Chapter 4

Sonocrystallization of Lactose from Whey

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Additional information is available at the end of the chapter

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Abstract

Whey is a by-product obtained from the cheese-making industry. This by-product is the primary source of high-value products such as whey protein concentrates and lactose. The partial removal of water from the whey is the first step in the recovery of lactose. Then, lactose in the concentrated whey is forced to crystallize through a cooling stage. This conventional process of crystallization is very slow up to 72 h accompanied by the generation of a mixture of lactose types (α, β, and amorphous) and low yield of lactose. These issues have been addressed through the seeding of lactose, the antisolvent crystallization, and more recently, by the crystallization of lactose assisted with low-frequency power ultrasound. Sonocrystallization is known to have a number of specific features that include the enhancement of the primary and secondary nucleation, as well as the development of smaller crystals with more uniform sizes and higher purity. Nowadays, there are a number of studies that provide relevant information on the effects of ultrasound on lactose crystallization, although some of these effects are still not fully understood. This book chapter discusses the current knowledge on lactose sonocrystallization and describes the basic principles of lactose crystallization and sonocrystallization.

Keywords: lactose, whey, crystallization, sonocrystallization

1. Introduction

The composition of whey varies according to the cheese process, but in general, this contains 0.8–1.0% soluble proteins, 0.05–0.5% fat, >1% salts, 5–6% lactose, and up to 93% of water [1–4]. The volume of whey recovered from a cheese process makes up 70–90% of the original

volume of milk [3]. Therefore, it is estimated that more than 80 million tons of whey are produced annually all over the world [4, 5].

Most of the small-scale dairy companies dispose of the whey into the municipal sewage, rivers, lakes, or use this by-product as fertilizer and animal feed [4, 5]. The disposal of cheese whey into water bodies and lands should be strongly discouraged because it produces serious environmental problems. The bacterial degradation of whey causes a depletion of oxygen in the water and soil killing aerobic organisms, such as fish, insects, plants, and microorganisms. The high biological and chemical oxygen demand (BOD: 30–50 g L^{-1}; COD: 60–80 g L^{-1}) of the whey arise from its large content of carbohydrates, chiefly lactose (5–6%) [2, 6–8]. In consequence, the removal of lactose reduces more than 80% of the BOD and COD of whey, which minimizes the negative environmental impact of this by-product [9, 10].

Besides the ecological benefits of lactose removal from whey, this by-product also has a great relevance for the food and pharmaceutical industries [11]. It is estimated that 400,000 tons of crystalline lactose are worldwide produced each year. In comparison with other carbohydrates, lactose has a low caloric value, low glycemic index, good plasticity, compressibility, and low level of sweetness. This sugar is used in the food industries in a wide variety of products such as instant coffee, infant formula, and baked foods. Meanwhile, lactose is used as an excipient for tablets and dry powder inhalers in the pharmaceutical industry [11, 12]. The general steps in the recovery of lactose from the whey involve a step for the partial removal of water followed by a crystallization step. Some of the challenges to overcome in the recovery of lactose from the whey are the long crystallization times, low yields, and low quality of lactose crystals. These problems on lactose crystallization have been approached through the seeding of lactose, the use of antisolvent, and more recently, by the sonocrystallization of lactose [1, 3, 5]. In the last years, the number of research studies of the crystallization of lactose assisted with ultrasound has increased considerably. Hitherto, it has been established that sonocrystallization decreases the size of crystals and improves the crystal size distribution but also might speed up the crystallization process or enhance the purity of lactose crystals. However, the effect that ultrasound has on lactose crystallization is by far not fully understood. This chapter discusses the current knowledge on lactose sonocrystallization (fifth section) but also addresses the basic principles of lactose crystallization (second section) and sonocrystallization (fourth section). Furthermore, the conventional process of lactose recovery from whey is described in the third section of this chapter.

2. Crystallization of lactose

Lactose is the principal carbohydrate in the milk of mammalians, which is a reducing disaccharide made up of galactose and glucose joint by a glycosidic bond (β 1–4). Lactose comprises of two stereoisomers α- and β-anomers. In solution, lactose opens and reforms the ring structure interchanging between α and β anomers (mutarotation). 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 highly 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}) [12, 14]. Therefore, the

α isomer will crystallize first in a supersaturated solution of lactose, like a whey concentrate. In this section, the three main phases of lactose crystallization are described: supersaturation, nucleation (appearance of crystals), and crystal growth [15].

2.1. Supersaturation

Supersaturation of lactose solutions is the first step in the crystallization process, since a non-equilibrium condition is required for the spontaneous birth of nuclei [16]. At any given temperature, a maximum amount of solute can be dissolved in a solvent. When a solution is saturated with a solute, this is considered being in a thermodynamic equilibrium. Any further increase in the concentration above the saturation (solubility) point disturbs the equilibrium and induces a pseudo-equilibrium state or supersaturation. The nucleation and hence crystallization won't occur at the supersaturation point (at least not spontaneously), since the energy available is insufficient to induce the nuclei formation. However, beyond the pseudo-equilibrium state (labile zone), nucleation takes place spontaneously. The region between solubility and supersolubility (supersaturation) is known as the metastable zone (MZ). The width of this region (MZW) is obtained by plotting the solubility and supersolubility of the solute as a function of temperature. From these curves, it is possible to establish the temperature and solute concentration required in a crystallization process [5, 17]. The conventional process of lactose crystallization has a wide MZW, which means that a very high supersaturation is necessary to induce nucleation [18, 19].

