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Effect of Ultrasound-Assisted Freezing on the Crystal Structure of Mango Sorbet

Anna Kamińska-Dwórznicka [1,*], Anna Kot [1], Ewa Jakubczyk [1], Magdalena Buniowska-Olejnik [2] and Małgorzata Nowacka [1,*]

[1] Department of Food Engineering and Process Management, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02776 Warsaw, Poland

[2] Department of Dairy Technology, Institute of Food Technology and Nutrition, University of Rzeszów, Ćwiklińskiej 2D St., 35601 Rzeszów, Poland

* Correspondence: anna_kaminska1@sggw.edu.pl (A.K.-D.); malgorzata_nowacka@sggw.edu.pl (M.N.)

Abstract: In this study, the effect of ultrasound-assisted immersion freezing (UAF) of mango sorbet in comparison to conventional freezer freezing, on freezing course and created crystal structure, was studied. The scope of work included the preparation of a sorbet mixture prepared on the basis of frozen mango fruit with the addition of locust bean gum (LBG), guar gum and a commercial mix of carrageenan without the addition of stabilizers, and freezing it using a conventional freezer and ultrasound-assisted freezing equipment, with variable operating parameters (21.5 kHz and 40 kHz—continuous or chopped mode). Then, the freezing time and the crystal structure of the frozen samples (a microscopy analysis) were examined. US-assisted freezing reduced the time of the process for stabilized samples of the sorbet. It was also proven that, proper stabilization with the combination of US treatment results in the formation of favorable crystal structure. Stabilized sorbet subjected to US action at a frequency of 21.5 kHz in chopped mode was characterized by the most uniform crystal structure, consisting of crystals with the smallest diameters among all the tested samples; the equivalent diameter was 9 µm, while for the stabilized control it was 25 µm.

Keywords: US-assisted freezing; sorbet; recrystallization; structure

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1. Introduction

Nowadays, the food industry is focusing on innovative methods that are simple, fast, non-toxic for the final product and also economic. Ultrasound use has been the subject of research for many years. Based on the commonly accepted definition, ultrasound is an acoustic wave with a frequency greater than 20 kHz, the threshold of human hearing. The mechanism of ultrasound is based on acoustic cavitation [1]. It takes place on the basis of an interaction between ultrasonic waves, liquid and dissolved gas. US-assisted freezing cavitation bubbles could be most beneficial as they reduce both the heat and the mass transfer resistance at the ice/liquid interface. US-assisted techniques can also be considered green technology due to the creation of an environmentally friendly process [2]. Additionally, ultrasound can be used to minimize processing or increase quality and improve processing efficiency, ensuring food safety while extending product shelf life [3–5]. The cavitation and mechanical effects produced by ultrasound can improve food's freezing rate and antibacterial effects, improving energy transfer and also reducing the costs of freezing [3,5].

Food drying and freezing are two of the most commonly used methods of preservation. Food freezing and cooling are essential not only in frozen food production but also as the main operation for ice cream production [6]. Crystallization is a process that leads to the formation of a crystalline lattice structure. When crystallization occurs at slow speed, the formed ice crystals are large—diameters cross the 50 µm range, causing cellular damage and great thawing loss in tissue products. For ice cream-type products, it results in coarse grains and an undesirable texture [4,7]. Fast freezing methods improve product quality by improving the heat transfer rate and reducing the formation of big ice crystals. A small and more uniform crystal structure after production also limits the recrystallization rate during storage [8]. However, quick freezing processes imply higher energy and economic costs. US technology promises to improve mass and energy transfer in processes, reducing costs [4,9]. During "sonocrystallization", US is able to change the crystal habit, crystal size distribution as well as promote or prevent coalescence processes [10,11]. Ice crystals in the created ice cream could be fractured during freezing and such fragmentation leads to crystal size reduction.

Most of the UAF studies describe the application of ultrasound in solid food technology [3,5,11–15] or examine how US treatment before freezing could affect the ice cream ingredients [9,16–18]. To the best of our knowledge, the effect of ultrasound application during ice cream production has not been studied yet. Ice cream is an exceptional type of multiphase product. Based on the official classification [19] of frozen desserts, the sorbet is a special type of ice cream that contains fruits and a real solution of sugar and water; it can also be described as a colloid due to hydrocolloid stabilizer content. It is also a crystal suspension, and all the components of this mix are necessary to form a proper crystal structure [6,20].

In ice cream (including sorbets), changes in the physical state of water cause undesirable changes in its stability, structure and texture during distribution or storage. Such modifications pose a problem, which result in quality loss at the final part of the cold chain due to recrystallization phenomena [21]. Recrystallization is a process related to changes in the characteristics of ice crystals such as shape, number, size, orientation or perfection. This process occurs due to two mechanisms: accretion and migration. The first one relies on joining two or more crystals together to form a single larger crystal. Migration is based on the melting process, which yields the movement of the melted liquid to the surface of larger crystals [7,22].

It was already reported that ultrasound homogenization has the potential to enhance the quality and performance of ice cream mixes [17]. The freezing of ice cream is mostly carried out in freezers (contact freezing)—this method also enables the proper aeration of the mixture during production [20]. This type of freezing, combined with the addition of a proper stabilizer mix, allows it to obtain a favorable crystal structure which is stable during longer storage, hence the recrystallization process [20,23]. Immersion freezing of the sorbet mixture creates a different type of crystal structure—even when the freezing rate is higher, the created crystal structure in a lack of air could be uneven and unfavorable. Therefore, the objective of the present work was to investigate the influence of ultrasound-assisted immersion freezing on mango sorbet crystal structure and compare it to the one created in a conventional freezer.

