Ferulic and Coumaric Acids, Total Phenolic Compounds and their Correlation in Selected Oat Genotypes

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Abstract

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Ferulic and coumaric acids were determined by high-performance liquid chromatography in 21 oat genotypes supplied from the gene bank of the Research Institute of Plant Production in Piešťany (Slovak Republic) with the aim of selecting some genotypes suitable for the preparation of functional foods. The content of coumaric acid was higher than that of ferulic acid in 61.9% of samples. The latter acid exceeded proportionally the former one in genotypes with a low content of phenolic acids, namely in the amount of up to 50 mg/100 g of grain, especially in chaffless oats. Ferulic acid content varied in the range from 16.50 mg/100 g (cultivar Jakub) to 149.36 mg/100 g of grain (cultivar Izak). The content of coumaric acid ranged between 8.05 mg/100 g (cultivar Detvan) and 210.27 mg/100 g of grain (cultivar Izak). The fact that the samples were grown in the same year (2003) in one locality (Vígľaš-Pstruša, Slovak Republic) minimises the influence of soil and climatic conditions and proves that remarkable differences between the content of ferulic and coumaric acids and the total content of phenolic compounds were caused by the genetic outfit of oats. A high correlation was found between the contents of the total phenolic compounds, ferulic acid, coumaric acid and their sum. The corresponding correlation coefficients *R* had the values of 0.9229, 0.9141, and 0.9211, respectively. This correlation enables one to employ a simple and inexpensive method for the determination of the total phenolic compounds using Folin-Ciocalteu reagent for a rapid assessment of differences detectable in the content of the sum of ferulic and coumaric acids in oat samples in view of the selection of genotypes suitable for the preparation of functional foods.

Keywords: oats; HPLC; ferulic acid; coumaric acid; total phenolic compounds

Phenolic acids are rather a small but important food component from the aspect of nutrition which is characterised by antioxidative activity (Graf 1992; Adom & Liu 2002; Kikuzaki *et al.* 2002; Kim *et al*. 2006). This basic property, which is very important for life, is associated with a number of biological functions such as antimutagenicity, anticarcinogenicity, deceleration of the organism ageing, and many others. The antioxidative activity of cereal extracts is very different and depends on the extraction agent, the kind of cereals and, to a certain extent, also on the cultivar and the morphological fraction (Zielinski & Kozlowska 2000). The phenolic compounds belonging to cinnamic acid derivatives (e.g. ferulic and coumaric acids studied by us) differ by a stronger antioxidative

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activity from the phenolic compounds of the group of benzoic acid derivatives (Velioglu *et al.* 1998). In accordance with the latest studies (Kim *et al.* 2006), ferulic, caffeic, and syringic acids are the strongest three antioxidants out of 8 phenolic acids tested for the antioxidative activity. The authors of the given publication explain this fact by the activity of the wheat-hull extracts containing as much as 2020 μg/g of ferulic acid.

Phenolic acids occur in a large number of plants and as secondary metabolites they are classified according to their importance into the group of substances following flavonoids. They differ from other phenols by an acid character. In plants, they can be esterified by other small molecules of aliphatic alcohols, phenols, phenolic acids and alkaloids. Using the carboxylic and hydroxylic groups, they can form bonds with starch and other polysaccharides via hydrogen or covalent bonds, and can create bridges and transverse linkages (Yu *et al.* 2001). Phenolic acids are localised in cellular walls and vacuoles. Most of phenolic acids exist in a binding form which represents as much as 70% in oats and wheat (Adom & Liu 2002). The dominant hydroxycinnamic acid in cereals is ferulic acid (Lachman & Capouchová 2006). This acid and its diferulates are present in large amounts in cereals, but in lower contents in fruits and vegetables (Bunzel *et al.* 2001). In the wheat grain, ferulic acid represents 0.5% (w/w) of dry matter and in the barley grain even 0.14% (w/w). In barley, this acid is concentrated mainly in the aleuronic grain-hull layer in the amount of 75% (Adom & Liu 2002), whereas in the endosperm it achieves only the amount of 10% (Sancho *et al*. 2001). Biologically accessible can be also diferulic acids present in cereal brans and built into the cellular walls of polysaccharides because the intestinal microflora exhibits an esterase activity able to release diferulic acid from the bound form (Andreasen *et al.* 2001).

