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Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas

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ABSTRACT

To better understand the role that dietary fibre plays in the health benefits of pulses, the cell wall polysaccharides of pulses were characterized. To allow comparison between this data and nutritional studies, cooked chickpeas, lentils, beans and peas were used rather than raw seeds. Insoluble fibre, soluble fibre, resistant starch and oligosaccharide fractions were isolated using digestive enzymes at 37 °C. Prebiotic galacto-oligosaccharides, including raffinose, stachyose and verbascose made up between 3.5 and 6.9% of the cooked pulses (dry weight basis). Pulse fibre is pectin rich, with most being found in the soluble fibre fraction. The soluble fibre fraction is a diverse mixture of polysaccharides of varying sizes and solutions of soluble fibre exhibit a range of viscosities depending on the pulse type. The sugars which make up the insoluble fibre fraction suggest a large cellulose component. Microscopic examination of cooked whole pulses showed that a large fraction of the starch was partially gelatinized and contained within intact cell walls whereas boiled flour pastes had few ungelatinized granules. After simulated upper-gut digestion, some starch remained inaccessible to digestive enzymes. Cell walls of pulses modulate starch gelatinization and reduce enzymatic hydrolysis, which may account for the low glycemic response attributed to pulses.

1. Introduction

Pulses, including beans, chickpeas, lentils and dry peas have long been recognised as nutritious because of their high quality protein (Boye, Zare, & Pletch, 2010) and nutrient density (Azarpazhooh & Boye, 2012). It has long been established that pulses are low glycemic index (Jenkins et al., 1983; Sievenpiper et al., 2009) and there is growing evidence that eating pulse foods regularly reduces serum cholesterol (Ha et al., 2014). Additional health benefits of pulses have been revealed through recent research. They have been shown to reduce blood pressure (Jayalath et al., 2014) and the high levels of polyphenols have good antioxidant properties (Azarpazhooh & Boye, 2012). As knowledge of the role of dietary fibre in preventing disease and enhancing health has increased, so too has interest in pulse crops. Pulses are a rich source of both soluble and insoluble dietary fibre (Tosh & Yada, 2010) and vegetable protein (Boye et al., 2010). These health benefits may be partially due to the composition and behaviour of the non-starch polysaccharides making up the cell walls of pulses. Therefore, it is important to understand the composition and physicochemical properties of the dietary fibre fraction. The galacto-oligosaccharide profiles of cooked pulses are also of interest. Previously, these small molecular weight carbohydrates were considered undesirable because they caused flatulence to people who did not consume them regularly. However, there is growing recognition that they have a prebiotic effect, in that they encourage the growth of probiotic bacteria which produce beneficial short chain fatty acids (Azarpazhooh & Boye, 2012).

Previous studies have shown that pulses are a significant source of dietary fibre. There is a wide degree of variation in the dietary fibre amounts and soluble to insoluble fibre ratios in pulses (Tosh & Yada, 2010). The total dietary fibre measurements in dried peas range from 14 to 26% (dwb) whereas beans were found to have 23–32% total dietary fibre. In order to better interpret the physiological function of pulse fibres, it is necessary to have better information about the characteristics of pulse varieties of economic interest. There are several types of fibre contained in pulses, including long chain soluble and insoluble polysaccharides, galacto-oligosaccharides and resistant starch. While insoluble fibre is typically associated with laxation, soluble fibre is linked with reducing cholesterol levels and ameliorating post-prandial blood glucose levels. All fibres can function as prebiotics, providing food for gut microorganisms.

Although some analyses of pulses have been conducted, most have focused on raw flours (Dalgetty & Baik, 2003) or hull materials (Ralet, Saulnier, & Thibault, 1993) which are not necessarily indicative of the whole pulses as eaten. In particular, the amount of resistant starch and the ratio of soluble to insoluble fibre are likely to change during processing.

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Understanding the positive health effects of pulses requires comprehensive characterisation of their component parts, including the different fibre sources. The objective was to characterize pulse fibres from commonly grown pulse varieties to identify and compare characteristics which may provide health benefits. Therefore, the soluble and insoluble fibre fractions from eight pulse varieties were extracted and characterized in terms of their composition and rheological behaviour. To that end, two varieties of four pulse types (pea, chickpea, beans and lentils) were analysed. Cooked whole pea, chickpeas, beans and lentils were analysed for galacto-oligosaccharides, starch and resistant starch content. Extracted soluble and insoluble dietary fibres were analysed for uronic acid content and monosaccharide profile. Extracted soluble fibre was analysed for molecular weight and viscosity. The fibre fractions collected were designed to be representative of the fibres in the condition they are present in the small intestine, where nutrients (sugars, protein, fats) are absorbed from food. This fractionation should give us the best comparison with potential health benefits such as lowering blood glucose and serum cholesterol. Additionally, the stability of the cell walls during digestion was investigated by subjecting cooked, finely diced pulses to a simulated digestion system and examining changes in the microstructure. This treatment was compared with pulse flours which were boiled to form a paste and also treated by simulated digestion.

2. Materials and methods

2.1. Materials

Green (CDC *Striker*) and yellow peas (CDC *Golden*), kabuli (CDC *Frontier*) and desi (CDC *Corinne*) chickpeas, green (CDC *Greenland*) and red lentils (CDC *Maxim*) and navy beans and pinto beans (CDC *Windbreaker*) were purchased from Saskatchewan Pulse Growers (Saskatoon, SK). Beans and chickpeas were soaked 16 h at a 1:4 pulse to deionised water ratio before being drained and added to a pot with sufficient water to achieve a 1:2.5 (chickpeas) or 1:2 ratio (beans). The beans and chickpeas were brought to a boil and then simmered for 22 min. Without soaking, lentils and peas were brought to a boil in deionised water after rinsing. Lentils were cooked at a 1:2.5 ratio, yellow peas at 1:3 and green peas at 1:3.25. Lentils were simmered for 30 min after boiling while yellow peas required 2 h and 5 min and green peas 1 h and 50 min. After cooking, all pulses were poured into shallow pans, cooled and freeze dried. Dried cooked pulses were milled to pass a 30 mesh screen and stored dry, in a desiccator.