2. Materials and Methods

2.1. Materials

Mango sorbet is the most popular among sorbets on the ice cream market, hence the choice of research material. The blended mango has a smooth texture, unlike strawberry mass. The following ingredients were used as a basis for sorbet preparation:

- Control Mixture: 66.5% of frozen mango (Hortex, mazowieckie, Poland), 13.8% of sucrose (Diamant, Przemyśl, Poland), 5.5% of mango syrup (Monin, Bourges, France), 13.9% of water
- Stabilized samples: a 0.3% of a mix of stabilizers was also added with the composition 0.1% of carrageenan (Fooding Shanghai, Shanghai, China), 0.1% of guar gum (Agnex, Białystok, Poland) and 0.1% LBG (*Locust Bean Gum*); (Fooding Shanghai, Shanghai, China). For each variant 1 L of the sorbet mix was prepared for further analysis.

2.2. Freezing in a Conventional Freezer

After mixing all ingredients and stabilizers, the sorbet (600 mL) was frozen in two small freezers, the G3 Ferrari G20035 Cremosa (Italy), to provide freezing for stabilized and non-stabilized samples at the same time under the same conditions. The process was monitored using two thermocouples to control the temperature in both freezers. Records were taken every 120 s (2 min) by the MPI-LAB temperature recorder (Metronic Instruments) connected to a PC. Sample temperatures after freezing—after 40 min of the process was at a level of −8 °C. Frozen variants of sorbets, named as follows: FWS—sample for freezer without stabilizers; FS—sample from freezer with stabilizers, were packed into 0.5 L boxes and transported for further microscopic analysis.

2.3. Freezing in Ultrasound-Assisted Freezing Equipment

An aluminum cylindrical can (0.05 m in diameter and 0.13 m high, 250 mL of potential capacity) containing 200 mL of the sorbet blend was placed in a bath of a two-chamber cryostat-type freezer, made of stainless steel, with an aggregate for freezing closed and open samples, filled with cooling liquid (standard coolant, propylene glycol, Borygo, Bernard, Poland). The device allows for the study of ultrasound-assisted freezing processes, on the principle of immersion in non-boiling liquid. The ultrasound frequency of the right bathtub was 21.5 kHz ± 10%, the left bathtub 40 kHz ± 10%, power 2.4 kW. Ultrasonic operation mode setting (continuous operation, chopped operation); (Figure 1).

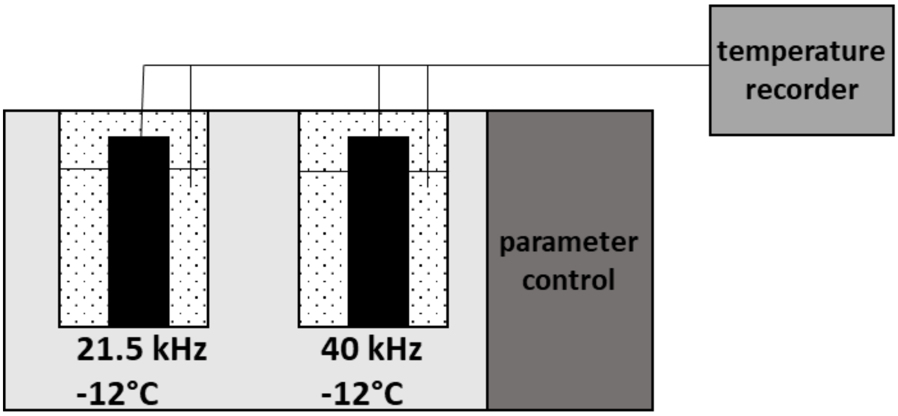


Figure 1. A diagram of the experimental set-up (own elaboration).

The process was carried out in two chambers at a temperature of −12 °C for the following variants:

- Bathtub without US for samples with (CS) and without stabilizers (CWS),
- Bathtub 21.5 kHz for samples with (CS21co) and without stabilizers (CWS21co), 10 US treatment, continuous operation,
- Bathtub 21.5 kHz for samples with (CS21cho) and without stabilizers (CWS21cho), US treatment, chopped operation,
- Bathtub 40 kHz for samples with (CS40co) and without stabilizers (CWS40co), US treatment, continuous operation,
- Bathtub 40 kHz for samples with (CS40cho) and without stabilizers (CWS40cho), US treatment, chopped operation.

The process was monitored using two thermocouples to control the temperature in both chambers. The temperature of the cooling liquid and the temperature of the solutions were tested to achieve −8 °C in the center of the vessel (the temperature was chosen according to the temperature of samples frozen in a conventional freezer). Records were taken every 120 s (2 min) by the MPI-LAB temperature recorder (Metronic Instruments, Mahadevapura, India) connected to a PC (Figure 1). In order to determine the effective time of freezing for both methods of freezing (US cryostat and conventional freezer), the recorded data were used to prepare freezing curves according to the method shown before [24]. Freezing processes and other measurements were performed in triplicate.

2.4. Cryoscopic Temperature Analysis

The cryoscopic temperatures of stabilized and non-stabilized sorbet samples were determined using a Marcel osm 3000 osmometer (Marcel, Waldenburg, Poland). The device measures freezing temperature with an accuracy of 0.002 °C. Measurements were performed in triplicate.

2.5. Microscopy Structure Analysis

To prepare slides for image analysis (after freezer and cryostat production), a small piece of sorbet was taken from the center of the aluminum can, from at least 3–5 different locations, a minimum of 3 cm away from the ice cream surface, and placed on an object slide by using a spatula and then covered with a cover slip placed on the top of the sample. Pressing the sample reduced the overlap of ice crystals to discern them individually and destroy the air cell structure to provide excellent clarity. Samples were prepared in a freezing chamber and transferred into a microscope with a cooling system (Linkam LTS420). This system occupied with a special, closed, liquid-nitrogen-cooled table, eliminated the influence of ambient temperature.

