damage. PPO can increase up to 33-fold upon germination (Kruger 1976), and the distribution of enzyme in the kernel may be dependent on the severity of sprouting, as has been found for the enzyme α-amylase (Kruger 1981). As such, results on wheats containing sprout damage could be quite different from those reported here.

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Ferulic Acid in Rye and Wheat Grain and Grain Dietary Fiber

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ABSTRACT

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The aim of the present work was to examine the effect of cross-linking of rye and wheat arabinoxylan by ferulic acid on grain nutritive value, measured in vitro by an enzymatic test. Determination of ferulic acid was based on spectrophotometric measurements of defatted samples at 320 nm. Approximately 80% of the *trans*-ferulic acid, the dominant phenolic acid of rye and wheat grain, was found in the bran of both species. Total content and extractability of free and esterified ferulic acid by water, ethanol, and alimentary enzymes (soluble dietary fiber) from grain meal were significantly higher in rye than in wheat. The activity of peroxidase, the enzyme thought to be responsible for the formation of diferulic bridges, was also significantly higher in rye. Most (85–90%) of the alkaline-soluble ferulic acid in grain was localized in the insoluble dietary fiber, and only about 5% was in the soluble fraction. In spite of the higher solubility of rye arabinoxylans and the higher arabinose-xylose ratio in rye than in wheat grain, the ratio of the number of arabinose residues per ferulic acid molecule was not significantly higher in the soluble fiber of rye. Thus, cross-linking of grain hemicellulose components by ferulic bridges does not appear to contribute to the known differences in the structure, molecular weight, and nutritive properties of soluble fiber of rye and wheat.

Ferulic acid and isoferulic acid (4-hydroxy-3-methoxy and 3-hydroxy-4-methoxy cinnamic acids) are the main phenolic acids of cell walls of monocots (Smith and Hartley 1983). *Trans*-ferulic acid is the dominant isomer and constitutes up to 90% of the total phenolic acids in wheat flour (Sosulski et al 1982). It is esterified to hemicellulosic components of plant cells, primarily to arabinosyl residues at 2-O or 3-O branches of the xylan backbone (Fry 1986, Mueller-Harvey et al 1986). Recently, feruloylated arabinoxylans have been isolated from various plants (Kato and Nevins 1985, Ahluwalia and Fry 1986, Mueller-Harvey et al 1986). Free or esterified ferulic acid could also polymerize with lignin, forming alkali-resistant bonds (Scalbert et al 1985). Cross-linking of pectins in the primary wall by ferulic acid, postulated in dicots, has not been found in monocots (Fry 1983). *N*-feruloylglycine has been detected as the terminal sequence of barley globulin

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(Van Sumere 1973), and recently *N*-feruloylglycine amidohydrolase was isolated from barley seeds (Martens et al 1988a,b). However, feruloylation of other protein amino acids was not reported, nor was the extent of protein acylation by ferulic acid established.

Hemicellulosic polysaccharides are components of dietary fiber, the level of which is commonly related to the nutritive value of plant products. Rye grain is inferior to wheat as a feed for young animals (Honeyfield et al 1983) and contains significantly higher amounts of soluble compounds not digestible in vitro by the alimentary enzymes (Boros et al 1985, Rakowska et al 1989, Rybka et al 1992). The content of these compounds is negatively correlated with grain protein digestibility in rats (Raczyńska-Bojanowska et al 1989, Rakowska et al 1992).

To check the possible effect of polysaccharide feruloylation on the nutritional properties of grain meals, including digestion of grain components by alimentary enzymes, our analysis included the alkali-labile ferulic acid esters in the grain and bran dietary fiber fractions of rye and wheat.

