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Production and characterisation of potato patatin–galactose, galactooligosaccharides, and galactan conjugates of great potential as functional ingredients

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ABSTRACT

Potato proteins are of high interest because of their high nutritional quality and multiple health benefits, but they are currently undervalued due to their limited solubility and stability. Glycated patatin (PTT) with galactose, galactooligosaccharides (GOSs) and galactan were produced through the Maillard reaction and characterised structurally and functionally. Fourier-transform infrared and fluorescence spectroscopy data revealed important changes in total secondary structures through glycation with GOSs (61.2%) and galactan (36.7%) and also significant tertiary structural changes leading to an exposure of tryptophan residues. These structural changes led to more heat stable forms of PTT with a higher unfolding temperature (70–90 °C) than the unmodified protein (50–70 °C) and with higher antioxidant activity. PTT:galactose conjugates exhibited similar thermal stability and pH-structural behaviour to native PTT. However, the high level of galactose conjugation to PTT and increased exposure of hydrophobic residues led to a significant increase in its emulsifying stability at pH 3.

1. Introduction

There is an increasing demand for the use of plant proteins as an alternative to animal proteins. Compared to proteins from other vegetable and cereal sources, potato proteins are of great potential as food ingredients because of their higher nutritional quality (Ralet & Gueguen, 2001), and their ability to regulate serum cholesterol levels (Liyanage et al., 2008), and to reduce food intake by increasing the circulation of cholecystokinin levels (Komarnytsky, Cook, & Raskin, 2011). Potato proteins include three major classes: (a) patatin (PTT, up to 40% w/w), (b) protease inhibitors (~40–50% w/w) and (c) other high molecular weight proteins (~10%) (Ralet & Gueguen, 2000). PTT is a glycoprotein with up to two carbohydrate chains and a molecular weight of approximately 40 kDa (Ralet & Gueguen, 2000). In addition to its antioxidant activity (Liu, Han, Lee, Hsu, & Hou, 2003), PTT has excellent foaming (Ralet & Gueguen, 2001), gelling (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2010), and emulsifying properties (Ralet & Gueguen, 2000). However, due to its very high exposed hydrophobicity (Creusot et al., 2010), PTT exhibits lower solubility when the ionic strength of the solution is increased. Furthermore, the denaturation temperature of PTT has been shown to be around 59 °C (Creusot et al., 2010), which is lower than those of other animal proteins being used as food ingredients. To make better use of PTT as functional ingredients and broaden its applications, the improvement of its solubility and its heat stability through structural modification is of great interest.

Among the various existing protein modification techniques, the glycation of proteins, *via* naturally-occurring Maillard reaction, led to the improvement of various functional properties of food proteins (Seo, Karboune, L'Hocine, & Yaylayan, 2013). The formation of protein-carbohydrate conjugates occurs during the early stage of the Maillard reaction, where the free amino group of protein reacts with the carbonyl group of carbohydrates to form Amadori products. During the advanced stages of the reaction, the products resulting from the degradation of the Amadori products can undergo numerous transformations *via* various pathways, giving rise to peptide-bound adducts, to protein cross-linking, and to the formation of brown and polymeric materials (Horvat & Jakas, 2004). To limit the Maillard reaction to its early stages, the Maillard reaction rate needs to be controlled. Our previous study (Seo, Karboune, Yaylayan, & L'Hocine, 2012) and other studies (Oliver, Melton, & Stanley, 2006) provided a good understanding of the effects of protein:carbohydrate ratio, temperature, incubation time, and water activity (a_w) as well as of their interactions on the glycation rate.

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The secondary and tertiary structural changes of proteins upon glycation through the Maillard reaction (Corzo-Martinez, Moreno, Olano, & Villamiel, 2008) and the improvements of their functional properties have been well investigated (Oliver et al., 2006). However, the relationships between the structural and functional properties of the protein conjugates have been overlooked and there is a need for more studies looking into structure–function relationships. The understanding of the mechanisms behind the improvements in functional properties of glycated food proteins is expected to result in a more effective production of targeted glycated proteins with enhanced functional properties.

As part of ongoing research, the objective of the present study was the investigation of the glycation of potato proteins, consisting mostly of PTT (75%), with galactose, galactooligosaccharides (GOSs) and galactan, through Maillard reaction under controlled conditions. The secondary/tertiary structural properties of purified PTT conjugates and their functional characteristics (heat stability, emulsifying activity, antioxidant activity) were studied and their relationships were discussed.

2. Materials and methods

2.1. Materials

Solanic 206P (≈75% PTT, ≈25% protease inhibitors) was kindly provided by Solanic (Foxhol, The Netherlands). Potato galactan (~280 kDa) was purchased from Megazyme (Wicklow, Ireland). Furosine standard was purchased from Neosystem Lab. (Strasbourg, France). All other chemicals were purchased from Sigma Chemical Co. (St-Louis, MO).

2.2. Preparation of galactooligosaccharides

GOSs were prepared from potato galactan according to our previous method (Seo et al., 2012). The average molar mass of GOSs was estimated to be 1990.9 g/mol through mass spectrometry analysis.

