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Effect of ultrasound treatment on particle size and molecular weight of whey proteins

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ABSTRACT

The aim of this study was to observe the effect of ultrasound on particle size and molecular weight of whey proteins. In this work high-intensity ultrasound (20 kHz probe and 40 kHz bath) were used. 10 wt.% protein model suspensions of whey protein isolate (WPI) and whey protein concentrate (WPC-60) were treated with ultrasound probe (20 kHz for 15 and 30 min) and ultrasound bath (40 kHz for 15 and 30 min). The results of particle size distribution have shown that, after treatment with an ultrasonic probe of 20 kHz, ultrasound caused a decrease in particle size, narrowed their distribution, and significantly increased the specific free surface in all samples. After treatment with ultrasonic bath of 40 kHz, there was a significant reduction in the size of particles. After treatment with probe of 20 kHz there was a significant decrease in molecular weight and protein fractionation. Ultrasonic bath treatment with 40 kHz ultrasound also showed significant changes in the composition of the molecular weight of protein fractions. Prolonged treatment of WPI with ultrasonic bath of 40 kHz encourages the formation of aggregates of molecules.

1. Introduction

Ultrasound frequency can mainly be classified into two fields: high frequency low energy diagnostic ultrasound in the MHz range, and low frequency high-energy power ultrasound in kHz range. 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).

The beneficial use of sound is realised through its chemical, mechanical, or physical effects on the process or product (Suslick, 1988). When particles of material in a liquid suspension are subjected to sonication a number of physical and mechanical effects can result (Mason, 1998). The cavitational effects, which are the basis of sonochemistry, are also the reason for the extremely effective uses of ultrasound (Mason et al., 1996). Application of the low frequency high-energy power ultrasound in the food industry has been explored for last 10 years. Various areas have been shown to be great potential for future development, e.g. microorganisms and enzymes inactivation, crystallization, drying, degassing, extraction, filtration, homogenisation, meat tenderization, oxidation, sterilization, etc. (Gennaro et al., 1999; Mason, 1998; Mason, 1990; McClements, 1995).

Ultrasound is used in food processing for a number of applications that are related to food preservation, modification of molecules, degassing and foam control, mixing, emulsification, meat tenderization, etc. 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. Chandrapala et al. (2011) studied the sonication-induced changes in the structural and thermal properties of proteins in reconstituted whey protein concentrate (WPC) solutions. The enthalpy of denaturation decreased when WPC solutions were sonicated for up to 5 min. Prolonged sonication increased the enthalpy of denaturation due to protein aggregation. Overall, the sonication process had little effect on the structure of proteins in WPC solutions which are critical to preserving functional properties. 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.

Arzeni et al. (2012) studied and comparatively explored the impact of high intensity ultrasound (HIUS) on the functionality of some of the most used food proteins at the industrial level: whey protein concentrate, soy protein isolate and egg white protein.

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The size of aggregates suffered an overall reduction for WPC and SPI. HIUS affected the studied functional properties differently depending on the size and nature of the protein. Jambrak et al. (2008) studied the effect of low-intensity ultrasound (500 kHz) and high-intensity ultrasound (20 kHz probe and 40 kHz bath), on solubility, emulsifying and foaming properties of whey protein isolate (WPI), whey protein concentrate (WPC) and whey protein hydrolysate (WPH). They observed that solubility increased significantly for all samples for 20 kHz probe, 40 kHz and 500 kHz baths except for WPC. Sağlam et al. (2013) studied heat stability and rheological properties of concentrated whey protein particle dispersions in different dispersing media. Whey protein particles were formed using a combination of two-step emulsification and heat induced gelation. Heat treatment did not significantly alter the zeta potential of the particles, whereas the size of the particles increased after heating due to swelling. The results show that swelling of the particles plays a significant role in the heat stability and rheological properties of these dispersions. Martini et al. (2010) studied power ultrasound (US) to decrease the turbidity of whey suspensions. This research shows an approximately 90% decrease in turbidity when US was applied. The greatest decrease in turbidity was observed when US was applied for 15 min using 15 W of electrical power at 60 °C.

Whey are widely used as ingredients in foods due to their unique functional properties, i.e. emulsification, gelation, thickening, foaming, and fat and flavour binding capacity (Bryant and McClements, 1998; McClements, 1995; Morr and Ha, 1993). They are used because of their high nutritive value and GRAS status (Bryant and McClements, 1998).

The aim of this study was to observe the effect of ultrasound on particle size and molecular weight of whey proteins as a function of ultrasound intensity and frequency of ultrasound, and also to compare sonication and its effects at a frequency of 40 kHz and 20 kHz.

2. Materials and methods

2.1. Materials

Protein powders were purchased as declared by manufacturer (Table 1):

Whey protein isolates (WPI, BiPRO®, Davisco Foods International, USA); Whey protein concentrates (WPC, »Meggle« GmbH, Wasserburg, Germany, WPC-60).

2.2. Sample preparation

The model systems marked as WPI or WPC were aqueous suspensions of powdered whey protein isolate and whey protein concentrate containing 10.0% of dry matter. For this purpose appropriate amount of WPI or WPC powder sample were dispersed in distiled water in volume of 100 mL. Each was dissolved in distiled water (temperature was 23 °C) by gentle magnetic stirring for 30 min to provide a 10% powder (w/w) dispersions and allowed to stand overnight at 4 °C for complete hydratation until homogenous suspensions were obtained. By measuring solubility it is possible to predict the protein/solvent, protein/protein and solvent/protein interactions which determine the functionality of whey proteins (Webb et al., 2002). The high values for solubility in water of control samples under investigation (89.2 g/100 g for WPC and 96.6 g/100 g for WPI, respectively) revealed the high proportion of native whey proteins warranted the broad commercial usage of investigated samples. The protein content is known as declared by manufacturer (Table 1). Temperature of samples was measured before and after ultrasound treatments.

Table 1
Protein powder specification declared by manufacturer.

Composition (%)	WPI	WPC
Protein	95	60
Fat	1	6
Carbohydrate–lactose	1	25
Ash	1	6
Moisture	2	3

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 & 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 power measurements

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 Maltsev, 1969; Margulis and Margulis, 2003).

$$P = m \cdot Cp \cdot (dT/dt)_{t=0} \quad (1)$$

where m is the mass of the sonicated liquid (g), Cp its specific heat at a constant pressure (J/gK), and dT/dt is the 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 labelled:

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).

2.4. Temperature changes

Before and after each treatment, temperature of samples has been measured with thermometer and then calculated average