Content of Phenolic Acids and Ferulic Acid Dehydrodimers in 17 Rye (*Secale cereale* L.) Varieties

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The contents of phenolic acids and ferulic acid dehydrodimers were quantified by HPLC analysis after alkaline hydrolysis in kernels of 17 rye (*Secale cereale* L.) varieties grown in one location in Denmark during 1997 and 1998. Significant variations ($P < 0.05$) with regard to the concentration of the analyzed components were observed among the different rye varieties and also between different harvest years. However, the content of phenolic acids in the analyzed rye varieties was narrow compared to cereals such as wheat and barley. The concentration of ferulic acid, the most abundant phenolic acid, ranged from 900 to 1170 μg g^{-1} dry matter. The content in sinapic acid ranged from 70 to 140 μg g^{-1} dry matter, *p*-coumaric acid ranged from 40 to 70 μg g^{-1} dry matter, and caffeic, *p*-hydroxybenzoic, protocatechuic, and vanillic acids were all detected in concentrations less than 20 μg g^{-1} dry matter. The most abundant ferulic acid dehydrodimer 8-O-4′-DiFA was quantified in concentrations from 130 to 200 μg g^{-1} dry matter followed by 8,5′-DiFA benzofuran form (50−100 μg g^{-1} dry matter), 5,5′-DiFA (40−70 μg g^{-1} dry matter), and 8,5′-DiFA (20−40 μg g^{-1} dry matter).

Keywords: *Ferulic acids; ferulic acid dehydrodimers; rye varieties; Secale cereale L.; HPLC*

INTRODUCTION

Bread made from whole rye flour (*Secale cereale* L.) is an important part of the diet in North and East European countries. Unlike wheat, a considerable part of rye is consumed as whole meal products. A high consumption of whole grain products may contribute to reduce the risk of chronic diseases such as cardiovascular disease and certain types of cancer (Slavin et al., 1997). The positive physiological effects have mainly been ascribed to dietary fiber, but other constituents, including phenolic acids and lignans, may contribute to the beneficial effects (Adlercreutz, 1984; Kroon et al., 1997; Slavin et al., 1997; Kroon and Williamson, 1999). Recently it has been shown that rye diets inhibit the growth of prostate cancer implants in rats (Landström et al., 1998). Free radicals and reactive oxygen species may give rise to initiating events in cancer, atherosclerosis, and cataracts in humans (Halliwell and Gutteridge, 1989). Dietary antioxidants may help in protecting the body from these reactive species (Deshpande et al., 1996). Phenolic acids have for some time been recognized as potent antioxidants (Graf, 1992; Natella et al., 1999), and recently several ferulic acid dehydrodimers have also been shown to exert antioxidant activity in different in vitro assays (Garcia-Cornesa et al., 1997a,b, 1999).

Previously, we quantified seven different phenolic acids in rye grain (cv. Esprit) after alkaline hydrolysis, with ferulic acid being the most abundant (ca. 1000 μg g^{-1} dm), followed in quantity by sinapic (ca. 130 μg g^{-1} dm) and *p*-coumaric acids (ca. 60 μg g^{-1} dm). Caffeic, vanillic, *p*-hydroxybenzoic, and protocatechuic acids were only detected in minor amounts (less than 20 μg g^{-1} dm) (Andreasen et al., 1999). The content of these phenolic acids in the rye bran fraction was ca. 15 times higher than that in the separated flour fraction (Andreasen et al., in press). Ferulic acid is also the most abundant phenolic acid in other types of cereals such as barley and wheat (Rybka et al., 1993; Zupfer et al., 1998; Lempereur et al., 1997). In wheat bran, maize bran, and barley (aleurone layer), where ferulic acid linkages in plant cell walls have been investigated, it appears that the main portion is linked to the heteroxylans via esterification at the O-5 position of arabinofuranose (Ishii, 1997). This may also be the case in rye kernels. Ferulic acid may also be linked by ester and ether bonds to lignin (Iiyama et al., 1990).

