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Bound phytophenols from ready-to-eat cereals: Comparison with other plant-based foods

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ABSTRACT

Whole-grain diets are linked to reduced risk of several chronic diseases (heart disease, cancer, diabetes, metabolic syndrome) and all-cause mortality. There is increasing evidence that these benefits are associated with the gut microbiota and that release of fibre-related phenolic metabolites in the gut is a contributing factor. Additional sources of these metabolites include fruits and vegetables, but the evidence for their protective effects is less well established. With respect to the availability of bound phytophenols, ready-to-eat cereals are compared with soft fruits (considered rich in antioxidants) and other commonly consumed fruits and vegetables. The results demonstrated that when compared with an equivalent serving of fruits or vegetables, a recommended portion of whole-grain cereals deliver substantially higher amounts of bound phytophenols, which are available for metabolism in the colon. The increased amount of these phenolic metabolites may, in part, explain the evidence for the protective effects of whole-grain cereals.

1. Introduction

Up until the pre-industrial revolution, the diet was predominantly composed of unrefined grains and vegetables with an overall substantially high fibre content. However, with the emergence of the industrial revolution came a dramatic change in human lifestyle with diet becoming increasingly processed, leading to decreased levels of fibre intake (Burkitt & Trowell, 1975; Cleave, Campbell, & Painter, 1969). Dietary fibre, the indigestible cell wall component of plant material, plays an important role in human diet and health (Adiotomre, Eastwood, Edwards, & Brydon, 1990; Montagne, Pluske, & Hampson, 2003; Smith & Tucker, 2011). Dietary fibre is a heterogeneous group of compounds that can be classified according to their solubility. Water insoluble fibre components include cellulose (a major component of the cell wall), hemicelluloses (cell wall polysaccharides soluble in dilute alkali found mainly in cereal grains) and lignin (non-carbohydrate cell wall components found in woody plants). Water soluble fibre components include pectin (found in fruits, vegetables, legumes, sugar beet and potatoes), gums (may be secreted by plants in a response to injury often found in leguminous seed plants and seaweed) and mucilages (synthesised by plants to prevent desiccation of seed endosperm) (Dhingra, Michael, Rajput, & Patil, 2012).

The 'fibre hypothesis' proposed in the 1970s suggested that foods rich in fibre, such as whole grains, fruits, and vegetables could deliver important health benefits (Trowell, 1972). Some important grains in the human diet include wheat, rice, maize and oats (Slavin, 2003), followed by rye, barley, triticale, millet and sorghum (Fardet, 2010). The physical structure of most cereal grains is similar and comprises the endosperm, germ and bran. The absolute amount of each of these components varies with the type of grain. For example, the bran content of maize is approximately 6% while that of wheat is around 16% (Slavin, 2003). Whole-grain cereals are also a rich source of compounds considered to be bioactive. For example, whole-grain wheat that contains approximately 13% dietary fibre, also contains at least 2% of bioactive compounds, whereas the bran and germ fractions (45% and 18% of dietary fibre, respectively) contains around 6–7% of these compounds. Removal of the bran and germ fractions from whole-grain wheat resulted in a significant reduction of phytochemicals and reported refining-related losses of fibre (by 58%), Mg (by 83%), Zn (by 79%), Se (by 92%), nicotinic acid (by 70%), folates (by 61%) and vitamin E (by 79%) (Truswell, 2002). This indicates that bioactive compounds present in whole wheat are mainly concentrated in the bran and

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germ fractions and refined cereal products are likely to contain substantially less protective compounds.

The majority of potential bioactives found in wheat bran are derived by the phenylpropanoid pathway and these include the phenolic acids (Mateo Anson, Hemery, Bast, & Haenen, 2012; Russell & Duthie, 2011). These can be divided into three fractions, depending on their potential interactions with other components in the food matrix. Soluble phenolic acids, directly measured in the supernatants of aqueous-organic extractions; esterified phenolic acids measured in the same supernatants after performing alkaline or acid hydrolysis; and insoluble phenolic acids, which remain in the residues following extraction and hydrolysis (Perez-Jimenez & Torres, 2011). Esterified phenolic acids, predominantly found in cereals, mostly consist of feruloylated oligosaccharides (e.g., feruloyl-arabinofuranosyl-xylopyranosylxylose) or sugar esters of ferulic acid (e.g., 5-O-feruloyl-L-arabinofuranose). Ferulic acid is linked by ester bonds to polymers in the plant cell wall, mostly to hemicelluloses (Faulds & Williamson, 1999). Ferulic acid is found to be linked to lignin through both ether and ester bonds and also mediates polysaccharide-protein cross-linking within the cell wall. Ferulic acid and other phytophenol moieties (constituting approximately 34% of the non-extractable compounds in wheat bran) (Sun, Sun, Wang, Zhu, & Wang, 2002; Verma, Hucl, & Chibbar, 2009), are also present in a wide variety of plant-based foods (Clifford, 1999).

Much research to date on phytophenols has focussed on the antioxidant potential of easily solubilised compounds. Recent evidence suggests that cereal grains could have a much more significant impact on total dietary intake of phytophenols than previously considered and that their biological effects could be more extensive than just their ability to inhibit oxidation (Anson, Havenaar, Bast, & Haenen, 2010; Russell et al., 2008). The total phytophenol content of grains had been commonly underestimated, as most of these compounds are present in the bound form (85% corn, 75% oats/wheat, 62% rice). Consequently, many of these compounds and their metabolites have remained largely undetermined and this fraction could provide a major contribution to the potential bioactivity (Adom, Sorrells, & Liu, 2003). To benefit both the rapidly growing food industry and the consumer, there is now an important need to identify the phytophenol profiles of fibre-containing foods. The aim of this work is to determine these important fibre-related and other bound metabolites in a variety of commercially available ready-to-eat cereals and compare these with those present in a range of soft fruits and commonly consumed fruits and vegetables.

