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Structural and rheological properties of amaranth protein concentrate gels obtained by different processes

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ABSTRACT

The heat-induced gelation of amaranth protein concentrates (APCs) by three processes was studied. The first was the conventional process for isolating protein (standard process-st), the second included an acid washing step prior to protein extraction (acid washing process-aw) and the third included heating (50 °C) during the alkaline extraction step (heat process-ht). The dispersions (12%, w/v) were heated to 55–90 °C and assessed by rheological measurements made under small deformations, whereas the gels obtained by heating at 70, 80 or 90 °C/30 min were subjected to uniaxial compression measurements (TPA and mechanical properties). The rheological parameters associated with the network structure, elasticity modulus (E) and storage modulus (G'), increased with increasing gelation temperature. For the APCst and APCht gels, protein aggregation occurred in two steps, whereas for APCaw, gelation occurred in a single step. The APCht gels showed the highest fracturability, hardness and adhesiveness, followed by the APCst and APCaw gels ($p < 0.05$). Similar results were obtained for the mechanical properties at fracture. Increasing the heat treatment temperature from 80 to 90 °C resulted in a more structured matrix with greater water-holding capacity as compared to gels obtained at 70 °C, and these properties were influenced by the extraction processes used to obtain the APCs. Heat extraction (APCht) improved the gelation and water-holding properties, whereas the acid treatment had the opposite effect.

1. Introduction

Amaranth proteins are alternative and complementary to conventional protein sources, with the potential for being used as food ingredients in products where gel formation is desirable (Avanza, Puppo, & Añón, 2005a, 2005b; Avanza & Añón, 2007).

Gelation is one of the most important functional properties of proteins, since it affects the texture and structure of foods. Heat-induced gelation of globular proteins involves the partial unfolding of the protein molecules, thus exposing sulfhydryl groups and non-polar internal regions, followed by the formation of aggregates via intermolecular interactions such as hydrophobic and electrostatic interactions, or hydrogen bonds and disulfide bonds (Ikeda & Nishinari, 2001; Clark, Kavanagh, & Ross-Murphy, 2001). In addition to the partial denaturation of the proteins, factors such as pH, ionic strength, temperature and the presence of non-protein components, also influence the gelation process (Avanza & Añón, 2007; Rubino, Arntfield, Nadon, & Bernatsky, 1996).

In a previous work, Bejarano-Luján and Netto (2010) showed that the addition of an acid washing step or mild heating during the alkaline extraction step to the standard process (protein solubilization at pH 9.0, precipitation at pH 4.5 and neutralization) led to different component losses and interactions. The results demonstrated that differences in processing conditions induced changes in the composition and in the extent of denaturation and aggregation/dissociation, which can modify the functionality. The acid washing and thermal processes resulted in concentrates with higher protein contents and whiteness indexes and lower phenolic compounds contents than the standard process. The protein concentrate from thermal process presented the lowest solubility, mainly because of protein aggregation due to heating during the alkaline extraction whereas the resulting concentrate from acid washing process showed the highest protein solubility. Therefore, the present work aimed to study the rheological properties under shear and uniaxial compression of amaranth protein concentrates obtained by alternative processes.

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2. Material and methods

2.1. Material

The amaranth seeds (*Amaranthus cruentus*, BR Alegria variety) were donated by EMBRAPA Cerrados – Planaltina – DF, Brazil. All the reagents used were of analytical or chromatographic grade.

2.2. Preparation of amaranth protein concentrates (APCs)

Whole amaranth flour (WAF) was obtained by grinding the seeds in a blade-mill (model MA630, Marconi, Piracicaba-SP, Brazil), followed by sieving to obtain flour with a granulometry of ≤425 μm. Defatted amaranth flour (DAF) was obtained from the whole flour by treating twice with hexane 1:3 (w/v) for 24 h at room temperature. The solvent was removed by centrifuging at 9000 × *g* for 30 min at 4 °C in a RC5C centrifuge (Sorvall® Instruments Dupont, Wilmington, Germany), and the flour was maintained at room temperature until the complete evaporation of the solvent, after which it was stored at 4 °C until used.

The three processes used to obtain the protein concentrates were described in details elsewhere (Bejarano-Luján & Netto, 2010). Briefly, the first was the conventional process for obtaining protein isolates, in which DAF was dispersed in water (1:10, pH 9.0) stirred for 2 h, the solubilized protein was precipitated at pH 4.5, neutralized, and freeze-dried. The APC resulting from this process was designated as APCst. The second, acid washing process, followed the same steps used in the conventional process, although the DAF was first dispersed (1:10) in water at pH 4.5, stirred for 30 min at room temperature, and this APC was designated as APCaw. The third, heat treatment process, followed the same steps used in the conventional process, however the alkaline extraction was carried out at 50 °C, and the APC obtained from this process was named APCht.

