Release of Covalently Bound Ferulic Acid from Fiber in the Human Colon

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The action of wheat bran as a potential colon anticarcinogen is related partly to its low fermentability in the large intestine. Phenolic acids are highly bioactive components and also limit degradability of wheat bran in ruminants. Therefore, the solubilization of covalently bound phenolic acids during the fermentation of wheat bran in a human model colon was examined. Phenolics were only partially and relatively slowly solubilized from wheat bran, but were rapidly and fully solubilized from sugarbeet fiber; the levels of free ferulic acid in fermentation liquors remained very low and did not reflect the amounts solubilized from fiber sources. Xylanase and ferulic acid esterase activities were shown to be present. The released ferulic acid did not appear to bind noncovalently to the residual wheat bran fiber. Thus, fermentation in the gut alters the partition of esterified phenolic acids from the insoluble residue and the soluble fraction, where microbial ferulic acid esterase(s) can potentially yield free ferulic acid.

Keywords: *Ferulic acid; phenolic acids; plant cell walls; fiber; wheat bran; sugarbeet pulp; colon fermentation; phenolic antioxidants; esterases; xylanase*

INTRODUCTION

Epidemiological studies have shown that consumption of wheat bran is associated with a reduced risk of colorectal and gastric cancers (Jacobs *et al.*, 1995). Studies on animal models or on human volunteers have indicated that the protective effect against carcinogenesis is greatest for the insoluble fibers and that the protective effect is modified by fermentation (Folino *et al.*, 1995; Harris and Ferguson, 1993; Reddy, 1987). The mechanism of this protection may be by reducing exposure to the colonic mucosa by binding potential carcinogens (Ryden and Robertson, 1995a) or dilution (Reddy *et al.*, 1989), increased fecal bulk and reduced transit times (Harris and Ferguson, 1993), lowering of the colon pH by short-chain fatty acids produced by bacterial fermentation, and modulation of cell proliferation by butyrate (Clausen, 1995).

Ferulic acid constitutes about 0.5% w/w of wheat bran (Ralet *et al.*, 1990) and 0.9% w/w of sugarbeet pulp (Kroon and Williamson, 1996). In wheat bran, the acid is esterified to arabinose residues, which form part of the arabinoxylan structure of the aleurone layer and pericarp of the wheat bran. In sugarbeet, ferulic acid is esterified to arabinose or galactose residues (about 50% each) in pectic side chains (Ralet *et al.*, 1994a). Free ferulic acid is a good antioxidant since it forms a resonance-stabilized phenoxy radical (Graf, 1992; Castelluccio *et al.*, 1995; Scott *et al.*, 1993), inhibits chemically induced carcinogenesis in animal models (Huang *et al.*, 1988; Tanaka *et al.*, 1993), protects against the formation of nitroso compounds (Stich *et al.*, 1984), and reduces carcinogen−DNA adduct formation in cultured cells (Wargovich *et al.*, 1985). The nitrite scavenging property of wheat bran is due to the ferulic acid content (Moller *et al.*, 1988).

The fermentation of ferulic acid rich material, such as wheat bran, sugarbeet fiber, tropical grasses, and rice endosperm, has been well studied in ruminants [see Besle *et al.* (1995) for a review] and involves the combined action of carbohydrate-degrading enzymes such as xylanases, cellulases, and pectinases. The release of free phenolics in ruminants is catalyzed by esterases. These enzymes have been isolated from several (anaerobic) ruminant microorganisms (Borneman *et al.*, 1991, 1992), soil bacteria (Faulds and Williamson, 1991), and aerobic fungi (Tenkanen *et al.*, 1991; Castanares *et al.*, 1992; Faulds and Williamson, 1993, 1994; Kroon *et al.*, 1996). Three cinnamoyl esterases have been isolated from the anaerobic ruminal fungus *Neocallimastix* (Borneman *et al.*, 1992) which, although efficient in releasing ferulic acid from small soluble feruloylated oligosaccharides, released very little from plant cell wall material. For all of the ferulic acid esterases (FAEs) so far studied, synergistic interactions have been demonstrated with other plant cell wall hydrolases for release of the free acid (Faulds and Williamson, 1994; Borneman *et al.*, 1992; Bartolomé *et al.*, 1995; Kroon and Williamson, 1996). However, the existence of these enzymes, secreted by the gut microflora in humans, has not been shown, although human fecal samples were able to release a little ferulic acid from methyl ferulate (Faulds and Williamson, 1995; Kroon *et al.*, 1996).

