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Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions

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Abstract

The aim of this study was to observe the effect of ultrasound and sonication on whey proteins in order to improve their functional properties. Effect of ultrasound treatment on physicochemical and functional properties was examined by pH, conductivity and solubility measurements and foaming properties.

In this work, low-intensity ultrasound (500 kHz) and the high-intensity ultrasound (20 kHz probe and 40 kHz bath) were used. 10 wt.% protein model suspensions of whey protein isolate (WPI); whey protein concentrate (WPC-60); and whey protein hydrolysate (HWP) were treated with ultrasound probe (20 kHz for 15 and 30 min) and ultrasound baths (40 kHz and 500 kHz for 15 and 30 min).

pH did not change significantly upon ultrasound treatments. Ultrasound affected functional properties (using 20 kHz probe) of whey proteins like solubility and foaming ability by sample exposure at high temperatures caused by sonication. Using ultrasound of 40 kHz frequency had less effect on protein properties and better results were obtained with 15 min treatment than with 30 min treatment. Ultrasound treatment with 500 kHz bath did not had effect on foaming properties of whey protein model solutions. Conductivity decreased for ultrasound treatments with 40 kHz and 500 kHz bath for all samples. Temperature of protein model solutions increased after all ultrasound treatments.

Keywords: Ultrasound treatment; Physicochemical and functional properties; Whey proteins

1. Introduction

Ultrasonic is a rapidly growing field of research and development for the food industry, which can mainly be classified into two fields: high frequency low energy diagnostic ultrasound in the MHz range, and low frequency high-energy power ultrasound. The high frequency ultrasound is usually used as an analytical technique for quality assurance, process control and non-destructive inspection, which has been applied to determine food properties, to measure flow rate, to inspect food packages, etc. (Floros and Liang, 1994; McClements, 1995; Mason et al., 1996; Mason, 1990). Application of the low frequency high-energy power ultrasound in the food industry is relatively new and has not yet been explored until recent years. Various areas have been identified with great potential for future development, e.g. crystallisation, drying, degassing, extraction, filtration, homogenization, meat tenderization, oxidation, sterilization, etc. (Floros and Liang, 1994; Gennaro et al., 1999; Mason, 1998, 1990; McClements, 1995). Ultrasound is also used in the emulsification and dispersing as well as to improve chemical reactions and

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surface chemistry (sonochemistry) or to influence crystallization processes (Knorr et al., 2002).

The beneficial use of sound is realized through its chemical, mechanical, or physical effects on the process or product. In fact, a new branch of chemistry called sonochemistry has been created to take advantage of the chemical effects of ultrasound (Suslick, 1988). General applications include acceleration of conventional and decomposition reactions, degradation of polymers, and polymerization reactions (Floros and Liang, 1994). When particles of material in a liquid suspension are subjected to sonication a number of physical and mechanical effects can result. The mechanical and physical effects of sound are utilized to improve cleaning of surfaces (Mason, 1998). The cavitational effects, which are the basis of sonochemistry, are also the reason for the extremely effective uses of ultrasound for the degassing of liquids. Power ultrasound has proved to be extremely useful in crystallization processes (Mason et al., 1996). The application of ultrasonic waves generating cavitation in suspensions, which contain micro-organisms and enzymes, often has a lethal result and deactivating action (Suslick, 1988).

At present, ultrasound is used in food processing for a number of applications that are not related to food preservation, such as degassing and foam control, mixing, emulsification and meat tenderization. One of the limitations of the use of ultrasound for preservation of foods is that the intensity of ultrasound required achieving microbial inactivation is such that can also have physical effects on foodstuffs. Ultrasound produces cell cavitation, localized heating and can lead to the formation of free radicals.

Ultrasound has been used for many years in the study of proteins (Owen and Simons, 1957; Conway and Verral, 1966; Pavlovskaya et al., 1992; Suzuki et al., 1996). These studies have been used to estimate protein hydration and to infer changes in protein conformation. These parameters may be related to functional properties of proteins in foods such as solubility, foaming capacity and flexibility (Gekko and Yamagami, 1991). Guzey (2001) reported that high-intensity ultrasonic processing improves emulsifying properties of whey protein isolate.

