Experimental Design and Optimization of Ultrasound Treatment: Functional and Physical Properties of Sonicated Ice Cream Model Mixtures

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Abstract

The aim of this study was to determine the effect of high power ultrasound on functional properties of ice-cream model mixtures. Mixture composed of sucrose, glucose, whole milk powder, whey protein concentrates (WPC) and distilled water was ultrasonically treated according to different parameters. Amplitude of ultrasounds, percentage of WPC in the sample and time of treatment are the three variables considered. Effect of ultrasound parameters on rheological properties (measurement of coefficient of consistency), thermal properties (measurement of initial freezing point) and foaming properties (measurement of maximal foam capacity) was observed. Experiment was designed using model called Central Composite Design (CCD) permitting to consider the significant factors for each property, and results were analyzed and process was optimized through response surface methodology-RSM. Through study, optimal conditions of ultrasound treatment (amplitude, treatment time and percentage of WPC) by which experiment should be performed were obtained. The factor “percentage of WPC” is significant from a rheological and thermal point of view. Regarding foaming properties, the significant factor that is to say affecting most the value of the maximum foam capacity is the duration of ultrasound treatment.

Keywords: High power ultrasound; Ice-cream model mixtures; Response surface methodology; Physical properties; Optimization

Introduction

Sonication technology can improve the process through reduced processing time, higher throughput and lower energy consumption [1-4]. Ultrasound technology is based on mechanical waves at a frequency above the threshold of human hearing (>16 kHz). These waves travel either through the bulk of a material or on its surface at a speed which is characteristic of the nature of the wave and the material through which it is propagating [1,5]. Ultrasound can be divided into different frequency fields: high frequency low energy diagnostic ultrasound in the MHz range and low frequency high-energy power ultrasound in kHz range [6]. Ultrasound can be used in conjunction with pressure treatment (manosonication), heat treatment (thermosonication) or both (manothermosonication) [6].

Usually, the high frequency ultrasounds are used as an analytical technique for quality assurance, process control or non destructive inspection. For example, high frequency ultrasounds is useful to control characteristic values like flow rate or to determine food properties [6]. For low frequency high-energy power ultrasounds, application in food industry is relatively new. The main use of this kind of ultrasound treatment is made in the field of extraction where ultrasounds permit to improve the yield and to decrease the necessary time. Innovative researches appeared since past years to propose new applications of ultrasounds in food industry [1,7-10]. Ultrasound effects on liquid systems are mainly related to the cavitation. This phenomenon is that acoustic cavitation also occurs and acts as nuclei for crystal growth or by the disruption of nuclei already present. In freezing, this phenomenon is thought to enhance both the nucleation rate and rate of crystal growth in a saturated or super cooled medium by producing a large number of nucleation sites in the medium throughout the ultrasonic exposure. Acoustic cavitation also occurs and acts as nuclei for crystal growth or by the disruption of nuclei already present. In freezing, this phenomenon would lead to fine ice crystals and shortening of the time between the onset of crystallization and the complete formation of ice, thus reducing damage to cellular structure. Ultrasound could be useful tool to obtain better physical and functional properties of treated material but caution should be taken care if oils of fats are present in the system. Chemat et al. [13] reported an increase in deterioration of the barrel freezer, malleability, and shape retention during meltdown, and smooth-eating texture [11]. The basic steps in the manufacturing of ice cream are generally as follows: blending, pasteurization, homogenization, aging the mix, freezing, packaging and hardening [12]. The temperature is a very important parameter to consider for the production of ice-cream. Cooling and/or freezing as a means of preservation of food has been used for hundreds of years through the use of natural ice or overwinter storage. One very important area related to freezing in the food industry is the formation of ice crystals during the freezing of water present in the food material. Sonication is thought to enhance both the nucleation rate and rate of crystal growth in a saturated or super cooled medium by producing a large number of nucleation sites in the medium throughout the ultrasonic exposure.