Further, it is not known whether free ferulic acid is released from bran in the human gut or whether it remains bound to the wheat bran and is excreted as part of the feces, although it is has been shown that the glucuronoarabinoxylans, which are cross-linked by phenolics, are resistant to degredation in the presence of human fecal bacteria (Stevens *et al.*, 1988). We have measured the amount of ferulic acid esterase activity in a model gut system and have examined the release of ferulic acid from wheat bran and compared it to the release from sugarbeet fiber. The results show that ferulic acid is released from both substrates, although much more rapidly and completely from sugarbeet fiber, and that the release may be partially catalyzed by microbial FAEs. The partitioning of ferulic acid be-

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tween insoluble and soluble phases and the implications in terms of potential antioxidant activity are discussed.

MATERIALS AND METHODS

Preparation of AIR and Bran Cell Wall Samples. The preparation of coarse and fine wheat bran cell wall samples (Ryden and Robertson, 1995a) and AIR of sugarbeet samples (Ryden and Robertson, 1995b) have been described.

***In Vitro* Fermentations.** *In vitro* fermentations were performed using a single-chamber fermenter (2 L wide-necked reaction vessel with four-bore flat flange lid) to simulate transit and substrate fermentation in the ascending colon under conditions of limiting substrate in an anaerobic cabinet, with 3 days of preadaptation of the fermenter to the test fibers (Ryden and Robertson, 1995a,b). Fecal samples were prepared from a pool originating from several normal adults and filtered through 100 μm nylon cloth prior to use. Following preadaptation, test samples (at least four for each fiber source, in sealed 10.6 μM mesh nylon bags, 9 cm length × 1.8 cm diameter) were added to the fermenter. Unfermented controls were obtained by incubating fiber sources in 10.6 μM bags in water at 37 °C for 18 h. After fermentation, sample bags were withdrawn and the residual fiber was recovered, washed over 10.6 μM mesh cloth, and freeze-dried. Duplicate samples were analyzed for fiber, as the constituent neutral sugars and uronic acids (Ryden and Robertson, 1995a,b). Samples for measurement of esterase and xylanase activity were prepared as follows: Fine wheat bran CWM (1.68 g) was fermented at 37 °C in 240 mL of 0.1 M sodium−phosphate buffer (pH 6.5) with fecal inoculum added to 10% (v/v) and the pH maintained at 6.5. A blank fermentation containing fecal inoculum but no wheat bran was performed as a control. Samples (10 mL) were removed from both the test and blank fermentation broths after 0, 1.5, 3, 6, and 24 h and frozen immediately (−20 °C).

Content of Ester-Linked Ferulic Acid in Insoluble Residues. Samples (10 mg) were assayed for total esterified ferulic acid by incubation with 1 M NaOH (1 mL) with constant agitation by rotation at 37 °C. After a 24 h incubation, which is sufficient to remove the ester-linked ferulic acid (Akin *et al*., 1992), hydrolyzed samples were diluted with 100 mM sodium acetate buffer (5 mL) and the pH was adjusted to 3.5−4.5 with a known volume of glacial acetic acid. Hydrolyzed samples were assayed for ferulic acid using HPLC (see below).

HPLC. Ferulic and other phenolic acids were measured by reversed phase HPLC (Kroon and Williamson, 1996). Samples (100 μL) were injected onto a C_{18} reversed phase analytical HPLC column (Spherisorb ODS-II, 10 × 240 mm, 5 μM particle size; Phenomenex) previously equilibrated with 28% methanol in 0.01% acetic acid (pH 3) and eluted at 1 mL min^{-1} with a four-step methanol gradient as follows: 28% methanol isocratic for 7 mL, 28−48% methanol in 8 mL, 48−100% methanol in 5 mL, 100% methanol isocratic for 5 mL. Elution of feruloylated material was monitored at 310 nm. The amount of ferulic acid eluting from the HPLC column was calculated by reference to phenolic (caffeic, *p*-coumaric, ferulic, sinapic, syringic, vanillic) acid standards. There was <10% variation between individual assays in a duplicate pair.

