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Dehydrated tofu whey as cryoprotectant in protein-stabilized oil-in-water emulsions

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ABSTRACT

The cryoprotective effect of dehydrated tofu whey (DTW) and sucrose on freeze–thaw stability of oil-in-water emulsions stabilized by soy protein isolate was comparatively evaluated at equivalent carbohydrate content (0.066, 0.33 and 1.5 g/100 g emulsion). The influence of storage time at -18 ± 2 °C for different periods (1–130 days) was evaluated. Emulsion stability was assessed by oiling off and particle size measurements. For unfrozen and freeze-thawed emulsions, flocculation index (FI) was determined from De Brouckere mean diameters ($D_{4,3}$) in the absence and presence of sodium dodecyl sulfate (SDS). Coalescence index (CI) was evaluated through variation of $D_{4,3}$ values before and after freeze-thawing. Control emulsion, without cryoprotectant, was unstable whatever the storage period. Both cryoprotectants improved emulsion stability by decreasing the amount of freezable water. However, the protective effect of DTW was better than that of sucrose. After a prolonged storage (130 days), a high stability to coalescence ($CI < 2$) was observed when DTW was added at the highest concentration. In contrast, sucrose only was effective as cryoprotectant at relatively short periods of storage (<30 days). This study has demonstrated that the addition of DTW is helpful to improve the stability of protein-stabilized o/w emulsions subjected to freeze-thawing.

1. Introduction

Proteins are important ingredients in food systems. Soy proteins play an important role in several food systems due to their high nutritional value and functional behavior (Molina, Papadopoulou, & Ledward, 2001). Soy protein isolates (SPI) are widely used as emulsifiers in food emulsions due to the surface active properties of their constitutive proteins, the storage globulins 7S (β-conglycinin, ~270 kDa) and 11S (glycinin, ~360 kDa). SPI are generally obtained in non-denatured state from isoelectric precipitation by acidifying (pH 4.5–4.8) an aqueous extract of defatted soy flour, and further solubilization and neutralization of precipitate (Sorgentini & Wagner, 1999; Yamauchi, Yamagishi, & Iwabuchi, 1991).

Tofu is generally made from a filtered water extract of whole soybean called soy milk. During the preparation of this product, a cooking process is necessary to eliminate off-flavor components and inactivate the antinutritional factors (Kunitz trypsin inhibitor, KTI and lectin, L). The curd is obtained by coagulation of 7S and 11S globulins, following by molding and pressing to remove whey. The tofu whey (TW) contains the majority of components that remains soluble after coagulation, mainly calcium, oligosaccharides and biologically active proteins (Sobral & Wagner, 2007). Espinosa-Martos and Ruperez (2006) analyzed the carbohydrate composition of TW, reporting the following contents (g/100 g): sucrose, 53, stachyose, 39, xylose, fructose and inulin, 1.0. At present, TW as residual liquid represent an important problem due to its negative environmental impact. A strategy to minimize this impact and allow the utilization of TW in formulated foods should include a concentration stage and the complete denaturation of KTI and L (Sobral & Wagner, 2007). Among the main components of TW, the soybean oligosaccharides have prebiotic effects and studies have shown that their consumption is related to several health benefits, such as lowering blood cholesterol, reducing blood pressure and

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preventing some types of cancer (Robertfroid, 2007). Moreover, thermal denatured KTI and L have a high biological value, similar to that of storage globulins 7S and 11S (Kishi & Inoue, 1987).

Freezing is one the most important preservation methods for maintenance of microbiological and chemical stability of food products (Xiong, 1997). Nevertheless, protein-stabilized oil-in-water (o/w) emulsions are highly destabilized when they are stored at temperatures where the water crystallizes (Ghosh & Coupland, 2008; McClements, 2004). In the absence of cryoprotectants, freeze–thaw stability of o/w emulsions prepared with native and thermally denatured soybean isolates depends on various factors such as protein concentration and sample aging (Palazolo, Sobral, & Wagner, 2011; Palazolo & Wagner, 2010). Indeed, cryoprotectants have been used to prevent cold protein denaturation and losses in functional properties in a wide variety of food systems (Xiong, 1997). In model o/w protein-stabilized emulsions, sugars and polyols improved the freeze–thaw stability (Ghosh, Cramp, & Coupland, 2006; Thanasukarn, Pongsawatmanit, & McClements, 2004). The addition of these low-molecular solutes increases the viscosity of continuous phase, depresses the freezing temperature and modifies both the amount of ice at given temperature and the glass transition. Sugars were reported to exert an inhibiting effect on ice crystal growth (Thiebaud, Dumay, & Cheftel, 2002). Moreover, Carpenter and Crowe (1988) proposed that the cryoprotection of sugars or polyols on isolated proteins can be accounted for by fact that these low molecular solutes are preferentially excluded from contact with the protein surface. Hence, any factor that alters protein stability in non frozen solution will tend to have the same qualitative effect during freeze-thawing (Arakawa, Prestrelski, Kenney, & Carpenter, 2001).

As a consequence of its high sucrose, oligosaccharides and protein contents, this article focuses on the cryoprotective effect of dehydrated tofu whey (DTW) on freeze–thaw stability of model o/w emulsions prepared with SPI and refined sunflower oil. A comparative analysis with sucrose at equivalent carbohydrate content was carried out. The effect of DTW or sucrose concentration in the continuous phase and frozen storage time was evaluated.