2.2. Nucleation

Nucleation has a major influence on crystallization and consequently on the quality properties of lactose crystals like its structure and size distribution [21]. The formation of a new solid phase from a supersaturated solution is called nucleation, and the nucleation rate is the change in the number of particles in solution with time [22]. There are two kinds of nucleations: the primary and secondary; the former occurs when a crystal is nucleated without an interphase in the solution. Nucleation in the absence of solid surfaces is called homogeneous nucleation, and if there is a foreign interphase in the solution, the process is referred as heterogeneous nucleation. In contrast, the secondary nucleation is induced by pre-existing crystals [13]. Two theories try to explain the nucleation mechanism, the Classical Nucleation Theory (CNT) and the Two-Step Nucleation Theory (TSNT). The basics of the CNT are that from a supersaturated solution, a number of ordered subcritical clusters of solute molecules are formed under certain temperature and concentration conditions. When the number of molecules in the cluster increases (until reaching a critical cluster size n^*) (~100 to 1000 atoms), the total free energy (ΔG) in the system rises. Above this n^*, the total free energy decreases continuously and the formation of a crystal nuclei becomes favorable. However, a cluster of size n^* has equal possibilities to form a crystal nucleus or to disaggregate. Therefore, the height of the free energy barrier for nucleation (ΔG^*) and the nucleation rate are determined largely by the n^* [16, 21]. The CNT gives some insights about the n^* and nucleation rate but does not provide information on the structure of aggregates or pathways leading to the formation of solid crystal from the solution [16]. On the other hand, the major difference between the CNT and the TSNT is

that the latter considers the formation of disordered (liquid-like) clusters instead of ordered subcritical clusters. Besides, the TSNT suggests the formation of a crystalline nucleus inside the liquid-like clusters beyond the n^* [16]. Although the theories of the nucleation process have advanced considerably in recent years, the particular ordering within the solid state via the nucleation process remains ambiguous. Moreover, some of the parameters described by these nucleation theories are difficult to verify experimentally, like the critical cluster size (n^*). The number of particles can be measured by methods such as light scattering, direct particle counting (microscopy), and turbidity measurements [22]. The problem arises from the fact that n^* typically falls in a range of 100–1000 atoms, which is hardly accessible to most of the current experimental methods [16].

2.3. Crystal growth

The growth of lactose crystals is controlled by several factors but the key variable determining the rate of nucleation is the supersaturation [23]. If nucleation is fast, many crystals form simultaneously and they will grow to approximately identical sizes. In contrast, if the nucleation is slow and fewer crystals nucleate at a time, the supersaturation in the solution drops slowly, the nucleation of new crystals continues, and the solution presents a wider crystal size distribution (CSD) [21]. Other variables that affect the crystal growth are the temperature, viscosity, pH, presence of salts, and whey proteins, which modify the levels of supersaturation and consequently the nucleation and crystal growth [24–26]. Speaking of impurities like salts and proteins, these can either accelerate or inhibit the crystal growth. The impurities induce a heterogeneous nucleation and are incorporated frequently into the crystal lattice. In addition, the presence of impurities affects the solubility and supersolubility of the substance being crystalized, modifying the nucleation and crystal growth. It is well established that salts may either increase or decrease the growth rate of lactose crystals. The presence of calcium chloride, calcium lactate, magnesium sulfate, and lithium chloride increases the crystallization velocity, at the difference of potassium phosphate [24]. In the same way, the whey proteins promote nucleation but slow down the growth of lactose crystals. This effect is attributed to its high water-binding capacity that creates areas of lactose supersaturation, which are favorable for nucleation [25].

3. Conventional recovering of lactose from whey

The process of recovery of lactose from cheese whey is described in **Figure 1**. Before whey processing, curd fines and fat are separated from the whey by centrifugation [27]. This clarified and defatted (0.07%) whey must be deproteinized in advance to the concentration step. The presence of whey proteins decreases the solubility of lactose [24], promotes nucleation, accelerates the lactose crystallization [25], and reduces the purity of lactose crystals [5]. Besides, proteins increase significantly the viscosity of the concentrated whey, hindering the recovery of lactose crystals [5]. The heat-acid precipitation of whey proteins is the easiest and cheapest method for whey deproteinization, although this method leaves between 0.1 and 0.2% of the residual protein in the whey [28]. Proteins can also be removed by ultrafiltration (UF) using membranes with a molecular weight cut-off (MWCO) ranging from 3 to 10 kDa. When UF is

