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Okara treated with high hydrostatic pressure assisted by *Ultraflo*® L: Effect on solubility of dietary fibre

E. Pérez-López [a], I. Mateos-Aparicio [b], P. Rupérez [a,*]

[a] Metabolism and Nutrition Department, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), Consejo Superior de Investigaciones Científicas (CSIC), José Antonio Novais 10, Ciudad Universitaria, E-28040 Madrid, Spain
[b] Departamento de Nutrición y Bromatología II, Bromatología, Facultad de Farmacia, Universidad Complutense de Madrid, Ciudad Universitaria, E-28040 Madrid, Spain

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ABSTRACT

Okara is an abundant and inexpensive by-product from soybean, rich in total dietary fibre (>55% dry weight), but poor in soluble dietary fibre (SDF, <5% dw). A combined method of high hydrostatic pressure (HHP) aided by the food grade enzyme *Ultraflo*® L was used for SDF maximization. At atmospheric pressure, incubation time was not a key factor, and a ratio of 1:40 (enzyme:Okara, *v/w*) was able to saturate the enzyme within 120–150 min. When HHP plus *Ultraflo*® L were applied, a synergy between both treatments was observed. Thus, at 600 MPa, 0.025% *Ultraflo*® L and 30 min treatment, soluble carbohydrate added up to 15.64 ± 0.32%, consisting of two peaks (9.14 ± 0.18 and 0.57 ± 0.01 kDa), determined by HPLC-ELSD. The combination of HHP plus *Ultraflo*® L on Okara improved the solubility of the dietary fibre, making it more suitable to be used in functional foods.
Industrial relevance: There is an increasing interest in finding new prebiotics from food industrial waste. Okara has been simultaneously treated with *Ultraflo*® L and HHP, taking into account the industrial costs of the procedure, and adjusting the quantity of enzyme and time of treatment to the minimum, by a Response Surface Methodology and an enzymatic activity assay. Besides, an HPLC-ELSD direct analysis (High Performance Liquid Chromatography with Evaporative Light Scattering Detector) was employed to monitor soluble fibre, as an easy and fast analytical method.

1. Introduction

Okara is the insoluble residue left during soybean milk or tofu production and the main by-product from this food industry (Mateos-Aparicio, Redondo-Cuenca, Villanueva, Zapata-Revilla & Tenorio-Sanz, 2010; O'Toole, 1999). Approximately, 1.2 kg of fresh Okara is produced from 1 kg of soybean seed in tofu manufacture, consequently tonnes of Okara are being generated worldwide, most especially in Asian countries like China, Japan and Korea (Li, Qiao, & Lu, 2012), where soybean seeds and derived products have been consumed for centuries. Nowadays, soybean is increasingly grown (FAO, n.d.) and consumed in Western countries (Espinosa-Martos & Ruperez, 2009; Prestamo, Ruperez, Espinosa-Martos, Villanueva, & Lasuncion, 2007).

The by-products generated after food processing, like Okara, are currently promising sources of functional compounds. Okara has a good nutritional value (Redondo-Cuenca, Villanueva, & Mateos-Aparicio, 2008), with a high dietary fibre content (>55% dry weight), but low in soluble dietary fibre (SDF, <5%), a considerable protein content (around 30%) and lesser amounts of fat, ash, isoflavones and soybean oligosaccharides, like stachyose and raffinose (Espinosa-Martos & Ruperez, 2009; Jimenez-Escrig, Alaiz, Vioque, & Ruperez, 2010, 2011; Jimenez-Escrig, Tenorio, Espinosa-Martos, & Ruperez, 2008; Li et al., 2012; Mateos-Aparicio, Mateos-Peinado, & Ruperez, 2010; Mateos-Aparicio, Redondo-Cuenca, Villanueva, Zapata-Revilla and Tenorio-Sanz, 2010; Villanueva, Perez-Cozar, & Redondo-Cuenca, 2013). Many of the beneficial health effects attributed to dietary fibre (DF) are related to SDF and soluble oligosaccharides, as for example the regulation of metabolic disorders related to obesity and reduction of cancer risk (Courtois, 2009; Charalampopoulos & Rastall, 2012). Okara is a potential weight loss supplement and prebiotic (Prestamo et al., 2007; Redondo-Cuenca et al., 2008) with beneficial effects on lipid metabolism (Villanueva, Yokoyama, Hong, Barttley, & Ruperez, 2011).

