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The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates

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ABSTRACT

The effect of ultrasound upon the physicochemical and emulsifying performance of wheat protein isolate (WhPI) and soy protein isolate (SPI) was investigated. Protein solutions (0.1–3 wt. %) were sonicated with an acoustic intensity of ~34 W cm^{-2} for 2 min. The physicochemical properties were assessed in terms of changes in protein aggregate size, hydrodynamic volume and molecular structure. The emulsifying performance of ultrasound treated WhPI and SPI was compared to their untreated counterparts, and a low molecular weight surfactant, Tween 80, for comparative purposes. Ultrasonic processing significantly reduced the aggregate size of both proteins, whilst no reduction in the primary structure molecular weight profile was observed in both instances, ascribed to insufficient energy to hydrolyse the peptide bond. Emulsions prepared with both untreated proteins yielded submicron emulsion droplets (~150 nm) at concentrations $\geq$ 0.75 wt. %. Emulsions fabricated with both sonicated proteins at concentrations <0.75 wt. % demonstrated significantly ($P < 0.05$) smaller emulsion droplets and long term emulsion stability in comparison to their untreated counterparts. This effect is consistent with the observed reduction in the equilibrium value of interfacial tension between untreated and ultrasound treated proteins.

1. Introduction

Proteins are ubiquitously utilised as functional ingredients within the food and pharmaceutical industries for emulsification, foaming, gelation and viscosity enhancement. The functionality of proteins is due to the chemical make-up of these molecules, their unique amino acid sequences (Walstra and van Vliet, 2003). Proteins are of particular interest in food formulations as emulsifying agents, due to their ability to adsorb and form viscoelastic films at oil-water interfaces (O'Connell and Flynn, 2007). Proteins provide several advantages for emulsion droplet stabilisation, such as protein–protein interactions at the interface, and electrostatic and steric stabilisation mechanisms due to the charged and bulky nature of these biopolymers, in comparison to low molecular weight surfactants (O'Sullivan et al., 2014).

Ultrasound is a mechanical pressure wave with a frequency greater than 20 kHz, the threshold for human auditory detection. Low frequency (20–100 kHz), high power ultrasound (10–1000 W cm^{-2}), commonly referred to as power ultrasound, is utilised for the alteration, generations or modification of food microstructures (O'Sullivan et al., 2014). The effects of power ultrasound upon food microstructures are attributed to ultrasonic cavitations, generated by localised pressure differentials over short periods of time (a few microseconds). Ultrasonic cavitations yield localised regions of high hydrodynamic shear and rises in temperature at the site of bubble collapse (~5000 °C) accounting for the observed effect of power ultrasound (O'Sullivan et al., 2016).

Ultrasound treatment has been related to the physicochemical modifications of food proteins. However, few studies detail the effect of ultrasound upon cereal proteins, other than that of Zhang et al. (2011) for wheat gluten and O'Sullivan et al. (2016) for rice protein isolate, both demonstrated that the acoustic energy provided insufficient energy to reduce the molecular weight profile of these cereal proteins. Zhang et al. (2011) studied the effect of ultrasound upon the rheologically behaviour of wheat gluten, both the storage (G′) and loss (G″) modulii decreased, and additionally the foaming capacity and emulsifying performance, both were enhanced. O'Sullivan et al. (2016) reported no significant reduction

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in aggregate size of rice protein isolate, ascribed to insufficient energy to achieve scission of disulphide bonds maintaining the structure of denatured aggregates. However, the effect of ultrasound treatment upon the physicochemical structure of wheat protein and relation to submicron emulsion formation and long term stability with respect to protein concentration has yet to be investigated.

Wheat protein isolate (WhPI) is of particular interest to the food industry, as it is the second most cultivated cereal crop (725 million metric tonnes) after maize (1100 million metric tonnes), and followed by rice (496 million metric tonnes) (FAO, 2015). WhPI is a highly functional ingredient utilised commonly within baked and process foods (Ahmedna et al., 1999). WhPI is extracted from *Triticum aestivum* and is primarily cultivated in the EU, China, India and USA (FAO, 2015). The major protein fractions in WhPI are polymeric glutenins and monomeric gliadins, with minor fractions of albumins and globulins (Kuktaite et al., 2004).

Soy protein isolate (SPI) a food ingredient of great importance, as it is the largest commercially available legume protein source owing to its high nutritional value, current low cost, and a highly functional ingredient due to its emulsifying and gelling capabilities (Achouri et al., 2012; Molina et al., 2002; Sorgentini et al., 1995). SPI, extracted from *Glycine max*, is an oilseed legume grown primarily in the United Sates, Brazil, Paraguay and Uruguay (Gonzalez-Perez and Arellano, 2009). The major protein fractions in oilseed legumes are albumins (2S) and globulins, the dominant fractions of which are glycinin (11S; 300–360 kDa) and β-conglycinin (7S; 150–190 kDa) (Shewry et al., 1995).