Ferulic acid units can undergo dimerization by oxidative coupling catalyzed by peroxidase (Geissmann and Neukom, 1973). The main dehydrodimers hitherto identified in plant material are 8-O-4′-DiFA, 8,5′-DiFA, 8,5′-DiFA benzofuran form, 5,5′-DiFA (Figure 1), and 8,8′-DiFA noncyclic form, with 8-O-4′-DiFA often being the most prominent (Ralph et al., 1994; Waldron et al., 1996; Garcia-Conesa et al., 1997b; Lempereur et al., 1998). By alkaline hydrolysis and extraction from rye bran, the 8-O-4′-DiFA was the most abundant dimer, followed in quantity by the 8,5′-DiFA benzofuran form, 8,5′-DiFA, and 5,5′-DiFA in decreasing relative amount (Andreasen et al., in press). The content of these dimers in the rye bran fraction is 10−15 times higher than that in the flour fraction (Andreasen et al., in press).

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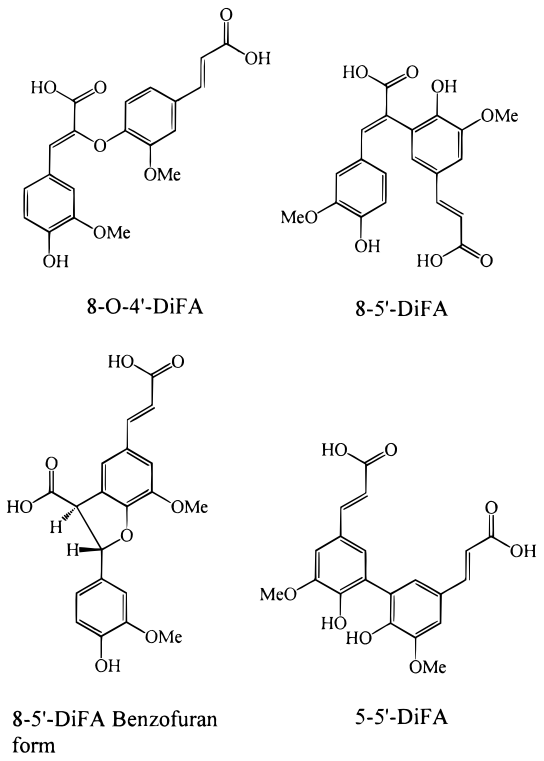

Figure 1. Chemical structures of ferulic acid dehydrodimers isolated from rye.

Ferulic acid units esterified to arabinoxylans can link adjacent arabinoxylan chains together through formation of diferulic acid bridges (Hatfield et al., 1999). Covalent cross-links between cell wall polymers can modify the mechanical properties of the cell wall, thereby affecting certain cell wall parameters such as extensibility (Faulds and Williamson, 1999). Cross-linked wheat arabinoxylans are capable of binding much more water per gram of polymer than purified arabinoxylan fractions and form viscous solutions or gels in aqueous solution (Izydorczyk et al., 1990). Oxidative gelation of arabinoxylans in wheat appears to affect both the rheological properties of dough and the final bread volume (Hoseney, 1984). Cross-linking of pentosans via ferulic acid dehydrodimers may therefore be an important determinant for dough handling and bread quality, including rye bread baking. Quantification of the level of ferulic acid dimers in rye flour could provide a basis for determining such a relation, and differences in levels among different rye varieties might indicate differences in baking performance.

To date, very little information is available on the genetic contribution and the effect of the cultivation conditions on the phenolic content of rye grain. The purpose of the present study was to investigate the genetic variability with respect to the content of phenolic acids and ferulic acid dehydrodimers in mature kernels of rye. The rye varieties used in this study are included in a research project in the Danish Cereal Network concerning rye quality and rye bread baking performance and quality.

MATERIALS AND METHODS

Rye Samples. Grain from 17 rye varieties consisting of 6 hybrid varieties (Rapid, Esprit, Amado, Komet, Avanti, and Apart) and 11 population varieties (PetkusII, Dominator, Motto, Quadriga, Nikita, Hacada, Amilo, Tsulpan3, Ponsi, Akusti, and Anna) were analyzed for their content of phenolic acid and ferulic acid dehydrodimers. The 17 rye varieties were grown under the same conditions in 1997 and 1998 at Ronhave Experimental Station (Danish Institute of Agricultural Sciences) in Denmark. Chemical fertilizer and pesticides were used according to normal practice. The samples were sown at the end of September 1996 and 1997 and harvested in the middle of August 1997 and 1998, respectively. After harvest the samples were stored under dry conditions at 10−15 °C until analysis in January 1999. All 17 rye varieties harvested in 1997 and the 4 rye varieties (Ponsi, Esprit, Motto, and Tsulpan3) harvested in 1998 were analyzed for the content of phenolic acids and ferulic acid dehydrodimers. The climate in 1997 was warm and dry compared to 1998 when the climate was cold and wet. The falling numbers, which is inversely proportional to the enzyme activity in the cereals, was on average, for rye varieties harvested in 1997, 240 ± 50 s and, for varieties harvested in 1998, 140 ± 40 s .