Assay for Esterase and Xylanase Activities. For esterase activity, fermentation liquor (0.1 mL) was incubated under rotation at 37 °C in the presence of methyl ferulate (MFA, 1 mM) in a final volume of 0.5 mL. Aliquots were removed after 75 min, boiled (5 min), and assayed for free ferulic acid using HPLC analysis (see above). Esterase activity was expressed as nanomoles of ferulic acid released per minute per milliliter of liquor. For xylanase activity, fermentation liquor (0.1 mL) was incubated under rotation at 37 °C in the presence of a soluble xylan (from oat spelts; 0.5% w/v final concentration) in a final volume of 0.5 mL. After 30 min of incubation, supernatants (13000*g* for 5 min) were assayed for reducing sugars by the dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of xylanase activity releases 1 μmol of xylose min^{-1} under the conditions described. For both assays, sterile-filtered (0.2 μm) liquor from unfermented samples was incubated to give a true zero time point; boiled liquor (100 °C for 5 min) was incubated as a control.

Gastric and Intestinal Enzymolyses. Enzymolyses were based on the method described by Robb *et al*. (1986) using porcine enzymes (all obtained from Sigma). Gastric enzymolyses were performed using a solution of 10 mg mL^{-1} pepsin in acidified saline (150 mM NaCl/20 mM HCl). Intestinal enzymolyses were performed using a solution containing 1.5 mg mL^{-1} trypsin, 1.5 mg mL^{-1} chymotrypsin, 5 mg mL^{-1} α-amylase, 1.84 mM cholic acid, and 1.84 mM deoxycholate in saline (150 mM NaCl). For both treatments, portions of the fine wheat bran CWM (10 mg) were incubated with 1 mL of enzymolysis fluid for 150 min at 37 °C while under constant rotation (5 rpm). Enzymolysis fluid was incubated in the absence of wheat bran as a control. Enzymolyses were terminated by boiling (5 min), and after centrifugation (13000*g* for 5 min), the supernatants were assayed for free and esterified ferulic acid by HPLC (see above). To allow estimation of levels of both free and esterified feruloyl groups, the HPLC column was also calibrated with *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX): this soluble feruloylated oligosaccharide was purified from wheat bran after enzymic treatment (Ralet *et al*., 1994b) and is well separated from ferulic acid by HPLC under these conditions. The release of esterified feruloylated material was therefore calculated in terms of FAXX equivalents.

Binding Studies. An *in vitro* method [see Ryden and Robertson (1995a)] was used to test for binding of ferulic acid to wheat bran. One milliliter of a solution of ferulic acid (2 μg mL^{-1} in 100 mM sodium−phosphate buffer, pH 5.5) was added to portions (range 0−10 mg) of fine wheat bran CWM in 1.5 mL plastic Eppendorf tubes. Tubes containing either ferulic acid solution but no fiber or fiber but no ferulic acid solution were incubated as controls. The tubes were incubated under constant rotation (5 rpm) at 37 °C for 2 h and centrifuged (13000*g* for 5 min), and the supernatants were assayed for ferulic acid by HPLC as described above.

RESULTS

Fermentation of Wheat Bran. Since the anticarcinogenic properties of bran may be related to their insolubility, we examined the release of ferulic acid from wheat bran in a model human gut. The carbohydrate and lignin composition of the unfermented and fermented wheat bran samples used here has been described elsewhere (Ryden and Robertson, 1995a). To test if ferulic acid was solubilized in the small intestine prior to fermentation in the colon, fine wheat bran samples were subjected to *in vitro* gastric and small intestinal enzymolyses. Gastric and small intestinal enzymolytic treatment released (respectively) 0.41 and 2.46 nmol of free ferulic acid and 6.91 and 4.70 nmol of esterified ferulic acid (FAXX equivalents), the sum of which accounts for only 2.6% of total feruloyl groups in the fiber. These results indicate that only a small proportion of esterified feruloyl groups is released from wheat bran prior to fermentation in the colon.

