Comparative Study on Heat Stability and Functionality of Camel and Bovine Milk Whey Proteins

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ABSTRACT

Heat stability, emulsifying, and foaming properties of camel whey have been investigated and compared with that of bovine whey. Camel whey is similar to bovine whey in composition, but is deficient in β -lactoglobulin (β -LG), a major component of bovine whey. Whether the deficiency in β -LG will affect stability and functional properties is not yet known. Substantial information on the functional properties of bovine milk whey proteins is available; however, there is little research done on functional properties of camel whey proteins. Therefore, the objective of this study was to investigate the heat stability, emulsifying, and foaming characteristics of camel whey proteins. Calorimetric studies showed no significant difference in heat stability between bovine and camel whey proteins in liquid form. Upon drying, thermograms indicated that the 2 proteins are different in composition and thermal stability. The difference is represented in the absence of β -LG and the occurrence of protein denaturation peak at a lesser temperature in camel whey. The first marginal thermal transition in bovine whey appeared at 81°C, followed by 2 other transitions at 146 and 198°C. For camel whey, the transitions appeared at 139, 180, and 207°C respectively. The first marginal denaturation peak in bovine whey is due to β -LG, which is essentially absent in camel whey, while the second peak is due to the mixture of α -lactalbumin, serum albumin, and possibly part of the partially stabilized β -LG structure during the denaturation process. Because camel whey is deficient in β -LG, the denaturation peak at 139 must be due to the mixture of α -lactalbumin and camel serum albumin. In both proteins, the highest thermal transition is due to sugars such as lactose. The solubility study has shown that camel whey is more sensitive to pH than bovine milk whey and that heat stability is lowest near the isoelectric point of the proteins at pH 4.5. The sensitivity to pH resulted in partial denaturation and increased tendency to aggregate, which caused poor and unstable

emulsion at pH 5. Both bovine and camel whey proteins have demonstrated good foaming properties; however, the magnitudes of these properties were considerably greater in bovine milk for all of the conditions studied.

Key words: calorimetry, denaturation temperature, β -lactoglobulin, α -lactalbumin

INTRODUCTION

Tons of whey proteins are produced annually as by-product of the cheese and casein manufacturing industry. In the past, whey was considered a waste product—suitable only for livestock feed or dumping into rivers, a practice that creates environmental havoc. Like bovine milk, the protein of camel milk is constituted in the casein and whey fractions. The role of bovine whey protein ingredients in the formation of emulsion-based food products such as beverages, frozen desserts, ice creams, sport supplements, infant formula, and salad dressing is well known. Another useful property of whey proteins is the ability to form a gel on heating (Singh and Hevea, 2003). The proteins in these ingredients assist in the formation of small oil droplets during homogenization by decreasing the interfacial tension and, by so doing, increase the stability of the droplet formed (Demetriades et al., 1997).

Whey is a complex mixture of different proteins. In general, the main components include β -LG ($\approx$ 55%), α -LA ($\approx$ 24%), serum albumin (SA, $\approx$ 5%) and immunoglobulins ($\approx$ 15%) (Swaigood, 1996). However, there could be variation in the protein composition and the relative concentration of these components in milk, depending on the type of the animal which produced the milk. The variation in composition and relative concentration of the protein components may have some influence on the functional and physical properties of the whey protein ingredients. Compared with bovine whey, camel whey is reported to contain greater content of antimicrobial factors such as lactoferrin and immunoglobulin (Elagamy et al., 1992). Another major difference between camel and bovine whey is the absence of β -LG which is found in many livestock ruminants' milk. The main components of camel whey include SA, α -LA, immunoglobulins, lactoferrin, and

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peptidoglycan recognition protein (Farah, 1993; Kappler et al., 2004). In addition to the difference in composition, camel milk is also different from bovine milk in its physical characteristics. It is less viscous and whiter, but it contains all of the essential nutrients in bovine milk (Elagamy, 2000). On average, camel milk contains more whey protein than bovine milk (Farah, 1993; Walstra et al., 1999). This variation is primarily because of the greater content of albumin and lactoferrin in camel milk (Farah, 1993). The type of protein and its relative concentrations may affect the way these proteins behave and interact with other food ingredients in a complex formulation.

