Food
Processing & Technology

Jambrak et al., J Food Process Technol 2012, 3:3
http://dx.doi.org/10.4172/2157-7110.1000145

Research Article Open Access

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

**Anet Režek Jambrak[1]*, Doriane Lerda[2], Ranko Mirčeta[1], Marina Šimunek[3], Vesna Lelas[1], Farid Chemat[2], Zoran Herceg[1] and Verica Batur[1]**

[1]*Faculty of Food Technology and Biotechnology, University of Zagreb, 10000 Zagreb, Pierottijeva 6, Croatia*
[2]*UMRA 408 INRA, University of Avignon, Safety and Quality of Products of plant origin, 33 Rue Louis Pasteur, 84029 Avignon cedex, France*
[3]*Vindija D.O.O, Medimurska 6, 42000 Varazdin, Croatia*

**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 ultrasound is propagated via a series of compression and rarefaction waves induced on the molecules of the medium passed through [5].

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

***Corresponding author:** Anet Režek Jambrak, Faculty of Food Technology and Biotechnology, University of Zagreb, 10000 Zagreb, Pierottijeva 6, Croatia, Tel: +385-1-4605-035; Fax: +385-1-4605-072; E-mail: arezek@pbf.hr

**Received** December 10, 2011; **Accepted** January 21, 2012; **Published** January 24, 2012

**Citation:** Jambrak AR, Lerda D, Mirčeta R, Šimunek M, Lelas V, et al. (2012) Experimental Design and Optimization of Ultrasound Treatment: Functional and Physical Properties of Sonicated Ice Cream Model Mixtures. J Food Process Technol 3:145. doi:10.4172/2157-7110.1000145

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 energy 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) and Glucose. Each sample was prepared in a 400 mL glass and homogenized by vigorous hand mixing followed by a magnetic stirring for 15 min.

The final volume of samples was 300 mL.

### Experimental methodology

In this study, experiment was designed in STATGRAPHICS Centurion (StatPoint technologies, Inc, VA 20186, USA) software. Experiment consists of 16 experimental trials (Table 2). The variables were percentage (%) of whey protein concentrate (WPC),-$X_1$ (%), amplitude-$X_2$ (%) and treatment time-$X_3$ (min). The operating variables were considered at three levels namely, low (-1), central (0) and high (1). Central composite design has been created: $2^3$+ star which study the effects of 3 factors in 16 runs. The design has been run in a single block. The order of the experiments has been fully randomized. This provides protection against the effects of lurking variables.

Repetition experiments were carried out after other experiments followed by order of runs designed by program. Response (output) values were for rheological properties: values of consistency coefficient, for thermal properties: values of initial freezing point and for foaming properties: values of maximal foam capacity.

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]:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j}^{3} \beta_{ij} X_i X_j \quad (1)$$

where $\beta_0$ is the value of the fixed response at the central point of the experiment which is the point (0, 0, 0); $\beta_i$, $\beta_{ii}$ 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

| RECIPES | A | B | C |
|---|---|---|---|
| Sucrose | 10. 0% | 10. 0% | 10. 0% |
| Glucose | 8.0% | 8.0% | 8.0% |
| Whole Milk Powder (WMP) | 8.0% | 6.0% | 4.0% |
| Whey Protein Concentrate (WPC) | 10.0% | 12.0% | 14.0% |
| Water | 64.0% | 64.0% | 64.0% |

**Table 1:** Composition of the different samples.

| Samples | % of WPC | Amplitude (%) | Time (min) | Power (W) | Energy (J) | $T_i$ (°C) | $T_f$ (°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 | 29.627 | 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 ($T_i$) and after treatment ($T_f$).