Sample Preparation. Whole rye grains were ground in a Retch mill (model ZM1) through a 0.5-mm screen at 10.000 rpm before analysis. Moisture content was determined by heating at 130 °C for 2 h (ICC standard no. 110/1). The analytical results are expressed as dry matter. Rye sample (2.0 g) and the internal standards (2 mg of anisic acid and 0.5 mg of cinnamic acid) were combined with 75 mL of 0.08 M phosphate buffer (pH 6.0) and treated with 0.1 mL of α-amylase (15 min, 100 °C, pH 6.0) (Sigma, A-3306) to degrade starch to avoid gelatinization of the sample during saponification. The sample was saponified for 1 h with 25 mL of 2 M NaOH at 25 °C (agitation, under nitrogen, and in the dark). The pH was adjusted to pH < 2 with 2 M HCl, and the sample was then centrifuged (10000*g*, 10 min). The pellet was saponified for 1 h with 50 mL of 2 M NaOH at 25 °C (agitation, under nitrogen, and in the dark). The pH was then adjusted to pH < 2 with 2 M HCl. The supernatant and the pellets were extracted with ethyl acetate (3 × 50 mL). Centrifugation (10000*g*, 10 min) was used between each extraction to separate the aqueous and organic phases. The combined ethyl acetate fractions were dried with anhydrous Na_2SO_4 and evaporated under reduced pressure at 30 °C. The dry residue was then dissolved in 10 mL of methanol + 10 mL of 0.02 M phosphate buffer (pH 2.15) and filtered through a 0.45-μm nylon filter before HPLC analysis. The described sequential saponification procedure was tested to be as effective as 18 h of saponification with 1 M NaOH (data not shown).

Separation and Quantification of Phenolic Acids by HPLC. Samples were analyzed by HPLC using the method of Andreasen et al. (1999). Separations were performed on a RP-18 column (LiCroCART 100, Merck) at 35 °C, by gradient elution with solvent A (0.02 M phosphate buffer, pH 2.15) and solvent B (methanol:0.02 M phosphate buffer, pH 2.15, 40:60, v/v) using the following elution profile: 0−50 min isocratic 75% A, 25% B, linear gradient over 50 min to 100% B, held isocratic at 100% B for further 20 min. Flow rate: 1 mL min^{-1}. Injection volume: 40 μL. Cis and trans isomers of ferulic acid were separated by using the following gradient: 0−50 min isocratic 75% A, 25% B, linear gradient over 70 min to 100% B, held isocratic at 100% B for further 20 min. Phenolic acids were identified and quantified by comparison with authentic compounds. The following compounds were used: *trans*-ferulic acid, *trans*-*p*-coumaric acid, *trans*-sinapic acid, *trans*-cinnamic acid, *trans*-caffeic acid, vanillic acid, *p*-hydroxybenzoic acid, and protocatechuic acid. The *cis*-hydroxycinnamic acids were prepared by radiation with UV light according to Waldron et al. (1996). All standards (Sigma) were dissolved in methanol (1 g L^{-1}) and diluted with 0.02 M phosphate buffer (pH 2.15) making external standard curves in the following concentration range: ferulic and anisic acids, 10−60 mg L^{-1}; cinnamic and sinapic acids, 3−20 mg L^{-1}; *p*-coumaric acid, 1−6 mg L^{-1}; caffeic, vanillic, *p*-hydroxybenzoic and protocatechuic acids, 0.4−2.4 mg L^{-1}. Detection limits: cinnamic acid, 0.05 mg L^{-1}; ferulic, *p*-coumaric, and caffeic acids, 0.1 mg L^{-1}; sinapic, protocatechuic, vanillic, and anisic acids, 0.2 mg L^{-1}.

