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Ultrasound-assisted crystallization of lactose in the presence of whey proteins and κ-carrageenan

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ABSTRACT

The conventional process of lactose crystallization is prolonged, hardly controllable and the crystals have low quality. In this work, the effect of ultrasound on the crystallization of lactose in an aqueous system was assessed. Additionally, it was studied how the presence of whey proteins (which are a common impurity) and κ-carrageenan (that possess high water-binding capacity) could modify the process of lactose crystallization. Lactose solutions at 25% were sonicated in a continuous flow chamber at two different energy densities (9 and 50 J mL^{-1}) before the start of crystallization. Some of these lactose solutions were previously added with κ-carrageenan (0, 150 and 300 mg L^{-1}), with whey proteins (0.64%) or with both at the same time. Ultrasound sped up the rate of crystallization, decreased the crystal's size and narrowed the crystal size distribution (CSD). The presence of whey proteins accelerated the process of crystallization but induced the formation of amorphous lactose. Likewise, the rate of lactose crystallization was improved by the addition of 150 mg L^{-1} of carrageenan. Whereas, the combination of carrageenan and whey proteins generated the smallest crystals (6 μm), the narrowest CSD and minimized the formation of amorphous lactose.

1. Introduction

The primary carbohydrate in the milk of mammalians is lactose, which is a reducing disaccharide composed of galactose and glucose joined by a glycosidic bond (β 1–4). Through a hemiacetal link, these saccharides form a ring structure with a chiral center that gives the possibility of two isomeric forms, α and β [1]. In solution, lactose opens and re-forms the ring structure interchanging between α and β anomer (mutarotation) [2,3]. The mutarotation equilibrium of lactose at 20 °C is attained when the ratio of β/α isomers is 1.70 (63:37), although this proportion is strongly dependent on temperature. In equilibrium, the isomer β form is more abundant and more soluble (500 g L^{-1}) than α-lactose isomer (70 g L^{-1}) [1]. Therefore, in a concentrated solution of lactose (like a concentrated cheese whey), it is more feasible that the less soluble α-lactose became supersaturated and further crystallized [2].

Lactose is recovered chiefly from cheese whey, and it is estimated that 400,000 tons of crystalline lactose is produced annually [1]. The cheese whey contains typically 5–6% of lactose, 0.8–1% of protein and 0.06% of fat [4–6]. The conventional process for lactose recovery from cheese whey has as a first step the partial removal of water to obtain 50–70% of total solids (most commonly 40–65%) [6]. Secondly, the proteins are removed from the cheese whey by heat coagulation. This procedure usually left a residual content of proteins between 0.1 and 0.2%, but this concentration may fluctuate depending on the extent of water removal [7]. The concentrated whey is rapidly cooled to 30 °C and then is slowly cooled (1–3 °C h^{-1}) to decrease enough the lactose solubility and to favor the formation and growth of nuclei that further develop into lactose crystals [8]. Finally, the resulting crude crystals of lactose are removed by centrifugation. Under these conditions, the process for lactose recovering is prolonged (up to 72 h), hardly controllable and the lactose crystals used to have poor quality (yield, size, purity) [3,6,9,10]. Consequently, the crude crystals of lactose must be redissolved, subjected to demineralization and removal of whey proteins, and recrystallized, which is time and resource consuming [6,11].

There are different approaches to improving the lactose crystallization. The oldest and most used method is the addition of small lactose crystals in the concentrated whey just before the start of crystallization (seeding of nuclei). An addition of lactose crystals at the appropriate time may induce a secondary nucleation that accelerates the crystallization process [10]. However, if the addition of lactose crystals is done too soon or too late, this will not produce an effect on lactose crystallization or the effect will be too small [12]. Another approach is the addition of non-solvent compounds that sharply decrease

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the solubility of lactose (anti-solvent crystallization). This change in the lactose solubility narrows the metastable zone and reduces the induction times of nucleation, leading to smaller crystal sizes [13,14]. The main drawbacks of anti-solvent crystallization are the large amounts of solvent used, and the expensive separation and purification steps required to remove the anti-solvent from the product [10,15]. Moreover, the process of crystallization is widely variable when an anti-solvent is used due to the rapid formation of supersaturation which leads the formation of agglomerates and crystals with irregular shapes [10]. Recently, it has been explored the crystallization of lactose assisted with low-frequency power ultrasound (sonocrystallization) [10]. In general, it has been observed that ultrasound may induce primary nucleation at much lower supersaturation levels. Likewise, it has been proposed that ultrasound promote secondary nucleation by disruption of crystals or by loose agglomerates already formed [12,16]. There are some theories about how the transient [10,12,16] and stable cavitation [17,18] affect the primary and secondary nucleation. However, as the whole crystallization process is very complex, the precise mechanism of ultrasound action over the crystallization remains ambiguous [18,19].

