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Comparative study of high intensity ultrasound effects on food proteins functionality

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ABSTRACT

The objective of this work was to comparatively explore 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 (WPC), soy protein isolate (500E) and egg white protein (EW).

10% w/w solutions at pH 6.5–7.1 were treated with HIUS for 20 min, in an ultrasonic processor. The operating conditions were: 20 kHz, 4.27 ± 0.71 W and 20% of amplitude.

Before and after the HIUS treatment, the size of protein particles was measured by static light scattering. The amount of sulfhydryl groups was determined with Ellman's reagent and the surface hydrophobicity by a fluorescence technique.

The effects of HIUS on samples viscosity were determined. The evolution of the elastic (G') and viscous (G'') moduli as well as tan δ were registered upon time and temperature in a controlled stress rheometer.

In general, HIUS promoted a decrease in the consistency index of all protein solutions, mainly of soybean isolate. The gelation performance of EW was not modified by HIUS. However, WPC presented a higher elastic character, but 500E did not show changes upon heating, as it was already denatured before HIUS treatment. The size of aggregates suffered an overall reduction for WPC and 500E, but a slight increase for EW. Sulfhydryl content was unchanged for all proteins after HIUS application but surface hydrophobicity was greatly increased after treatment for all proteins. HIUS affected the studied functional properties differently depending on the size and nature of the protein. This technology could be used to obtain improved functional properties in some protein samples.

1. Introduction

Due to the increasing consumer demand for high quality food, new safe and effective methods of food processing and preservation are being developed. Sound waves are generally considered safe, non-toxic, and environmentally friendly – this gives the use of ultrasound a major advantage over other techniques (Kentish and Ashokkumar, 2011). For the food industry, this new technology could be of significant interest, as it allows "green" chemistry to be conducted, i.e. conducting chemical reactions using environmentally benign solvents and reactants (Weiss et al., 2011). High-intensity ultrasound (HIUS), with a frequency range between 16 and 100 kHz, and 10 and 1000 W/cm^2 of power, has immense potential for a wide variety of applications. The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence (Knorr et al., 2004; O'Donnell et al., 2010). It may cause chemical and physical changes in a viscous medium by cyclic generation and collapse of cavities. The increased pressure and temperature in the vicinity of these cavities is the basis for the observed chemical and mechanical effects. The rapid bubble collapse produces shear forces in the surrounding bulk liquid and these shear forces are strong enough to break covalent bonds in polymeric materials that are dissolved in the bulk phase (Güzey et al., 2006).

HIUS has been applied to accelerate mass transport processes in mixing, drying and extraction, degassing of liquid foods, for the induction of oxidation/reduction reactions, for extraction of proteins, for microbial and enzyme inactivation, for the induction of nucleation for crystallization and is also one of the few methods that allow the preparation of submicron emulsions (Bhaskaracharya et al., 2009; Camino et al., 2009; Knorr et al., 2004). One of the limitations of the use of ultrasound for preservation of food is that the intensity of ultrasound required to achieve microbial inactivation is such that can also have physical effects on foodstuffs (Jambrak et al., 2008). Riener et al. (2009) found that the rubbery aroma

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Nomenclature

HIUS	high intensity ultrasound
WPC	whey protein concentrate
500E	soy protein isolate
EW	egg white protein
G'	storage modulus (Pa)
G''	loss modulus (Pa)
G'_h	storage modulus at heating period (Pa)
G'_c	storage modulus at cooling period (Pa)
tan δ	G''/G'
T_g	gelling temperature (°C)
t_g	gelling time (min)
SH	free sulfhydryl groups (μmol/g soluble solids)
H_0	surface hydrophobicity (a.u.)
DSC	differential scanning calorimetry
T_{onset}	onset temperature (°C)
T_{peak}	peak temperature (°C)
T_{end}	endset temperature (°C)
m	consistency index (mPa.s)
n	flow behavior index
D_{32}	$\Sigma n_i\ d_i^3/\Sigma n_i\ d_i^2$ = volume-surface mean diameter or Sauter diameter (μm)
D_{43}	$\Sigma n_i\ d_i^4/\Sigma n_i\ d_i^3$ = volume-mean diameter or De Brouker diameter (μm)
PI	polydispersity index
S	solubility (%)
f	frequency (Hz)
n	frequency dependence of the gel solid character

imparted to milk by sonication was less intense on reducing the sonication power from 400 to 100 W. Thus, ultrasound-induced reactions must be taken into account for the successful introduction of HIUS technology for processing in the food industry.

