



# Modification of the soy protein isolate surface at nanometric scale and its effect on physicochemical properties



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## ABSTRACT

The objective of this research was to modify at nanoscopic scale the surface of soy protein isolate (SPI) to improve its physicochemical properties. Nanostructured soy protein isolate (NSPI) was obtained by freezing SPI in liquid nitrogen and subsequent freeze drying. AFM images showed a rough surface for NSPI with valleys and cavities with diameters ranging from 8 to 15 nm. SPI presented a smooth surface and cavities with diameters in the order of 50–150 nm. According to the thermographic analysis, the homogeneous nanocavities formed on the surface by the nanostructuring process, allowed for an orderly arrangement of water molecules and hence a better heat distribution in the NSPI than in SPI. The spin–lattice relaxation times of adsorbed water molecules were lower in NSPI than in SPI at the whole water activity range. Gelling, water holding and oil absorption capacity were higher for NSPI than SPI, and both materials had similar emulsifying capacity.

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## 1. Introduction

Recent research has shown the potential of nanotechnology in a wide range of food applications (Fabra et al., 2013; Quintanilla-Carvajal et al., 2010; Gutiérrez et al., 2008). All major food companies are consistently looking for ways to improve production efficiency, food safety and food characteristics with the ultimate goal of gaining competitive advantage and market segments; therefore for an industry where competition is intense and innovation is vital, nanotechnology has been an aid to have advances in the production and improve the food quality with new functional properties (Cushen et al., 2012). Nanotechnology focuses on the characterization, manufacture, and manipulation of biological and non-biological structures smaller than 100 nm. Food related applications of nanotechnologies offer a wide range of benefits to the consumer. These include a possible reduction in the use of preservatives, salt, fat and surfactants in food products; development of new or improved tastes, textures and mouth sensations through nano-scale processing of foodstuffs (Chaudhry and Castle, 2011). Consequently, interest and activities in this

research area have greatly increased over the past years (Weiss et al., 2006).

Control of food microstructure and structure impacts on many food attributes, including, nutrient accessibility, microbial, chemical and biochemical stability of food, mass transfer and transport phenomena, and food texture (Aguilera, 2000, 2005). There are different studies about particle size reduction in food that have demonstrated unique and novel functional properties (Weiss et al., 2006); however, it is also important to analyze the benefits of the modification of food surface at nanometric scale when creating nanocavities without reduction of particle size. It has been found that the surface energy increases with the inverse of the cavity size, nanostructure with small holes can allow certain molecules to move in the pores and enhance chemical reactions (Ouyang et al., 2009). Pascual-Pineda et al., in 2014 encapsulated carotenoids by using a nanostructured material prepared with alginate/zeolite valfor and another that was non-nanostructured prepared with alginate. The nanostructured material retained more carotenoids than the non-nanostructured one and protected the carotenoids at higher water activities.

The advent of nanotechnology, introducing control over matter at the nanoscopic scale, has produced a new class of materials with novel properties, creating new possibilities in a diversity of domains (Granda-Valdés et al., 2009). In food industry, soy

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## Nomenclature

SPI	soy protein isolate	LGC	lowest gelling capacity
NSPI	nanostructured soy protein isolate	$a_w$	water activity
AFM	Atomic Force Microscopy	Fmax	maximum force
$\Delta T$	maximum temperature–minimum temperature	EC	emulsifying capacity
T1	longitudinal relaxation time	S	solubility
WHC	Water Holding Capacity	$g_n$	standard acceleration commonly used to express the centrifugal acceleration
NMR	Nuclear Magnetic Resonance		
OAC	oil absorption capacity		

proteins have been widely used due to their very mild off-flavor, low cost, high nutritional value and interesting functional properties (Belloque et al., 2002), such as water and oil absorption, ability to form gels, foaming capacity among others. In addition to its high nutritional quality and its excellent processing properties, soy protein has a hypocholesterolemic potential when compared with animal proteins, such as casein, and thus decreases the risk of atherosclerosis (Anderson et al., 1995; Potter, 1995; Sirtori et al., 1995).

Soy proteins contain four protein fractions that are classified according to their sedimentation properties into: 2 s, 7 s, 11 s and 15 s which represent, respectively, 8 g/100 g, 35 g/100 g, 52 g/100 g and 5 g/100 g of the total protein content (Bara et al., 2004; Kinsella, 1979). Soy protein isolate contains 90% protein, its major components being 7 s globulin or  $\beta$ -conglycinin and 11 s globulin or glycinin (Petruccelli and Añon, 1995; Belecicu and Moraru, 2013).