In this work, wheat protein isolate (WhPI) and soy protein isolate (SPI) were investigated in order to assess the significance of power ultrasound for the improvement of emulsifying performance. The objectives of this research were to discern the effects of ultrasound treatment upon WhPI and SPI in terms of differences to physicochemical properties, measured in terms of aggregate size, molecular structure and hydrodynamic volume. Additionally, the emulsifying efficacy of WhPI and SPI before and after ultrasound treatment was assessed in terms of initial emulsion droplet size, long term stability and interfacial tension. Oil-in-water emulsions were prepared with either untreated or ultrasound treated WhPI and SPI at different concentrations, and compared between them and to a low molecular weight surfactant, Tween 80.

2. Materials and methodology

2.1. Materials

Wheat protein isolate (Prolite® 100; WhPI) and soy protein isolate (Pro-Fam® 781; SPI) were both kindly provided by Archer Daniels Midland (ADM; Decatur, USA). The protein content of WhPI and SPI was 90 wt. % and 86 wt. %, respectively. The pH of WhPI and SPI at a protein concentration of 1 wt. % was 4.2 ± 0.1 and 6.9 ± 0.1, whereby WhPI possessed a cationic charge (17.4 ± 0.4 mV) and SPI an anionic charge (−35.5 ± 0.6 mV). Tween 80 and sodium azide were purchased from Sigma Aldrich (UK). The oil used was commercially available rapeseed oil. The water used in all experiments was passed through a double distillation unit (A4000D, Aquatron, UK). All materials were used with no further purification or modification of their properties.

2.2. Methods

2.2.1. Preparation of emulsifier solutions

WhPI, SPI and Tween 80 were dispersed in water to obtain solutions within a protein concentration range of 0.1–3 wt. %, and Tween 80 was soluble at the range of concentrations, whereas WhPI and SPI possessed an insoluble component regardless of hydration time. Sodium azide (0.02 wt. %) was added to the solution to mitigate against microbial activity.

2.2.2. Ultrasound treatment of protein solutions

An ultrasonic processor (Viber Cell 750, Sonics, USA) with a 12 mm diameter stainless steel probe was used to ultrasound treat 50 ml aliquots of protein solution in 100 ml plastic beakers, which were placed in an ice bath to reduce heat gain. The protein solutions were sonicated with a frequency of 20 kHz and amplitude of 95% (wave amplitude of 108 μm at 100% amplitude) for up to 2 min. This yielded an ultrasonic power intensity of ~34 W cm^{-2}, which was determined calorimetrically by measuring the temperature rise of the sample as a function of treatment time, under adiabatic conditions. The acoustic power intensity, I_a (W cm^{-2}), was calculated as follows (Margulis and Margulis, 2003):

$$I_a = \frac{P_a}{S_A}, where \quad P_a = m.c_p\left(\frac{dT}{dt}\right) \tag{1}$$

Where P_a (W) is the acoustic power, S_A is the surface area of the ultrasound emitting surface (1.13 cm^2), m is the mass of ultrasound treated solution (g), c_p is the specific heat of the medium (4.18 kJ/gK) and dT/dt is the rate of temperature change with respect to time, starting at t = 0 (°C/s).

The temperature of protein solutions was measured before and after sonication by means of a digital thermometer (TGST3, Sensor-Tech Ltd., Ireland), with an accuracy of ±0.1 °C. Prior to ultrasound treatment, the temperature of protein solutions were within the range of 5–10 °C. After ultrasonic irradiation, the temperature raised to approximately ~45 °C.

2.2.3. Characterisation of untreated and ultrasound treated protein solutions

2.2.3.1. Microstructure characterisation. The size of untreated and ultrasound treated WhPI and SPI were measured by laser diffraction using the Mastersizer 2000 (Malvern Instruments, UK). Protein size is reported as a size distribution. The protein size distributions are reported as the average of three repeat measurements.

2.2.3.2. Molecular structure characterisation. The molecular structure of untreated and ultrasound treated WhPI and SPI was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using a Mini-Protean 3 Electrophoresis System (Bio-Rad, UK). 100 μL of protein solution at 1 wt. % concentration were added to 1 mL of native sample buffer (Bio-Rad, UK) in 2 mL micro tubes and sealed. A 10 μL aliquot was taken from each sample and loaded onto a Tris-acrylamide gel (Bio-Rad, UK; 4–20% Mini Protean TGX Gel, 10 wells). A protein standard (Bio-Rad, UK; Precision Plus Protein™ All Blue Standards) was used to determine the molecular weight of the samples. Gel electrophoresis was carried out initially at 55 V (I > 20 mA) for 10 min, then at 155 V (I > 55 mA) for 45 min in a running buffer (Bio-Rad, UK; 10× Tris/Glycine/SDS Buffer). The gels were removed from the gel cassette and stained with Coomassie Biosafe stain (Bio-Rad, UK) for 1 h and de-stained with distilled water overnight.

2.2.3.3. Hydrodynamic volume characterisation. The intrinsic viscosity (*i.e.* hydrodynamic volume) of untreated and ultrasound treated WhPI and SPI were determined by a double extrapolation to a zero concentration method, as described by Morris et al. (1981), using the models of Huggins' and Kraemer, as follows: