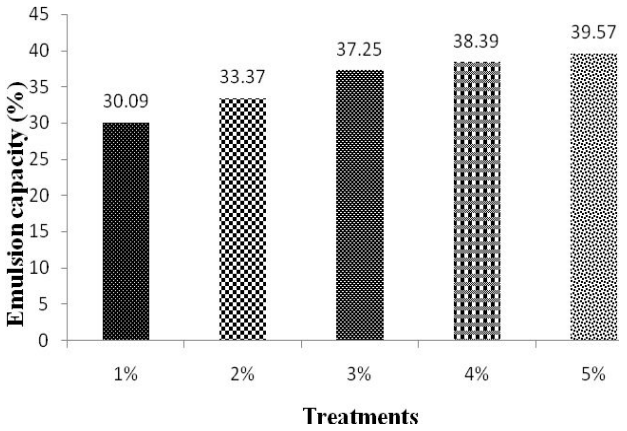

Figure 3. Effect of treatments on emulsion capacity (%) of phosphocaseinate powder

The mean values for emulsion stability of phosphocaseinate treatments; 1%, 2%, 3%, 4% and 5% were 54.73±2.73, 59.87±2.99, 64.85±1.00, 69.57±0.98 and74.49±0.98%, respectively (Figure 4).

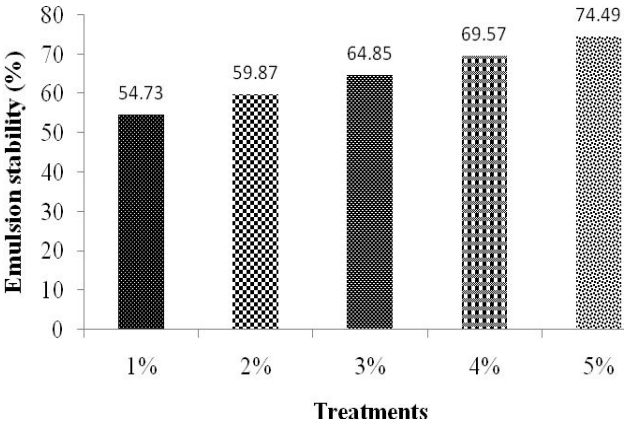

Figure 4. Effect of treatments on emulsion stability (%) of phosphocaseinate powder

Protein solubility: The treatments showed significant effect on protein solubility of phosphocaseinate powder as mentioned in Table 7. The protein solubility of phosphocaseinate based treatments including 1%, 2%, 3%, 4% and 5% were 17±0.68, 20.34±1.01, 24.98±0.99, 30.05±1.20 and 37.09±1.48%, correspondingly (Figure 5).

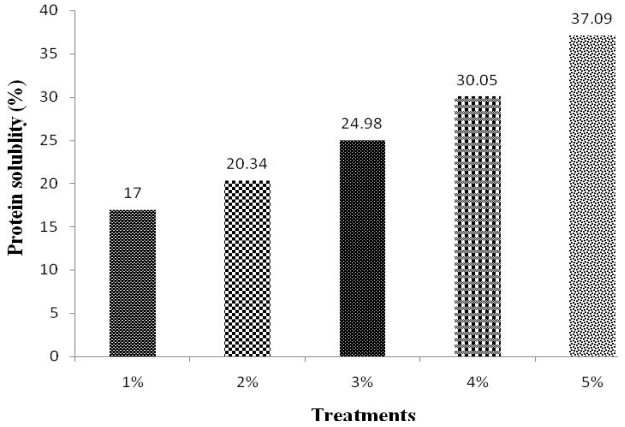

Figure 5. Effect of treatments on protein solubility (%) of phosphocaseinate powder

Effect of acidification on particle size distribution: Effect of acidification on particle size distribution in 3% phosphocaseinate suspension is shown in Figure 6. At pH 6, maximum peak volume was measured in contrast to other acidified solutions. In this context, pH 4 has elucidated maximum particle size distribution as compared to pH 6 and 5. The effect of different acidifying pH on particle size distribution in 4% phosphocaseinate suspension is illustrated in Figure 7.

