Influence of innovative technologies on rheological and thermophysical properties of whey proteins and guar gum model systems

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Summary

The aim of this study was to examine the effect of high-power ultrasound (US) and high-pressure processing (HP) on model systems composed of whey protein concentrate (WPC) and whey protein isolate (WPI) with or without guar gum addition. This kind of systems can be found in food production industry so the aim was to use novel food processing technologies to be utilized as a method for products development. Aqueous suspensions (10 g kg^{-1}) of powdered whey proteins were treated with either ultrasound or high pressure. The treatment conditions were as follows: US: frequency of 30 kHz, for 5 and 10 min; HP: pressure intensity 300-600 MPa, for 5 and 10 min. Rheological and thermophysical properties were analyzed after guar gum addition (0.5 g kg^{-1}). Ultrasound treatment showed a significant influence on all examined properties through protein denaturation caused by cavitation and microstreaming effects. High pressure caused significant increase in viscosity and consistency coefficients of model systems with and without guar addition. Significant decrease of initial freezing and initial thawing temperature was observed in all samples. With this research the direct influence of ultrasound and high-pressure treatment on the rheological and thermophysical properties of whey protein isolate and concentrate model systems with or without guar gum was demonstrated.

Key words: high-power ultrasound (US), high-pressure processing (HP), whey proteins, guar gum, rheology, thermophysical properties

Introduction

In food technology today, scientists are investigating the application of new innovative technologies, such as ultrasound and high-pressure treatment, either for new product development or to improve existing technological processes. These newly developed non-thermal food-processing technologies usually focus on the preservation and modification of the functional properties of food components, while keeping food quality attributes.

Ultrasound represents mechanical waves with frequencies above the human hearing threshold (ca. 18 kHz) (Mason, 1998). This method can be classified into 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 a frequency range of 5-10 MHz (Mason, 1998). It is generally used in the diagnostic analysis of food materials. At high intensities (the high-intensity ultrasound uses much higher power

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levels, typically 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 as a food preservation treatment (Entezari et al., 2004). High-intensity ultrasound is used also in food processing operations such as: emulsification, extraction, degassing, filtration, drying, and enhancement of oxidation (Leadley and Williams, 2002; Mason, 1998). High intensity ultrasound, generated by periodic mechanical motions of a probe, transfers ultrasonic energy into a fluid medium and triggers extremely high alterations in pressure, leading to the formation of small rapidly growing bubbles (cavities) (Mason, 1990). These bubbles expand during the negative pressure excursion and implode violently during the positive excursion, generating high temperatures, pressures and shear forces at the probe tip (Suslick, 1988). This phenomenon is known as cavitation. During implosion, very high temperatures (approximately 5500 K) and pressures (approximately 50 MPa) are reached inside these bubbles (Mason, 1990; Mason, 1998; Suslick, 1988), consequently causing several reactions around imploding bubbles. There are four types of cavitation based on the mode of generation: acoustic, optic, particle and hydrodynamic. In food processing application, only acoustic and hydrodynamic cavitation are found to be efficient (Gogate and Kabadi, 2009) because they produce chemical or physical changes in the treated material. There are few papers describing the influence of ultrasound on whey proteins. Several researchers have studied the effect of ultrasound on specific properties of whey proteins (Wang et al., 2008; Jambrak et al., 2008). Some are engaged in the investigation of whey proteins' aggregation (Bryant and McClements, 1998), while others have studied changes in rheological (Meza et al., 2009) or thermophysical properties of whey proteins (Krešić et al., 2008). There are very few papers that examine the effect of ultrasound on the functional properties of whey suspensions with hydrocolloids. For example, group of authors (Gancz et al., 2006) studied the flocculation of whey protein-stabilized emulsions induced by addition of high methoxyl pectin. Since it was shown that ultrasound could be successfully used for modifying solubility, foaming and other functional properties of whey proteins (Jambrak et al., 2008) or soy proteins (Jambrak et al., 2009), it could be

expected that this innovative technology might also be introduced in industrial application for manufacturing creams, pastes and other kinds of products based on different protein sources. The possible usage of this technology in food industry has a good outlook in the future due to the fact that it is less time and energy consuming compared to traditional methods.

High-pressure processing (HP) is a method of food processing in which food is subjected to elevated pressures (up to 87,000 pounds per square inch or approximately 6,000 atmospheres), with or without the addition of heat. It has been used to achieve microbial inactivation (Donsì et al., 2009) or to alter the food attributes with the aim of achieving consumer-desired qualities (Oey et al., 2008). High pressure processing causes minimal changes in the "fresh" characteristics of foods by elimination of thermal degradation. Consequently, in comparison with thermal processing, HP results in foods with fresher taste, and better appearance, texture and nutrition value (Oey et al., 2008). High-pressure processing can be conducted at ambient or refrigerated temperatures, thereby eliminating thermally induced cooked off-flavours.

There has been much research over the past ten years involving the high pressure processing of whey proteins. HP treatment denatures the two most abundant whey proteins, α-lactalbumin (α-la), at pressures higher than 400 MPa, and β-lactoglobulin (β-lg), at pressures higher than 100 MPa, respectively. The majority of denatured β-lg in HP-treated milk associates with the casein micelles, although some denatured β-lg remains in the serum phase or is attached to the milk fat globule membrane; HP-denatured α-la is also associated with the milk fat globules (Huppertz et al., 2005). This treatment dissociates large protein aggregates leading to the unmasking of the buried hydrophobic groups without affecting protein solubility. However, to the best of our knowledge there has not been much research related to high-pressure treated whey proteins in combination with hydrocolloids in which rheological and phase changes properties were investigated.