The recrystallization process was analyzed based on the images of ice crystals taken after preparation. A microscope (Olympus BX53), with the cooling system Linkam LTS420 (temperature range −196 °C to 420 °C) and a camera (Olympus SC50), were used. The obtained images were then analyzed using NIS Elements D software. From 300 to 500 crystals were marked for a particular sample, and then the area, equivalent diameter and standard deviation were calculated using NIS Elements D Imaging software (ver. 5.30.00, Nikon), according to the aforementioned method [7,25].

2.6. Statistical Analysis

To determine the effect of our study, the one-way ANOVA test was used. Further tests to detect the significance of differences between particular groups were found using Student's t-test applied to compare independent samples in pairs, and variance analysis (ANOVA) was used for more than two groups. Detailed analysis was based on Tukey's confidence intervals. All statistical tests were carried out at a significance level of $\alpha = 0.5$. The statistical processing of results was performed using Statistica 13.1.

The frequency distribution of crystal size was computed using Microsoft Excel 2019 macro-data analysis. The relative frequency of any class interval was calculated as the number of crystals in that class (class frequency) divided by the total number of crystals and expressed as a percentage. The parameter X_{50} was analyzed as a mean diameter (D_A) for 50% of crystals in the sample. The mean diameter (D_A) and standard deviations (SD) of each class were also calculated in accordance with the aforementioned method described [6,20,25].

3. Results and Discussion

3.1. Freezing Curves Analysis

3.1.1. Freezing in a Conventional Freezer

Freezing with both methods (freezer and cryostat) started at a temperature of −2 °C (the samples were prepared from frozen mango). Sensors were placed in the geometrical center of the samples. In relation to the cryoscopic temperature (Table 1) and also after a course of freezing curves (Figures 2–4) analysis, we can claim that the phase change and final freezing stage occurred simultaneously.

Table 1. The cryoscopic temperature of stabilized and non-stabilized mango sorbet.

Stabilized Sample	Non-Stabilized Sample
−2.9 [a]	−3.2 [b]

[a,b] means in the same row indicated by different letters are significantly different (p value < 0.05).

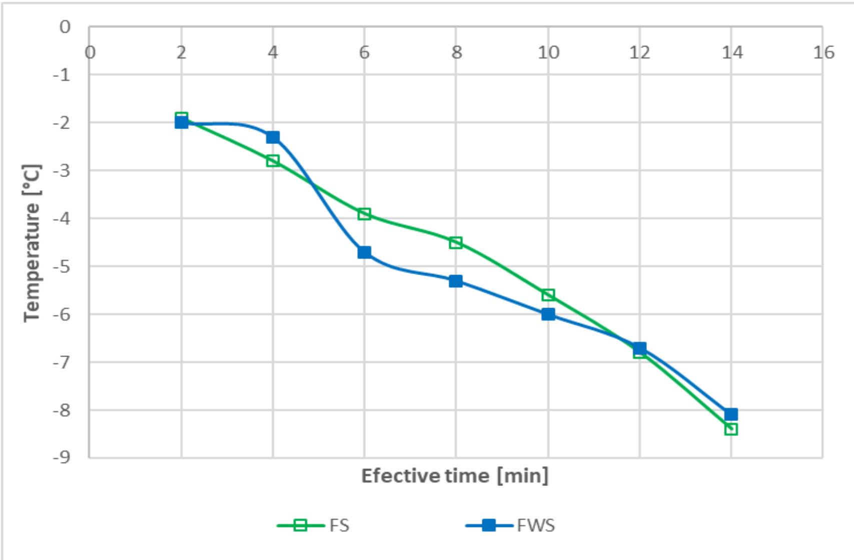


Figure 2. Freezing curves for the sorbet sample without (FWS) and with (FS) stabilizers frozen in a conventional freezer (temperature changes in the thermal center of the sample during the freezing process).

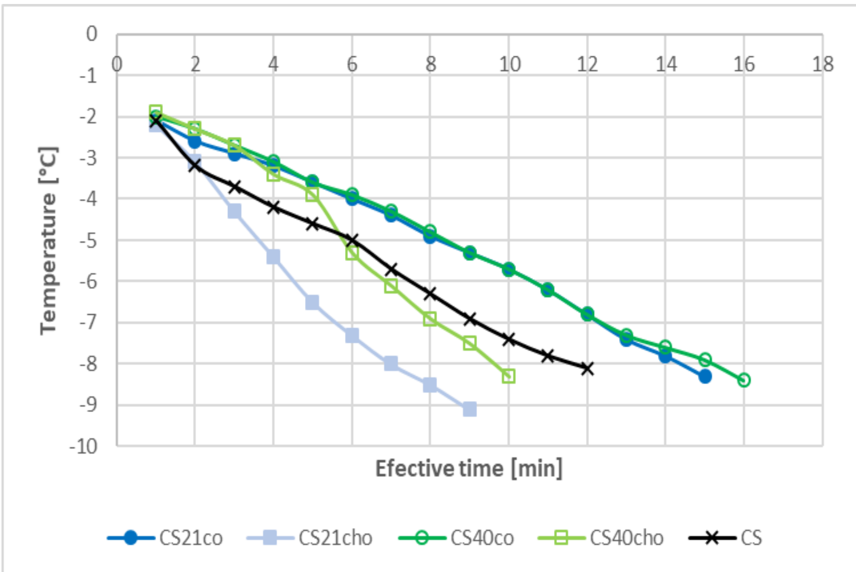


Figure 3. Freezing curves for the stabilized sorbet sample frozen in a cryostat without US (CS), US-assisted 21.5 kHz and 40 kHz continuous operation (CS21co and CS40co) and chopped operation (CS21cho and CS40cho) (temperature changes in the thermal center of the sample during the freezing process).

