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EFFECT OF pH AND HEAT TREATMENT ON PHYSICOCHEMICAL CHARACTERISTICS OF SODIUM PHOSPHOCASEINATE POWDER

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ABSTRACT

The present study was conducted to check the effect of alkalinization (pH 8 to 11), acidification (pH 6- 4) and temperature (60, 65, 70, 75, 80, 85 and 90ºC) on size, charge and viscosity of sodium phosphocaseinate powder after rehydrated in deionized water @ 3, 4 and 5% concentration at room temperature. The particle size distribution was determined by master sizer, while, size and charge of different phosphocaseinate solutions were assessed through zeta sizer. Moreover, the viscosity was monitored by viscometer. Alkalinization significantly affected the size of casein micelles; with minimum size noted at pH 11 in the tested solutions. Moreover, charge of casein micelles decreased with the progressive rise in pH of various solutions. The mean values at pH 11 for 3, 4 and 5% solutions were -28.35, -20.55, -29.09 mV, correspondingly. Acidification resulted in increased size of casein micelles. Heat treatment resulted in decreased size and charge of casein micelles. The mean values for casein micelles size at 90ºC for 3, 4 and 5% solutions were 255.50, 260.55 and 289.71 nm, respectively. Likewise, charge was observed as -15.25, -15.85 and -14.85 mV in respective manner. The decreasing trend of pH from 6.9 to 5.5 elucidated maximum viscosity 1.59-1.80 cP in 5% solution as compared to 3% (1.28 to 1.60 cP) and 4% (1.47 to 1.74 cP). Conclusively, the physicochemical conditions such as low pH or high temperature cause conformational changes in protein, leading to aggregation and precipitation. These information will help processers to formulate innovative protein enriched dairy and non-dairy products.

Key words: Acidification, Alkalinization, Viscosity, Size, Charge

INTRODUCTION

Caseins are the major phosphoproteins in the cow and buffalo milk accounting for 80% of the total milk protein. The caseins are groups of proteins including α_{s1}- α_{s2}-, β- and κ-caseins present @ 24.8, 11.9, 24.9 and 10.8% (D' Ambrosio *et al.,* 2008) in the micellar form (Morris, 2000; McMahon *et al.*, 2009). The micelle is composed of 94% protein and 6% inorganic materials (Gaucheron, 2005). The size of micelle varies from 194 to 222 nm with hydration properties 2.5-2.7 H_2O/g and zeta potential of -20.4 mV. The buffalo milk casein micelles are relatively bigger in size as compared to cow (Famelart *et al.,* 2009; Ahmad *et al.,* 2009).

The structure of casein is tremendously elastic and mostly unfolded. The amphiphilic character is based on the hydrophilic & hydrophobic regions as well as polypeptide chain characteristics. The caseins has the ability to associate through hydrophobic and electrostatic interactions (DeKruif and Holt, 2003; Martin *et al*., 2003). The casein micelles are sterically stable due to hair like structure of κ-casein thus considered as polyelectrolyte brush in a medium of high ionic strength. The micelles exhibit stability against high temperature, ethanol and salt medium in the absence of colloidal calcium phosphate (CCP). The concentrations of these salts play a vital role in the functionality and stability of milk and dairy products. The casein can be separated from milk either by acid precipitation or rennet coagulation. However at industrial scale it is prepared by acidification method which later on after dissolving in saturated solution of NaOH changed to sodium caseinate. The sodium caseinate in powder form is available for various foods and non food uses (Kamizake *et al.*, 2003). Earlier, Anema and Li (2003) observed an increased size of casein micelles during heating of skim milk from 75-100°C. The heat treatment induces reactions that modify the stability of casein micelles. The denatured whey proteins are adsorbed on the surface of micelles thereby make complexes resulting increased size. When milk is heated at 90ºC for 30 min, micelle size is increased from 25-30 nm such expansion improves the whiteness of treated milk as compared to raw (Walstra *et al*., 2006). However, the size of casein micelles of buffalo milk is 190 nm after heating @ 140ºC for 10 sec which ultimately consolidates 200 nm particles within 4-10 min of heating. The aggregation is initially reversible that become irretrievable with progressive heating. The aggregation rate declines with decreasing concentration of heating time. This lack of aggregation is generally associated with the absence of calcium and phosphate (Panouille *et al.,* 2004; Murphy *et al.,* 2004; Kennedy *et al.,* 2006).

