LWT - Food Science and Technology 43 (2010) 254–262

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Ultrasonic effect on physicochemical and functional properties of α-lactalbumin

Anet Režek Jambrak [a,*], Timothy J. Mason [b], Vesna Lelas [a], Greta Krešić [c]

[a] Faculty of Food Technology and Biotechnology, University of Zagreb, 10000 Zagreb, Pierottijeva 6, Croatia
[b] Sonochemistry Centre, Faculty of Health and Life Sciences, Coventry University, Priory Street, Coventry CV1 5FB, UK
[c] Faculty of Tourism and Hospitality Management, Department of Food and Nutrition, University of Rijeka, Primorska 42, P.O. Box .97, 51410 Opatija, Croatia

ARTICLE INFO

Article history:
Received 15 December 2008
Received in revised form
24 August 2009
Accepted 1 September 2009

Keywords:
Ultrasound treatment
α-Lactalbumin
Physicochemical properties
Functional properties
Particle size
Molecular weight

ABSTRACT

Ultrasound is the sound whose frequency is too high for humans to hear which is within the frequency range of 20 Hz–20 kHz, and the frequency of ultrasound is above 20 kHz. The aim of this study was to observe the effect of ultrasound and sonication on α-lactalbumin (α-LA) with a view to improving its physicochemical and functional properties. In this work both low-intensity ultrasound (500 kHz bath) and the high-intensity ultrasound (20 kHz probe and 40 kHz bath) were used. Ten per cent wt ($g\,g^{-1}$ dry matter) protein model suspensions of α-lactalbumin (α-LA) 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).

Changes in pH values, electrical conductivity, solubility measurements, foaming properties, as well as rheological and freezing-thawing properties have been examined. The protein fractions of α-lactalbumin were analyzed before and after ultrasound treatment by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

The result showed that pH did not change significantly upon ultrasound however conductivities increased significantly after 20 kHz sonication. Electrical conductivity decreased significantly for ultrasound treatments in baths at 40 kHz and 500 kHz for all samples. Solubility increased significantly for all samples at 20 kHz. Foam capacities and foam stabilities were improved after ultrasound treatments for both 20 kHz and 40 kHz treatments. Foaming properties were not improved for protein model suspensions for 500 kHz treatments. The molecular weight of the protein decreased significantly after ultrasound treatments both using a 20 kHz probe and 40 kHz bath. The flow behaviour of α-lactalbumin was observed to be shear-thickening after all treatments. Apparent viscosity data calculated with power law equation ($R^2 = 0.983–0.999$) have not been changed significantly after all treatments. A remarkable decrease of initial freezing point was obtained after 20 kHz treatments.

1. Introduction

Application of the low frequency high-energy power ultrasound (10–1000 W cm^{-2}, with the frequency range from 20 to 100 kHz) in the food industry is relatively new and has not yet been explored until recent years (Mason, 1998; McClements, 1995). Various areas have been identified with great potential for future development, e.g. freezing (Aparicio, Otero, Guignon, Molina-García, & Sanz, 2008), drying (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008), extraction (Vilkhu, Mawson, Simons, & Bates, 2008), sterilization (Cameron, McMaster, & Britz, 2008) etc. For these reasons high-intensity ultrasound is considered to be a potential unit operation for the non-thermal processing of food. In broader applications ultrasound is also used in emulsification and dispersion, to improve chemical reactions and surface chemistry (sonochemistry) and to influence crystallization processes (Knorr, Ade-Omowaye, & Heinz, 2002).

Ultrasound is able to produce these effects through the physical, mechanical and chemical results of acoustic cavitation a process which involves the formation, growth and violent collapse of small bubbles in liquid as a result of acoustic pressure fluctuation. Cavitation can accelerate chemical reactions, increase diffusion rates, disperse aggregates, break down small particles and polymeric materials such as enzymes and destroy microorganisms. The lower frequency range of ultrasound is normally used to produce cavitation because at very high frequencies, i.e. above 1 MHz, cavitation becomes more difficult and above 2.5 MHz cavitation does not occur (Sala, Burgos, Condon, Lopez, & Raso, 1995).

These higher frequencies (5–10 MHz) are of use as an analytical technique for quality assurance, process control and non-destructive inspection and have been applied to determine food properties,

* Corresponding author. Tel.: +385 1 4605 035; fax: +385 1 4605 072.
E-mail address: arezek@pbf.hr (A.R. Jambrak).

0023-6438/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2009.09.001

to measure flow rate, to inspect food packages, etc. (Mason, 1998; Mason, Paniwnyk, & Lorimer, 1996; McClements, 1995; Mizrach, 2008; Yebra, Cancela, & Cespón, 2008). One example is in the study of proteins (Gekko & Yamagami, 1991; Jambrak et al., 2008; Krešić, Lelas, Jambrak, Herceg, & Brnčić, 2008) with the aim 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 & Yamagami, 1991).