Whey are widely used as ingredients in foods due to their unique functional properties, i.e. emulsification, gelation, thickening, foaming, and fat and flavor binding capacity (Bryant and McClements, 1998; McClements, 1995; Mason, 1998). They are used because of their high nutritive value and GRAS status (Bryant and McClements, 1998). Molecular changes occurring during protein hydrolysis may result in modified techno functional behavior of the hydrolysates compared to the intact protein such as altered solubility, viscosity, sensory properties and foam properties (Panyam and Kilara, 1996; Nielsen, 1997; Caessens et al., 1999).

Solubility is the most practical measure of protein denaturation and aggregation, and, hence, a good index of protein functionality. Generally, proteins that initially exist in a denatured, partially aggregated state often exhibit impaired ability to participate effectively in gelation, emulsification, and foaming (Kinsella, 1976).

Foam formation is governed by three factors: transportation, penetration and reorganization of the molecules on the air/water interface. These processes depend on size, surface hydrophobicity and structural flexibility of the surfactants (Wilde and Clark, 1996).

The aim of this study was to observe the effect of ultrasound and sonication on whey proteins in order to improve their functional properties. Effect of ultrasound treatment on physicochemical and functional properties was examined by pH, conductivity and solubility measurements and foaming properties.

2. Materials and methods

2.1. Materials

Protein powders were purchased as declared by manufacturer (Table 1): whey protein isolate (WPI, BiPRO®, Davisco Foods International, USA); whey protein concentrate (WPC, "Meggle" GmbH, Wasserburg, Germany, WPC-60); whey protein hydrolysate (HWP, BioZate 5®, Davisco Foods International, USA).

2.2. Sample preparation

The model systems marked as WPI, WPC or HWP were aqueous suspensions of powdered whey protein isolate, whey protein concentrate and whey protein hydrolysate containing 10.0% of dry matter. For this purpose appropriate amount of sample were dispersed in distilled water in volume of 100 ml by vigorous hand mixing until homogenous suspensions were obtained. For solubility determination samples were prepared as described in Section 2.5. The protein content is known as declared by manufacturer (Table 1). Temperature of samples was measured before and after ultrasound treatments.

2.3. Ultrasound treatment

2.3.1. Ultrasound treatment with 20 kHz probe

Samples for ultrasound treatment with probe (20 kHz) were placed in 100 ml flat bottom conical flask. Samples were treated for 15 and 30 min with power ultrasound, high-intensity and low frequency, 20 kHz probe (Sonics

Table 1
Protein powder specification declared by manufacturer

Composition (%)	WPI	WPC	HWP
Protein	95	60	94
Fat	1	6	1
Carbohydrate – lactose	1	25	1
Ash	3	6	5
Moisture	5	3	5.5

& Materials Inc., Danbury, CT, USA, Model: V1A, power 600 W) attached to the transducer so that high power intensity can be obtained (Jencons Scientific Ltd. – Ultrasonic processor). Probe has a vibrating titanium tip 1.2 cm and is immersed in the liquid and the liquid is irradiated with an ultrasonic wave directly from the horn tip. In this ultrasonic experiment the ultrasonic intensity was 43–48 W/cm^2, as measured by calorimetry by thermocouple Hanna Instruments, model: HI 9063.

2.3.2. Ultrasound treatment with 40 kHz bath

Samples were placed in 100 ml flat bottom conical flask for ultrasound treatment with bath (40 kHz). Samples were treated for 15 and 30 min, where Erlenmeyer flask was immersed into a 40 kHz bath (Sonomatic, Model SO375T, HF-Pk-power 300 W – overall dimensions: 370 × 175 × 250 mm; internal dimensions: 300 × 150 × 150 mm). An ultrasonic transducer is attached to the outer surface of the liquid container and the liquid is irradiated with an ultrasonic wave from the surface of the liquid container. A standing wave of an ultrasonic wave is formed inside the liquid. The typical acoustic amplitude in a standing-wave type sonochemical reactor is much smaller than that in a horn-type sonochemical reactor (Tuziuti et al., 2002). In this ultrasonic experiment the ultrasonic intensity was 1 W/cm^2, as measured by calorimetry by thermocouple Hanna Instruments, model: HI 9063.

2.3.3. Ultrasound treatment with 500 kHz bath

Samples (100 ml) were placed in 250 ml Erlenmeyer conical flask for ultrasound treatment with high frequency bath (500 kHz). Samples were treated for 15 and 30 minutes with 500 kHz (512 kHz) bath (Undatim Ultrasonics, Model ES01/06/92, power 100 W). In all ultrasonic experiments the ultrasonic intensity do not exceed 0.5 W/cm^2, as measured by calorimetry by thermocouple Hanna Instruments, model: HI 9063.

Ultrasonic power, which is considered as mechanical energy, would partly lose 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).

$$P = m \cdot C_p \cdot (dT/dt) \quad (1)$$

where: m – the mass of the sonicated liquid (g); C_p – its specific heat at a constant pressure (J/gK); and dT/dt – slope at the origin of the curve.

It 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).

Treatments were labeled: No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).

2.4. Temperature changes, pH determination and conductivity determination

Before and after each treatment, temperature of samples has been measured with thermometer and than calculated average increase in temperature after treatment. During ultrasound treatment temperature has been controlled by thermocouple Hanna Instruments, model: HI 9063.

pH of protein model solutions were determined before and after ultrasound treatments for 20 kHz probe, 40 kHz bath and 500 kHz bath, by pH METER, Pye Model 292, Pye Unicam.

Conductivity of protein model solutions was determined before and after ultrasound treatments for 20 kHz probe, 40 kHz bath and 500 kHz bath, by Conductometer (PTI-8 Digital Conductivity Meter), Scientific Industries International Inc., UK. Instrument was calibrated with chemicals for which we knew conductivity.

2.5. Solubility determination

After ultrasound treatment whey protein 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 and the pH adjusted with either 6 N NaOH or 6 N HCl to 7.0. Temperature of samples was 23 °C. Suspensions were centrifuged at 20,000g for 15 min, at 23 °C, and absorbance was measured at 280 nm on a sample aliquot diluted 1:10 (vol/vol) in dissociating buffer (50 mM EDTA, 8 M urea at pH 10). The same procedure was performed for suspensions treated with ultrasound. Solubility was obtained from the absorbance ratio of the supernatant and the suspension before centrifugation (Britten et al., 1994).

2.6. Foaming properties

For foaming properties determination samples were prepared as described in Section 2.2 and than ultrasonically treated as described in Section 2.3. Suspensions were whipped at room temperature with blender (Morphy Richards Go Cordless Rechargeable Multi Tool, Argos, UK) equipped with a wire whip beater at maximum speed setting for up to 15 min to determine maximum foam expansion. Whipping was interrupted after 5 min intervals to determine foam expansion. Foam expansion was determined by level-filling a 100 ml plastic weighing boat with foam and weighing to ±0.01 g. Foam expansion was calculated using the expression:

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

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 (Morr and Foegeding, 1990).

2.7. Statistical analyses

The whole study was repeated and each value represents the mean of three measurements from three 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% ($\alpha = 0.05$), was carried out to assess whether the different treatments conducted to statistically different results for those variables evaluated. The values statistically different are accompanied by the same letter (a) and the values not statistically different with another letter (b).

3. Results and discussion

3.1. pH, conductivity and temperature changes

Values of pH did not change significantly ($p > 0.05$) upon ultrasound treatment with probe and baths (Table 2).

Conductivities were changed significantly ($p < 0.05$) especially for 20 kHz probe treatment, and generally it increased for all samples for 20 kHz treatments (Table 3). For WPI conductivity increased from 1.61 to 1.92 mS/cm, for WPC from 3.87 to 4.62 mS/cm and for HWP from 4.44 to 5.05 mS/cm.

There are many reports, which have proved the formation of hydroxyl radicals during sonication (Petrier et al., 1992; Makino et al., 1983; Hart and Henglien, 1985) which causes increase in conductivity. The high local temperatures and pressures that result from cavitation lead to formation of free radicals and other compounds, so ultrasound can induce oxidant species. This can improve some analytical procedures based on oxidation reactions, as some electron acceptor species can be generated in the sonicated solution (e.g. thermal dissociation into H atoms and OH radicals – the latter forming hydrogen peroxide). In the special case of electrodes, they can be subjected to ultrasound-assisted cleaning either before use or during the electroanalytical step (Banks and Compton, 2003).

Table 2
pH of samples before and after ultrasound treatment

Samples	WPI	WPC	HWP
A	7 ± 0.07	6.1 ± 0.05	7.3 ± 0.01
B1	7.1 ± 0.05^{b}	6.1 ± 0.05^{b}	7.2 ± 0.02^{b}
B2	7.1 ± 0.05^{b}	6.1 ± 0.04^{b}	7.2 ± 0.02^{b}
C1	7.1 ± 0.04^{b}	6.2 ± 0.03^{b}	7.2 ± 0.03^{b}
C2	7.2 ± 0.03^{b}	6.2 ± 0.03^{b}	7.2 ± 0.01^{b}
D1	7.2 ± 0.04^{b}	6.2 ± 0.05^{b}	7.2 ± 0.02^{b}
D2	7.2 ± 0.04^{b}	6.2 ± 0.04^{b}	7.3 ± 0.01^{b}

[a] Significant differences vs control (A) at $p < 0.05$.
No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).
[b] Significantly not different vs control (A) at $p < 0.05$.

Table 3
Conductivity (mS/cm) of samples before and after ultrasound treatment

Samples	WPI	WPC	HWP
A	1.61 ± 0.22	3.87 ± 0.62	4.44 ± 0.61
B1	1.72 ± 0.23^{a}	4.20 ± 0.58^{a}	5.05 ± 0.56^{a}
B2	1.92 ± 0.21^{a}	4.62 ± 0.60^{a}	4.80 ± 0.49^{a}
C1	1.29 ± 0.20^{b}	3.08 ± 0.49^{b}	3.66 ± 0.58^{b}
C2	1.27 ± 0.19^{b}	3.71 ± 0.45^{b}	4.28 ± 0.62^{b}
D1	1.22 ± 0.22^{b}	2.80 ± 0.57^{a}	3.12 ± 0.60^{a}
D2	1.46 ± 0.23^{b}	3.47 ± 0.56^{b}	3.89 ± 0.58^{b}

No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).
[a] Significant differences vs control (A) at $p < 0.05$.
[b] Significantly not different vs control (A) at $p < 0.05$.

Conductivity decreased for ultrasound treatments with 40 kHz and 500 kHz bath for all samples (Table 3). For WPI conductivity decreased from 1.61 to (1.27 mS/cm for 40 kHz bath and 1.22 mS/cm for 500 kHz bath), for WPC from 3.87 to (3.08 mS/cm for 40 kHz bath and 2.80 mS/cm for 500 kHz bath) and for HWP from 4.44 to (3.66 mS/cm for 40 kHz bath and 3.12 mS/cm for 500 kHz bath).

The decrease in conductivity is due to the presence of ion aggregates which do not take part in the conduction process, along with an increase in viscosity which would also result in lower conductivity (Bohnke et al., 1993; Southall et al., 1996). Also, this can be explained on the basis of the fact that the active cavitational area in the case of bath is much more (surface area of US irradiating face of bath is 56 times more than the surface area of the irradiating face of horn) than in case of horn. The major result of ultrasound treatment is generation of free radicals and decomposition of water where created ions take part in reactions. Higher values of conductivity for 30 min treatments results from longer exposure time of sample to the ultrasound.

Measurements of samples temperature are shown in Table 4. One can observe that highest temperature of sample is obtained after ultrasound treatment with 20 kHz probe where temperature increased up to 43–45 °C that is significantly lower than denaturation temperature of proteins (Giroux and Britten, 2004). For 40 kHz bath and 500 kHz bath temperature increased up to 27–30 °C. This is logical because highest input of energy is with 20 kHz probe, and excess energy is liberated as increase in temperature. From Table 4, one can observe that highest average

Table 4
Temperature of samples (°C) before and after ultrasound treatment, and average increase in temperature

Samples	WPI	WPC	HWP	Average increase
A	23 ± 0.02	23 ± 0.01	23 ± 0.02	
B1	42 ± 0.01[a]	42 ± 0.02[a]	43 ± 0.03[a]	19.3
B2	43 ± 0.01[a]	45 ± 0.03[a]	45 ± 0.01[a]	21.3
C1	28 ± 0.01[b]	25 ± 0.03[b]	28 ± 0.01[b]	4.0
C2	34 ± 0.03[b]	31 ± 0.02[b]	30 ± 0.03[b]	8.7
D1	24 ± 0.03[b]	24 ± 0.01[b]	27 ± 0.01[b]	2.0
D2	27 ± 0.02[b]	28 ± 0.02[b]	29 ± 0.02[b]	5.0