Caseins are generally rehydrated before using in the products. The conditions like acidification, alkalinization and heat treatments during rehydration influence the various properties of sodium caseinate. The extensive studies have been conducted on the properties of the cow milk casein (Walstra *et al.,* 2006; Sauer and Moraru, 2012; Anema, 2008), but few studies were available regarding the buffalo milk casein. Furthermore no work was available on the behavior of buffalo milk sodium caseinate under different conditions during rehydration. Considering these points, a study was design to investigate the changes in sodium caseinate powder under different condition of pH and heating temperature. The sodium phosphocaseinate powder was dissolved in deionized water at different concentrations to study the impact of pH and temperature on its physicochemical properties.

MATERIALS AND METHODS

The present study was conducted at School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, Australia.

Procurement of raw material: The sodium phosphocaseinate powder of buffalo milk was procured from Lab UMR-STLO (Science and Technology of Milk and Egg), Rennes-France.

Modifications through pH and heat treatment: Na Phosphocaseinate powder (3, 4 and 5% concentration) was 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 continuously agitated for 3 hr followed by centrifugation at 1000*g* for 10 min to separate non-dissolved solution. The solution was filtered through glass fiber filter paper before using 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 solutions. These solutions were acidified with 1 mol/L HNO_3 and alkalinized by adding 1 mol/L NaOH at pH 7, 8, 9, 10, 11 under vigorous stirring at 25ºC (Mimouni *et al.,* 2009). For modification through heat treatment, the solutions were heated at different temperatures *i.e.* 60, 65, 70, 75, 80, 85 and 90ºC in water bath for 10 min (Mimouni *et al.,* 2009).

Particle size distribution: The size of casein micelles in phosphocaseinate solutions was determined following protocol of Khalid *et al.* (2000) using master size 2000 laser particle sizer. For measurement, the sample was directly injected into the dispersion cell containing 700 mL distilled water with agitation @ 2000 rpm. The refractive index of dispersion medium was set at 1.33 and particle size 1.57. Furthermore, 12 mL sample was dispersed into apparatus circulating cell containing 700 mL 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.

Size and charge: The size and charge of casein micelles in different phosphocaseinate solutions were assessed by zeta sizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom) following the method of Silva (2013).

During analysis, 700 µL sample was added in cuvette for determination of average diameter of casein micelles through zetasizer software. Moreover, 600 µL sample was added in cell for the assessment of charge.

Viscosity: For viscosity determination of aforementioned solutions, then pH was decreased to 6.9, 6.4, 6 and 5.5 simultaneously by using 1 M HNO_3. Viscosity of solutions was measured through Brookfield DV-11+Viscometer + Spindle# 0 at 100 rpm following the guidelines of Silalai and Roos (2010). All analysis was done in triplicate form.

Statistical analysis: The resulting data were subjected to statistical analysis using two factor factorial under complete randomized design. Means and standard deviations (SD) were calculated for all the samples with three replicates.

RESULTS AND DISCUSSION

Effect of alkalinization on particle size distribution: The diameters were measured at 3% solution as D (v, 0.5) 0.12, D (v, 0.1) 0.06 and D (v, 0.9) 0.24. The volume weighted mean D (4, 3) and surface weighted mean D (3, 2) of the casein micelles have been shown in Table 1. The mean diameter of largest particle size D (4,3) was recorded as 0.89 for 3% solution at pH 11.The results indicated that pH 6.86 (control) exhibited maximum peak volume whilst, minimum value for this trait was observed at pH 9 and 10.

Table 1. Effect of pH on volume and surface weighted of casein micelles at different concentration of phosphocaseinate solutions.

Concentrations	Volume weighted mean D (4, 3)	Surface weighted mean D (3,2)	Concentrations	Volume weighted mean D (4, 3)	Surface weighted mean D (3,2)
3% solution at pH 6.86	0.14	0.10	**3% solution at pH 6.86**	0.19	0.10
pH 8	0.16	0.19	**pH 7**	29.17	0.18
pH 9	0.19	0.21	**pH 6**	5.11	0.12
pH 10	0.71	0.23	**pH 5**	101.77	37.12
pH 11	0.89	0.26	**pH 4**	607.81	251.95
4% solution at pH 6.86	0.18	0.10	**4% solution at pH 6.86**	0.18	0.10
pH 8	0.20	0.21	**pH 7**	0.19	0.25
pH 9	0.27	0.22	**pH 6**	0.23	0.11
pH 10	0.29	0.26	**pH 5**	101.77	37.12
pH 11	0.32	0.28	**pH 4**	747.39	288.46
5% solution at pH 6.86	0.18	0.11	**5% solution at pH 6.86**	0.18	0.11
pH 8	0.23	0.17	**pH 7**	0.19	0.17
pH 9	0.26	0.21	**pH 6**	0.21	0.11
pH 10	0.28	0.25	**pH 5**	186.47	57.36
pH 11	0.31	0.28	**pH 4**	529.67	125.12