Whey proteins are used in many food products because of their good functional and physical properties like gelling, emulsification, foaming etc. Whey proteins are also used due to their nutritive value and functional properties. The most important

Table 1. Characteristics of whey protein concentrate (WPC) and whey protein isolate (WPI),
expressed on a dry basis, declared by the manufacturers

Characteristics	WPC	WPI
Component (g kg^{-1})		
Protein	61.3±0.2	97.8±0.2
Ash	4.5±0.1	1.7±0.1
Lipids	7.4±0.2	0.5±0.2
Lactose	26.8±0.2	-
Property		
pH[a]	6.4±0.1	7.1±0.1

[a]reconstituted in distilled water, at 20 °C, to 10 g kg^{-1} protein

functional properties of whey proteins are solubility, viscosity, water holding capacity, emulsification and foaming properties (Bryant and McClements, 1998). Hydrocolloids have a wide application in the production of frozen desserts, ice creams and yoghurts due to their great ability to increase viscosity, to prevent syneresis, to improve foaming properties, and to prevent undesirable crystal growth during freezing (Goff and Sahagian, 1996).

The aim of this study was to examine the effect of ultrasound and high-pressure processing on model systems composed of whey protein concentrate or whey protein isolate and guar gum, and to investigate interactions between proteins and hydrocolloid. Since this kind of system could be commercially used in the food industry, the idea is to propose a possible future application of these new emerging technologies for improving the rheological and thermophysical properties of whey proteins-based model systems.

Materials and Methods

Materials

Protein powders, namely: Whey protein isolates (WPI, BiPRO®, Davisco Foods International, USA) and Whey protein concentrates (WPC, "Meggle" GmbH, Wasserburg, Germany, WPC-60) were purchased as declared by the manufacturer (Table 1). Guar gum (E 412, Supreme Gums Pvt. Ltd., Jaipur, India) was imported and kindly provided by IREKS AROMA d.o.o., Zagreb, Croatia.

Sample preparation

The model systems that were marked as whey protein isolates (WPI) or whey protein concentrate (WPC) consisted of aqueous suspensions of whey proteins containing 10.0 % (w/w) of dry matter. For the preparation of samples before ultrasound or high-pressure treatment, appropriate amounts of samples were dispersed in distilled water in a volume of 100 mL by vigorous hand mixing until homogenous suspensions were obtained. The treatment conditions were as follows: Ultrasound: frequency of 30 kHz, for 5 and 10 min; High pressure: pressure intensity 300-600 MPa, for 5 and 10 min. For ultrasound treatment model systems were labelled by adding 30US mark to WPI or WPC, and 5 or 10 for treatment time. Similar labels 300HP5, 400HP5, 500HP5 and 600HP5 denote high-pressure treatments for 5 or 10 minutes. After high-pressure treatment, when guar gum (0.5 % w/w) was added, samples were labelled by adding +G to the initial label (i.e. WPI300HP5+G or WPC300HP5+G). For ultrasound treated whey protein samples, newly formed model systems with guar gum have been labelled with WPI30US5 or 10+G or WPC30US5 or 10+G. With US and HP treated samples, rheological (section 2.5) and thermophysical (section 2.6) analyses were conducted. Labelling of model systems is summarized in Table 2.

Ultrasound treatment

Ultrasound treatment with 30 kHz probe

Three-hundred mL of sample were placed in 400 mL flat bottom flask and were treated for 5 minutes (30US5) or 10 minutes (30US10) with

Table 2. Composition of model systems prepared with untreated, ultrasound (US) and high pressure (HP) treated whey protein isolate (WPI) and whey protein concentrate (WPC) with or without guar gum addition

Materials	Ingredients (g kg^{-1})				
Samples	Ultrasound treatment 30 kHz (min)	High pressure treatment min (MPa)	Whey protein concentrate (WPC)	Whey protein isolates (WPI)	Guar gum (G)
WPI	untreated			10	--
WPI+G	untreated			10	0.5
WPI30US5	5			10	--
WPI30US10	10			10	--
WPI30US5 + G	5			10	0.5
WPI30US10 + G	10			10	0.5
WPI300HP5		5 (300)		10	--
WPI400HP5		5 (400)		10	--
WPI500HP5		5 (500)		10	--
WPI600HP5		5 (600)		10	--
WPI300HP10		10 (300)		10	--
WPI400HP10		10 (400)		10	--
WPI500HP10		10 (500)		10	--
WPI600HP10		10 (600)		10	--
WPI300HP5+G		5 (300)		10	0.5
WPI400HP5+G		5 (400)		10	0.5
WPI500HP5+G		5 (500)		10	0.5
WPI600HP5+G		5 (600)		10	0.5
WPI300HP10+G		10 (300)		10	0.5
WPI400HP10+G		10 (400)		10	0.5
WPI500HP10+G		10 (500)		10	0.5
WPI600HP10+G		10 (600)		10	0.5
WPC	untreated		10		--
WPC+G	untreated		10		0.5
WPC30US5	5		10		--
WPC30US10	10		10		--
WPC30US5 + G	5		10		0.5
WPC30US10 + G	10		10		0.5
WPC300HP5		5 (300)	10		--
WPC400HP5		5 (400)	10		--
WPC500HP5		5 (500)	10		--
WPC600HP5		5 (600)	10		--
WPC300HP10		10 (300)	10		--
WPC400HP10		10 (400)	10		--
WPC500HP10		10 (500)	10		--
WPC600HP10		10 (600)	10		--
WPC300HP5+G		5 (300)	10		0.5
WPC400HP5+G		5 (400)	10		0.5
WPC500HP5+G		5 (500)	10		0.5
WPC600HP5+G		5 (600)	10		0.5
WPC300HP10+G		10 (300)	10		0.5
WPC400HP10+G		10 (400)	10		0.5
WPC500HP10+G		10 (500)	10		0.5
WPC600HP10+G		10 (600)	10		0.5

power ultrasound. Ultrasound of high intensity and low frequency, 30 kHz probe (Hielscher-Ultrasound Technology, Hielscher Ultrasonics, GmbH, Warthestrasse 21D-14513, Teltow, Germany, UP100H (100W, 30 kHz)) was used for treatments. The probe had a vibrating titanium tip of 10 mm in diameter and it was immersed in the liquid in the depth of maximum 20 mm. The liquid was irradiated with an ultrasonic wave directly from the horn tip. The temperature of the model systems was 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 being 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 following equation (Trujillo and Knoerzer, 2009).