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:

$$P = m \times C_p \times \frac{\partial T}{\partial t} \quad (2)$$

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:

$$\text{Foam expansion (\%)} = \frac{\text{Unwhipped suspension wt (g) - foam wt (g)}}{\text{Unwhipped suspension wt (g)}} \times 100 \quad (3)$$

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 done in triplicates for each sample. The shear rate versus shear stress was interpreted using the Rheometric computer program. The values for n and k were obtained from plots of log shear stress versus log shear rate, according to the power law equation:

$$\log \tau = \log k + n \log \gamma \quad (4)$$

Where τ is the shear stress (Pa); γ is the shear rate ($s^{-1}$); n is the flow behavior index, and k is the consistency index (Pa $s^n$).

Apparent viscosity ($\eta_{app}$) was calculated at 1290 $s^{-1}$ using Newtonian law, in addition with linear least square method for regression analysis.

$$\tau = \eta_{app} \gamma \quad (5)$$

### Determination of thermophysical properties of ice-creams model mixtures

Parameters of thermophysical properties were determined using data logger apparatus (KTT 300, Kistock, KIMO) that measures temperature of referent material (quartz sand) and examined material (ice-cream model mixtures). Instrument is connected and operated through computer with software (Kilog, KIMO, France). 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) were 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

| 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 |

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

% 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 microstreming 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 samples. 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 as compared to untreated ones (Tables 6), because of the formation of foams with larger foam lamellas what makes them more fragile [8]. On contrary, for samples A13, A14 and A15 there is increase in foam stability index after ultrasound treatment. This could be explained for prolonged ultrasound treatment time and highest amplitude for these samples. Ultrasound applied might is causing

| 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 | 0.2875 |
| BC | 0.03125 | 1 | 0.03125 | 0.22 | 0.6554 |
| CC | 0.205674 | 1 | 0.205674 | 1.45 | 0.2738 |
| Total error | 0.85094 | 6 | 0.141823 | | |
| Total (corr.) | 2.5375 | 15 | | | |
| $R^2$ = 66.4654 %. $R^2$ (adjusted for df) = 16.1636 %. | | | | | |

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

| Source | Sum of Squares | Df | Mean Square | F-Ratio | P-Value |
|---|---|---|---|---|---|
| A : WPC | 13.225 | 1 | 13.225 | 0.03 | 0.8794 |
| B : Amplitude | 766.325 | 1 | 766.325 | 1.45 | 0.2734 |
| C : time | 16849.4 | 1 | 16849.4 | 31.95 | 0.0013 |
| AA | 651.208 | 1 | 651.208 | 1.23 | 0.3090 |
| AB | 2.53125 | 1 | 2.53125 | 0.00 | 0.9470 |
| AC | 5.88245 | 1 | 5.88245 | 0.01 | 0.9193 |
| BB | 1289.19 | 1 | 1289.19 | 2.44 | 0.1690 |
| BC | 1.7672 | 1 | 1.7672 | 0.00 | 0.9557 |
| CC | 1414.53 | 1 | 1414.53 | 2.68 | 0.1526 |
| Total error | 3164.66 | 6 | 527.443 | | |
| Total (corr.) | 24878.1 | 15 | | | |
| $R^2$ = 87.2793 %. $R^2$ (adjusted for df) = 68.1983%. | | | | | |

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

| Sample | Foam capacity (%) | | | Foam stability index (sec) | Maximum foam stability (min) |
|---|---|---|---|---|---|
| | Mixing time | | | | |
| | 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* | | | | | |
| Ab | 78.01 | 78.58 | 81.07 | 14 | 2.7 |
| A5* | | | | | |
| A6 | 78.90 | 83.80 | 84.10 | 120 | 24 |
| A7 | 81.65 | 83.36 | 83.73 | 329 | 56.81 |
| A8* | | | | | |
| A9* | | | | | |
| A10 | 71.78 | 76.74 | 82.04 | 30 | 2.23 |
| A11 | 76.94 | 78.26 | 78.42 | 24 | 4.45 |
| A12 | 73.79 | 76.91 | 77.40 | 22 | 3.28 |
| A13 | 80.22 | 83.35 | 83.78 | 342 | 53.45 |
| A14 | 80.30 | 83.15 | 81.68 | 360 | 60.58 |
| A15 | 80.58 | 81.74 | 82.55 | 141 | 22.32 |
| A16* | | | | | |

