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Development and evaluation of rheological and bioactive properties of rice protein-based gels

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ABSTRACT

The rice industry produces a large amount of by-products daily. In fact, it is estimated that approximately 100 million tons of rice residues and by-products are generated each year. However, new protein sources are demanded for human food because of the continuous increase in the global population coupled with the almost total inability to increase global food production.

A rice protein concentrate (RPC), which is a by-product of the rice industry, and two different hydrolysates (RPH_{25} and RPH_{120}) obtained from RPC, have been used in order to evaluate the potential of RPC to form a gel-like food product 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 understand the gel structure. Finally, an antioxidant characterisation was carried out using three different reagents: DDPH, ABTS and Folin-Ciocalteu.

A proper degree of hydrolysis and an optimal pH value seem to be key factors in the manufacturing of gels, showing a remarkable influence on both rheological properties and antioxidant activities.

1. Introduction

The continuous increase in the global population involves a huge challenge related to the growing need for food worldwide. The scientific community would be wise to explore all good ideas in order to find new alternative sources of human nutrition. Thus, the use of surpluses with high nutritional quality from the food industry has been postulated as an interesting alternative for the formulation of new food products.

Although the world's rice production is mostly located in Asia, the production of this crop in Europe involves 410,000 ha, being located mainly in the Mediterranean (Ferrero and Tinarelli, 2007). Unfortunately, the rice industry typically produces a large amount of by-products that may yield a negative environmental impact. In fact, approximately 100 million tons of rice residues and by-products are generated every year (Li et al., 2010). The present work uses rice husks, which are used in low-value-added applications, since they are mainly incinerated for energy purposes or used for animal feeding (Njie and Reed, 1995), however they are not used for human food because of the lack of protein functionality. To improve upon this, a proper hydrolysis may be a very interesting alternative to increase protein functionality (*i.e.*, by increasing solubility or mechanical properties) and antioxidant activity (obtaining bioactive peptides) (Wang et al., 2009). Despite the fact that the rice bran and its hydrolysates is deeply studied for the formulation of food products (Adebiyi et al., 2008; Cao et al., 2009), the literature related to the improvement of techno-functional properties is non-existent form rice husks. In fact, authors do not found previous research works of bioactive food products made from rice husks. However, nowadays, these by-products are called to be used as new protein sources for the manufacture of food products such gels, which should also exhibit excellent nutritional quality. However, this huge challenge requires an extensive exploration and implementation of protein-based food products (Urbonaite et al., 2015).

Over the last decade, there has been increasing interest in the bioactive properties of proteins and hydrolysates used in food products. Antioxidants have a relevant place in the daily diet of humans, as free radicals are continuously produced during human metabolism and may induce damage to organic biomolecules that may promote changes in DNA and, as a result, serious health problems. Therefore, antioxidants are proposed to be antiaging

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agents (Irakli et al., 2015). Antioxidant activity has been found in numerous proteins and peptides from both animal and vegetable sources (Zhai et al., 2015).

The aim of this work was to develop rice protein-based gels and to evaluate the rheological properties and antioxidant activities of gels made from rice by-products, 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 performed. Subsequently, rheological measurements of aqueous protein dispersion were performed by means of temperature ramp tests in order to follow the gelation process and frequency sweep tests were also carried out. To understand the mechanical response obtained, the chemical nature of each protein interaction was also determined. Finally, the antioxidant activities of the different gels were evaluated using various methods.

2. Material and methods

2.1. Materials

Rice protein concentrate from rice surpluses (RPC) was delivered from Remy Industries (Leuven-Wijgmaal, Bélgica).

RPC 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 RPH_{25} and RPH_{120} for 25 or 120 min of hydrolysis, respectively. The degree of hydrolysis was determined by the formol titration method.

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 x 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.

2.2.2. Free and total sulfhydryls

Free and total sulfhydryl groups of all samples were determined using the method developed by Beveridge et al. (1974) and Thannhauser et al. (1984), respectively. Samples were suspended (10 mg/mL) in buffer containing 0.086 mol/L Tris-HCl, 0.09 mol/L glycine, 4 mmol/L EDTA and 8 mol/L urea, pH 8.0. Dispersions (3 mL reactive solutions and 0.5 mL protein solution) were stirred at 25 °C for 10 min at 500 rpm in a thermomixer and centrifuged at 15,000*g* (10 min, 10 °C). The supernatant was incubated in the dark at room temperature for 25 min with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNT) (4 mg DTNB/mL methanol) and 1 mL NTSB (2-nitro-5-thiosulfobenzoate) was used in the case of the total sulfhydryls. Absorbance at 412 nm was measured in a Genesys-20 spectrophotometer (Thermo Scientific, USA). The molar extinction coefficient of NTB (13,600 L mol^{-1} cm^{-1}) was used. The protein concentration of the extracts was determined by the Lowry method.

2.2.3. Surface hydrophobicity of proteins (H_0)

Hydrophobicity (H_0) of soluble proteins in protein extracts (pH 8.0) was measured 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 hydrophobicity (H_0).

2.2.4. Amino acid characterisation

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.

Reverse phase HPLC by precolumn fluorescence derivatization with o-phtaldialdehyde (SIL-9A Auto Injector, LC-9A Liquid Chromatograph, RF-530 Fluorescence HPLC Monitor, all parts from Shimadzu Corporation, Japan) was performed using a NovaPak C18 cartridge (Waters, Milford, MA, USA). Glycine/arginine and methionine/tryptophan were determined together, as their peaks merged. The analysis was performed once on each sample. By this procedure, it is only possible to determine alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), histidine (His), serine (Ser), glycine (Gly), arginine (Arg), threonine (Thr), tyrosine (Tyr), methionine (Met), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu) and lysine (Lys). The other amino acids were not included in the results of this study because they are completely destroyed by acid hydrolysis or cannot be directly determined from acid-hydrolysed samples.

2.2.5. Protein solubility

Protein solubility was determined as a function of pH in the range of 2–10. Aqueous dispersions at 1 mg/mL were prepared and the pH of different aliquots was adjusted with 6 M NaOH and 6 M HCL for alkaline and acid pH values, respectively. Samples were homogenized and centrifuged for 15 min at 15,000 × *g*. The protein content was determined in quadruplicate by the Lowry method.

2.3. Gelation process

Aqueous dispersion of 12 wt % protein concentration were subjected to thermal gelation in three different stages: (i) The first step was carried out at a constant heating rate (5 °C/min) from 20 °C to 90 °C; (ii) After the first step, an isothermal step was performed at 90 °C for 30 min; (iii) Subsequently, a cooling stage was carried out at a constant cooling rate (5 °C/min) from 90 °C to 20 °C. Gels were performed at three different pH values: 2.0, 6.5 and 8.0.

2.4. Characterisation of gels

2.4.1. Viscoelastic measurements of gels

Small-amplitude oscillatory shear (SAOS) measurements were performed in a controlled-stress rheometer (Kinexus Ultra +) from Malvern Instruments (Malvern, Worcestershire, United Kingdom). In a preliminary experiment, stress sweep tests were performed in order to establish the linear viscoelastic range. In fact, all tests were carried out at a stress clearly lower than the critical value for linear viscoelasticity. The gelation process was simulated *in situ* in the rheometer, following the same thermal treatment described in the