Food Chemistry 128 (2011) 404–409

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of bioprocessing of wheat bran in wholemeal wheat breads on the colonic SCFA production *in vitro* and postprandial plasma concentrations in men

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ARTICLE INFO

Article history:
Received 17 September 2009
Received in revised form 7 February 2011
Accepted 8 March 2011
Available online 21 March 2011

Keywords:
Wheat bran
Bioprocessing
Butyrate
Fibre

ABSTRACT

The health benefits of whole grain consumption can be partly attributed to the inclusion of the bran or outer-layers of the grain rich in dietary fibre. Fibre is fermented in the colon, leading to the production of beneficial metabolites, such as short-chain fatty acids (SCFA). The effect of five different types of bread on the SCFA production was studied in an *in vitro* model of human colon. Additionally, the postprandial effects of two selected breads on the SCFA plasma concentrations were investigated in men. A higher *in vitro* production of butyrate was induced by wholemeal wheat bread with bioprocessed bran than by native bran. The increase in butyrate seemed to be in exchange for propionate, whilst the total SCFA production remained similar. However, differences between the two breads in the postprandial butyrate concentrations could not be detected in peripheral blood of men, probably due to an effective utilisation by colonocytes.

1. Introduction

Whole grain consumption has been associated with a reduced risk of type-2 diabetes (de Munter, Hu, Spiegelman, Franz, & van Dam, 2007), cardiovascular disease (Jensen et al., 2004) and some types of cancer: colonic cancer (Larsson, Giovannucci, Bergkvist, & Wolk, 2005; Schatzkin et al., 2007), pancreatic cancer (Chan, Wang, & Holly, 2007), and small intestinal cancer (Schatzkin, Park, Leitzmann, Hollenbeck, & Cross, 2008). The health benefits of whole grains versus refined grains may be attributed to the inclusion of the outer layers of the grain, the bran. It is in the bran, that most of the micronutrients, phytochemicals and fibre of the grain are located (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007; Mateo Anson, van den Berg, Havenaar, Bast, & Haenen, 2008).

Fibre intake is reported to decrease intestinal transit time and increase stool bulk, reduce levels of total and/or LDL cholesterol in blood, and reduce concentrations of postprandial blood glucose and insulin (Anderson et al., 2009). The health benefits of fibre are linked to the formation of metabolic end-products by the colon microbiota, such as the short-chain fatty acids (SCFA). SCFA formation is beneficial for the microbiota that colonise the large intestine to obtain energy for maintenance and multiplication, and for the host to maintain pH and deliver energy to the colonic cells (Macfarlane & Gibson, 1997). Particularly, butyrate is the preferred source of energy for the colonocytes and it has been reported to have antiproliferative activities and to modulate gene expression and immunogenicity (Hamer et al., 2008).

The metabolic effects of fibre depend on the physicochemical properties, the degree of polymerisation, the arabinose/xylose ratio, the distribution of side chains, the degree of cross-linking, and the extent of digestion in the small intestine (Napolitano et al., 2009). The fibre in wheat bran is mainly composed of the cell wall polysaccharides: arabinoxylan (~64%), cellulose (~29%), and non cellulosic glucan (~6%) (Fincher & Stone, 1986). The structure of these polysaccharides is cross-linked by small phenolic acids, such as ferulic acid. A high degree of cross-linking increases the molecular size of the polysaccharide and reduces its solubility (Izydorczyk & Biliaderis, 1992).

It has been shown that processing can increase the bioavailability of nutrients and other compounds through chemical or enzymatic reactions that hydrolyse or release them from the food matrix (Mateo Anson et al., 2009; Watzke, 1998). Similarly, bioprocessing may result in structural modifications of the fibre affecting the fermentation properties in the colon. In the present study, the effect of bran addition to wholemeal wheat bread and the effect of the bran bioprocessing, by fermentation or by hydrolytic enzymes combined with fermentation, on the production of short chain fatty acids (SCFA) are studied in an *in vitro* model of colon.

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doi:10.1016/j.foodchem.2011.03.043

Additionally, the postprandial effects of wholemeal wheat breads, with native bran or bioprocessed bran, on the plasma concentration of SCFA are investigated in men.

2. Materials and methods

2.1. Chemicals

Acetic acid, propionic acid, iso-valeric acid, L-lactic acid and D-lactic acid were obtained from Sigma–Aldrich (The Netherlands). Butyric acid, iso-butyric acid, and 2-ethyl butyric acid were obtained from Fluka AG (Switzerland). All chemicals were of analytical grade.