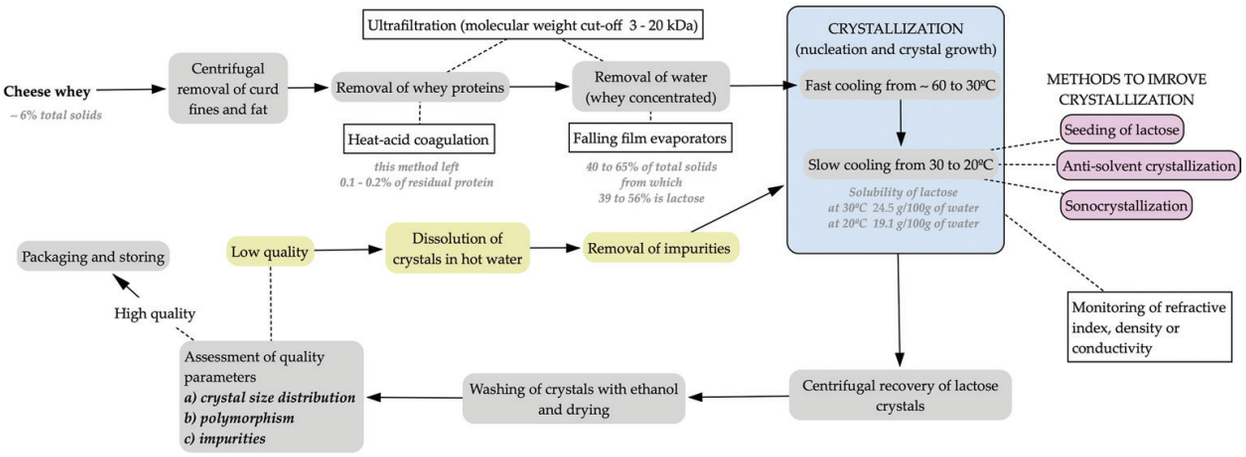

Figure 1. Schematic procedure for the recovery of lactose from whey.

carried out with this MWCO, the fat and protein fraction are retained in retentate; meanwhile, permeate keeps the lactose, vitamins, and minerals. The major drawback of this technology is the high cost of UF equipment and membranes because most of the small and medium-scale dairy processors cannot afford it [4]. Finally, if the deproteinized whey is not evaporated immediately, this must be pasteurized to avoid the fermentation of lactose by microorganisms.

The clarified, defatted, and deproteinized whey is sent to the evaporators for concentration. Evaporation is performed under reduced pressure in falling film (single and multiple effect) evaporators. These evaporation units allow the concentration of total solids in the whey by nearly ten folds (concentration factor Q = 9.5). The content of dry matter in the whey is measured during the whole evaporation process through the refractive index *n*. When the whey reaches 40 to 65% of dry matter, the evaporation process is stopped. The temperature of the final concentrate is ~60°C, and the lactose content ranges from 39 to 56%. At this point, lactose is supersaturated in the whey concentrate but won't crystallize as the temperature is high [1, 4, 28, 29]. The whey concentrate (still being hot) is then transferred into a large stirred tank where it is cooled fast enough to induce crystallization of lactose. Once in the crystallizer, the whey is first cooled rapidly from 60 to 30°C and then slowly from 30 to 20–25°C (1–3°C h^{-1}) [17, 26, 29]. The nucleation and crystallization of lactose will occur spontaneously just beyond the metastable zone (MZ), that is a region between the supersaturation point where nucleation occurs and the saturation equilibrium of lactose [14, 30]. This MZ is attained mostly during the second cooling stage when the temperature drops below 30°C, and the lactose supersaturation rises considerably [26]. The progress of crystallization can be followed measuring the changes of lactose concentration in the liquor either by refractive index, density, or conductivity [31]. The complete process of crystallization is prolonged and may take up to 48 h. Crude lactose crystals are separated from the liquor by centrifugation, filtration, or both and then washed with a nonsolvent compound (such as ethanol) to remove impurities and water. The resulting crystals are air dried and further characterized by its size distribution and purity. The yield of crystallization depends upon many variables, but typically 65% of lactose is recovered from this process [12, 17, 29]. Crude lactose is further recrystallized, if some quality parameters are not achieved such as the crystal size distribution (CSD), form,

and purity. For this lactose refining, the crystals are re-dissolved, treated with charcoal to remove impurities (salts and proteins), and recrystallized as previously described [4, 31].

The process of lactose crystallization is very slow (up to 72 h), the quality of lactose is usually poor, and the yields of crystallization are very low. One of the oldest methods used to improve the process of crystallization is the seeding of lactose. This approach consists in the addition of small lactose crystals into whey concentrate (seeding of nuclei) just before the second cooling step. The addition of lactose crystals may induce a secondary nucleation that accelerates the crystallization process and reduces the CSD [5]. However, this method has low reproducibility because its success depends on the addition of crystals in the appropriate timing [13]. More recently, alternative methods such as the use of antisolvent or sonocrystallization have been explored to assist the crystallization of lactose. The addition of nonsolvent compounds into whey concentrate (antisolvent crystallization) decreases the solubility of lactose, narrows the metastable zone, and reduces the induction times of nucleation. In general, the antisolvent crystallization improves the yield of crystallization and reduces the size of lactose crystals [20, 32]. The main drawbacks of antisolvent crystallization are the large amounts of solvent used, and the expensive separation and purification steps required to remove the antisolvent from the product [5, 9]. The crystallization of lactose assisted with low-frequency power ultrasound (sonocrystallization) is discussed later in the chapter.

4. General principles of ultrasound and sonocrystallization

Sonochemistry and sonoprocessing have a wide range of applications in food technology, medicine, nanotechnology, chemical synthesis, materials extraction, polymerization, phase separation, surface and water cleaning, catalysis, enhancing the enzyme activity, and so on. Sonication of a liquid generates acoustic cavitation depending upon the experimental conditions used. Strong physical effects and highly reactive radicals are generated during acoustic cavitation [33]. An overview of the general principles of ultrasound is discussed in this section.

4.1. Ultrasound

Ultrasound refers to sound waves of a frequency that cannot be detected by human ear. The ultrasonic frequency ranges from 20 kHz to >10 MHz within which they are further divided into low frequency (20–100 kHz), intermediate frequency (100 kHz–1 MHz), and high frequency (1–10 MHz) regions. The interaction of ultrasound gas bubbles in liquids can lead to the generation of chemical reactions and physical forces. The driving force behind such forces is acoustic cavitation [34].