Ice cream is an aerated water and suspension of crystallized fat in highly concentrated sugar solution containing hydrocolloids, casein micelles and proteins. The importance of the fat structure and colloidal aspects of ice cream are widely recognized today, as fat structure is the underlying explanation for dryness of ice cream at extrusion from the barrel freezer, malleability, and shape retention during meltdown, and smooth-eating texture [11]. The basic steps in the manufacturing of ice cream are generally as follows: blending, pasteurization, homogenization, aging the mix, freezing, packaging and hardening [12]. The temperature is a very important parameter to consider for the production of ice-cream. Cooling and/or freezing as a means of preservation of food has been used for hundreds of years through the use of natural ice or overwinter storage. One very important area related to freezing in the food industry is the formation of ice crystals during the freezing of water present in the food material. Sonication is thought to enhance both the nucleation rate and rate of crystal growth in a saturated or super cooled medium by producing a large number of nucleation sites in the medium throughout the ultrasonic exposure. Acoustic cavitation also occurs and acts as nuclei for crystal growth or by the disruption of nuclei already present. In freezing, this phenomenon would lead to fine ice crystals and shortening of the time between the onset of crystallization and the complete formation of ice, thus reducing damage to cellular structure. Ultrasound could be useful tool to obtain better physical and functional properties of treated material but caution should be taken care if oils of fats are present in the system. Chemat et al. [13] reported an increase in deterioration of...
sonicated sunflower oil samples processed for 2 min (20 KHz, 150 W). The ultrasound assisted lipid oxidation is attributed to the cavitation phenomenon, which affected structural and functional components leading to lipid oxidation. The collapse of cavitation bubbles in the emulsions result in formation of oxygen accumulated hot spots having temperatures above 5000°C and pressure of 500 MPa which caused lipid oxidation by mechanisms like the thermal effect, due to free radicals generated during sonication and mechanical forces created by micro-streaming and shock waves.

Furthermore intense mechanical stress and agitation owing to shear and turbulence during sonication lead to oxygen inclusion and distribution within the material which will increase the rate of lipid oxidation. Furthermore it has shown that oxygen availability in the system is related to an increase in lipid oxidation during the time.

The objective of this study was to investigate the effect of high power ultrasound and ice-cream model mixtures on functional and physical properties. Effect of ultrasound processing parameters (amplitude, treatment time and percentage of WPC) on rheological properties (measurement of coefficient of consistency), thermal properties (measurement of initial freezing point) and foaming properties (measurement of maximal foam capacity) was observed.

Materials and Methods

Samples

Samples were prepared according to three different recipes resumed in Table 1. There was 3 different mixtures varied according percentage (%) of whey protein concentrate (WPC), and denoted A (10% WPC), B (12% WPC) and C (14% WPC). Model mixture, composed by sucrose, glucose, whole milk powder, whey protein concentrate (WPC) and distilled water, was ultrasonically treated according to different parameters. Experiment has been designed by Central Composite Design (CCD). Untreated samples were denoted as A, B and C, and ultrasound treated one as A1-A16. Composition of Whey Protein concentrate-WPC (“Meggle” GmbH, Wasserburg, Germany) is: protein: 60%; water: 3.0%; ashes: 6.0%; lactose: 25.0%, fat: 6.0%; Whole Milk Powder - WMP (“DUKAT”, Zagreb, Croatia) is: protein: 26.3%; fat: 26.1%; lactose: 39.8%; ashes: 3.0%; water: 4.8%; Sucrose (Viro Tvornica Secera, Virovitica, Croatia) is: protein: 60%; water: 3.0%, ashes: 6.0%, lactose: 25.0%, fat: 6.0%; Whole Milk Powder - WMP (“DUKAT”, Zagreb, Croatia).