Control= pH 6.86

In case of 4% solution, D (v, 0.5), D (v, 0.1) and D (v, 0.9) were quantified as 0.12, 0.06 and 0.23, respectively. At pH 11 4% solution depicted more volume and surface weighted means *i.e.*0.32 and 0.28, correspondingly. Additionally, 4% phosphocaseinate solution showed monomodal graph at different pH levels by using average particle size and volume weighted mean. At 5% filtered solution, the diameters were D (v, 0.5) 0.125, D (v, 0.1) 0.069 and D (v, 0.9) 0.226. Furthermore, 5% filtered solution at pH 9 exhibited highest average particle size as compared to other alkalinization conditions. The unimodal graph depicted same peak volume at pH 8, 10 and 11 whereas at pH 9 least peak volume was observed.

Earlier, Ahmad *et al.* (2009) recorded that majority of the casein molecules (90 to 95%) were combined to form casein micelles at pH 6.7. They also revealed that a variety of molecular forces are involved in the structure and stability of casein micelles depending on the balance between electrostatic repulsion and hydrophobic interaction. Furthermore, calcium phosphate is cross linked with casein molecules and neutralizes the negatively-charged phosphoseryl groups, allowing hydrophobic interaction between caseins. In milk, the micellar calcium phosphate is in equilibrium with the aqueous phase *i.e.* supersaturated.

Effect of alkalinization on charge of casein micelles: The charge on casein micelles decreases gradually with increasing pH in different solutions of Na phosphocaseinate powder. Mean values for 3% solution at pH 6.86 (control), 8, 9, 10 and 11 were -13.93, -16.53, -21.6, -24.2 and -28.35 mV, respectively (Table 3). The 4% solution at pH 11 showed minimum charge of casein micelle -20.55 mV as compared to pH 6.86 (control). Moreover, the charge of casein micelles in 5% solution at pH 6.86 (control), 8, 9, 10 and 11 were observed as -14.01,-16.35, -21.62, - 26.99and -29.09 mV, accordingly (Table 2).

Table 2. Effect of alkalinization on charge of casein micelles.

Concentrations	Charge (mV)
3% solution at pH 6.86	-13.93± 4.00
pH 8	-16.53±2.36
pH 9	-21.6±1.41
pH 10	-24.2±1.13
pH 11	-28.35±4.03
4% solution at pH 6.86	-12.65±1.20
pH 8	-13.88±0.19
pH 9	-14.8±1.15
pH 10	-16.06±0.98
pH 11	-20.55±1.35
5% solution at pH 6.86	-14.01±1.39
pH 8	-16.35±1.41
pH 9	-21.62±1.62
pH 10	-26.99±3.21
pH 11	-29.09±1.27

pH 6.86= Control

Vaia *et al.* (2006) concluded that the alkalinization of milk has opposite effect *i.e.* by increasing the level of colloidal calcium phosphate at pH 7.5, the micelles become stabilized due to strong electrostatic attraction in negatively charged caseins.

The casein ionization depends on alkalinization ultimately modifying the protein structure and molecular organization of casein micelles. The underlying mechanism is based on an increase in anionic charge of casein micelles under alkaline environment. Furthermore, increase in phosphate ion concentration was also found during alkalinization at pH 9.7 or 8.6 either in cow or buffalo milk, leading to the higher affinity of Ca to demineralize casein hence disrupt interactions between protein and mineral content. Conclusively, increase in the negative charge ensures smaller size of casein micelles (Bian, 2012). It has been documented that alkalinization and mineral addition directly affect the solubilization property of casein. The Ca-phosphate acts as an important moiety in the binding of casein micelles. On the other hand, reduction in Ca decreases cohesive forces and hydrophobic linkages between casein molecules thus increase solubility. Furthermore, κ-casein at outer face of micelles govern the final size distribution thus keep the structure in tightly bounded form, leading to higher tendency for reduced size. However, the increase in negative charge density is also considered as a critical factor in small size of casein micelles during alkaline conditions (Ahmad *et al.,* 2009).

