Food Hydrocolloids 74 (2018) 115–123

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Comparative behaviour of solutions and dispersions of amaranth proteins on their emulsifying properties

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ARTICLE INFO

Article history:
Received 17 May 2017
Received in revised form
25 July 2017
Accepted 28 July 2017
Available online 2 August 2017

Keywords:
Emulsions
Protein solutions
Protein dispersions
Amaranth

ABSTRACT

In the present work the effect of the presence of soluble and insoluble protein on the stability of oil-water emulsions prepared with amaranth protein isolates (API) was analyzed. For this purpose, four types of emulsions were prepared: API-pH2 and API-pH6.3 dispersions and solutions. At pH 2.0 the amaranth proteins present higher solubility, are denatured and partially hydrolyzed; while at pH 6.3 its solubility is lower and its structure is more similar to the native protein. The soluble proteins present in API-pH2 and API-pH6.3 reduce with equal intensity the interfacial tension. However, the proteins present in API-pH2 are adsorbed twice as fast as those present in API-pH6.3, with equal rearrangement rate at the oil/water interface. Both, solutions and dispersions of API-pH2 and API-pH6.3 allow the formation of oil-in-water emulsions. Flocculation phenomena are evident, particularly in the case of API-pH6.3 dispersions. The calculated creaming-flocculation constant demonstrates that stability of emulsions increase with protein concentration and with the decrease of pH. The behaviour of API-pH2 and API-pH6.3 solutions was similar to that corresponding to the dispersions discarding a negative effect of the insoluble protein on the emulsifying properties of amaranth proteins.

1. Introduction

According to prospective studies carried out by international organisations, during the next decades, the world population will continue to increase; and along with this, there will also be an increase in food demands, which will have to be fulfilled in a scenario of fresh water shortages, reduced areas of cultivable lands and profound climate changes (United Nations, 2013; Alexandratos & Bruinsma, 2012). In addition, there is also increasing awareness of the relationship between the type of foods people consume and their health status and well-being (Council of the European Union, 2007; Udenigwe & Aluko, 2012). In this context, alternative plant cultivations, such as those of amaranth, which have long been cultivated by the Inca, Mayans and Aztec peoples, emerges as an interesting alternative, especially for their ability to fix higher amounts of carbon dioxide, as every C4-type plant, for its agricultural features and the nutritional bioactive properties of is proteins (Janssen et al., 2016; Kiegel, 1994). The amaranth grains storage proteins have an excellent amino acid balance and are known to have antihypertensive, antioxidant, antithrombotic, antiproliferative, cholesterol lowering and immunomodulatory properties (Caselato-Sousa, & Amaya-Farfán, 2012; Fritz, Vecchi, Rinaldi, & Añón, 2011; Montoya-Rodriguez, Gomez-Favela, Reyes-Moreno, Millan-Carrillo, & Gonzalez de Mejía, 2015; Moronta, Smaldini, Docena, & Añón, 2016; Moronta, Smaldini, Fossati, Añón, & Docena, 2016; Orsini Delgado, Galleano, Añón,& Tironi, 2015; Quiroga, Barrio, & Añón, 2015; Sabbione, Scilingo, & Añon, 2015). One of the main drawbacks of functional foods is that its biologically active peptides do not always reach the target organs due to the sensitivity of such peptides to hydrolysis by gastrointestinal proteases.

Several studies have demonstrated that amaranth storage proteins also have good emulsifying, foaming, gelifying and film-forming properties, as well as a good water retention capacity (Avanza, Puppo, & Añón, 2005; Bolontrade, Scilingo, & Añón, 2013; Bolontrade, Scilingo, & Añón, 2016; Shevkani, Singh, Rana, & Kaur, 2014; Silva-Sánchez, González-Castañeda, De León-Rodríguez, & Barba de la Rosa, 2004; Ventureira, Martinez, & Añón, 2010, 2012a, 2012b).

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http://dx.doi.org/10.1016/j.foodhyd.2017.07.042
0268-005X/

Food emulsions have been proposed as bioactive compound transporters (Adjonu, Doran, Torley, & Agboola, 2014; McClemens, 2010). It has been demonstrated that protein hydrolysates, many of which are known to contain physiologically active peptides, can form emulsions; however, these emulsions are not stable; particularly when such hydrolysates contain peptides of low or very low molecular mass (Scherze & Muschiolik, 2001). In addition, there exist controversies about the deleterious effects that the presence of insoluble proteins may entail, particularly as regards the protein emulsifying properties.

It is well known that amaranth proteins present reduced solubility at pH values near the neutrality and under low ionic strength conditions (Bolontrade et al., 2013). These conditions are very frequent in the food industry and therefore, represent a limitation for the use of such proteins. Several studies have demonstrated that the solubility of these vegetal proteins can be increased by working at pHs lower than 4.5, a value that corresponds to the average isoelectric point (pI) of amaranth storage proteins (Bolontrade et al., 2013; Shevkani et al., 2014; Ventureira et al., 2010). Even though the latter is a possibility to increase the solubility, this strategy has limited use due to the slightly acidic or nearly neutral pH of foods.

Taking into account this restriction imposed by the protein solubility and, considering that amaranth proteins have the capacity to form and stabilise emulsions that could act as potential transporters of bioactive ingredients, the aim of this work was to analyse the effect of the insoluble protein content in the emulsifying properties of amaranth protein isolates at acidic pH (API-pH2) and at nearly neutral pH (API-pH6.3).