*no foaming of samples

**Table 6:** Foaming properties.

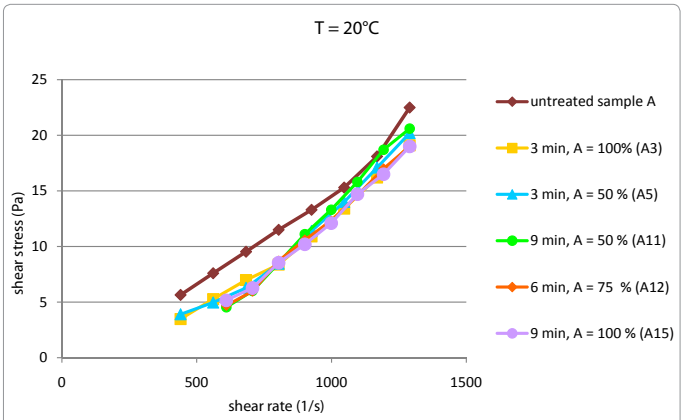


**Figure 1:** Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 10% of WPC.

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).

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

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 ($R^2$). The predicted values were within the 95% prediction limit.

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

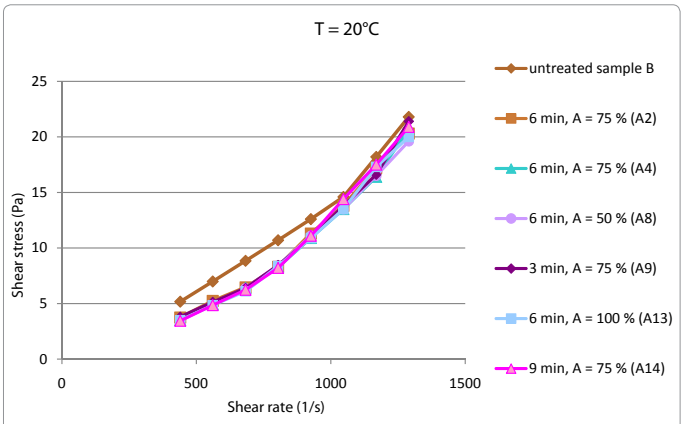


**Figure 2:** Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 12% of WPC.

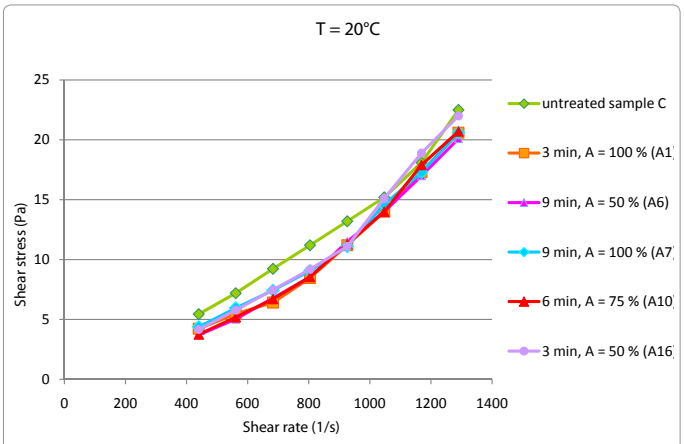


**Figure 3:** Shear stress versus shear rate behavior of untreated and ultrasound treated samples prepared with 14% of WPC.

| 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 | 1.88277E-8 | 1 | 1.88277E-8 | 1.95 | 0.2120 |
| CC | 8.0738E-9 | 1 | 8.0738E-9 | 0.84 | 0.3957 |
| Total error | 5.79096E-8 | 6 | 9.6516E-9 | | |
| Total (corr.) | 3.55415E-7 | 15 | | | |
| $R^2$ = 83.7065 %. $R^2$ (adjusted for df) = 59.2662%. | | | | | |