Table 1: Composition of the different samples.

| Samples % of WPC | Amplitude (%) | Time (min) | Power (W) | Energy (J) | T1 (°C) | T2 (°C) |
|-----------------|---------------|------------|-----------|------------|---------|---------|
| A1              | 14            | 100        | 3         | 100        | 19.145  | 19      | 34      |
| A2              | 12            | 75         | 6         | 82         | 30.954  | 19      | 43      |
| A3              | 10            | 100        | 3         | 103        | 18.884  | 20      | 34      |
| A4              | 12            | 75         | 6         | 79         | 29.467  | 20      | 42      |
| A5              | 10            | 50         | 3         | 62         | 11.041  | 20      | 28      |
| A6              | 14            | 50         | 9         | 60         | 34.423  | 20      | 45      |
| A7              | 14            | 100        | 9         | 99         | 60.125  | 19      | 61      |
| A8              | 12            | 50         | 6         | 62         | 23.048  | 20      | 37      |
| A9              | 12            | 75         | 3         | 83         | 15.101  | 18      | 30      |
| A10             | 14            | 75         | 6         | 78         | 28.627  | 19      | 41      |
| A11             | 10            | 50         | 9         | 57         | 32.674  | 19      | 42      |
| A12             | 10            | 75         | 6         | 78         | 29.168  | 19      | 40      |
| A13             | 12            | 100        | 6         | 102        | 37.825  | 20      | 48      |
| A14             | 14            | 75         | 9         | 76         | 42.952  | 20      | 50      |
| A15             | 10            | 100        | 9         | 92         | 54.358  | 18      | 56      |
| A16             | 14            | 50         | 3         | 63         | 11.436  | 19      | 28      |

Table 2: Treatments, values of three parameters: amplitude, time and percentage of WPC; power, energy input and temperature before (T1) and after treatment (T2).

In order to optimize the ultrasound treatment and investigate effects of above variables on rheological properties, thermal properties and foaming properties, central-composite face centered design with the variables at three levels was used in the experiment (Table 2). Design matrix for the experiment and the regression model proposed for response was given below [14]:

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j \]

where \( \beta_0 \) is the value of the fixed response at the central point of the experiment which is the point (0, 0, 0); \( \beta_i \), \( \beta_{ij} \) and \( \beta_{ij} \) are the linear, quadratic and cross-products coefficients, respectively. While demonstrating the significant effects 3-dimensional fitted surfaces were drawn [15-16]. The model was fitted by multiple linear regressions (MLR). Experimental results were analyzed by response surface methodology (RSM). Calculations were done at 95% of confidence level. Analysis of variance (ANOVA) was carried out to determine any significant differences (p<0.05) among the applied treatments.

Ultrasound treatments

Three parameters were varied according STATGRAPHICS computer software, namely, amplitude of ultrasound (50, 75 and 100%), percentage of WPC (10%, 12% and 14%) in the sample and time of treatment (3, 6 or 9 minutes) (Table 2). After sample homogenization, samples were treated by ultrasounds 20 kHz probe: S-4000 (Misonix Sonicators, Newtown, Connecticut, SAD) and samples were denoted as A1-A16. The probe with a titanium tip of 12.7 mm in diameter was...
introduced in the sample (300 mL) so that the same part of probe was immersed in the liquid (about 2 cm) and the tip was at the “center” of the mix.

**Determination of acoustic power**

The most widely accepted method for determining the power from an acoustic horn into an aqueous solution is the calorimetric technique described by Margulis and Maltsev [17]. This method involves taking a known volume of sample and applying ultrasound (for ca.3 min) while monitoring the change in temperature with time for various ultrasonic amplitudes. The ultrasonic power can be readily determined from the following equation:

\[ P = m \cdot C_p \cdot \frac{dT}{dt} \]

where \( P \) is the ultrasonic power (W), \( m \) is the mass of the sample (kg), \( C_p \) is the specific heat capacity and \( dT/dt \) is change in temperature during sonication of sample in time.

