Journal of Food Engineering 166 (2015) 38–44

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Amaranth protein films prepared with high-pressure treated proteins

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ARTICLE INFO

Article history:
Received 15 November 2014
Received in revised form 4 May 2015
Accepted 9 May 2015
Available online 20 May 2015

Keywords:
Protein films
Amaranth proteins
High-pressure treatment
Thermal treatment
Protein cross-linking

ABSTRACT

This work studies the effect of using high-pressure modified amaranth proteins in the preparation of edible film and compares the efficiency of high pressure and thermal treatment on the functionality of amaranth protein films. This films were prepared by casting using glycerol as plasticizer from protein dispersions submitted to high pressure treatments of different intensity (0.1, 200, 400 and 600 MPa). Protein dispersions treated with high-pressure were able to form uniform films with better mechanical properties, lower water solubility and water vapor permeability than those prepared from non-treated protein dispersions without modifying its thickness, color and water content, but somewhat more opaque. This could be attributed to structural changes by high-pressure treatment, which favored protein unfolding, increasing protein surface hydrophobicity and the amount of free SH, that were re-associated during film formation producing a higher crosslinking of matrixes that was denoted in a better functionality of films. These films also showed better properties than those prepared with amaranth protein isolates thermally treated.

1. Introduction

In the last decades the technology based on the use of hydrostatic high pressure (HP) has been shown to constitute an adequate option for satisfying the high demand of high quality and minimally processed foods, free of additives and microbiologically safe (Gould, 1995). HP treatment (100–1000 MPa) is known to modify the functional proper- ties of alimentary macromolecules (Farr, 1990; Hayashi, 1995; O'Reilly et al., 2001). In particular HP produces a variable degree of protein denaturation that depends mainly on the pressure level used, leading to aggregation and dissociation of polypeptides, and modifying their surface hydrophobicity, solubility, etc. These effects depend on extrinsic factors such as pH, temperature and ionic strength of the medium (Puppo et al., 2004; Speroni et al., 2010), as well as on intrinsic factors such as the nature and the concentration of the protein. In this way, the effect of high-pressure treatment (100–1000 MPa) on the structure of globular proteins has been widely studied for soy, lupin, wheat, milk and egg albumin proteins (Bouaouina et al., 2006; Chapleau and de Lamballerie-Anton, 2003; Puppo et al., 2005; Van der Plancken et al., 2007; Zhang et al., 2005). Furthermore it has been reported that some of these modified proteins, showed a remarkable improvement in their functionality for example, in its ability to form and stabilize foams, emulsions and gels (Bouaouina et al., 2006; Speroni et al., 2009; Puppo et al., 2005).

Condés et al. (2012) has reported that amaranth isolate proteins were very sensitive to HP treatment, more than others such as soybean proteins, since they suffered a higher degree of denaturation at 200 and 400 MPa, which was accompanied by a decrease in protein solubility and protein dissociation and aggregation via hydrophobic interactions and disulfide bonds.

In a previous work we reported that protein films from native amaranth protein isolates had interesting water vapor permeability but poor mechanical properties, but these last properties could be improved by denaturing proteins partially or totally by thermal treatments prior film formation. The resulting films showed higher tensile strength and lower water solubility but also higher WVP, due to the higher crosslinking of these proteins through hydrogen and disulfides bonds (Condés et al., 2013).

Considering that amaranth protein didn't gel during high-pressure treatment at protein concentrations of filmogenic dispersions (≈5% w/v) (Condés et al., 2012) and that large volumes of sample could be handled by HP, protein modification may be carried out on film forming dispersions, avoiding having to lyophilize and redisolve proteins (as was necessary with heat-treated proteins), what would be a major advantage in processing.

There is no information in the literature on edible film preparation from amaranth protein previously treated with high-pressure.

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http://dx.doi.org/10.1016/j.jfoodeng.2015.05.005
0260-8774/

Therefore, the aim of this work was to study the effect of using high-pressure modified amaranth protein in the preparation of edible film and to compare HP treated protein films functionality with those of thermally treated ones.

2. Materials and methods

2.1. Plant materials

Seeds of *Amaranthus hypochondriacus*, (cultivar 9122) used in this work were obtained from Estación Experimental del Instituto Nacional de Tecnología Agropecuaria (INTA), Anguil, La Pampa, Argentina.

2.2. Flour preparation

Seeds were ground and screened by 0.092 mm mesh. The resulting flour was defatted with hexane at 25 °C for 5 h (100 g/L suspension) under continuous stirring. After drying at room temperature, the flour was stored at 4 °C until used.

2.3. Preparation of amaranth protein isolates

Amaranth protein isolate used in this study was prepared according to Martínez and Añón (1996). Briefly, defatted flour was suspended in water (100 g/L) and pH adjusted to 11.0 with 2 mol/L NaOH. The suspension was stirred for 60 min at room temperature and then centrifuged 20 min at 9000*g* at 15 °C. The supernatant was adjusted to pH 5.0 with 2 mol/L HCl and then centrifuged at 9000*g* for 20 min at 4 °C. The pellet was suspended in water, neutralized with 0.1 mol/L NaOH and lyophilized. Amaranth protein isolate was stored in hermetic containers in a chamber at 4 °C until used. Its protein content, determined by Kjeldahl (AOAC 920.53, 1995), $N = 5.85$ (Segura-Nieto et al., 1994) was 91.1 ± 0.2% w/w of proteins (d.b.), and process yield was 14.1 (2.0 g isolate per 100 g of defatted flour.