Analysis of Ferulic Acid Dehydrodimers. Ferulic acid dehydrodimers were identified and quantified according to

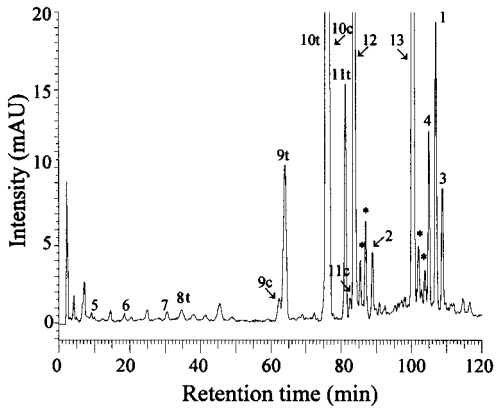

Figure 2. HPLC elution profile of phenolic monomers and dimers present in saponified extracts from rye (cv. Rapid), detection at 280 nm, injection volume 40 μL: (**1**) 8-O-4′-DiFA; (**2**) 8,5′-DiFA; (**3**) 8,5′-DiFA benzofuran form; (**4**) 5,5′-DiFA; (**5**) protocatechuic acid; (**6**) *p*-hydroxybenzoic acid; (**7**) vanillic acid; (**8t**) *trans*-caffeic acid; (**9c**) *cis*-*p*-coumaric acid; (**9t**) *trans*-*p*-coumaric acid; (**10c**) *cis*-ferulic acid; (**10t**) *trans*-ferulic acid; (**11t**) *trans*-sinapic acid; (**11c**) *cis*-sinapic acid; (**12**) anisic acid (internal standard); (**13**) *trans*-cinnamic acid (internal standard); (*) unidentified compounds having UV−vis spectra corresponding to ferulic acid dehydrodimers.

Andreasen et al. (in press) using the following response factors (RF): 8-O-4′-DiFA, RF 0.14; 8,5′-DiFA, RF 0.18; 8,5′-DiFA benzofuran form, RF 0.12; 5,5′-DiFA, RF 0.21. Detection limits for the dimers were 0.4 mg L^{-1}. The response factors were calculated under the chromatographic conditions used by comparing the response area of the compounds on HPLC with the response area of *trans*-cinnamic acid at 280 nm. Determinations of the response factors were based on gravimetric quantification using pure standards. The response factors were in accordance with those reported by Waldron et al. (1996). However, a precise determination of the response factor of 5,5′-DIFA was not possible due to very small amounts available of this compound. The response factor of 5,5′-DIFA was therefore adapted from Waldron et al. (1996).

Statistics. The experiments were run as randomized block designs using blocks consisting of 6 samples/day (Montgomery, 1997). Different rye varieties were analyzed in double replica for rye samples harvested in 1997 and triple replica for samples harvested in 1998. The single determinations for a specific rye variety were performed on different days. The SAS 6.12 software package (1989−1996, SAS Institute Inc., Cary, NC) was used for statistical analysis. Contents were compared by an ANOVA *F*-test using the General Linear Models procedure and expressed by a *P*-value (the *P*-value is defined as the smallest level of significance that would lead to rejection of the null hypothesis H_0). Furthermore the Ryan−Einot−Gabriel−Welsch multiple range test (*SAS/STAT Guide*, 1987) was used to control the type I experimental error rate.

RESULTS

Variation in the Content of Phenolic Acids in Rye Grain. A typical HPLC chromatogram of rye grain extract is shown in Figure 2. HPLC analysis confirmed that ferulic acid was the dominant phenolic acid in rye. The level of ferulic acid ranged from 941 to 1174 μg g^{-1} dm for rye harvested in 1997 (Table 1). The content of sinapic acid ranged from 93 to 153 μg g^{-1} dm, and the content of *p*-coumaric acid ranged from 37 to 65 μg g^{-1} dm (Table 1). In all the 17 rye varieties harvested in 1997 the contents of vanillic, *p*-hydroxybenzoic, caffeic, and protocatechuic acids were less than 20 μg g^{-1} dm.