Pulses were also cooked after grinding for microscopic imaging. Raw pulses were milled first in a laboratory mill and then a ball mill (Retsch, Haan, Germany) to pass a 45 mesh screen. Ground pulse flour and water were mixed at a 1:4.5 ratio (g:ml), brought to a boil and then simmered, covered for 10 min.

2.2. Total and resistant starch content

Total starch of raw and cooked pulses was measured according to AOAC method 996.11. The resistant starch of cooked pulses was measured according to AOAC method 2002.02. Starch and resistant starch were measured using commercially available assay kits (Megazyme International, Bray, Co. Wicklow, Ireland). Total and resistant starches were calculated as a percentage of the total dry matter (% dwb).

2.3. Extraction of ethanol soluble mono-, di- and oligosaccharides

Dry, ground, cooked pulses were extracted in 50% ethanol for 30 min at 23 °C using 1 g of pulse solids per 10 ml. This solvent has previously been used to extract sugars and oligosaccharides while inhibiting co-extraction of water soluble polysaccharides (Bainy, Tosh, Corredig, Poysa, & Woodrow, 2008). Extracted mono-, di- and oligosaccharides were separated from the non-ethanol soluble material by centrifugation (10,000 ×*g* for 10 min) and the supernatant was reduced in a rotary evaporator. The extract was diluted to 10 ml and analysed on an HPLC-PAD system as outlined below.

2.4. Extraction of soluble and insoluble fibre

Soluble and insoluble fibres were extracted from each pulse using a modification of AOAC method 991.43, total soluble and insoluble dietary fibre in foods. Dry, ground pulse flour was mixed with 80 ml of MES/Tris buffer (0.5 M, pH 8.2), 300 U thermostable α-amylase (Megazyme International, Bray, Co. Wicklow, Ireland) and heated at 95–100 °C for 35 min. After the addition of 20 ml deionised water and cooling to 60 °C, 60 U of protease (Megazyme International, Bray, Co. Wicklow, Ireland) was added and the mixture was stirred for 30 min at 60 °C. The pH was adjusted to between 4.1 and 4.8 with the addition of HCl and 3260 U of amyloglucosidase (Megazyme International, Bray, Co. Wicklow, Ireland) was added, followed by stirring at 60 °C for 30 min. The solution was boiled for 10 min to deactivate enzymes and then centrifuged for 15 min at 10000 ×*g*. The insoluble residue was kept and washed twice with 10 ml of 95% ethanol and then a third time with 10 ml acetone on a fritted glass filter. The supernatant was decanted and mixed with 4 times the supernatant volume of 95% ethanol to precipitate the water soluble fibre. The mixture was centrifuged 15 min at 10000 ×*g* to recover the soluble fibre. This residue was also washed with 10 ml of 95% ethanol and then acetone. Both the soluble and insoluble fibres were dried in an air oven at 60 °C, milled to a powder and then dried in a vacuum oven at 80 °C. Dried powders were stored in a desiccator.

2.5. Spectrophotometric measurement of galacturonic acid

Galacturonic acid measurement was carried out according to the colourometric method of Blumenkrantz and Asboe-Hansen (1973) using *m*-hydroxydiphenyl. Measurements were carried out on a Cary 300 spectrophotometer (Agilent Technologies Canada, Mississauga, ON) using galacturonic acid monohydrate (Sigma-Aldrich Canada Co., Oakville, ON) to create a calibration curve ranging from 10 to 100 μg/ml.

2.6. HPLC analysis of mono-, di- and oligosaccharides

Mono-, di- and oligosaccharides present in the soaking water from beans and chickpeas and extracted from cooked, ground pulse flours using ethanol were analysed by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (DX600, Thermo Scientific Dionex, Sunnyvale, CA) using a CarboPac PA1 column (4 × 250 mm) and guard (3 × 25 mm). Samples were eluted in 10 mM NaOH for 25 min and then another 20 min in 100 mM NaOH. Standard solutions between 5 and 80 μg/ml were prepared using pure sugars (glucose, galactose, sucrose, fructose, raffinose, stachylose and verbascose) and these were used to quantify extracted sugars. Pulse potentials (E, volts) and durations (t, ms) were $E_1 = 0.05$, $t_1 = 480$, $E_2 = 0.6$, and $t_2 = 180$; $E_2 = 0.6$, $t_2 = 180$, $E_3 = -0.6$, and $t_3 = 60$ with a 1.0 s detector response time. Standard solutions between 5 and 80 μg/ml were prepared using pure sugars (glucose, galactose, rhamnose, arabinose, xylose and mannose) and these were used to quantify sugar residues released by hydrolysis.

2.7. HPLC analysis of monosaccharide profiles of polysaccharides

Soluble and insoluble fibres were accurately weighed (~20 mg) into glass test tubes and stirred with 0.5 ml 72% H_2SO_4 for 1 h at room temperature. Another 5.5 ml of deionised water was added to each tube and they were stirred 3 h at 100 °C. After cooling, the hydrolysates were diluted to 10 ml with water in a volumetric flask and then stored at −20 until further analysis.