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# Study on the Influence of Ultrasound Homogenisation on the Physical Properties of Vegan Ice Cream Mixes 

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#### Abstract

This study investigated the effect of ultrasound homogenisation on the physical properties of vegan ice cream mixes. Samples were prepared based on vegan recipes with different sorts of stabilisers such as iota carrageenan and iota carrageenan's acid and enzymatic hydrolysates. Ice cream mixes were compared for stability, particle size distribution, rheological properties and morphological structure. All mentioned analyses were conducted before and after 24 h of maturation at $4^{\circ} \mathrm{C}$. It was found that the ultrasound treatment decreased the size of particles and, in conjunction with the maturation stage, a significant reduction was visible (the lowest value was at $9.76 \mu \mathrm{~m}$ ). The addition of the hydrolysates of iota carrageenan had a considerably better effect in reducing the size of particles than iota carrageenan. The range of TSI values was from 1.7 to 4.2 . Additionally, two sorts of destabilisation occurred: sedimentation and coalescence, during the maturation of ice cream mixes, which was also visible in the images. According to the rheological properties, ice cream mixes, with the addition of stabilisers, showed non-Newtonian shear-thinning (pseudoplastic) behaviour. Moreover, the effect of ultrasound treatment on the consistency index was only pivotal for ice cream mixes with an addition of iota carrageenan and with enzymatic $\beta$-galactosidase hydrolysates of iota carrageenan.


Keywords: ultrasound; homogenisation; vegan; ice cream mix; iota carrageenan; stabilisers

## 1. Introduction

There is a myriad of elements of different nature such as sugar, fats, stabilisers, water and others, which constitute the compounds of the ice cream mix. All of them have to be correctly blended and emulsified together so there is less possibility of reducing the quality of the final product. The knowledge of the characteristics of compounds and the relationship between them is pivotal to obtaining the desirable quality of ice cream. According to that, the ice cream mix determines the sensorial characteristics, structure, resistance to melting, hardness or viscoelastic behaviour of ice cream [1-3]. Not only do ingredients contribute to the ice cream structure, but also the manufacturing process, which includes the homogenisation step.

In food engineering, homogenisation is a process that is used to reduce the size of particles and globules in the product, narrowing the size distribution or providing a stable emulsion. Consequently, there is a possibility of achieving greater stability of fat globules during the ice cream mix maturation $[2,4,5]$. However, as a result of the need to improve traditional food processing in recent decades, alternative methods are being implemented. The search for alternative processes has drawn attention to ultrasound [6].

In the food industry, ultrasound has been the subject of research for many years and the application of this method has now become widely used. Overall, ultrasound is defined as an acoustic wave with a frequency greater than 20 kHz , the threshold for human auditory detection. The mechanism of ultrasound is based on acoustic cavitation. It occurs because of the interaction between ultrasonic waves, liquid, and dissolved gas. Consequently, the
implosion of cavitation bubbles generates an extremely high temperature and pressure which later produces high shear energy waves and turbulence. Moreover, the ultrasound technique is an innovative and up-and-coming technology in the food industry owing to the fact that it is relatively cheap, very simple, really fast, non-toxic and energy-saving. It can also be considered a green technology on the grounds that it creates an environmentally friendly process. Additionally, the ultrasound can be used to minimize processing or increase quality and improve processing effectiveness and efficiency, providing food safety while extending the shelf life of the product [7-12]. Currently, ultrasound is commonly used for instance in the activation or deactivation of enzymes, homogenisation, emulsification, stabilisation, crystallization, or even ultrasound-assisted drying [10,13].

Ultrasound waves yield an efficient homogenising procedure compared to traditional homogenisation. Therefore, the use of ultrasound during the process of the production of ice cream may be a new promising approach [9]. In the presented study, ultrasound homogenisation was used as an alternative method to conventional homogenisation. According to the current research, it was proven that ultrasound homogenisation may produce particles with a narrow particle size distribution. Moreover, the ultrasound may improve stability and contribute to the adsorbing protein of the emulsion. Consequently, such improved stability and smaller particle size contributed to considerably better results during the freezing process due to the fact that such favourable conditions lead to creating a superior ice crystal structure and obtaining smaller ice crystals in comparison to the use of traditional homogenisation [8,14,15].