On the basis of the ferulic acid contents obtained for 1997 samples, four of the varieties were also analyzed after harvest in 1998. Ponsi (high content 1997), Motto (low content 1997), and Tsulpan3 and Esprit (medium contents in 1997) were chosen. The level of ferulic acid analyzed in these four varieties harvested in 1998 ranged from 895 to 1107 μg g^{-1} dm (Table 1). The content in sinapic acid ranged from 72 to 104 μg g^{-1} dm, and the content in *p*-coumaric acid ranged from 53 to 61 μg g^{-1} dm (Table 1). Vanillic, *p*-hydroxybenzoic, caffeic, and protocatechuic acids were found in less than 20 μg g^{-1} dm in all four rye varieties.

Table 1. Content (μg g^{-1} dm) of Ferulic, Sinapic, and *p*-Coumaric Acids and 1000 Kernel Weight (g) for Rye Grain Varieties[a]

varieties	ferulic acid	sinapic acid	*p*-coumaric acid	1000 kernel weight
		1997 Harvest		
Ponsi	1174 ± 15^{a}	144 ± 30^{ab}	50 ± 1^{cd}	25.7
Nikita	1114 ± 12^{ab}	140 ± 8^{ab}	44 ± 0^{cdef}	32.8
Dominator	1104 ± 26^{ab}	139 ± 2^{ab}	44 ± 3^{cdef}	28.8
Akusti	1103 ± 32^{ab}	136 ± 29^{ab}	54 ± 1^{bc}	27.0
Esprit	1092 ± 58^{ab}	121 ± 8^{ab}	48 ± 3^{cde}	29.9
Quadriga	1091 ± 0^{ab}	153 ± 9^{a}	38 ± 2^{f}	28.6
Amando	1082 ± 50^{ab}	149 ± 5^{ab}	65 ± 6^{a}	27.3
Tsulpan3	1082 ± 50^{ab}	102 ± 23^{ab}	45 ± 3^{cdef}	30.5
Anna	1067 ± 36^{abc}	141 ± 17^{ab}	60 ± 0^{ab}	28.7
PetkusII	1057 ± 57^{abc}	121 ± 12^{ab}	44 ± 2^{cdef}	31.1
Komet	1040 ± 5^{abc}	101 ± 1^{ab}	37 ± 1^{f}	29.4
Avanti	1018 ± 4^{bc}	95 ± 5^{b}	39 ± 0^{ef}	31.0
Amilo	1003 ± 13^{bc}	138 ± 4^{ab}	41 ± 0^{def}	31.8
Rapid	1000 ± 1^{bc}	125 ± 9^{ab}	50 ± 7^{cd}	31.6
Apart	988 ± 17^{bc}	126 ± 12^{ab}	49 ± 0^{cd}	32.1
Hacada	945 ± 59^{c}	93 ± 16^{b}	38 ± 1^{f}	29.3
Motto	941 ± 38^{c}	118 ± 4^{ab}	42 ± 1^{def}	33.1
P-value	0.0002	0.006	0.0001	
$n' = 2^{b}$	109	45	8	
$n' = 17^{b}$	139	57	11	
σ	35	14	3	
		1998 Harvest		
Ponsi	1107 ± 73^{l}	104 ± 17^{l}	61 ± 4^{l}	
Tsulpan3	1076 ± 11^{l}	88 ± 16^{l}	57 ± 4^{l}	
Esprit	1018 ± 23^{l}	72 ± 10^{l}	59 ± 3^{l}	
Motto	895 ± 61^{m}	77 ± 17^{l}	53 ± 2^{l}	
P-value	0.003	0.13	0.21	
$n' = 2^{b}$	110	35	11	
$n' = 4^{b}$	129	41	13	
σ	49	16	3.5	
P-Values for Ponsi, Tsulpan3, Esprit, and Motto (1997 and 1998)				
varieties	0.0001	0.08	0.02	
year	0.04	0.0003	0.0001	
varieties*year	0.7	0.2	0.99	

[a] Data represent means ± standard deviation (1997, $n = 2$; 1998, $n = 3$). Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$). ^{abcdef}REGW grouping ($\alpha = 0.05$) for harvest year 1997. ^{lm}REGW grouping ($\alpha = 0.05$) for harvest year 1998. [b] Critical range in REGW test, n' = number of means; σ = root mean square error; SD < 0.5 rounded to SD = 0.