In contrast, 5% suspension of phosphocaseinate powder at varying pH showed that the control (pH 6.86) and pH 6 had the same peak volume. Moreover, pH 5 and 4 showed maximum particle size distribution ranged from 10 to 1000µm (Figure 8).

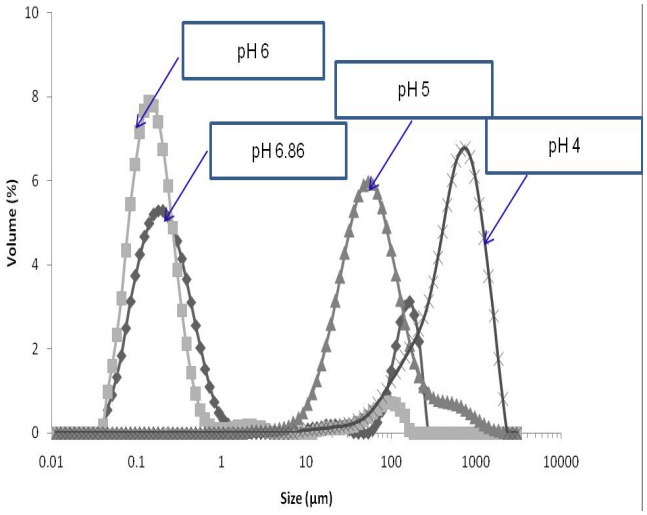

Figure 6. Effect of acidification on particle size distribution in 3% suspension of phosphocaseinate powder at different pH

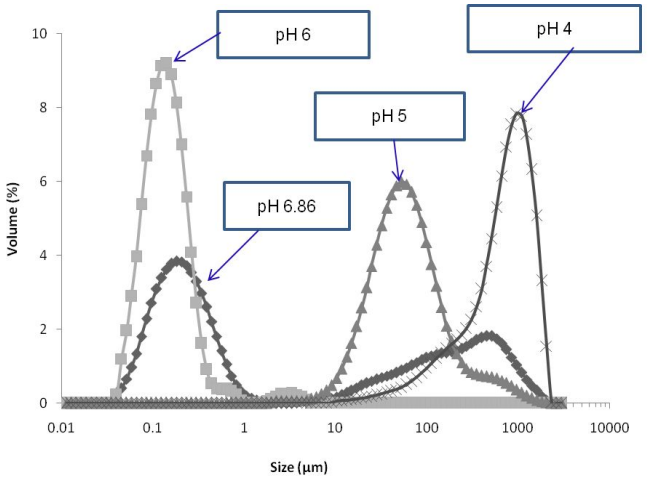

Figure 7. Effect of acidification on particle size distribution in 4% suspension of phosphocaseinate powder at different pH

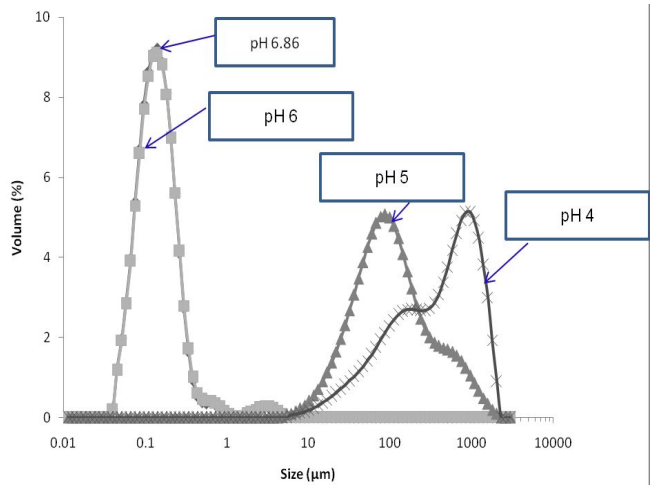

Figure 8. Effect of acidification on particle size distribution in 5% suspension of phosphocaseinate powder at different pH

DISCUSSION

The results of the present research investigation showed slight variations in compositional analysis of phosphocaseinate powder depending on milk source & composition, manufacturing procedure and storage response. Previously, Gaiani *et al.* (2007) highlighted phosphocaseinate as highly proteinous source with casein 80±5.32%, moisture 7.8±0.2% and ash 9.7±0.1%. Earlier, Schuck *et al.* (2002) indicated that the phosphocaseinate powder contains 89% protein.