Whey and its derivatives are used as ingredients in foods due to their unique functional properties, i.e. emulsification, gelation, thickening, foaming, and fat and flavor binding capacity (Bryant & McClements, 1998). One of major constituents of whey proteins is α-lactalbumin (21 g per 100 g) and in cow's milk varies from 1.2 to 1.5 g L^{-1}. It has good emulsifying and foaming properties, but poor gelation ability (Guzey & Weiss, 2001; İbanoğlu & İbanoğlu, 1999). It demonstrates a range of immune-enhancing properties as anti-carcinogenic agent and as precursor for bioactive peptides. There are studies on the effect of ultrasound on the functionality of β-lactoglobulin (Jambrak et al., 2008) and whey proteins (Krešić et al., 2008). However, little is known on the effect of ultrasound on the functional properties of α-lactalbumin, which is the subject matter of this paper. Here ultrasonic treatment has been examined in terms of its effects on pH values, electrical conductivity, solubility, foaming properties, emulsifying properties and particle size measurements, as well as on its rheological and thermo-physical properties.

Industrial relevance of ultrasound processing is cost effective, low time consuming and processing is at lower temperature what favors retention of product quality. Aim of ultrasound application is to homogenize product and to obtain better product properties without deterioration its' quality.

2. Materials and methods

2.1. Materials

Protein powder alpha-lactalbumin (α-LA, BioPURE-Alpha-lactalbumin™, Davisco Foods International, USA) was purchased as declared by manufacturer. According to the manufacturers, the typical composition of this powder was 95 g per 100 g protein, 1 g per 100 g fat, 1 g per 100 g carbohydrate (lactose – whey), 1.5 g per 100 g ash and 1.5 g per 100 g moisture.

2.2. Sample preparation

The model systems marked as α-LA were aqueous suspensions of powdered α-lactalbumin containing 10.0 per cent weight (g g^{-1} dry matter) of dry matter. For solubility and emulsifying properties determination samples were prepared as described in Section 2.5.

The following treatment regimes were used in the experiments:

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**).

2.3. 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 (Model V1A, power 600 W, Sonics & Materials Inc., Danbury, CT, USA) attached to the transducer so that high power intensity can be obtained (Ultrasonic processor, Jencons Scientific Ltd., UK). 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 39–44 W cm^{-2}, as measured by calorimetry by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK). Amplitude of the sonotrode was set to 50 per cent.

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) and treated for 15 and 30 min (Model SO375T, HF-Pk-power 300 W- overall dimensions: 370 × 175 × 250 mm; internal dimensions: 300 × 150 × 150 mm, Sonomatic, Warrington, UK). An ultrasonic transducer was attached to the outer surface of the liquid container and the liquid was irradiated with an ultrasonic wave from the surface of the liquid container. In this ultrasonic experiment the ultrasonic intensity was 1–2 W cm^{-2}, as measured by calorimetry by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK).

2.3.3. Ultrasound treatment with 500 kHz bath

The method was the same as in 2.3.2 except that the bath was 512 kHz (Model ES01/06/92, power 100 W, Undatim Ultrasonics, Nivelles, Belgium). In all ultrasonic experiments the ultrasonic intensity did not exceed 1 W cm^{-2}, as measured by calorimetry by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK).

2.3.4. Ultrasound power measurement using calorimetry

Ultrasound transfers through a medium by mechanical vibrations and part of that energy will be lost in the form of heat (Thompson & Doraiswamy, 1999). This provides a method of measuring ultrasonic energy by recording the temperature rise of the medium as a function of time leading to the acoustic power estimation (in W) using Eq. (1) (Margulis & Malt'sev, 1969; Margulis & Margulis, 2003).

$$P = m \cdot c_p \cdot \left(\frac{dT}{dt}\right) \quad (1)$$

where: m is the mass of the sonicated liquid (g), c_p its specific heat at a constant pressure (J $(g\,K)^{-1}$) and dT/dt is the slope of the curve at time 0.

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 $(mL)^{-1}$).

2.4. Temperature changes, pH determination and electrical conductivity determination

Before and after each treatment, temperature of samples has been measured with thermometer and then calculated average increase in temperature after treatment. During ultrasound treatment temperature has been controlled by thermocouple (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK).

The pH values of protein model solutions during treatment were determined using a pH meter (Pye Model 292).

Changes in electrical conductivity were determined using a calibrated PTI-8 Digital Electrical conductivity Meter (PTI-8 Digital Electrical conductivity Meter, Scientific Industries International Inc., UK).