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Ultrasound improves the renneting properties of milk

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ABSTRACT

The effects of ultrasound application on skim milk (10% w/w total solids at natural pH 6.7 or alkali-adjusted to pH 8.0) prior to the renneting of milk at pH 6.7 were examined. Skim milk, made by reconstituting skim milk powder, was sonicated at 20 kHz and 30 °C (dissipated power density 286 kJ kg^{-1}) in an ultrasonic reactor. The rennet gelation time, curd firming rate, curd firmness, and the connectivity of the rennet gel network were improved significantly in rennet gels made from milk ultrasonicated at pH 8.0 and re-adjusted back to pH 6.7 compared to those made from milk sonicated at pH 6.7. These renneting properties were also improved in milk sonicated at pH 6.7 compared to those of the non-sonicated control milk. The improvements in renneting behavior were related to ultrasound-induced changes to the proteins in the milk. This study showed that ultrasonication has potential to be used as an intervention to manipulate the renneting properties of milk for more efficient manufacturing of cheese.

1. Introduction

A significant amount of milk is converted to cheese. The first step in the conversion of milk to cheese is renneting. During renneting, the cleavage of κ-casein by chymosin promotes the aggregation of casein micelles and the formation of a gel network in milk, which will become the cheese curd [1]. The renneting properties of milk are defined by an array of measures including gelation time, gel firmness, curd firming rate and gel network connectivity. These properties are influenced by a number of compositional and physicochemical factors in the milk including pH, total solid concentration, protein content, free and micellar calcium content and calcium phosphate state, which control the state of association of casein proteins into micellar structures and the mechanism of casein micelle aggregation [2–8].

Low frequency ultrasound mainly promotes predominantly physical effects in food materials [9,10] resulting from shear-induced effects due to microjets near the solid surface, pressure pulse emitted into the surroundings (shockwave propagation) and turbulence, which are created during the asymmetric collapse of cavitation bubbles formed in a reactor, as a result of sound pressure rarefaction cycles [11,12]. At low frequency ultrasound, large cavities are formed with relatively low sonochemical activity and fewer bubbles are in the active resonance size range. Hence the total yield of radicals are less at low frequency [13]. The linear resonance size equation and Blake Threshold Radius equation specify the boundaries for active bubble implosion [14].

Low frequency high power ultrasound processing can change the particle size of casein micelle particles in milk [15,16]. Others have shown that ultrasound at 20 kHz either caused a small decrease in the casein micelle size in skim milk [17,18] or no change in casein micelle size [19], with major effects being on the reduction in fat globule size. Ultrasound also causes denaturation of whey proteins [17,18]. In our previous study, we have shown that 20 kHz ultrasonication of skim milk at pH 8.0 caused significant changes in casein micelles including size reduction and alteration of the casein partitioning between the micellar and serum phase [20]. The potential use of ultrasound in the manufacture of a range of dairy products has been previously highlighted. Ultrasound processing, which reduces the size of caseinate particles [21,22], improves the elasticity and density of acid-induced gels [23]. Milk ultrasonicated at low frequency was reported to produce yoghurt gels with more interconnected chains and more homogenous porosity, which were attributed to a decrease in casein micelle size from physical effects of unstable cavitation [24].

The ultrasonication of milk for cheese manufacturing has received little attention in the literature although the modification of the cheese curd microstructure upon direct application of low frequency ultrasound has been shown previously [25]. Ultrasound effects on oxidation, cheesemaking and sensory properties of raw milk have also been investigated [26]. In this work, we systematically examine the renneting properties of milk subject to ultrasound at the natural pH of milk and high pH (pH 8).

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The sonophysical and sonochemical effects of the ultrasound treatment are considered.

2. Materials and methods

2.1. Materials

Low-heat skim milk powder (4% moisture, 33.6% total protein) was purchased from Tatura Milk Industries Limited (Tatura, VIC 3616, Australia). Rennet was obtained from Chymax Plus, CHR Hansen Australia (Bayswater, VIC 3153, Australia). Sodium azide, sodium chloride, calcium chloride, Tris buffer, and ammonium bicarbonate were obtained from Sigma–Aldrich Pty Ltd. (Castle Hill, NSW 2154, Australia).

2.2. Milk sample preparation

Low-heat skim milk powder was dispersed in deionised water at 20% (w/w) total solids (TS) and the solution was stirred for 5 h at ~23 °C prior to overnight storage at 4 °C. The reconstituted skim milk solution was preserved by adding sodium azide (0.02% w/w). Reconstituted skim milk (10% w/w TS) at pH 6.7 and 8.0 were prepared by dilution of the 20% TS stock solution and adjustment with 0.1 M NaOH. The unadjusted pH of the milk (10% TS) at 20 °C was pH 6.68 ± 0.05. Prepared milk samples (pH 6.7 and 8.0) were stored at 4 °C overnight and a portion of these samples was used the next day for ultrasound processing. Non-sonicated and sonicated milks at pH 8.0 were re-adjusted to pH 6.7 with 1 M HCl prior to renneting.