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

where: m - is the mass of the sonicated liquid (g), c_p - specific heat of medium at a constant pressure is dependent on composition and temperature of tested liquid (J (gK)$^{-1}$), dT/dt - slope at the origin of the curve.

Ultrasonic intensity has been measured using calorimetry by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK). Ultrasound intensity was 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}).

High-pressure treatment

For high pressure (HP) treatment, a WPC or WPI dispersion was put into a PET bottle (170 mL, 45´ 135 mm) with a screw cap (internal diameter of 30 mm) and treated with high pressure of 300, 400, 500 and 600 MPa for a holding time of 5 min and 10 min, at 40±2 °C, with a single processor machine (LAB 50, SIG Simonazzi, Parma, Italy) whose details are described elsewhere (Krešić et al., 2006). After the pressure was released, the pressurized sample was quickly removed from the vessel, fro-

zen in a blast freezer at -50 °C and then freeze-dried at 10^{-2} Pa, for 48 h (Pittia et al., 1996). Protein powder was transferred in plastic bags, hermetically closed and stored over dried silica gel until analyzed.

Determination of rheological properties of model systems

Torque measurements were carried out on the model systems using a Rheometric Viscometer (Model RM 180, Rheometric Scientific, Inc., Piscataway, USA) with the spindle (no. 3; $\varnothing$=14 mm; l=21 cm). Shear stress against the increasing shear rates from the lowest value of 0 s^{-1} to 1290 s^{-1}, as well as downwards, was applied. The 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 the 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:

$$\log \tau = \log k + n \log \gamma \qquad (2)$$

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).

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

$$\tau = \eta_{app} \, \gamma \qquad (3)$$

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). The instruments were interfaced with a standard PC and software for data analysis (STEP7/ Win 32, Siemens Energy and Automatisation Inc., Alpharetta, GA, USA). Measurements were performed continuously in the temperature range from -30 °C to 0 °C, with intervals of measurements of 0.01 °C. The apparatus had a high frequency of sampling (10 measurements per second). Distilled water was used as a calibration substance for the static correction (0.88 °C) of the initial freezing point. As

a result, the following temperatures were obtained: initial freezing, initial thawing, peak of the freezing curve and start of the melting.

Statistical analyses

The whole study was repeated, and each value represents the mean of three measurements from two independent treatments. Analysis of variance (ANOVA), using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL) was carried out to assess whether the different treatments were conducted to statistically different results for evaluated variables. The level of significance was 0.05 ($\alpha=0.05$).

Results and discussion

Ultrasound intensity

In ultrasonic treatment with 30 kHz probe, the ultrasonic intensity was 73-78 W cm^{-2}, as measured calorimetrically. The very high ultrasound intensity that was measured resulted in unfolding of protein and breaking of peptide bonds by hydrolysis (Morel et al., 2000). Cavitation phenomena caused the denaturation of proteins in combination with the formation of aggregates that most likely occurs with the formation of intermolecular disulphide bridges through SH/S-S interchange (Jambrak et al., 2008).

Influence of high power ultrasound (US) and high pressure processing (HP) on rheological properties

From the results of rheological properties showed in Tables 3a and 3b, it is obvious that apparent viscosity of untreated model systems at maximal shear rate is small (7.0 mPa s). This could be explained by its compact globular structure, due to which natural whey proteins form suspensions of low viscosity even at concentrations from 40-45%. This gives them great potential to be used in food beverages production (Mulvihill, 1991).

Rheological properties are expressed with consistency coefficient (k) and flow behaviour indices (n), and are adequately described with Ostwald de Wale's power law. After 30 kHz ultrasound treatment of whey protein model systems without guar gum addition (Table 3a), there have been neither significant changes ($p>0.05$) in apparent viscosity nor

in flow behaviour indices (n). But values for consistency coefficients (k) decreased for whey protein isolates model systems after ultrasound treatment for 5 and 10 minutes, (0.041 mPa s^n to 0.0265 mPa s^n) and (0.041 mPa s^n to 0.0203 mPa s^n), respectively. It could be concluded that the ultrasound treated whey protein isolates showed different behaviour, which could be explained by denaturation due to the rapid molecule movement induced with cavitation, followed by microstreaming and unfolding of protein chains which also occurred. Different behaviour was obvious in model systems of whey protein concentrate, where consistency coefficients (k) increased after ultrasound treatment for 5 minute (0.036 mPa s^n to 0.0432 mPa s^n), and then decreased after ultrasound treatment for 10 minutes (0.036 mPa s^n to 0.0254 mPa s^n). These systems showed different behaviour due to different chemical composition, namely the presence of fat, lactose and sucrose, which exhibited a protective effect of cavitation on the denaturation of protein molecules (Dumay et al., 1994). Whey protein isolates (WPI) are composed mainly of proteins (97.8 %), while in whey protein concentrates, (WPC) the share of proteins was about 60 % (Table 1).

