Hot topic: Sonication increases the heat stability of whey proteins

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ABSTRACT

The thickening or gelling of protein-based dairy streams and ingredients upon exposure to heat has been an ongoing problem in dairy processing for many decades. This phenomenon can restrict the range of dairy product options and reduce manufacturing efficiencies by limiting the type and extent of heat treatment that can be used. In this report, we outline a novel approach to overcoming this problem. The use of preheating treatments to induce whey protein aggregate formation in whey products is well known in the field. However, we show that the application of ultrasound for a very short duration after such a heating step breaks down these aggregates and prevents their reformation on subsequent heating, thereby reducing the viscosity increase that is usually associated with this process. This novel technique has the potential to provide significant economic benefit to the dairy manufacturing industry.

Key words: ultrasound, whey protein, heat stability, sonication

In the dairy industry, the stability of dairy proteins toward heat treatment is a major processing issue. Exposure of whey proteins to temperatures in excess of 70°C causes denaturation, which in turn leads to protein aggregation through both hydrophobic interactions and the formation of intermolecular disulfide bonds (Wang et al., 2006). This can result in excessive thickening or gelling during processing of the dairy product and later, upon storage (Morr and Richter, 1999). Several pretreatment procedures have been developed to improve the heat stability of dairy proteins, including forewarming (Deysher et al., 1929) and pH adjustment (Singh, 2004). We have developed a simple and efficient ultrasonic treatment that appears highly effective in this regard.

Although ultrasonic processing technology appears to work for several dairy systems, our research to date has shown that effects on the heat stability of whey

protein solutions and concentrates are the most impressive. Whey, a by-product of cheese making, contains significant amounts of β -lactoglobulin, α -lactalbumin, and serum albumin, along with lactose and smaller amounts of minerals, lipids, and lactic acid. In a dairy factory, whey proteins are concentrated by membrane filtration, evaporation, and spray drying for application in a range of processed foods, including protein supplements and infant formulas. During this manufacturing process, the aqueous whey protein solutions containing significantly high levels (4 to 15% by weight) of protein are subjected to heat treatment. Issues with thickening and gelation of the protein solution ultimately limit the extent to which heat treatment is applied, the total solids concentration that can be used, or both.

The ultrasonic process involves a short preheat treatment followed by sonication of the whey protein solution for a short time at 20 kHz. The general experimental procedure is outlined in the scheme shown in Figure 1. In a typical experiment, an aqueous solution containing 4 to 15% total protein (by weight) was preheated to 80°C and held for 1 min. The preheated solution was then subjected to high-intensity, low-frequency ultrasound for less than 5 min. A post-heat-treatment in an 85°C water bath for 20 min was then used to assess the effectiveness of the ultrasonic treatment.

In the specific experiments presented here, whey protein concentrate (**WPC**) powder containing 80% protein derived from industrial Cheddar cheese whey was obtained from Warrnambool Cheese and Butter Factory (Victoria, Australia). This powder was reconstituted to 6.4% protein (by weight) in deionized water (>18.2 M Ω -cm, MilliQ, Millipore, North Ryde, Australia) for 1 h and held overnight at $<5^{\circ}\text{C}$. Whey protein retentate containing 27% protein was obtained as the retentate from the ultrafiltration of sweet whey from the same source. This retentate was diluted to 4% protein.

In bench-top sonication trials, preheating was carried out on 200-g samples placed in sealed, stainless steel tubular containers (73 mm diameter, 135 mm deep). Each container was fitted with an automated stirring arm and a thermometer. The apparatus was immersed

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Figure 1. A typical experimental plan for a protein solution; control (C), preheating (PreH), sonication (US), and postheating (PostH). Color figure available in the online version of this article.

in a water bath preheated to 80°C. The solutions were stirred continuously and reached 80°C in 120 ± 20 s. The samples were held at this temperature for a further 1 min and then transferred to an ice bath for rapid cooling to 20 to 25°C. The preheating step for the pilot-scale trials was deliberately designed to match the bench-top sonication trials as closely as possible. A temperature-controlled, steam-jacketed kettle containing approximately 40 L was used with manual stirring. The heating temperature was set to achieve a come-up time to 80°C of 120 ± 30 s, similar to the bench-top process. The whey protein retentate was then held at this temperature for a further 1 min before being manually transferred to stainless steel containers and rapidly cooled to 20 to 25°C using an ice water bath.