MATERIALS AND METHODS

Grain Fractions

Grain and bran of the commercial cultivars of rye (cvs. Dańkowskie Złote, Modesz, and Motto) and wheat (cvs. Grana, Parada, and Jawa) and grain of two rye S_4 inbred lines of different fiber content were ground to pass a 40-mesh sieve. Flour and bran from cvs. Dańkowskie Złote and Grana were obtained from the Institute of Cereal Milling, Warsaw. An alcohol-soluble fraction was obtained by boiling ground grain or bran for 30 min in 80% ethanol (1:100, w/v). A water-soluble fraction was obtained by extraction of ground grain with water for 1 hr at 10°C (1:5, w/v), and the water-insoluble fraction was sedimented by centrifugation.

The arabinoxylan-rich fraction of the water extract was prepared by ethanol precipitation of the heat-deproteinized extract (Meuser and Suckow 1986). Soluble and insoluble dietary fiber was isolated from rye and wheat grains by the enzymatic gravimetric method of Asp et al (1983) on the basis of successive digestion of grain meal with pepsin and pancreatin. Soluble indigestible fiber components (enzyme-extractable) were precipitated with 4 vol of 96% ethanol. The soluble fiber value was not corrected for protein (Raczyńska-Bojanowska et al 1989). Insoluble, nondigested fiber was collected by filtration after enzymatic digestion. To determine indigestible components of the water extract, the content of soluble fiber was determined in the water extract and the water-insoluble remnant in grain of rye inbred lines using the same Asp procedure.

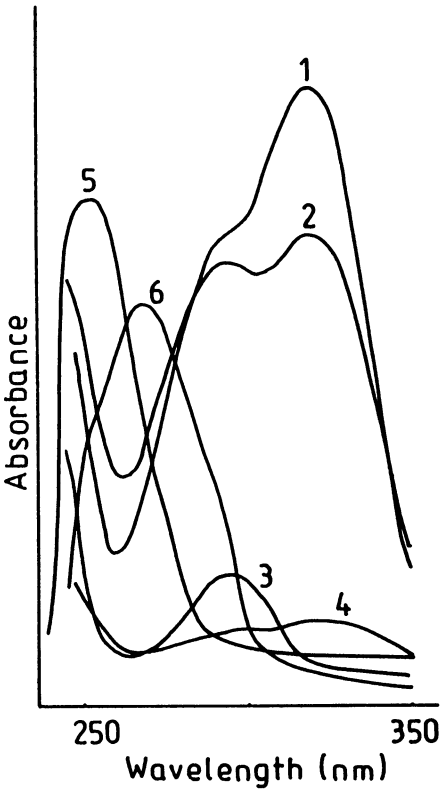

Fig. 1. Molar absorption spectra of phenolic acids in chloroform. 1, ferulic acid; 2, isoferulic acid; 3, *m*-hydroxybenzoic acid; 4, caffeic acid; 5, *p*-hydroxybenzoic acid; 6, syringic acid.

Determination of Ferulic Acid

Ground grain and bran defatted with hexane (Krygier et al 1982) were extracted with 1% NaOH containing 0.5% $NaBH_4$ for 20 min (1:100, w/v) (Huang et al 1986). The extracts were acidified with HCl to pH 2.5 and extracted three times with 2 vol of chloroform. The content of ferulic acid was determined spectrophotometrically at 320 nm; the molar extinction coefficient of ferulic acid in chloroform was 22.85. The recovery of ferulic acid added to the alkaline extract was 95%. Ferulic acid in the grain fiber fractions was determined either directly by chloroform extraction at pH 2.0 (free) or after saponification with 2*N* NaOH (esterified).

Thin-Layer Chromatography

Thin-layer chromatography of phenolic acids extracted from rye and wheat grains was performed on silica gel plates (60 F 254, Merck) using benzene-methanol-acetic acid (88:8:4). The acids were detected under ultraviolet light and by spraying with sulfanilamide-nitrite reagent (Randerath 1971); they were identified by comparison with the appropriate standards.

Determination of Pentosans

Total pentosan content was measured with aniline acetate (Raczyńska-Bojanowska et al 1983) and arabinose and xylose as their aldol acetate derivatives (McGinnis 1982) by gas chromatography after hydrolysis in 2*N* trifluoroacetic acid for 1 hr at 121°C (Albersheim et al 1967).