Effect of acidification on size and charge of casein micelles: The maximum size of casein micelles was observed at pH 4 in 3, 4 and 5% solutions as 2007.5, 3062, 1183 nm, respectively. The charge on casein micelles in 3, 4 and 5% Na phosphocaseinate solution at pH 6.86 was - 13.93, -12.65 and -14.01mV, correspondingly (Table 3). Whilst, 3, 4 and 5% solution at pH 6 presented the charge on casein micelles as - 23.69, -25.26 and -26.35 mV, in respective manner.

Raouche *et al.* (2008) investigated the size of casein micelles in skim milk at pH 5.2 and 5.5 and noticed the values 192±2 and 194±4 nm, respectively. They further concluded that the charge on casein micelles decreased during acidification. Previously, Horne (2003) determined the average size of casein micelles as 200 nm. The above results concluded that the size of casein micelles increases as the charge decreases during acidification. The present results are also in agreement with Liu and Guo (2008), they observed that size of casein micelles decreased during acidification.

Hence, the intact casein micelles were observed at pH 5.5. The increase in viscosity due to higher concentration is directly dependent on distance between casein micelles. Afterwards, Orlien *et al.* (2006) studied smaller casein micelles size at lower pH due to the dissociation of casein micelles and dissolution of colloidal calcium phosphate. However, the swelling of micelles was noticed with increase in hydration. The subsequent increase in temperature up to 40°C was found effective in shifting the dissolved caseins and calcium phosphate back to micellar structure. On the other hand, smaller micelles were associated in the form of larger micelles thus responsible for increased turbidity at 40°C and varying pH levels. The change in pH can alter the protein and hydration level of micelles.

Table 3. Effect of acidification on size and charge of casein.

Concentrations	Size (nm)	Charge (mV)
3% solution at pH 6.86	258.15±10.35	-13.93±4.00
pH 6	268.45±12.43	-23.69±0.76
pH 5	276.55±15.06	ND
pH 4	2007.5±18.12	ND
4% solution at pH 6.86	265.65±9.14	-12.65±1.20
pH 6	274.4±9.53	-25.26±0.50
pH 5	518.8±18.41	ND
pH 4	3062±20.06	ND
5% solution at pH 6.86	259.2±9.69	-14.01±1.39
pH 6	267.65±5.77	-26.35±0.62
pH 5	275.7±8.40	ND
pH 4	1183±24.29	ND

ND= Not determined
pH 6.86 = Control

At acidified conditions, pH changes the intramicellar interaction in the initial level of the micelles. The loss of micelle structure is due to increased level of hydration and dissolution of colloidal calcium phosphate & caseins at pH 5.5 (Orlein *et al.,* 2004). Françoise *et al.* (2009) noticed the removal of calcium-casein linkages during acidification as well as re-association of casein molecules with the micelles. As a result, increase in casein micelles and decrease in charge was analyzed during acidification of milk from 6.70 to 4.80. Likewise, Philippe *et al.* (2003) and Famelart *et al.* (2003) observed the zeta potential for skim milk as -22 and -19 mV, respectively. In the present study, slight variations were noticed in the charge of casein micelles due to lower ionic concentration of deioninzed water.

Effect of heat treatment on size and charge of casein micelles: The result expounded that heat treatment showed a declining trend in both size and charge of casein micelles. At 60ºC, the size of casein micelles was 192.93, 211.05 and 229.02 nm at 3, 4 and 5% solutions, respectively. Likewise, the mean sizes of casein micelles were 255.50, 260.55 and 289.71 nm for 3, 4 and 5%

solution at 90ºC, correspondingly. Whilst, the charge on casein micelles for respective solutions at 60ºC and 90ºC were recorded as -18.85, -18.70 & -17.43 mV and -15.25, -15.85& -14.85 mV, accordingly (Table 4).

Table 4. Effect of heat treatments on size and charge of casein micelles.