2.4. High pressure treatment (HP)

Aqueous dispersions of amaranth protein isolate at 5% w/v were vacuum conditioned in a polyethylene bag and subjected to high-pressure treatment at 200, 400, and 600 ± 5 MPa for 5 min in a 2.0 L reactor unit model FPG 9400:922 (Stansted Fluid Power Ltd, UK) equipped with temperature and pressure regulation. A mixture of propylene glycol and water (30:70) was used as pressure-transmitting medium. The target pressure was reached at 6.5 MPa/s and released at 20 MPa/s. The adiabatic heating was manifested as an increase in temperature that was maximal for 600 MPa. In that case, a transient increase was verified up to 33.5 °C. Conditions of HP processing were chosen in accordance to Chapleau and de Lamballerie-Anton (2003).

2.5. Characterization of high-pressure treated isolates

2.5.1. Differential Scanning Calorimetry (DSC)

A TA Instrument DSC Q100 V9.8 Build 296 (New Castle, DE, USA) was used for these studies. Temperature and heat flow calibration of the equipment were carried out according to ASTM standards, using lauric and stearic acid and indium as standards, respectively. Hermetically sealed aluminum pans containing 10–15 mg of samples (20% w/v of amaranth protein isolates) were prepared and scanned at 10 °C/min over the range of 20–120 °C. Denaturation enthalpies (ΔH_d) and temperatures in the minimum signal of the peak (T_d in °C) were taken from the corresponding thermograms (Universal Analysis V4.2E, TA Instruments, New Castle, DE, USA). Enthalpy values ($\Delta \underline{H}_d$) were expressed as J/g protein, taking into account the dry weight (determined by perforating the pans and heating overnight at 105 °C) and the protein content of sample (Molina et al., 2004).

2.5.2. Determination of protein solubility

Non-treated and HP-treated isolate dispersions were centrifuged at 10000*g* for 20 min at 20 °C. Protein solubility was calculated as:

$$\text{Solubility} = (P/P_{\text{total}}) \times 100 \quad (1)$$

where P is the protein content (mg/mL) of supernatants determined by the Bradford procedure (Bradford, 1976) using bovine serum albumin (p.a., Sigma Chemical Co., St. Louis, MO) as standard; and P_{total} is the total protein content determined by Kjeldahl method (AOAC 920.53, 1995), $N = 5.85$.

2.5.3. Free sulfhydryls

Free SH groups were determined according to the procedure described by Beveridge et al. (1974). Non-treated and HP-treated isolates dispersions were dissolved in a specific buffer (0.086 mol/L Tris buffer, 0.09 mol/L glycine, 0.004 mol/L EDTA, and 8 mol/L urea, pH 8.0), and after 30 min the samples were centrifuged for 20 min at 10,000*g* at 20 °C. Forty μL of Ellman's reagent (4 mg of 5,5′-dithio-bis(2-nitrobenzoic acid)/mL in methanol) (p.a., Sigma Chemical Co.) were added to 1 mL aliquots of the supernatant. Absorbance at 412 nm was determined at different times until the maximum absorbance was reached. A molar extinction coefficient of 13,600 mol/L cm was used. Protein concentration was determined by the Bradford method. Determinations were performed at least twice. The concentration of SH groups was expressed as μmol SH/g of protein.

2.5.4. Surface hydrophobicity

Surface hydrophobicity (H_0) of non-treated and HP-treated isolates dispersions was measured according to Kato and Nakai (1980) using 0.008 mol/L 1-anilino-8-naphthalene-sulfonate (ANS, p.a., Aldrich Chemical Co.) as probe. Protein dispersions were diluted (0.1 g/L) in water. Fluorescence intensity (FI) was measured with an Aminco-Bowman SPF 100 fluorescence spectrometer (Sylver Spring, Maryland, EEUU) at 450 nm (excitation) and 540 nm (emission) wavelengths. The initial slope of the fluorescence intensity versus protein concentration plot was used as an index of H_0. Measurements were performed in duplicate.

2.6. Film formation

Films were prepared by dispersing amaranth protein isolate (5% w/v) and glycerol (1.25% w/v, Anedra, Argentina) in distilled water, or directly adding the same concentration of glycerol to amaranth protein dispersions treated at 200, 400 or 600 MPa. All dispersions were magnetically stirred for 1 h at room temperature, their pH was adjusted to pH 10.5 with 2 mol/L NaOH, and they were stirred again for additional 20 min. Ten mL of each film forming dispersion were poured onto polystyrene Petri dishes (64 cm^2) and dried at 60 °C for 3 h in an oven with air flow and circulation (Yamato, DKN600, USA). The dry films were conditioned at 20 °C and 58% relative humidity in desiccators with saturated solutions of NaBr for 48 h before being peeled from the casting surface for characterization.