Functional properties such as emulsifying and foaming properties of bovine milk whey proteins have been thoroughly studied and reported. However, there is little information about these properties of camel whey proteins. Understanding these functional properties is essential to predict the perception of consumers and how they will react to particular food product due to its textural properties. Taste and perception of a food product are highly influenced by the structures that are formed by the ingredients used to create it. Therefore, understanding the behavior of these ingredients in a complex system is crucial for creating the desired structures and maintaining stability. Once the product exits the factory, its structure may continue to change as a result of thermal or mechanical stress or biological action. Controlling these physical or chemical changes is part of the art and constitutes a challenge to the processor as he strives to provide food structures which are not only desirable but also stable enough to withstand changes during the storage period. Therefore, the objective of this study was to investigate the heat stability and functional properties such as emulsion and foaming characteristics of camel whey proteins.

MATERIALS AND METHODS

Sample Preparation

Fresh bovine milk was supplied by the UAE University farm and camel milk was donated by Al-Ain Dairy (Al-Ain, United Arab Emirates). The fat was removed from the milk by centrifugation at $15,000 \times g$ for 10 min. The whey was separated from the casein by addition of rennet enzyme and coagulated at 37°C for 3 h followed by centrifugation at $15,000 \times g$ for 10 min. Part of the whey was concentrated using a vacuum evaporator at 50°C for 60 min, followed by drying in a vacuum oven for 12 h at 70°C for differential scanning calorimetry application. The rest was frozen at -18°C until used for emulsion formation and heat stability tests. Sunflower oil was purchased from a local supermarket and used

without further purification. Deionized water was used for the preparation of all solutions.

Differential Scanning Calorimetry Measurements

A DSC Q100 differential scanning calorimeter (TA Instruments Inc., New Castle, DE) equipment with nitrogen refrigeration cooling system (RCS) and aluminum hermetic pans were used. Differential scanning calorimetry was conducted on both liquid and dry whey obtained from bovine and camel milk. For the liquid whey, a 7-mg sample was pipetted into the aluminum pan and hermetically sealed. The temperature was raised from 20 to 160°C at a rate of 5°C/min. For dry whey scanning, an 8-mg sample was put in the aluminum pan and sealed. The temperature was increased from 20 to 250°C at a rate of 5°C/min.

Heat Stability Test

Heat stability testing was conducted using the method outlined in Chevalier et al. (2001) with some modifications. The protein content of bovine and camel whey was first determined using the Kjeldahl method (AutoKjeldahl Unit K-370, Büchi Labortechnik AG, Flawil, Switzerland). Fresh whey with a protein ratio of 2 mg/mL was diluted in 0.1 mol/L sodium acetate buffer at pH 4, 4.5, and 5, and 0.1 mol/L sodium phosphate buffer for pH 7. After solubilization in the appropriate buffer, samples were heated in a water bath at different temperatures (60, 70, 80, 90, and 100°C) for up to 1 h. Heated samples were cooled immediately at 4°C and centrifuged at $2,575 \times g$ for 15 min. The UV absorption at 280 nm of the supernatant were measured with a Spectronic Genesys 5 spectrophotometer (Milton Roy Company, Ivyland, PA) to estimate the dissolved protein and the results compared with the untreated sample (2 mg/mL).

Emulsion Preparation

The emulsifying properties were determined by the method of Chevalier et al. (2001) with some modifications. The required concentration (2 mg/mL) was prepared by measuring 100 mL of bovine whey (77 mL in case of camel whey) in a graduated measuring cylinder. The oil-in-water emulsions were prepared by blending 15 wt% sunflower oil (75 mL) and 85 wt% (425 mL) whey protein solutions using an Ultra-Turrax T25 high-speed mixer (IKA Labortechnik, Staufen, Germany) at 20,000 rpm for 30 s. The mixture was then homogenized in an APV-1000 pressure homogenizer (Invensys APV Products, Albertslund, Denmark) at inlet and outlet pressures of 45,000 and 5,000 kPa, respectively.

Optical Microscopy

One milliliter of the emulsion was taken and diluted with 9 mL of 0.1% SDS. Emulsions were gently shaken in a Falcon tube (Becton Dickinson, Franklin Lakes, NJ) before analysis to ensure homogeneity. A drop of emulsion was mounted on a microscope slide and then covered with a cover slip. The microstructure of the emulsion was then viewed using a transmitted light microscope (Zeiss Model K300, Kontron Elektronik GmbH, Carl Zeiss Inc., Munich, Germany) connected to digital image processing software.