Determination of Peroxidase

Peroxidase activity in rye grain immediately after harvest was determined spectrophotometrically at 485 nm using guaiacol as a substrate (Patykowski et al 1988). One unit of activity has been defined as a change in absorbance of 1.0.

Statistics

Standard deviation and analysis of variance were performed on the basis of analytical results from replicate experiments.

RESULTS AND DISCUSSION

The dominant form of phenolic acid in both rye and wheat grain was ferulic acid, although isoferulic, coumaric, syringic, and caffeic acids were detected in minor amounts by thin-layer chromatography. In addition, *p*-hydroxybenzoic acid was present in rye grain. In the water-soluble fraction of rye grain, the spots corresponding to caffeic and syringic acids were relatively more intense than those in the whole grain. All of the aforementioned phenolic acids also were found in trace amounts in the soluble dietary fiber fractions.

Quantification of ferulic acid was based on measuring the absorbance at 320 nm (Ahluwalia and Fry 1986) of chloroform extracts of the defatted samples, saponified under nitrogen in the presence of borohydride to stabilize ferulic acid. Absorbance of other phenolic acids at this wavelength, with the exception of caffeic acid, was negligible (Fig. 1). However, it has been found that during the saponification step in our experimental procedure, caffeic acid is oxidized (Krygier et al 1982, Huang et al 1986). The absorbance spectra of chloroform extracts from both the grain and the soluble dietary fiber from rye and wheat indicate clearly the 320-nm maxima attributed to ferulic acid (Fig. 2).

Our determinations of ferulic acid in cereal grain dealt only

with the free acid and the esterified form released by alkali treatment. The alkali-stable ferulic acid bound to lignin or found in *N*-feruloylglycine, as in barley globulin, constitutes an unknown proportion of the ferulic acid bound to the cell wall constituents of cereal grains.

The content of ferulic acid in three cultivars of rye grain was significantly higher than that in three cultivars of wheat (Table I), although all cultivars were grown under the same conditions. A larger variation was observed among the rye inbred lines, with ferulic acid contents as high as 1,220.7 μg/g and a coefficient of variation of 16% (J. Sitarski, *unpublished data*).

The higher content of ferulic acid in rye than in wheat was associated with a higher activity of peroxidase, an enzyme thought to be responsible for the formation of diferulic bridges linking cell polysaccharides (Fry 1986). The difference between the two cereals was significant at $P < 0.001$ (Table I). In both cereal species about 80% of the alkali-labile ferulic acid was located in the bran (Table II).

Most of the ferulic acid in cereal grain is extractable with 80% ethanol (Table III), i.e., 3,857 and 2,495 μg/g of ethanol-extractable fractions from rye and wheat, respectively. In 80% aqueous methanol, ferulic acid esters of sugar carboxylic acids were detected in rye by Strack et al (1986). The content of ferulic acid extracted with alcohol does not appear to be associated with the nutritive value of cereal grain, since neither the extraction of rye grain with ethanol nor the addition of the extracted compounds to casein had any effect on the nutritive value (M. Rakowska, *personal information*). On the other hand, extraction of rye grain with water significantly improved the growth rate and the feed-to-gain ratio in birds, and the addition of the lyophilized extract to wheat or triticale diets negatively affected both of these nutritional parameters (Boros et al 1985). Moreover, it has been reported previously that only the content of soluble fiber was correlated with the digestibility of rye grain protein in rats (Raczyńska-Bojanowska et al 1989). Only 11–12% of the total alkali-soluble ferulic acid of rye and wheat grain was extracted with water, less than 6% was found in soluble fiber, and over 85% in insoluble fiber (Tables III and IV). Ethanol extracted the ferulic acid remaining in the insoluble fiber that had not been extracted by the enzymes. On the other hand, indigestible compounds of water extract (soluble fiber of water extract) constituted 40–50% of the soluble fiber of the whole grain (K. Rybka, *unpublished data*).