2. Materials and methods

2.1. General reagents

Standard phytophenols and general laboratory reagents were purchased from Sigma-Aldrich (Gillingham, England). Compounds which were not commercially available (including the ferulic acid dimers) were synthesised as described previously (Russell, Burkitt, Forrester, & Chesson, 1996; Russell, Burkitt, Scobbie, & Chesson, 2003).

2.2. Cereals, fruits and vegetables

The ready to eat cereals analysed were breakfast cereals normally consumed without further preparation, except for addition of milk, with or without heating. These included: (1) wheat bran (87%) based (fibre content = 27 g 100 g^{-1}), (2) whole wheat (65%) and wheat bran (21%) based (fibre content = 15 g 100 g^{-1}), (3) maize (unspecified%) based (fibre content = 3 g 100 g^{-1}), (4) mixed whole-grain based (oats, wheat, barley, rice, maize; 77.8%) based (fibre content = 7.1 g 100 g^{-1}), (5) whole wheat (94.9%) based (fibre content = 9.9 g 100 g^{-1}) and (6) rolled oats (100%) based (fibre content = 9 g 100 g^{-1}). These were selected to contain a variety of cereals in a wide concentration range. The fruits and vegetables were blackberry (*Rubus fruticosus*; Sleeping beauty; Mexico), raspberry (*Rubus idaeus*; Maravilla; Morocco), blueberry (*Vaccinium cyanococcus*; Kirra; Morocco), red cherry (*Prunus avium*; Kirra; Spain), cranberries (*Vaccinium oxycoccus*; USA), goji berry (*Lycium barbarum*; China), tomato (*Solanum lycopersicum*; Sanita Cherry; Spain), grape (*Vitis vinifera*; Summer Royal Seedless; Italy), pear (*Pyrus communis*; Conference; Holland), carrot (*Daucus carota*; UK), apple (*Malus domestica*; Royal Gala; New Zealand) and broccoli (*Brassica oleracea*, UK). All products were purchased form a local supermarket. All fruits and vegetables were considered to be ripe and were prepared in accordance with the predominant method of consumption.

2.3. Extraction of phytophenols from cereals, fruits and vegetables

Cereals were analysed directly and the moisture content determined independently. All fruits and vegetables were washed, weighed and stored at −80 °C. They were then lyophilised (Heto Lab Equipment; Allerød; Denmark) and the moisture loss recorded. All samples were freeze-milled (Spex 6700; Edison; USA) and the powder stored in a desiccator prior to extraction. In order to extract bound phenolic acids from the cell wall (polysaccharides, lignins, or proteins) it is necessary to hydrolyse using acid, alkaline, or enzymatic procedures (Barberousse et al., 2008). These methods have been shown to release phenolic compounds from various plant sources (Bunzel, Ralph, Bruning, & Steinhart, 2006; Bunzel et al., 2003 Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001; Navarro-Gonzalez, García-Valverde, García-Alonso, & Periago, 2011). Samples (approx. 0.1 g dry weight; $n = 3$) were suspended in HCl (0.2 mol dm^{-3}; 3 cm^3), extracted into ethyl acetate (EtOAc; 5 cm^3) and the layers were separated by centrifugation (5 min; 1800×*g*; 4 °C). The extraction was repeated three times and the EtOAc extracts combined. The organic layer was left to stand over sodium sulphate (anhydrous) for 1 h and filtered. The combined organic layers were then evaporated to dryness under reduced pressure at temperature not exceeding 40 °C and stored in a desiccator prior to analysis by HPLC. The pH of the aqueous fraction was increased to pH 7 with NaOH (4 mol dm^{-3}). NaOH (4 mol dm^{-3}) was added to give a final concentration of 1 mol dm^{-3} and the sample stirred at room temperature for 4 h under nitrogen. The pH was reduced to pH 2 with HCl (6 mol dm^{-3}) and the samples extracted into EtOAc (5 cm^3 × 3) and processed as described above. The pH of the aqueous fraction was then increased to pH 7 with NaOH (4 mol dm^{-3}). HCl (10 mol dm^{-3}) was added to give a final concentration of 2 mol dm^{-3} and the sample incubated with stirring at 95 °C for 30 min. Cooled and extracted with EtOAc (5 cm^3 × 3) and processed again as described above. The extracts were then re-dissolved in methanol (0.5 cm^3) and an aliquot (20 μl) transferred to an eppendorf. Internal standard 1 for negative mode mass spectrometry (IS1; ^{13}C benzoic acid; 2 ng μl^{-1} in 0.02% acetic acid in 75% methanol; 20 μl), internal standard 2 for positive mode mass spectrometry (IS2; 2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline; 0.5 ng μl^{-1} in 0.02% acetic acid in 75% methanol; 20 μl) and acidified (0.4 mol dm^{-3} HCl) methanol (40 μl) were added, vortexed and centrifuged (12,500×*g*; 5 min; 4 °C).

2.4. Metabolite analysis

Liquid chromatography separation of a wide range of phenolic metabolites (Russell et al., 2011) was performed on an Agilent