The content of ferulic acid in the rye varieties harvested in 1997 can be categorized into three overlapping groups (a−c, Table 1) by using the Ryan−Einot−Gabriel−Welsch multiple range test ($\alpha = 0.05$). Likewise, the varieties harvested in 1998 can be categorized into two groups ($\alpha = 0.05$). As expected ANOVA analysis on the four varieties from 1997 and 1998 showed significant ($P < 0.0001$) differences in ferulic acid content between rye varieties. Furthermore significant differences between the two harvest years were observed ($P = 0.04$), where rye harvested in 1997 had a higher content of ferulic acid than rye harvested in 1998.

Variation in the Content of Ferulic Acid Dehydrodimers in Rye Grain. Varieties with high ferulic acid content also had high levels of ferulic acid dehydrodimers. Ponsi, Nikita, and Dominator had the highest content of ferulic acids and the ferulic acid dehy-

Table 2. Content (μg g^{-1} dm) of Ferulic Acid Dehydrodimers for Rye Grain Varieties[a]

variety	total identified FA dimers[b]	8,5′-DiFA	5,5′-DiFA	8-O-4′-DiFA	8,5′-DiFA benzofuran form
		1997 Harvest			
Dominator	409 ± 73^{a}	38 ± 8^{a}	69 ± 7^{a}	200 ± 41^{a}	102 ± 18^{a}
Ponsi	387 ± 27^{ab}	37 ± 2^{ab}	68 ± 5^{a}	188 ± 7^{ab}	95 ± 13^{a}
Nikita	355 ± 15^{abc}	31 ± 1^{abc}	65 ± 3^{ab}	161 ± 5^{abc}	98 ± 6^{a}
Tsulpan3	353 ± 31^{abc}	33 ± 2^{abc}	61 ± 6^{ab}	169 ± 34^{abc}	90 ± 11^{a}
Akusti	351 ± 5^{abc}	33 ± 2^{abc}	58 ± 5^{ab}	158 ± 2^{abc}	102 ± 0^{a}
Quadriga	351 ± 0^{abc}	27 ± 6^{abc}	65 ± 0^{ab}	171 ± 1^{abc}	89 ± 5^{a}
Esprit	350 ± 14^{abc}	32 ± 1^{abc}	56 ± 8^{ab}	160 ± 13^{abc}	79 ± 1^{a}
Komet	348 ± 6^{abc}	32 ± 2^{abc}	67 ± 2^{ab}	175 ± 1^{abc}	74 ± 3^{a}
Anna	343 ± 24^{abc}	29 ± 1^{abc}	63 ± 4^{ab}	166 ± 14^{abc}	85 ± 6^{a}
Amando	333 ± 13^{abc}	30 ± 4^{abc}	57 ± 5^{ab}	159 ± 0^{abc}	88 ± 4^{a}
Avanti	329 ± 16^{abc}	31 ± 2^{abc}	63 ± 8^{ab}	162 ± 1^{abc}	73 ± 9^{a}
Rapid	326 ± 16^{abc}	28 ± 1^{abc}	54 ± 9^{ab}	151 ± 11^{abc}	92 ± 5^{a}
PetkusII	312 ± 24^{abc}	28 ± 0^{abc}	56 ± 7^{ab}	145 ± 13^{abc}	83 ± 4^{a}
Amilo	306 ± 6^{bc}	27 ± 3^{abc}	60 ± 1^{ab}	146 ± 2^{abc}	74 ± 6^{a}
Apart	305 ± 15^{bc}	26 ± 1^{bc}	49 ± 0^{ab}	145 ± 7^{abc}	86 ± 7^{a}
Hacada	283 ± 31^{c}	26 ± 0^{bc}	46 ± 6^{b}	128 ± 10^{c}	84 ± 15^{a}
Motto	278 ± 28^{c}	25 ± 3^{c}	50 ± 6^{ab}	135 ± 10^{bc}	68 ± 8^{a}
P-values	0.008	0.01	0.01	0.02	0.02
$n' = 2^{c}$	81	9	17	47	27
$n' = 17^{c}$	104	12	22	60	34
σ	26	3	6	15	9
		1998 Harvest			
Ponsi	332 ± 21^{l}	32 ± 6^{l}	58 ± 9^{l}	167 ± 11^{l}	74 ± 4^{l}
Tsulpan3	291 ± 18^{m}	23 ± 2^{l}	56 ± 8^{l}	150 ± 11^{l}	62 ± 3^{m}
Esprit	282 ± 1^{m}	21 ± 5^{l}	51 ± 2^{l}	146 ± 4^{l}	63 ± 2^{m}
Motto	241 ± 18^{n}	22 ± 7^{l}	41 ± 3^{l}	125 ± 10^{m}	53 ± 3^{n}
P-values	0.001	0.14	0.17	0.004	0.0005
$n' = 2^{c}$	37	13	20	21	8
$n' = 4^{c}$	43	15	23	25	9
σ	16	5	7	10	3
		P-Values for Ponsi, Tsulpan3, Esprit, and Motto (1997 and 1998)			
varieties	0.0001	0.02	0.03	0.001	0.0009
year	0.0001	0.006	0.06	0.02	0.0001
varieties*year	0.7	0.05	0.9	0.9	0.5