**Foaming properties of ice-creams model mixtures**

For determination of foaming properties samples were prepared as described in section 2.1 and then ultrasonically treated as described in section 2.3. After ultrasound treatment, suspensions were whipped at room temperature with blender (TIP 3228, Gorenje, Slovenia) equipped with a wire whip beater at maximum speed setting for up to 15 min to determine maximum foam expansion. Whipping was interrupted every 5 minutes during the run in order to determine foam expansion. Foam expansion was determined by level-filling a 100 mL plastic weighing boat with foam and then weighed. Foam expansion was calculated using the expression:

\[ \text{Foam expansion} = \frac{\text{Unwhipped suspension wt (g) - foam wt (g)}}{\text{Unwhipped suspension wt (g)}} \times 100 \]  

Foam stability was determined by transferring 100 mL of maximum expansion foam into a pyrex filter funnel with dimensions of 7.5 cm inner top diameter, 0.4 cm inner stem diameter and 7.0 cm stem length. A small plug of glass wool was placed in the top of the funnel stem to retain the foam but allow drainage of the liquid. The time required (min) for drainage of the entire foam was determined for index of foam stability [18].

**Determination of rheological properties of ice-creams model mixtures**

Torque measurements were carried out on the 10% (w/w) model dispersions using a Rheometric Viscometer (Model RM 180, Rheometric Scientific, Inc., Piscataway, USA) with the spindle (no. 3; Ø=14 mm; l=21 cm). Shear stress against the increasing shear rates from lowest value of 0s⁻¹ to 1290s⁻¹ as well as downwards was applied. Volume of the beaker was 36 mL. The samples were kept in a thermostatically controlled water bath for about 15 minutes before measurements in order to attain desirable temperature of 25°C. Measurements were performed continuously in the temperature interval from -30°C to 0°C, with intervals of measurements of 0.01°C. The apparatus had high frequency of sampling (10 measurements per second). Distilled water was used as calibration substance for the static correction (0.88°C) of the initial freezing point. As results, initial freezing temperature and initial thawing temperature for each sample were obtained.

**Results and Discussion**

The influence of ultrasound parameters (amplitude and treatment time) and percentage of WPC on rheological, thermophysical (DTA) and foaming properties of ice cream model mixtures have been analyzed. Different results were interpreted according to Central Composite Design (CCD) and response surface methodology (RSM) permitting to consider the significant factors for each property.

Actual power of ultrasound in the study was from 57-103 W cm⁻² (Table 1).

In order to test the model signification and suitability, analysis of variance (ANOVA) was carried out. In Table 3 phase transition temperatures (initial freezing and melting temperatures) for ice-cream model mixtures are shown. There is obvious significant influence (p<0.05) of percentage of whey protein concentrate in ice-cream model mixtures, on initial freezing and melting temperatures. There is statistically significant influence (p<0.05) in decreasing of initial freezing temperatures for ice-cream model mixtures containing 14

**Table 3:** Initial and final freezing and melting temperatures of ice-cream model mixtures (untreated and treated).

| Sample | Initial freezing temperature (°C) | Top of freezing curve (°C) | Initial melting temperature (°C) | Top of melting curve (°C) |
|--------|----------------------------------|---------------------------|----------------------------------|--------------------------|
| A      | -2.7                             | -8.5                      | -7.9                             | -1.4                     |
| B      | -2.7                             | -8.0                      | -7.0                             | -2.4                     |
| C      | -1.6                             | -7.7                      | -6.8                             | -0.8                     |
| A1     | -1.4                             | -7.3                      | -7.1                             | -1.3                     |
| A2     | -1.7                             | -8.0                      | -7.1                             | -1.3                     |
| A3     | -2.5                             | -7.7                      | -6.8                             | -1.4                     |
| A4     | -2.7                             | -7.9                      | -6.1                             | -1.7                     |
| A5     | -2.3                             | -7.0                      | -6.7                             | -1.8                     |
| A6     | -2.0                             | -7.5                      | -6.7                             | -1.9                     |
| A7     | -1.7                             | -8.1                      | -7.3                             | -1.2                     |
| A8     | -2.6                             | -8.1                      | -6.5                             | -1.6                     |
| A9     | -1.6                             | -8.0                      | -7.4                             | -1.2                     |
| A10    | -1.6                             | -7.6                      | -6.6                             | -0.8                     |
| A11    | -2.6                             | -7.0                      | -6.2                             | -1.7                     |
| A12    | -2.4                             | -7.4                      | -6.4                             | -1.8                     |
| A13    | -2.0                             | -8.1                      | -7.2                             | -1.7                     |
| A14    | -1.9                             | -7.8                      | -7.5                             | -1.5                     |
| A15    | -1.9                             | -7.5                      | -6.7                             | -1.7                     |
| A16    | -2.1                             | -8.0                      | -6.7                             | -1.6                     |
% of WPC. Amplitude of ultrasound and treatment time have not shown significant influence (p>0.05) in changes of initial freezing temperatures (Table 4). Ultrasound can promote ice nucleation due to cavitations and enhance heat and mass transfer due to microstreaming agitation [7]. This might be due to the accumulated thermal effect which is proportional to acoustic duration.