4.2. Acoustic cavitation

Acoustic cavitation is the phenomenon of formation, growth, and violent collapse of microbubbles in a liquid medium under the influence of acoustic field (**Figure 2**). Bubbles which are inherently present as small nuclei will grow to a critical size under the applied ultrasonic energy. The growth of the acoustic bubbles is due to the phenomenon called "rectified

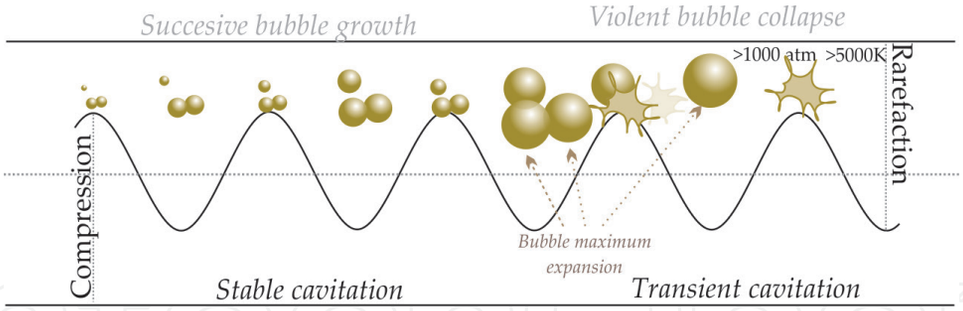

Figure 2. Schematic representation of acoustic cavitation.

diffusion" which is defined as slow growth of the acoustic bubble as a function of time due to unequal mass transfer across the air/water interface [35]. There are two types of cavitation bubbles that exist depending upon the ultrasonic intensity, i.e., transient cavitation bubbles and stable cavitation bubbles. When the ultrasonic intensity is very high, transient cavitation bubbles last for a few acoustic cycles. On the other hand, stable cavitation bubbles can oscillate for many acoustic cycles. The size of stable cavitation bubbles grows over time due to coalescence and also by rectified diffusion until the size is reached, where the coupling of bubble's resonance frequency and driving frequency of the ultrasound occurs. In a multibubble cavitation field, bubbles with a range of size are generated and grown toward the critical size. Miinaert's equation (Eq. 1) provides a relationship between linear resonance radius (critical size) of the bubble with frequency [36].

$$R_{res} = \frac{3}{f} \tag{1}$$

where R_{res} is the linear resonance radius (m) and f is the ultrasonic frequency (Hz).

Transient cavitation bubbles dominate at the lower frequency where they can grow rapidly above a threshold size during the rarefaction cycle. The nature of cavitation bubble is controlled by numerous parameters, such as acoustic pressure, frequency, type of reactor, and bubble size.

4.3. Chemical and physical effects of ultrasound

The collapse of cavitation bubble leads to the generation of a very high temperature of >5000 K and pressure (>1000 atm) within the bubble (**Figure 2**) [34, 36, 39]. The collapse of the bubble takes place in a very short period of time, and thermodynamically, the work done leads to a near adiabatic heating of the bubble contents, which lead to extreme conditions [36–38]. The maximum temperature and pressure generated within the cavitation bubble can be theoretically calculated using Eqs. (2) and (3), respectively, to a near adiabatic heating of the bubble contents which lead to extreme conditions [36–38].

$$T_{max} = T_0 \left\{ \frac{P_m(\gamma - 1)}{P_v} \right\} \tag{2}$$

where T_0 is the temperature of the solution, P_m is the pressure inside the liquid, γ is the ratio of specific heat of gas-vapor mixture, and P_v is the pressure of the bubble when it has maximum size.

$$P_{max} = P_v \left\{ \frac{P_m(\gamma - 1)}{P_v} \right\}^{\frac{\gamma}{(\gamma-1)}} \quad (3)$$

The extreme conditions generated on bubble collapse results in: (1) light emission—sonoluminescence, (2) radical generation, and (3) shock waves, microjetting, microstreaming, shear forces, and microturbulence [40]. The violent collapse of cavitation bubbles can sometimes lead to the emission of light called sonoluminescence [41]. The intensity of light emitted and the sonochemical yield depends on different factors, such as amount and type of dissolved gases in a liquid, ultrasonic frequency and power, hydrostatic pressure, and addition of some solutes. When ultrasound is passed through a liquid medium, the formation of standing waves takes place. Mostly, active bubble formation occurs at the pressure antinodes; therefore, the number of bubbles increases with an increase in the antinodes formed in a liquid. The increase in ultrasonic frequency leads to (i) an increase in number of antinodes and active cavitation bubbles and (ii) a decrease in size and collapse intensity of cavitation bubbles [40].

In air-saturated water, different radicals and molecular products such as H_2O_2, HO_2, H, and OH radicals are generated. These radical are formed through the following reactions: (i) $H_2O \rightarrow H^{\cdot} + OH^{\cdot}$, (ii) $OH^{\cdot} + OH^{\cdot} \rightarrow H_2O_2$, (iii) $H^{\cdot} + O_2 \rightarrow HO_2^{\cdot}$. Primary radicals can be used to initiate a number of chemical reactions such as polymerization, synthesis, and degradation. The detailed information on the fundamentals and applications of the ultrasound could be found in literature [42–44]. The physical and chemical effects of ultrasound can be utilized and applied in a number of fields such as material synthesis, water treatment, and crystallization. In the following section, various reported investigations dealing with crystallization processes under ultrasound and possible mechanisms involved are summarized.