Furthermore, the ultrasound treatment would replace food additives and emulsifiers, for example in the preparation of dairy-based emulsion. The results of the study by Aslan and Dogan [16] show that ultrasound treatment could eliminate additives such as emulsifiers in the preparation of food emulsions. Not only can the findings of this study be beneficial for the economy but also for commercial aspects. Moreover, this knowledge can then be used in the ice cream mix preparation to, for instance, extend the shelf life of the ice cream. So, based on that, the power of ultrasound is a promising tool in the preparation of the ice cream mix $[15,16]$.

The aim of this study was to develop the previous study by Kot et al. [17], conducted on vegan ice cream mixes, to compare the physical properties of ice cream mixes that were affected by different sorts of homogenisation. For this purpose, ice cream mixes with the same vegan recipe were produced but instead of traditional homogenisation, ultrasound homogenisation was used. Stability, particle size, rheological properties and morphology analysis of all the ice cream mix samples were performed.

## 2. Materials and Methods

### 2.1. The Preparation of the Hydrolysates of Iota Carrageenan

How the hydrolysis of iota carrageenan was conducted was meticulously described in the paper by Kot et al. [17]. In the mentioned research, acid and enzymatic hydrolysates of iota carrageenan were obtained. To perform acid hydrolysis, 0.1 M hydrochloric acid was used. While for enzymatic hydrolysis two sorts of enzymes were applied: $\beta$-galactosidase and its cheaper and commercial equivalent-lactase.

### 2.2. The Preparation of Ice Cream Mixes

### 2.2.1. The Materials for the Recipe for Ice Cream Mixes

The ingredients used to prepare the ice cream mixes were: $66.5 \%$ roasted almond original drink (Enerbio, Rossmann, Hanover, Germany), 16\% almond syrup (Monin, Bourges, France), $12 \%$ inulin (Orafti BENEO, Tienen, Belgium), $5 \%$ pea protein (Nuturalys S85 plus, Roquette, Lestrem, France), 0.4\% emulsifier E471 (Fooding Shanghai, Shanghai, China), $0.08 \%$ LBG-Locust Bean Gum (Fooding Shanghai, Shanghai, China), $0.02 \%$ xanthan gum (Fooding Shanghai, Shanghai, China), $0.01 \%$ iota carrageenan (Fluka, Sigma-Aldrich, St. Louis, MI, USA) or $0.005 \%$ newly obtained: the acid hydrolysates of iota carrageenan and enzymatic hydrolysis by $\beta$-galactosidase and enzymatic hydrolysis by commercial
lactase. The description of abbreviations of prepared ice cream mixes was characterised in Table 1.

Table 1. The description of abbreviations of samples.

| Sample | Description |
| :---: | :--- |
| $\mathbf{C}$ | The control sample |
| $\mathbf{C U}$ | The control sample after ultrasound homogenisation |
| $\mathbf{I}$ | The sample with stabilisers (the combination of iota carrageenan, LBG and xanthan <br> gum) |
| $\mathbf{I U}$ | The sample with stabilisers (the combination of iota carrageenan, LBG and xanthan <br> gum) after ultrasound homogenisation |
| $\mathbf{A}$ | The sample with stabilisers (the combination of acid hydrolysates of iota carrageenan, <br> LBG and xanthan gum) |
| AU | The sample with stabilisers (the combination of acid hydrolysates of iota carrageenan, <br> LBG and xanthan gum) after ultrasound homogenisation |
| B | The sample with stabilisers (the combination of enzymatic $\beta$-galactosidase hydrolysates <br> of iota carrageenan, LBG and xanthan gum) |
| BU | The sample with stabilisers (the combination of enzymatic $\beta$-galactosidase hydrolysates <br> of iota carrageenan, LBG and xanthan gum) after ultrasound homogenisation |
| L | The sample with stabilisers (the combination of enzymatic commercial lactase <br> hydrolysates of iota carrageenan, LBG and xanthan gum) |
| LU | The sample with stabilisers (the combination of enzymatic commercial lactase <br> hydrolysates of iota carrageenan, LBG and xanthan gum) after ultrasound <br> homogenisation |

### 2.2.2. The Production of Ice Cream Mixes

According to the recipe, dry and liquid ingredients were weighed separately. After it, all components were mixed using a Bosch MaxoMixx 750W blender (Bosch, Gerlingen, Germany). Then, the pasteurization process was performed by using a Vorwerk thermomixer used at a temperature of $85^{\circ} \mathrm{C}$ within 1.5 min and cooled to $25^{\circ} \mathrm{C}$.