Both coarse and fine bran preparations were fermented, and each reached a maximum extent after 18 h (Table 1). The amounts of insoluble residue (percent by weight of initial substrate) after fermentation for 24 h were 64.4 and 60.8 for the fine and coarse bran, respectively (i.e. about 40% of the fiber was considered fermentable). Nonlinear least-squares regression analysis of the data over the time period 3−18 h indicated that rates of carbohydrate fermentation were about 20 and 27% h^{-1} for the course and fine bran, respectively (Table 2), and that after 24 h, some 60% of the CWM would remain in the colon.

The coarse and fine wheat bran preparations contained 0.53 and 0.48% (w/w) alkali-extractable (=esterified) ferulic acid, respectively (Table 1). Fer-

Table 1. Ester-Linked Phenolic Acid Composition of Sugarbeet AIR and Wheat Bran CWM and of the Insoluble Residues Remaining after *in Vitro* Fermentation Using a Human Fecal Inoculum[a]

sample	substrate recovered[b] (% unfermented)	ferulic acid[c] (mg/g of residue)	total ferulic acid in residue (mg)	contribution of other phenolic acids[d] (%, w/w)
sugarbeet AIR, fermented				
0 h	100	9.24 ± 0.20	3.23	nd
3 h	51.1−55.9	0.33 ± 0.02	0.116	N/D
7 h	29.3−33.5	nd	nd	N/D
18 h	16.2−19.4	nd	nd	N/D
24 h	8.1−10.2	nd	nd	N/D
fine bran CWM, fermented				
0 h	100	5.33 ± 0.06	1.87	0.021
6 h	71.2−73.6	4.07 ± 0.25	1.43	N/D
18 h	64.0−65.9	2.44 ± 0.23	0.854	N/D
24 h	63.5−65.2	2.29 ± 0.09	0.802	N/D
coarse bran CWM, fermented				
0 h	100	4.80 ± 0.80	1.68	0.023
6 h	74.7−76.9	2.72 ± 0.48	0.952	N/D
18 h	59.9−62.0	2.68 ± 0.68	0.938	N/D
24 h	60.3−61.3	1.78[e]	0.315	N/D

[a] *In vitro* fermentations were performed using a single-chamber fermenter to simulate transit and substrate fermentation in the ascending colon under conditions of limiting substrate in an anaerobic cabinet, with 3 days preadaptation of the fermenter to the test fibers (Ryden and Robertson, 1995a,b). Fecal samples were prepared from a pool originating from several normal adults and filtered through 100 μm nylon cloth prior to use. Following preadaptation, test samples (at least four for each fiber source, 350 mg per bag) were added to the fermenter. Unfermented controls were obtained by incubating fiber sources in water at 37 °C for 18 h. [b] The data for recovery of fermented substrate are given as the 95% confidence intervals (at least four samples analyzed in duplicate for each fiber source). [c] These data are accompanied by the standard deviation ($n = 3$). [d] The AIR and CWM samples were analyzed for esterified caffeic, *p*-coumaric, sinapic, syringic, and vanillic acids. Data are the mean values ($n = 3$) for the total contribution of these phenolics. nd, not detected. N/D, not determined. [e] The standard deviation was not calculated since insufficient material was available to allow more than one alkaline extraction.

Table 2. Rate and Extent of Fermentation and the Phenolic Content of Various Cell Wall Materials

fiber source	av rate of fermentation[a]	extent of fermentation[b]	total cell wall phenolics [mg (g of CWM)$^{-1}$]
apple	60%/h (3−9 h)	>95%	nd[c]
cabbage	32%/h (3−9 h)	>95%	0.086
carrot	20%/h (3−9 h)	>95%	0.021
sugarbeet	37%/h (3−9 h)	>95%	9.24
fine bran	28%/h (3−18 h)	40%	5.53
coarse bran	19%/h (3−18 h)	40%	5.03

[a] Values were obtained by nonlinear least-squares regression for the linear fermentation period (indicated in parentheses). [b] Values indicate the extent of fermentation at the end of the fermentation period. [c] nd, not detected.

mentation led to decreases in both the total amount of ferulic acid remaining in the insoluble residue and in the proportion (percent, w/w) of ferulic acid in the insoluble residue. Hence, the extent of solubilization of esterified feruloyl groups was proportionally higher than the extent of fermentation of the bran. After 18 h of fermentation, there was little further release of ferulic acid from fine bran. For both the coarse and fine bran, the contribution of other phenolic acids (vanillic, syringic, *p*-coumaric), by weight, was about 0.02% (Table 1) and was not significant compared to the contribution from ferulic acid.