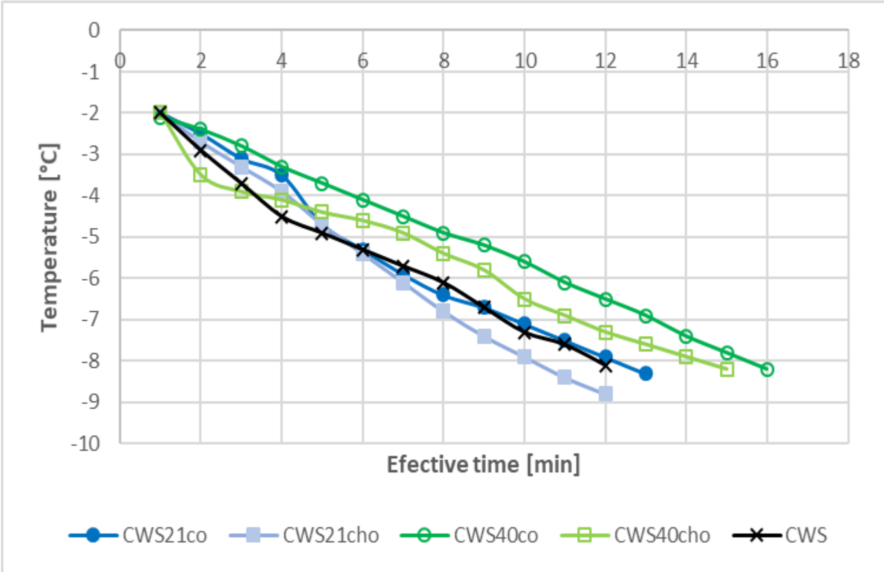


Figure 4. Freezing curves for the non-stabilized sorbet sample frozen in a cryostat without US (CS), US-assisted 21.5 kHz and 40 kHz continuous operation (CS21co and CS40co) and chopped operation (CS21cho and CS40cho) (temperature changes in the thermal center of the sample during the freezing process).

The total times of sorbet freezing in a conventional freezer for stabilized and non-stabilized samples were similar (Figure 2); however, visible temperature fluctuation during the process was noticed for the non-stabilized sample. The addition of stabilizers should not significantly affect the cryoscopic temperature value and heat transfer conditions; however, it can influence the water molecule organization in a product due to stabilizer (hydrocolloids) properties. Based on that theory, we can suppose that there is an influence on the size and number of ice crystals. It was already proven that some of the hydrocolloids used in ice cream can modify the crystal structure of ice cream samples [6,20,21,23,26].

3.1.2. Freezing in Ultrasound-Assisted Freezing Equipment

While freezing in US-assisted equipment (US-assisted cryostat), a visible difference in effective freezing time was noticed; however, not for all of the samples and types of US treatments (Figures 3 and 4). As observed for the stabilized samples (Figure 3), the chopped operation of US at both 21.5 and 40 kHz frequency allowed for reducing the effective freezing time by 32.5% (8.1 $\pm$ 0.5 for the CS21cho sample vs. 12.1 for the CS sample). This was already proven for milk ice cream after treatment with 20 kHz US—there was a 35% of total freezing time reduction [9]. This effect was also observed during the US freezing of solid products [10,15,27], for example chicken breast freezing [3], when the time reduction was over 15%. It is also worth highlighting that this effect was nearly the same for different US intensities. Continuous US operation caused the elongation of freezing time when compared to the freezing time of the reference CS sample, due to temperature fluctuations during US action, which was also already confirmed for US-assisted water freezing [28].

It was also surprising that for non-stabilized samples (Figure 4), significant freezing time reduction was not visible even for the samples frozen with chopped US operation. The shortest time of freezing was noticed for the sample frozen without US, and it was equal to the freezing time of the sample treated with 21.5 kHz US in chopped operation. For the rest of the samples, a longer freezing period occurred. The longest freezing period was observed for the sample treated with a 40 kHz continuous cycle and it was nearly 16 min. To summarize, the addition of stabilizers could affect the freezing time of sorbet frozen with US chopped operation regardless of US intensity.

3.2. Crystal Structure Analysis

In frozen food, small ice crystals are desired, especially in such desserts as sorbet. Ice crystal diameters bigger than 25 µm become perceptible and influence consumer assessment [6,20,29–31]. For both tested methods of freezing (without US), it was found that, in stabilized sorbets (FS and CS), equivalent crystal diameter was at a level of 17 µm, while for non-stabilized samples (FWS and CWS) we could observe differences between the sample frozen in a conventional freezer (23 µm) and in the cryostat (30 µm); (Figures 5 and 6, Table 2).

Table 2. A comparison of ice crystal size distribution in sorbet samples frozen in a conventional freezer and cryostat with and without US.

Variants	Minimum Size of Ice Crystals [µm]	Maximum Size of Ice Crystals [µm]	Average Diameter in the Class with the Highest Frequency [µm]
FS	10.18	24.06	16.59 ± 1.33^{d}
FWS	12.75	39.01	23.44 ± 2.44^{j}
CS	10.43	25.44	17.28 ± 1.97^{e}
CWS	19.51	46.04	30.41 ± 1.91^{k}
CS21co	11.59	24.32	16.72 ± 2.01^{d}
CWS21co	12.02	38.26	22.55 ± 1.76^{i}
CS21cho	6.68	19.07	12.11 ± 2.07^{a}
CWS21cho	14.51	32.78	21.36 ± 1.44^{h}
CS40co	14.15	41.12	20.42 ± 2.31^{g}
CWS40co	16.12	54.69	19.98 ± 2.05^{f}
CS40cho	8.59	32.70	13.36 ± 1.97^{b}
CwS40cho	11.68	35.96	15.29 ± 2.15^{c}

a–k means in the same row indicated by different letters are significantly different (p value < 0.05).