The content of phenolic acids is influenced by the cultivar of factors. The production of *trans*-cinnamic acid and of a wide range of secondary metabolites with the basic C_6–C_3 carbons skeleton of phenylalanine is induced by various biotic and abiotic stresses such as, for example, an attack of pathogens, UV-radiation, low temperature, low iron, phosphorus, and nitrogen contents, the application of herbicides, etc. (Weidner *et al.* 2000; Orsák *et al*. 2001).

The fact that nutrition plays an important role in the prevention of chronic diseases, such as cardiovascular diseases, diabetes, and Alsheimer's disease, is evidenced by the results of many epidemiologic studies (Adom & Liu 2002). It is therefore necessary to produce special foods, the so-called nutraceutics (functional foods and nutritional complements), which have favourable physiological effects on the human organism. For the proven physiological effects of these foods is responsible a certain group of substances – phytochemical compounds which include also phenolic acids. The biological activity of some phytochemicals is attributed to the antioxidative activity. Hydroxycinnamic acids are antioxidant polyphenols common in the human diet although their potential health benefits depend on their bioavailability. Hydroxycinnamic acid can be metabolised by the liver as suggested by the results obtained using HepG2 cells as a hepatic model system (Mateos *et al*. 2006). Phenolic acids have significant biological and pharmacological properties and some of them have demonstrated a remarkable ability to alter sulfate conjugation. Phenolic acids can alter sulfate conjugation and the antioxidant capacity in living systems (Yeh & Yen 2006). Sulfate conjugation by phenolsulfotransferases is an important process in the detoxification of xenobiotics and endogenous compounds. Phenolic acids are known to increase the activities of phenolsulfotransferases (Yeh *et al*. 2004). Phenolic acid derivatives may exert their anti-inflammatory action through inhibiting superoxide generation (Lee *et al*. 2005). Phenolic acids exert a direct antiproliferative action, evident at low concentrations, comparable with those found in biological fluids after the ingestion of foods rich in phenolic acids (Kampa *et al*. 2004).

The aim of our work was the evaluation of ferulic and coumaric acids in selected oat genotypes known up to now, and also in new ones suitable for the design and preparation of functional foods.

MATERIAL AND METHODS

Oat samples. 21 oat varieties from the Research Institute of Plant Production in Piešťany, Section of the Genetic Plant Sources, which had been grown at the Research Breeding Station Vígľaš-Pstruša (Slovak Republic), were milled on the electronic grinder (Gorenje SMK 102, HR) and sifted through a 0.3 mm-mesh sieve.

Isolation of free and bound phenolic acids. According to the literature (Adom & Liu 2002) phenolic acids were isolated with slight alterations. Two grams of the milled sample were extracted

twice during 10 min with 20 ml of 80% methanol on the shaker (LTI, Kavalier Votice, Czech Republic). The supernatants were separated by centrifugation (3500 min^{-1}, 10 min). The combined supernatants were evaporated to dryness on the rotary vacuum evaporator of the type 350 (Unipan, Poland) and the evaporation residue was dissolved in 10 ml methanol. The extract obtained in this way was used for the determination of free phenolic acids. The residue after the methanol extraction was hydrolysed with 40 ml 2M NaOH at room temperature in dark for 1 h under shaking. After neutralisation with 12M of hydrochloric acid the phenolic compounds were extracted with ethyl acetate using a five-step extraction (5 × 40 ml). After distilling off ethyl acetate on the rotary vacuum evaporator and dissolving the evaporation residue in methanol, the combined ethyl ester layers were used for the determination of bound phenolic acids.

Determination of total phenolic compounds. The total phenolic compounds were determined spectrophotometrically in the visible region at the wave length λ = 725 nm by applying the Folin-Ciocalteu reagent (Velioglu *et al.* 1998). The calibration curve was constructed in the concentration range from 0.02 to 0.065 mg/ml. Ferulic acid was used as the standard.

HPLC analysis. Ferulic and coumaric acids were estimated using HPLC, the pump LCP 4000, the programmer gradient GP 3, and the UV detector LCD 2082.2 (ECOM Prague, Czech Republic) on the column Phenomenex Maxsil C18 (250 × 4.6 mm) using the detection at λ = 280 nm. For the isocratic elution the mixture of water:acetonitril:acetic acid (88:10:2) as the mobile phase (Weidner *et al*. 2000) at the flow rate of 0.5 ml/min was used. The results were evaluated by means of the Apex programme, Version 2.