However, for ultrasound treated model systems with whey proteins and guar gum, significant increase in apparent viscosity ($p<0.05$) after treatment was observed in comparison with model systems prepared with untreated WPI (Tables 3a and 3b). Ultrasound treatment caused the denaturation of proteins due to very high ultrasound intensity. This can be explained by the phenomenon of swelling of unfolded globular proteins, which thereby led to an increase in the hydrodynamic radius of protein molecule and to greater intermolecular spinning. Consequently, all these caused viscosity increase (Rattray and Jelen, 1995). This phenomenon is reinforced with the presence of guar gum. Guar gum acts as a thickener in food products, such as sauces, salad dressings, and serves as a stabilizer in ice cream. This hydrocolloid increases viscosity through bonding of a high amount of water.

On the contrary, a statistically significant ($p<0.05$) decrease in the consistency coefficient (k) has been observed in model systems prepared with whey protein isolates and guar gum (69.51 mPa s^n to 6.69 mPa s^n) and whey protein concentrates and guar gum (31.0 mPa s^n to 2.61 mPa s^n) (Table 3b).

Table 3a. Values of rheological parameters for model systems prepared with untreated, ultrasound (US) or high pressure (HP) treated model systems of whey protein isolate (WPI) and whey protein concentrate (WPC)

Treatment	Apparent viscosity* μ_{app} (mPa s)	Consistency coefficient k (mPa s^n)	Flow behavior index n	Regression coefficient R^2	Statistical analysis
WPI	7.0±0.2	0.041±0.013	1.724±0.040	0.989	
WPI30US5	6.0±0.1	0.0265±0.010	1.764±0.021	0.995	
WPI30US10	6.0±0.1	0.0203±0.011	1.791±0.024	0.992	
WPI300 HP5	7.4±0.2	0.082±0.012	1.719±0.034	0.997	
WPI400 HP5	8.3±0.1	0.158±0.010	1.547±0.026	0.997	$p<0.05$
WPI500 HP5	9.1±0.1	0.199±0.012	1.536±0.034	0.996	$p<0.05$
WPI600 HP5	10.6±0.2	0.223±0.009	1.508±0.037	0.998	$p<0.05$
WPI300 HP10	7.8±0.2	0.115±0.009	1.693±0.027	0.978	
WPI400HP10	9.5±0.2	0.177±0.011	1.619±0.029	0.997	$p<0.05$
WPI500HP10	10.4±0.1	0.224±0.010	1.567±0.031	0.997	$p<0.05$
WPI600HP10	12.2±0.2	0.264±0.013	1.539±0.034	0.995	$p<0.05$
WPC	7.0±0.1	0.036±0.012	1.739±0.036	0.994	
WPC30US5	7.0±0.2	0.0432±0.012	1.703±0.035	0.993	
WPC30US10	7.0±0.1	0.0254±0.013	1.784±0.036	0.995	
WPC300HP5	8.1±0.2	0.112±0.011	1.612±0.040	0.996	
WPC400HP5	8.5±0.1	0.115±0.010	1.594±0.034	0.995	$p<0.05$
WPC500HP5	9.2±0.2	0.162±0.008	1.521±0.033	0.992	$p<0.05$
WPC600HP5	10.6±0.2	0.189±0.009	1.444±0.025	0.992	$p<0.05$
WPC300HP10	8.3±0.1	0.132±0.007	1.555±0.026	0.999	
WPC400HP10	8.7±0.2	0.153±0.008	1.508±0.027	0.997	$p<0.05$
WPC500HP10	10.0±0.1	0.207±0.009	1.507±0.031	0.996	$p<0.05$
WPC600HP10	11.0±0.2	0.243±0.010	1.500±0.032	0.991	$p<0.05$

*at 1290 s^{-1}

Under the turbulent conditions, which occurred during ultrasound treatment (i.e. homogenization), motion favoured the adsorption of proteins. Consequently, the formation of aggregates prevailed, leading to a decrease of the consistency coefficient (*k*).

After ultrasound treatment, flow behaviour indices increased and the model system remained non-Newtonian. However, the type of flow changed from pseudoplastic (*n*<1) to dilatant (*n*>1). Loss of tertiary structure of globular proteins during denaturation caused by cavitation brought about an increase in the volume that was taken by proteins. This increase in consistency coefficients can also be explained by the ability of newly formed whey protein globules to bind greater amounts of water, because hydrophilic parts of amino acids are oriented towards the outer part of protein molecules (Ipsen et al., 2000).

Treatment with high pressure results in changes of rheological properties of all samples, which is one of the indicators of conformational changes in protein molecules. Kanno et al., (1998) showed that changes in viscosity for whey protein isolate can be used as an index of denaturation caused by high pressure treatment. It was previously shown that loss of tertiary structure of globular proteins, occurring during this treatment condition, was manifested in

Table 3b. Values of rheological parameters for model systems prepared with untreated,   ultrasound (US) or high pressure (HP) treated model systems of whey protein isolate (WPI) and whey protein concentrate (WPC) with guar gum addition