### 2.2.3. Ultrasound Homogenisation

The next step in preparing ice cream mixes was to treat chosen samples by ultrasound by using a homogeniser Ultrasonic Liquid Processor VCX 500 (Sonics \& Materials, Inc., Newtown, CT, USA) with a diameter probe (Model CV334). 250 mL of ice cream mixes for each trial were used to homogenise. The frequency of 20 kHz and exposure time of 5 min was used. Then ice cream mixes were allowed to mature for 24 h at $4^{\circ} \mathrm{C}$.

### 2.3. The Ice Cream Mixes' Physical Analysis

### 2.3.1. Stability Analysis of Ice Cream Mixes

The stability of ice cream mixes was conducted by using Turbiscan Lab Expert (Formulation SA, Toulouse, France). To record the date of backscattered (BS) light during measurements, Turbisoft 2.0.0.33 software was used, which allowed comparing stability based on the Turbiscan Stability Index (TSI). Analysis of the stability of ice cream mixes was performed before and after maturation (for 24 h ; at $4^{\circ} \mathrm{C}$ ).

### 2.3.2. The Analysis of Particle Size Distribution

The Cilas 1190 (Cilas, Orléans, France)—a laser diffraction instrument was used to establish the particle size of the ice cream mixes. The emulsions of ice cream mixes were suspended in water at an obscuration of $10 \%$. The obtained results were expressed as the median diameter of $\mathrm{D}_{50}$ and as diagrams of particle size distribution. The analysis was performed before and after maturation (for 24 h ; at $4^{\circ} \mathrm{C}$ ).

### 2.3.3. The Morphology of the Ice Cream Mix

The morphology of ice cream mixes was determined according to the conducted before and after maturation step (for 24 h ; at $4^{\circ} \mathrm{C}$ ). Firstly, samples of ice cream mixes were prepared using a small amount and then put on the slide using a spatula, covered with a slipped glass. Photos of the ice cream mixes were taken using the Olympus BX 43F microscope (Nikon, Shanghai, China) equipped with the Olympus CAM-SC 50 (Nikon, Tokyo, Japan).

### 2.3.4. The Rheological Analysis of the Ice Cream Mix

Experiments were carried out to measure the rheological properties of the ice cream mix before and after maturation, according to the methodology developed by Kot et al. [17]. Rheological tests were performed using a Haake Mars 40 rheometer (Thermo Scientific Inc., Karlsruhe, Germany) in rotational mode within a shear rate of $0-100 \mathrm{~s}^{-1}$. All experiments were performed in triplicate to verify repeatability. The Herschel-Buckley model, Ostwald de Waele model and Bingham model were used to predict the flow properties of ice cream samples. A comparison of the correlation coefficient (R) and chi-square ( $\chi 2$ ) of the analysed models showed that the Herschel-Buckley model (1) was adequate to describe the rheological properties of all ice cream samples.

$$
\begin{equation*}
\eta_{a p p}=\tau_{0}(\dot{\gamma})^{-1}+K \dot{\gamma}^{n-1} \tag{1}
\end{equation*}
$$

where: $\eta_{\text {app }}$-the apparent viscosity (Pa s), $\dot{\gamma}$-the shear rate $\left(\mathrm{s}^{-1}\right), \tau_{0}$ —the yield stress ( Pa ), K—the consistency index ( $\mathrm{Pa} \mathrm{s}^{\mathrm{n}}$ ), and n -flow behaviour index (dimensionless).

