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Antioxidant and anti-inflammatory capacity of bioaccessible compounds from wheat fractions after gastrointestinal digestion

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ABSTRACT

Wholegrain consumption is associated with several health benefits, in contrast to the consumption of refined grains. This can partly be related to the antioxidant compounds in the outer parts of the grain kernel. The bioaccessibility of these antioxidant compounds from the wholegrain matrix during gastrointestinal digestion is crucial for their absorption and bioavailability. In the current study, the bioaccessible compounds from aleurone, bran and flour were obtained from a dynamic *in vitro* model of the upper gastrointestinal tract. They were collected at 1 h time intervals to assess their antioxidant capacity (TEAC assay) and also their anti-inflammatory effect (TNF-α reduction in U937 macrophages stimulated with LPS). The bioaccessible compounds from aleurone had the highest antioxidant capacity and provided a prolonged anti-inflammatory effect, shown by the TNF-α reduction of a relatively late time-interval (3–4 h after start of digestion). The contribution of ferulic acid to those effects was minor due to its low bioaccessibility. Aleurone seems a promising wheat fraction for cereal products with a healthy added value.

1. Introduction

Wholegrain consumption has been associated with a reduced risk for the development of the metabolic syndrome (Esmaillzadeh and Azadbakht, 2006; Sahyoun et al., 2006; Slavin, 2004). The metabolic syndrome increases, among others, the development of cardiovascular disease and type 2 diabetes. There are some indications that chronic inflammation and oxidative stress may play a central role in the aggravation of these disorders (Förstermann, 2008; Hansel et al., 2004; Van Guilder et al., 2006).

It has been suggested that the outermost component of the grain, i.e. the bran, is the main one responsible for the health benefits associated with wholegrain (Jensen et al., 2004). Bran contains many bioactive compounds, such as micronutrients (vitamins and minerals), antioxidants (phenolic compounds), and other phytochemicals (phytic acid, sterols). Most of these compounds are particularly concentrated in the aleurone layer, which is a monolayer of cells overlying the endosperm and adhering to the pericarp (Hemery et al., 2009). The aleurone cells play a crucial role in plant physiology, since the aleurone cells host hormonal signaling processes that are necessary for the seed germination. Some of these processes involve reactive oxygen species, whose production in the cell is regulated by antioxidant and oxidant enzymes (Fath et al., 2001).

Dry fractionation techniques have been developed to obtain fractions of the wheat grain that can be used to produce cereal products of a healthy added value. The different fractions of wheat grain have been characterized for chemical composition in previous studies (Hemery et al., 2007). A high content of biologically active compounds can be used as the first criterion for selection of a wheat fraction. However, the compound needs to be able to reach its primary site of action in order to be biologically active; in other words, it needs to be bioavailable. The first factor limiting the bioavailability of a compound is the release and solubility of the compound from the food matrix. Only then it becomes available for intestinal absorption. This concept has been termed bioaccessibility.

An *in vitro* model of upper gastrointestinal tract, the TIM system, has been previously used to assess the bioaccessibility of some

Abbreviations: ABAP, (2,2-azobis(2-aminopropane) hydrochloride; ABTS, 2,2′ azino-bis(3-ethylbenzthiazoline)6-sulfonic acid; FA, Ferulic Acid; LDH, Lactate Dehydrogenase; LPS, lipopolysaccharide; NF-κB, Nuclear Factor kappa B; SEM, Standard Error of the Mean; TDF, Total Dietary Fibre; TEAC, Trolox Equivalent Antioxidant Capacity; TIM, TNO Intestinal Model; TNF-α, Tumor Necrosis Factor -alpha.

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bioactive compounds in food, such as minerals (Eklund-Jonsson et al., 2008; Haraldsson et al., 2005; Larsson et al., 1997), folic acid (Verwei et al., 2006) and ferulic acid (Mateo Anson et al., 2009). Ferulic acid has been identified as the major antioxidant compound in wheat (Mateo Anson et al., 2008) and it has been suggested as marker of antioxidant compounds in wheat grain. The bioavailability of ferulic acid from wheat grain has been determined by its bioaccessibility, which could be well estimated *in vitro*. The same *in vitro* model is used in the current study to obtain the bioaccessible compounds from the wheat fractions aleurone, bran, and flour. The aim of the present study was to investigate the antioxidant and anti-inflammatory capacities of the respective bioaccessible compounds in order to identify the most promising wheat fraction for a possible health effect.

2. Experimental

2.1. Chemicals

Lipopolysaccharide (LPS, *E. coli* 0111:B4), ABTS (2,2′ azinobis(3-ethylbenzthiazoline)6-sulfonic acid), Trolox (6-hydeoxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), protease (P-5147), α-amylase (A-6211), pepsin (P-7012) and bile (porcine bile extract, P-8631) were obtained from Sigma (St. Louis, MO, USA). ABAP (2,2-azobis(2-aminopropane) hydrochloride) was obtained from Polyscience (Warrington, PA). Pancreatic juice from porcine pancreas (Pancreax V powder) was obtained from Paines & Byrne (Greenford, United Kingdom). Rhizopus lipase (150,000 units/mg F-AP 15) was obtained from Amano Enzyme, Inc. (Nagoya, Japan). RPMI-1640 medium, fetal calf serum and L-glutamine were purchased from Life Technologies (Breda, The Netherlands). Phorbol myristate acetate (PMA) was obtained from Omnilabo (Breda, The Netherlands). All chemicals used were of analytical grade quality.

