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Rheological, functional and thermo-physical properties of ultrasound treated whey proteins with addition of sucrose or milk powder

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Summary

Ultrasound represents a non-thermal food processing technique and has great potential to be used in the food industry. The objective of this research was to observe ultrasound impact on physical properties of model systems prepared with whey protein isolates (WPI) or whey protein concentrates (WPC) with or without sucrose or milk powder addition. This kind of systems is often used in milk beverages and milk based products. Model systems with protein and milk powder or sucrose addition were treated with high power ultrasound (HPU) probe of 30 kHz frequency for 5 and 10 minutes. After sonication several properties were determined and examined: solubility, emulsifying and foaming properties, rheological and thermophysical properties. Ultrasound treatment showed severe influence on all examined properties, caused by protein denaturation as a consequence of cavitation and microstreaming effects. Ultrasound treatment caused decrease in protein solubility for whey protein isolate and whey protein concentrates model systems, compared to untreated sample. There was statistically significant increase in foam volume of model systems, prepared with sucrose or milk powder and WPI after ultrasound treatment. Statistically significant decrease in emulsion activity and emulsion stability indices was observed for model systems prepared solely with isolates and concentrates. After treatment of whey protein model systems (with or without milk powder or sucrose) with 30 kHz ultrasound, the changes in consistency coefficients (k) were observed, but there were no significant changes in flow behaviour indices (n). After addition of milk powder or sucrose, statistically significant decrease in initial freezing and melting temperatures was observed due to the ultrasound treatment.

Key words: whey proteins, ultrasound, sucrose, milk powder, functional properties, rheological properties

Introduction

Hence in food technology ultrasound is relatively new non-thermal technology and has been much used in research nowadays. General properties of ultrasound to be used in food processing are the fact that power of ultrasound can cause changes in some properties (chemical, functional, physical etc.) that may be interesting as technological benefit. Ultrasound represents mechanical waves, i.e. a variation of pressure or density with frequencies above the human hearing threshold (ca. 18 kHz) (Mason, 1998).

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Ultrasound can be classified in two categories: low intensity (high frequency-low power), and high intensity (low frequency-high power) ultrasound. The low-intensity ultrasound uses very small power levels, typically less than 1 W cm^{-2}, with the frequency range of 5-10 MHz (McClements, 1995; Mason, 1998). It is generally used in diagnostic analysis of food materials. At high intensities (typically power levels in the range of 10-1000 W cm^{-2}, with the frequency of 20-100 kHz (Mason, 1998)), ultrasound has a lethal effect on microorganisms, and as such has a potential in food preservation treatments (Entezari et al., 2004; Piyasena et al., 2003; Zenker, 2004). High-intensity ultrasound could be used in many food applications, such as emulsifycation, sterilization, extraction, degassing, filtration, drying, and enhancing oxidation (Leadley and Williams, 2002; Mason, 1998). The phenomenon during ultrasound treatment is known as cavitation. During implosion of growing bubbles, very high temperatures (approximately 5500 K) and pressures (approximately 50 MPa) are reached inside these bubbles (Mason, 1990; Mason, 1998; Suslick, 1988) what could consequently cause several reactions around imploding bubble.

There are four types of cavitation based on the mode of generation: acoustic, optic, particle and hydrodynamic. In processing application, like in food processing industry only acoustic and hydrodynamic cavitation are found to be efficient (Gogate and Kabadi, 2009) since they produce chemical or physical changes in the treated material. There are few papers which describe influence of ultrasound on whey proteins. One is investigating rheological (Meza et al., 2009) or thermophysical properties of whey proteins (Krešić et al., 2008). Several researchers studied effect of ultrasound on specific properties of whey proteins (Wang et al., 2008; Jambrak et al., 2008; Guzey et al., 2006). There are also very few papers dealing with addition of hydrocolloids of other compounds in whey proteins suspensions in order to study ultrasound effect on functional properties.

The objective of this study was to examine the effect of ultrasound treatment on physical properties of whey proteins in combination with addition of sucrose or milk powder. Solubility, rheological, foaming and emulsifying properties, as well as thermal analysis have been measured with the aim to examine the influence of ultrasound treatment on selected mixture.