2.3. Ultrasound processing

A 20 kHz horn-transducer ultrasound processing unit (UPI1000hd, Hielscher Ultrasonics GmbH, Warthestr. 21, 14513 Teltow, Germany) was used to process milk held in a custom-made stainless steel treatment chamber. The 20 kHz horn-transducer (cross-section 3.1 cm^2) was made of titanium and was operated for 15 min with 101 kW/m^2 net power via radial vibration (mode of vibration). The sonication conditions (power flow, sonication time, sample volume) were arranged to provide 286 kJ kg^{-1} in a sample volume of 100 mL. These conditions were chosen based on previous studies [20] where temperature was maintained below 30 °C by immersion of the chamber in an ice-water bath. The calorimetric power dissipation was estimated by measuring the increase of the milk temperature induced by ultrasound processing in a foam insulated trapezoidal chamber (bottom diameter 85 mm; top diameter 80 mm; height 75 mm). In this case, the energy lost to ambient air was assumed negligible due to the chamber insulation and the energy absorbed by the metallic chamber walls was also considered negligible. The power input was therefore calculated using the following equation: $P = mC_p\ (\Delta T/\Delta t)$, where P is power input in watts (W), C_p is the specific heat capacity of the water (4.18 kJ $°C^{-1}$ kg^{-1}), m is the mass of water (g), and $\Delta T/\Delta t$ is the increase of temperature of the sample in a defined processing time (120 s). The specific energy input (or power density in the system) was calculated as follows: $Es = Ptm^{-1}$, where Es is specific energy (kJ kg^{-1}), P is power input (W), t is the processing time (s) and m is the mass of sample (g). All ultrasound processing treatments were carried out at least in duplicate.

The maximal pressure level reached at low frequency in the reactor vessel was measured using a hydrophone probe (model TC4038, Reson, Goleta, USA) connected to an oscilloscope (TDS 2022C, Tektronix, Beaverton, USA). The cavitational yield (based on free radical formation [12] and hydrogen peroxide formed) was determined in a water matrix media by using the colorimetric method described elsewhere [27].

2.4. Particle size measurement

The sizes of particles in non-sonicated and sonicated milk were measured using two methods: dynamic light scattering and size exclusion chromatography.

2.4.1. Light scattering

The mean particle size of casein micelles in non-sonicated and sonicated milk was measured by dynamic light scattering (Malvern Zetasizer Nano ZS, Malvern, Worcestershire, UK). Milk was diluted for measurement with permeate obtained by ultracentrifugation and filtration following the method by Hemar et al. [28]. Measurements were made using a scattering angle of 173° and laser with a wavelength 633 nm. A cumulate analysis correlation function was used to obtain the intensity mean diameter (z-average diameter) of each sample by using a viscosity of 1 mPa.s and a refractive index of $n = 1.343$.

2.4.2. Size exclusion chromatography

Size exclusion chromatography of unfiltered milk samples was performed on Sephacryl S-1000SF. Milk samples were diluted 1:1 with elution buffer (50 mM NaCl, 20 mM Tris, 3 mM $CaCl_2$, 0.2 g/L NaN_3, pH 6.7) and applied to the column of Sephacryl S-1000SF (1.6 cm diameter × 98 cm long, Amersham Bioscience Pty Ltd., Baulkham Hills, NSW, Australia). Milk was then eluted at 0.5 mL/min. The column was run by a SCL-10A system controller (Shimadzu, USA MFG Inc.), LC-10AD pump (Shimadzu, USA MFG Inc.), SIL-10AD auto injector (Shimadzu, USA MFG Inc.) and SPD-10A US–VIS detector set at 280 nm. The total running time was 450 min.

2.5. Examination of the formation of soluble aggregates and monomeric proteins by SEC

Milk was adjusted to pH 6.7 and separated into its micellar phase and into its serum phase (supernatant) by high speed centrifugation. The supernatant was obtained by centrifuging milk at 55,000×g at 25 °C for 90 min (Beckman-Coulter Optima L-90K ultracentrifuge with a type 55.2 Ti rotor, Beckman Instruments Australia Pty Ltd., Gladesville, NSW, 2111, Australia). The milk supernatant was then filtered through a 0.2 μm disposable syringe filter unit and soluble aggregates and monomeric proteins were separated by SEC and quantified following the method of Chandrapala et al. [29].

2.6. Rheological properties

The rennet-induced gelation profiles were followed using an Anton Paar-Physica stress controlled rheometer (MCR 301, Anton Paar Physica, Physica Meßtechnik GmbH, Stuttgart, Germany) with a cup and bob geometry (CC27/s, Serial # 1805, diameter 26.661 mm, length 39.985 mm) using low-amplitude dynamic oscillation measurement. A portion of the non-sonicated and sonicated milks (20 mL) were pre-warmed in glass beakers to 31 °C in a water bath for 10 min. A 3.5 IMCU/mL rennet solution was prepared by diluting 875 μL stock rennet solution (200 IMCU/mL) to 50 mL using a volumetric flask. 0.2 mL rennet solution (3.5 IMCU/mL) was added to 20 mL pre-warmed milk to achieve a final strength of rennet 0.035 IMCU/mL while stirring for 1 min. The milk-rennet mixture (~ 17 mL) was then immediately transferred into the cup. The renetting measurements were carried out at a constant frequency of 1 Hz and a constant shear strain of 2.5% at constant temperature 31 °C as described elsewhere [30]. Data points were collected at 30 s intervals for 60 min. The gelation time was defined