Treatment	Apparent viscosity* μ_{app} (mPa s)	Consistency coefficient k (mPa s^n)	Flow behavior index n	Regression coefficient R^2	Statistical analysis
WPI+G	27.3±0.1	99.41±0.011	0.749±0.028	0.998	
WPI30US5 + G	18.0±0.2	69.51±0.014	0.813±0.034	0.998	$p<0.05$
WPI30US10 + G	16.0±0.2	6.69±0.012	1.113±0.012	0.997	$p<0.05$
WPI300HP5+G	30.2±0.1	144.43±0.011	0.737±0.026	0.999	
WPI400HP5+G	31.4±0.1	197.42±0.010	0.702±0.024	0.997	
WPI500HP5+G	32.8±0.2	261.85±0.009	0.667±0.031	0.979	$p<0.05$
WPI600HP5+G	35.4±0.2	356.72±0.008	0.636±0.032	0.989	$p<0.05$
WPI300HP10+G	31.3±0.1	153.61±0.008	0.712±0.024	0.989	
WPI400HP10+G	31.9±0.2	206.37±0.007	0.689±0.027	0.998	
WPI500HP10+G	33.6±0.2	287.16±0.009	0.664±0.026	0.999	$p<0.05$
WPI600HP10+G	36.5±0.1	358.97±0.010	0.625±0.024	0.999	$p<0.05$
WPC+G	21.4±0.2	45.51±0.011	0.864±0.031	0.999	
WPC30US5 + G	16.0±0.2	31.0±0.008	0.905±0.012	0.992	$p<0.05$
WPC30US10 + G	15.0±0.2	2.61±0.008	1.231±0.013	0.993	$p<0.05$
WPC300HP5+G	24.1±0.2	57.83±0.009	0.831±0.021	0.997	
WPC400HP5+G	24.8±0.1	134.91±0.010	0.742±0.024	0.998	
WPC500HP5+G	26.2±0.2	203.44±0.011	0.667±0.026	0.979	$p<0.05$
WPC600HP5+G	27.8±0.2	319.42±0.011	0.632±0.017	0.988	$p<0.05$
WPC300HP10+G	25.1±0.2	64.15±0.012	0.871±0.015	0.989	
WPC400HP10+G	26.3±0.1	162.21±0.012	0.743±0.016	0.999	
WPC500HP10+G	26.9±0.1	231.72±0.011	0.623±0.021	0.996	$p<0.05$
WPC600HP10+G	28.4±0.2	319.84±0.010	0.635±0.018	0.998	$p<0.05$

*at 1290 s^{-1}

the partial unfolding of protein helix thereby releasing and deliberating newly formed places for water binding. All these described changes were the major reason of viscosity increase (Krešić et al., 2008). Additionally, it could be expected that the increase in viscosity and consistency coefficient (k) would be proportional to amount of denatured proteins, as was confirmed in this paper. The data presented in Table 3a indicate viscosity increase of model system prepared with either WPC (from 7.0 mPa s of untreated WPC to 11.0 mPa s of WPC600HP10) or WPI (from 7.0 mPa s to 12.2 mPa s of WPI600HP10). Interestingly, in systems prepared with proteins itself (WPI or WPC) after high pressure treatment

flow behaviour indices (n) showed non-Newtonian behaviour with dilatant properties ($n>1$), whereas in model systems with guar gum after high pressure treatment flow behaviour indices showed non-Newtonian pseudoplastic behaviour ($n<1$). It could be concluded that high-pressure treatment alone does not change the type of flow behaviour. However, the addition of guar gum caused significant ($p<0.05$) changes in flow behaviour. Also, the observed effect was related to the intensity of applied pressure and treatment time, because a remarkable increase in apparent viscosity and consistency coefficients (k) was achieved by increasing pressure.

Guar gum caused viscosity increase due to its gelling property and its ability to bind a great amount of water (Hansen, 1994). In a protein-polysaccharide complex, polysaccharides often have the role of anionic component, while proteins represent the cationic component of the system. Model systems prepared with guar gum addition into high pressure treated suspensions of whey proteins showed rheological behaviour that can be described as complete compatibility of the ternary system: water-two biopolymers.

The model system prepared with WPC treated at 600 MPa for 10 min and the addition of guar gum had a viscosity of 28.4 mPa s, which was greater compared with model systems consisting of untreated WPC and guar gum whose viscosity was 21.4 mPa s (Table 3b). As presented in Table 3b, the value of the consistency coefficient in the mentioned systems increased almost seven fold (from 45.06 mPa s^n to even 319.84 mPa s^n). A significant increase in viscosity and consistency coefficient can partially be explained by an increase in the viscosity of proteins solely, due to the opening of globular structure caused by high-pressure activity.

The positive cooperation of hydrocolloids has a greater effect due to change in the conformation of protein molecules that affects biopolymer interaction. Taking into account that the carbohydrate structure of guar gum assumes there are no hydrophobic groups, it could be accepted that this hydrocolloid operates through the mechanism of changing rheological properties of water phase between dispersed particles (Dickinson, 2003). Grinberg and Tolstoguzov (1997) have showed that self-organization of proteins assumes that water becomes a poorer solvent for proteins, but remains good for polysaccharides. Therefore, conformational changes and formation of aggregates as a result of high pressure processing (which is to a greater extent expressed in samples containing higher protein amount and treated at higher pressures) has resulted in the presence of a greater amount of water for hydrocolloids interactions. Obviously, this fact further reinforces the previously described compatibility for ternary systems.

From the examination of flow behaviour, it could be concluded that all model systems, ultrasound or high pressure treated, with whey protein isolates and concentrates without guar gum addition have non-Newtonian, dilatant ($n>1$) behaviour. The shear stress *vs*. shear strain relationship is evident in Figures 1 and 2. From the results shown and discussed before, it can be concluded that an increase in shear stress of the model systems prepared with WPI and WPC and guar gum occurred during ultrasound treatment.

Influence of high-power ultrasound (US) and high-pressure processing (HP) on thermophysical properties

Untreated whey protein concentrate (WPC) has a lower initial freezing temperature (-1.18 °C) in comparison with protein isolate (WPI) (-1.01 °C) (Table 4a), due to the greater amount of lactose in dry matter. It is well known that the presence of sugars causes the viscosity increase of the system by modifying the amount of frozen water at a specific temperature, as well as by modifying the temperature of phase changes and inhibiting crystal growth. All these behaviours are consequently the cause of the phenomenon confirmed in our paper as well: that freezing could start at lower temperatures (Goff and Sahagian, 1996). Also, the increase in viscosity is also because of presence of fat which is after ultrasound treatment homogenized thereby causing alternations in rheological properties (Luque de Castro and Priego-Capote, 2007).