Okara is a readily available and cheap by-product but its high moisture content (≈80%) and easy putrefaction make it difficult to preserve. Common ways of preserving fresh Okara, like oven-drying or freeze-drying, are industrially expensive (Mateos-Aparicio, 2011; Mateos-Aparicio, Redondo-Cuenca, & Villanueva, 2012; Villanueva et al., 2011). On the other hand, Okara is rich in total dietary fibre but has a low SDF content. Therefore, there is a need to explore novel treatments which are capable to increase the solubility of Okara as well as to extend its shelf life.

* Corresponding author. Tel.: +34 915492300; fax: +34 915493627.
E-mail address: pruperez@ictan.csic.es (P. Rupérez).

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Commonly used treatments to increase the amount and availability of SDF polysaccharides are mainly chemical (Mateos-Aparicio, Mateos-Peinado, Jimenez-Escrig, & Ruperez, 2010) or enzymatic (Reid, Novak, & Lewandowski, 2006). At atmospheric pressure, the use of enzymes to increase SDF content is reported in soybean (Nakamura, Furuta, Maeda, Nagamatsu, & Yoshimoto, 2001; Napolitano et al., 2009; Vega-Paulino & Zuniga-Hansen, 2012). Also, a food-grade hydrolytic enzyme (*Ultraflo*® L) degrades wheat (Marcos et al., 2013; Min et al., 2006) and Okara's insoluble dietary fibre attained by the AOAC's method (Kasai, Murata, Inui, Sakamoto, & Kahn, 2004; Ruperez, Perez-Cozar, Redondo-Cuenca, & Villanueva, 2011; Villanueva et al., 2013).

More recently, high hydrostatic pressure (HHP) has been used for SDF maximization (Li et al., 2012; Mateos-Aparicio, Mateos-Peinado, & Ruperez, 2010) and food preservation. HHP technology (Vervoort et al., 2012) has the advantage to minimise undesirable changes, produced by traditional thermal treatments, which affect the nutritional and sensory quality of foods (San Martín, Barbosa-Cánovas, & Swanson, 2002) and it keeps longer the organoleptic attributes of fresh food (Ferrari, Maresca, & Ciccarone, 2010; Lambert, Demazeau, Largeteau, & Bouvier, 1999).

The combined effect of HHP and degrading enzymes could be even more effective for SDF maximization and preserving foods and agrofood by-products. HHP (400–600 MPa) inactivates some enzymes that cause product spoilage, but enhances the activity of polyphenoloxidase and α- and β-amylases (hydrolases) among others (San Martín et al., 2002). HHP assisted by hydrolases has been previously applied for the extraction of functional ingredients from cactus, ginseng (Hoon et al., 2013; Kim, Park, Yu, Imm, & Suh, 2014) and fermented rice bran (Kim & Han, 2012).

However, to the best of our knowledge, the combined effect of HHP and food-grade enzymes has not been applied before to soybean or Okara. Therefore, our aim was to assay the effect of high hydrostatic pressure – assisted by a hydrolytic enzyme (*Ultraflo*® L) – on the soluble and total dietary fibre content of Okara, and most especially on its soluble oligosaccharide and polysaccharide content. Additionally, as no previous report is noticed about the kinetics of this specific enzyme on Okara as substrate, a useful HPLC-ELSD method is proposed to monitor soluble carbohydrates from dietary fibre, which could be very useful in case HHP is applied at an industrial scale.

2. Materials and methods

2.1. Raw material, enzyme and carbohydrate standards

Fresh Okara, obtained as a by-product from soybean [*Glycine max* (L.) Merr] was provided by a local food processing industry (Toofu-Ya S.L., Arganda del Rey, Madrid, Spain). It was previously freeze-dried (Virtis Bench Top 3 L, Hucoa-Erlöss S.A., Madrid, Spain) and then defatted by extraction with ethylic ether in a Soxtec System (Tecator, Höganäs, Sweden). Before any enzymatic or HHP treatment, freeze-dried Okara was hydrated in water (1–5%, *w*/*v*) at room temperature with constant shaking in a Heidolph Reax 2 rotatory shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) overnight. Enzymatic treatment was performed with *Ultraflo*® L (Novozymes Spain, S.A., Pozuelo de Alarcón, Madrid, Spain), a commercial food-grade Beta-glucanase (endo-β-1,3(4)-), with both cellulase and xylanase activities.