The protein contents of the flours and protein concentrates, as determined by the semi-micro Kjeldahl (AOAC, 2005) method using a conversion factor of 5.85, were 14.9 and 16.5%, respectively, for WAF and DAF. The concentrates showed protein contents of 73.6% for APCst, 75.2% for APCht and 77.8% for APCaw. The fat (Bligh & Dyer, 1959) and ash (AOAC, 2005) contents of all the APCs were similar: 3.0 and 3.5%, respectively. The APCs showed (in dry basis) differences in total carbohydrates 19.6, 15.7, and 17.6%, in starch content (Instituto Adolfo Lutz, 1985); 4.9, 3.3, and 6.4%; and in total phenolics content (Tsaliki, Lagouri, & Doxastakis, 1999) 0.8, 0.5, and 0.4 mg EAG/g of dry matter, for APCst, APCaw and APCht, respectively.

2.3. Differential scanning calorimetry (DSC)

The thermal behavior of the APCs was analyzed using a DSC 2920 (TA Instruments, New Castle, USA) calorimeter. The equipment was calibrated with indium at a heating rate of 10 °C/min. Hermetically sealed aluminum pans were used as sample holders, containing 10 mg of the concentrate suspended in water (20% w/v), and a double empty pan was employed as the reference. The capsules were scanned at 10 °C/min in the range from 20 to 120 °C. The denaturation temperature (T_d) and enthalpy (δH) were obtained by analyzing the thermograms using a Universal Analysis Instrument, version 4.3A. The measurements were carried out in duplicate.

2.4. Preparation of APC dispersions

Dispersions of the concentrates (12% w/v protein) were prepared by dispersing in distilled water for 30 min at room temperature using a magnetic stirrer, followed by deaeration by centrifugation at 1200 × *g* for 2 min at 10 °C. The dispersions were used for oscillatory rheological measurements and also to obtain gels at different storage temperatures, which were later analyzed for uniaxial compression, water-holding capacity and microstructure.

2.5. Oscillatory rheology

The gelation kinetics was assessed via oscillatory rheological tests in a rheometer (Carri-Med CSL^2 500, TA Instruments, Surrey, England) with temperature control by a Peltier system. Stainless-steel cone plate geometry was used, with a diameter of 6 cm and an angle of 1°. In order to avoid sample dehydration, the plate edges were covered with low viscosity silicone.

A temperature sweep was initially performed under the following experimental conditions: heating from 55 to 90 °C at 2 °C/min, frequency of 1 Hz and 1% strain. After the temperature sweep, the rheological properties were monitored at 90 °C for 20 min to reach the steady state values. Values for the storage modulus (G'), loss modulus (G''), and phase angle (tan δ) as a function of the temperature and time were obtained. The samples gel point was determined by differential variation of the storage modulus (G') as a function of temperature ($dG'/dT > 0.1$ Pa/°C). The measurements were carried out in duplicate.

2.6. Physical properties of the heat-induced gels

2.6.1. Gel preparation

To obtain the gels, the APC dispersions were transferred to metal tubes (2 cm diameter × 4 cm height) and heat treated in a water bath at different temperatures (70, 80 or 90 °C) for 30 min. Immediately after heat treatment, the tubes were cooled in an ice bath and stored at 10 °C for 15 h before analyzing the gels.

2.6.2. Uniaxial compression

In the compression tests, the APC gels (2 cm diameter and 2 cm height), previously equilibrated at 10 °C, were assessed in a TA-XT2 Texture Analyzer (Stable Microsystems Ltd., Surrey, England) using a cylindrical probe (SMSP/55) with a diameter of 5.5 cm. The gels were compressed at a constant speed of 1 mm/s in the TPA tests and to determine their mechanical properties.

Gel texture was analyzed via two-cycle uniaxial compression, with a 20s interval between cycles (Steffe, 1996). The final compression height was 50% of the initial height. The parameters fracturability, hardness and adhesiveness, were obtained from the force vs. time curves (Bourne, 2002).

Mechanical properties were determined from the uniaxial compression of the gels (in one cycle) to 80% of their original height. The force and height values were transformed into Hencky stress (σ_H)-Hencky strain (ε_H) curves (Steffe, 1996). Fracture stress and strain were obtained at the maximal point of the stress–strain curve, whereas work at fracture (W_f) was calculated by integrating the area under the stress–strain curve, and the elasticity modulus (E) was determined based on the slope of the initial linear region of the curve.

2.6.3. Water-holding capacity (WHC)

WHC was determined according to Puppo, Lupano, and Añón (1995). Gel samples (0.8–1.3 g), cut in the shape of a disk, were wrapped in Whatman no. 1 filter paper and placed in the middle position of a 50 mL centrifuge tube. Loss of water was determined from the gel weight before and after centrifugation at 1200 × *g* for 5 min at 15 °C (model RC5C centrifuge, Sorvall® Instruments Dupont, Wilmington, Germany). WHC was expressed as the