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

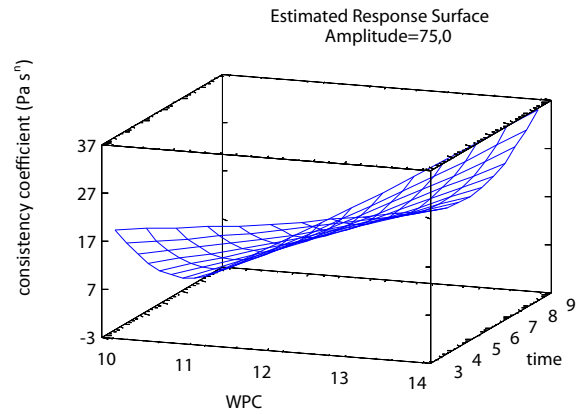


**Figure 4:** Surfaces obtained with RSM study: rheological parameter (consistency coefficient Pa $s^n$) behavior in function of time of treatment, % of WPC and estimated response surface at amplitude = 75%).

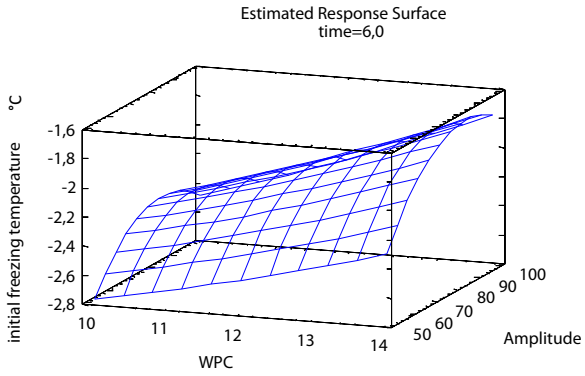


**Figure 5:** Surfaces obtained with RSM study: initial freezing temperature behavior in function of amplitude applied, % of WPC and estimated response surface (time = 6 min).

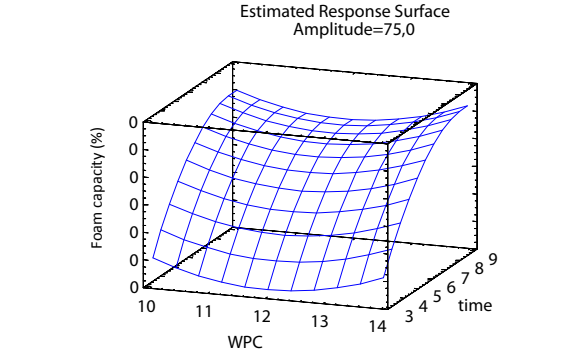


**Figure 6:** Surfaces obtained with RSM study: foaming properties behavior in function of time, % of WPC and estimated response surface at fixed amplitude (75%).

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%;

| a) rheological parameter (optimum value of consistency coefficient = 0,000562348 Pa $s^n$) | | | |
|---|---|---|---|
| Factor | Low | High | Optimum |
| WPC | 10.0 | 14.0 | 14.0 |
| Amplitude | 50.0 | 100.0 | 100.0 |
| Time | 3.0 | 9.0 | 9.0 |
| Optimum value was obtained with the following treatment: Percentage of WPC = 14%; – Amplitude = 100%; Time = 9 min. | | | |
| b) Thermophysical parameters (optimal value of initial freezing temperature = -1,36436°C | | | |
| Factor | Low | High | Optimum |
| WPC | 10.0 | 14.0 | 14.0 |
| Amplitude | 50.0 | 100.0 | 84.7005 |
| Time | 3.0 | 9.0 | 3.0 |
| Optimum value was obtained with the following treatment: Percentage of WPC = 14%; – Amplitude = 84.7005%; Time = 3 min. | | | |
| c) Foaming properties (optimal value of maximal foam capacity = 105,915%) | | | |
| Factor | Low | High | Optimum |
| WPC | 10.0 | 14.0 | 14.0 |
| Amplitude | 50.0 | 100.0 | 79.8809 |
| Time | 3.0 | 9.0 | 8.8885 |
| Optimum value was obtained with the following treatment: Percentage of WPC = 14%; Amplitude = 79.8809%; Time = 8.885 min. | | | |

**Table 8:** Optimized values of rheological (a) thermo physical (b) and foaming properties (c) at optimal processing parameters (ultrasound amplitude and treatment time and % of WPC).

