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Dry-fractionation of wheat bran increases the bioaccessibility of phenolic acids in breads made from processed bran fractions

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ABSTRACT

This study evaluated the potential of using ultra-fine grinding and electrostatic separation of wheat bran as methods to improve the bioaccessibility of para-coumaric acid (pCA), sinapic acid (SA) and ferulic acid (FA) from bran-rich breads. Bran fractions were produced and used to bake white bread, whole-grain bread, and seven different bran-rich breads. The influence of bran particle size and bread composition on the bioaccessibility of pCA, SA and FA was studied using a dynamic computer-controlled *in vitro* gastro-intestinal model. The amount of bioaccessible phenolic acids was higher in whole-grain bread and bran-rich breads than in white bread, and the finer the bran particles in bran-rich breads, the more bioaccessible the phenolic acids. The highest amounts of bioaccessible phenolic acids were observed for two of the fractions obtained by electrostatic separation of ground bran. Only the free and conjugated phenolic acids forms were found to be bioaccessible, and the bioaccessibility of SA was much higher than that of FA, due to the higher solubility of SA. This study demonstrated that the use of bran fractionation to reduce the particle size, or to include only some parts of the bran in foods, can help developing grain-based products with increased nutritional potential.

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1. Introduction

Epidemiological studies have shown that the consumption of whole grain foods has a protective effect against the development of diet-related disorders such as cardiovascular disease, type 2 diabetes, and certain types of cancers, this beneficial influence being linked to the high phytochemical content of whole grains (Anderson, 2004; Jacobs, Marquart, Slavin, & Kushi, 1998; McKeown, Meigs, Liu, Wilson, & Jacques, 2002). In wheat grain (*Triticum aestivum* L.), the majority of the health-beneficial phytochemicals are present in bran and germ. For example, the bran/germ fraction contains 83% of total grain phenolic content (Liu, 2007). Among wheat grain phytochemicals, phenolic acids are of particular interest, with ferulic acid (FA) being the most common phenolic acid found in wheat grain and bran, and sinapic acid (SA) and para-coumaric acid (pCA) being minor compounds (Liu, 2007). One of the best documented biological activities of phenolic acids is their antioxidant properties (Fardet, Rock, & Rémésy, 2008; Zhao & Moghadasian, 2008). Indeed, FA, SA and pCA have been reported to prevent peroxidation of lipids and proteins through radical scavenging activities (Kikuzaki, Hisamoto, Hirose, Akiyama, & Taniguchi, 2002; Niwa, Doi, Kato, & Osawa, 1999), to exert anti-inflammatory effects *in vivo* (Luceri et al., 2007; Yun et al., 2008) and to display breast cancer chemopreventive activity (Hudson, Dinh, Kokubun, Simmonds, & Gescher, 2000). SA was also found to exert cerebral protective and anxiolytic properties in mice (Karakida et al., 2007; Yoon et al., 2007), and pCA to exhibit antimelanogenic properties (An, Lee, Choi, Moon, & Boo, 2008).

Wheat bran is a complex material composed of several layers, which are characterized by distinct structures and compositions. The outer pericarp and inner pericarp are mostly made of

Abbreviations: FA, ferulic acid; pCA, para-coumaric acid; SA, sinapic acid; GI, gastrointestinal; DM, dry matter; FES, fraction from electrostatic separation; D_{50}, median particle size; TEAC, Trolox equivalent antioxidant capacity.

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branched heteroxylans, cellulose and lignin, characterized by numerous cross-links between the polymer chains (Fincher & Stone, 1986; Pomeranz, 1988). The testa is rich in lignin, whereas the hyaline layer is mainly composed of poorly cross-linked arabinoxylans (Barron, Surget, & Rouau, 2007; Evers & Reed, 1988). The aleurone layer is made up of thick cell walls composed of relatively linear arabinoxylans and β-glucans, enclosing intra-cellular bioactive compounds (Fincher & Stone, 1986; Pomeranz, 1988). In bran layers, ferulic and para-coumaric acids are mostly insoluble due to ester-bonds with cell wall components such as arabinoxylans and lignin, whereas a high proportion of sinapic acid is soluble, esterified to sugars and other small compounds (Li, Shewry, & Ward, 2008; Pomeranz, 1988).

Phenolic acids need to be absorbed during digestion to play their protective role in the human body. Previous rat and human trials have reported the low bioavailability of ferulic acid from bran-rich foods (Adam et al., 2002; Kern, Bennett, Mellon, Kroon, & Garcia-Conesa, 2003), probably due to the structure of the food matrix effect restricting the release of FA in the gastrointestinal tract (Adam et al., 2002; Vitaglione, Napolitano, & Fogliano, 2008). Indeed, the bioavailability of ferulic acid has been said to be greatly influenced by its bioaccessibility in the small intestine (Mateo Anson, van denBerg, Havenaar, Bast, & Haenen, 2009b), i.e. by its release from the food matrix and availability for intestinal absorption (Parada & Aguilera, 2007). Therefore, modifying the structure and/or the composition of the food matrix could be a good way to improve the bioavailability of phenolic acids from whole grain foods.