Foaming Properties

Twenty milliliters of whey was added to 20 mL of sodium phosphate buffer (pH 7) in a measuring cylinder. The mixture was mixed at different speeds (8,000, 9,500, and 13,000 rpm) using the Ultra Turrax mixer. The content was mixed for 2 min and the volume of the foam directly read in the measuring cylinder.

Statistical Analysis

Analysis of variance was carried out using Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Differential Scanning Calorimetry Measurements

Unfolding of globular proteins during thermal denaturation demands absorption of heat to break intramolecular bonds (noncovalent and, in some cases, disulfide) and is therefore endothermic. The combined thermogram of liquid camel and bovine whey is shown in Figure 1. There is no difference in thermal denaturation between the 2 whey proteins. The peak denaturation temperature in camel whey was at 109°C, whereas that of bovine whey was at 106°C. Contrary to our expectation, no individual thermal transition was observed for major individual proteins in bovine whey such as β -LG, α -LA, and SA. This means that the peaks of the individual proteins overlapped and a single peak for the mixture was observed. This observation is in agreement with what has been previously reported by Paulsson et al. (1985). In a calorimetric study of thermal stability of a pure protein mixture of α -LA, β -LG, and bovine SA at pH 6.6, only a single thermal transition of the mixture was observed. In Paulsson et al. (1985), the thermal denaturation temperature of the mixture was below 80°C, whereas in this study the denaturation temperature was 109°C for camel and 106°C for bovine

Figure 1. Differential scanning calorimetry traces of liquid camel and bovine milk whey.

whey. Bearing the processing cost in mind, this study was conducted on unpurified proteins, whereas in the previously mentioned studies pure and desalted proteins were used. It is possible that salts and other impurities influenced our results.

To investigate the effect of drying on the stability of the whey, differential scanning calorimetry was conducted on dry whey and the thermograms are displayed in Figure 2. Unlike liquid bovine whey, a marginal transition extending from 60 to 85°C in dry bovine whey suggests the presence of β -LG in the mixture. Similar observation has been reported by previous investigators (Boye and Alli, 2000; Fitzsimons et al., 2007). Aside from the overlapping of the transition peaks in the liquid form, the absence of any thermal transition in liquid form may be due to the fact that the sample was too small. Only 7 mg of the sample was placed in the aluminum pan to avoid spilling of the contents during the sealing stage and to minimize pressure build up when the temperature was raised. Because camel whey is deficient in β -LG, the appearance of a single and broad transition extending from 110 to 135°C suggests overlapping of thermal denaturation peaks of α -LA and camel SA as was the case for liquid whey. The observed shift in the denaturation temperature can be attributed to the partial stabilization effect on the protein structures during the denaturation process. This partial stabilization phenomenon following the destabilization induced by breakdown of disulfide bonds was also reported by De Wit and Klarenbeek (1981) as a possible reason for the shift of denaturation temperature of residual β -LG structure in the range of 130 to 140°C. A similar argument can be presented to explain the observed high denaturation temperature in the dry bovine whey denaturation process. Other tran-

Figure 2. Differential scanning calorimetry traces of dry camel and bovine whey.

sitions close to 200°C can be assigned to sugars such as lactose that are also present in whey. Considering the complex nature of proteins, further studies on isolated individual proteins may be needed to reach a final and more reliable conclusion on their stability. However, the preliminary findings indicate that camel whey is relatively heat stable, as reported by Farah and Atkins (1992), and the absence of β -LG can be considered as an important difference in chemical compositions as reported by Elagamy et al. (1998).

Heat Stability Test

Heat stability in liquid whey was measured indirectly by measuring the solubility at different temperatures and pH. It was assumed that after heating the liquid whey, the denatured fraction would precipitate, leaving the undenatured part dispersed in the liquid medium. The effect of temperature on the denaturation process is shown in Figure 3 and 4 for both camel and bovine whey. In general, greater temperature increases the denaturation process and makes the proteins significantly less soluble. For bovine whey, solubility decreases from 81 to 56% as the temperature is raised from 60 to 100°C, while for camel whey, it decreases from 76 to 55% in the same range. The ANOVA results suggest that bovine whey is more soluble within the pH and temperature range tested. However, it appears that the effect of the temperature depends on the pH level. At pH 7, the effect of temperature is relatively small and the curve remains flat at around 70% solubility in both bovine and camel whey. The major change in solubility occurred at pH 4.5. At this pH, solubility decreases drastically, especially at greater temperatures (80, 90, and 100°C) and the shape of the graph suggests that

Figure 3. Effect of temperature on bovine whey protein solubility.

there is an interaction between temperature and pH. In both figures, we can see that solubility is less at pH 4.5 within the temperature range 80 to 100°C. This point represents the isoelectric point of many proteins.