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 = 8.885 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.

### References

1. Chemat F, Zill-e-Huma, Khan MK (2011) Applications of ultrasound in food technology: Processing, preservation. and extraction. Ultrason Sonochem 18: 813-835.
2. Mason TJ, Paniwnyk L, Lorimer JP (1996) The uses of ultrasound in food technology. Ultrason Sonochem 3: S253-S260.
3. Piyasena P, Mohareb E, McKellar RC (2003) Inactivation of microbes using ultrasound: a review. Int J Food Microbiol 87: 207-216.
4. Patist A, Bates D (2008) Ultrasonic innovations in the food industry: From the laboratory to commercial production. Innov Food Sci Emerg Technol 9: 147-154.
5. Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21: 323-331.
6. Režek Jambrak A, Lelas V, Krešić G, Badanjak M, Rimac Brnčić S, et al. (2011) Rheological, functional and thermo-physical properties of ultrasound treated whey proteins with addition of sucrose or milk powder. Dairy 61: 79-91.
7. Leonelli C, Mason TJ (2010) Microwave and ultrasonic processing: Now a realistic option for industry Review Article. Chemical Engineering and Processing: Process Intensification 49: 885-900.
8. Krešić G, Lelas V, Režek Jambrak A, Herceg Z, Rimac Brnčić S (2008) Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. J Food Eng 87: 64-73.
9. Vilkhu K, Mawson R, Simons L, Bates D (2008) Applications and opportunities for ultrasound assisted extraction in the food industry-A review. Innov Food Sci Emerg Technol 9: 161-169.
10. De la Fuente-Blanco S, Riera-Franco de Sarabia E, Acosta-Aparicio VM, Blanco-Blanco A, Gallego-Jurez JA (2006) Food drying process by power ultrasound. Ultrason 44: e523-e527.
11. Mortazavi A, Tabatabaie F (2008) Study of Ice Cream Freezing Process after Treatment with Ultrasound. World Appl Sci J 4: 188-190.
12. Goff HD (2008) 65 years of ice cream science. Int Dairy J 18: 754-758.
13. Chemat F, Grondin I, Costes P, Moutoussamy L, Sing AS, et al. (2004) High power ultrasound on lipid oxidation of refined sunflower oil. Ultrason Sonochem 11: 281-285.
14. Lu CH, Engelmann NJ, Lila MA, Erdman JW Jr (2008) Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology. J Agric Food Chem 56: 7710-7714.
15. Kuehl R (2000) Design of Experiments: Statistical Principles of Research Design and Analysis. (2nd Ed.), Duxbury Press, Pacific Grove CA, 2-225.
16. Khuri AI, Cornell JA (1996) Response Surfaces: Design and Analyses. (2nd Ed.), Marcel Dekker, New York.
17. Margulis MA, Margulis IM (2003) Calorimetric method for measurement of acoustic power absorbed in a volume of a liquid. Ultrason Sonochem 10: 343-345.
18. Morr CV, Foegeding EA (1990) Composition and functionality of commercial whey and milk protein concentrated and isolates: A status report. Food Technol 44: 100-112.
19. Jambrak AR, Mason TJ, Lelas V, Herceg Z, Herceg Lj I (2008) Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. J Food Eng 86: 281-287.
20. Montgomery DC (2001) Design and Analysis of Experiments. (5th Ed.), New York, Wiley.
21. Myers RH, Montgomery DC (2002) Response Surface Methodology: Process and Product Optimization Using Designed Experiments. (2nd Ed.), John Wiley & Sons, USA.