Fermentation of Sugarbeet. The release of phenolics was also tested from a readily fermentable substrate, sugarbeet AIR. The non-starch polysaccharide composition of the unfermented and fermented sugarbeet residues used here has been described elsewhere (Ryden and Robertson, 1995b). The extent of fermentation was much greater than for wheat bran, with degradation of most of the pectic polysaccharides after 24 h (only 9% AIR remained as water-insoluble residue; Table 1). Nonlinear least-squares regression analysis of the data over the time period 3−9 h indicated that rates of carbohydrate fermentation were about 37% h^{-1} and the extent of fermentation was >95% (Table 2). The insoluble residue remaining after 24 h of fermentation consisted mainly of glucose (57%), xylose (25%), and uronic acid (11%). The contents of arabinose and galactose in the insoluble residue were reduced from 32 and 7% (w/w) in the unfermented sample to 0.6 and 4% (w/w), respectively, after 24 h of fermentation. This indicates that the pectic side chains were almost fully fermented and agrees with the complete solubilization of feruloyl groups we have shown here.

The sugarbeet AIR contained 0.92% (w/w) alkali-extractable ferulic acid (Table 1). Ferulic acid was rapidly solubilized during fermentation and was not detected in insoluble residues after 7, 18, or 24 h of fermentation. Hence, the rate of solubilization of the feruloyl groups is faster than overall solubilization of the sugarbeet polysaccharides. Ferulic acid was the only phenolic acid detected in the sugarbeet AIR.

Hydrolytic Enzyme Activities. It has been demonstrated that efficient enzymic release of ferulic acid from wheat bran requires *endo*-xylanase and ferulic acid esterase activities (Faulds and Williamson, 1995a; Bartolomé *et al.*, 1995). We assayed liquor samples obtained from a control (−wheat bran) and a test (+wheat bran) fermentation using human fecal inoculum and fine bran for FAE and xylanase activity.

Methyl ferulate has been developed as a substrate for the assay of ferulic acid esterase activity (Faulds and Williamson, 1991), and we have demonstrated that incubation of human fecal slurry at 37 °C with methyl ferulate results in time-dependent (up to 2 h) release of free ferulic acid (Faulds and Williamson, 1995b). Ferulic acid release from methyl ferulate was assayed for both nonboiled and boiled liquor samples after a 75 min incubation. Incubations were also performed using sterile-filtered unfermented liquors (+ or −WB) to give a better indication of zero-time activities, since control (−WB) activities were variable and the assay period (75 min) is sufficient to allow for enzyme induction.

First, the fine bran fermentation liquor was assayed for the presence of free and esterified phenolic acids. We were unable to detect feruloyl groups in liquor samples from control fermentations (without wheat