From the results shown in Table 4a, it can be noted that, after ultrasound or high pressure treatment of whey protein isolates and whey protein concentrates, the initial freezing and initial thawing temperatures have decreased. This was accompanied by a drop in the temperature at which the peak of the freezing curve occurs and a drop in peak temperature at which melting begins (*data not shown*). This phenomenon was enhanced by all process parameters, namely by pressure increase (from 300 to 600 MPa) or by prolonged treatment time (5 to 10 minutes) in both processing methods. After ultrasound treatment for 10 minutes (30US10) of whey protein isolates (WPI), there was a slight decrease in initial freezing temperature (-1.01 to -1.25 °C) and melting temperature (-6.35 to -6.78 °C).

After guar gum addition into model suspensions of whey protein isolates (WPI) and whey protein
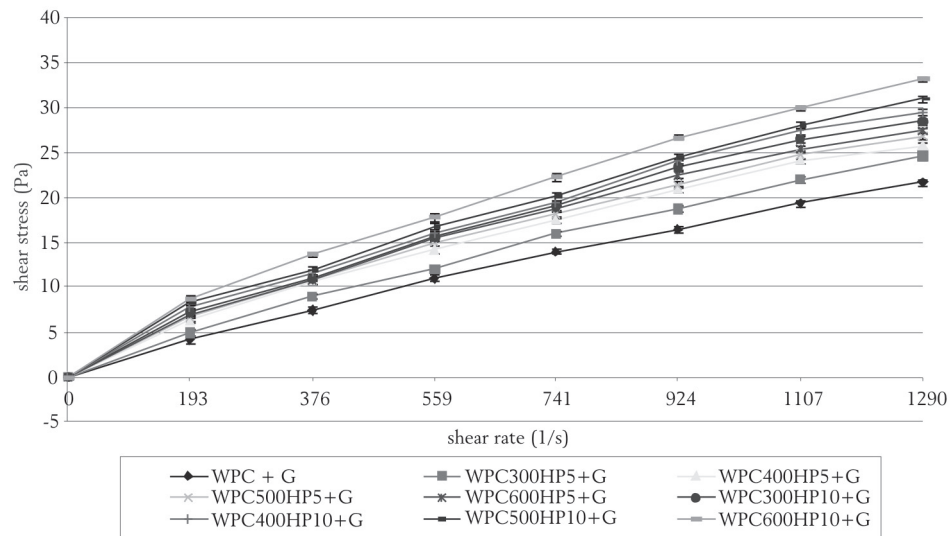
Figure 1a. Shear rate and shear stress relationship of untreated and high pressure treated whey protein concentrate model systems with guar addition
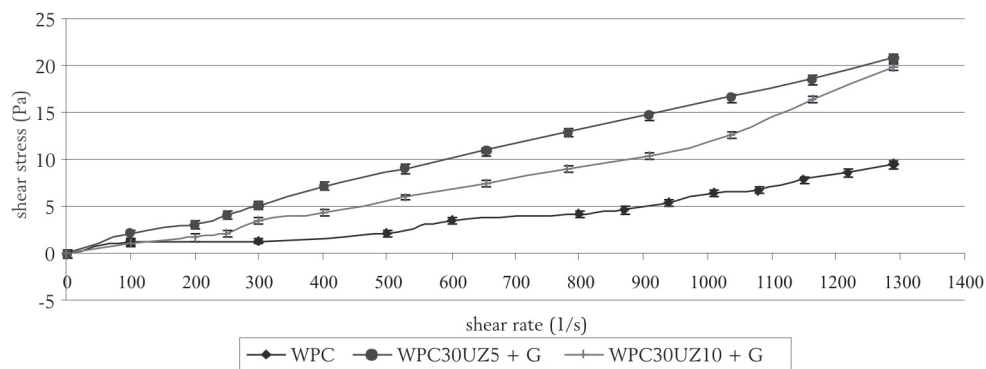

Figure 1b. Shear rate and shear stress relationship of untreated and ultrasound treated whey protein concentrate model systems with guar addition

concentrates (WPC), there was an obvious decrease in initial freezing and thawing temperature as compared with model systems prepared with untreated whey proteins (Table 4b). Also, the values of the mentioned temperatures further decreased with increasing treatment times and increasing intensity of applied pressure. For high-pressure treated WPI suspensions (5 min) with guar gum, there was a decrease in initial freezing temperatures, from 300 MPa to 600 MPa (-2.06 °C to -2.59 °C), and for WPC with guar, from 300 MPa to 600 MPa (-2.34 °C to -2.92 °C) (Table 4b). The shift in initial freezing and melting temperatures was slightly lower for WPC due to the presence of lactose, as lactose showed cryoprotective effect during treatments.