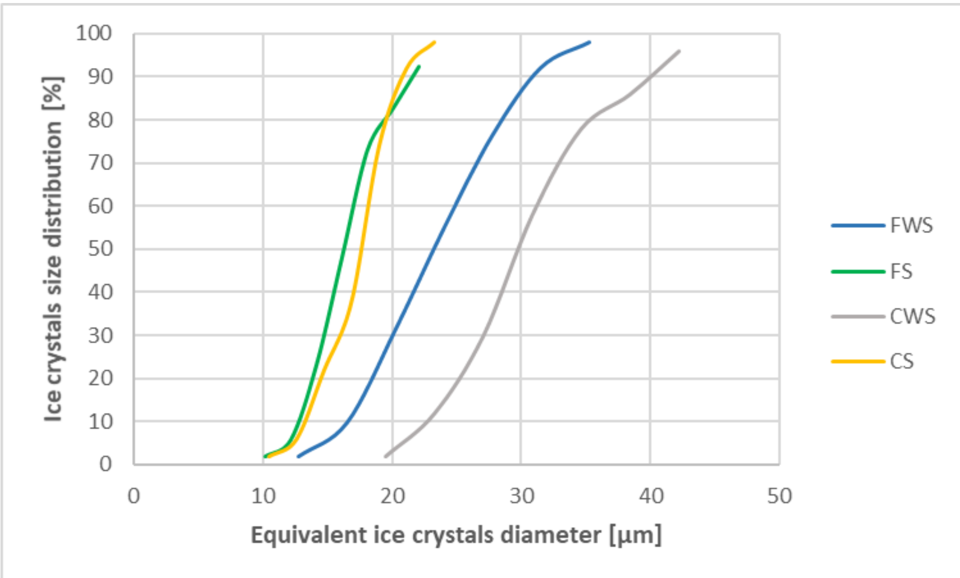
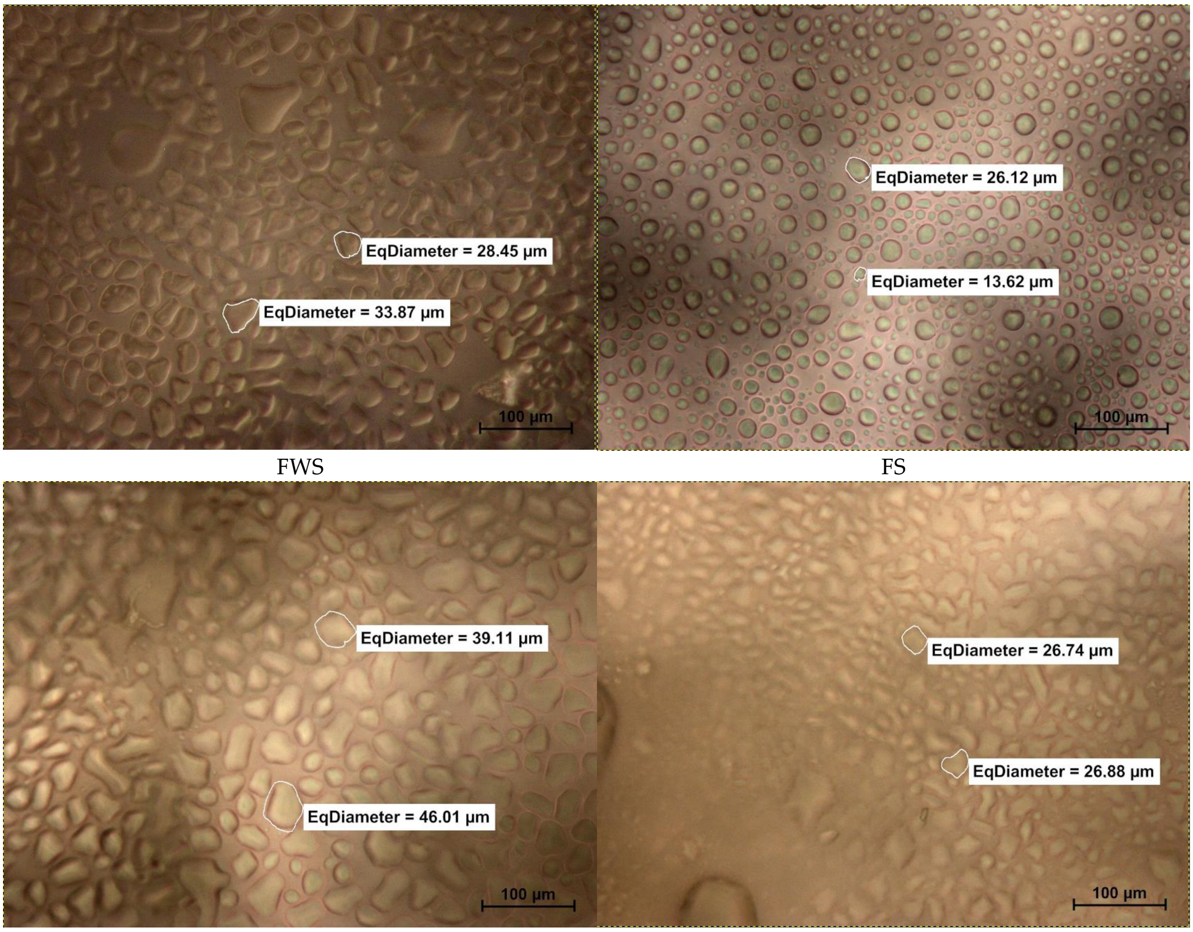


Figure 5. Ice crystal size distribution stabilized and non-stabilized sorbet sample frozen in a conventional freezer (FS and FWS) and cryostat without US (CS and CWS).