RESULTS AND DISCUSSION

In view of the fact that free phenolic acids constitute in cereals only a slight part of the total content of phenolic acids (Matilla *et al*. 2005), and that in the oat samples as much as 97.8% of the ferulic acid exist in, the bound form (Adom & Liu 2002) (the major part of ferulic and coumaric acids being bound to water-non-extractable arabinoxylans) (Glitso & Knudsen 1999; Faulds *et al.* 2003; Rao & Muralikrishna 2004), the correlations between the contents of ferulic and coumaric acids, and between their sum and the content of the total phenolic compounds were expressed by us for the sum of both free and bound acids estimated after alkaline hydrolysis. Both ferulic and coumaric acids are dealt with in our study for two reasons: because from the aspect of the content of phenolic acids they belong to the prevailing acids in cereals (Hernanz *et al.* 2001; Peterson 2001), and also because they show the antioxidative activity associated with a wide spectrum of biological effects. The compiled results from the measurements carried out with 21 oat genotypes obtained from the gene bank of the Research Institute of Plant Production in Piešťany (Slovak Republic) are indicated in Tables 1 and 2.

Among 21 oat genotypes studied, coumaric acid had a dominant position in as much as 13 genotypes, which represent 61.9%, while the remaining 38.1% were found to have a higher content of ferulic acid (Table 1). In contrast to coumaric acid, the higher content of ferulic acid in the oat grain was measured by Peterson (2001). In accordance with literature sources, all other cereal varieties contain higher amounts of ferulic acid than coumaric acid, especially wheat bran (Kim *et al.* 2006), rye (Glitso & Knudsen 1999; Weidner *et al.* 2000), wheat (Faulds *et al.* 2003; Rao & Muralikrishna 2004), and barley (Hernanz *et al.* 2001). In the wheat, rye, and barley straw, the content of ferulic acid was found to be 1.86 times higher than that of coumaric acid (Run-Cang Sun *et al*. 2001). The contents of ferulic and coumaric acids determined in our samples, which had been improved by Slovak and Czech breeders and which are saved in the gene bank of the Research Institute of Plant Production in Piešťany (Slovak Republic), were relatively high. For comparison, ferulic acid was the most abundand hydroxycinnamate in barley with the concentration ranging from 35.90 mg/100 g to 62.40 mg/100 g. The coumaric acid levels ranged from 7.9 mg/100 g to 26.00 mg/100 g (Hernanz *et al.* 2001). In rye, the concentration of ferulic acid ranged from 9.00 mg/100 g to 117.00 mg/100 g. The content of coumaric acid ranged from 4.00 mg/100 g to 7.00 mg/100 g (Andreasen *et al*. 2000a). Peterson (2001) determined 27.60 mg/100 g of ferulic acid in oatmeal by the isotope dilution assay. In the red wheat bran, the content of ferulic acid amounts to 202.00 mg/100 g and that of coumaric acid to 4.66 mg/100 g (Kim *et al.* 2006).

From the large extent of values obtained by determining the amount of the investigated phenolic acids

Table 1. The content of ferulic and coumaric acids and of the total phenolic compounds in selected oat genotypes[a]