Data for foaming properties are given in Table 4. For untreated samples, for increase in percentage of WPC in ice-cream model mixture there is increase in foam capacity (%) for 14% WPC samples. There is increase in foam capacity (%) for prolonged mixing time (5 to 15 min). Also, increase in foam stability index (sec) and maximum foam stability (min) for higher WPC percentage (14%). After ultrasound treatment of ice-cream model mixtures, there is significant reduction in foam capacity (%) for all treatments. There are interesting data where no foaming properties could be observed for samples A1, A5, A8, A9 and A16 after ultrasound treatment. From Table 5 there is statistically significant (p<0.05) influence of treatment time on foaming capacity. Whey protein concentrates exhibited different foaming properties due to the presence of carbohydrates and fat in its composition. Foaming values for both foam capacity and stability were generally lower because of WPC composition. Lactose and fat amount reduced the ability of whey proteins to propagate at air-water interface. Foam stabilities are reduced for all treatments and all model systems after ultrasound treatment of ice-cream model mixtures, there is significant reduction in foam capacity (%) for prolonged mixing time (5 to 15 min). Also, increase in foam stability index (sec) and maximum foam stability (min) for 14% WPC samples. There is increase in foam capacity (%) for 14% WPC samples, for increase in percentage of WPC in ice-cream model mixture which is proportional to acoustic duration. This might be due to the accumulated thermal effect due to ultrasound treatment [7]. This might be due to the accumulated thermal effect due to ultrasound treatment [7].

### Table 4: Analysis of variance (ANOVA) for initial freezing temperature.

| Source | Sum of Squares | DF | Mean Square | F-Ratio | P-Value |
|--------|----------------|----|-------------|---------|---------|
| A: WPC | 0.841          | 1  | 0.841       | 5.93    | 0.0508  |
| B: Amplitude | 0.441        | 1  | 0.441       | 3.11    | 0.1283  |
| C: time | 0.004          | 1  | 0.004       | 0.03    | 0.8721  |
| AA     | 0.00226489     | 1  | 0.00226489  | 0.02    | 0.9036  |
| AB     | 0.03125        | 1  | 0.03125     | 0.22    | 0.6554  |
| AC     | 0.03125        | 1  | 0.03125     | 0.22    | 0.6554  |
| BB     | 0.193174       | 1  | 0.193174    | 1.36    | 0.2876  |
| BC     | 0.03125        | 1  | 0.03125     | 0.22    | 0.6554  |
| CC     | 0.205674       | 1  | 0.205674    | 1.45    | 0.2738  |
| Total  | 0.85094        | 6  | 0.141823    |         |         |
| Total (corr.) | 2.5375    | 15 |        |         |         |

R² = 66.4654 %. R² (adjusted for df) = 16.1636 %.

