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Antioxidant properties of soy protein–fructooligosaccharide glycation systems and its hydrolyzates

María D. Mesa [a], Jose M. Silván [b], Josune Olza [a], Ángel Gil [a], María D. del Castillo [b,*]

[a] Instituto de Nutrición y Tecnología de Alimentos, Universidad Granada, Ramón y Cajal 4, 18071 Granada, Spain
[b] Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Juan de la Cierva 3, 28006 Madrid, Spain

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ABSTRACT

The present research aimed to assess how heating at 95 °C for 1 h followed by proteolysis affects the antioxidant properties of Maillard reaction mixtures constituted by soy protein isolate (SPI) and fructooligosaccharides (FOS) in 0.5 M phosphate buffer pH 7.4. Solutions of protein and sugars were also heated alone as controls. Glycation and cross-linking of protein was estimated by *o*-phaldialdehyde assay. Samples were hydrolyzed *in vitro* mimicking gastro-intestinal conditions and subsequently submitted to analysis. *In vitro* antioxidant properties were determined by LDL oxidation and oxygen radical absorbance capacity assays. Simultaneously, undigested reaction mixtures constituted by SPI and sugars were heated, fractioned by ultrafiltration and the fractions were characterized. Although Maillard reaction might give rise antioxidants, present data seem to indicate that neoantioxidants able to prevent LDL oxidation and to scavenge peroxyl-alkyl radicals were majority formed by thermal degradation of FOS (caramelization). Peptides derived from soy protein scavenged peroxyl radicals and did not protect LDL against cupper oxidation.

1. Introduction

A reappraisal of the beneficial effect of legume seed dietary intake is currently taking place. Soy proteins have been widely used as a functional ingredient in many processed foods because of their ability to form gels with high nutritional, sensory, and physiological qualities (Junfeng et al., 2006). Soy intake is of the current interest to human health because of its potential to decrease the risk of chronic diseases such as heart diseases and cancer. Protein and peptides are responsible for most of the biological/functional activities of the legume seeds (Scarafoni, Chiara, & Duranti, 2007). Several studies have been devoted to asses the antioxidant potential of the soy protein fractions as well as the structural characterization of the most active peptides. The amino acid sequences determine the antioxidant power. Processing conditions to obtain protein isolates as well as the type of protease and the degree of hydrolysis affect the antioxidant activity of native soy protein and its hydrolyzates, respectively (Moure, Domínguez, & Parajó, 2006).

Maillard reaction (MR) has been employed for improving functional properties, such as solubility, heat stability, emulsifying properties, and anti-allergenicity of proteins. This reaction has also resulted reliable for obtaining neoglycopeptides exhibiting enhanced functional (gel forming and emulsifying) properties and antioxidative activity from soy protein hydrolyzates and curdlan, which is a polysaccharide having attractive functional and biological properties and an approved food additive by US FDA (Junfeng et al., 2006). This complex food chemical event, considered the most common food reaction occurring during food processing and storage, is initiated by natural condensation of the ε-amino groups of proteins with reducing-end carbonyl group of carbohydrates giving rise numerous compounds named as Maillard reaction products (MRP). We are constantly exposed to MRP since they are present in daily diet at considerably amounts. MRP may result beneficial (Lindenmeier, Faist, & Hofmann, 2002; Somoza, 2005), as antioxidant, chemopreventive and antimutagenic agents, or harmful (Bengmark & Gil, 2007) for human health.

FOS (fructooligosaccharides) is a mixture of oligosaccharides possessing unique nutritional and functional properties. FOS are very well-known and proved prebiotics "indigestible food ingredient(s) that beneficially affects host health by selectively stimulating the growth and/or activity of one limited number of bacteria in the colon" (Gibson & Robertfroid, 1995). FOS preparation employed in the present study (Raftilose® P95) contains mostly repeating fructose subunits (FF_n) with the terminal fructose having reducing capacity and able to participate in MR (Huebner, Wehling, Parkhurst, & Hutkins, 2008; Trofimova & de Jongh, 2004; Van de Lagemaat, Silván, Moreno, Olano, & del Castillo, 2007). Soy protein and FOS may react under heating conditions forming new products with different biological properties than those showed by the

* Corresponding author. Tel.: +34 915622900; fax: +34 915644853.
E-mail address: delcastillo@ifi.csic.es (M.D. del Castillo).

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native protein. Since both food additives, soy protein and FOS, are health promoting compounds, a successful soy protein–FOS conjugate might preserve some of their beneficial properties. It is also possible that during thermal treatment, FOS could have full or partial degradation to fructose or have formation of other compounds like caramelization products, possessing new biological properties (Huebner et al., 2008). Very few are known related to the antioxidant products of the latter compounds (Tsai, Hsieh, & Huang, 2004).

The present study was undertaken to investigate how Maillard type conjugation between soy protein and FOS followed by proteolysis may affect the antioxidant properties of this food protein. The contribution of each compound forming the glycation mixture to its overall antioxidative activity was also assessed.