This finding can be observed more clearly in Figures 5 and 6, which show the effect of pH on solubility. After heating the protein for 1 h, we observed a decrease in solubility of about 55% at pH 4.5 and 100°C for camel and 52% for bovine whey at the same pH and temperature. This shows that the 2 whey proteins are significantly different in terms of heat stability. The dependence of solubility on pH has been studied and reported by Bernal and Jelen (1985). They observed a transition from soluble to insoluble states to fall within a narrow pH range of 3.7 to 3.9. Below the critical pH region (pH 3.9), the high solubility is attributed to high electrostatic repulsion between the unfolded proteins molecules combined with the absence of disulfide interchange reaction at such a low pH. A similar effect

Figure 4. Effect of temperature on camel whey protein solubility.

Figure 5. Effect of pH on bovine whey protein solubility.

may occur at the higher pH range (pH 5 to 7). Between pH 3.9 to 4.5, the net charge is small and electrostatic repulsive force is less; protein-protein interaction is more likely to happen and heavy protein precipitation is observed. This can adequately explain the greater thermal stability of camel whey at pH 5 compared with pH 4.5.

For comparison between bovine and camel whey, the observed increase in stability can be associated with the presence of β -LG which is the most abundant and most stable among the 3 major whey proteins in bovine whey (β -LG, α -LA and SA). The denaturation temperature of the 3 proteins in their pure forms have been reported as 81°C for β -LG, 74°C for SA and 61°C for α -LA (Bernal and Jelen, 1985). The thermal denaturation temperatures of the individual proteins can be used to explain the observed change in the trend at pH 4.5 when the temperature was kept at 60 and 70°C. At these low temperatures, the heat had little effect on the proteins and very little precipitation was noticed even after centrifugation, resulting in high absorbance at the selected wavelength (280 nm). The ANOVA results indicate that camel whey is more sensitive to the effect

Figure 6. Effect of pH on camel whey protein solubility.

of pH on the solubility of proteins than bovine whey because it contains α -LA which is highly susceptible to acid denaturation (Paulsson et al., 1985). As the pH is decreased from 7 to 4, solubility decreases by about 16% in camel and 9% in bovine whey. At neutral pH, the aggregation process is inhibited by electrostatic repulsion between the unfolding globules, as a result, solubility is less near the isoelectric point of the protein where there is no net electric charge and the proteins tend to precipitate. This is in agreement with previous studies reported by Chevalier et al. (2001). Therefore, one way of enhancing aggregation is to reduce the charge on the protein molecules by decreasing the pH toward the isoelectric point (Boye et al., 1995).

Emulsifying Properties

The purpose of this test was to compare the microstructures and stability of emulsion formed from bovine and camel whey proteins. Emulsifying properties of bovine and camel whey measured at pH 5 and 7 in the presence of 15% sunflower oil were evaluated by measuring the mean diameter of the oil droplet size and distribution using a conventional optical microscope connected to digital image processing software installed on a computer. The electronic microscopy images are shown in Figure 7 and the oil droplet size distributions are shown in Figure 8. As can be seen in Figure 8, about 80% of the droplets in the emulsion formed from bovine whey have diameters less than 2 μ m and only 1.5% of the droplets have diameters greater than 3 μ m. No droplet diameter greater than 3.5 μ m was present. In the case of camel whey at the same pH, 88% of the droplets have diameters larger than 2 μ m and only 12% are smaller than 2 μ m in diameter.