No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).
[a] Significant differences vs control (A) at $p < 0.05$.
[b] Significantly not different vs control (A) at $p < 0.05$.

increase in samples temperature is after ultrasound treatment with 20 kHz probe for 30 min and it is 21.3 °C, than with 40 kHz bath for 30 min (4.0–8.7 °C) and the lowest is after treatment with 500 kHz bath (2–5 °C). This can be explained by the fact that suspension were treated with 20 kHz probe are the most exposed to high power that those treated with 500 kHz bath and 40 kHz bath. Also, the way of treatment is different. At probe treatment horn is inserted in suspension which favors contact between tip and sample, whereas at baths suspensions were inserted in baths with flask so there was not direct contact with irradiating surface.

3.2. Solubility determination

Solubility increased significantly for all samples for 20 kHz probe, 40 kHz and 500 baths except for WPC. Highest increase was after 20 kHz probe and 40 kHz treatment for 15 min, for WPI from 66.8% to 85% and 84%, and after 40 kHz bath treatment for 15 min for HWP from 72.1% to 82% (Table 5). Solubility didn't change significantly for WPC samples because of different powder composition (Table 1) and probably due to significant amount of lactose in WPC, which similar to other disaccharides exhibited protective effect like during pressurization treatment (Dumay et al., 1994).

Table 5
Solubility (%) before and after ultrasound treatment

Samples	WPI	WPC	HWP
A	66.8 ± 1.8)	92.2 ± 1.45	72.1 ± 1.14
B1	85 ± 1.68[a]	96 ± 1.64[b]	71 ± 1.34[b]
B2	68 ± 1.23[b]	95 ± 1.56[b]	79 ± 1.36[b]
C1	84 ± 1.45[a]	94 ± 1.34[b]	82 ± 1.22[a]
C2	79 ± 1.56[b]	93 ± 1.05[b]	72 ± 0.32[b]
D1	70 ± 1.34[b]	93 ± 0.96[b]	74 ± 1.64[b]
D2	71 ± 1.18[b]	93 ± 1.16[b]	73 ± 1.56[b]

No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).
[a] Significant differences vs control (A) at $p < 0.05$.
[b] Significantly not different vs control (A) at $p < 0.05$.

The high-intensity ultrasound enhances protein solubility by changing protein conformation and structure in the way that hydrophilic parts of amino acids from inside are opened toward water (Morel et al., 2000; Moulton and Wang, 1982; Wang, 1975). This treatment also led to decreases in protein molecular weight implying that larger area of protein is covered by molecules of water (Morel et al., 2000). The increased temperature after treatment also contribute to enhanced solubility since in general protein solubility increases with temperature between 40°C and 50°C which is the case using ultrasound horn (probe).

The high degree of solubility implies that the solubility of any of the tested whey products should not affect any of the other functional properties. It was revealed (Cheftel, 1992) that the major protein component can primarily determine the functional behavior of WPI. β-lactoglobulin is less affected under the same heating conditions, and α-lactalbumin is the most resistant of the whey protein fraction and they are both constituents of whey proteins.

The solubility increase could be attributed also to the changes in the three-dimensional structures of globular protein resulted in increased number of charged groups (NH_4^+, COO^-) confirmed with higher electrical conductivity than that of control sample. In those conditions the protein–water interactions increase, because the electrostatic forces are higher and more water interacts with the protein molecules.

3.3. Foaming properties

Foam capacities and foam stabilities were improved after ultrasound treatments for both 20 kHz and 40 kHz treatments for whey proteins (Table 6). Foam capacities were increased significantly after 20 kHz probe treatment for 15 min, and 40 kHz bath/15 min, for WPI (132% to 235% and 220%) and WPC (124% to 221% and 209%) model samples. No improvement in foaming properties for protein model suspensions for 500 kHz treatments was observed. Foam stabilities were improved for all samples after 20 kHz probe, having highest increase for WPI (68.3–98.4 min) and WPC (55.6–89.5 min) samples, and after 40 kHz bath treatments having highest increase for HWP (65.7–85.6 min) samples. For 500 kHz bath no improvement were observed (Table 6).

