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Identification and characterisation of water and alkali soluble oligosaccharides from hazelnut skin (*Corylus avellana* L.)

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ABSTRACT

Hazelnut skins are a good example of agricultural by-product with the potential to become a valuable source of functional ingredients. In this work, the fibre from hazelnut skins was extracted by using water and alkali solution and characterised by a suite of analytical tools (MALDI-FTICR, nano LC-Chip-Q-ToF and gas chromatography). Over thirty complex free oligosaccharides, composed mainly of galacturonic acid and N-acetylgalactosamine, were characterised for the first time in the present study. Their concentration ranged between 16 and 34 mg per g of extract. The oligosaccharides isolated from this agricultural by-product are mainly hexose oligosaccharides (potentially galacto-oligosaccharides,) and xyloglucans. The identified composition could justify the bioactive activity of the extracts, namely prebiotic activity, previously demonstrated.

1. Introduction

Hazelnut is considered a nontraditional food and has been receiving an increasing attention due its nutritional and nutraceutical properties. Among nut species, hazelnut plays a major role in human nutrition and health because of its specific composition in fat (mainly oleic acid), protein, carbohydrate, dietary fibre vitamins, minerals, phytosterols (mainly β-sitosterol) and antioxidant phenolics (Alasalvar, Shahidi, Liyanapathirana, & Ohshima, 2003). Dietary fibre has important therapeutic implications for certain health conditions such diabetes, hyperlipidemia and obesity, and may exhibit a protective effect against hypertension, chronic heart diseases, cholesterol, colorectal/prostate cancer and intestinal disorder (Anderson & Bridges, 1998; Tariq et al., 2000). Hazelnut seed skin (perisperm) is a fibre-rich pellicle spontaneously detached from the seed as a consequence of roasting; its accumulation makes it a waste/underutilised by-product for the food industry, despite previous works reporting high content in antioxidant polyphenols.

Generally, plant cell wall is primarily composed of polysaccharides, which can be classified as cellulose and cell wall matrix components (namely pectin), hemicellulose and xyloglucans. Cell wall polysaccharides are already largely used by the food industry as thickeners, stabilizers, gelling agents and, in some cases, emulsifiers (Imenson, 1996). When fibre is added to food there are certain organoleptic, technological and nutritional properties, as well as considerations of shelf life, that must be satisfied (Gourgue, Champ, Lozano, & Delortlaval, 1992). The hydrolysis of soluble fibres and starch often generates oligosaccharides that have a degree of polymerisation (DP) between 2 and 25 monosaccharide units. Conventional structural characterisation of oligosaccharides has been achieved by glycosyl and linkage analyses with techniques such as nuclear magnetic resonance (NMR) or mass spectrometry (Hopkins & Macfarlane, 2003; Macfarlene, Steed, & Macfarlene, 2008). The development matrix-assisted laser desorption ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR) (Park & Lebrilla, 2005) and micro-chip liquid chromatography combined with high-performance mass spectrometry

Abbreviations: AcDP, acetone dry powders; ACN, acetonitrile; ASF, alkali soluble fraction; AUC, area under curve; BPC, base peak chromatogram; CID, collision induced dissociation; DHB, 2.5-dihydroxybenzoic acid; DP, degree of polymerisation; EIC, extracted ion chromatogram; FID, flame ionization detector; FOS, fructooligosaccharides; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; GC, gas chromatography; GCC, graphitized carbon cartridges; GOS, galactooligosaccharides; Hex, hexose; HexNAc, N-acetyl-hexosamine; Nano HPLC-Chip-Q-ToF, high pressure liquid chromatography chip quadrupole time of flight; MALDI-FTICR, matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry; OS, oligosaccharides; PGC, porous graphitized carbon; SPE, solid phase extraction; TFA, trifluoroacetic acid; TMS, trimethylsialyl; WSF, water soluble fraction; XOS, xylooligosaccharides; XyGs, xyloglucans.

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(Agilent 6520 Accurate-Mass-Quadrupole-Time-of-Flight) (Barile et al., 2010; Ninonuevo et al., 2006) have improved carbohydrate research with mass spectrometry. These are sensitive and robust analytical techniques used to achieve rapid and comprehensive oligosaccharide characterisation in biological samples without requiring chemical derivatization (Ninonuevo et al., 2006).

The following oligosaccharides are commercially available and have been suggested as having some degree of prebiotic potential: lactulose, fructooligosaccharides (FOS), galactooligosaccharides, soybean oligosaccharides, lactosucrose, isomaltooligosaccharides (IMO), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) (Gibson, Berry Ottaway, & Rastall, 2000). Moreover, arabinogalactooligosaccharides, arabinoxylooligosaccharides, arabinooligosaccharides, galacturonan oligosaccharides and rhamnogalacturonan oligosaccharides have been added for the fermentation in pure cultures of intestinal bacteria; particularly in *Bifidobacteria* spp., *Lactobacilli* spp., *Bacteroides* spp., *Clostridium* spp., *Escherichia coli* and *Klebsiella* spp. cultures (Barreteau, Delattre, & Michaud, 2006). The prebiotics properties of carbohydrates are likely influenced by oligosaccharide degree of polymerisation, monosaccharide composition (e.g. glucose, galactose, xylose, fructose, fucose, mammnose, arabinose, rhamnose, glucosamine and galacturonic acid) and by the glycosidic linkages (Rastall et al., 2005). Improved knowledge of the composition and structure of polysaccharides from plant cell wall, could result in the development of novel prebiotics. These polysaccharides are available in large amounts notably from the food industry as by-products. Additionally, the use of optimal hydrolysis condition may lead to more effective process for oligosaccharide production (Barreteau et al., 2006). It was therefore determined the exact composition of oligosaccharides in hazelnut skins and analysed their molecular structure in order to understand their technological and bioactive properties. In the present work, hazelnut skins were used as a model for optimisation of fibre extraction with the consequent oligosaccharides isolation. Their characterisation and quantification was performed by accurate mass spectrometry combined with gas chromatography (GC).