4.4. Sonocrystallization (ultrasound-assisted crystallization)

Ultrasound is found to influence crystallization process, which is referred to as sonocrystallization. Ultrasound has been used from a long time to initiate nucleation and control growth during cooling and antisolvent crystallization process, but the mechanism behind sonocrystallization is still debatable and unclear. It is generally accepted that the physical effects of acoustic cavitation are responsible for the effects observed during sonocrystallization [13]. Sonocrystallization is known to have a number of specific features. For most materials, such features include enhancing the primary nucleation, due to uniform mixing throughout the liquid medium; relatively easier nucleation in some systems which are otherwise hard to nucleate under conventional procedures; ultrasound also has the tendency to initiate secondary nucleation and formation crystals with uniform and small size with high purity. Supersaturation is the driving force behind crystallization process, which is accompanied by nucleation and growth of the crystals, and ultrasound can affect both processes. Active pharmaceutical ingredients are found in a variety of crystalline solid forms, which include polymorphs, hydrates, salts, co-crystals, and amorphous solids. Such different solid forms exhibit different and unique

physical as well as chemical properties that can affect the bioavailability, solubility, stability, and other characteristics of the drug. Reduction in particle size can significantly enhance the bioavailability and solubility in most of the pharmaceutical drugs. Therefore, production of smaller size particles with uniform size distribution and desired properties is very important in the development of pharmaceutical drugs. Applying ultrasound during crystallization also results in a number of other benefits, such as nucleation at lower level of supersaturation, narrowing of metastable zone width, highly repeatable and predictable crystallization, reduction in the induction time, improved morphology, and polymorphs selectivity [45–47].

Ultrasound has also shown the tendency to significantly influence the agglomeration of the particles. There are different physical effects generated by ultrasound that can contribute to reduce the agglomeration. This includes shock waves generated due to acoustic cavitation, which can decrease the time of contact between particles that can hinder the interaction of particles together. Also, sometimes agglomeration occurs at the stage of nucleation. Nuclei usually have high surface area to volume ratio, which can lead to high surface tension and nuclei tend to lower the surface tension by interacting to one another. Then, the surface tension tends to drop during the crystal growth when the particles become more stable, which can prohibit agglomeration [48]. Lastly, the uniform mixing of the sonication mixture due to physical forces of the ultrasound can help to reduce agglomeration by locally controlling the nucleus population [48].

Hunt and Jackson [49] demonstrated that nucleation occurs during the collapse of a cavitation bubble rather than when it expands. They have demonstrated this by slowing down the formation and collapse of cavitation bubble in pure liquid sealed in U-tube during isolated cavitation events. The pressure variations and very high pressures generated when the cavity collapses tend to lower the crystallization temperature of the liquid, which results in nucleation [50]. Another reason may be the rapid cooling that occurs after the bubble collapse around the collapsing bubble, thus creating a region of high supersaturation. According to another report [36], nucleation occurs due to the negative pressures generated during the collapse of cavitation bubbles. A possible nucleation mechanism, proposed during ice crystallization, is that the concentration and agglomeration of ice clusters can occur near the bubble surface because of the diffusion of species from low- to high-pressure zones. Louisnard et al. [52] have also suggested that high-pressure gradients are required, for pressure diffusion to be effective and that can only be attained during the collapse of the bubbles, and thus, stable cavitation can also act as a potential nucleation initiator. It has been provided different physical mechanisms that can possibly influence crystallization process with sonication. It was suggested that high pressures due to cavitation, agitation intensive mixing of the liquid medium by ultrasound, supercooling at the bubble surface, and enhanced heterogeneous nucleation are the possible factors responsible for the observed benefits of the sonocrystallization [52]. Author [53] has suggested that high pressure generated is strong enough to initiate the nucleation, as it increases the melting point of the liquid; therefore, cavitation bubble is important to initiate the crystallization. The enhancement of heterogeneous nucleation occurred as the ultrasound can lead to production of different nuclei from the single seed [53]. On the other hand, Virone et al. [54] determined the physical mechanism of ultrasound-induced crystallization based on the bubble dynamics for the first time. The authors correlated the nucleation

rate to the maximum pressure reached inside the cavitation bubble. To correlate such factors, they used numerical simulations on bubble dynamics.

Since sonocrystallization has many benefits over the conventional crystallization, numerous studies have been reported on the impact of various ultrasonic parameters on crystallization process for a variety of solutes such as acetylsalicylic acid, sodium acetate, sucrose, glycine, lactose, adipic acid, carbamazepine, NaCl, KCl, benzoic acid, and paracetamol. Various parameters investigated include sonication time, frequency and power, horn diameter, and supersaturation ratio. Besides affecting the MZW, crystal size distribution (CSD), and yield, ultrasound also provides control over polymorph forms of some solutes. It was shown that sonication can influence the primary nucleation and crystal growth of roxithromycin during antisolvent crystallization. With intensive amount of shear generated, ultrasound helped to reduce agglomeration and change the roxithromycin crystal morphology from a hexagonal to rhombus shape [55]. Further study by Hatkar et al. [51] on salicylic acid clearly established that ultrasound can be effectively used to control the antisolvent crystallization process in terms of the mean size of obtained crystals and size distribution. During sonocrystallization experiments, ultrasound-related variables like irradiation time and power of ultrasound were found to affect the crystal size distribution, whereas frequency did not have much effect over the range of frequencies investigated. It was found that irradiation time and power of ultrasound decreased the average particle size, as well as a reduction in the agglomeration was observed [36].

5. Sonocrystallization of lactose

Sonocrystallization of lactose has aroused great interest in the last decade. Consequently, there are a number of studies that provide relevant information on the effect of ultrasound on lactose crystallization, although such effect is not completely understood yet. The current information concerning the sonocrystallization of lactose is condensed in **Tables 1** and **2**.