FWS FS

CWS CS

Figure 6. Images from a stabilized and a non-stabilized sorbet sample frozen in a conventional freezer (FS and FWS) and cryostat without US (CS and CWS).

When analyzing images, it is obvious that the stabilizer addition affects the formation of a more uniform crystal structure; this is especially visible in the case of the sorbet prepared with a conventional freezer (Figure 6—FS). The ice crystals are more regular and rounded in shape. Even if they are of different sizes, the shape is similar.

It was also clear (Figures 5 and 6, Table 2) that in the freezer, independent from the stabilization, smaller crystals were formed. It was already noticed in previous research [20,32,33] that mixing generates a large presence of air bubbles and because of that it creates excellent conditions for smaller ice crystal aggregation. This was shown using the example of strawberry sorbet [20] frozen under similar conditions as in our study.

The proper stabilization of sorbet usually prevents samples from extensive crystallization during production and from extensive recrystallization during storage. It was already proven that the mix of carrageenans could create a crystal structure with crystals smaller than 17 µm [6,20]. Proper stabilization plus US-assisted freezing could help to create a favorable crystal structure with ice crystal diameters lower than 13 µm, which is clearly visible from the results (Figures 7–10 and Table 2). When analyzing ice crystal diameter at 50% of the cumulative distribution of the sample (X_{50}), it is clear that the smallest ice crystals were formed in stabilized samples treated with US at 21.5 kHz—CS21cho and CS21co, (Figures 7 and 8 and Table 2) achieving an X_{50} parameter in the range of 12–17 µm, while for untreated samples, 20 to even 30 µm was achieved (Table 2). A similar effect was already shown in gelatin gel samples [34], where US treatment (20 kHZ for 180 s) caused a significant reduction of X_{50} parameters from 280 to 47 µm. This effect was also observed for a dough sample [35]. US treatment promoted the formation of a large number of ice crystals; however, they were very small in diameter.

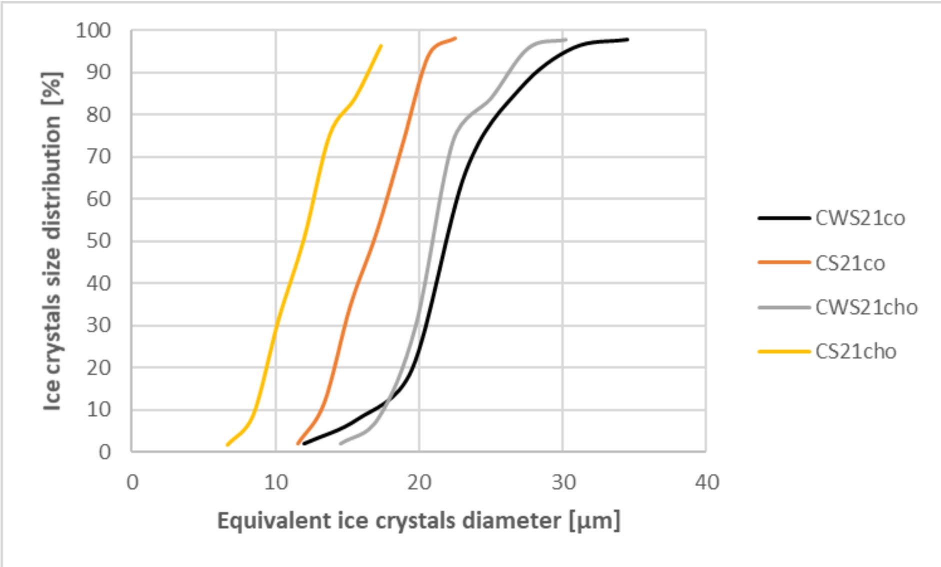


Figure 7. Ice crystal size distribution in a stabilized and a non-stabilized sorbet sample frozen in a cryostat, US-assisted at 21.5 kHz with continuous operation (CS21co and CWS21co) and chopped operation (CS21cho and CWS21cho).

