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ARTICLE

Bioprocessing of Wheat Bran Improves in vitro Bioaccessibility and Colonic Metabolism of Phenolic Compounds

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Ferulic acid (FA) is the most abundant phenolic compound in wheat grain, mainly located in the bran. However, its bioaccessibility from the bran matrix is extremely low. Different bioprocessing techniques involving fermentation or enzymatic and fermentation treatments of wheat bran were developed aiming at improving the bioaccessibility of phenolic compounds in bran-containing breads. The bioaccessibility of ferulic acid, *p*-coumaric acid, and sinapic acid was assessed with an in vitro model of upper gastrointestinal tract (TIM-1). Colonic metabolism of the phenolic compounds in the nonbioaccessible fraction of the breads was studied with an in vitro model of human colon (TIM-2). The most effective treatment was the combination of enzymes and fermentation that increased the bioaccessibility of FA from 1.1% to 5.5%. The major colonic metabolites were 3-(3-hydroxyphenyl)propionic acid and 3-phenylpropionic acid. Bran bioprocessing increases the bioaccessibility of phenolic compounds as well as the colonic end metabolite 3-phenylpropionic acid.

KEYWORDS: Ferulic acid; wheat bran; phenylpropionic acid; bioprocessing; bioaccessibility

INTRODUCTION

Epidemiological studies have linked the consumption of whole grain with reduction of diet-related disorders such as cardiovascular disease, type 2 diabetes, and some types of cancer (*1*). Part of the health effect derived from whole grain foods could be attributed to the phenolic compounds in the bran. In the plant kingdom, phenolic compounds are essential molecules against oxidative damage, as they have UV-absorption properties and radical-scavenging activities. Therefore, the majority of the phenolic compounds are located in the most external tissues of the plant (*2*). In wheat grain, most of the phenolic compounds are located in the bran, which constitutes the outermost parts of the grain. Traditionally, the milling of the wheat grain aimed at removing the bran or outer layers of the grain to obtain the refined white flour. Nowadays, it is well-known that the outer layers contain phytochemicals with potential bioactivities, suggesting the use of wheat grain as whole instead of refined (*3*).

One of the most abundant phenolic compounds in wheat grain, especially in wheat bran, is ferulic acid (FA), accounting for 90% of the total polyphenols in wheat grain (*4*). In the bran, FA is largely located as a structural component of the cell walls of aleurone and pericarp (*3*). Most of the FA is covalently bound to complex polysaccharides in the cell walls, mainly arabinoxylans (*5*). The potential health effect of FA has been partly attributed to its antioxidant properties (*6*). FA was also identified as the major contributor to the antioxidant capacity of aleurone, which is the fraction of highest antioxidant capacity in wheat grain (*7*). However, in order to evaluate its biological activity, the bioavailability of this compound should be first addressed.

Bioaccessibility, which is defined as the release of the compound from its natural matrix to be available for intestinal absorption, is the first limiting factor to the bioavailability (*8*). In a previous in vitro study, it was found that the bioaccessibility of FA from aleurone, bran, and bread enriched with aleurone was extremely low ($<1\%$) (*9*). Combining these results with in vivo data from other studies, it was concluded that the bioavailability of FA from cereal products was limited by its bioaccessibility. In vivo, some esterase activity has been reported for epithelial cells of small intestine. However, the esterase activity in the luminal contents of large intestine was 10-fold higher than that of extracts from epithelial cells of small intestine (*10*). Moreover, when FA was administered as feruloyl arabinoxylans purified from bran, the major FA release took place in large intestine, while no significant FA release was detected during passage to ileum (*11*). Thus, release of FA and possibly other compounds bound to cell wall polysaccharides will mainly occur in the large intestine by bacterial esterases. In the large intestine, the free compounds may exert their activity locally or by bioconversion into colonic

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Table 1. Enzymatic Activities of the Enzyme Preparations Used for Bran Bioprocessing

enzyme preparation	endoglucanase (cellulase)	xylanase	β-glucanase	α-amylase	ferulic acid esterase
Veron CP [a]	91	200	435	1	0
Grindamyl A1000 [b]	0	0	0	12	0
Depol 740 L [a]	13	200	100	ND[c]	0.44

[a] Enzyme dosages calculated based on the xylanase activity; xylanase dosage per gram of bran was 200 nkat. [b] Enzyme dosages calculated based on the α-amylase activity; α-amylase dosage for bran was 0.01% (w/w), i.e., 12 nkat/g of bran. [c] ND = not determined.

metabolites. Metabolism of FA to 3-(3-hydroxyphenyl)propionic acid (3OHPPA) has been shown by ruminal microbiota (*12*) and recently by human microbiota (*13*), but colonic human bioconversion requires further verification.

The development of innovative processing techniques seems a promising approach to improve the bioaccessibility of health-promoting compounds in cereal grains. In the current study, bioprocessing strategies to release bound phenolic compounds from wheat bran have been used, such as the use of enzymes targeting specific linkages in wheat bran or the use of fermentation systems as sources of these enzymes. Five different wheat breads were prepared: white bread, whole-meal bread, whole-meal bread with native bran, whole-meal bread with fermented bran, and whole-meal bread with fermented and enzymatic treated bran. Differences in the bioaccessibility of the major phenolic compounds in the breads were studied with the use of a computer-controlled model of the upper gastrointestinal tract (TIM-1 system). Additionally, the formation of colonic metabolites derived from these phenolic compounds was investigated. This was studied with the use of an in vitro model of large intestine (TIM-2 system), which is inoculated with complex microbiota of human origin in high density.