5.1. Effects on lactose supersaturation and nucleation

It has already been discussed that sonication favors the formation of supersaturation and modifies the metastable zone [13, 14, 30]. The effect of ultrasound on lactose supersaturation has been scarcely documented chiefly because almost all the studies on lactose sonocrystallization have been done in combination with nonsolvents that greatly modify the solubility of lactose (**Table 1**) [4, 9, 18, 20, 28, 32]. The antisolvents used for these studies include ethanol, propanol, glycerol, and acetone, all of which decrease the solubility of lactose sharply and speed up the attainment of supersaturation [5]. On the other hand, there are a couple of studies that have been conducted in the absence of nonsolvent compounds (**Table 2**) [15, 22, 55]. From these works, it is reported that ultrasound energy densities of up to 0.15 W g^{-1} (at 20 kHz) narrow the MZW of lactose [22].

On the other hand, the effect of ultrasound on nucleation is not entirely clear, but it is generally accepted that sonication increases the rate of nucleation [15]. The long time of crystallization

	Effect on	Antisolvent	Experimental setup	Key outcomes	Ref.
Model solution system	Crystal growth rate	85% n-propanol	12–18% lactose, 20 kHz, 120 W	Growth rates from 0.007 to 0.027 μm s^{-1}	[32]
	Size and morphology	80% Acetone	12–16% lactose, 120 W	The crystal diameter decreased from 4 to 2.48 μm. Appearance of rod shaped crystals	[20]
		Ethanol	20–30% lactose, 20 kHz, 10–30 W	There was no correlation between ultrasound and particle size. Appearance of rods, needles and tomahawks shape.	[19]
		85% n-propanol	12–18% lactose, 20 kHz, 120 W	Mean diameter from 12 to 15 μm. Appearance of elongated and rod/needle-shaped lactose crystals in sonicated samples and tomahawk shape for stirring samples.	[32]
		85% Ethanol	11.5–17.5% lactose, 22 kHz, 12.3 W	Smaller crystals with more uniform shape than those obtained in the absence of sonication (stirring). Tomahawk and needle-shaped crystals were observed.	[28]
	Yield	85% Ethanol	11.5–17.5%lactose, 22 kHz, 12.3 W	Lactose recovery after sonication treatment (91.48%) was much higher that nonsonicated samples (14.63%)	[28]
	Purity	Ethanol	20–30% lactose, 20 kHz, 10–30 W	Decreases β-lactose incorporation.	[19]
Concentrated cheese whey	Size and morphology	65–85% Acetone	5–15% of lactose content, 120 W	Narrow crystal size distribution (2.5–6.5 μm) at pH 6.5, 15% of lactose concentration and 75% acetone concentration. Appearance of needle shaped crystals.	[58]
		85% Ethanol	22–33 kHz, 40–120 W	An increase in power from 40 to 120 W and frequency from 22 to 33 kHz had a marked reduction in particle size.	[6]
	Yield	85% Ethanol	22–33 kHz, 40–120 W	The yield without sonication was 74.7% and increased to 97.6% with 40 W of ultrasonic power. In a range between 60 and 120 W the yield decreased from 98.6 to 75.6%. An increase in frequency from 22 to 33 kHz did not change the lactose recovery (94 to 92%)	[6]
	Purity	85% Ethanol	22–33 kHz, 40–120 W	An increase in dissipation power (40 to 120 W) decreases the lactose purity from 96.9 to 88.7%. An increase in frequency from 22 to 33 kHz increased the purity from 79.5 to 91.5%.	[6]

Table 1. Reported effects of ultrasound in combination with antisolvents on lactose crystallization.

	Effect on	Experimental setup		Ref.
Model solution system	Supersaturation	60% lactose, 0.46 W g^{-1}	Ultrasound affected the heterogeneous nucleation.	[22]
			More prominent effect of ultrasound at low supersaturation between 1.6 and 2.1	
	Nucleation	30–50% lactose, 20 kHz, 750 W	Sonication showed a very rapid nuclei induction.	[18]
	Induction time	60% lactose, 0.46 W g^{-1}	Induction time was faster than stirring but this decrease with an increasing power from 0.15 to 1.15 W g^{-1}. Sonication resulted in a significantly faster nucleation rates than stirring, 5.3×10^5 and 1.6×10^4 mL^{-1} min^{-1}	[22]
	Crystal growth rate	60% lactose, 0.46 W g^{-1}	Did not change growth rate between ultrasound and stirring (0.14 μm min^{-1})	[22]
	Size and morphology	30–50% lactose, 20 kHz, 750 W	Particles from 15 to 30 μm. Production of rod-shaped lactose crystals with high elongation ratio. Appearance of rod-shaped crystals with high elongated ratio.	[18]
		60% lactose, 0.46 W g^{-1}	Number of crystals mL^{-1} was 2.8×10^6 and 4.6×10^5 for sonicated and nonsonicated samples.	[22]
		33% lactose, 20 kHz, 10-70 W, (oscillatory)	Batch produces bigger crystals than continuous treatment. An increase in power produces smaller crystals. Tomahawk crystals were observed.	[55]
	Yield	30–50% lactose, 20 kHz, 750 W	Yield of 84% with 5 min of sonication	[18]
		33% lactose, 20 kHz, 10-70 W, (oscillatory)	Sonication power from 0.10 to 0.15 W g^{-1} increased the yield from 17.4 to 25.1% (2.5 h of crystallization; and 19.7 to 28.3% after 4 h).	[56]
Concentrated cheese whey	Crystal growth rate	3 to 16 J mL^{-1}, 20 kHz	A faster rate of crystallization were obtained for whey sonicated at a flow rate of 11 L min^{-1} and applied energy density of 3.3 J mL^{-1}	[15]
	Size and morphology	3 to 16 J mL^{-1}, 20 kHz	Narrow distribution of crystal sizes (38 ± 10 μm) than stirred solutions (57 ± 17 μm).	[15]
	Yield	3 to 16 J mL^{-1}, 20 kHz	Yield obtained from 75 to 85% after 24 h.	[15]