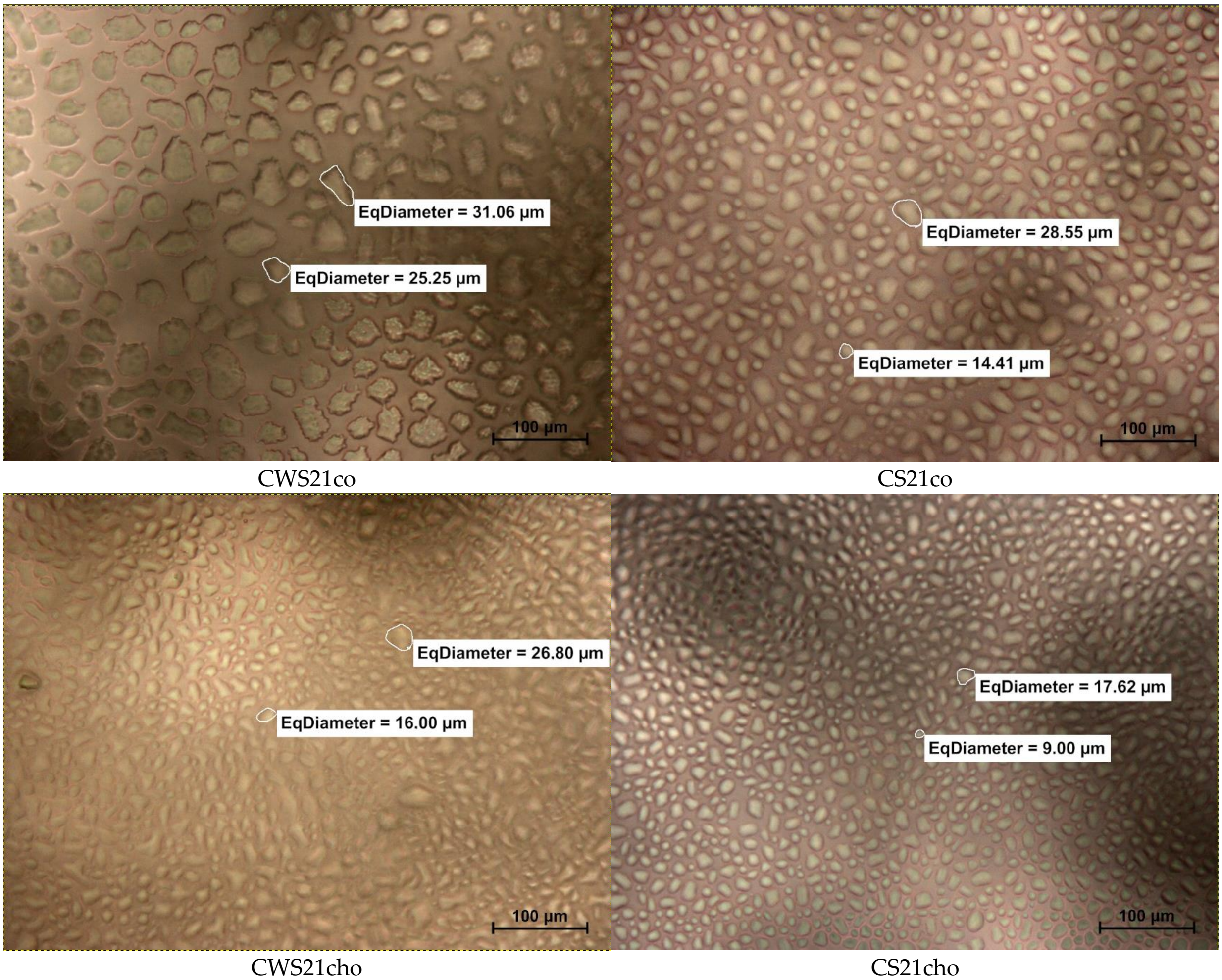


Figure 8. Images from a stabilized and a non-stabilized sorbet sample frozen in a cryostat, US-assisted at 21.5 kHz with continuous operation (CS21co and CWS21co) and chopped operation (CS21cho and CWS21cho).

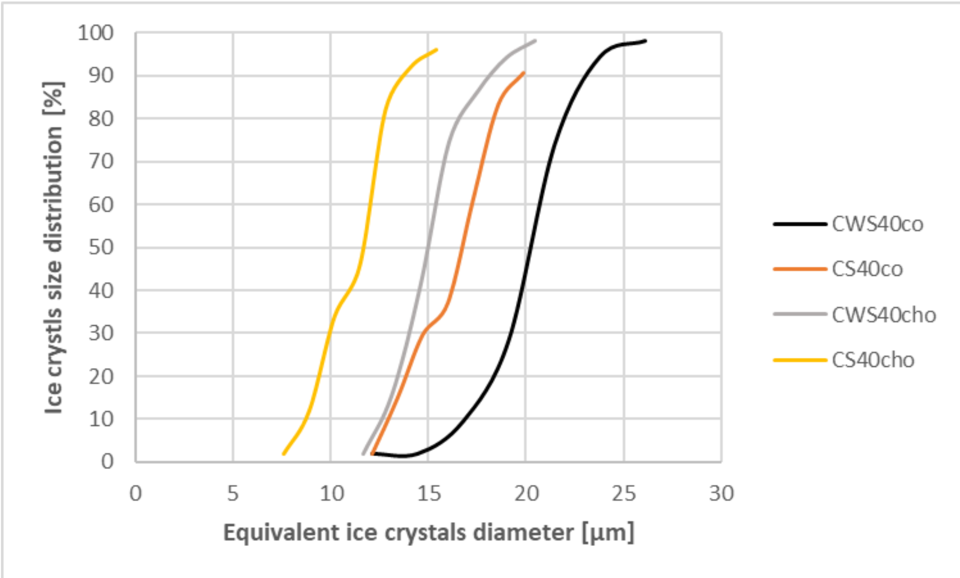


Figure 9. Ice crystal size distribution in a stabilized and a non-stabilized sorbet sample frozen in a cryostat, US-assisted at 40 kHz with continuous operation (CS40co and CWS40co) and chopped operation (CS40cho and CWS40cho).