### Table 5: Analysis of variance (ANOVA) for foaming properties.

| Sample | Foam capacity (%) | Mixing time | Foam stability index (sec) | Maximum foam stability (min) |
|--------|-------------------|-------------|----------------------------|-----------------------------|
|        | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| A      | 98.01 | 100.02 | 103.2 | 75   | 63.51  |
| B      | 110.20| 115.20 | 118.6 | 78   | 68.62  |
| C      | 120.03| 121.4 | 128.3 | 98   | 70.35  |
| A1     |        |        |        |      |        |
| A2b    | 74.31 | 76.19  | 78.89  | 20   | 3.2    |
| A3*    | 78.01 | 78.58  | 81.07  | 14   | 2.7    |
| A5*    | 78.90 | 83.80  | 84.10  | 120  | 24     |
| A6     | 81.65 | 83.36  | 83.73  | 329  | 56.81  |
| A7     |        |        |        |      |        |
| A8*    |        |        |        |      |        |
| A9*    | 71.78 | 76.74  | 82.04  | 30   | 2.23   |
| A10    | 76.94 | 76.26  | 78.42  | 24   | 4.45   |
| A11    | 73.79 | 76.91  | 77.40  | 22   | 3.28   |
| A12    | 80.22 | 83.35  | 83.78  | 342  | 53.45  |
| A13    | 80.30 | 83.15  | 81.68  | 360  | 60.58  |
| A14    | 80.58 | 81.74  | 82.55  | 141  | 22.32  |
| A15    |        |        |        |      |        |
| A16*   |        |        |        |      |        |

Table 6: Foaming properties.

![Figure 1: Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 10% of WPC.](image)

Combined 3D and contour plots representing the linear and quadratic effects of the independent variables are shown in Figures 4, 5 and 6. The analysis of variance (ANOVA) showed that the resultant quadratic polynomial models adequately represented the experimental homogenization effect. Mechanical homogenization process tended to increase the foaming power. The homogenization effect of ultrasound usually disperses the protein and fat particles more evenly, which may improve the foaming stability [19]. This could later prevent collapse of foam and promote production of more stable foam. In Figures 1, 2 and 3 shear stress versus shear stress relationship is shown for samples containing 10, 12 or 14% of WPC. From calculations, and from flow behavior values it could be concluded that all systems (non-treated and ultrasound treated) had dilatant (n>1), non-newtonian behavior (data not shown). In Table 7 analysis of variance (ANOVA) showed that percentage of WPC had statistically significant (p<0.05) influence on rheological parameter (consistency coefficient).

| Sample | Foam capacity (%) | Mixing time | Foam stability index (sec) | Maximum foam stability (min) |
|--------|-------------------|-------------|----------------------------|-----------------------------|
|        | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| A      | 98.01 | 100.02 | 103.2 | 75   | 63.51  |
| B      | 110.20| 115.20 | 118.6 | 78   | 68.62  |
| C      | 120.03| 121.4 | 128.3 | 98   | 70.35  |
| A1     |        |        |        |      |        |
| A2b    | 74.31 | 76.19  | 78.89  | 20   | 3.2    |
| A3*    | 78.01 | 78.58  | 81.07  | 14   | 2.7    |
| A5*    | 78.90 | 83.80  | 84.10  | 120  | 24     |
| A6     | 81.65 | 83.36  | 83.73  | 329  | 56.81  |
| A7     |        |        |        |      |        |
| A8*    |        |        |        |      |        |
| A9*    | 71.78 | 76.74  | 82.04  | 30   | 2.23   |
| A10    | 76.94 | 76.26  | 78.42  | 24   | 4.45   |
| A11    | 73.79 | 76.91  | 77.40  | 22   | 3.28   |
| A12    | 80.22 | 83.35  | 83.78  | 342  | 53.45  |
| A13    | 80.30 | 83.15  | 81.68  | 360  | 60.58  |
| A14    | 80.58 | 81.74  | 82.55  | 141  | 22.32  |
| A15    |        |        |        |      |        |
| A16*   |        |        |        |      |        |
data. The significance of experimental factors which affect the treatment process may be quantified from the model coefficients, multiple determinations and probabilities that were generated from STATGRAPHICS software. The predicted response models were significant for all examined parameters (p<0.0001) and fitted well with the experimental data with low RMSE and high regression coefficients (R²). The predicted values were within the 95% prediction limit.