2. Materials and methods

2.1. Food samples

Samples of hazelnut skins (perisperm from *Corylus avellana* L., "Tonda Gentile Trilobata" cultivar from Piedmont, Italy) were obtained from Elah–Dufour S.p.A. (Novi Ligure, Alessandria, Italy) as a by-product of the conventional industrial roasting process (hot air convective plant).

2.2. Chemicals

All reagents and standard chemicals (D-(+) glucose, D-(+) galactose, D-(+) mannose, D-(+) fucose, D-(+) arabinose, D-(+) xylose, D-(+) rhamnose, D-(+) galacturonic acid, D-glucuronic acid, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and D-allose using for the determination of total sugar content were purchased from Sigma–Aldrich (Ontario, Canada). All solvents, chemicals and enzymes used in all analysis were of reagent-grade level and purchased either from Sigma–Aldrich (Ontario, Canada). 2.5-dihydroxybenzoic acid (DHB) was purchased from Sigma–Aldrich (Ontario, Canada).

2.3. Oligosaccharides extraction

The procedure is exemplified in Fig. 1. Hazelnut skin was first defatted as described in the AOAC Official Methods (AOAC, 1995),

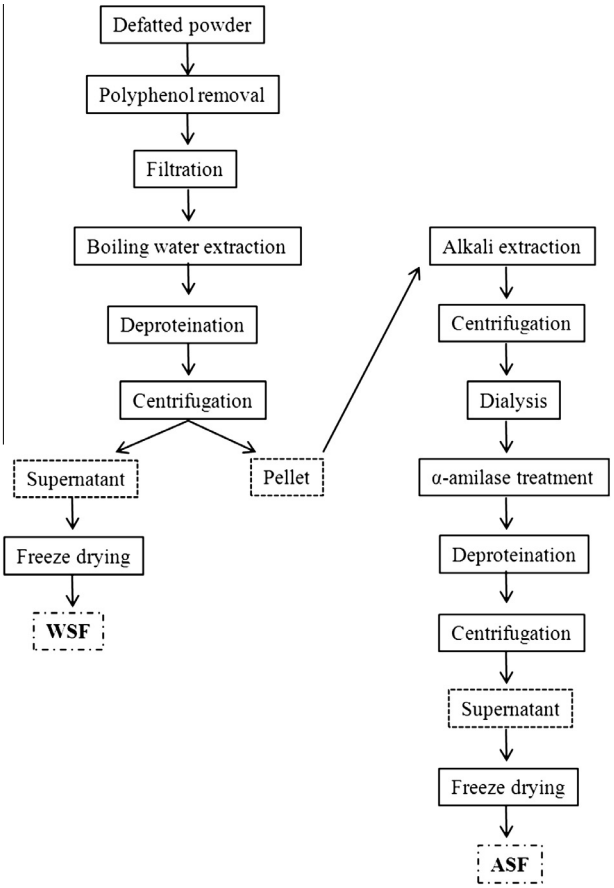

Fig. 1. Representative illustration of the extraction procedure.

and was then subjected to phenolic compounds extraction using the method described by Voigt, Wramr, Heinrichs, and Biehl (1994). Briefly, 10 g of sample were extracted in an aqueous solution (twice 180 mL of 80% acetone solution; once 180 mL of 70% acetone solution; once 120 mL of 100% acetone solution and once 180 mL of 100% methanol solution) under magnetic stirring at 4 °C (1 h for each step). About 5 g of solid residue (acetone dry powders or AcDP) were obtained after the extraction and were washed twice with 60 mL of 80% (w/w) ethanol to inactivate the enzymes and to remove residual polyphenols according to the method described by Synytsya et al. (2009). The mixture was filtered and the residue was washed with distilled water and extracted with 200 mL of boiling water for 6 h. The temperature was adjusted to 37 °C and the suspension was incubated with 150 mL of α-amylase (5 mg/mLin 3.6 mM $CaCl_2$; 1:250 v/v) for 1 h to remove α-glucans. Ethanol precipitation (70%, three volumes) was applied to remove proteins. The sample was centrifuged (8000*g* for 15 min) and the supernatant containing the water soluble fraction (WSF) was recovered and lyophilized (HETO mod. Drywinner 8: pre-freezing −20 °C for 2 h; primary drying −10 °C for 10 h and 0 °C for 8 h; secondary drying 10 °C for 4 h and 20 °C for 2 h). The pellet was treated with 50 mL of sodium hydroxide solution (1 M). This suspension was centrifuged, dialyzed and the supernatant was incubated with 150 mL of α-amylase solution (5 mg/mL in 3.6 mM $CaCl_2$; 1:250 v/v) at the same conditions above described. Ethanol precipitation (70%, three volumes) was applied to remove proteins. The sample was centrifuged (at 8000*g* for 15 min) and the supernatant containing the alkali soluble fraction (ASF) was collected and lyophilized, as described above. The extraction process was conducted in triplicate.