The application of high intensity ultrasound to modify biopolymers is increasingly being studied. Several works focus on the ability of ultrasound to depolymerise polysaccharides such as dextran, xanthan, lambda-carrageenan, chitosan and starch (Chen et al., 1997; Eschette and Norwood, 2003; Iida et al., 2008; Kardos and Luche, 2001; Liu et al., 2006; Lorimer et al., 1995), which directly impacts on their functional properties. Kardos and Luche (2001) found that ultrasonic radiation offers important potential for the conversion of biomass raw materials, such as polymeric carbohydrates, to useful lower molecular weight particles. Gordon and Pilosof (2010) succeeded in controlling particle size with HIUS by combining different treatment times, temperatures and concentrations of whey protein isolate (WPI) solutions or gels. Zisu et al. (2010) could achieve a reduction in viscosity and an improvement in gelling properties of reconstituted WPC, whey protein retentate prepared by evaporation and ultrafiltration, milk protein retentate and calcium caseinate by sonication in pilot scale reactors operating at a frequency of 20 kHz and varying amplitudes. However, the effects of high-intensity ultrasound on the structural and functional properties of food proteins need to be further studied.

Whey protein concentrates (WPC) and isolates (WPI) are important food ingredients because of their desirable functional properties, such as gelation, foaming and emulsification. Whey proteins are a significant source of functional protein ingredients for many traditional and novel food products (Mishra et al., 2001). The main proteins in whey are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA) and they account for 70% of total whey proteins (Cayot and Lorient, 1997). These proteins are responsible for the functional properties of WPC and WPI, such as solubility in water, viscosity, gelation, emulsification, foaming, color, flavor and texture enhancement and offer numerous nutritional advantages to formulated products (Krešić et al., 2008).

Soybean proteins are widely used in many foods as functional and nutritional ingredients (Vohra and Kratzer, 1991). These proteins are used in a wide range of food applications, including processed meat, nutritional beverages, infant formulas, and dairy product replacement. Glycinin and β-conglycinin, the major components of soybean protein, account for approximately 70% of the proteins in soybeans (Nielsen, 1985). Most of the previous studies have been done using native soy protein isolate, glycinin and β-conglycinin, which give important information on these systems but are not so representative of the behavior of commercially available soy isolates.

Egg white contains as many as 40 different proteins, among them, ovalbumin (54%), conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%) are the main proteins involved in the heat induced gelation (Cheftel et al., 1989). Of the major proteins in egg white, conalbumin is the most heat sensitive. Thus, denaturation of conalbumin, which occurs at approximately 63 °C, is ordinarily the process that limits the heat-treatment to be given to egg white since, upon denaturation, conalbumin becomes insoluble. The thermolability of conalbumin may be ascribed to the absence of disulfide bonds in the molecule. On the other hand, the higher stability of lysozyme might be due to the compactness of this protein, since it has four disulfide cross-linkages and no free thiol group (Perez and Pilosof, 2004). Ovalbumin is the main constituent responsible for the gelling properties of egg white (Mine, 1995). This protein is a monomeric phosphoglycoprotein of 45 kDa, containing 1 disulfide bond and 4 SH groups buried within the core of the protein that become exposed upon heating, leading to intermolecular reactions that stabilize the gel structure. The denaturation temperature of ovalbumin is close to 84 °C (Fernandez-Diaz et al., 2000).

In the present study, the tests were conducted on commercial samples of proteins which are often used by food manufacturers as ingredients of many food products in order to assess the industrial and technological relevance of the application of ultrasound on complex food protein systems. On the other hand and from a more basic point of view, the knowledge regarding the impact of HIUS application on commercial protein ingredients is really scarce and these protein isolates and concentrates may behave quite differently from native proteins. Therefore, the objective of this work was to offer a comparative study of the HIUS effects on the molecular and functional properties of three commonly used protein sources in the food industry.

2. Materials and methods

2.1. Materials

The following protein products were used: whey protein concentrate (WPC), from Milkaut, Santa Fe, Argentina; a commercial soybean protein isolate (500E), from Solae, St. Louis, USA; and egg white powder (EW), from Ovoprot, Buenos Aires, Argentina. The protein content (total basis) of each sample was 85.10 ± 1.07; 94.94 ± 0.79, and 88.93 ± 1.18, respectively (N × 6.25) (AOAC, 1980).

WPC, 500E and EW powders were reconstituted in double distilled water at room temperature to obtain 10% w/w solutions. Sodium azide (0.02% w/w) was added in order to prevent microbial