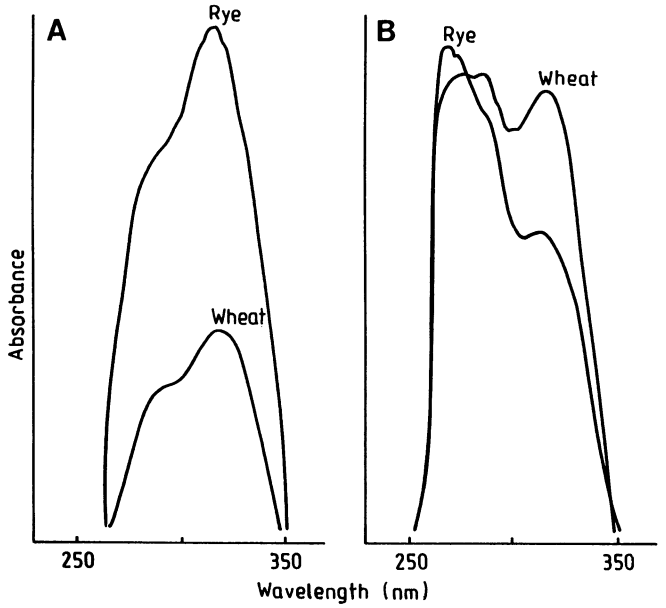

Fig. 2. Ultraviolet absorption spectra in the chloroform extracts of rye and wheat grain (**A**) and soluble fiber extracted from rye and wheat grain (**B**) by the alimentary enzymes in vitro.

TABLE I
Ferulic Acid Content and Peroxidase Activity in Whole Grain of Winter Rye and Wheat Cultivars[a]

Species and Cultivar	Ferulic Acid ($\mu g\ g^{-1}$)	Peroxidase Activity (units $g^{-1}\ hr^{-1}$)
Rye		
Dańkowskie Złote	1,006	74.0
Modesz	1,138	87.4
Motto	1,046	79.2
$\bar{x}_1$	1,063	80.2
Wheat		
Grana	783	43.9
Parada	772	60.0
Jawa	982	66.6
$\bar{x}_2$	846	56.8
Difference: $\bar{x}_1 - \bar{x}_2$	217***[b]	23.4***

[a] Results are expressed as the mean of two separate extractions. The means of both species were evaluated by the analysis of variance.
[b] *** = $P < 0.001$.

The composition of the water-soluble fractions from the grain of both cereals differed significantly (Table V). Both the water extract and the soluble fiber from rye grain contained significantly higher amounts of pentoses than the corresponding fractions from wheat grain. The arabinose-xylose ratio was lower in the rye fractions than in the corresponding wheat fractions, which may indicate higher branching of wheat arabinoxylan (Fry 1986). However, the molar arabinose-ferulic acid ratios from rye and wheat (Table VI) were similar in the whole grain and corresponding soluble fractions, i.e., water extract and grain soluble dietary fiber fractions, which lower the nutritive value of rye (Boros et al 1985, Raczyńska-Bojanowska et al 1989). The number of arabinosyl residues per mole of ferulic acid increased from approximately 50 in the whole rye and wheat grain to 450 in their soluble fiber fractions, despite the different extractability of arabinoxylan from rye and wheat. Therefore, one might conclude that feruloylation of hemicellulose (Table III) does not affect extractability of the indigestible grain components by the alimentary enzymes. This is confirmed by the analysis of the two rye inbred lines differing in the content of soluble dietary fiber (48 and 60 mg/g of grain meal) (Table IV). The content of alkali-labile ferulic acid did not differ among particular dietary fiber fractions from the two rye inbred lines. However, the rye inbred lines differed in nutritive value, as estimated in vitro (Raczyńska-Bojanowska et al 1989). The highest content of ferulic acid and the highest number of feruloyl residues per arabinose molecule were found in the insoluble dietary fiber (not extracted by the alimentary enzymes in the in vitro test), i.e., in the fraction that is not related to protein digestibility (Raczyńska-Bojanowska et al 1989, Rakowska et al 1992). Ahluwalia and Fry (1986) calculated that the degree of feruloyl esters in the water-insoluble