Cultivar	Country of origin	Acid	c_{FEA} [b]	$\Sigma(c_{FA} + c_{CA})$ [c]	c_{TPC} [d]
			(mg/100 g grain)		
00782	Slovak Republic	FA	19.44 ± 0.41	33.92	149.80 ± 4.27
		CA	14.48 ± 0.162		
Abel	Czech Republic	FA	21.50 ± 1.74	31.53	220.48 ± 1.57
		CA	10.03 ± 0.24		
Adam	Czech Republic	FA	17.76 ± 3.63	28.63	176.54 ± 3.53
		CA	10.87 ± 0.58		
Ardo	Czech Republic	FA	81.64 ± 1.01	201.96	346.39 ± 3.85
		CA	120.32 ± 1.66		
Auron	Czech Republic	FA	91.98 ± 1.54	218.32	420.84 ± 1.83
		CA	126.34 ± 8.91		
Azur	Czech Republic	FA	112.26 ± 5.37	253.40	503.59 ± 0.71
		CA	141.14 ± 1.27		
Cyril	Czech Republic	FA	112.72 ± 7.31	258.64	553.36 ± 2.77
		CA	145.92 ± 2.30		
Dalimil	Czech Republic	FA	101.11 ± 6.06	233.44	363.55 ± 6.15
		CA	132.33 ± 10.49		
Detvan	Slovak Republic	FA	19.62 ± 0.95	27.67	223.20 ± 3.97
		CA	8.05 ± 0.66		
Euro	Austria	FA	103.58 ± 5.31	276.79	479.86 ± 4.12
		CA	173.21 ± 3.75		
Expander	Austria	FA	95.45 ± 1.48	248.39	482.92 ± 2.40
		CA	152.94 ± 2.41		
Flämingsstern	Germany	FA	103.41 ± 6.15	260.05	408.16 ± 3.78
		CA	156.64 ± 12.11		
Izak	Czech Republic	FA	149.36 ± 1.51	359.63	451.15 ± 1.24
		CA	210.27 ± 8,85		
Jakub	Czech Republic	FA	16.50 ± 0.99	25.61	225.00 ± 1.83
		CA	9.11 ± 0.64		
Neklan	Czech Republic	FA	123.67 ± 1.76	313.32	439.03 ± 2.77
		CA	189.65 ± 11.14		
PS-100	Slovak Republic	FA	114.40 ± 0.82	299.62	495.10 ± 3.77
		CA	185.22 ± 13.10		
PS-106	Slovak Republic	FA	18.89 ± 3.20	32.52	167.30 ± 2.51
		CA	13.63 ± 1.25		
PS-90	Slovak Republic	FA	19.89 ± 0.72	30.29	110.47 ± 4.73
		CA	10.40 ± 0.71		
Roxton	Canada	FA	96.57 ± 9.52	205.60	456.38 ± 3.09
		CA	109.03 ± 8.81		
SV-5	Slovak Republic	FA	31.44 ± 0.42	48.83	157.88 ± 1.33
		CA	17.39 ± 0.24		
Zvolen	Slovak Republic	FA	84.10 ± 1.05	191.76	345.16 ± 3.83
		CA	107.66 ± 8.28		

[a]means ± standard deviation are calculated from three determinations; [b]the amount of phenolic acids detected by the HPLC method; [c]the total amount of ferulic and coumaric acids; [d]the content of the total phenolic compounds determined by Folin-Ciocalteu reagent; FA – ferulic acid; CA – coumaric acid

Table 2. The proportions of ferulic and coumaric acids in the total phenolic compounds in selected oat genotypes

Cultivar	P_{FA} (%)	P_{CA} (%)
Izak	33.11	46.61
Neklan	28.17	43.20
Dalimil	27.81	36.40
Flämingsstern	25.34	38.38
Zvolen	24.37	31.19
Ardo	23.58	34.74
PS-100	23.11	37.41
Azur	22.29	28.03
Auron	21.86	30.02
Euro	21.58	36.10
Roxton	21.16	23.89
Cyril	20.37	26.37
SV-5	19.91	11.01
Expander	19.77	31.67
PS-90	18.00	9.41
00782	12.98	9.67
PS-106	11.29	8.15
Adam	10.06	6.16
Abel	9.75	4.55
Detvan	8.79	3.61
Jakub	7.33	4.05

P_{FA} – proportion of ferulic acid in the content of the total phenolic compounds expressed in %

P_{CA} – proportion of coumaric acid in the content of the total phenolic compounds expressed in %

in various oat genotypes (ferulic acid – 16.50 mg/100 g of grain in cv. Jakub and 149.36 mg/100 g of grain in cv. Izak; coumaric acid – 8.05 mg/100 g of grain in cv. Detvan and 210.27 mg/100 g of grain in cv. Izak) is it obvious that the contents of ferulic and coumaric acids in the oat grain is influenced by a large number of factors including the genetic outfit, which should be borne in mind in the production of oat-based foods.

The oat samples with the prevailing amount of ferulic acid are shown in Table 2 in the last positions, which means that they have a low proportion of ferulic acid within the content of the total phenolic compounds (7.33–19.91%). Only the cultivar Adam and SV-5 belong to chaffy oats while the other ones belong to chaffless oats. It follows that ferulic acid is contained in higher amounts than coumaric acid in oat genotypes with a low content of phenolic acids (up to 50.0 mg/100 g grain) (Table 3), especially in chaffless oats. Consequently, the Slovak cultivars of chaffless oats PS-90, PS-106, and Detvan are insignificant from the aspect of the content of ferulic acid.

Ferulic and coumaric acids are contained in oats in relatively narrow ratios. According to our calculations, the average ratio value of ferulic and coumaric acids in individual oat genotypes is 0.71 in the case of coumaric acid prevailing, and 1.81 when the oat samples contain more ferulic than coumaric acid.