In Table 8 optimized values for each examined factor is given.

![Figure 2](image2.png) **Figure 2:** Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 12% of WPC.

![Figure 3](image3.png) **Figure 3:** Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 14% of WPC.

| Source         | Sum of Squares | DF | Mean Square | F-Ratio | P-Value |
|----------------|----------------|----|-------------|---------|---------|
| A : WPC        | 1.34282E-7     | 1  | 1.34282E-7  | 13.91   | 0.0097  |
| B : Amplitude  | 3.42576E-8     | 1  | 3.42576E-8  | 3.55    | 0.1086  |
| C : time       | 1.27092E-8     | 1  | 1.27092E-8  | 1.32    | 0.2949  |
| AA             | 1.51862E-10    | 1  | 1.51862E-10 | 0.02    | 0.9043  |
| AB             | 2.25888E-8     | 1  | 2.25888E-8  | 2.34    | 0.1769  |
| AC             | 4.11989E-8     | 1  | 4.11989E-8  | 4.27    | 0.0843  |
| BB             | 6.62778E-9     | 1  | 6.62778E-9  | 0.69    | 0.4390  |
| BC             | 1.80378E-8     | 1  | 1.80378E-8  | 1.95    | 0.2120  |
| CC             | 8.03786E-9     | 1  | 8.03786E-9  | 0.84    | 0.3957  |
| Total error    | 5.79068E-8     | 6  | 9.65106E-9  |         |         |
| Total (corr.)  | 3.55415E-7     | 15 |             |         |         |

R² = 83.7065 %. R² (adjusted for df) = 59.2662%.

**Table 7:** Analysis of variance (ANOVA) for rheology.

Optimum value is obtained and predicted according data obtained. Optimum value for consistency coefficient was obtained with the following treatment: percentage of WPC = 14%; amplitude = 100%; time = 9 min. Optimum value for initial freezing temperature was obtained with the following treatment: percentage of WPC = 14%;

![Figure 4](image4.png) **Figure 4:** Surfaces obtained with RSM study: rheological parameter (consistency coefficient Pa s⁻¹) behavior in function of time of treatment, % of WPC and estimated response surface at amplitude = 75%.

![Figure 5](image5.png) **Figure 5:** Surfaces obtained with RSM study: initial freezing temperature behavior in function of amplitude applied, % of WPC and estimated response surface (time = 6 min).

![Figure 6](image6.png) **Figure 6:** Surfaces obtained with RSM study: foaming properties behavior in function of time, % of WPC and estimated response surface at fixed amplitude (75%).
amplitude = 84.7005%; time = 3 min. Optimum value for foaming capacity was obtained with the following treatment: percentage of WPC = 14%; amplitude = 79.8809%; time = 8.8885 min.

Conclusion

Amplitude of ultrasound, percentage of WPC in the sample and time of treatment are the three variables considered to effect rheological properties (measurement of coefficient of consistency), thermal properties (measurement of initial freezing point) and foaming properties (measurement of maximal foam capacity). Experiment was adequately designed using model called Central Composite Design (CCD) and results were analyzed and process was optimized through Response surface methodology-RSM.

There is obvious significant influence of percentage of whey protein concentrate in ice-cream model mixtures, on initial freezing and melting temperatures. There are interesting data where no foaming properties could be observed for samples A1, A5, A8, A9 and A16 after ultrasound treatment. There is statistically significant influence of treatment time on foaming capacity. Percentage of WPC had statistically significant influence on rheological parameter (consistency coefficient). Optimum value is obtained and predicted according data obtained. Optimum value for consistency coefficient, for initial freezing temperature and for foaming capacity was obtained with statistical analysis.

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