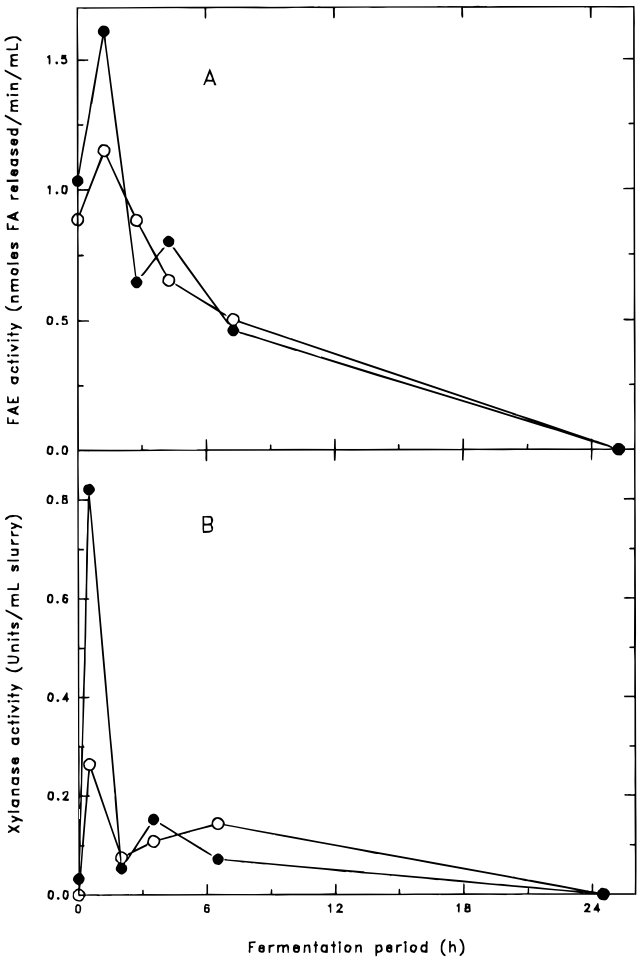


Figure 1. Enzyme activities in liquors after fermentation of human fecal inoculum either in the presence (●) or absence (○) of fine bran CWM. FAE and xylanase were measured using methyl ferulate or soluble oat spelts xylan, respectively, as substrate. Enzymic activity was calculated using the difference between unboiled and boiled liquors, and the zero time points using sterile-filtered liquors. Data are the means of duplicate assays. Variation between replicates was <4%.

bran). Free ferulic acid was detected only in the zero [0.245 nmol (mL of liquor)$^{-1}$] and 1.5 h [0.085 nmol (mL of liquor)$^{-1}$] samples from test (plus wheat bran) fermentations.

Free ferulic acid was released from added methyl ferulate in all of the incubation mixtures (Figure 1A), including those containing boiled and/or sterile-filtered fermentation liquor, although at lower rates than for those containing nonboiled or unsterilized liquor. This indicates either that enzymic FAE activity is not completely destroyed by boiling or that methyl ferulate is also hydrolyzed nonenzymically under these conditions. Enzymic FAE activities were calculated using the difference between nonboiled and boiled liquor samples after a 75 min incubation, and activity was detected in all of the samples tested except for the 24 h samples (Figure 1A). FAE activity was rapidly induced (1.5-fold) by wheat bran to 1.61 nmol min^{-1} (mL of liquor)$^{-1}$] after 75 min, but activities rapidly returned to control levels within 3 h. The significance of these findings is discussed below.

Xylanase activity was induced 3-fold by wheat bran [0.82 unit (mL of liquor)$^{-1}$ compared to 0.26 unit (mL of liquor)$^{-1}$] (Figure 1B). Xylanase activity was considerably lower in the 1.5−6 h samples and undetectable from 24 h samples, both in the presence and in the absence of wheat bran. Hence, the observed induction of xylanase occurs very rapidly but the activity apparently persists for only a short time. It is possible that xylanase enzymes secreted by gut microorganisms bind to the wheat bran; indeed, it is known that several xylanases possess cellulose or xylan binding domains (Coughlan and Hazlewood, 1993; Black *et al.*, 1995).

The rate of fermentation of wheat bran appears to be proportional to the levels of both xylanase and FAE activities in the fermentation liquor. Hence, the highest rate of degradation of wheat bran occurs in the first 6 h (Table 1) when the levels of both FAE (Figure 1A) and xylanase (Figure 1B) are also at their highest levels.

Binding Studies. Our results indicate that significant quantities of ferulic acid are released from (insoluble) cereal fiber into the soluble phase by fermentation in the human colon. To test if the de-esterified ferulic acid was likely to remain in solution, or whether it would associate with the fiber and become insoluble again, we incubated ferulic acid (2 μg mL^{-1}) with various concentrations of fine wheat bran (0−10 mg mL^{-1}) and determined the level of free acid remaining in solution. We were unable to detect binding of ferulic acid to fine wheat bran even at a concentration of up to 10 mg of fiber mL^{-1}, indicating that ferulic acid solubilized from fiber should remain available in solution.