At pH 7 and room temperature, whey protein is 100% soluble and a stable emulsion with small droplet size was formed by both bovine and camel whey and the mean diameters were 1.07 and 1.3 μ m for bovine and camel whey, respectively. The difference of 0.24 μ m suggests a relative superiority of emulsifying properties of bovine whey. At pH 5, the mean diameter of the oil droplet size increased considerably. The increase in the droplet size was accompanied by the formation of a creamy layer within 1 h and the creamy layer is more noticeable in camel milk. The observed increase is more than 2-fold (2.86 μ m) for camel milk and slightly above half (1.7 μ m) for bovine whey. The low stability of the emulsion at pH 5 is believed to be due to the aggregation of the protein molecules which exhibit low solubility at pH 5. The high increase in the aggregation of the camel milk whey protein at lower pH implies that camel milk is more sensitive to pH than bovine milk because of the high content of α -LA. This phenomenon can be

explained by the decrease in the net electric charge on the droplets. At pH values close to the isoelectric point of the protein, the magnitude of the electrical charge on the droplets decreased. Therefore, the droplet charge is insufficient to generate an electrostatic repulsive force that overcomes the various attractive droplet-droplet interactions such as Van der Waals and hydrophobic forces and as a result, the droplets aggregate to form larger flocculates (McClements, 2004).

Foaming Properties

Foam is a dispersed system often found in foodstuffs. Whey proteins are surface-active agents that are widely used as functional ingredients for the formation and

stabilization of the system. The simultaneously polar and nonpolar regions found in these molecules give them surface active properties. When a solution containing whey protein is mechanically agitated it absorbs and forms gas bubbles, creating foam which is protected against collapsing. Systematic understanding of the structure and the mechanical properties of the air-water interface seem to be essential for controlling the behavior of the foam system. In the present study, native proteins were able to produce foam by beating, as measured by the increase in the foam volume after beating for 2 min. Results revealed that bovine milk whey produced greater foam volume for all conditions studied, as clearly seen in Figure 9. However, the difference is not statistically significant.

Camel whey pH 5

Camel whey pH 7

Cow whey pH 5

Cow whey pH 7

Figure 7. Microscopic images of bovine and camel whey emulsion, magnification $\times 1,000$. Bar = 20 μm .

Figure 8. Oil droplet size distribution of camel and bovine whey pH 5.

Foam stability at different mixing speeds is shown in Figure 10. As expected, greater mixing speed increases the foam volume and stability. It is well known that bulk properties such as viscosity play a vital role in the stabilization of dispersed systems. The viscosity of a protein solution is influenced by the structure and rigidity of the protein constituents. Graham and Phillips (1980) reported that flexible and disordered macromolecules such as caseins form films with very low viscoelastic properties, whereas globular proteins (lysozyme, β -LG, bovine SA, ovalbumin) form films with relatively high rigidity. The considerable high viscoelastic behavior of globular proteins (especially β -LG) layer can be attributed to the high packing density and strong intermolecular interactions, as compared with loose dynamic structure of casein layer (Dickinson, 1999). In the formation of whey-stabilized foam and emulsions, a protein network is created in the presence of globular proteins via intermolecular interactions

Figure 10. Effect of mixing speed on camel whey foam volume at pH 7.

Figure 9. Effect of whey type on the foam volume and stability at pH 7.

(hydrogen bonds, electrostatic, and hydrophobic interactions). Bearing in mind that camel milk lacks β -LG, which is the principal globular protein in bovine milk whey protein, this may represent a genuine argument for the relative inferiority of camel-whey-stabilized foam and emulsions.

CONCLUSIONS

The current study has shown that, like bovine whey, camel whey has the potential for a wide range of applications in product development and food formulations, especially in food products where low acidity is a desirable characteristic. In addition, this study has demonstrated the ability of camel whey to produce stable foam and emulsions at pH 7 and this can motivate cheese manufacturing companies to increase the utilization of this by-product in developing functional foods of high nutritional value and aesthetic properties.

In all conditions studied, bovine milk whey gave better emulsion and foam properties when compared with camel-whey-stabilized dispersed systems. Camel whey's relatively inferior foam and emulsion properties may be attributed to the absence of β -LG, which constitutes more than 50% of the total protein in bovine whey. The calorimetric study showed no difference in heat stability between camel and bovine whey in liquid form. However, the preliminary differential scanning calorimetry traces in dry whey and solubility studies indicate that camel milk whey proteins are slightly more susceptible to heat denaturation than bovine whey proteins. To reach more concrete conclusions regarding heat stability, further studies are needed on pure individual whey protein components to establish their heat stability under different buffering conditions.

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