Table 2. Reported effects of ultrasound on lactose crystallization (without antisolvents).

is one of the major issues to deal with during the recovering of lactose. Therefore, ultrasound has been used in lactose crystallization chiefly to accelerate nucleation and consequently to reduce the crystallization time [5]. The theoretical effects of ultrasound on nucleation can be summarized as follows:

- during the stable cavitation, the bubbles remain without collapsing for a number of ultrasonic cycles. The movement of these bubbles or flow streams enhances the mass and heat

transfers, as well as the aeration, so does the nucleation rate [5, 14]. The stable bubbles may act as nucleation centers, since the rapid growth of bubbles during the acoustic cycles drops the temperature locally and increases the supersaturation nearby the bubbles [5]. Besides, the pressure gradient around the cavitation bubbles induces a controlled diffusion of particles or embryos (segregation effect) that also favor the nucleation process [57].

- during the transient cavitation, the vigorous collapse of bubbles releases shockwaves and creates local zones of high pressure and temperature. These release of energy promotes mass transfer, molecular collisions, and supply the driving force for instantaneous nucleation (ΔG^*) [5, 13, 30]. Moreover, cavitation bubbles tend to locate themselves near the boundaries of the earlier formed crystals. When these bubbles collapse, the crystals are disrupted in many small fragments promoting the secondary nucleation [14].

Only a few works have addressed the effect of ultrasound on lactose nucleation, like in that reported by Dincer et al. [22]. In such study, it was observed that sonication of lactose solutions (60%) with an ultrasonic power density of 0.46 W g^{-1} (at 20 kHz) enhances the nucleation rate (5.3×10^5 crystals mL^{-1} min^{-1}) as compared to simple stirring at 300 rpm (0.16×10^5 crystals mL^{-1} min^{-1}). The ultrasound affected primarily the heterogeneous nucleation, and this effect was improved at low levels of lactose supersaturation (1.6–2.1). This study also reported that sonication decreased nearly ten-fold the induction time of nucleation. Induction time has been used to determine the nucleation rate, and this is defined as the time elapsed from the creation of supersaturated solution and the appearance of the first crystals [13]. The conventional crystallization of lactose exhibits a long induction time, which makes the process uneconomical [32]. Therefore, the reduction of induction time by lactose sonocrystallization becomes relevant.

5.2. Effects on the crystal size distribution (CSD) and yield

In contrast to nucleation, there is no consensus to the effect of ultrasound on crystal growth. Although, it is theorized that ultrasound might promote the growth of crystals through the mass, and heat transfers enhancement [14]. Patel and Murthy [32] reported crystal growth rates between 0.007 and 0.027 μm s^{-1} for a crystallization process of lactose assisted with ultrasound (120 W) and antisolvents (n-propanol 85%). Meanwhile, Dincer et al. (2014) reported a growth rate of 0.14 μm min^{-1} for a lactose solution (60%) sonicated at 0.46 W g^{-1}. Nevertheless, these authors did not observe a difference between the growth rates of sonication and stirring.

The size of lactose crystals commonly falls between 2 and 50 μm. The desired lactose crystal size varies depending on the specific use. For instance, when lactose is employed as an excipient in dry powder inhalers, its size must range between 2 and 6 μm for an optimum drug delivery to the lung. When lactose is destined to the food industry, the size of crystals is regularly bigger than 20 μm. No matter the intended use, a narrow crystal size distribution (CSD) is always preferable [5, 19]. The principal factors that affect the crystal size of lactose are (a) initial levels of saturation, (b) presence of salts and proteins, (c) rate of nucleation/crystallization, and (d) extent of secondary nucleation [5, 24, 32, 58]. Similarly, the addition of nonsolvents, as well as the seeding of lactose, decreases the size of lactose crystals and narrows the CSD [13, 18].

The shape and size of lactose crystals are also modified by sonication. The tomahawk shape (characteristic of α-lactose monohydrate) is the most reported in sonocrystallized lactose [5], although elongated shapes are described in some reports [9]. According to Dhumal et al. [18], applying ultrasound causes some faces of the lactose crystals grow faster than other producing elongated rod-shaped crystals. Besides, ultrasound increases the surface roughness of lactose crystals by reducing the incorporation of β-lactose into the crystal lattice [9, 18].