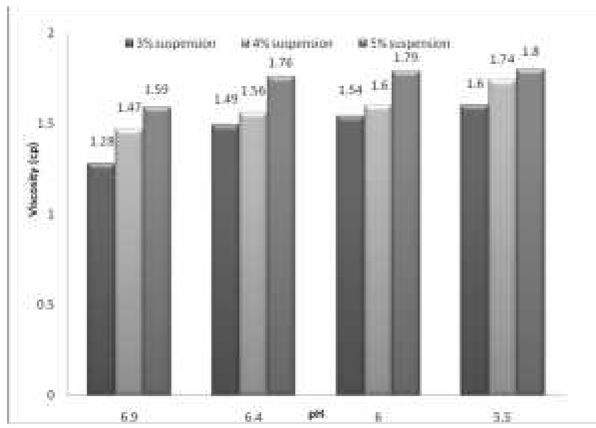
Concentrations	Size (nm)	Charge (mV)
3% Solution at 60 °C	192.93±9.64	-18.85±0.94
65 °C	198.052±9.90	-17.80±0.89
70 °C	203.10±10.10	-16.70±0.83
75 °C	220.50±11.25	-16.30± 0.81
80 °C	230.55±11.52	-16.61±1.32
85 °C	245.16±12.25	-15.99±1.02
90 °C	255.50±12.77	-15.25±0.76
4% Solution at 60 °C	211.05±10.55	-18.70±0.93
65 °C	222.65±11.13	-17.61± 0.88
70 °C	231.15±11.55	-16.95±0.84
75 °C	241.12±12.05	-16.70±1.01
80 °C	245.82±12.29	-15.35± 0.92
85 °C	250.05±12.50	-15.85±0.79
90 °C	260.55±13.02	-15.82±0.94
5% Solution at 60 °C	229.02±11.45	-17.43±0.87
65 °C	238.32±11.91	-16.45±0.98
70 °C	243.25±12.16	-15.13±1.05
75 °C	252.71±12.63	-15.90±1.27
80 °C	267.75±13.38	-15.23±0.76
85 °C	275.15±13.75	-15.95±0.79
90 °C	289.71±14.48	-14.85±0.60

Control = pH 6.86

The current results are in accordance with the findings of Sauer and Moraru (2012). They explored the dependence of casein micelles stability on temperature and documented that gradual rise in temperature is the main reason of increase in particle size of casein micelles. They further enumerated that the particle size of casein micelles was 193±16.1 for control and 229.25.3nm at 150°C heated solution. Earlier, Anema and Li (2003) measured the size of casein micelles in heat reconstituted skim milk and reported an increase from 199 to 260 nm at 70 to 100°C for 10 min. The initial integrity of micelles is dependent on the temperature and pH however, dissolution of colloidal calcium phosphate and weaker hydrophobic bonds also affect the disruption of casein micelles differently (Anema, 2008). It is thereby concluded that casein aggregate size plays an important role in the formation of stronger gel. Silva *et al.*, 2013 investigated the decrease the charge of casein micelles on cow milk ranged from -15.6 to -14.6

Effect of acidification on the viscosity: The protein hydration tends to increase the viscosity in various dairy products. Viscosity is an important parameter to determine air incorporation, creaming, flow rate, flowing conditions and casein miceller aggregation (Bhandari, 2001).

The Na phosphocaseinate solutions (3, 4 and 5%) were prepared by lowering the pH from 6.9 to 5.5 and maximum viscosity was observed in 5% solution (1.59 to 1.80 cP) as compared to 3% (1.28 to 1.60 cP) and 4% solution (1.47 to 1.74 cP) (Figure 1).



Control = pH 6.86

Figure 1. Effect of acidification on viscosity (cp) of solution of phosphocaseinate powder at different concentration

The present results are in harmony with the findings of Aaltonen (2012). He noticed a significant increase in viscosity when the pH was decreased from 6.9 to 5.6. Previously, Anema *et al.* (2004) found higher viscosity and size of casein micelles in acidified and heat treated skim milk. They further indicated that whey protein was easily bound to casein micelles at reduced pH level.

Conclusions: The main conclusion of this study is that 5% solution of sodium phosphocaseinate powder showed better acidification, alkalinization and heat treatment behavior on physicochemical characteristics such as size, charge and viscosity as compared to other solutions (3 and 4%). These results were affected by the factors like conformational stability and hydrophobic properties of the rehydrated sodium phosphocaseinate powder. From the increase in temperature was find out that the sodium caseinate with stand up to 90°C here in best temperature good solubility with reform to the change 70-75°C. These findings will help processor for pH and heat treatment effects on physic chemical characteristics with rehydrated conditions of sodium phosphocaseinate powder to formulated new innovative protein enriched dairy and non dairy products.

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