2. Materials and methods

2.1. Materials and reagents

2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroma-2-carboxylic acid (trolox), bovine serum albumin (BSA), $CuSO_4$, EDTA, butylated hydroxytoluene (BHT), furfural, 5-hydroxymethyl-2-furaldehyde (HMF), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), Triton X-100, sodium azide, H_2O_2 30% (w/v), sodium dodecyl sulfate (SDS), thiobarbituric acid, pepsin and pancreatin-bile extract were purchased from Sigma Chemical Co. (Germany). NaCl, HCl, $NaHCO_3$ NaH_2PO_4, Na_2HPO_4, $(NH_4)_2MoO_4$, $Cl_3C_2O_2H$, and KBr were obtained from Panreac (Spain), while KI was purchased from Merk (Germany). Phosphate buffer saline (PBS) was from Gibco, Invitrogen (UK). Commercial FOS (Raftilose® P95) was kindly provided by Orafti España S.L. SPI 90% purity was purchased at Manuel Riesgo, S.A. (Madrid, Spain).

2.2. Glycation of soy protein with FOS and fructose

SPI (25 mg/ml) and commercial FOS (500 mg/ml) were dissolved in 0.5 M phosphate buffer pH 7.4 in a molar ratio of primary amino groups to FOS 1: 52 (SPI-FOS). The mixtures were transferred to well-capped vials and heated in a water-bath at 95 °C for 1 h with constant stirring. Since commercial FOS contains a significant amount (6% w/w) of fructose, which could be an important competitor of the reducing FOS in the reaction, a SPI-fructose control system containing SPI (25 mg/ml) and fructose (38 mg/ml) in a weight ratio equivalent to that present in the SPI–FOS system, which corresponded to a molar ratio of primary amino groups to fructose 1: 16.8 (SPI–F), was included in the study. Solutions of SPI, fructose and FOS alone were also prepared and employed for controlling the occurrence of other thermal degradation reactions besides MR. After sampling, fractions were cooled immediately in an ice water-bath followed by storage at −20 °C.

2.3. Extent of glycation

The degree of lysine loss due principally to glycation was estimated with the fluorogenic *o*-phtaldialdehyde (OPA) assay described in principle by Goodno, Swaisgood, and Catignani (1981) as modified by Ramirez-Jimenez, Garcia-Villanova, and Guerra-Hernandez (2004).

2.4. Sample fractionation

Heated and unheated samples (SPI, FOS, fructose, SPI-FOS, and SPI-F) were submitted to ultrafiltration. This step of sample preparation allowed a preliminary characterization of the molecular weight of new compounds occurring in the samples. Briefly, two milliliters of samples (1 mg/ml) were placed in the sample reservoir of a Centricon YM-10 Centrifugal Filter Devices (Millipore) and centrifuged at room temperature, 5000*g* for 1 h. Proteins and other compounds possessing molecular weight higher than 10 kDa were retained (HMW), while those exhibiting lower mass were filtered (LMW). Filters were washed with water and concentrated samples recovered and dissolved in a final volume of 2 ml. The filtrates containing compounds having molecular weight lower than 10 kDa that were obtained by washing of the samples were mixed, freeze-dried, reconstituted with 2 ml of distilled water and stored at −20 °C until analysis. Protein concentration of the fractions obtained by ultrafiltration was determined by the micro BCA Protein Assay Kit (Pierce Biotech, Rockford, USA) (Smith et al., 1985).

2.5. In vitro gastrointestinal digestion mimicking physiological conditions

Samples of native SPI, heated SPI, heated SPI-F, and heated SPI-FOS were hydrolyzed. Since after consumption as part of the diet, proteins derived compounds are digested, absorbed and metabolized, we decided to investigate the production of antioxidant peptides from native and glycated soy proteins and their potential effect on key plasma targets like LDL. *In vitro* digestion was performed as described by Rubio and Seiquer (2002). A gastric digestion followed by an intestinal phase was carried out at 37 °C and pH 2.0 and 7.5, respectively. The degree of digestibility of the samples was estimated as total nitrogen remaining in solution after *in vitro* digestion, which was determined by Kjeldalh (Lynch & Barbano, 1999).

2.6. In vitro antioxidant assessments

Changes in antioxidant activity induced by thermal treatment and proteolysis of the samples were evaluated by means of two different methods known as *in vitro* LDL oxidation and oxygen radical absorbance capacity employing fluorescein ($ORAC_{FL}$).

2.7. In vitro LDL oxidation

2.7.1. LDL isolation

Fasting blood samples were collected into pre-chilled EDTA-coated tubes from healthy volunteers. Plasma was immediately separated by centrifugation at 1750*g* for 15 min and 4 °C. LDL was isolated by single discontinuous density-gradient ultracentrifugation in a vertical rotor VTI-50 employing a vacuum Beckman ultracentrifuge, at 242,000*g* during 2.5 h and 4 °C (Chung, Wilkinson, Geer, & Segrest, 1981). Isolated LDL was exhaustively dialyzed overnight against 150 mM NaCl, pH 7.4–7.6, at 4 °C. Fresh LDL isolates were used for the oxidation experiments in the following four days. LDL protein was measured employing BCA reagent kit.

2.7.2. LDL oxidation

LDL isolate was oxidized in PBS at 37 °C with 20 μM $CuSO_4$ as described below. The oxidation carried out in the absence (oxidation control) or presence of the samples was assayed. LDL oxidation was followed up by measuring conjugated dienes, lipid hydroperoxides, and thiobarbituric acid substances (TBARS) which are formed at different LDL oxidation stages. Trolox concentrations of 0.13, 0.53, 1.3 and 2.6 μg/ml were also assayed as a reference. Kinetic of LDL oxidation in presence of *in vitro* digested samples was followed by analysis of those three markers, while the effect of both undigested samples (whole protein) and their fractions containing molecules with higher and lower molecular masses than 10 kDa was assayed by analysis of conjugated dienes. Prior