The present results regarding mineral composition are in accordance with Gaucheron (2005). They reported that phosphocaseinate powder contains sodium ranging from 5 to 9 mg/100g and potassium 2 to 7 mg/100g. Similarly, Carazo and Jaurez (2002) determined sodium, potassium, calcium and magnesium as 6, 9.7, 11 and 0.3 mg/100g in casein, correspondingly. Later, Noël *et al.* (2008) noticed 0.47±0.03 magnesium, 12±0.5 calcium and 0.04± 0.5 mg/100g potassium in phosphocaseinte powder. Another group of researchers, Guzman *et al*. (2000) found that sodium caseinate powder of buffalo milk contains sodium, calcium and magnesium as 11.58, 1.82 and 0.4g/kg, respectively.

The results regarding foaming capacity of phosphocaseinate powder showed significant variation within treatments. Likewise, Marinova *et al.* (2009) reported significant rise in foaming capacity and foaming stability with the increase of protein powder in deionized water. They have also affirmed the importance of two main parameters *i.e.* bubbling and stirring for the achievement of foaming stability in powder suspensions. The present trend is also similar to the findings of Roman (2003). The underlying mechanism proved that foaming capacity of protein is related to its tendency of lowering the interfacial tension between hydrophobic and hydrophilic components in dairy products.

In a previous research study, Philippe *et al.* (2003) explained that high foaming capacity of phosphocaseinate powder was due to the pronounced surface activity of its soluble proteins. Later, Banavara *et al.* (2013) analyzed the foaming stability & capacity of whey proteins ranging from 10 to 90%.

The results of emulsifying properties are in concordance with Roman and Sgarbieri (2006) showed that casein micelles perform better emulsifying properties at its isoelectric pH. The lipid-protein interaction helps to promote the emulsion stability against coalescence during storage by reducing conformational stability and hydrophilic nature of protein. Afterwards, Moro *et al.* (2011) described that the surface hydrophobicity has an impact on foamability and emulsifying activity. Nevertheless, surface hydrophobicity of β-lactoglobulin was recognized as the main influential factor to enhance foamability.

Earlier, Raghavendra and Raghavarao (2010) documented that milk heated at 90 °C exhibits 49±0.34% emulsion stability while the control sample showed 27.7±0.58%. Previously, Peamprasart and Chiewchan (2006) concluded that some proteins are denatured at 80 °C resulting in the aggregation of oil droplet.

Current findings regarding protein solubility of milk powder are in agreement with Hojilla *et al.* (2009) and Haque *et al.* (2012). They documented the solubility of milk protein concentrate as 34.0±0.5%. According to Moure *et al.* (2006), the protein solubility of milk powder was 40.02%. Likewise, Choi *et al.* (2008) found minimum protein solubility 46.34% at pH 4 and maximum 92% at pH 10. Additionally, protein showed good solubility in both acidic and alkaline conditions; an important consideration in dairy food formulations.

The present results regarding particle size of phosphocaseinate powder are supported by the work of

Wade *et al.* (1996), they found that the size of casein micelles increases as the pH of milk protein concentrate decreases.

Similarly, Du *et al.* (2007) and Tuinier *et al.* (2000) observed an increasing trend in the apparent size of casein micelles at pH 5.0 due to the formation of unstable flocculation. Afterwards, Attia *et al.* (2000) also noticed higher solubilization of casein and phosphorus during acidified conditions. They found an increasing tendency in casein release from pH 5.5 to 5.0 hence maximum expansions in micelle structure as well as volume.

Conclusion:The phosphocaseinate powder of buffalo milk contains the highest content of minerals such as calcium, phosphorus, sodium and potassium. The results showed that 5% solution has the highest foaming stability, foaming capacity, emulsion capacity and emulsion stability and protein solubility as compared to the other solutions. These results were affected by the factors like conformational stability, and hydrophobic properties of the phosphocaseinate powder. It is concluded that phosphocaseinate powder of buffalo milk can be used in different food products to enhance the functional properties.

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