TABLE II
Content of Alkali-Labile Ferulic Acid in Bran and Flour from the Grain of Rye (cv. Dańkowskie Złote) and Wheat (cv. Grana)

	Rye		Wheat	
	Flour	Bran	Flour	Bran
Ferulic acid, $\mu g\ g^{-1}$	334 ± 24	1,684 ± 137	199 ± 9	1,964 ± 105
Yield, mg $100\ g^{-1}$ of whole grain	17 ± 1	84 ± 7	13 ± 1	65 ± 3
Ferulic acid, % of total grain ferulic acid	17	83	17	83

TABLE III
Proportion of Dry Matter, Ferulic Acid, and Pentoses in the Whole Grain of Rye (cv. Dańkowskie Złote) and Wheat (cv. Grana) Extracted Alternately with Ethanol, Water, and Alimentary Enzymes

	Rye			Wheat		
Fraction	Dry Matter	Ferulic Acid	Arabinose + Xylose	Dry Matter	Ferulic Acid	Arabinose + Xylose
Whole grain	100	100	100	100	100	100
80% EtOH extract	9	38	<1	8	32	<1
Water extract	17	12	30	12	11	16
Arabinose-xylose fraction[a]	4	2	15	2	1	7
Soluble fiber	6	4	40	3	2	17

[a]Obtained as described in Materials and Methods.

TABLE IV
Contents of Arabinose, Xylose, and Ferulic Acid and the Arabinose-to-Xylose and Arabinose-to-Ferulic Acid Molar Ratios in Two Rye Inbred Lines[a]

	Contents, μmol 100 mg^{-1}			Ratios	
	Arabinose	Xylose	Ferulic Acid	Arabinose-Xylose	Arabinose-Ferulic Acid
Line A					
Soluble fiber in					
Water extract	141 ± 6	193 ± 9	0.33 ± 0.01	1:1.4	432:1
Water-insoluble remnant	91 ± 4	131 ± 5	0.57 ± 0.02	1:1.4	159:1
Insoluble fiber	113 ± 4	165 ± 7	3.32 ± 0.11	1:1.5	35:1
Line B					
Soluble fiber in					
Water extract	146 ± 7	234 ± 12	0.30 ± 0.01	1:1.6	495:1
Water-insoluble remnant	113 ± 5	180 ± 9	0.56 ± 0.02	1:1.6	201:1
Insoluble fiber	123 ± 4	180 ± 6	3.16 ± 0.14	1:1.5	39:1

[a]In lines A and B, the contents of grain soluble dietary fiber were 48 and 60 mg/g, respectively, and those of insoluble fiber 163 and 182 mg/g, respectively.

TABLE V
Content of Ferulic Acid (Free and Esterified) and Pentoses in Each of the Soluble Fractions of Rye and Wheat Grains (cvs. Dańkowskie Złote and Grana)

	Ferulic acid, μg g^{-1}		Pentoses, mg g^{-1}	
Fraction	Free	Esterified	Arabinose	Xylose
Rye				
Water extract	560 ± 25	81 ± 4	72 ± 4	99 ± 4
Arabinose-xylose fraction[a]	ND[b]	460 ± 20	134 ± 8	244 ± 13
Soluble fiber	ND	611 ± 24	173 ± 3	284 ± 9
Wheat				
Water extract	397 ± 15	324 ± 10	44 ± 3	50 ± 2
Arabinose-xylose fraction[a]	ND	370 ± 15	99 ± 2	118 ± 2
Soluble fiber	ND	395 ± 8	93 ± 4	112 ± 6

[a]Obtained as described in Materials and Methods.
[b]Not determined.

