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Effect of ultrasound pretreatment on rennet-induced coagulation properties of goat's milk

Lily Zhao [1], Shuwen Zhang [1], Hankie Uluko, Lu Liu, Jing Lu, Haixiao Xue, Fanhua Kong, Jiaping Lv *

Institute of Agro-products Processing Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China

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ABSTRACT

The effects of ultrasound (US) pretreatment on goat milk before rennet-induced coagulation were studied in order to improve the milk coagulation properties. Skimmed goat milk was subjected to US at 800 W for different times (0–20 min) and various parameters were evaluated. The particle sizes in US pretreated goat milk under the transmission electron microscopy were smaller than in untreated samples. For US pretreated samples, the degree of whey protein denaturation, contents of soluble calcium and phosphorus increased by 9.57%, 16.90% and 13.68%, respectively. The gel firmness, coagulum strength, final storage modulus, cohesiveness, water holding capacity and cross-linking of gels demonstrated marked increase. The turbiscan stability index (TSI) also confirmed the improvement of goat milk coagulation properties with increasing duration of US pretreatment, whereas the gelation time was prolonged.

1. Introduction

Milk coagulation is one of the primary steps in the production of most fermented dairy products, like cheese and yoghurt. And the clotting by rennet is a normal practise in cheese making. When the enzyme chymosin is added to milk, it induces the hydrolysis of kappa-casein that causes the destabilization of casein micelles. After sufficient destabilization is brought about, an aggregation reaction begins and leads to the formation of a net structure and space-filling gel (Horne & Banks, 2004). And cheese yield and gelation properties are strongly affected by many factors, such as the physico-chemical properties of milk itself, coagulation process condition, like enzyme concentration, temperature, pH and concentration of Ca^{2+}.

Goat milk has been reported to have higher digestibility and lower allergenic properties than cow milk, and also have certain therapeutic values, which makes it attractive to consumers (Haenlein, 2004). However, the coagulation ability of goat milk is so poor that the gel has less hardness, more whey separation and more fragile than cow milk (Abou-Dawood & El-Sawaf, 1977). Poor coagulation properties can lead to more protein particle loss in whey, lower cheese yield and less textural integrity. The type of goat milk cheeses is less than cow milk cheeses, and most cheeses made from goat milk fell into the group of fresh or white unripened cheeses and soft cheeses. This is mostly related to the poor mechanical properties of goat milk curd, which is generally two soft to resist the applied mechanical forces during curd treatment in semi-hard and hard cheese manufacture (Medina & Nuñez, 2004). The renneting kinetics of goat milk which is different from cow milk, could explain the shorter coagulation time and the poor gelation properties (Remeuf, Lenoir, & Duby, 1989). Previous studies have shown that this problem is due to the lower casein content, particularly the low proportion of α_{s1}-casein and higher degree of casein micelle dispersion (Remeuf & Lenoir, 1986; Vegarud et al., 1999). Differences in casein micelle composition, size, hydration (Remeuf, 1992), the mineral concentration, the mineral distribution in soluble phase and colloidal phase between two species could also affect its coagulation properties. Moreover, αs1-casein polymorphism has a significant influence on coagulation properties (Remeuf, 1992). Various methods have been proposed in order to obtain a satisfactory curd. For example, increasing the content of non-fat solid by concentration of milk using membrane processes such as ultrafiltration (UF) significantly increased the rennet curd firmness and rate of firming (Catarino, Martins, Duarte, Prudêncio, & Pinho, 2013), addition of whey protein concentrate (WPC) could promote firmness, hardness and adhesiveness as well as reduce syneresis (Herrero & Requena, 2006; Martín-Diana, Janer, Peláez, & Requena, 2003). While other methods, like mixing goat milk with cow milk (Kücükcetin, Demir, Asci, & Comak, 2011; Uysal, Kilic, Kavas, Akbulut, &

* Corresponding author. Address: Institute of Agro-Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), No. 2 Yuan Ming Yuan West Road, Haidian District, P.O. Box 5109, Beijing 100193, PR China. Tel./fax: +86 10 62819421.
E-mail address: lvjp586@vip.sina.com (J. Lv).
[1] Co-first authors.

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Kesenkas, 2003; Vargas, Chafer, Albors, Chiralt, & Gonzalez-Martinez, 2008), a combination of dry matter supplement, heating and protein cross-linking with transglutaminase (Ardelean, Otto, Jaros, & Rohm, 2012; Rodriguez-Nogales, 2006), have also been applied to improve goat milk acid gels and not rennet curd. Other physical methods including heat treatment (Lucey, Tamehaba, Singh, & Munro, 2001), high-pressure treatment (Pandey, Ramaswamy, & Gelais, 2000), and ultrasound treatment (Loveday, Sarkar, & Singh, 2013; Marchesini et al., 2012; Nguyen & Anema, 2010; Riener, Noci, Cronin, Morgan, & Lyng, 2009; Vercet, Oria, Marquina, Crelier, & Buesa, 2002) have been applied to cow milk to improve curd properties, but rarely to goat milk.