DISCUSSION

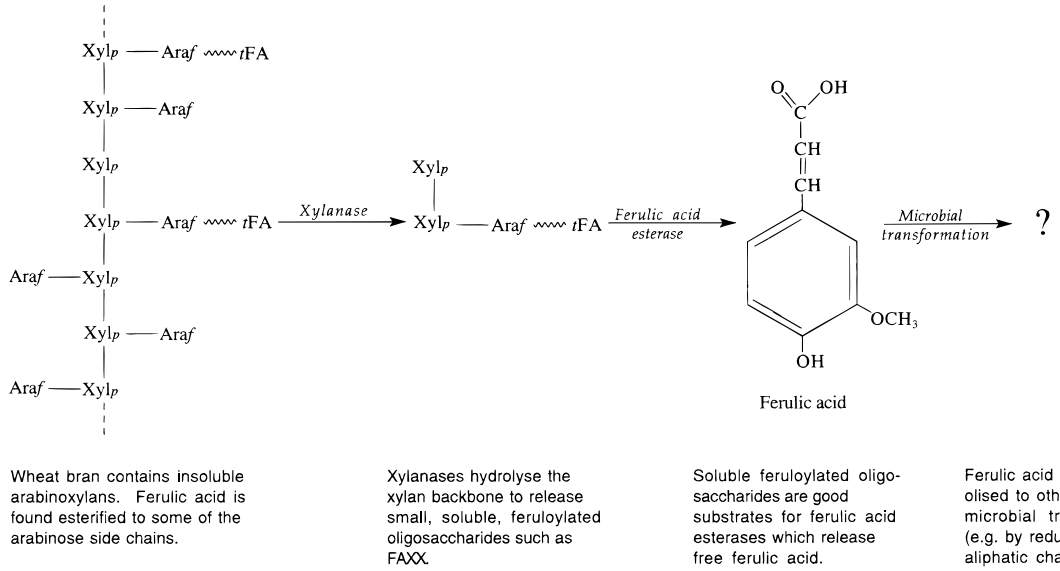
We estimate that the daily intake of three portions of fresh fruit [apple, plum, and peach (100 g each)], together with wheat bran (20 g; twice the U.K. average) from bread, breakfast cereal, etc., would result in ingestion typically of 210 mg of ester-linked hydroxycinnamic acid, of which about half would be ferulic acid, mainly from wheat bran (Herrmann, 1989). The significance of such a substantial intake of hydroxycinnamic acids is poorly understood, although they are known to be good antioxidants (Scott *et al.*, 1993).

Esterified ferulic acid that is covalently bound to an insoluble wheat bran matrix may play a different antioxidant role to soluble forms of esterified ferulic acid and also to the free acid. For example, it has been demonstrated that soluble feruloylated oligosaccharides obtained from corn bran were just as effective as the free acid in (*in vitro*) antioxidant assays, but neither were as active as a soluble fraction of high molecular weight which may have contained ferulate dimers (Ohta *et al.*, 1994). To begin to address this question, we have investigated how the passage of wheat bran and sugarbeet fiber through the human gastrointestinal tract changes the partitioning of feruloyl groups (i) from the insoluble phase to the soluble phase and (ii) from esterified forms to free acid.

Degradation of cell wall polymers and release of ferulic acid during fermentation is due to the action of several hydrolytic enzymes. For degradation of wheat bran fiber, xylanase is the most important hydrolytic activity (Bartolomé *et al.*, 1995), while FAE activity is required to cleave ferulic acid−sugar linkages with concomitant release of free acid (Faulds and Williamson, 1995a). Several hydrolytic enzymes are also required for degradation of sugarbeet fiber, and a different esterase is required to cleave ferulic acid−sugar linkages and hence release free acid (Kroon and Williamson, 1996).

Our results indicate that, in humans, over 95% of the total release of feruloyl groups probably takes place during fermentation in the colon: only small amounts

Scheme 1. Solubilization of Esterified Feruloyl Groups from Insoluble Fiber Such as Wheat Bran[a]



[a] Abbreviations: Xyl*p*, D-xylopyranose; Ara*f*, L-arabinofuranose; *t*FA, *trans*-ferulate; FAXX, *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose. Relevant references can be found in Table 3.