### 2.4. Statistical Analysis

For the particle size of distribution and rheological properties, a statistical analysis was performed. The STATISTICA 13.3 software (Statsoft Polska, Kraków, Poland) was used to perform the analysis of variance (ANOVA). The significance of the test is set at $\alpha=0.05$. The presented data are expressed as a mean with standard deviations ( $\pm \mathrm{SD}$ ) and also the differences between groups were evaluated using the Tukey HSD test.

## 3. Results

### 3.1. The Analysis of the Stability of Ice Cream Mixes

The results of the stability of ice cream mixes were determined as values of the TSI factor (Turbiscan Stability Index) in Figure 1 and as Back Scattering (Figure 2a,b). According to the obtained results, it was noted that the ultrasound treatment had a vital influence on the stability of ice cream mixes. The TSI value had a range from 1.7 to 4.2.


Figure 1. The turbiscan stability index (TSI) of ice cream mixes.


Figure 2. The variation of the backscatter at maturation time at $4{ }^{\circ} \mathrm{C}$ (a) sedimentation; (b) coalescence).
In a group of samples without ultrasound treatment, the lowest result of the TSI value (1.7) was observed in the sample with the addition of enzymatic (lactase treatment) hydrolysates of iota carrageenan (L). Additionally, samples with the addition of acid hydrolysates of iota carrageenan (A) and iota carrageenan (I), as a stabiliser, had a lower value of the TSI factor, close to the control sample (C), around 2 (Table 1 and Figure 1). Only samples with the addition of enzymatic ( $\beta$-galactosidase treatment) hydrolysates of iota carrageenan (B) achieved a value of 3.6.

In the case of a group of ice cream mixes with ultrasound treatment, the same tendency was visible. The lowest TSI value (2.2) was reported for sample (LU) with the addition of enzymatic (lactase treatment) hydrolysates. In addition, diminished stability in the ice cream mixes with the addition of enzymatic ( $\beta$-galactosidase treatment) hydrolysates of iota carrageenan (BU) was at 4.2 (Table 1 and Figure 1). Overall, the samples without ultrasound treatment achieved the lowest TSI value, which contributed to the highest stability of ice cream mixes. Based on that, it may be concluded that the effect of ultrasound cavitation declined the stability of ice cream mixes.

When it comes to the results of Back Scattering (BS\%), two different phenomena of destabilisation occurred: coalescence and sedimentation (Figure 2a,b). Based on the presented results, it was observed that the addition of stabilisers contributed to the observed effects on the grounds that coalescence occurred in the samples with the addition of a stabiliser regardless of ultrasound treatment. Sedimentation was visible in the control sample without stabilisers also independently regardless of ultrasound treatment.

### 3.2. Analysis of Particle Sizes of Ice Cream Mixes

An analysis of particle size of ice cream mixes was presented as the mean diameter in Table 2 and as particle size distribution in Figure 3a,b before and after 24 h of maturation at $4^{\circ} \mathrm{C}$.

Table 2. The value of median $D_{50}$ of ice cream mixes before and after maturation (for 24 h ; at $4{ }^{\circ} \mathrm{C}$ ).

| Sample | $\mathrm{D}_{50}$ before Maturation | $\mathrm{D}_{50}$ after Maturation |
| :---: | :---: | :---: |
| C | $15.65 \pm 0.62^{\text {a }}$ | $17.23 \pm 0.49^{c}$ |
| CU | $14.68 \pm 0.17{ }^{\text {a }}$ | $9.97 \pm 0.23{ }^{\text {a }}$ |
| I | $30.40 \pm 0,44{ }^{\text {e }}$ | $28.50 \pm 0.43{ }^{\text {e }}$ |
| IU | $28.70 \pm 0.16^{\text {d }}$ | $23.73 \pm 0.62^{\text {d }}$ |
| A | $38.36 \pm 0.49^{\text {c }}$ | $11.41 \pm 0.97{ }^{\text {ab }}$ |
| AU | $35.70 \pm 0.50{ }^{\text {b }}$ | $11.24 \pm 1.41^{\text {a }}$ |
| B | $42.15 \pm 0.19 \mathrm{~g}$ | $11.29 \pm 0.53{ }^{\text {ab }}$ |
| BU | $34.29 \pm 0.14{ }^{\text {f }}$ | $9.76 \pm 0.36^{\text {a }}$ |
| L | $38.31 \pm 0.66^{\text {c }}$ | $13.20 \pm 0.45{ }^{\text {b }}$ |
| LU | $36.92 \pm 0.56{ }^{\text {b }}$ | $10.59 \pm 0.09^{\text {a }}$ |

The different superscript letters in the table represent significant differences in the means of the same parameter ( $p<0.05$ ). Values represent means $\pm$ standard deviations.