[a] Data represent means ± standard deviation (1997, $n = 2$; 1998, $n = 3$). Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$). [abc]REGW grouping ($\alpha = 0.05$) for harvest year 1997. [lmn]REGW grouping ($\alpha = 0.05$) for harvest year 1998. [b] Total identified FA dimers (8,5′-DiFA, 5,5′-DiFA, 8-O-4′-DiFA, and 8,5′-DiFA benzofuran form). [c] Critical range in REGW test, n' = number of means; σ = root mean square error; SD < 0.5 rounded to SD = 0.

drodimers, while Apart, Hacada, and Motto had the lowest content. The total content of ferulic acid dehydrodimers ranged from 278 to 409 μg g^{-1} dm for rye harvested in 1997 (Table 2). 8-O-4′-DiFA was the most abundant dimer in all rye varieties. The content ranged from 128 to 200 μg g^{-1} dm for varieties harvested in 1997. 8,5′-DiFA benzofuran form was found in concentrations from 68 to 102 μg g^{-1} dm, 5,5′-DiFA in concentrations from 46 to 69 μg g^{-1} dm, and 8,5′-DiFA was found in concentrations from 25 to 38 μg g^{-1} dm (Table 2). 8-O-4′-DiFA was also the most abundant dimer in all rye varieties harvested in 1998, when the content ranged from 125 to 167 μg g^{-1} dm. 8,5′-DiFA benzofuran form was found in concentrations from 53 to 74 μg g^{-1} dm, 5,5′-DiFA in concentrations from 41 to 58 μg g^{-1} dm, and 8,5′-DiFA in concentrations from 21 to 32 μg g^{-1} dm. For all four dimers there were significant differences between varieties and between harvest year ($P < 0.05$). In 1998 Ponsi was the variety with the highest content of all four dimers while Motto had the lowest content.

Measurements of 1000 kernel weight (Table 1) were made in order to determine which cultivars had larger kernels, since smaller kernels have a higher surface-to-volume ratio, and as a result, they might have a higher percentage of seed coat, aleurone, and germ cells and consequently have a higher ferulic acid concentration (Zupfer et al., 1998). A comparison between kernel weight (Table 1) and ferulic acid concentration gave a correlation coefficient (R^2) of 0.38, revealing a negligible relationship.

DISCUSSION

Variation in the Content of Phenolic Acids and Ferulic Acid Dehydrodimers in Rye Grain. Ferulic acid was the most abundant phenolic acid in all 17 rye varieties investigated, followed in quantity by sinapic and *p*-coumaric acid. The three hydroxycinnamates can exist in two isomeric forms: a trans form and a cis form. We determined that ca. 10% of the ferulic acid was in the cis form; likewise ca. 15% of the *p*-coumaric and sinapic acids were in the cis form. The trans form of ferulic acid originally formed in the metabolic pathway from phenylalanine or tyrosine can be partially converted to the cis form by cis−trans isomerase in vivo and by ultraviolet light in vitro (Caccamesa et al., 1979) and probably also in vivo.