Carbohydrate standards with different average molecular weight (MW) were used for retention time (RT) calculation and calibration curve: Dextran T-70 (70 kDa) and Dextran T-10 (10 kDa) were obtained from Pharmacia Biotech Europe GmbH (Barcelona, Spain), Inulin (5.93 kDa, estimated), Stachyose (0.67 kDa) and Raffinose (0.52 kDa, estimated) were obtained from Sigma, Alcobendas, Madrid, Spain, D (+)-Glucose (0.18 kDa), D (+)-xylose (0.15 kDa) and α-Cellulose were acquired from Merck, Darmstadt, Germany. α-Cellulose from Sigma (Alcobendas, Madrid, Spain), was also employed to study the enzyme activity.

All other reagents used were of chromatography grade. All solutions, including, dilutions and mobile phases were prepared with ultrapure water (Resistivity 18.2 MΩ cm at 25 °C; Milli-Q Integral 5 Water Purification System from Millipore, Merck KGaA, Darmstadt, Germany).

2.2. Optimization of Ultraflo® L treatment at atmospheric pressure

Prior to the HHP plus *Ultraflo*® L treatment of Okara, the enzyme procedure was optimized at atmospheric pressure by assaying the activity of *Ultraflo*® L, aided by a Response Surface Methodology (RSM) and analysing the enzymatic kinetic of *Ultraflo*® L.

2.2.1. Enzymatic activity of Ultraflo® L on Okara and cellulose as substrates

Okara sample and commercial α-Cellulose (5.5 mg mL^{-1}) were treated with *Ultraflo*® L (0.05%, 0.1% and 0.2% *v*/*v*) and incubated for 5, 15, 30 and 45 min at 37 °C. The results were expressed as fungal beta glucanase (FBG) units being a β-glucanase unit (BG) the amount of enzyme which, under standard conditions, liberates glucose or other reducing carbohydrates with a reduction power corresponding to 1 μmol of glucose per minute. Density of the enzyme was assessed to express the results in FBG g^{-1}. Experimental results were compared with the information on product data of *Ultraflo*® L. All reducing carbohydrates (both neutral sugars and uronic acid) were spectrophotometrically measured by the 3,5-dinitrosalicylic acid assay (DNS) for reducing sugars (Miller, 1959) conveniently adapted to microplate by us.

2.2.2. Response Surface Methodology (RSM)

As three different variables can affect the release of soluble carbohydrates (oligo- and polysaccharides) from the insoluble dietary fibre of Okara during the enzymatic treatment with *Ultraflo*® L, a RSM using Box–Behnken and central composite designs were used to elucidate and predict its response. The following factors and ranges were established as independent variables: Time 15–90 min, Okara 1–5% (*w*/*v*) and *Ultraflo*® L 0.5–1.5% (*v*/*v*). The response was obtained as soluble carbohydrates (16.0–6.2 kDa MW) derived from dietary fibre by quantification after High Performance Liquid Chromatography with Evaporative Light Scattering Detector, HPLC-ELSD method performance (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Condezo-Hoyos, Perez-Lopez, & Ruperez, 2015) as in 2.4.

Minitab 16 software was used to design the Response Surface experiments (Box–Behnken Design) and to perform the Response Surface Regression and the analysis of Variance ($P < 0.05$) as previously described by our group (Condezo-Hoyos et al., 2015).

2.2.3. Enzymatic kinetic on Okara as substrate

Enzymatic kinetic of *Ultraflo*® L was assessed using pre-hydrated Okara (1%, *w*/*v*) as substrate and different concentrations of *Ultraflo*® L (0.05%, 0.025%, 0.01% and 0.005%, *v*/*v*). The control was prepared using 0.01% *Ultraflo*® L shocked by heat. The incubation was made in a water bath at 37 °C with constant shaking and aliquots were taken at 15, 30, 90, 120, 150 and 180 min. After incubation, samples were immediately frozen to stop enzyme activity. Then, samples were filtered through 0.45 μm syringe filters (cellulose acetate, 25 mm diameter, Análisis Vínicos, Tomelloso, Toledo, Spain) before injection (50 μL) into the HPLC-ELSD system and response was measured as in 2.2.2. and 2.4.

2.3. HHP treatment assisted by Ultraflo® L of Okara

Pre-hydrated Okara solution 1% (*w*/*v*) was treated simultaneously with *Ultraflo*® L (concentration of 0.025%) and high hydrostatic pressure (400 and 600 MPa) with the subsequent untreated controls (0.1 MPa, atmospheric pressure at 22 ± 1 °C) for 15 or 30 min at