Different authors have explored the crystallization of lactose assisted with ultrasound. Nevertheless, most of these studies have been focused on the combination of ultrasound with anti-solvent crystallization [6,7,9,11,13–16,20,21], and only a few works have been conducted in aqueous systems in the absence of non-solvent [3,22]. The sonocrystallization of lactose in the presence of large amounts of anti-solvents like ethanol, propanol, glycerol or acetone makes difficult the interpretation of the real effect of ultrasound on lactose crystallization. On the other hand, the use of such non-solvents is not an environmentally friendly alternative and has the technological disadvantages previously described. Instead, biopolymers with a high water-binding capacity (like carrageenan) could be used to decrease the lactose solubility and improve the whole process of lactose crystallization. So far, it is only known that polysaccharides like κ-carrageenan may control the growth of lactose crystals through the formation of gel-type aggregates [23]. The κ-carrageenan is an anionic polysaccharide which has one negative charge per disaccharide repeating unit [24]. At temperatures higher than 50 °C κ-carrageenan exist in solution as a random coil, but after cooling this polymer undergoes a double-helical transformation [25]. The presence of certain cations (K^+, Rb^+, Cs^+, and $NH_4{}^+$) and a sufficient carrageenan concentration promotes the aggregation of helices, leading to the formation of a gel [26]. In this work, it was assessed the effect of low-frequency power ultrasound on the crystallization of lactose in an aqueous system free of anti-solvent. In addition, it was studied how the presence of whey proteins (which are a common impurity) or κ-carrageenan (that possess high water-binding capacity) can modify the process of lactose crystallization.

2. Material and methods

2.1. Materials

The standard β-lactose (≤30% α-lactose), anhydrous lactose (Fluka-analytical) and κ-carrageenan (with traces of λ-carrageenan) were purchased from Sigma-Aldrich (St. Louis, MO). The whey protein concentrate (WPC) was acquired from a local producer (WPC, A&N, Chih, Mex). This WPC had 33.49% w/w of protein, according to the micro-Kjeldahl method[27]. The deionized water (> 18 MΩ cm) was obtained from an ultra-pure water purification system (Thermo Scientific, D8611, Dubuque, IO).

2.2. Preparation of lactose solutions

Lactose solutions were prepared from anhydrous lactose at 25% (w/v). Some lactose solutions were added with κ-carrageenan and whey proteins (see the experimental design). After mixing the ingredients, the solutions were heated at 60 ± 2 °C, cooled to 30 °C and filtered through 0.45 μm pore size (Merck Millipore, Billerica, MA) to remove undissolved lactose crystals. The solutions were adjusted (if necessary) to pH 7 with NaOH (1N). Lactose solutions were kept at 30 °C in a water bath circulator (Julabo, Allentown, PA) until the sonication step. Those treatments that did not include a sonication step were stirred mechanically with a probe of 1.3 cm (diameter) at 3500 rpm for 107 s (IKA, Ultra-Turrax T 18 basic, Nara, Japan) and then crystallized.

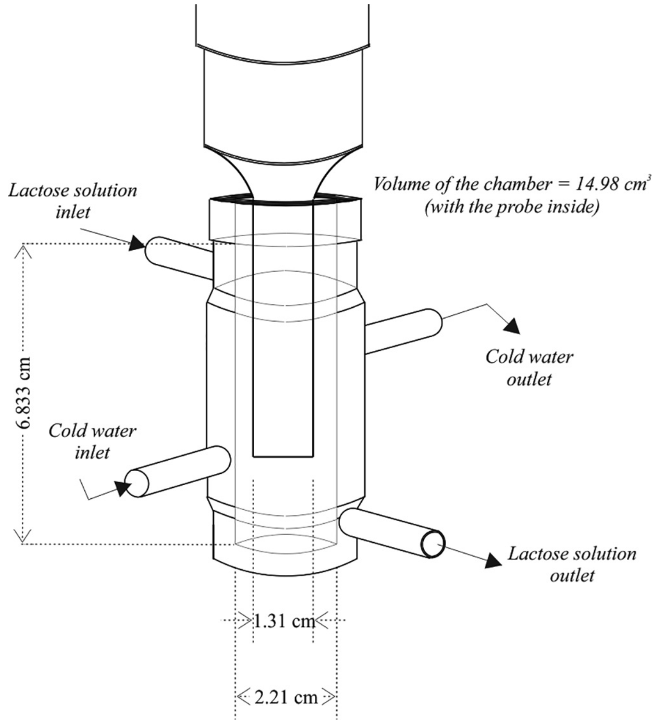

Fig. 1. Schematic diagram of the stainless steel continuous flow chamber and ultrasound probe used for sonication of lactose solutions.

2.3. Sonication of lactose solutions

Sonication of the solutions was carried out with an ultrasonic processor (20 kHz, GEX750, Sonic, Newtown, CT) and a probe of 1.3 cm diameter. The probe was placed in a stainless steel continuous flow chamber at 1.57 cm from the bottom (Fig. 1). The lactose solutions at 30 °C were pumped through the chamber at a flow rate of 10 mL min^{-1} with a peristaltic bomb (EP1- Econo pump, Biorad, Hercules, CA). The actual volume of the chamber (with the probe inside) was 14.98 mL and the residence time of the solutions in the chamber was 107 s. Water was pumped through the jacket of the chamber from a water bath (Julabo, Allentown, PA) to maintain the temperature of the solutions constant (± 3 °C). The ultrasound was applied at two different energy densities: 9 and 50 J mL^{-1} (0.083 and 0.467 W mL^{-1}), which was attained by varying the vibrational amplitude of the probe at 40 and 70% respectively. These energy densities (Q) in J s^{-1} were calculated multiplying the heat capacities (*Cp*) of the lactose solutions by the rise of temperature (*T*) during the sonication time (*t*) (Eq. (1)). The *Cp* of each lactose solution was estimated knowing the mass (*m*) of each component in the solution (water, lactose, carrageenan, WPC) and its corresponding *Cp* (0.45, 4.181, 0.237 and 1.64 kJ kg^{-1} $°C^{-1}$) [22]. The energy densities (*Q*) were finally expressed as J mL^{-1} considering the residence time and volume of the solution in the continuous chamber.

$$Q = \sum_{t=1}^{n} = m_i Cp_i \left(\frac{dT}{dt}\right) \tag{1}$$