2.3. Preparation of conjugates

Potato proteins and carbohydrates (galactose or GOSs or galactan) at the molar ratio of 1:9 were dissolved (10 mg of Solanic 206P mL^{-1}) in 0.05 M sodium phosphate buffer (pH 7). After freeze drying at −25 °C, the mixtures were incubated in sealed glass desiccators at 48 °C for 1–7 days under controlled a_w value of 0.65 using a saturated solution of potassium iodide. Control experiments were performed with only potato proteins incubated under the same conditions.

2.4. Determination of the extent of glycation of PTT with selected carbohydrates

2.4.1. Furosine analysis

To estimate the degree of glycation, ε-N-2-(furoylmethyl)-L-lysine (furosine) was determined using a modified method of Seo et al. (2012). Five milligrams of incubated potato protein and carbohydrate mixture were added to 2.5 mL of 8 N HCl in vials, capped under nitrogen, and incubated at 110 °C for 23 h. The hydrolysates were centrifuged at 13,000 × *g* for 15 min and 0.5 mL of the supernatant were applied to an activated Sep-pak C18 cartridge (Waters Corp., Milford, MA). Furosine was eluted with 3 mL of 3 N HCl, and the eluate was evaporated until dryness under nitrogen and resolubilised in 1 mL of water:acetonitrile:formic acid mixture (95:4.5:0.5, v:v:v). Furosine concentration was quantified using a Beckman high-performance liquid chromatography (HPLC) system equipped with a programmable solvent module (model 126), a photodiode array detector and 32 Karat software for data collection. The separation was performed on a Waters Symmetry Shield RP8 3.5 μm (4.6 × 50 mm) column using an isocratic elution of 5 mM octanesulphonic acid in a mixture of water:acetonitrile:formic acid (79.8:20:0.2, v:v:v) for 30 min at a flow rate of 0.3 mL min^{-1}. Injected sample volume was 20 μL and the detection of furosine was performed at 280 nm. The calibration curve was constructed using a furosine standard. All assays were run in triplicates.

2.4.2. Measurement of proportion of free amino groups

The proportion of free amino groups of potato proteins and of their corresponding conjugates was assayed using trinitrobenzene sulphonic acid (TNBS) method, according to a modified method described by Seo et al. (2013). All assays were run in triplicates.

2.4.3. Determination of percentage of blocked lysine

The percentage of blocked lysine due to glycation and not to protein cross-linking was indirectly estimated from the furosine content and the initial total lysines of potato protein. The Amadori compound tagatosyl-lysine formed during the Maillard reaction between lysine residues and galactose moieties was reported to generate about 42% furosine upon 8 N acid hydrolysis (Krause, Knoll, & Henle, 2003). The estimated concentations of furosine were, therefore, adjusted accordingly. There are 24 lysines on PTT and 10–14 lysines on the major protease inhibitors (accession number, Q2MY50, P58514, Q41448, P16348, UniProtKB). By considering the proportions of PTT (75%) and of protease inhibitors (25%) present in potato proteins (Solanic 206P), 21 free amino groups were estimated. By taking the above information into account, the percentage of blocked lysine was estimated according to the following equation.

$$\%\ \text{Blocked lysine} = (1/0.42 \times \text{furosine}) \times 100/(\text{total lysine}) \quad \text{(a)}$$

2.4.4. Measurement of the protein aggregation index

The extent of protein inter/intramolecular cross-linking was expressed as the protein aggregation index, obtained spectrophotometrically by measuring the turbidity of protein–carbohydrate conjugates (10 mg mL^{-1}) at 600 nm according to a method of Wang and Ismail (2012). All assays were run in triplicates.

2.5. Purification of the conjugates

Potato proteins and their respective galactose- and GOSs-conjugates, recovered after 1 and 3 days of glycation, respectively, were purified through anionic exchange chromatography on MonoQ 5/50 GL (GE Healthcare, Piscataway, NJ) using an ÄKTApurifier system (GE Healthcare). PTT:galactan conjugates were first ultrafiltered using a stirred ultrafiltration unit (Amicon system; Millipore, Billerica, MA) fitted with a 300 kDa molecular mass cut-off membrane to remove unbound galactan, protease inhibitors, and unconjugated PTT; the retentate was further subjected to purification on MonoQ. Protein–carbohydrate conjugate mixtures (2–3 mg protein) were loaded on the column equilibrated with 50 mM potassium phosphate buffer (pH 6). PTT conjugates were eluted using the same buffer containing 1 M of sodium chloride at a flow rate of 1 mL min^{-1}. The gradient system was 0–2 min, 0% B; 2–12 min, 0–25% B; 12–17 min, 25% B; 17–22 min, 25–40% B; 22–27 min, 40% B; 27–32 min, 40–100% B; 32–37 min, 100% B. The elution profile was monitored by UV-absorbance detection at 280 nm. Fractions of 1 mL were collected and subjected to total carbohydrate analysis using the phenol–sulfuric acid method. The fractions containing purified glycated PTT were collected and dialysed against distilled water for 48 h at 4 °C. The purified