In bench-top sonication trials, 60-mL samples were sonicated in a custom-made glass vessel equipped with a cooling jacket, using a 20-kHz, 450 W ultrasonic horn (19 mm diameter, Branson Sonifier 450, Danbury, CT) at an amplitude of 50% for between 5 s and 20 min. The actual power delivered to the solution was determined using calorimetry to be 31 W. During sonication chilled water was circulated continuously to maintain the sample temperature below 10°C. In the pilot-scale trials whey protein solutions (<20°C) were pumped at a flow rate of 300 mL/min through a 20-kHz, 1 kW flow-through sonication unit (Hielscher UIP 1000hd, Teltow, Germany). The sonication was carried out at 60% amplitude, giving a calorimetric power delivery of 270 W or 54 J/mL. Temperature in the sonication unit was maintained by circulating ice water through an external jacket using a peristaltic pump.

For postheating, aliquots of sonicated and unsonicated solutions were placed either as 1-mL samples in 1.5-mL Eppendorf tubes or as 33-g samples in 70-mL polypropylene sample containers. These were then immersed in an 85°C water bath for 20 min. The temperature reached 70°C within the first 10 min of this postheat cycle and hence the samples were exposed to temperatures >70°C for at least 10 min. The containers were then transferred to an ice bath for rapid cooling. Some samples were frozen at -18°C and then freeze-dried in a Dynavac freeze dryer, Model FD-5 (Dynavac

freeze dryer, model FD-5, Melbourne, Australia). The freeze-dried samples were gently milled to produce flakes, which were stored in tightly sealed plastic containers at room temperature.

The size distribution of protein agglomerates was determined at room temperature (approximately 22°C) using a Malvern Mastersizer 2000 laser diffraction system (Malvern Instruments Ltd., Malvern, UK). Samples were shaken vigorously by hand and dispersed directly into circulating deionized water to obtain an obscuration of 10%. Measurements were made using a refractive index of 1.456 for the protein agglomerates and 1.33 for the dispersant (H₂O) with an absorption coefficient of 0.001. Mie theory (Van De Hulst, 1957) was used to analyze the data, taken as the average of 3 measurements.

The viscosity of the treated samples was measured using a Universal Stress Rheometer SR5 (Rheometric Scientific Inc., Piscataway, NJ). A controlled steady stress sweep was carried out using 40-mm parallel plate geometry with a set gap of 1 mm at 25°C. The stress was varied to obtain the viscosity data between the shear rates of 50 to 200 s⁻¹. The data presented in Figure 2 is at a shear rate of 100 s⁻¹.

Figure 2 shows typical experimental results observed with a reconstituted WPC (6.4% protein by weight). The viscosity of the preheated sample increased significantly compared with that of the control sample. This viscosity then increased further upon a second heat treatment. At higher protein concentrations, these heat treatments can lead to gel formation. However, the sample subjected to sonication for even 5 s showed a significant decrease in viscosity, and the postheating process had only a minimal effect on the viscosity of the solution. Similar results were achieved for solutions of whey protein retentate sampled directly from an industrial separation process. As can be seen in Figure 3, continuous flow through processing provided similar changes in the viscosity of the solution caused by sonication. Results at other protein concentrations were also similar. This functionality (low viscosity) was preserved even after freeze or spray drying and then reconstitution into aqueous solution (Figure 3).

Figure 2. The effects on solution viscosity for a 6.4% protein (by weight) solution reconstituted from whey protein concentrate and sonicated with a 20-kHz horn at a calorimetric power of 31 W in a batch mode. Dark gray (blue) bars represent solutions without sonication; light gray (pink) bars indicate sonicated solutions. PreH = preheating; PostH = postheating; US = sonication. Color figure available in the online version of this article.

It is important to note that these effects were observed only when sonication was used following a heat treatment. Other researchers observe protein aggregation (Stathopoulos et al., 2004; Gülseren et al., 2007) and viscosity increases (Kresic et al., 2008) in similar dairy solutions when sonication alone is used. Indeed, in the present work, sonication in the absence of preheat treatment resulted in gel formation when a relatively severe treatment was applied. For example, a 4% protein solution sonicated in the bench-top cell for 60 min gelled upon exposure to an 80°C water bath for 12 min, whereas the unsonicated control sample did not gel in 20 min.