2. Materials and methods

2.1. Materials

Defatted soy flour was provided by Solae Latin America (Barueri, SP, Brazil). The soy flour contains 95 g dry solids per 100 g powder and the composition (in dry basis, as given by the producer) was: 56.0 g/100 g crude protein (N × 6.25), 7.0 g/100 g ash, 3.5 g/100 g total lipids and 14.0 g/100 g dietary fiber. Refined sunflower oil (Molinos Cañuelas, Argentina) was purchased in a local supermarket. Tofu whey (TW: 21.2 ± 0.3 g dry matter/L, pH 5.8 ± 0.1) was provided by Soyana S.H. (San Martín, Argentina). Sudan III (CI number 26100) was purchased from Chroma Gesselschaft (Schmidt GmbH & Co., Germany). The chemical reagents used in this work were all of analytical grade.

2.2. Preparation of soybean protein isolates

Soy protein isolates (SPI) was prepared according to experimental procedure reported by Sorgentini and Wagner (1999). The defatted soy flour was extracted for 2 h at 20 °C at pH 8.0 with deionized water (water: flour ratio: 10:1). The mixture was centrifuged at 10,400× *g* for 15 min at 20 °C (Beckman Coulter Avanti J25 centrifuge, Beckman Coulter, USA). The supernatant was adjusted to pH 4.5 with HCl, then kept for 2 h at 4 °C and subsequently centrifuged at 10,400× *g* for 20 min in the same conditions. The precipitate was washed with water, resolubilized in water by neutralization at pH 8.0 with NaOH at room temperature, freeze-dried and finally ground. SPI was stored as a freeze-dried powder at 4 °C and was rapidly utilized in further experiments for avoid the sample aging. The crude protein (N × 6.25), ash and moisture contents (g/100 g) of SPI sample were 90.25, 3.08 and 2.10, respectively.

2.3. Preparation of dehydrated tofu whey (DTW)

Tofu whey (TW) was concentrated using a rotavapor (R-124, Büchi Labortechnik, Switzerland) at 50 °C. In this device, a final pressure of 8.10^3 Pa was reached. The obtained syrup was dried at 50 °C under vacuum (400 Pa) for 4 h. Then, the sample, obtained as sticky scales, was frozen in liquid N_2, ground and dried consecutively at room temperature in desiccators containing silica gel (5 days), CaO (7 days) and finally P_2O_5 (5 days). A fine yellow powder of dehydrated tofu whey (DTW) was finally obtained. The chemical composition of dehydrated tofu whey sample (DTW, g/100 g) was: total carbohydrates, 38.6 ± 3.2, crude protein (N × 6.25), 14.5 ± 0.1, ash, 17.2 ± 0.2 and total calcium, 1.9 ± 0.1. A DSC analysis of proteins isolated from DTW by cold acetone precipitation revealed that KTI and L are totally denatured (Sobral, Palazolo, & Wagner, 2010; Sobral & Wagner, 2009). For DTW, the antitryptic activity (AA) was negligible. AA was determined through the inhibitory effect of an acid extract of the sample (0.05 mol/L HCl, 24 h) on trypsin activity (from porcine pancreas, Sigma Co, USA) at pH 8.0 and 37 °C, using denatured hemoglobin as substrate (Sobral & Wagner, 2009).

2.4. Preparation of aqueous dispersions

SPI aqueous dispersions (2.0 g/100 g) were prepared in 0.1 mol/L sodium phosphate buffer pH 7.0 by magnetic stirring for at least 5 h at room temperature to ensure complete dispersion and hydration. Sodium azide (0.03 g/100 mL) was added in order to avoid the microbial spoilage.

DTW sample was dispersed in 0.1 mol/L sodium phosphate buffer pH 7.0 at 0.4, 2.0 and 9.0 g/100 g by using a magnetic stirrer. The free calcium content was negligible due to phosphate ions (mainly as HPO_4^{2-}) are in excess respect to divalent ion. In the same buffer, three solutions of sucrose were also prepared at an equivalent concentration of total carbohydrates (TCH) respect to those of DTW. These sucrose concentrations were 0.13, 0.66 and 3.0 g/100 g, respectively.

2.5. Preparation of o/w emulsions

A two-step process was used to prepare the stock o/w emulsion. First, SPI aqueous dispersion were mixed with refined sunflower oil (oil mass fraction, $\phi_m = 0.33$) in a high-speed blender (Ultraturrax T-25, IKA Labortechnik, Germany) at 20,000 rpm for 60 s. Coarse emulsion was then re-circulated three times through a twin-stage valve high pressure homogenizer (Panda 2K, GEA Niro Soavi, Italy) to finally obtain the stock o/w emulsion. The homogenization pressure was 40 and 4 MPa in the first and second valve, respectively. Then, diluted o/w emulsions without and with cryoprotectant were prepared using vertical containers with plastic caps. Aliquots (10 g) of stock emulsion were gently mixed with 10 g of 0.1 mol/L sodium phosphate buffer pH 7.0 (control emulsion) or 10 g of aqueous dispersions containing sucrose or DTW at equivalent TCH content (Section 2.4). For diluted emulsions ($\phi_m = 0.165$), the cryoprotectant concentration (g/100 g emulsion) were the following: DTW: 0.2, 1.0 and 4.5; sucrose: 0.066, 0.33 and 1.5, respectively. On these diluted emulsions, the freeze–thaw stability was evaluated.