Materials and Methods

Materials and sample preparation

The model systems marked as WPI (Whey protein isolates -WPI, BiPRO®, Davisco Foods International, USA; composition: proteins 95 %, fat 1 %, lactose 1 %, ash 1 % and moisture 2 %) and WPC (Whey protein concentrates - WPC, "Meggle" GmbH, Wasserburg, Germany, WPC-60; composition: proteins 60 %, fat 6 %, lactose 25 %, ash 6 % and moisture 3 %), were aqueous suspensions of powdered WPI and WPC contained 10.0 % of dry matter. For samples prepared with sucrose and WPI or WPC, 10.0 % (w/w) of sucrose has been added to the systems containing 10 % (w/w) of WPI or WPC, and dispersed in distilled water in volume of 100 mL by vigorous hand mixing until homogenous suspensions were obtained. In total these systems contained 20 % (w/w). These systems were labelled as WPI+S; WPI5+S; WPI10+S (the number means the time of ultrasound treatment), or WPC+S; WPC5+S; WPC10+S. For samples prepared with full fat milk powder (DUKAT, Zagreb, Croatia, composition: fat 26.1 %, lactose 39.8 %, proteins 26.3 %, water 4.8 % and ash 3.0 %) and WPI or WPC, 5.0 % (w/w) of milk powder has been added to the systems containing 10 % (w/w) of WPI or WPC, after which, the system was dispersed in distilled water in volume of 100 mL by vigorous hand mixing until homogenous suspensions were obtained. In total these systems contained 15 % (w/w). These systems were labelled as WPI+MP; WPI5+MP; WPI10+MP (the number means the time of ultrasound treatment), or WPC+MP; WPC5+MP; WPC10+MP.

For preparation of model systems containing proteins with milk powder or sucrose addition, samples were prepared as shown in Table 1. For solubility determination samples were prepared as described in section *Foaming properties of whey protein model systems*, and for emulsifying properties as described in section *Determination of rheological properties of whey protein model systems*.

Ultrasound treatment

Ultrasound treatment with 30 kHz probe

Samples for ultrasound treatment were placed in 400 mL flat bottom flask and treated with power ultrasound, high intensity and low frequency, 30 kHz probe (Hielscher-Ultrasound Technology,

Table 1. Model system composition

Materials	Ingredients (%) (w/w)					
Samples	Ultrasound treatment 30 kHz (min)	Whey protein isolates (WPI)	Whey protein concentrate (WPC)	Water	Sucrose (S)	Milk powder (MP)
WPI	--	10		90	--	--
WPI5	5	10		90	--	--
WPI10	10	10		90	--	--
WPI+S	--	10		80	10	
WPI+MP	--	10		85		5
WPI5+S	5	10		80	10	
WPI10+S	10	10		80	10	
WPI5+MP	5	10		85		5
WPI10+MP	10	10		85		5
WPC	--		10	90	--	--
WPC5	5		10	90	--	--
WPC10	10		10	90	--	--
WPC+S	--		10	80	10	
WPC+MP	--		10	85		5
WPC5+S	5		10	80	10	
WPC10+S	10		10	80	10	
WPC5+MP	5		10	85		5
WPC10+MP	10		10	85		5

Hielscher Ultrasonics, GmbH, Warthestrasse 21D-14513, Teltow, Germany, UP100H (100W, 30 kHz), diameter of the probe was 10 mm, cycle was put on maximum and amplitude was 100 %). Samples were treated for 5 and 10 minutes so that high power intensity can be obtained. Probe has a vibrating titanium tip of 10 mm and is immersed in the liquid and the liquid is irradiated with an ultrasonic wave directly from the horn tip. Temperature of the model systems have been measured by laser thermometer (RAYTEK, Minitemp FS, European Headquarters Raytek GmbH, Berlin, Germany).

Determination of ultrasound power and intensity

Ultrasonic power, which is considered as mechanical energy, would partly be lost in the form of heat when ultrasound passes through the medium (Thompson and Doraiswamy, 1999). Since the ultrasonic irradiation of a liquid produces heat, recording the temperature as a function of time leads to the acoustic power estimation (in W) by the equation (Margulis and Malt'sev, 1969; Margulis and Margulis, 2003).

$$P = m \cdot c_p \cdot (\frac{dT}{dt}) \qquad (1)$$

Where: m - is the mass of the sonicated liquid (g), c_p - specific heat of medium at a constant pressure dependent on composition and volume of medium (J $(gK)^{-1}$), dT/dt - slope at the origin of the curve. Ultrasound intensity is expressed in watts per unit area of the emitting surface (W cm^{-2}), or in watts per unit volume of the sonicated solution (W cm^{-3}).

Temperature increase during ultrasound treatment has been measured by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK) and afterward ultrasound intensity is expressed in Wcm^{-2}.

Determination of whey proteins solubility

After ultrasound treatment whey proteins were lyophilized in freeze dryer (ChemLab Instruments Ltd., Hornchurch, Essex, UK; Model SB6CB) by freezing for a minimum of 3 h to temperature of -45 °C. Lyophilized protein powders were dispersed (1 % w/w) in deionized water. The solubility of protein was determined at pH 7.0 by the method described by Smith et al. (1985). The concentration of proteins was determined using bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Stock solutions (0.1 % protein (w/w)) of control and treated whey proteins were prepared and allowed to hydrate overnight at 5 °C. Aliquots of the solutions (1 mL) were centrifuged at 12.500 g for 25 min at 20 °C. 100 μL of diluted supernatant sample (1/100) was added to 2 mL of BCA reagent. The tubes were incubated at 37 °C for 30 min and then cooled to 20 °C before measurement using a spectrophotometer at 562 nm (Helios ß, Unicam, UK). Protein content was determined after calibration using bovine serum albumin dilution series of five concentrations as external standard and the protein solubility was calculated as the percentage of soluble protein in the supernatant relative to the total protein content in the sample. Measurements of protein solubility have been done for protein model systems without sucrose or milk powder addition.

Foaming properties of whey protein model systems

For determination of foaming properties samples were prepared as described in section *Ultrasound treatment* and then ultrasonically treated as described in section *Determination of whey proteins solubility*. 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. Procedure is described elsewhere in details (Jambrak et al., 2008). Foam expansion was calculated using the expression:

$$\text{Foam expansion (\%)} = \frac{\text{Unwhipped suspension wt (g) - foam wt (g)}}{\text{Unwhipped suspension wt (g)}} \times 100 \quad (2)$$

The time required (min) for drainage of the entire foam was determined for foam stability (Morr and Foegeding, 1990).

Emulsifying properties of whey protein model systems

For emulsifying properties determination samples were prepared as described in section *Ultrasound treatment* and then ultrasonically treated as described in section *Determination of whey proteins solubility*. After ultrasound treatment, protein suspensions were analyzed by the turbidometric technique for emulsion activity index (EAI) and emulsion stability index (ESI) as previously described (Webb et al., 2002). Emulsions were prepared with 3 % protein dispersions (w/v) using 10 mL of sunflower oil (Zvijezda d.d, Zagreb, Croatia) in ratio 1:3 (10 mL oil/30 mL of protein dispersion), by mixing for 90 sec in a blender. The absorbance of the diluted emulsions was measured by spectrophotometer (Helios-b, Pye Unicam Ltd, Cambridge, UK) at 500 nm in 1 cm path length cuvettes. The whole procedure is described elsewhere in details (Jambrak et al., 2008).

The emulsifying activity index (EAI) was determined by the turbidimetric method of Pearce and Kinsella (1978). The emulsion activity index (EAI) was then calculated, and also emulsion stability, what is explained in details elsewhere (Jambrak et al., 2008).

Determination of rheological properties of whey protein model systems

Torque measurements were carried out on the model dispersions using a Rheometric Viscometer (Model RM 180, Rheometric Scientific, Inc., Piscataway, USA). The system is composed of round shaped bob (no. 3; Ø=14 mm; l=21 mm) which is inserted in the tube (no. 3; Ø=15.18 mm). Shear stress against the increasing shear rates from lowest value of 0 s^{-1} to 1290 s^{-1} as well as downwards was applied. Measurements were done in triplicates for each sample (Krešić et al., 2008; Jambrak et al., 2009). 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:

$$\log \tau = \log k + n \log \gamma \quad (3)$$

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

Determination of thermophysical properties

Parameters of thermophysical properties were determined using DTA apparatus (MP DT-Pt-L, Elektron, d.d., Stubičke toplice, Croatia) which is suitable for this type of measurement (Krešić et al., 2008; Herceg et al., 2006). The instruments were interfaced with a standard PC and software for data analysis was used (STEP7/Win 32, Siemens Energy and Automatisation Inc., Alpharetta, GA, USA). 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.

Statistical analyses

The whole study was repeated and each value represents the mean of three measurements from two independent ultrasound treatments. The effect of ultrasound treatment on tested parameters was determined by analysis of variance, using statistical analyses with SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL). Analysis of variance (One-Way ANOVA), significant level used was 5 % (α=0.05), was carried out to assess whether the different treatments conducted to statistically different results for those variables evaluated. The values not statistically different are accompanied by the letter (a) and the values statistically different with the letter (b).

Table 2. Whey protein isolate (WPI) and whey protein concentrate (WPC) solubility (%) in water of suspensions prepared with untreated and treated proteins by 30 kHz frequency probe set ultrasound

Treatment	Solubility (%)
WPI	96.5[a]
WPI5	54.6[b]
WPI10	64.6[b]
WPC	91.0[a]
WPC5	60.1[b]
WPC10	76.9[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

Results and discussion

Influence of ultrasound treatment on whey proteins solubility

In ultrasonic treatment with 30 kHz probe the ultrasonic intensity was 73-78 W cm^{-2}, as measured calorimetrically. Ultrasound treatment with 30 kHz ultrasound probe has showed the largest decrease in protein solubility ($p<0.05$) for WPI (96.5 % to 54.6 %) after 5 minute treatment, and (96.5 % to 64.6 %) after 10 minute treatment (Table 2).

For WPC model systems, decrease in solubility[1] it could be observed[2] (91 % to 60.1 %) after 5 minute treatment ($p<0.05$), and (91 % to 76.9 %) after 10 minute treatment, respectively, compared to untreated sample (Table 2). The main reason for the decrease in protein solubility is in the fact that during ultrasound treatment large number of cavitation bubbles produces large increase in local temperature and pressure in the surrounding area of collapsing bubble (Galazka et al., 2000; Cheftel et al., 1985). Also, there is difference in composition of WPC and WPI, where for WPC there is higher amount of lactose and fats present in the systems that is oxidized and target of ultrasound so proteins are partially protected (Dumay et al., 1994). The increase in solubility after 10 min ultrasound treatment can be explained with the fact that for prolonged treatment time, protein chains are reorganizing and high ultrasound intensity (73-78 W cm^{-2}) that have been measured, results in unfolding of protein, opening of hydrophilic parts on the outer side of protein and breaking of peptide bonds due to the hydrolysis occurred (Morel et al., 2000; Moulton and Wang, 1982).

Influence of ultrasound treatment on foaming properties

Results for foaming properties of model systems prepared with WPI and WPC and with or without addition of sucrose or milk powder addition are shown in Tables 2 or 3. Statistically significant ($p<0.05$) changes in foam capacity (%) for model systems prepared solely with isolates can be ob-

Table 3. Foam properties of untreated and treated whey protein isolate (WPI) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Foam capacity (%)			Foam stability index (sec)	Foam stability (min)
	Mixing time				
	5 min	10 min	15 min		
WPI	299.02±1.21[a]	448.30±1.70[a]	491.30±1.14[a]	132±0.9[a]	290±0.9[a]
WPI5	318.92±1.03[a]	322.44±1.46[b]	314.91±1.44[b]	90±0.7[b]	150±1.0[b]
WPI10	367.13±1.33[b]	361.82±1.66[b]	447.12±1.67[a]	60±0.8[b]	75±1.0[b]
WPI+S	326.59±1.06[b]	461.86±1.51[a]	513.21±1.22[a]	156±0.9[a]	320±0.9[a]
WPI+MP	320.78±1.56[a]	380.33±1.62[b]	412.03±1.45[b]	102±0.8[a]	250±1.1[a]
WPI5+S	877.35±1.45[b]	824.01±1.33[b]	906.86±1.12[b]	300±0.7[b]	1440±1.0[b]
WPI10+S	793.66±1.38[b]	802.69±1.78[b]	789.95±1.81[b]	300±0.8[b]	1440±1.0[b]
WPI5+MP	357.97±1.54[b]	320.52±1.57[b]	402.99±1.81[a]	10±0.8[b]	90±1.2[b]
WPI10+MP	790.85±1.46[b]	856.93±1.74[b]	876.43±1.75[b]	240±0.9[b]	1200±1.1[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

served. For prolonged mixing time, decrease (491.3 % to 314.91 %) in foam volume after 5 minute ultrasound treatment (WPI5) occurred. For the model systems prepared with WPC there was no significant ($p>0.05$) changes in foam volume, except for ultrasound treatment for 5 minutes (WPC5) where foam volume decreased (370.28 % to 316.13 %). After ultrasound treatment of model systems prepared with addition of sucrose or milk powder to WPI, statistically significant increase ($p<0.05$) in foam volume (%) was observed after all treatment times and for prolonged mixing time, compared to untreated model systems prepared with addition of sucrose or milk powder to WPI (Table 3).

After addition of sucrose there is no statistically significant increase in foam capacity (491.3 % to 513.21 %), on contrary to decrease in foam capacity after milk powder addition (milk powder; 491.3 % to 412.03 %) compared to model system prepared with just isolate. The fact that sucrose and polysaccharides do not have affinity for air-water interface is well known (Bos and van Vliet, 2001), but they are fostering protein-protein interactions which could lead to the formation of multilayer film on the interface. This could later prevent collapse of foam and promote production of more stable foam (Adebowale and Lawal, 2003). After ultrasound treatment of model systems prepared by addition of sucrose to WPI or concentrate, statistically significant increase ($p<0.05$) in foam capacity occurred (Tables 3 and 4). The positive protein-protein interactions are evident and consequently opening of protein chains which are now fragmented, and had the ability to propagate at air-water interface leading to better foaming properties of these systems (Jambrak et al., 2009; Mishra et al., 2001).