Table 3. Summary of the Antioxidant Behavior of Fermentation Products from Wheat Bran in the Human Colon

treatment	important product(s)	physical phase; location	reported antioxidant properties	comments	refs
none	wheat bran fiber (0.5% w/w ferulic acid)	insoluble; throughout GI tract	nitrite scavenging	fermentation is slow and incomplete	Faulds and Williamson (1995a); Ryden and Robertson (1995a); Jacobs *et al.* (1995)
enzymic hydrolysis (e.g. xylanase for wheat bran)	feruloylated oligosaccharides (e.g. FAXX)	soluble; colon	inhibits lipid peroxidation	short half-life in colon	Faulds and Williamson (1995a); Ohta *et al.* (1994)
de-esterification (by ferulic acid esterases)	free ferulic acid	soluble; colon	inhibits lipid peroxidation; scavenges $OH^{\bullet}$ and $ABTS^{\bullet}$	does not non-covalently bind to residual fiber	Scott *et al.* (1993); Faulds and Williamson (1995a)
microbial transformation (by gut microorganisms)	unknown	phase unknown; likely formed in colon	unknown		Besle *et al.* (1995)

were released by gastric and small intestinal treatments. Once the insoluble fiber reaches the colon, where it is fermented, there is significant loss of covalently bound feruloyl groups from the insoluble phase, with the rate and extent of solubilization dependent on the fiber source (Table 1). Results shown here and elsewhere (Faulds and Williamson, 1995a) have indicated that release of free ferulic acid from wheat bran proceeds predominantly as shown in Scheme 1; that is, the insoluble wheat bran fiber is hydrolyzed by xylanase activity which releases feruloylated oligosaccharides (e.g. FAXX) to the soluble phase where they are efficiently hydrolyzed by FAE activity to release the free acid. Solubilization of sugarbeet fiber proceeds by a similar mechanism; several hydrolytic activities are also required for efficient degradation of the insoluble polymers (Ralet *et al.*, 1994a), but different esterases are required for subsequent release of free acid (Kroon and Williamson, 1996). However, sugarbeet fiber is more readily fermentable than bran (see Table 1).

Levels of feruloylated compounds in the liquors of wheat bran fermentations remained very low, and far lower than the amounts of feruloyl groups released from the insoluble fiber (<5% of that released; see above). This indicates that, once released to the soluble phase by fermentation in the human colon, feruloyl groups are either rapidly converted to another form or bound noncovalently to the residual fiber. Since small, soluble feruloylated oligosaccharides are resistant to degradation by carbohydrases due to hindrance from the phenolic moiety (Hespell and O'Bryan, 1992; E. Luonteri, VTT Biotechnology and Food Research, Finland, unpublished results, 1995), we presume that free ferulic acid is released from all of the feruloylated material solubilized from the fiber. Free ferulic acid does not appear to bind noncovalently to wheat bran fiber *in vitro* (see above), although it has been suggested this occurs for other dietary phenolics (Tew *et al.*, 1996). Hence, the low levels of ferulic acid observed in fermentation liquors indicate that the free acid is either rapidly utilized by gut microorganisms or transformed to other phenolic forms while remaining in solution. It is known that most monomeric aromatic acids can be fully degraded (to CO_2, CH_4, aliphatic acids, volatile fatty

acids) by rumen microorganisms (mainly bacteria) by several mechanisms [see Besle *et al*. (1995) for a review], but it is not known how readily similar transformations can take place in the human colon. The potential for antioxidant behavior of fermentation products from wheat bran is summarized in Table 3.

In conclusion, we have shown, using an *in vitro* assay, (1) that significant amounts of esterified feruloyl groups can be solubilized from plant fiber in the large intestine, (2) that microbial ferulic acid esterase and xylanase activities present in the human gastrointestinal tract may be responsible for the solubilization, (3) that the rate of solubilization of esterified feruloyl groups from insoluble plant cell wall materials is dependent on the plant source, (4) that the levels of feruloyl groups in solution in fermentations in our model colon remain very low and do not reflect the amounts released from the fiber source, and (5) that ferulic acid does not bind to wheat bran fiber.

ABBREVIATIONS USED

AIR, alcohol insoluble material; CWM, cell wall material; FAE, ferulic acid esterase; HPLC, high-performance liquid chromatography.

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