This phenomenon could be explained by the fact that the addition of carbohydrates into the suspension influenced nucleation and formation of ice crystals (Goff and Sahagian, 1996; Tolstoguzov, 2002). Also, the action of ultrasound on model systems caused a decrease in the particle size of proteins (Jambrak et al., 2009) as well as in fat globules, consequently leading to the binding of a greater amount of water onto liberated hydrophilic parts of amino acids. Afterwards this could lead to a decrease of the initial freezing temperature (Xie and Timasheff, 1997).
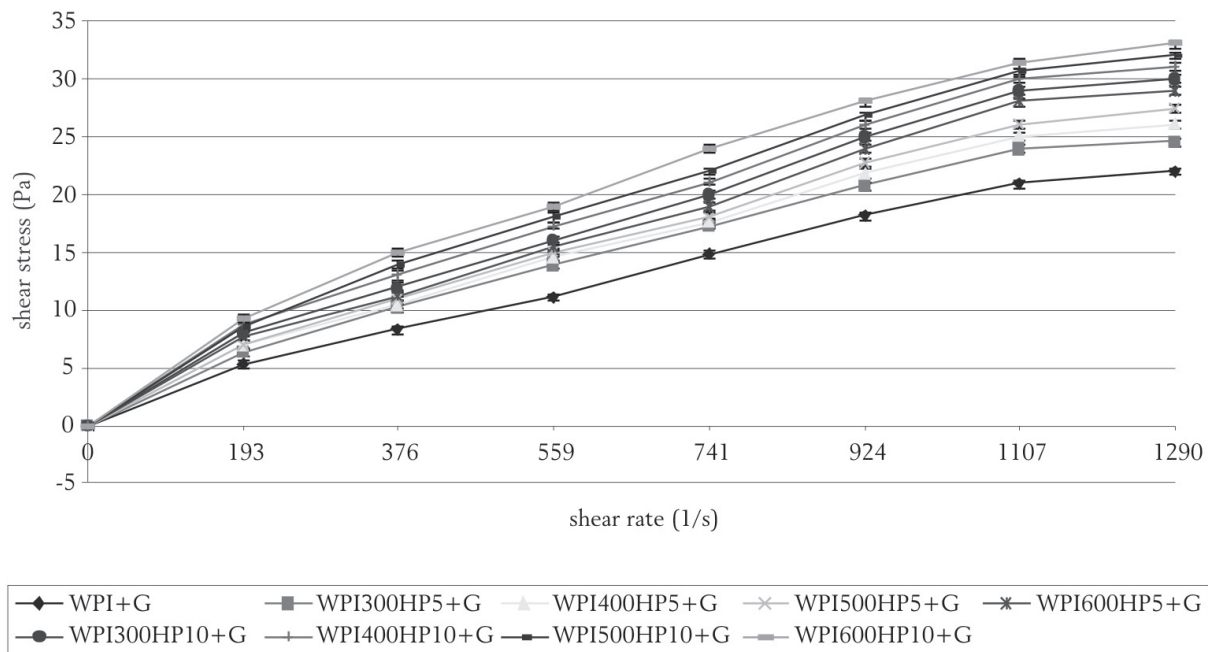
Figure 2a. Shear rate and shear stress relationship of untreated and high pressure treated whey protein isolate model systems with guar addition
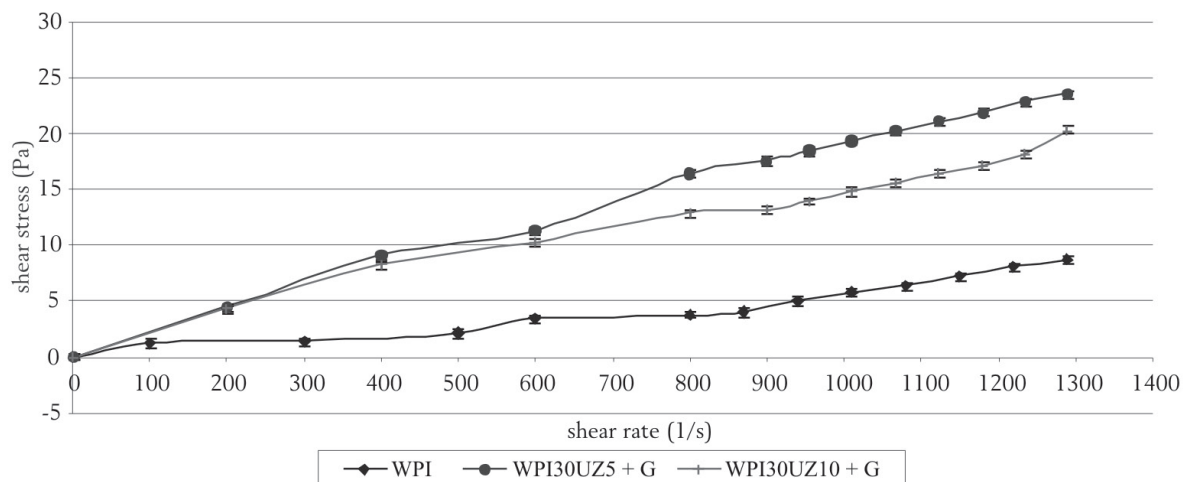

Figure 2b. Shear rate and shear stress relationship of untreated and ultrasound treated whey protein isolate model systems with guar addition

Conclusions

With this research the direct influence of ultrasound and high-pressure treatment on the rheological and thermophysical properties of whey protein isolate and concentrate model systems with or without guar gum was demonstrated. Ultrasound treatment showed a great influence on all examined properties due to protein denaturation through cavitation and microstreaming effects. Consequently, this led to a decrease in consistency coefficients. High-pressure treatment also caused an increase in viscosity and consistency coefficients of all examined model systems. Loss of tertiary structure of globular proteins during high-pressure treatment was obvious through the partial unfolding of protein helix thereby releasing and deliberating newly formed places for water binding.

Table 4a. Freezing and thawing temperature of model systems prepared with untreated, ultrasound (US) or high pressure (HP) treated model systems of whey protein concentrate (WPC) and whey protein isolate (WPI)