Before the maturation process (directly after preparing the ice cream mixes), the mean diameter of $D_{50}$ ranged from 14.68 to $42.15 \mu \mathrm{~m}$ (Tables 1 and 2). The lowest value was noted for the control sample, CU, with the ultrasound treatment. For all ice cream mixes with the addition of stabilisers, the mean diameter was significantly higher (from 28.70 to $42.15 \mu \mathrm{~m}$ ) than the previously mentioned CU sample. Nonetheless, it was observed that the ultrasound treatment reduced the size of particles in all samples no matter the addition of stabilisers. For instance, sample B (with enzymatic ( $\beta$-galactosidase) hydrolysates of iota carrageenan)) without ultrasound homogenisation, had a $\mathrm{D}_{50}$ at $42.15 \mu \mathrm{~m}$ (it was the highest result), while the same sample but with ultrasound treatment achieved a level of $34.29 \mu \mathrm{~m}$. According to that, the decrease in the size of the particle by acoustic cavitation was beneficial. The statistical analysis resulted in significant differences between samples before maturation time, so based on that it may be said that the ultrasound and use of stabilisers influenced the particle size of ice cream mix particles (Tables 1 and 2).

Taking into consideration the maturation time of ice cream mixes, which is crucial in ice cream production, the same analysis was conducted after 24 h . A noticeable reduction in $D_{50}$ was noted in comparison to the results before maturation. The range of particle size was from 9.76 to $28.50 \mu \mathrm{~m}$. Additionally, the same tendency of lower value, as before maturation, was visible for samples with ultrasound treatment. The samples with the addition of hydrolysates of iota carrageenan with ultrasound treatment had the lowest value of $\mathrm{D}_{50}$ (Tables 1 and 2). For instance, the sample BU (with the enzymatic ( $\beta$-galactosidase) hydrolysates of iota carrageenan)) achieved $9.76 \mu \mathrm{~m}$, sample LU (with enzymatic (lactase) hydrolysates of iota carrageenan)) at $10.59 \mu \mathrm{~m}$ and AU (with acid hydrolysates of iota carrageenan) at $11.24 \mu \mathrm{~m}$ (Tables 1 and 2). Moreover, according to statistical appraisal, the three mentioned samples with the control sample, CU , are in the same homologous group. It may be concluded that ultrasound treatment was a more crucial contribution to reducing the size of particles than the use of stabilisers. Only the sample with the addition of iota carrageenan with and without ultrasound treatment (I and IU) had the highest value of particle size at $28.5 \mu \mathrm{~m}$ and $23.73 \mu \mathrm{~m}$.



Figure 3. The distribution of particle size: (a) before the maturation of ice cream mixes; (b) after the maturation of ice cream mixes (for 24 h ; at $4^{\circ} \mathrm{C}$ ).

Considering the size of the particles, the particle size distribution of the ice cream mix before and after maturation was analysed (Figure 3a,b). Before maturation, the trimodal or fourfold particle size distribution were noted (Figure 3a, Table 2).

Additionally, looking at the range of peaks in the figures, samples with the addition of stabilisers (regardless of ultrasound treatment) and control samples ( $C$ and $C U$ ) were approximately the same. Only samples I and IU (with the addition of iota carrageenan) had distinctive distributions of peaks in the figure. After 24 h of maturation, in all ice cream mixes, the four-fold particle size distribution was observed based on four characteristic peaks in the figure (Figure 3b, Table 1). Only sample I (with the iota carrageenan) did not change the sort of distribution of particles. Moreover, the distribution of characteristic peaks in the figure was the same for samples with the addition of hydrolysates of iota carrageenan. For the control samples ( C and CU ) and for the sample with the addition of
iota carrageenan (I and IU) the range of peaks was different in comparison to the previously mentioned samples.