2.2. Wheat fractions

The wheat fractions (*Triticum aestivum* L.) were obtained from Bühler A.G. (Uzwil, Switzerland) as milled fractions: aleurone 2 (50% TDF), coarse bran (60% TDF), and 76% flour from pearling (3% TDF). The fractions were of the wheat cultivar Tiger, harvested in 2005, Germany. The aleurone fraction (99% aleurone) was obtained from coarse bran (42% aleurone) (Hemery et al., 2009) as described in the Bühler A.G. patent applications (Bohm et al., 2003; Bohm and Kratzer, 2005). The pure native wheat starch was obtained from AVEBE (Latenstein, The Netherlands) and it was used as blank. The wheat fractions were stored at −20 °C until use.

Before the start of each experiment, 23 g (fresh weight) of aleurone, bran, flour or starch were mixed with artificial saliva, which consisted of 100 ml electrolyte solution, 30 ml citrate buffer (pH = 6) and amylase (9600 units). Milli-Q water was added to the mixture up to a final volume of 300 ml. This final mixture was introduced in the gastric compartment of the TIM system as described below and the digestion was started.

2.3. The dynamic computer-controlled gastrointestinal model (TIM system)

The gastrointestinal model has been previously described in detail (Minekus et al., 1995). The model comprises four compartments that represent the stomach, duodenum, jejunum and ileum. The secretion of digestive juices and pH adjustment in each section are simulated according to physiological data (Minekus et al., 1995). The composition of the different digestive juices used in the model has been previously described (Larsson et al., 1997). Each compartment consists of a glass exterior with flexible, inner silicon tubing, connected by peristaltic valves that determine the transport rate of the food through the successive compartments. All parameters are computer-controlled using set-points for a moderate transport time of food to simulate accurately the gastrointestinal conditions of human adults after the intake of a semi-solid meal. The half-time of stomach emptying was 70 min. The jejunal and ileal compartments are connected with semi-permeable hollow fibre membrane units of cellulose diacetate (DICE-90 high performance cellulose diacetate dialysers, Baxter SA, US). These dialysis units mimic the absorption of water and digested compounds of low molecular weight that are in solution, which are referred to as the bioaccessible compounds from the wheat fractions. Dialysate samples from the jejunal and ileal compartments were collected in 1 h intervals for 6 h. Dialysate samples were stored at −20 °C until analysis. Gastrointestinal *in vitro* digestions were performed in duplicate.

2.4. Antioxidant capacity assay

The Trolox Equivalent Antioxidant Capacity Assay (TEAC) was used to determine the antioxidant capacity of the wheat fractions and their bioaccessible compounds, obtained from the TIM system. The TEAC assay determines the ability of antioxidants to scavenge ABTS radicals. This was performed as described by van den Berg et al. (1999) with some modifications. Briefly, ABTS radicals were produced by incubating a solution of 0.23 mM ABTS and 2.3 mM ABAP in 100 mM sodium phosphate buffer, pH 7.4 at 70 °C for 10 min and cooled down in ice water. The absorption of the solution reached 0.7 ± 0.02 at 734 nm. During the experiment, the $ABTS^{\bullet}$ solution was stored in ice. A fresh solution was prepared each day. The bioaccessible compounds in the dialysate collected from TIM were added to the $ABTS^{\bullet}$ solution and the reduction in absorbance was measured after 5 min. The TEAC of the bioaccessible compounds corresponds to the concentration of a Trolox solution that causes an equal decrease in absorbance at 734 nm. The antioxidant capacity of the bioaccessible compounds from the wheat fractions was expressed in absolute values, μmol Trolox Equivalents (TE), which was calculated by multiplying the concentration of TE by the total volume of dialysate collected from the model in that 1 h period. The cumulative antioxidant capacity of the bioaccessible compounds was calculated by the successive addition of the antioxidant capacity of each dialysate sample over time. The antioxidant assay was performed in duplicate. Results are expressed as mean and error (half the range between duplicates).

2.5. Macrophage activation

Human monocyte-like histiocytic lymphoma cells U937 obtained from the ATCC (CRL-1593.2, Manassas, VA, USA) were grown in RPMI-1640 medium, supplemented with 10% (v/v) fetal calf serum and 2 mM L-glutamine at 37 °C, 5% CO_2 in a humidified atmosphere (Sündstrom and Nilsson, 1976). U937 monocytic cells were differentiated into macrophages using phorbol myristate acetate (PMA, 10 ng/ml overnight) as described previously (Verhoeckx et al., 2004). U937 macrophages were cultured at a concentration of 1×10^6 cell/well in 24-well cell culture plates. The PMA-differentiated macrophages were allowed to recover from PMA treatment for 48 h, during which the culture medium was replaced daily. At the third day after PMA treatment, the macrophages were exposed to LPS (E. coli, 1 μg/ml) for 4 h in the presence of dialysate containing the bioaccessible compounds or control. The tested dialysate was collected from the jejunal compartment of the TIM system in the time periods: 0–1, 1–2, 2–3, and 3–4 h of gastrointestinal digestion of aleurone, bran, flour and starch. The dialysate