The objective of the present study was to modify the surface at nanometric scale of soy protein isolate (SPI) in order to improve its physicochemical properties.

## 2. Material and methods

### 2.1. Material

Soy protein isolate was acquired from ZAVE S.A. de C.V. (Xalapa, Ver., México). The composition of the soy protein isolate is: 90 g protein/100 g dry solids, 5 g fat/100 g dry solids, 5 g ash/100 g dry solids. Ethanol 99.5% was acquired from REPROQUIFIN S. A. de C. V. (México, D.F.). Liquid nitrogen was obtained in GENEXA (Xalapa, Ver., Mexico).

### 2.2. Nanostructuring of SPI

First, the soy protein isolate was treated with absolute ethanol (99.5%) in a SPI: ethanol ratio of 1:3 (w/v) for 1 h. Then, distilled water was added to the dispersion and it was homogenized at 180 rpm for 5 min, this dispersion was centrifuged (Hettich Zentrifugen, Universal 32R, Germany) at  $1852 \times g_n$  for 10 min three times, in each case the supernatant was discarded and distilled water was added; then, the precipitate was frozen with liquid nitrogen and finally freeze-dried at 0.1 mBar and  $-50^\circ\text{C}$  (Labconco, Look Lyph 4.5, USA) for two days to obtain a dry powder (NSPI).

### 2.3. Water Holding Capacity (WHC)

Water holding capacity was determined using the method of Naczki et al. (1985): 2 g protein sample and 16 mL of distilled water were mixed at room temperature for 5 min by a vortex (Scientific industries, Vortex-2 genie, USA) in a 50 mL centrifuge tube. After

mixing, the pH was adjusted to values of 2, 4, 6, 8 and 10 by addition of NaOH 1N and HCl 1N. The tubes were stirred for 30 min and then centrifuged at  $1361 \times g_n$  for 10 min and the water separated in their supernatants was removed carefully. The water holding capacity was determined by weighing the tubes. WHC was expressed as g of water absorbed per g of protein.

### 2.4. Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was assessed in triplicate using the procedure of Naczki et al. (1985). Five protein samples (2 g) were mixed with canola oil (12 mL) by a vortex during 5, 10, 15, 20 and 25 min in a pre-weighed 50-mL graduated centrifuge tube. After centrifugation at  $1361 \times g_n$  for 15 min, the supernatant was discarded, and the tubes were reweighed. The OAC was expressed using the following equation:

$$\text{OAC} = \frac{(\text{g of protein} + \text{oil}) - \text{g of protein}}{\text{g of protein}} \quad (1)$$

### 2.5. Emulsifying capacity (EC)

The method used was according to Chau et al. (1997). Portions of 100 mL of 2% (w/v) protein suspension were homogenized at 2000 rpm for 2 min using a homogenizer (IKA Eurostar, Euro-STPCV 6000 S1, USA). Afterwards, 100 mL of canola oil were added and homogenized for 1 min. The emulsions were centrifuged in 50 mL-graduated centrifuge tubes at  $605 \times g_n$  for 5 min, and the volume of the emulsion was measured. Emulsifying capacity (EC) was calculated as follows:

$$\text{EC}(\%) = \left( \frac{\text{volume of emulsified layer}}{\text{total volume}} \right) 100 \quad (2)$$

### 2.6. Gel formation capacity

The gel formation capacity of protein was determined by finding the least gelling concentration (LGC) through the method of Aydemir and Yemenicioglu (2013) after modification. For this purpose, protein solutions were prepared in distilled water with concentrations between 1 and 14 g/100 g. All protein solutions were prepared at room temperature in test tubes (1.46 cm in diameter) and heated in a water bath at  $85^\circ\text{C}$  for 30 min. The tubes were then cooled immediately to room temperature and incubated for 2 h at  $5^\circ\text{C}$  for gel formation. The gel formation was detected by observing the flow characteristics of the tube contents when they were turned upside down. The LGC corresponds to the lowest protein concentration (g/100 g) that gave place to hard gel with no falling or slipping of the content by gravity when tubes were turned upside down.

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