From the Table 4 for model systems prepared with WPC and addition of sucrose or milk powder, there is significant increase ($p<0.05$) in foam volume (%) for all treatment times and for prolonged mixing time, compared to untreated model systems prepared with WPC and addition of sucrose or milk powder. Exception can be observed with the decrease in foam volume (%) for ultrasound treatment for 5 minutes (304.96 % to 151.92 %) of model system prepared with WPC and milk powder (WPC5+MP). 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 for WPC because of lactose and fat amount which 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 2 and 3), because of the formation of foams with larger foam lamellas what makes them more fragile (Krešić et al., 2008). This was not the case in model systems prepared with addition of sucrose or milk powder to isolate after ultrasound treatments. It had more stable foams compared to untreated one (Table 3).

Table 4. Foam properties of untreated and treated whey protein concentrate (WPC) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Foam capacity (%)			Foam stability index (sec)	Foam stability (min)
	Mixing time				
	5 min	10 min	15 min		
WPC	334.88±1.92[a]	356.62±1.46[a]	370.28±1.29[a]	30±0.3[a]	210±0.8[a]
WPC5	321.56±1.45[a]	325.55±1.74[a]	316.13±1.38[b]	42±0.6[a]	23±0.4[b]
WPC10	313.53±1.86[a]	371.84±1.87[a]	386.28±1.25[a]	73±0.6[b]	39±0.8[b]
WPC+S	338.55±1.72[a]	376.92±1.56[a]	380.68±1.78[a]	60±0.8[a]	230±0.8[a]
WPC+MP	324.57±1.71[a]	311.80±1.43[a]	304.96±1.32[b]	30±0.6[a]	5±0.7[a]
WPC5+S	697.41±1.57[b]	682.88±1.73[b]	701.94±1.36[b]	150±0.9[b]	150±0.3[b]
WPC10+S	625.87±1.64[b]	590.37±1.27[b]	604.83±1.78[b]	300±0.7[b]	120±0.6[b]
WPC5+MP	124.95±1.75[b]	128.60±1.23[b]	151.92±1.44[b]	150±0.9[b]	5±0.6[a]
WPC10+MP	624.07±1.46[b]	700.86±1.43[b]	753.18±1.25[b]	195±0.4[b]	100±0.4[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

Influence of ultrasound treatment on emulsifying properties

Under turbulent conditions that are occurring during ultrasound treatment (like homogenization), movements favor the adsorption of proteins and formation of aggregates predominates (Walstra, 1983). These emulsions have been prepared after ultrasound treatments of model systems where whey protein are opened are reoriented toward oil-water interface.

Statistically significant decrease ($p<0.05$) in emulsion activity (EAI) and emulsion stability indices (ESI) could be observed for model systems prepared with isolates and concentrates by itself (Table 5 and 6).

Also, it is evident that for protein samples with addition of sucrose or milk powder prior ultrasound treatment, there was increase ($p<0.05$) in EAI compared to proteins itself without ultrasound treatment. After addition of sucrose in model systems which were not treated with ultrasound, EAI value

Table 5. Emulsion properties of untreated and treated whey protein isolate (WPI) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Emulsion activity index EAI ($m^2\ g^{-1}$)	Emulsion stability index ESI (h)
WPI	148.20±1.37[a]	70.50±0.9[a]
WPI5	83.89±1.89[b]	13.92±1.03[b]
WPI10	93.57±1.58[b]	20.92±1.16[b]
WPI+S	153.60±1.28[a]	73.50±0.84[a]
WPI+MP	165.03±1.47[a]	65.30±0.86[a]
WPI5+S	63.49±1.53[b]	33.12±0.81[b]
WPI10+S	61.16±1.58[b]	29.54±0.89[b]
WPI5+MP	152.46±1.46[a]	34.78±0.94[b]
WPI10+MP	143.66±1.43[b]	28.97±0.95[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

Table 6. Emulsion properties of untreated and treated whey protein concentrate (WPC) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Emulsion activity index EAI ($m^2 g^{-1}$)	Emulsion stability index ESI (h)
WPC	114.50±1.53[a]	65.50±1.12[a]
WPC5	99.79±1.13[b]	14.88±1.10[b]
WPC10	113.43±1.26[a]	16.98±0.61[b]
WPC+S	124.30±1.68[a]	67.2±0.78[a]
WPC+MP	134.60±1.45[a]	64.3±0.96[a]
WPC5+S	112.00±1.83[b]	21.98±0.79[b]
WPC10+S	88.15±1.29[b]	18.11±0.69[b]
WPC5+MP	161.26±1.82[b]	28.44±0.90[b]
WPC10+MP	151.29±1.38[b]	24.51±0.95[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