Ultrasound (US) transmits high intensity and frequency sound waves through the liquid food material that causes periodic high and low pressure cycles. Vacuum bubbles can be formed during a low pressure and violently collapse during high pressure. The collapse results in rapid and marked increase in temperature and pressure, which ultimately results in violent collision between particles in liquid food (Nguyen & Anema, 2010). Applications of US in milk industry include extending the shelf life of milk; homogenization, particles sizes reduction and uniformity improvement. US can also improve the coagulation properties, but only a few have been reported on rennet gel properties, especially on goat milk which has weaker coagulation properties than cow milk. Therefore, this study was conducted to determine the effects of US pretreatment on rennet coagulation properties of goat milk and their possible improvements. The US pretreated samples were compared with cow milk and goat milk without US pretreatment.

2. Materials and methods

2.1. Goat milk sample

Fresh goat milk was obtained from a ranch in Shanxi province (China). Broad spectrum microtabs (0.045%) were added to the milk samples as a preservative. Then the milk was stored in a refrigerator at 2–4 °C before use.

2.2. US pretreatment of skim goat milk and formation of rennet gels

Fresh goat milk was skimmed by a disc type cream separator (FT-15, Armfield Ltd., Ringwood, England) and samples of skim goat milk were placed in a beaker jacketed with ice and treated by a 13 mm ultrasonic probe placed at the centre and to a depth of about 3 cm. US was performed at a frequency of 20 kHz and a power of 800 W for treatment times of 0–20 min using a cell disruptor (JY92-IIN, Ningbo Scientz, Zhejiang, China). The milk samples were left to reach an equilibrium at 38 °C and then rennet-induced gels were formed according to the following steps: first adjusting the pH to 5.8 ± 0.2, then adding calcium chloride (0.03% w/w), and lastly adding 0.003% (w/w) chymosin (Chr Hansen, Denmark) which was sourced from 2% stock solution at 38 °C, and incubation for 40 min.

2.3. Turbiscan measurement

Rennet-induced coagulation was monitored using a Turbiscan MA2000 (Formulaction, Ramonville St. Agne, France). The apparatus comprised of a detection head equipped with a near-infrared light source (880 nm). The light source scans the sample (placed in borosilicate glass tubes 12 mm inner diameter and 25 mm high) at 1 min intervals from top to bottom and measures the percentage of light backscattered or transmitted during 40 min at 38 °C incubation. For the opaque milk and during coagulation, only the backscattering can offer useful results. Diversity in the levels of backscattering are associated with changes in particle size and concentration of the rennet-induced coagulation. An increase of backscattering intensity is associated with an increase in particle concentration and size. The rate and size of the particle aggregation, and the extent of syneresis can be monitored which cannot be evaluated by the naked eye. The turbiscan stability index (TSI) are calculated according to backscattering changes that indicate the protein particles aggregation and dynamic migration by Turbisoft 2.0. Therefore, the TSI could be used to evaluate the degree of particle aggregation.

2.4. Rheological measurements

The viscoelastic properties of the rennet coagulation were recorded continuously using controlled-strain rheometer (Physica MCR 301, Anton Paar Company, Austria) with low amplitude oscillation. The sample (10 mL) was placed in a cylinder after adjusting pH, adding calcium and chymosin, then mixing them using a glass bar immediately for 5 s with temperature maintained at 38 °C. The interval between mixing and lowering the probe was about 10 s, and then measurement was started. A layer of liquid paraffin was placed onto the surface of sample to prevent evaporation during coagulation. The oscillation mode was set at a frequency of 1 Hz and a constant strain of 1% after the stress sweep experiment. Each sample was measured for 40 min. The parameters determined were the storage modulus G', the loss modulus G″ and $\tan\theta = G''/G''$. The rennet coagulation time (RCT, the time where $\tan\theta = 1$), the maximum coagulation strength by storage modulus G' (G'max) and the curd firming rate (CFR, the greatest slope of G' curve) were selected as descriptors of the coagulation properties (Frederiksen et al., 2011).

2.5. Texture measurements

Texture measurements were performed on samples that had been rennetted in beakers in 38 °C water bath for 40 min. The coagulation firmness (g), cohesiveness ($g\ s^{-1}$) and springness were measured using a texture analyser (TA-XT21, Stable Micro System Company, UK). The probe (SMS P/35) was used at a speed of 2.0 mm s^{-1} and a depth of 10 mm into the curd. The distance between the sample surface and bottom of probe was essential to ensure that the cohesiveness can be calculated (Awad, 2007). Tests were performed on samples at 38 ± 2 °C that were heat preserved at 38 °C before testing, and then taken from the water bath for testing. Curves of force versus time were analysed by an in-built software.

2.6. Water holding capacity, undenatured whey content and syneresis rate determination

Water holding capacity (WHC) was determined using the centrifugation method described by Parnell-Clunies, Kakuda, and Deman (1986). Briefly, the samples were centrifuged at 4000×g for 10 min after coagulation formation. The supernatant was drained and the remaining pellets were weighted then Eq. (1) was used to calculate the WHC:

$$\mathrm{WHC}\ (\%) = \left(\frac{\text{weight of drained gels}}{\text{weight of sample}}\right) \times 100. \quad (1)$$

Un-denatured whey content was measured using Kjeldahl method. The whey was collected after pH was adjusted to 4.2, then NaCl was added until saturation and then the un-denatured whey was collected.

Syneresis rate was measured as described by Arango, Trujillo, and Castillo (2013) with some modifications. Samples with enzyme and calcium were poured into a centrifuge tube, which