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Development of thermally processed bioactive pea protein gels: Evaluation of mechanical and antioxidant properties

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ABSTRACT

Thermal processing of plant proteins (e.g. from pea) is highly interesting due to its potential in the generation of new textures in food products. This processing route is particularly attractive when it is combined with the revalorization entailed by the bioactive properties of the processed gels. In fact, the antioxidant activity of food products, which has been found in numerous proteins and peptides from protein hydrolysates, is widely demanded by the society since it prevents the development of serious diseases.

This work is focused on the evaluation of mechanical and antioxidant properties from a Pea Protein Concentrate (PPC) and two hydrolysates obtained from PPC (PPH_{25} and PPH_{120}) at three different pH values (2.0, 6.5 and 8.0). The gelation process was monitored and, subsequently, mechanical spectra were obtained. In addition, protein interactions (ionic interactions, hydrophobic interactions, hydrogen bonds and disulphide bonds) were also determined in order to elucidate their contribution to the formation of the gel network. Finally, the characterisation of the antioxidant activity of the gels was carried out using three different reagents: DPPH, ABTS and Folin–Ciocalteu. Results put forward that each variable studied, the degree of hydrolysis and pH, are key factors over the thermal processing of gels, showing a remarkable influence on both mechanical properties and antioxidant activity.

1. Introduction

Dry legumes or pulses are the edible seeds of leguminous crops. Legumes are considered the second most important source of human food after cereals. They are an inexpensive source of proteins and other nutrients such as starch, dietary fibre, vitamins, minerals and polyphenols. Food industry, in general, and legume industry, in particular, produce a huge amount of by-products with an important percentage of protein (Graham and Vance, 2003). Proteins have been used frequently in food products, especially, for non-nutritional purposes. In fact, the most common role has been related to provide stabilization the structure of food products, such as gels and emulsions (Banerjee and Bhattacharya, 2012; Foegeding and Davis, 2011; McClements, 2004).

The gelation of proteins (mainly globular proteins) in order to produce food products has received considerable attention for some years because of the potential applications that can be found in the food industry (Clark et al., 2001; Dickinson and McClements, 1995; Foegeding and Davis, 2011; Galanakis et al., 2010a; Zhou et al., 2015). Usually, the gelation of proteins has been carried out through heat treatment. The thermal gelation of globular proteins is a multi-step process that involves partial unfolding of the native protein, aggregation and further formation of a gel-network which is able to hold water into clusters (Clark et al., 2001). The rearrangement of protein chains changes the interactions among them, however the forces which are responsible for the gel structure are mainly the same as initially: hydrophobic, Van der Waals, hydrogen bonding, and covalent interactions (Chen et al.,

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2006). Furthermore, gels can exhibit different microstructural properties which depend on preparation technique, and have been strongly related to the aggregation of proteins (Bonnaillie and Tomasula, 2012; Lefevre and Subirade, 2000).

Traditionally, this ability of proteins to provide kinetic stabilization in food products has been called functional properties. However, one of the most important requirement of any food product, the nutritional quality, has not been deeply considered (Foegeding and Davis, 2011; Galanakis et al., 2010b). This bio-functionality is precisely one of the most relevant issues in the last few years because it assesses the importance of the nutrition in the human health (Kitts et al., 2003; Kitts and Weiler, 2003). Moreover, Foegeding and Davis (2011) stated that the focus of all development of new food products should be the maintenance of nutritional and bioactive properties of proteins after the generation of food structures.

It has been demonstrated that the absorption of bioactive proteins or, and more specifically, peptides by the human body involves positive effects, which are added to the typical nutrition caused by food products (Jensen et al., 2013; Torbica et al., 2016). The bioactivity of food products has been related to the ability of proteins towards antihypertensive, immunomodulatory, anti-inflammatory, antioxidant and hypocholesterolemic effects (Cudennec et al., 2016; Park and Nam, 2015; Sakanaka et al., 2005; Thompkinson et al., 2014). However, the effectiveness of nutraceutical products in preventing diseases strongly depends on the preservation of the bioavailability of the active ingredients (Chen et al., 2006).

Antioxidants as bioactive compound are quite important in the daily diet of humans, because free radicals are continuously produced during the human metabolism and may induce damages to organic biomolecules that may promote changes in DNA and, as a result, serious health problems (Gey, 1993; Li and Shah, 2014). In this sense, antioxidants have been postulated as antiageing agent in the human body (Brown, 2005). The antioxidant activity has been found in numerous proteins and peptides from protein hydrolysates (Elias et al., 2008; Gomez-Guillen et al., 2011; Sarmadi and Ismail, 2010; Vastag et al., 2010). Despite the fact that the exact mechanism for the antioxidant capacity found in proteins is not completely known, several paths have been suggested. Some of them are: interactions with pro-oxidative metals, adducting lipid-derived volatile aldehydes, and non-radical reduction of lipid hydroperoxides among others (Elias et al., 2008).