MATERIALS AND METHODS

Chemicals. Standards for the analysis of phenolic acids: sinapic acid was purchased from Fluka (Buchs, Switzerland), and *p*-coumaric and ferulic acids were purchased from Extrasynthése (Genay, France). Standards for the analysis of phenolic metabolites: benzoic acid (BA), 3-hydroxybenzoic acid (3OHBA), 3-(4-hydroxyphenyl)propionic acid (4OHPA), and 3-(3,4-dihydroxyphenyl)propionic acid (3,4diOHPA) were products from Aldrich (Steinheim, Germany). 4-Hydroxybenzoic acid (4OHBA), 2-(3-hydroxyphenyl)acetic acid (3OHAA), and 2-(3,4-dihydroxyphenyl)acetic acid (3,4diOHAA) were purchased from Sigma (St. Louis, U.S.A.); 3-phenylpropionic acid (3PA) and 3,4-dihydroxybenzoic acid (3,4diOHBA) were from Fluka (Buchs, Switzerland); and 3-(3-hydroxyphenyl)propionic acid (3OHPA) was purchased from Alfa Aesar (Karlsruhe, Germany). 2,2,2-Trifluoro-*N*-methyl-*N*-trimethylsilyl-acetamide (MSTFA) from Pierce (Rockford, U.S.A.) was used as the silylation reagent. Protease (P-5147), α-amylase (A-6211), pepsin (P-7012), and bile (porcine bile extract, P-8631) were purchased from Sigma (St. Louis, U.S.A.). Pancreatic juice from porcine pancreas (Pancreax V powder) was obtained from Paines & Byrne (Greenford, United Kingdom). Rhizopus lipase (150 000 units/mg of F-AP 15) was obtained from Amano Enzyme, Inc. (Nagoya, Japan).

All compounds are named by IUPAC nomenclature or the given abbreviation. All chemicals were of analytical grade.

Experimental Breads. The wheat flours used for the bread making were white flour (76% flour from peeled wheat grains, variety Tiger, harvest of year 2006) and whole-meal 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: (1) white bread, (2) whole-meal bread, (3) whole-meal bread with native bran, (4) whole-meal bread with fermented bran, and (5) whole-meal bread with fermented and enzymatic treated bran.

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 bran was applied along with the yeast fermentation using an enzyme mixture of 0.01% (w/w) Grindamyl A1000 (Danisco), 0.36% (w/w) Depol 740 L (Bioacatalysts), and 0.14% (w/w) Veron CP (Rohm Gmbh). The enzyme mixture contained a variety of hydrolytic enzymes, mainly xylanase, β-glucanase, α-amylase, cellulase, and also ferulic acid esterase (**Table 1**). The activity profiles of the enzymes were determined using standard assay methods: β-glucanase as described by Bailey and Linko (*14*), xylanase as described by Bailey et al. (*15*), α-amylase using Megazyme Ceralpha method, cellulose as described by IUPAC (*16*), and ferulic acid esterase by spectrophotometric method (*17*).

For the dough preparation, wheat flour, yeast, and salt were mixed with water. The proportion of the ingredients in the mixture was 1% yeast, 1% salt, and 98% white or whole-meal flour. For the breads enriched with bran (breads 3, 4, and 5), 16% of the mixture was bran and 82% was whole-meal flour. In the breads with bioprocessed bran (breads 4 and 5), xylanase also was used (0.05%). The use of white flour (76% flour) provided a low amount of phenolic acids in the bread (bread 1). In the whole-meal bread (bread 2), the phenolic acid content is derived from the use of whole-meal flour (100% flour) instead (**Table 2**). In the breads with bran (breads 3, 4, and 5), it has been estimated that approximately one-half of the total phenolic acid content in the bread can be attributed to the addition of bran.

All doughs were kneaded with spiral kneader (Diosna SP 12 F, Dierks & Sohne, GmbH, Osnabruck, Germany) for 2 min at a low speed (100 rpm), followed by 5 min at a high speed (200 rpm). After the intermediate proof (45 min, 28 °C, 70% relative humidity), the dough was divided into 400 g pieces and molded. The molded dough pieces were proofed at 37 °C with 70% relative humidity for 55 min. The loaves of 400 g were baked for 10 min at 220 °C and 20 min at 200 °C (Rack Oven 9000, Sveba Dahlen AB, Sweden). Steam was added for 20 s during the initial baking phase.

The basic chemical composition of the breads was determined: protein content by Kjeldahl method, total dietary fiber (TDF) by Enzymatic-Gravimetric method (*18*), fat by Fat in Flour−Mojonnier method (*19*), arabinoxylans (*20*) and digestible starch (*21*). The moisture content of fresh breads was also measured (**Table 2**).

TIM-1 System. The gastrointestinal model has been previously described in detail (*22*). The model comprises four compartments that represent the stomach, duodenum, jejunum, and ileum (**Figure 1**). Secretion of digestive juices and pH adjustment in each section are simulated according to physiological data (*22*). The composition of the different digestive juices used in the model was previously described (*23*). All parameters are computer controlled, and a protocol of medium transport time of food was chosen in the study to simulate a semisolid meal.

Table 2. Phenolic Acid Composition: Ferulic Acid (FA), *p*-Coumaric Acid (*p*-CA), and Sinapic Acid (SA), and Chemical Composition of the Experimental Breads; Results Are Mean of Triplicate Determinations (Relative Standard Deviation < 5%)

	bread [a]				
	1	2	3	4	5
	Phenolic composition (μg/g of DM)				
ferulic acid					
free	3.6	13	12	42	100
total	86	810	1300	1300	1300
p-coumaric acid					
free	0.8	0.9	1.2	1.5	3.0
total	2	20	40	40	40
sinapic acid					
free	0.9	3.5	4.6	9.6	9.9
total	9	70	130	130	130
	chemical composition (g/100 g)				
moisture	37	39	41	40	40
fat	0.8	1.4	1.7	1.9	1.9
protein	8.5	9.6	9.7	9.9	10
TDF	2.9	6.1	9.7	10	9.2
ash	0.9	1.4	1.9	2.0	2.0
starch	50	42	35	35	36

[a] (1) White wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran.

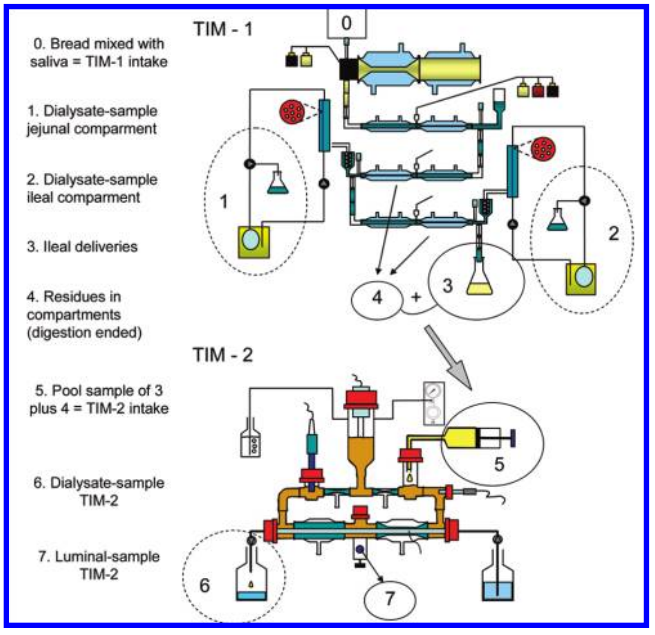

Figure 1. Schematic overview of the experimental setup of the in vitro model of upper gastrointestinal tract (TIM-1) and the in vitro model of human colon (TIM-2).

The half time of stomach emptying was 70 min. The jejunal and ileal compartments are connected with a semipermeable hollow fiber membrane units of cellulose diacetate (DICEA-90 high performance dialysers, Baxter SA, U.S.A.). This dialysis system removes the water and digested products. For the TIM-1 experiments, 35 g of freeze-dried bread was mixed with artificial saliva that contained 9600 units of amylase, 30 mL of citrate buffer (pH = 6), and 100 mL of electrolyte solution. Breads were freeze-dried in order to facilitate the posterior grinding. This procedure was chosen in order to obtain a standardized homogeneous mixture of the bread with the artificial saliva. Milli-Q water was added to the mixture up to a final volume of 300 mL. This mixture (TIM-1 intake) was introduced in the gastric compartment representing the stomach, and the digestion was started (**Figure 1**). The digestion took 6 h; dialyzate samples were collected in 2 h aliquotes, containing the released and dialyzed phenolic acids. This represents the bioaccessible fraction of the bread. The ileum deliveries, ileal material that exits the model over time, were also collected and pooled with the residues in the compartments by the end of the digestion experiment (3 and 4 in **Figure 1**). This represents the nonbioaccesible fraction of the breads in the upper gastrointestinal tract. This pooled sample was freeze-dried and subsequently reconstituted in water to a fixed volume and used as starting material for TIM-2 experiments (TIM-2 intake). All TIM experiments were performed in duplicate.

TIM-2 System. The colonic fermentation experiments were performed in a dynamic model of human large intestine (TIM-2) explained in detailed by Minekus et al. (*24*). The model was inoculated with a standardized pool of active microbiota from healthy volunteers (four men and five women; aged 21−35 years). They were nonsmokers and had not used antibiotics, prebiotics, or laxatives at least 3 months prior to the donation. The model and the preparation of the feacal inoculum were performed under strict anaerobic conditions. After the adaptation of the microorganisms to the standard medium for 16 h, 10 mL of this medium was replaced by 10 mL of the TIM-2 intake, a mixture of the collected TIM-1 ileal deliveries and residues (**Figure 1**). During the first 6 h of colonic fermentation, 50 mL of TIM-2 intake was gradually added at a flow speed of 0.15 mL/min. From 6 to 24 h, the standard medium was gradually added at a flow speed of 0.045 mL/min as substrate for the microbiota. TIM-2 standardized medium was prepared according to the ileal delivery medium described by Gibson et al. (*25*) with modifications (g/L): 4.7 arabinogalactan, 4.7 pectin, 4.7 xylan, 4.7 amylopectin, 23.5 casein, 39.2 starch, 23.5 bactopeptone, 17 Tween 80, and 0.4 bile (oxoid). After the 24 h experiment, a wash-out period of 20 h was performed by feeding standard medium before starting the duplicate TIM-2 experiment. Samples were collected from lumen and dialysis fluids as shown in the schematic design.

Determination of Phenolic Acids in Breads and TIM-1 Samples. The content of phenolic acids (ferulic acid, *p*-coumaric acid, and sinapic acid) in the breads was determined as free and total phenolic acids as previously described by Bartolomé and Gómez-Cordovés (*26*). For the determination of the free phenolic acids, 50 mg of freeze-dried bread were first thoroughly mixed with 2 mL of water and then the suspension was acidified with HCl to reach final HCl concentration of 0.35 M (pH < 1.5). This mixture was extracted twice using ethyl acetate (2 × 5 mL). The extracts were pooled and evaporated to dryness. The residue was dissolved to 0.5 mL of 50% methanol/water and filtrated through a 0.2 μm filter before injection to high-performance liquid chromatography (HPLC). For the determination of total phenolic acids (free and esterified), the samples were hydrolyzed with 2 M NaOH for 16 h in the absence of light and under N_2 atmosphere before the extraction with ethylacetate (2 × 5 mL). The analytical quantification of the phenolic acids was performed by HPLC and diode array detector as described by Mattila et al. (*27*).

Determination of Phenolic Metabolites in TIM-2 Samples. In luminal and dialyzate samples from TIM-2, besides ferulic acid, *p*-coumaric acid, and sinapic acid, the following phenolic metabolites were determined: 3-phenylpropionic acid (3PPA), 3-(3-hydroxyphenyl)propionic acid (3OHPPA), 3-(4-hydroxyphenyl)propionic acid (4OHPPA), 3-(3,4-dihydroxyphenyl)propionic acid

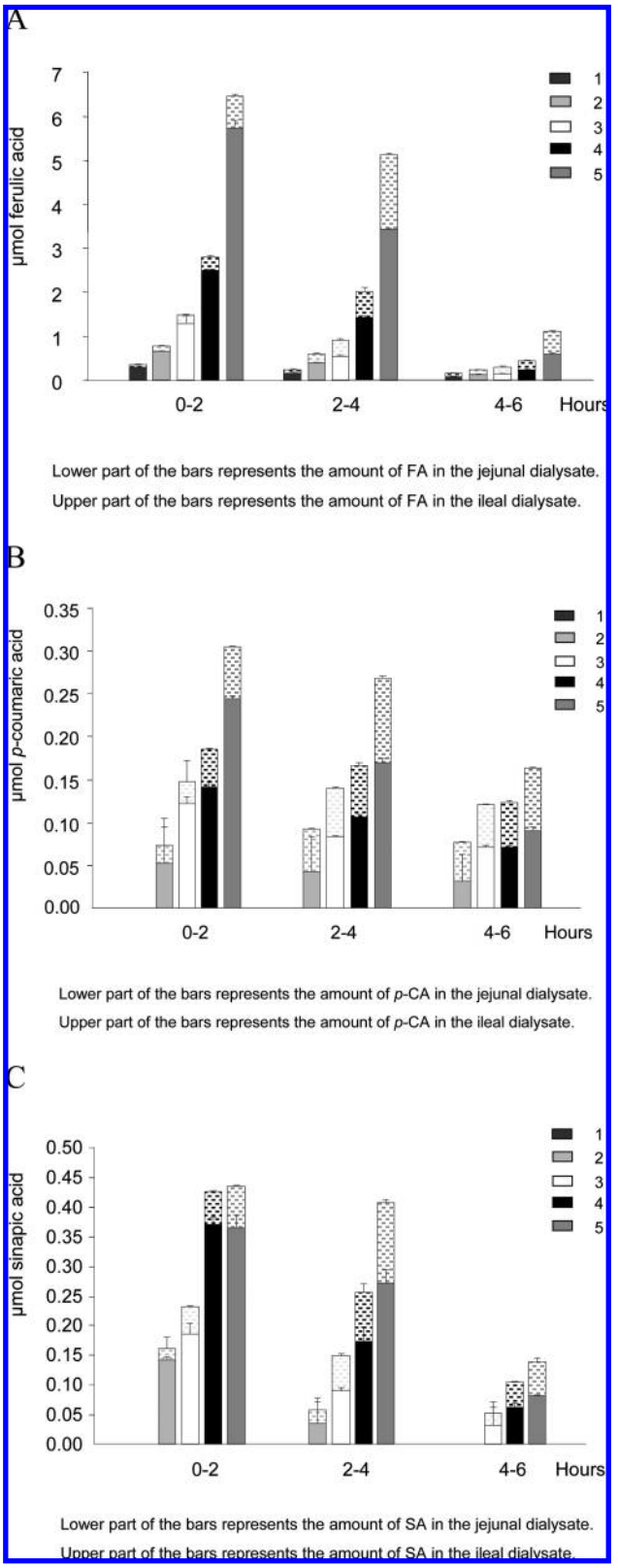

Figure 2. Phenolic acids: (**A**) ferulic acid (FA), (**B**) *p*-coumaric acid (*p*-CA), and (**C**) sinapic acid (SA) in the bioaccessible fraction (TIM-1 dialysate-samples) of the different breads: (1) white wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran.

(3,4diOHPPA), 2-(3-hydroxyphenyl)acetic acid (3OHPAA), 2-(3,4-dihydroxyphenyl)acetic acid (3,4diOHPAA), benzoic acid

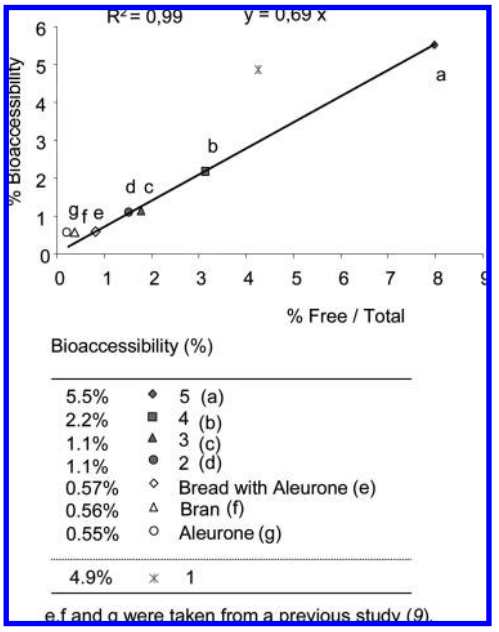

Figure 3. Correlation between the proportion of free ferulic acid in the breads and the bioaccessibility (%). (1) White wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran.

(BA), 3-hydroxybenzoic acid (3OHBA), 4-hydroxybenzoic acid (4OHBA), and 3,4-dihydroxybenzoic acid (3,4diOHBA). Luminal and dialysate samples were acidified by addition of HCl to a final concentration of 0.35 M (pH < 1.5), and the phenolic metabolites were extracted twice using ethyl acetate (2 × 5 mL). Luminal samples were hydrolyzed with 2 M NaOH during 16 h as described above to determine the amount of total ferulic acid (free and esterified). Hydrolysis was stopped by addition of HCl, to a final concentration of 2.8 M (pH < 1.5). The extraction was performed twice with ethyl acetate (2 × 5 mL). The extracts were evaporated to dryness under nitrogen, dissolved in 100 μL of dichloromethane, and silylated with 30 μL of MSTFA (5 min, 50 °C). The analytical determination was performed by GC-MS as described by Aura et al. (*28*).

Calculations. The bioaccessibility (%) of ferulic acid, *p*-coumaric acid, and sinapic acid were calculated as the sum of the free phenolic acid in the jejunal dialyzates and ileal dialyzates for the 6 h of digestion, divided by the total content of phenolic acid (free and esterified) in the bread (TIM-1 intake) times 100.

The phenolic metabolites quantified in the TIM-2 samples are expressed as the sum of the free phenolic metabolite in the dialyzate sample and in the luminal sample. They are expressed cumulative over the 24 h of colonic fermentation.

RESULTS

The bioprocessing of wheat bran increased the content of free phenolic acids in the bran-containing breads, breads 4 and 5 compared to bread 3, which contained native bran (**Table 2**). In all breads, ferulic acid (FA) was the most abundant phenolic acid. The total content in FA (free and esterified) was approximately 10-fold and 40-fold higher than that of total sinapic acid (SA) and total coumaric acid (*p*-CA), respectively. Bran fermentation increased the amount of free FA in the bread by approximately 3-fold. The combination of fermentation and enzymatic treatment of bran increased 8-fold the amount of free FA in the bread, from 12 to 100 μg/g of dry matter (DM). These bioprocessing

techniques also increased the free form of the other two major phenolic acids in the bread, *p*-CA and SA (**Table 2**).

To determine the bioaccessibility of the phenolic acids in the breads, each of the five experimental breads was digested in the TIM-1 system that simulates the upper gastrointestinal tract (**Figure 1**). The dialyzate samples that were collected from the model contain the fraction of the compound that is released from the food matrix and consequently available for absorption. The bioaccessible amounts of FA, *p*-CA, and SA are shown in **Figure 2**. Most of the bioaccessible phenolic acid was found in the dialysate samples from the jejunal compartment and especially in the dialyzate sample collected during the first 2 h interval (**Figure 2**). FA was the major phenolic acid in the bioaccessible fraction of the breads (**Figure 2**).

There was a large variation in the bioaccessibility of FA in the different breads (**Figure 3**). Combination of fermentation and enzymatic treatment increased the bioaccessibility of FA 5-fold as compared to the bread with native bran, i.e., from 1.1% in bread 3 to 5.5% in bread 5. A strong correlation was found between the bioaccessibility of FA and the percentage of free FA in the bread matrix, except for the white bread, which was excluded (**Figure 3**). The bioaccessibility of *p*-CA and SA in the breads were also increased by the bioprocessing of bran, although the increase was smaller compared to FA. The bioaccessibility of *p*-CA and SA were increased by around 2-fold by the bioprocessing of bran: *p*-CA bioaccessibility was increased from 5.2% (bread 3) to 9.9% (bread 5) and SA bioaccessibility was increased from 2.1% (bread 3) to 5.0% (bread 5). Similarly to FA, the increase in bioaccessibility of *p*-CA and SA could be related to the increase in the proportion of free phenolic acid in the bread.

Table 3. Phenolic acids: Total Ferulic Acid, Total Sinapic Acid, and Total *p*-Coumaric Acid Calculated in the Starting Material for the TIM-1 (TIM-1 Intake) and TIM-2 (TIM-2 Intake) Experiments

	bread [a]				
	1	2	3	4	5
TIM-1 intake (μmol)					
ferulic acid	15	140	240	240	230
p-coumaric acid	0.47	4.9	7.8	8.0	7.4
sinapic acid	1.4	11	21	21	20
TIM-2 intake (μmol)					
ferulic acid	5.1	50	91	93	52
p-coumaric acid	0.12	1.6	2.9	3.0	1.6
sinapic acid	0.13	0.93	2.0	2.4	1.6

[a] (1) White wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran.

Despite the increase in the bioaccessibility of FA, most of the FA in the breads was recovered in the ileal deliveries and residues in the TIM-1 model (**Figure 1**) after the digestion was completed. Most of this FA was not free (98−99%). FA covalently bound to other structures was not bioaccessible from the breads during the simulation of upper-gastrointestinal transit. In order to study the colonic features on the nonbioaccessible fraction of the breads, the ileal deliveries and residues from the TIM-1 system were pooled and used as starting material for the TIM-2 system (TIM-2 intake) as described in Material and Methods (**Figure 1**).

During the first 6 h, the TIM-2 intake (**Table 3**) was gradually introduced in the TIM-2 model. This resulted in a gradual increase in the amount of total FA (free and esterified) present in the colonic model during the first 9 h (**Figure 4**). From the 9 h until the end (24 h), the amount of total FA gradually decreased. In **Figure 3**, the bars at the 24 h show the residual amount of total FA (free and esterified) that was not metabolized after the 24 h of colonic fermentation. Most of this FA was bound; **Table 4** shows the amount of FA that was free. The amount of free FA remained low for the entire colonic fermentation, while the total FA decreased, which indicates a rapid metabolism of free FA.

The main phenolic metabolites detected during the TIM-2 experiment were phenylpropionic acid derivatives, namely, 3-phenylpropionic acid with different grades of hydroxylation. The metabolites 3-(3-hydroxyphenyl)propionic acid (3OHPPA) and 3-phenylpropionic acid (3PPA) were the highest in amount, while phenylacetic acid and benzoic acid derivatives were in much lower quantities (< 5 μmol) (**Table 4**). Regarding the time-course formation of the phenylpropionic metabolites: 3,4-dihydroxyphenylpropionic acid (3,4diOHPPA) increased over time until the 9 h, and from then it decreased (**Figure 4**); 3-hydroxyphenylpropionic acid (3OHPPA) increased longer over time, namely, until the 12 h, since then it also decreased (**Figure 4**). The only metabolite that increased continuously over time for the entire 24 h experiment was 3-phenylpropionic acid (3PPA) (**Figure 4**).

Table 4. Phenolic Metabolites Determined in Samples from the Colonic Experiment (TIM-2); Results Are the Cumulative Amount in μmol at the End of the Experiment (24 h) and Are Expressed As Mean ± Half of the Range between Duplicates

	bread [a]				
cumulative (μmol)	1	2	3	4	5
benzoic acid	4 ± 2	2 ± 0.9	5 ± 2	3 ± 0.6	1 ± 2
3OHBA[b]	0.5 ± 0.02	0.4 ± 0.1	0.7 ± 0.3	0.4 ± 0.02	0.3 ± 0.1
4OHBA[b]	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.02 ± 0.01	0.01 ± 0.02
3,4diOHBA[b]	0.5 ± 0.06	0.2 ± 0.01	0.5 ± 0.01	0.5 ± 0.2	0.2 ± 0.1
3OHPAA[b]	0.1 ± 0.08	0.1 ± 0.03	0.1 ± 0.03	0.4 ± 0.01	0.2 ± 0.2
3,4diOHPAA[b]	0.3 ± 0.09	0.6 ± 0.08	0.6 ± 0.03	0.5 ± 0.05	0.5 ± 0.1
3PPA[b]	10 ± 0.07	20 ± 3	20 ± 8	50 ± 7	50 ± 2
3OHPPA[b]	30 ± 20	70 ± 6	100 ± 10	60 ± 8	40 ± 4
4OHPPA[b]	5 ± 0.2	9 ± 0.1	8 ± 0.5	3 ± 0.3	2 ± 0.2
3,4diOHPPA[b]	0.4 ± 0.04	1 ± 0.2	2 ± 0.4	0.9 ± 0.01	0.9 ± 0.1
ferulic acid	3 ± 0.6	0.6 ± 0.7	3 ± 2	2 ± 0.7	2 ± 0.5
p-coumaric acid	1 ± 0.3	0.6 ± 0.2	1 ± 0.4	0.6 ± 0.1	0.9 ± 0.03
sinapic acid	4 ± 3	2 ± 0.5	5 ± 3	2 ± 0.2	1 ± 1

[a] (1) White wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran. [b] 3OHBA, 3-hydroxybenzoic acid; 4OHBA, 4-hydroxybenzoic acid; 3,4diOHBA, 3,4dihydroxybenzoic acid; 3OHPAA, 2-(3-hydroxyphenyl)acetic acid; 3,4diOHPAA, 2-(3,4-dihydroxyphenyl)acetic acid; 3PPA, 3-phenylpropionic acid; 3OHPPA, 3-(3-hydroxyphenyl)propionic acid; 4OHPPA, 3-(4-hydroxyphenyl)propionic acid; 3,4diOHPPA, 3-(3,4-dihydroxyphenyl)propionic acid.

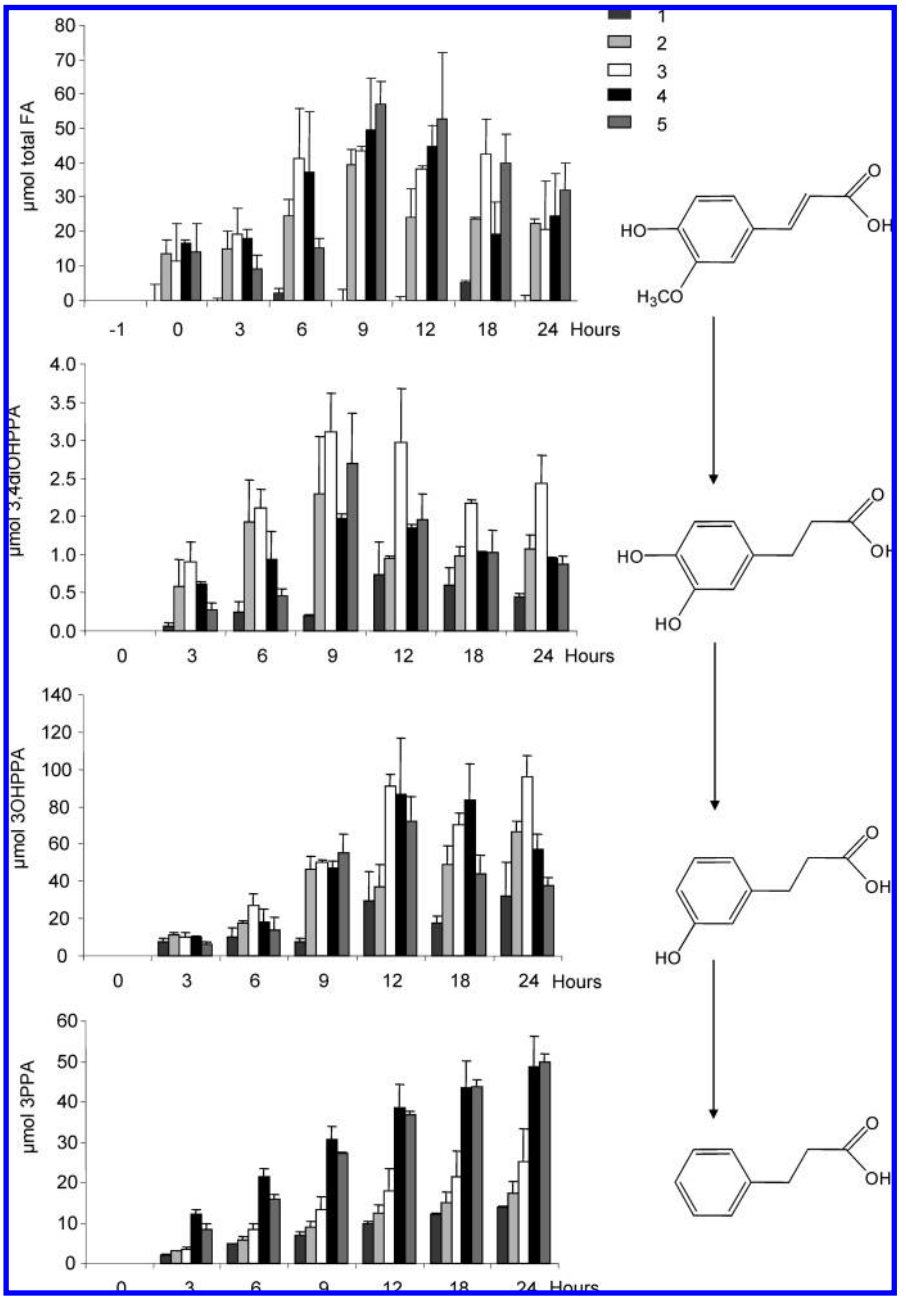

Figure 4. Total ferulic acid (FA) and major identified colonic metabolites: 3,4-dihydroxyphenylpropionic acid (3,4diOHPPA), 3-hydroxyphenylpropionic acid (3OHPPA), and 3-phenylpropionic acid (3PPA). The proposed sequence of reactions, based on the results and chemical structures, is also given. (1) White wheat bread, (2) whole-meal wheat bread, (3) whole-meal wheat bread with native wheat bran, (4) whole-meal wheat bread with fermented wheat bran, and (5) whole-meal wheat bread with fermented and enzymatic treated wheat bran.

This time-course of phenolic metabolite formation was similar for all the tested wheat breads. In the breads containing bioprocessed bran, either by fermentation or the combination of enzymatic and fermentation treatment, 3PPA formation was enhanced compared to the bread containing native bran and the other breads.