increased due to the newly formed interactions with proteins (Dickinson and Galazka, 1991). After addition of sucrose or milk powder and ultrasound treatment, decrease in EAI and ESI was observed, compared to model systems without treatments. The exception was systems with WPC and milk powder where increase ($p>0.05$) in EAI compared to untreated samples happened (134.60 $m^2 g^{-1}$ to 161.26 $m^2 g^{-1}$ and 151.29 $m^2 g^{-1}$). Ultrasound caused denaturation of proteins and thereby proteins lost the ability for emulsion formation. In untreated WPI samples proteins are capable to produce emulsions because of the ability to act as surface active compound (Jambrak et al, 2009). For model systems prepared with WPC and addition of sucrose after ultrasound treatment for 5 and 10 min there was statistically significant reduction ($p<0.05$) in emulsion activity index (EAI) (124.3 $m^2 g^{-1}$ to 112.00 $m^2 g^{-1}$ and 88.15 $m^2 g^{-1}$). For milk powder addition there was increase in EAI (134.6 $m^2 g^{-1}$ to 161.26 $m^2 g^{-1}$ and 151.29 $m^2 g^{-1}$). In this case ultrasound treatment influenced emulsion formation due to the sonication of lactose and fats that were in the systems. They were first molecules exposed to ultrasound treatment, and thereby they showed protective effect on proteins (Murray, 1997).

Emulsion stability index (ESI) is estimation of protein capacity for staying at water/oil interface after emulsion storage or heating (Mohanty et al., 1988). From the data it statistically significant decrease ($p<0.05$) for all systems and all treatment conditions is evident. This disruption of emulsion happened because of lack of surface acting compounds that are capable to reduce surface tension of the formed emulsion, and also because of partial aggregation of oil droplets (Dickinson and Galazka, 1991).

Influence of ultrasound treatment on rheological properties

Rheological properties are expressed with consistency coefficient (k) and flow behaviour indices (n), and adequately described with Ostwald de Wale's power law. After 30 kHz ultrasound treatment of whey protein model systems with or without milk powder or sucrose addition, there have been statistically significant ($p<0.05$) changes in consistency coefficients (k), except for WPI10 and WPI+S model systems. There were no significant changes ($p>0.05$) in flow behaviour indices (n). All model systems have increased consistency coefficients (k) after 5 min ultrasound treatment, on contrary for systems that were treated for 10 min where it decreases (Table 7).

For WPI with addition of milk powder, consistency coefficient decreased after 10 min ultrasound treatment (0.0904 mPa s^n to 0.0525 mPa s^n), and for WPC (Table 8) with milk powder addition after 5 and 10 min ultrasound treatment (0.2178 mPa s^n to 0.1169 mPa s^n and 0.1171 mPa s^n).

Table 7. Rheological properties of untreated and treated whey protein isolate suspensions (WPI) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Consistency coefficient ***k*** (mPa s^n)	Flow behaviour index ***n***	Regression coefficients r^2
WPI	0.0175±0.012[a]	1.8333±0.014[a]	0.991
WPI5	0.0265±0.013[b]	1.7645±0.013[a]	0.995
WPI10	0.0203±0.010[a]	1.7917±0.017[a]	0.992
WPI+S	0.0198±0.017[a]	1.8489±0.015[a]	0.997
WPI+MP	0.0904±0.018[b]	1.6181±0.014[a]	0.994
WPI5+S	0.1613±0.019[b]	1.5476±0.015[a]	0.996
WPI10+S	0.0631±0.020[b]	1.6777±0.014[a]	0.993
WPI5+MP	0.1722±0.021[b]	1.5220±0.012[a]	0.995
WPI10+MP	0.0525±0.019[b]	1.6879±0.011[a]	0.992

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

Loss of tertiary structure of globular proteins during denaturation caused by cavitation, lead to increase in volume which is taken by proteins. Values of consistency coefficients increased also because of increase in viscosity due to the existence of protein aggregates in the systems. This can be explained with the fact that unfolded globular proteins swelled and thereby lead to increase in hydrodynamic radius of protein molecule and greater intermolecular spinning (Rattray and Jelen, 1995). This increase in consistency coefficients also can be explained with the ability of newly formed whey protein globules to bind higher amount of water molecules as hydrophilic parts of amino acids are oriented towards outer part of protein molecule (Ipsen at el., 2000). From the flow behaviour values it could be concluded that all systems (non-treated and ultrasound treated) had dilatant ($n>1$), non-newtonian behaviour. From the results shown before, one can conclude that during ultrasound treatment there was increase in shear stress of the systems in the case of sucrose or milk powder addition to WPI. There is different influence of ultrasound treatment of isolates, where they have been denatured under rapid molecule movement due to cavitation (probe) and microstreaming and unfolding of protein chains occurred. This was not the case for systems prepared with WPC with sucrose (WPC5+S and WPC10+S) where there was statistically significant ($p<0.05$) decrease and totally different shear stress to shear strain behaviour compared to WPI systems. These systems showed different behaviour because of different composition and the presence of fats, lactose and sucrose in the system that show protective effect of cavitation on protein molecule to denaturation (Dumay et al., 1994; Murray, 1997).