Different processing methods can be employed to modify the bran structure and composition to enhance the bioaccessibility of phenolic acids, by using either wet-fractionation processes such as enzymatic treatments and fermentation (Katina et al., 2005; Moore, Cheng, Su, & Yu, 2006), or dry-fractionation processes such as ultra-fine grinding, air-classification and electrostatic separation (Antoine, Peyron, Lullien-Pellerin, Abecassis, & Rouau, 2004; Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007). The use of wheat bran dry-fractionation as a mean to improve the bioaccessibility of phenolic acids from whole grain foods has never been studied. Ultra-fine grinding, by drastically decreasing bran particle size, could make phenolic acids more easily accessible to enzymes (during both fermentation before baking, and digestion), either by increasing the particle surface area of cell walls, or by breaking the aleurone cells and thus increasing the release of intra-cellular contents. This might favor the release of phenolic acids from the food matrix in the gastrointestinal (GI) tract. Furthermore, fractionation processes such as electrostatic separation allow to dissociate the different bran layers to produce bran fractions displaying different compositions (Hemery, Rouau, et al., 2009b). Thus, bran fractions with high phenolic content can be obtained and may be used to produce high phenolic content cereal foods.

The purpose of the present study was to evaluate the potential of using bran ultra-fine grinding and bran electrostatic separation as a way to improve the bioaccessibility of pCA, SA and FA from a standard food matrix such as whole-grain bread. Therefore, various bran fractions and flours were produced using these dry-fractionation processes, and used to bake white bread, whole-grain bread, and seven different bran-rich breads. The use of a dynamic computer-controlled *in vitro* gastro-intestinal model (TNO intestinal model) made it possible to study the release of phenolic acids from the breads during gastrointestinal transit, and to assess the influence of bran particle size and bread composition on the bioaccessibility of para-coumaric, sinapic and ferulic acids.

2. Materials and methods

2.1. Chemicals

Pepsin (P-7012), bile (P-8631), trypsin (P-5147), α-amylase (A-6211), and 2,3,5 trimethoxy-(E)-cinnamic Acid (TMCA, T-4002) were obtained from Sigma–Aldrich (St. Louis, USA). Rhizopus lipase (150,000 units/mg, F-AP 15) was obtained from Amano Enzyme Inc. (Nagoya, Japan), and pancreatin (Pancreax V powder) was obtained from Paines & Byrne (Greenford, UK).

2.2. Preparation of bran fractions

All flours and bran fractions were produced from grains of wheat (*T. aestivum* L.) cultivar Tiger, harvested in 2006 in Germany. A Bühler laboratory mill (Bühler A.G., Uzwil, Switzerland) was used to produce white flour (0.55% ash content) and whole meal flour (containing 100% of the wheat grain). Coarse bran was obtained after a large scale conventional milling process, and was further processed in an impact mill into fractions named "Amb. medium" and "Amb. fine", according to a Bühler A.G. Patent (Bohm, Bogoni, Behrens, & Otto, 2003). The "Amb. ultrafine" fraction was produced by carrying out three successive grinding steps with a selection grid of 0.3 mm (the material was milled to pass through the grid). The fraction named "Cryo. ultrafine" was obtained by cryogenic grinding at −100 °C by combining a cryogenic screw feeder (Micronis, Agen, France) with liquid nitrogen supply to the hammer mill, in order to make the bran more brittle by lowering the temperature, resulting in the bran breaking up in smaller particles.

A pilot electrostatic separator (TEP System, Tribo Flow Separations, Lexington, USA) was used for the production of fractions with various compositions, as described by Hemery, Holopainen, et al. (2010), using ultrafine bran (obtained by cryogenic grinding) as a starting material. The particles were charged by impacting against each other and against the walls of the charging line, and the charged bran particles were then introduced in a separation chamber with two high voltage electrodes (15,000 V), where the positively charged particles were attracted by the negative electrode and *vice versa*. A particles recovery system allowed to separately collect two fractions, containing the positively or negatively charged particles. These two fractions underwent a second separation step, giving four different fractions. The two "purest" samples were kept, giving the "FES positive" and the "FES negative" fractions, and their two by-products were mixed, giving the "FES middle" fraction.

The particle size distribution of the samples was measured by laser diffraction using a Coulter LS 230 granulometer (Coulter, Miami, USA) at room temperature, using ethanol as carrier. The particles median diameter (D_{50}) and the granulometric distribution (expressed in % of total particles volume) were determined to characterize the fractions.

2.3. Breadmaking

The fractions and flours were used to bake white, whole, or bran-rich breads. The proportion of bran to be included in bran-rich breads was calculated, based on the amount of total phenolic acids in the flours and bran, in order to have the same amount of phenolic acids in the mixture of white flour + bran, than in the whole meal flour. Thus, the bran-rich breads (made from the Amb. medium, Amb. fine, Amb. ultrafine, and Cryo. ultrafine fractions) contained 15.9% bran and 80.4% white flour (DM basis), while the white and whole-grain breads contained 96.3% white flour and whole meal flour, respectively. The same recipe was kept

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