### 3.3. Analysis of Rheological Properties of Ice Cream Mixes

The relation between shear rate and apparent viscosity can be described by different rheological models (Ostwald de Waele, Bingham, Power Law, Herschel-Buckley). Data obtained from the ice cream mixes (before and after maturation) showed that the lowest values of the chi-square (from 15 to 22,143 ) and the highest values of the correlation coefficient (from 0.9981 to 1) were observed for the Herschel-Buckley model. For this reason, this model was selected to characterise the flow behaviour of ice cream mix samples.

The increase in consistency K was observed for ice cream samples with the addition of stabilisers in comparison to control samples (Figure 4a). However, the consistency values of samples prepared with iota carrageenan (I, IU) were four- to seven-fold higher than those observed for ice cream mixes obtained with the addition of other stabilisers. The effect of ultrasound treatment on the consistency index was only significant for ice cream mixes with the enzymatic $\beta$-galactosidase hydrolysates of iota carrageenan ( $\mathrm{B}, \mathrm{BU}$ ) and iota carrageenan (I, IU). The consistency index of these samples decreased after ultrasound treatment. The consistency and viscosity of ice cream mixes with iota carrageenan without ultrasound treatment (I) increased after 24 h of maturation. A similar effect was observed for the ice mix, BU, after maturation. Additionally, the high-value standard deviation and coefficient of variation ( $15 \%$ ) of the K parameter may indicate that the sample with iota carrageenan after maturation was less homogenous than other mixes, which was also visible on the images (Figure 4-IU b). The consistency index did not differ for other ice cream mixes after maturation.


Figure 4. The parameters of the Herschel-Buckley model of the ice cream mix before and after maturation (for 24 h ; at $4^{\circ} \mathrm{C}$ ): (a) the consistency index, (b) yield stress, (c) the flow behaviour index. The different superscript letters represent significant differences in the means of the same parameter ( $p<0.05$ ).

### 3.4. The Microstructure of Ice Cream Mixes

To support previous analysis and results, microscopic photos were also taken. The microscopic analysis is presented in Figure 5 with regard to the maturation process.


Figure 5. Cont.


Figure 5. Cont.


Figure 5. The microscopy analysis of ice cream mixes before (a) and after maturation (b) (for 24 h ; at $4^{\circ} \mathrm{C}$ ).

Overall, the ice cream mixes were described by an irregular distribution of fat particles and the agglomeration of particles were visible; however, the images prove that both controls with and without ultrasound treatment look different from samples with the addition of the stabiliser. As already pointed out, those samples also present the highest stability with behaviour similar to Newtonian fluids. Taking into consideration the ultrasound treatment in ice cream mixes, a reduction in particles and different orders in the structure were observed.

For instance, in the control sample before the maturation with and without ultrasound homogenisation, the changes were visible. In the sample before maturation creating the double emulsion was noticed. Before the maturation, the particles were surrounded by bigger agglomerates (Figure 5). After the maturation, the number of agglomerates decreased in comparison to single smaller particles which also was confirmed by the TSI value (Figure 1). This is owing to the fact that the control sample characterised the lowest TSI value and the same better stability of the emulsion. Additionally, in the sample CU (Tables 1 and 2) after the maturation, the $\mathrm{D}_{50}$ was significantly smaller than before the maturation step.

Additionally, the addition of stabilisers affected the structure of ice cream mixes. After microscopy analysis, we can suppose that all undesirable changes occurred. Looking at samples before maturation: with iota carrageenan or with its hydrolysates in comparison to the control sample, no matter of ultrasound treatment, the distinctive conformation of particles was noticed. For samples, I and IU (Tables 1 and 2) before and after the maturation time, the size of particles did not change significantly, however, the sample IU stood out among more uniform structures than other samples. According to the Back Scattering (Figure 2a,b), the destabilisation of samples occurred which can be explained by agglomerates in all mentioned samples. In samples with the addition of hydrolysates of iota carrageenan, the reduction in particles was observed no matter the ultrasound treatment. The particles before the maturation were characterised by bigger size which was confirmed by median $\mathrm{D}_{50}$ (Table 2). After maturation, a significant size reduction was observed in the presented photos which also was proved by the $\mathrm{D}_{50}$ parameter. Moreover, the bigger
particles were observed based on the microscopic analysis after the maturation, but it can be the effect of destabilisation according to the Back Scattering (Figure 2a,b). We can also suppose based on the images that flocculation and aggregation occurred.