Wastes from proteins surpluses has been postulated previously to be used for high-value added food products (Galanakis et al., 2014). In fact, surpluses or by-products from crayfish, rice, albumen, oil mill or carob have been previously used in food applications (Aguilar et al., 2010; Bengoechea et al., 2008; Felix et al., 2016; Galanakis et al., 2010c; Romero et al., 2012, 2009). One of the features previously studied for the pea protein has been the emulsifying properties which are very interesting characteristics for the food industry (Peng et al., 2016; Stone et al., 2015; Tamm et al., 2016). In addition, the gelation and the effect of processing conditions on thermal and textural properties of heat-induced pea protein-based gels have been also reported (Shand et al., 2007; Sun and Arntfield, 2010). In addition, the hydrolysis of this protein may result in the exposure of hydrophobic groups (Damodaran et al., 2007), as well as increase the molecular flexibility of the proteins, which enables a faster unfolding of the proteins (Tamm et al., 2016) or the antioxidant activity (Elias et al., 2008).

Some authors have studied the use of pea protein in food gels (Munialo et al., 2015; Sun and Arntfield, 2012a,b). However, no references to the evaluation of bioactive properties of pea protein-based gels or hydrolysates have been found, despite the fact that pea proteins may contribute fairly attractive features to food products. Among these features is its lack of genetic modifications in commercial species, as well as its relatively low allergenicity and associated rates of feeding intolerance (Directive 2001/18/EC, 2001).

The aim of this work has been to develop pea protein-based gels and to evaluate the mechanical properties and antioxidant activities of these gels, as well as to analyse the effect of the degree of hydrolysis and pH value. To achieve these objectives, a physicochemical characterisation of the different systems was carried out (PPC, PPH_{25} and PPH_{120}). Subsequently, rheological measurements of aqueous protein dispersion were performed by means of temperature ramp tests in order to follow the gelation process as well as frequency sweep tests were also carried out. To understand the mechanical response obtained, the chemical nature of each protein interaction (ionic interaction, hydrophobic interaction, hydrogen bond and disulphide bond) were also determined. Finally, the antioxidant activities of the different gels were evaluated against three different reagents: ABTS, DDPH and Folin–Ciocalteu.

2. Material and methods

2.1. Materials

Pea protein concentrate (PPC) was delivered by Roquette (Lestrem, France). PPC was subjected to a hydrolysis process using pancreatic trypsin. The enzyme/substrate ratio (E/S) was set at 1:100 (w/w). The pH of the dispersion was kept constant at 8.0 during the entire period of hydrolysis (25 or 120 min) by the addition of 6 M NaOH. The resulting hydrolysates were heated in boiling water for 10 min to inactivate the enzyme, and centrifuged at 5000 rpm for 15 min to separate any impurities. Subsequently, the supernatant was freeze-dried in order to obtain a protein-hydrolysate powder. As a consequence of the different times of hydrolysis, two different hydrolysates were obtained and named PPH_{25} and PPH_{120} for 25 or 120 min of hydrolysis, respectively. The degree of hydrolysis was determined by the formol titration method (Taylor, 1957), obtaining degrees of hydrolysis of 12.2 ± 0.7 and 26.2 ± 1.7 for each system, respectively.

All other reagents used were of analytical grade, purchase from Sigma-Aldrich Chemical Company (St. Louis, USA). Milli-Q ultrapure water was used for the preparation of all solutions.

2.2. Protein powder characterisation

2.2.1. Elemental characterisation

The protein content of all studied systems (concentrate and hydrolysates) was determined in quadruplicate as $\%N \times 6.25$ using a LECO CHNS-932 nitrogen micro analyser (Leco Corporation, St. Joseph, MI, USA). In the same way, lipid, moisture and ash contents were determined according to A.O.A.C. methods (2000).

2.2.2. Surface hydrophobicity of proteins (H_0)

Surface hydrophobicity (H_0) of soluble proteins in protein extracts (pH 8.0) was measured according to Kato and Nakai (1980), using the fluorescent probe 1-anilino-8-naphtalene-sulfonate (ANS). Protein extracts were diluted with 0.05 M phosphate buffer (pH 8.0) to obtain protein concentrations ranging from 5 to 0.005 mg/mL. Then, 40 μL of ANS (8.0 mM in the same buffer) were added to 2 mL of sample. Fluorescence intensity (FI) was measured with a Tucan Infinite 200 PRO Microplate Reader (Tecan Group Ltd., Männedorf, Switzerland), at wavelengths of 365 nm (excitation) and 484 nm (emission). The initial slope of fluorescence intensity versus protein concentration was used as an index of protein surface hydrophobicity (H_0).

2.2.3. Amino acid characterization

RPC samples were dissolved in 6 M hydrochloric acid and incubated in an oven at 110 °C for 24 h. After hydrolysis, the pH was adjusted to 7 using 6 M NaOH and the samples were filtered through a Whatman glass microfibre filter (GF/C). Finally, samples were diluted (1:500) by adding doubly distilled water.