Influence of ultrasound treatment on thermophysical properties

After ultrasound treatment for 5 minutes of WPI without additions of milk powder or sucrose there have been slight decrease in initial freezing temperature (-0,98 to -1.09 °C) and melting temperature (-6.30 to -6.62 °C). Statistically significant ($p<0.05$) decrease was observed after 10 minutes treatment for initial freezing temperature (-0.98 to -1.25 °C), and melting temperature (-6.30 to -6.78 °C) (Table 9).

After sucrose or milk powder addition into model suspensions of WPI without ultrasound, there is obvious decrease in initial freezing temperature in comparison with suspension prepared with isolate proteins itself. There was also decrease in freezing and melting temperatures (Table 10) of model systems prepared with WPC and sucrose (-1.21 °C to -1.25 °C and 1.22 °C), or milk powder addition (-1.62 °C to -1.78 °C and -1.80 °C).

This phenomenon is already very well known because addition of carbohydrates or molecules into

Table 8. Rheological properties of untreated and treated whey protein concentrate suspensions (WPC) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Consistency coefficient ***k*** (mPa s^n)	Flow behaviour index ***n***	Regression coefficients r^2
WPC	0.0369±0.021[a]	1.7394±0.021[a]	0.994
WPC5	0.0432±0.023[b]	1.7039±0.012[a]	0.993
WPC10	0.0254±0.012[b]	1.7840±0.024[a]	0.995
WPC+S	0.0274±0.017[a]	1.7731±0.029[a]	0.998
WPC+MP	0.2178±0.015[b]	1.5085±0.028[a]	0.995
WPC5+S	0.2147±0.014[b]	1.5202±0.030[a]	0.997
WPC10+S	0.1184±0.016[b]	1.5973±0.031[a]	0.998
WPC5+MP	0.1169±0.017[b]	1.5938±0.028[a]	0.997
WPC10+MP	0.1171±0.011[b]	1.5824±0.027[a]	0.998

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

suspension is influencing nucleation and formation of ice crystals (Goff and Sahagian, 1996; Hartel, 1998; Tolstoguzov, 2002). Also, the action of ultrasound on model systems causes decrease in particle size and molecular weight of proteins (Jambrak et al., 2009) as well as in fat globules. This lead to binding of higher amount of water on liberated hydrophilic parts of amino acids, what can afterwards lead to decrease of initial freezing temperature (Arakawa et al, 1990; Xie and Timasheff, 1997).

Conclusions

In this paper direct influence of ultrasound probe treatment on protein model systems prepared with or without addition of sucrose or milk powder is shown. Ultrasound treatment showed great influence on all examined properties due to the protein denaturation caused by cavitation and microstreaming effects. Ultrasound treatment caused decrease in protein solubility for WPI

Table 9. Initial freezing and initial thawing temperatures of untreated and treated whey protein isolate suspensions (WPI) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Initial freezing temperature (°C)	Top of the freezing curve (°C)	Start of melting (°C)	Initial thawing temperature (°C)
WPI	-0,98±0.11[a]	-4,20±0.15[a]	-6,30±0.14[a]	-1,09±0.10[a]
WPI5	-1,09±0.12[a]	-4,59±0.12[a]	-6,62±0.15[a]	-1,12±0.15[a]
WPI10	-1,25±0.09[b]	-5,15±0.15[b]	-6,78±0.14[b]	-1,23±0.11[b]
WPI+S	-1,30±0.14[a]	-6,34±0.15[a]	-7,03±0.16[a]	-1,36±0.14[a]
WPI+MP	-1,69±0.11[a]	-6,63±0.11[a]	-7,46±0.17[a]	-1,59±0.12[a]
WPI5+S	-1,38±0.13[a]	-6,43±0.17[a]	-7,15±0.15[a]	-1,39±0.14[a]
WPI10+S	-1,42±0.12[b]	-6,57±0.12[b]	-7,21±0.14[b]	-1,43±0.17[b]
WPI5+MP	-1,73±0.11[a]	-6,74±0.15[a]	-7,63±0.13[b]	-1,73±0.15[b]
WPI10+MP	-1,77±0.13[b]	-6,83±0.14[b]	-7,71±0.16[b]	-1,75±0.12[b]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

Table 10. Initial freezing and initial thawing temperatures of untreated and treated whey protein concentrate suspensions (WPC) with 30 kHz frequency probe set ultrasound with or without sucrose or milk powder addition