TABLE VI
Molar Ratios of Arabinose to Xylose and Arabinose to Ferulic Acid in Rye and Wheat

	Arabinose-Xylose		Arabinose-Ferulic Acid	
Fraction	Rye	Wheat	Rye	Wheat
Grain	1:1.6	1:1.3	48:1	54:1
Water extract	1:1.4	1:1.2	130:1	110:1
Soluble fiber	1:1.6	1:1.2	456:1	430:1

arabinoxylan in barley grain was 1 per 138 arabinose residues. According to our data, the ratio was approximately 1 per 40 arabinose residues in rye inbred lines.

Another conclusion might be drawn from the similarity in the ratio of ferulic acid molecules to arabinose residue in soluble rye and wheat fibers. Since a dramatic difference was observed in the response of rats (coefficients of true digestibility of 44.0 and 73.2, respectively) to the equivalent amounts of rye and wheat soluble fiber (Rakowska et al 1989), the binding of ferulic acid in dietary fiber appears to be associated with neither the solubility of arabinoxylan nor its nutritional properties.

The high solubility of rye arabinoxylan, including its extractability by alimentary enzymes, does not result from the size of the polymer, nor is it related to its lower degree of branching than that found in wheat (Fincher and Stone 1986). It may be due, as suggested by those authors, to the differences in the asymmetry of the molecule caused by a different distribution of arabinose substituents in the xylan backbone.

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Flow Behavior of Wheat Flour-Water Dough Using a Capillary Rheometer. I. Effect of Capillary Geometry[1,2]

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ABSTRACT

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A capillary extrusion rheometer was employed for detailed investigation of the flow behavior of wheat flour-water dough. Dough was extruded at ambient conditions through capillaries of different lengths and diameters. In the shear rate range of 9–5,000 sec^{-1}, the dough exhibited shear thinning with an average flow behavior index of 0.34 and consistency coefficient of 2,395 Pa-$sec^{0.34}$. The flow curves, corrected for end effects and for effect of die diameter on shear rates, were independent of capillary dimensions. The capillary rheometer technique was found to be a reliable and repeatable method for determining flow parameters of viscous materials such as dough.

As with most foods, rheological properties are important in breadmaking. The final quality of the bread can be related to the rheological properties of its flour dough, which explains the amount of research being conducted in this area. Researchers have addressed different aspects of dough rheology and have described the methods used to evaluate the rheological properties (Bloksma 1975; Hibberd and Parker 1975; Matsumoto et al 1975; Matsuo and Irvine 1975; Rasper 1975; Bushuk 1985; Dick 1985; Faubion et al 1985; Hoseney 1985; Nagao 1985; Dreese et al 1988a,b; Fitzgerald et al 1988; Refai et al 1988). Their work concentrated on the qualitative evaluation of flours. Instruments such as the farinograph or mixograph were used to obtain optimum mix time and absorption data. These instruments provide useful information about dough, but they cannot generate numerical data to characterize flow behavior. In automated bakeries, knowledge of dough viscosity plays an important role in production control and equipment design. Dough viscosity may relate to the quality of the baked product and may control that quality in some instances. On-line methods to sense dough consistency require knowledge of dough flow behavior over a wide range of flow conditions. Dough can be pumped from the point of mixing to the fermentation chamber and then to the oven, but suitable pumps cannot be designed or specified unless the dough's rheological parameters and flow behavior properties are known. None of the studies referenced above give the basic information on flow behavior needed for engineering design. Basic information on flow behavior of wheat flour-water dough based on a capillary extrusion rheometer was reported by Sharma et al (1990). They found it was possible to describe the flow behavior of dough using the capillary rheometer results. Because only one size capillary (3.21 mm diameter) with different lengths was used, the reliability of the technique needs verification at other capillary diameters. Therefore, this study was undertaken to investigate the effects of capillary geometry on the flow behavior of wheat flour-water dough.

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