2. Materials and methods

2.1. Amaranth seeds and flour

Seeds of *Amaranthus hypochondriacus* were obtained from INDEAR (Instituto de Agrobiotecnología de Rosario, Argentina). The flour was obtained by grinding the seeds as described previously. Crude protein of flour was 18.7 ± 0.4% (dry basis); as determined by the Kjeldahl method (AOAC, 1984) using a factor of 5.85 (Scilingo, Molina Ortiz, Martinez, & Añón, 2002). The content of carbohydrate, lipids and ash were 68.2 ± 1.6% w/w, 7.4 ± 0.7% w/w and 3.2 ± 0.1% w/w, respectively (all results are expressed in dry basis).

2.2. Preparation of amaranth protein isolates (API)

API were obtained by alkaline extraction, pH 9.0, and isoelectric precipitation, pH 5.0, as previously described by Martínez and Añón (1996). The protein content of isolates was 84.7 ± 4.2% (Nx5.85) (dry basis), as determined by the Kjeldahl method. The isolates also contain 3.3 ± 0.3% w/w of ash and 10% w/w of carbohydrate, basically soluble fiber (results are expressed in dry basis).

2.3. Protein solubility

API were solubilized in phosphate buffer pH 2.0 (API-pH2) (0.052 M H_3PO_4, 0.048 M KH_2PO_4, 0.052 M NaCl, ionic strength 0.1) and pH 6.3 (API-pH6.3) (0.047 M KH_2PO_4, 0.013 M K_2HPO_4, ionic strength 0.1). Dispersions (total protein: soluble + insoluble fractions) of a protein concentration of 0.1% w/v were stirred for 1 h at room temperature. Dispersions were centrifuged at 15,000 × g for 15 min at 20 °C and the supernatants corresponded to the protein soluble fraction (API-pH2 and API-pH6.3 solutions). The protein content in the supernatant was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). The protein solubility (PS%) was calculated as the ratio between the protein content in the supernatant (Ps) and the total protein content (Pt) determined by the Kjedahl method (N x 5.85).

$$PS\% = Ps \times 100/Pt \quad (1)$$

2.4. Differential scanning calorimetry (DSC)

Dispersions and solutions at 2% w/v of API-pH2 and API-pH6.3 were frozen at − 80 °C and lyophilized. Hermetically sealed aluminium pans were prepared to contain 10–15 mg of either lyophilized API-pH2 or API-pH6.3 dispersions or solutions in distilled water (20% w/v). A hermetically pan containing lyophilized API-pH7.5 was also prepared under the same conditions. The value of the enthalpy of this sample was selected as reference for the calculation of the degree of denaturation attained by API-pH2 and API-pH6.3. A double empty pan was employed as reference. Capsules were heated from 20 to 120 °C at a rate of 10 °C min^{-1}. DSC measurements were performed in a TA Q100 calorimeter (TA-Instruments, USA). The equipment was calibrated at a heating rate of 10 °C min^{-1} by using indium, lauric acid, and stearic acid (p.a.) as standards. The dry matter content of samples was determined by leaving the pans overnight in an oven at 105 °C and then weighed. The denaturation temperature (Td, °C) and the enthalpy of transition (ΔHd, J g^{-1} dry protein) were obtained by analyzing the thermograms with the Universal Analysis 2000 Software.

2.5. Electrophoresis

SDS-PAGE was carried out under reducing conditions according to Laemmli (1970) in stacking, resolving and spacer gels that contained 12, 4 and 10% w/v acrylamide respectively. Runs were performed in minislabs (Bio-Rad Hercules, CA, USA). Phosphorylase b (94 kDa), bovine serum albumin (67 kDa); ovoalbumin (43 kDa); carbonic anydrase (30 kDa); trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) were used as molecular markers.

2.6. Zeta-potential measurements

The zeta-potential values of API suspended in water at pH 2.0 and 6.3 were measured by a dynamic laser light scattering using a Nano Particle analyzer SZ-100 (Horiba Scientific Inc., UK) at 25 °C. The zeta-potential was determined by measuring the direction and rate of droplet movement in a well-defined electric field. 500 μL of diluted sample (0.05% w/v) was put in the electrophoresis cell.

2.7. Preparation of oil-water emulsions

Emulsions were prepared with either the total protein (API-pH2 and API-pH6.3 dispersions) or with the soluble fraction of API (API-pH2 and API-pH6.3 solutions). For dispersions, the sample concentration was 0.1, 0.4, 0.8 and 1.7% w/v and for solutions were 0.8 and 1.7% w/v of total proteins corresponding to: 0.7 and 0.2% w/v and 1.4 and 0.5% w/v of API-pH2 and API-pH6.3 respectively. Samples were suspended in the buffers described in Section 2.3, stirred 1 h at room temperature and then centrifuged at 7000 × g for 15 min at 20 °C. Emulsions were prepared by homogenizing 4 mL of refined sunflower oil and 16 mL of the protein dispersion or solutions (20% oil v/v) with an ULTRA-TURRAX T25 rotor/stator (Janke & Kunkel GmbH, Staufen, Germany) homogenizer at a rate of 20.000 rpm for 1 min, to produce coarse emulsions. Samples were then further homogenized with an ultrasound homogenizer (SONICS Vibra Cell VCX750) at a power level of 50%, applying pulses of 30 s each with the standard tip immersed 2/3 in a 28 mm