Treatment	Initial freezing temperature (°C)	Top of the freezing curve (°C)	Start of melting (°C)	Initial thawing temperature (°C)
WPC	-1,18±0.12[a]	-4,53±0.12[a]	-6,13±0.18[a]	-1,31±0.15[a]
WPC5	-1,19±0.18[a]	-4,54±0.18[a]	-6,15±0.17[a]	-1,37±0.15[a]
WPC10	-1,22±0.14[a]	-4,57±0.16[a]	-6,16±0.14[a]	-1,38±0.14[a]
WPC+S	-1,21±0.12[a]	-6,28±0.18[a]	-7,05±0.16[a]	-2,09±0.13[a]
WPC+MP	-1,62±0.12[a]	-6,46±0.19[a]	-7,24±0.15[a]	-1,92±0.13[a]
WPC5+S	-1,25±0.13[a]	-6,37±0.18[a]	-7,15±0.15[b]	-2,12±0.19[a]
WPC10+S	-1,22±0.13[a]	-6,40±0.19[a]	-7,12±0.14[b]	-2,18±0.18[a]
WPC5+MP	-1,78±0.12[a]	-6,67±0.21[b]	-7,33±0.13[b]	-2,01±0.18[a]
WPC10+MP	-1,80±0.15[b]	-6,71±0.23[b]	-7,37±0.12[b]	-2,02±0.19[a]

Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b). The values are compared along the columns

and WPC model systems, compared to untreated sample. The main reason for the decrease in protein solubility was the fact that during ultrasound treatment large number of cavitation bubbles produced large increase in local temperature and pressure in the surrounding area of collapsing bubble, therefore causing protein denaturation. There was statistically significant increase in foam volume of model systems prepared with addition of sucrose or milk powder to WPI after ultrasound treatment. With doubled ultrasound treatment time (10 min) there was increase in foaming capacity. Prolonger ultrasound treatment caused better propagation of proteins on the interface, which is blocked in the case of shorter treatment because of lactose and fats that are present in milk powder. There is statistically significant decrease in emulsion activity and emulsion stability indices for model systems prepared with isolates and concentrate itself. After 30 kHz ultrasound treatment of whey protein model systems with or without milk powder or sucrose addition there have been changes in consistency coefficients (k), but there were no significant changes in flow behaviour indices (n). Loss of tertiary structure of globular proteins during denaturation caused by cavitation lead to increase in volume which is taken by proteins and as a consequence increase in consistency coefficients have been observed. After addition of milk powder or sucrose with ultrasound treatment there is obvious statistically significant decrease in initial freezing and melting temperatures.

Reološka, funkcionalna i termofizička svojstva sustava proteina sirutke tretiranih ultrazvukom s dodatkom saharoze ili mlijeka u prahu

Sažetak

Ultrazvuk je novija netoplinska metoda tretiranja hrane, te ima veliki potencijal primjene u prehrambenoj industriji. Svrha ovog istraživanja je utvrditi utjecaj ultrazvuka na fizikalna svojstva modelnih sustava pripremljenih s izolatima (WPI) i koncentratima proteina sirutke (WPC), sa ili bez dodataka saharoze ili mlijeka u prahu. Takvi se sustavi obično koriste kod mliječnih napitaka na bazi mlijeka. Modelni sustavi s proteinima sirutke i dodacima saharoze ili mlijeka u prahu tretirani su ultrazvukom visoke snage (HPU) frekvencije 30 kHz kroz 5 i 10 minuta. Različiti parametri su ispitivani kao što su: topljivost proteina, svojstva pjenjenja i emulgiranja, te termofizikalna svojstva. Tretman ultrazvukom pokazao je veliki utjecaj na sva ispitivana svojstva, zbog denaturacije proteina uzrokovane kavitacijom i učinkom

mikrostrujanja. Pokazalo se smanjenje u topljivosti proteina kod WPI i WPC sustava, u usporedbi s netretiranim uzorcima. Pokazano je značajno povećanje u svojstvima pjenjenja nakon tretmana ultrazvukom kod sustava pripremljenih sa saharozom ili mlijekom u prahu, te WPI-a. Također je pokazano smanjenje emulgirajućih svojstava kod sustava pripremljenih samo s izolatima i koncentratima. Nakon tretmana sustava proteina sirutke (sa ili bez dodataka mlijeka u prahu ili saharoze), zamijećene su promjene u koeficijentu konzistencije (k), ali nisu uočene promjene u indeksu tečenja (n). Nakon dodataka mlijeka u prahu ili saharoze, primijećeno je značajno smanjenje inicijalnih temperatura smrzavanja i odmrzavanja uslijed tretmana ultrazvukom

Ključne riječi: proteini sirutke, ultrazvuk, saharoza, mlijeko u prahu, funkcionalna svojstva, reološka svojstva

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