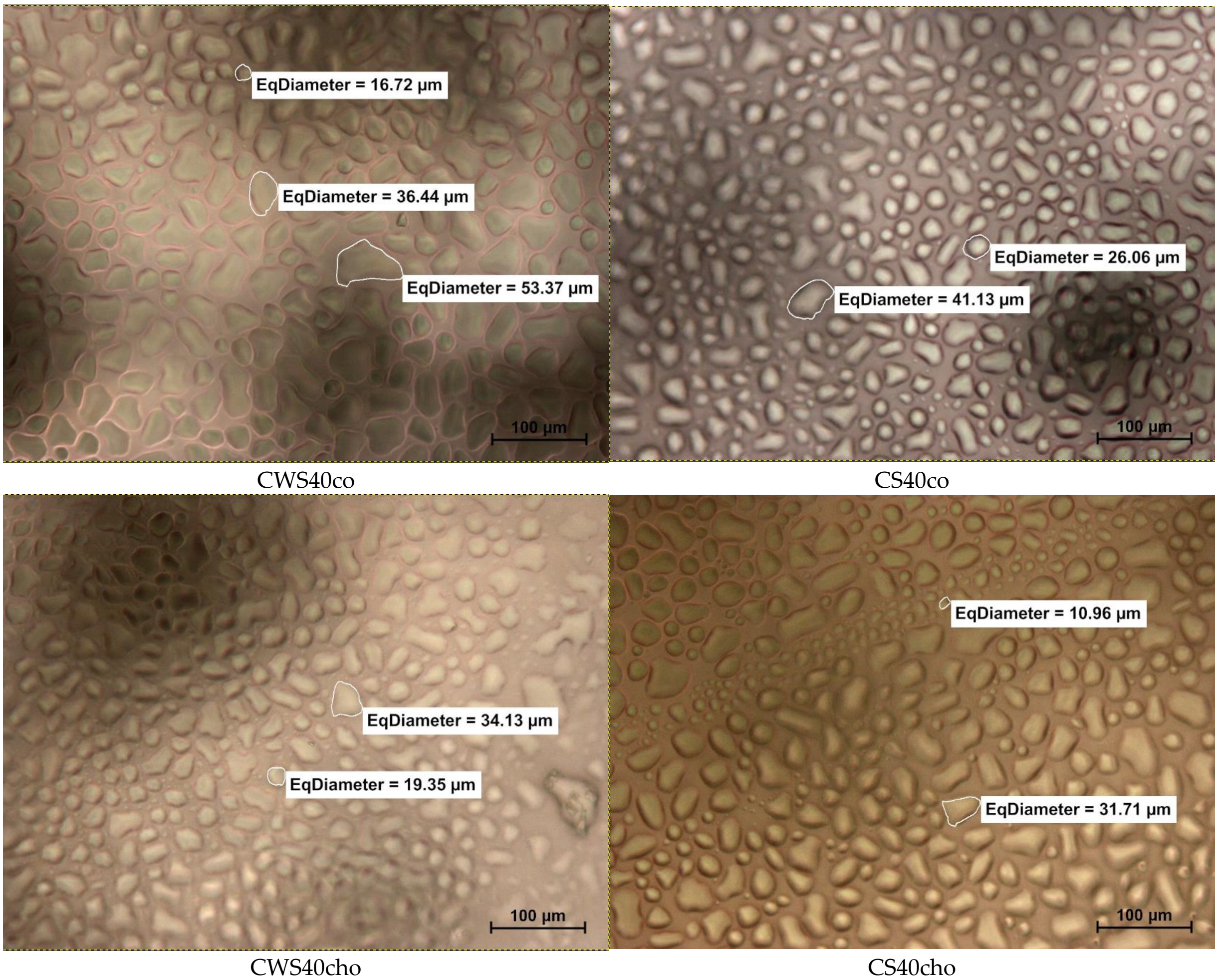


Figure 10. Images from a stabilized and a non-stabilized sorbet sample frozen in a cryostat, US-assisted at 40 kHz with continuous operation (CS40co and CWS40co) and chopped operation (CS40cho and CWS40cho).

Great effects were also presented for US-assisted immersion freezing of different types of plant tissue [10,36], mushrooms [11], carp tissue [37] and animal tissue [13,38]. In the frozen pork tissue sample, for US-assisted freezing (30 kHz, 180 W) the examined average radius of ice crystals was 6 times lower than for the control sample [38]. It was also proven that US treatment during freezing influences the recrystallization rate during pork tissue storage [13]. In frozen mushrooms (*Agaricus bisporus*), the crystal structure was described by cryo-microscopy and also SEM photos [11]. Ice crystal sizes after US treatment were almost 2.5 times lower than in the control samples. It was also proven that US-assisted freezing changes the shapes of ice crystals. New shapes of crystals prevent the crystal lattice from joining the free molecules of water that after freezing could increase crystal sizes.

When comparing two types of US treatments (continuous and chopped operation), it was clear that chopped US was better for the samples—it resulted in a reduction in the total time of freezing and smaller ice crystals after production (Figures 7–10 and Table 2). When comparing two different US frequencies (21.5 and 40 kHz), the differences in crystal diameters seem not to be significant (Table 2); however, when comparing images, there are noticeable differences in crystal shapes and arrangements (Figures 9 and 10). The stabilized sorbet treated with US at 21.5 kHz with chopped operation(CS21cho), was characterized by the most unified structure, consisting of crystals similar in shapes and sizes with the smallest diameters among all given samples. A similar effect was already noticed in frozen pork tissue (US-assisted immersion freezing; [38]) and in frozen mushroom [11]—US treatment unified the shapes and sizes of created ice crystals. What was visible on the image from the sample that was stabilized, treated with US at 40 kHz under chopped operation (CS40cho), ice crystals were less unified and bigger than in samples from a lower frequency (CS21cho). However, the characteristic path of small and more rounded in shape ice crystals was also noticed (Figure 10—CS40cho).

4. Conclusions

The application of US-assisted freezing during the freezing of mango sorbet has been studied to improve the quality of the final product. It was already proven that common ice cream stabilizer addition did not affect the freezing course. However, in this study, the effect of US treatment on freezing time was only visible for stabilized samples (even over a 30% time reduction) and was independent of the US frequency.

US-treated samples were characterized by the most unified structure, which was even more favorable than after conventional freezer treatment. The smallest diameters of ice crystals (even lower than 10 μm) were noticed for the sorbet sample treated with US at a frequency of 21.5 Hz, using chopped operation. This result was also visible in the images. This experiment showed that the US technique can be used industrially as a potential method to reduce ice crystal diameters in the ice cream sorbet type, and in this way improve product quality and acceptability.

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