## 4. Discussion

### 4.1. Analysis of the Stability of Ice Cream Mixes

Based on the TSI value, it may be concluded that the effect of ultrasound cavitation declined the stability of ice cream mixes. In the research of Kot et al. [17], the homogenisation process also contributed to exacerbating the stability of vegan ice cream mixes. The TSI value for samples after homogenisation treatment ranged from 3.0 to 6.5 . According to the results in the presented paper, the TSI value for samples after the ultrasound homogenisation did not achieve more than 4.2. Based on that, it may be deduced that ultrasound homogenisation is a more beneficial method for the stability of ice cream mixes than traditional homogenisation owing to the fact that the air bubbles which occur during the homogenisation may be the reason for decreasing the stability of the emulsion in the ice cream mix. According to the research of O'Sullivan et al. [18], the ultrasound treatment contributed to enhancing the stability of the emulsion prepared based on the pea protein isolate. Additionally, an improvement in the stability of the emulsion contributed to the improvement in the interfacial layer after ultrasonic irradiation. The authors also concluded that not only did the ultrasound treatment influence this parameter, but it also affected the time of treatment, before or after emulsification. So, based on the mentioned examples, it may be inferred that the influence of ultrasound on stability depends on various factors. Moreover, in the event of ice cream mixes, which is the multiphase product, the choice of parameters in this method may be more difficult and also hard to control.

As was mentioned in the part of the results, two different phenomena of destabilisation have occurred: coalescence and sedimentation. Coalescence often occurs due to particle size increases, while sedimentation is explained as the migration of particles [19]. For instance, in the research by Voronin et al. [20] milk ice cream mixes, after high-pressure jet processing, were prone to creaming and rapid separation was visible. On the other hand, in the work by Aslan and Dogan [16], it was found that ultrasound treatment was resistant to the coalescence of the emulsion owing to the fact that the smaller droplet size, created by ultrasound, was associated with the creaming index. The emulsion was also more stable when the creaming index was low.

### 4.2. An Analysis of Particle Sizes of Ice Cream Mixes

In the result part, it was highlighted that the size of particles was decreased. Additionally, in the study by O'Sullivan et al. [21], a pivotal reduction in particle size in the emulsion, based on the pea protein isolate with the ultrasound treatment, was observed. This phenomenon was attributed to the disruption of non-covalent associative forces, such as electrostatic and hydrophobic interactions and hydrogen bonding. Such mentioned forces maintained aggregates of protein in the solution through high levels of hydrodynamic shear and turbulences during ultrasonic cavitations. On the other hand, in comparison to the research of Kot et al. [17], where traditional homogenisation was used in vegan ice cream mixes, the reduction in the particle size, at the lowest value of $\mathrm{D}_{50}$ after maturation, was $6.33 \mu \mathrm{~m}$. In the presented study, the lowest particle size for samples after the ultrasound treatment was $9.76 \mu \mathrm{~m}$. Furthermore, in the study by Sert and Mercan [22], a different sort of homogenisation was used-high-pressure homogenisation, for preparing the sheep milk ice cream mix. The higher the pressure of homogenisation the lower and more satisfying the particle size achieved. The lowest $D_{50}$ value was at a level of $6.49 \mu \mathrm{~m}$. Based on the mentioned studies, ultrasound homogenisation did not reduce the particle size significantly, like other sorts of homogenisation. However, the sizes of the particles in the ice cream mix do not play a crucial role in forming a crystal structure. The uniform structure of the prepared emulsion is considered more important [14]. Nonetheless, it must not be forgotten that ultrasound is much more economical than conventional high-pressure
homogenisation or other mixing methods. Ultrasound is believed to be energy-saving and at the same time, offers more flexibility in its implementation [23].