2.2. Experimental breads

The wheat flours used for the bread-making were; white wheat flour (76% flour from peeled wheat grains, variety Tiger, harvest of year 2006) and wholemeal wheat flour (100% flour made of peeled (3.5%) wheat grains). The bran fraction used for enrichment was commercial wheat bran from peeled grains. All flour and bran fractions were supplied by Bühler AG (Switzerland). Five different breads were prepared: wheat bread (WB), wholemeal wheat bread (WWB), wholemeal wheat bread with native wheat bran (WWB + B), wholemeal wheat bread with fermented wheat bran (WWB + FB) and wholemeal wheat bread with fermented and enzyme-treated wheat bran (WWB + FEB). The bran fermentation was performed by mixing 22% (w/w) bran and 0.27% (w/w) Baker's Yeast (Finnish Yeast Ltd.) with water. The fermentation mixture was kept at 20 °C for 20 h. The enzymatic treatment of the bran was applied, along with the yeast fermentation, using an enzyme mixture of 0.01% (w/w) Grindamyl A1000 (Danisco, Denmark), 0.36% (w/w) Depol 740L (Bioacatalysts, UK) and 0.14% (w/w) Veron CP (Rohm Gmbh, Germany). The enzyme mixture contained a variety of hydrolytic enzymes; mainly, xylanase, β-glucanase, α-amylase, cellulase and also ferulic acid esterase. The activity profiles of the enzymes were determined using standard activity assay methods: β-glucanase, as described by Bailey and Linko (1990), xylanase, as described by Bailey, Biely, and Poutanen (1992), α-amylase using the Ceralpha method (Megazyme, Ireland), cellulose, as described in the IUPAC method by Ghose (1987) and ferulic acid esterase by a spectrophotometric method (Forssell et al., 2008). For the dough preparation, wheat flour, yeast, salt and water were mixed and, for the breads with added bran, the treated bran or native wheat bran was mixed to get a bran content of 9% (as dry matter) in the total dough. The basic chemical composition of the breads was determined: protein content by the Kjeldahl method, total dietary fibre (TDF) by the enzymatic–gravimetric method of AOAC 985.29 (1990), fat by the fat in flour–Mojonnier method of AOAC 922.06 (2000), arabinoxylans (Douglas, 1981) and digestible starch (McCleary, Solah, & Gibson, 1994).

2.3. In vitro study

2.3.1. Experimental design with the TIM-1 and TIM-2 systems

The test breads were first digested in the TIM-1 system, which is a dynamic model of the upper gastro-intestinal tract (Minekus, Marteau, Havenaar, & Huis In'T Veld, 1995). A sample of 35 g of freeze-dried bread was digested for 6 h in duplicate. During that time, the ileum effluent, i.e. the material that exits the ileum compartment of the model over time, was collected on ice and pooled with the residue of the ileum compartment at the end of the 6 h digestion. This non-digested material of the bread from duplicate experiments was pooled and used as input material for the colonic fermentation in the TIM-2 system. The TIM-2 system is a dynamic model of human colon that has been previously described in detail (Minekus et al., 1999; Venema, van Nuenen, Smeets-Peters, Minekus, & Havenaar, 2000). The compartments of the model were inoculated with metabolically active intestinal microbiota (pooled stools), freshly collected from healthy volunteers (four men and five women, aged 21–35 years). The donors were non-smokers and had not used antibiotics, prebiotics, probiotics or laxatives for at least 3 months prior to the donation. The preparation of the feacal inoculum and the inoculation of the TIM-2 system were performed under strict anaerobic conditions. During an adaptation period of 16 h, the microorganisms were fed with the standardised colonic medium described elsewhere (Minekus et al., 1999; Venema et al., 2000), which was gradually introduced into the colonic compartment (0.045 ml/min). At the start of the experiment, 10 ml of the content in the colonic compartment was replaced by 10 ml of the TIM-2 input material, collected from the TIM-1 system as explained above. The rest of it (60 ml) was gradually added to the colonic compartment (0.15 ml/min) for approximately 6 h to simulate the *in vivo* passage of the ileum content to the colon via the ileo-caecal valve. After that and until the end of the experiment (period from 6 to 24 h), the colonic medium was gradually added (0.045 ml/min) as substrate for the microbiota. Samples were collected from lumen and dialysate at regular time intervals and immediately frozen in liquid N_2 and stored at −80 °C for analyses. After the 24 h experiment, a wash-out period of 20 h was performed by feeding the colonic medium to the microbiota before starting the next TIM-2 experiment.

2.3.2. Determination of neutral sugars in the input for the TIM-2 system

The non-digested fraction of the bread, the TIM-2 input material, was hydrolysed for 1 h in 2 M H_2SO_4 in a water bath at 100 °C. Glucose (Glu), galactose (Gal), arabinose (Ara), xylose (Xyl), rhamnose, and fructose were determined by high performance anion-exchange chromatography (Dionex Corporation, Sunnyvale, CA) with pulsed amperometric detection (PAD-II, Dionex) as described elsewhere (Samuelsen, Cohen, Paulsen, Brüll, & Thomas-Oates, 1999). Rhamnose and fructose were below the quantification limit (<0.2 μg/ml). The coefficient of variation of the analysis was less than 5%.

2.3.3. Determination of fermentation metabolites in the TIM-2 samples

Acetate, propionate, butyrate, valerate, iso-valerate and iso-butyrate were analysed in lumen and dialysate samples collected from the TIM-2 system. Samples were centrifuged (3000*g* for 5 min) and 50 μl of supernatant were added to 650 μl of a mixture of formic acid (20%), methanol and 2-ethyl butyric acid (internal standard, 2 mg/ml in methanol) at a ratio of 1:4.5:1. A 0.5 μl sample was injected on the GC-column (Stabilwax-DA, length 15 m, ID 0.53 mm, film thickness 0.1 μm; Resteck, Bad Homburg, Germany) in a Chrompack CP 9001 gas chromatograph using an automatic liquid sampler. The column was heated at 2 °C/min from 125 °C to 140 °C according to the method described by Jouany (1982). Peaks were detected with a flame ionisation detector and integrated using MAITRE software (Varian). L-Lactate and D-lactate were determined in the supernatants of lumen and dialysate samples by an enzymatic assay (based on the Boehringer, UV-method, Cat. No. 1112821) with a Cobas Mira plus autoanalyser (Roche). Ammonia was also quantified in the supernatants of lumen and dialysate samples by enzymatic spectrophotometric determination with the Cobas Mira Plus autoanalyser, using NH_4Cl as standard.

The results were expressed as cumulative sums of mmol equivalents in the lumen and dialysate samples over time, except for Fig. 2 (see below). Differences between duplicate experiments were less than 15% for all breads for the 24 h period, except for