Treatment	Initial freezing temperature (°C)	Initial thawing temperature (°C)	Statistical analysis
WPI	-1.01±0.11	-1.09±0.10	
WPI30US5	-1.09±0.12	-1.12±0.13	
WPI30US10	-1.25±0.11	-1.23±0.11	$p<0.05$
WPI300HP5	-1.17±0.14	-1.24±0.10	
WPI400HP5	-1.40±0.13	-1.66±0.10	$p<0.05$
WPI500HP5	-1.74±0.12	-1.98±0.09	$p<0.05$
WPI600HP5	-2.18±0.11	-2.13±0.09	$p<0.05$
WPI300HP10	-1.23±0.10	-1.49±0.08	
WPI400HP10	-1.51±0.11	-1.84±0.11	$p<0.05$
WPI500HP10	-1.92±0.12	-2.02±0.12	$p<0.05$
WPI600HP10	-2.25±0.14	-2.26±0.14	$p<0.05$
WPC	-1.18±0.10	-1.31±0.11	
WPC30US5	-1.19±0.11	-1.37±0.13	
WPC30US10	-1.22±0.13	-1.38±0.11	
WPC300HP5	-1.39±0.12	-1.41±0.10	$p<0.05$
WPC400HP5	-1.48±0.14	-1.54±0.10	$p<0.05$
WPC500HP5	-1.80±0.13	-1.65±0.13	$p<0.05$
WPC600HP5	-2.27±0.14	-2.27±0.12	$p<0.05$
WPC300HP10	-1.52±0.12	-1.68±0.10	$p<0.05$
WPC400HP10	-1.76±0.10	-1.70±0.11	$p<0.05$
WPC500HP10	-2.08±0.11	-1.83±0.14	$p<0.05$
WPC600HP10	-2.48±0.10	-2.35±0.15	$p<0.05$

After ultrasound or high-pressure treatment of whey protein isolates and whey protein concentrates with or without guar gum, a decrease in initial freezing and initial thawing temperature occurred. This phenomenon is enhanced by the extension of treatment times (from 5 to 10 minutes) and by increasing the intensity of high-pressure treatment (from 300 MPa to 600 MPa).

Results obtained confirmed that these two new and innovative technologies have strong potential to improve the usage of whey proteins as functional ingredients in many formulations in food processing.

Utjecaj inovativnih tehnika na reološka i termofizička svojstva modelnih sustava proteina sirutke i guar gume

Sažetak

Svrha ovog istraživanja je ispitati utjecaj ultrazvuka visoke snage (US) i procesiranja visokim tlakom (HP) na modelne sustave sastavljene od koncentrata proteina sirutke (WPC) i izolata proteina sirutke (WPI) sa ili bez dodatka guar gume. Ovi sustavi mogu se naći u prehrambenoj industriji, tako da je cilj ovog rada koristiti nove tehnike procesiranja kao metodu koja se može koristiti u razvoju novih proizvoda. Vodene suspenzije (10 g kg^{-1}) proteina sirutke su tretirane s ultrazvukom ili

Table 4b. Freezing and thawing temperature of model systems prepared with untreated, ultrasound (US) or high pressure (HP) treated model systems of whey protein concentrate (WPC) and whey protein isolate (WPI) with guar gum addition

Treatment	Initial freezing temperature (°C)	Initial thawing temperature (°C)	Statistical analysis
WPI+G	-1.56±0.11	-1.59±0.09	
WPI30US5 + G	-1.47±0.12	-1.59±0.10	$p<0.05$
WPI30US10 + G	-1.61±0.13	-1.63±0.11	
WPI300HP5+G	-2.06±0.11	-1.79±0.12	$p<0.05$
WPI400HP5+G	-2.14±0.10	-1.89±0.11	$p<0.05$
WPI500HP5+G	-2.43±0.12	-2.12±0.10	$p<0.05$
WPI600HP5+G	-2.59±0.14	-2.28±0.13	$p<0.05$
WPI300HP10+G	-2.09±0.12	-1.84±0.11	$p<0.05$
WPI400HP10+G	-2.24±0.13	-1.96±0.12	$p<0.05$
WPI500HP10+G	-2.69±0.11	-2.19±0.10	$p<0.05$
WPI600HP10+G	-2.78±0.10	-2.33±0.10	$p<0.05$
WPC+G	-1.98±0.10	-1.56±0.11	
WPC30US5 + G	-2.03±0.12	-1.60±0.12	
WPC30US10 + G	-2.10±0.11	-1.62±0.12	
WPC300HP5+G	-2.34±0.12	-2.19±0.13	$p<0.05$
WPC400HP5+G	-2.52±0.13	-2.30±0.11	$p<0.05$
WPC500HP5+G	-2.83±0.10	-2.41±0.11	$p<0.05$
WPC600HP5+G	-2.92±0.09	-2.60±0.15	$p<0.05$
WPC300HP10+G	-2.39±0.10	-2.23±0.13	$p<0.05$
WPC400HP10+G	-2.69±0.11	-2.42±0.14	$p<0.05$
WPC500HP10+G	-2.87±0.12	-2.62±0.12	$p<0.05$
WPC600HP10+G	-2.94±0.13	-2.64±0.11	$p<0.05$

visokim hidrostatskim tlakom. Uvjeti tretiranja su bili sljedeći: US: frekvencija od 30 kHz, kroz 5 i 10 min.; HP: intenzitet tlaka 300-600 MPa, kroz 5 and 10 min. Reološka i termofizička svojstva analizirana su nakon dodatka guar gume (0,5 g kg⁻¹). Ultrazvučnim tretmanom pokazan je značajan utjecaj na sva ispitivana svojstva kroz denaturaciju proteina sirutke uzrokovanu putem kavitacije i učinaka mikrostrujanja. Tretman visokim hidrostatskim tlakom uzrokovao je značajno povećanje viskoznosti i koeficijenta konzistencije modelnih sustava sa ili bez dodatka guar gume. Također, zamijećeno je značajno smanjenje početne temperature smrzavanja i odmrzavanja kod svih uzoraka. S ovim istraživanjem pokazan je direktan utjecaj tretmana ultrazvukom i visokim hidrostatskim tlakom na reološka i termofizikalna svojstva modelnih sustava izolata i koncentrata proteina sirutke sa ili bez dodatka guar gume.

Ključne riječi: ultrazvuk visoke snage (US), procesiranje visokim hidrostatskim tlakom (HP), proteini sirutke, guar guma, reologija, termofizička svojstva

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