DISCUSSION

Ferulic acid (FA) is considered the most abundant phenolic compound in wheat grain; however, its bioavailability from the natural cereal matrix is rather low. In a previous study, it was shown that the bioavailability of FA is determined by its low bioaccessibility, which could be assessed in vitro (*9*). A low bioaccessibility means that most of the FA is not released from the food matrix during gastrointestinal transit and, consequently, will not be available for intestinal absorption. The objective of the current study was to investigate whether bioprocessing techniques, such as fermentation and enzymatic treatments, could enhance the bioaccessibility of FA from wheat bran.

FA, besides being the major phenolic compound in wheat grain, was also found to be the most abundant phenolic compound in the bioaccessible fraction of the wheat breads.

Bioprocessing of wheat bran by fermentation or by the combined action of hydrolytic enzymes and fermentation promoted the release of phenolic acids and increased their free fraction in the wheat breads. Bioprocessing significantly increased the bioaccessibility of the phenolic acids. The most effective bioprocessing technique was the combination of

fermentation and enzymatic treatment of wheat bran, which increased FA bioaccessibility by 5-fold compared to native bran.

The enzyme preparations used for the treatment of wheat bran had various cell-wall-degrading activities, mainly xylanase, cellulase, and β-glucanase (**Table 1**). The combined action of these enzymes enables the hydrolysis of different wheat polymers, thus improving the solubility and breaking down of the complex cell-wall structures in the bran. One of the enzyme preparations used in our study (Depol 740 L) also contained ferulic acid esterase activity (**Table 1**), which is able to cleave the ester-bound FA of the cell-wall polymers in wheat. It has been reported that ferulic acid esterase can release FA more efficiently in combined action with cell-wall-degrading enzymes, especially with xylanases (*26*, *29*). Besides free FA, feruloyl oligosaccharides may have some biological activity (*30*).

Despite the substantial increase in the bioaccessibility of phenolic compounds achieved by bioprocessing, the major part of the phenolic acids remained in the nonbioaccessible fraction that will enter the colon. In the colon, fermentation of the cell-wall structures by the action of bacterial enzymes is expected to facilitate the release of phenolic acids that were not accessible in small intestine.

In the colonic model (TIM-2 system) used in our study, total FA (free and esterified) was decreased over the time (9−24 h) (**Figure 4**), while no substantial increase in free FA was detected (**Table 4**). Instead, other colonic metabolites were identified, mainly phenylpropionic acids with different grades of hydroxylation, namely, 3-(3-hydroxyphenyl)propionic acid (3OHPPA) and 3-phenylpropionic acid (3PPA). This indicates that FA is being rapidly metabolized upon release. On the basis of the pattern of appearance in time and the structures of these phenolic metabolites, the sequence of reaction has been proposed as indicated in **Figure 4**. These metabolic reactions involving FA demethylation and dehydroxylation have been also described in other studies (*12*, *13*, *31*). Monohydroxylated phenylpropionic acids have also been identified as colonic metabolites of proanthocyanidins (*32*), hydroxycinnamates (*33*, *34*), flavanones, and flavanols (*34*). Also diferulic acids and other phenolic compounds contained in the breads are likely to be metabolized to phenylpropionic acids. This is the first study that identifies 3OHPPA and 3PPA as the major metabolites of the human colonic metabolism expected after consumption of whole-wheat bread. Hydroxylated phenylacetic acids are mainly colonic metabolites of quercetin and isorhamnetin (*31*, *35*), and benzoic acid derivatives have been proposed as a result of β-oxidations of phenylpropionic acids (*31*, *33*) or ring-fission of anthocyanins (*36*). In the present study, 3PPA was identified as the end product of the colonic metabolism of ferulic acid, since this was the only metabolite increasing continuously over time during the entire experiment. The breads with bioprocessed bran led to the highest formation of 3PPA. In the bioprocessed bran, the cell-wall polymers binding the phenolic compounds were already partially degraded by the bran fermentation and enzymatic treatments. Consequently, the colonic enzymes might have displayed a higher activity to the partially hydrolyzed material via an increase in solubility of the substrate and the accessibility of the enzymes to the substrate. As a consequence, release and metabolism of phenolic acids in colon was more pronounced.

Future investigations addressing the biological activities of these colonic metabolites are still needed. So far, the recent study of Russell et al. (*13*) has shown that some of the colonic metabolites derived from FA, like 3,4diOHPPA and 3OHPPA, could reduce prostanoid production in cells, indicating possible anti-inflammatory properties.

From the findings in our study we can conclude that (i) bioprocessing of wheat bran can significantly improve the bioaccessibility of phenolic acids in whole-meal breads in intestine and moreover (ii) bioprocessing can also enhance the colonic release and conversion of phenolic acids into their metabolites. Among all the phenolic compounds in the daily diet, phenolic acids have been estimated to be the predominant group; in Finnish adults they were 75% of the total phenolic intake. The main foods contributing to the intake of phenolic acids were coffee and bread (*37*). Therefore, increasing the bioaccessibility of phenolic compounds from a daily consumed food such as bread can have an important impact on the uptake of phenolic compounds, their circulating metabolites, and possible health benefits.

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