Regarding the crystal size, all the works agree that ultrasound increases the number of lactose crystals and produces smaller crystals with more homogeneous sizes [6, 15, 18, 20, 22, 32, 56]. Only one study has reported that there is no correlation between ultrasound (10–30 W, 20 kHz) and the size of lactose crystals [9]. The effects on the number and size of lactose crystal are primarily attributed to the increase in the number of nuclei that promotes the ultrasound, whether during the primary or secondary nucleation [56]. The extent of size reduction that is attained through sonocrystallization depends upon the ultrasound energy density (or power), time of sonication, and frequency applied (**Tables 1** and **2**). For example, we have observed that ultrasound energy densities of 9 J mL^{-1} were enough to decrease the size of lactose crystals by half and to significantly narrow the CSD Nevertheless, a higher energy density (50 J mL^{-1}) did not produce a further change in the size of crystals or the CSD (**Figure 3A**). On the other hand, the effect of different ultrasonic frequencies on the size of lactose crystals has been hardly reported, because nearly all the studies have used the similar frequencies (20–22 kHz). So far, only the work of Gajendragadkar and Gogate [6] has explored an ultrasonic frequency of 33 kHz. According to these authors, a frequency increase from 22 to 33 kHz reduced the crystal size and improved the lactose purity but decreased the yield of crystallization.

The process of lactose crystallization is conventionally carried out in presence of residual whey proteins (0.1–0.2%), which also decrease significantly the crystals size. The water-binding capacity of whey proteins creates supersaturation spots that favor nucleation [24, 25]. A few studies have addressed the effect of whey proteins on lactose sonocrystallization. Bund and Pandit [28] reported an increase in the crystal size of lactose sonocrystallized with ethanol (85%) in the presence of 0.4% of bovine serum albumin (BSA). Patel and Murthy [32] described that 0.2 to 0.8% of BSA widened the CSD of lactose sonocrystallized (120 W, 20 kHz) with n-propanol (85%). In contrast, we have noted that 0.64% of whey proteins decreased the

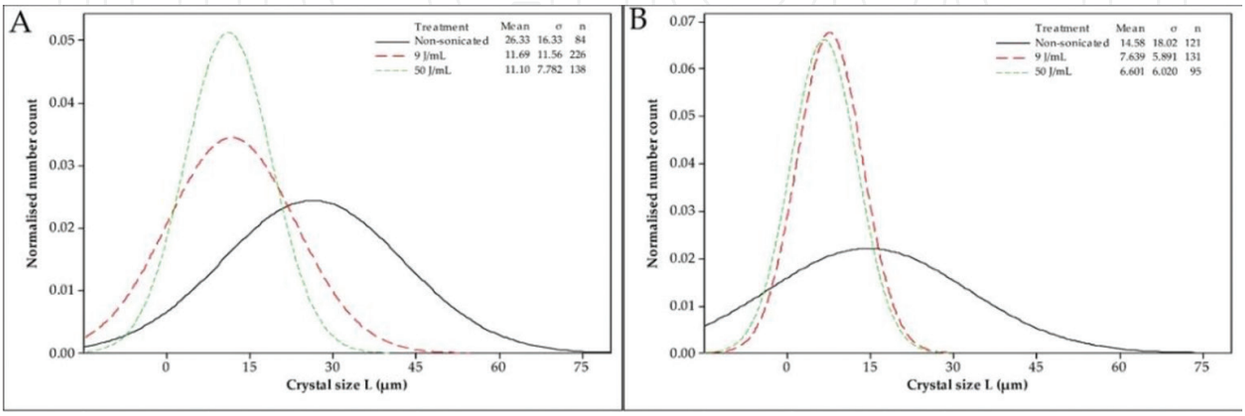

Figure 3. Effect of different ultrasound energy densities on the crystal size distribution (CSD) of lactose: (A) solutions saturated with 25% (w/v) of lactose; (B) solutions with 25% (w/v) of lactose and 0.64% (w/v) of whey proteins.

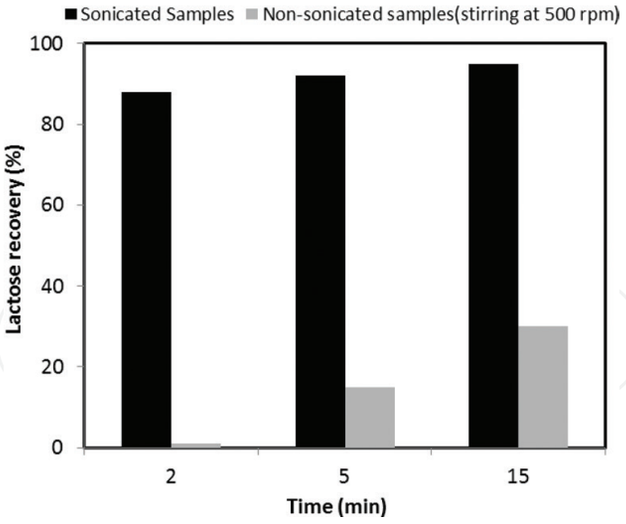

Figure 4. Effect of time on lactose recovery in sonicated and nonsonicated samples (reproduced from Bund and Pandit [28]).

size of crystal size significantly (9 J mL^{-1}) in an aqueous system without nonsolvents. Besides, the CDS was narrowed by the presence of whey proteins (**Figure 3B**).

Speaking of lactose recovering, there is a consensus that ultrasound improves the yield of lactose crystallization. **Tables 1** and **2** summarize the published data on crystallization yield obtained from lactose sonocrystallization with or without nonsolvents. Just to mention some examples, Bund and Pandit [28] showed that sonicated samples had higher lactose recoveries both in absence and presence of protein compared to the nonsonicated samples at different pH values with an antisolvent crystallization method. They recovered ~88% of lactose with 2 min of sonication as compared to 55–60% in 12 to 72 h with conventional lactose recovery (**Figure 4**). Similarly, the recovery of lactose was reported from the paneer whey with the use of ethanol as an antisolvent. The ultrasonic frequency and power utilized were 22 kHz and 120 W, respectively. Almost 90% of lactose was recovered in just 20 min with ultrasound.

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