Hydrolysis of whey protein generally resulted in increased foam-forming ability of the hydrolysates compared to the parental proteins (Britten et al., 1994; Ludwig et al., 1995; Lieske and Konrad, 1996; Caessens et al., 1999).

The larger increases of foaming power observed might be explained by the homogenization effect of ultrasound. 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 property. During ultrasound treatment proteins probably became partially

Table 6
Foaming properties: foam capacity (%) and foam stability FS (min) of samples before and after ultrasound treatment

Samples	WPI	WPC	HWP
	Foam capacity (%)		
A	132 ± 1.3	124 ± 1.43	168 ± 1.43
B1	235 ± 3.43[a]	221 ± 1.56[a]	175 ± 3.56[b]
B2	135 ± 2.40[b]	198 ± 2.64[b]	178 ± 3.24[b]
C1	220 ± 1.45[a]	209 ± 2.23[a]	197 ± 2.78[a]
C2	198 ± 1.36[b]	180 ± 1.45[b]	165 ± 2.23[b]
D1	140 ± 2.53[b]	167 ± 3.56[b]	178 ± 2.45[b]
D2	143 ± 1.35[b]	165 ± 3.47[b]	167 ± 3.23[b]
	Foam stability FS (min)		
A	68.3 ± 1.56	55.6 ± 2.02	65.7 ± 2.88
B1	98.4 ± 1.75[a]	89.5 ± 2.22[a]	78.6 ± 2.54[b]
B2	79.5 ± 1.24[b]	79.6 ± 2.45[a]	76.5 ± 2.58[b]
C1	84.6 ± 1.46[a]	86.7 ± 3.56[a]	85.6 ± 1.52[a]
C2	79.6 ± 1.32[b]	75.4 ± 3.45[b]	75.5 ± 1.69[b]
D1	67.8 ± 1.47[b]	57.6 ± 2.36[b]	67.4 ± 2.92[b]
D2	63.6 ± 1.08[b]	56.7 ± 2.65[b]	64.5 ± 2.62[b]

No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2); 40 kHz bath – 15 min (C1); 40 kHz bath – 30 min (C2); 500 kHz bath – 15 min (D1); 500 kHz bath – 30 min (D2).
[a] Significant differences vs control (A) at $p < 0.05$.
[b] Significantly not different vs control (A) at $p < 0.05$.

unfolded in structure which accompanies the increase in foaming power when creating foam afterward with blender. For adsorption on the air/water interface molecules should contain hydrophobic regions (Horiuchi et al., 1978; Turgeon et al., 1992). In whey proteins hydrophobic and hydrophilic amino acids are distributed quite uniformly over the entire protein (Schmidt et al., 1984).

Foam-forming abilities of whey protein concentrates depend on the protein concentration, with a reported optimum of ~10% (Schmidt et al., 1984). These solutions contain besides protein also fat, minerals and lactose. In studies concerning foam and other functional properties of whey protein concentrates and isolates it was shown that these components influence the functional behavior of whey protein (Morr and Ha, 1993; Zhu and Damodaran, 1994).

4. Conclusions

Results of using ultrasound in this work showed that ultrasound with high power (20 kHz) probe has major effect in changing whey protein's functional properties like solubility and foam ability by changing surrounding media of whey proteins like temperature and conductivity. Ultrasound of 40 kHz frequency had less effect on whey protein than 20 kHz probe. Major impact had treatment with 40 kHz bath for 15 min. It affected and decreased conductivity of protein sample, increased solubility and foaming ability of protein. Ultrasound of 500 kHz did not impact functional properties of whey protein like foaming ability, but it affected solubility and conductivity. Values of pH did not change significantly ($p > 0.05$) upon ultrasound treatment with probe and baths. Results of this work are showing several advantages and/or disadvantages of using sonication of proteins in food processing. One can observe that using ultrasound in food processing can lead to several advantages like increased protein solubility, foaming ability etc. Disadvantages may arise when using ultrasound without testing right power for treatment time that may lead to destructive effect of ultrasound like protein denaturation.

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