The changes in the size and distribution of particles, in this step of preparing ice cream, may contribute to creating a more or less desirable structure of ice crystals in the final product. In comparison to other research, for instance, in the research conducted by Warren and Hartel [24], a bimodal and a trimodal distribution of particle size were observed in the ice-cream mix. Such distribution may have reflected increased partial coalescence of fat droplets in ice cream mixes. In our research, the phenomena of coalescence also were noted, which may be connected with increasing diversity in particle distribution among ice cream mix samples. Moreover, in the study by Huppertz et al. [25], it was noted that the ice cream mix had a comparable particle size distribution, after high-pressure homogenisation. In the case of our study, ultrasound homogenisation contributed to more diversity in ice cream samples.

### 4.3. An Analysis of Rheological Properties of Ice Cream Mixes

The Hershey-Buckley model, which was used to describe the rheological properties in ice cream mixes, was used to describe the ice cream mixes obtained with different methods of homogenisation [17,26]. According to the obtained results, the higher values of yield stress may indicate the presence of firmer structures [27]. The addition of stabilisers to mixes caused a significant increase in yield stress.

The flow behaviour index characterises the type of fluids. The samples with an n value close to 1 show behaviour similar to Newtonian fluids. The smaller values of n (lower than 1) indicate a departure from Newtonian behaviour [28]. All ice cream mixes, with the addition of stabilisers, showed non-Newtonian shear-thinning (pseudoplastic) behaviour. After maturation, the low behaviour values index did not vary for the same type of ice cream mix with the exception of the BU sample. Moreover, the effect of ultrasound treatment on the n index was not significant for most samples. The differences between the two variants of control and the stabilized samples are also visible in the images (Figure 4).

### 4.4. The Microstructure of Ice Cream Mixes

Microscopic analysis was immensely pivotal to seeing changes in fat globules and the sort of destabilisation. Voronin et al. [20], proved that the destabilisation of fat aggregates was visible in the milk ice cream mixes. Overall, it may be concluded that maturation as a step in ice cream production displays a considerably pivotal role in creating the structure of ice cream mixes. Moreover, the reduction in the size of the particles was achieved, which may also have influenced conformation after maturation. According to research by Ahn et al. [29], the observation of the microstructure of an emulsion can provide a significant clue to the understanding of the structure of the emulsion and its relationship with stability. In this research, it was noticed that the interactions with proteins and phospholipids can modulate the stability of the emulsion, which may later contribute to functionality, such as the digestibility of dairy-based emulsions. Furthermore, according to the previous studies [30,31], the way of preparation but also the composition (including the chosen: stabilisers iota carrageenan and its hydrolysates) had concurrently a great impact on forming ice crystals structure of ice cream.

## 5. Conclusions

In the presented study, ultrasound homogenisation contributed to a decrease in the stability of ice cream mixes. The samples after the ultrasound treatment had a higher value of TSI. The elevated value of TSI was noted for sample IU (with the addition of iota carrageenan) and BU (hydrolysate of iota carrageenan) and was at a level of 4 . The addition of stabilisers in ice cream mixes improved stability during maturation. Moreover, the ultrasound treatment in ice cream mixes reduced the particle size; however, it was visible after maturation. Additionally, the combination of ultrasound and the use of stabilisers gave better results in reducing the size of the particle, than only using stabilisers and
the smallest particles were noticed in the BU sample-9.76 $\mu \mathrm{m}$. The effect of ultrasound treatment on the consistency index was only significant for ice cream mixes IU and BU and the consistency index of these samples decreased after ultrasound treatment. Based on the microscopic photos, the agglomeration of fats and considerable diversification was visible in all ice cream mixes. While analysing the microstructure, the effect of ultrasound treatment was not noticeable for most samples and only in the IU sample, after maturation, were the differences visible.

Due to the specific mechanism of ultrasound homogenisation, it may be contemplated as an interesting tool for ice cream applications. This method can be effectively used for reducing the size of particles. Moreover, it may be used in improving the stability of the emulsion in comparison to the traditional form of homogenisation. This method has the potential in enhancing the quality and performance of vegan ice cream mixes. However, although a plethora of advantages to using this method in production are acknowledged, wide-ranging research is still required to further industrial applicability.

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