The calculated *P*-values between harvest years (1997, 1998) show significant differences ($P < 0.05$) for all analyzed components (except sinapic acid). For all components it was also shown that there were significant variations ($P < 0.05$) within varieties harvest in 1997. For varieties harvested in 1998 there was significant differences ($P < 0.05$) in the content of ferulic acid, 8-O-4′-DiFA, 8,5′-DiFA benzofuran form, and the total

content of identified ferulic acid dimers. The content of ferulic acid in the rye varieties harvested in 1997 can be categorized into three overlapping groups (a−c, Table 1) thereby clearly indicating that the contents are genetic. Likewise, the varieties harvested in 1998 can be categorized into two groups ($\alpha = 0.05$).

The different climatic conditions during 1997 (warm and dry) and 1998 (wet and cold) only appeared to have a small influence on the content of the analyzed phenolic compounds. The total concentration of ferulic acid dehydrodimers in rye grain, which may reflect the extent of arabinoxylan cross-linking in the cell wall, was affected by the genotype (Table 2). The total content of the identified ferulic acid dehydrodimers varied from 241 to 409 μg g^{-1} dm, and there were significant ($P < 0.0001$) differences between rye varieties and between the two harvest years. Furthermore, the rye varieties harvested in 1997 and 1998 could be grouped into three overlapping groups (Table 2). The content of the four identified ferulic acid dehydrodimers (Table 2) showed significant differences among the varieties except in the content of 8,5′-DiFA benzofuran form for varieties harvested in 1997 and in the content of 8,5′-DiFA and 5,5′-DiFA for varieties harvested in 1998.

Saponified rye samples contain several additional compounds having UV−vis spectra resembling the spectra of ferulic acid dehydrodimers (Figure 2, peaks marked with an asterisk). The calculated total concentrations of the ferulic acid dehydrodimers in rye only included the four identified diferulic acids (8,5′-DiFA, 5,5′-DiFA, 8-O-4′-DiFA, and 8,5′-DiFA benzofuran form).

The order with respect to the content of ferulic acid and ferulic acid dehydrodimers in rye harvested in 1997 is the same as the order found between the four rye varieties harvested in 1998 (Tables 1 and 2), although the contents are significantly lower in 1998. These results indicate that the contents in ferulic acid and ferulic acid dehydrodimers are genetic. Since there was no correlation between 1000 kernel weight and ferulic acid content, the difference in ferulic acid concentration does not come from differences in the carypcosis structure as described in the Results section. However, the observed range in content of ferulic acid in rye, 895−1174 μg g^{-1} dm found in our experiment and 1006−1134 μg g^{-1} dm found by Rybka et al. (1993), is narrow compared to the range for contents of other cereals. In barley the variation in ferulic acid content ranged from 343 to 580 μg g^{-1} dm between different varieties (Zupfer et al., 1998). In durum wheat varieties, a very high genetic variability with respect to the content of ferulic acid has been found with concentrations ranging from 693 to 2443 μg g^{-1} dm (Lempereur et al., 1997). Different methods, such as acid hydrolysis versus alkaline hydrolysis, have been used to release the bound phenolic acids before extracting and analyzing the compounds and may have an impact on the results for the different cereals. Furthermore, rye is foreign-pollinated whereas barley and wheat are self-pollinated, and this could also explain the relatively small genetic variation with respect to the content of phenolic acids between rye varieties compared to those found between self-pollinated cereals.

This study shows that there are significant variations in the content of phenolic acids and ferulic acid dehydrodimers among the analyzed 17 rye varieties, although the variations between the different varieties are small. However, rye varieties such as Ponsi and Nikita have a higher content of potential beneficial components than varieties such as Hacada and Motto. Future studies will focus on understanding the significance of phenolic acids on rye dough handling and bread quality.

ABBREVIATIONS USED

8-O-4′-DiFA, (*Z*)-*β*-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-4-hydroxy-3-methoxycinnamic acid; 8,5′-DiFA benzofuran form, *trans*-5-[(*E*)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid; 5,5′-DiFA, (*E*,*E*)-4,4′-dihydroxy-5,5′-dimethoxy-3,3′-bicinnamic acid; 8,5′-DiFA, (*E*,*E*)-4,4′-dihydroxy-3,5′-dimethoxy-*β*,3′-bicinnamic acid; 8,8′-DiFA noncyclic form, 4,4′-dihydroxy-3,3′-dimethoxy-*β*,*β*′-bicinnamic acid; dm, dry matter; REGW, Ryan−Einot−Gabriel−Welsch.

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