Ultrasound is known to generate acoustic cavitation in liquids: the bubble nuclei present in the liquid medium grow and implode because of the pressure fluctuations caused by ultrasound. During acoustic cavitation, the liquid medium is subjected to extreme forces that include shear, turbulence, microstreaming, and heat. In addition, highly reactive radicals are generated (Ashokkumar and Mason, 2007). Considering these ultrasound-induced cavitation effects, the observed viscosity changes might have been caused by the physical or chemical effects of acoustic cavitation.

To investigate possible chemical effects due to radical generation, further experiments were carried out in which reconstituted WPC solutions were sonicated over a range of frequencies (20 kHz to 1 MHz). Previous studies (Ashokkumar et al., 2008) have shown that reactive radical generation is significantly greater at frequencies above 20 kHz. In this study, the WPC solutions sonicated at 20 kHz showed significant viscosity reduction, whereas those sonicated at higher frequencies did not show any viscosity reduction; this indicates that

the observed viscosity reduction is caused primarily by physical forces generated during acoustic cavitation.

Figure 4 shows that the protein aggregate size increased with heat treatment but reduced significantly under sonication. Similar observations were made for bench-top experiments involving WPC and across a range of protein concentrations. This again suggests that physical effects are responsible for the observed viscosity changes. Other authors have shown that similar changes in whey protein aggregate size may be induced by physical shear processes such as microparticulation, as used in the manufacture of Simplesse (Nutra Sweet Co. Ltd., St. Louis, IL) (Taylor and Fryer, 1994; Sanchez et al., 1997; Onwulata et al., 2002; Iordache and Jelen, 2003). However, to the best of our knowledge, there is only one comparable observation of increased heat stability (Dissanayake and Vasiljevic, 2009). These authors observed an increase in the heat coagulation time from 14.5 to 102.5 s following exposure of a 10% protein whey retentate to 90°C for 20 min and microfluidization for 5 passes at 140 MPa.

Although the mechanism is still not fully understood at the molecular level, we speculate that the observed viscosity reduction in whey protein solutions is primarily caused by the shear forces generated during acoustic cavitation. The initial increase in solution viscosity after heat treatment is due to heat-induced aggregation of whey proteins. During sonication, the shear forces generated by acoustic cavitation disrupt the hydrophobic interactions or the intermolecular disulfide bonds. It is known that the physical forces generated during acoustic cavitation are strong enough to break polymer chains (Price and Smith, 1993). Because further aggregation of protein particles occurs to a much

Figure 3. The viscosity of a whey protein retentate collected from the factory floor, diluted to 4% protein (by weight), and sonicated using a flow through a 20-kHz horn transducer at a calorimetric power of 270 W and a flow rate of 300 mL/min, after freeze drying and reconstitution to 15% solids. PreH = preheating; US = sonication. Color figure available in the online version of this article.

Figure 4. The agglomerate particle size distribution for the same whey protein retentate (Figure 3) before freeze drying. PreH = pre-heating; PostH = postheating; US = sonication. Color figure available in the online version of this article.

lesser extent during the post-heat-treatment process, we speculate that the functional groups responsible for such interparticle interactions such as free thiols are also deactivated during the heat treatment and sonication sequence. Other researchers have shown that heat-induced protein denaturation can cause irreversible changes to protein structure (Fox and McSweeney, 1998). Similarly, Gülseren et al. (2007) proposed that sonication can alter the functional properties of bovine serum albumin through the formation of an ultrasonically induced state that differs from a thermally, mechanically, or solvent induced state.

These remarkable effects form the basis of a completely new approach to controlling the viscosity and heat stability of milk proteins, which is expected to find wide application in the dairy processing industry. Further investigations on protein conformational changes as a function of ultrasound-induced shear forces will give more insight into the mechanism of action, which forms the basis of our continuing research. Ultimately, the relationship between the extent of denaturation of the major whey proteins and their heat stability after ultrasonication treatment will need to be determined.

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