Considering the health aspects of the presence of phenolic acids in foods with regard to the content of ferulic and coumaric acids, the most suitable cultivars for baking and meat industries are the following (sum of ferulic and coumaric acids): Izak 359.63 mg/100 g of grain, Neklan 313.32 mg/100 g of grain and PS-100 299.62 mg/100 g of grain. All these varieties have higher contents of coumaric acid than of ferulic acid. As for the varieties of the Slovak origin, the yellow-chaffy cultivar Zvolen, which covers about 70% of the cultivation areas in Slovakia, belongs, with respect to the genotypes examined, to the average ones (sum of ferulic and coumaric acids is 191.76 mg/100 g of grain). This cultivar contains 53.32% of ferulic and coumaric acids of the value of the Czech cultivar Izak which has the highest content of phenolic acids. The first

Table 3. The relation between the amounts of ferulic and coumaric acids and their ratio in selected oat genotypes

$\Sigma(c_{FA} + c_{CA})^{\dagger}$ (mg/100 g grain)	Number of cultivar for FA:CA < 1	Number of cultivar for FA:CA > 1
0–50	0	8
51–200	1	0
201–400	12	0

†the total amount of ferulic and coumaric acids; FA – ferulic acid; CA – coumaric acid

Slovak chaffless oat cultivar Detvan registered in 2002 contains a low amount of ferulic and coumaric acids (sum of the ferulic and coumaric acids is 27.67 mg/100 g of grain). When choosing the oat cultivar, one should realise that the antioxidative activity which is responsible for the health-beneficial effects of phenolic acids is much different with ferulic and coumaric acids, and that ferulic acid is essentially a stronger antioxidant (Kim *et al.* 2006). Thus, the oat antioxidative activity results from the synergistic action of ferulic and coumaric acids as well as from the other accompanying substances exhibiting an antioxidative effect.

By summarising the results it followed that although the sum of ferulic and coumaric acids participates in the total content of phenolic compounds in individual varieties by considerably different values, namely 11.38% in the cultivar Jakub and as much as 79% in the cultivar Izak (Figure 1), there exists a high correlation between the contents of individual acids as well as their sum and the total content of phenolic compounds. Oats are a source of many phenolic compounds which can be determined with Folin-Ciocalteu reagent, including fenolic and coumaric acids. In addition to ferulic and coumaric acids, traces of vanillic, sinapic, and *p*-hydroxybenzoic acids were also identified (Peterson 2001). Another phenolic compounds occurring only in oats are avenanthramides (Peterson *et al.* 2002; Chen *et al.* 2004; Liu *et al.* 2004; Dimberg *et al.* 2005; Matillla *et al.* 2005). In oat bran 13 mg/kg avenanthramides was found (Matilla *et al.* 2005). Oat avenanthramides are bioavailable and increase the antioxidant capacity

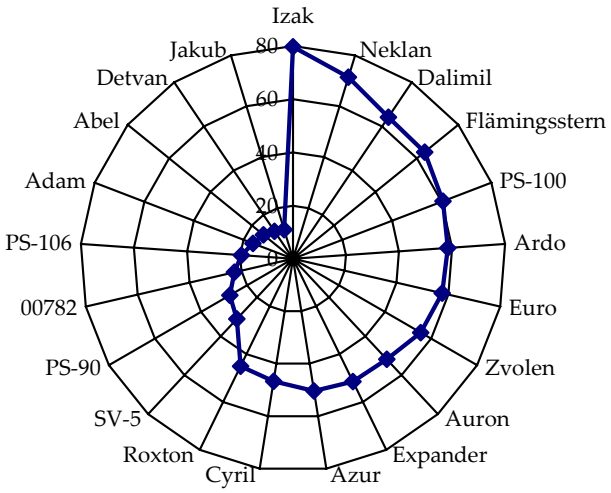

Figure 1. The percentage of the sum of ferulic and coumaric acids in the content of total phenolic compounds

in healthy older adults (Chen *et al.* 2007). The correlation coefficient *R* for the dependence of the total phenolic compounds = *f* (the sum of FA + CA) has the value 0.9211; for the total phenolic compounds = *f* (FA) has the value 0.9229, and for the total phenolic compounds = *f* (CA) has the value 0.9141. In contrast to the financially demanding and time consuming method of the high-performance liquid chromatography with the relatively high values of retention times for ferulic and coumaric acids (Andreasen *et al*. 2000a,b; Yu *et al*. 2001; Kim *et al*. 2006), the spectrophotometric method for the determination of the total phenolic compounds by means of a Folin-Ciocalteu reagent is rapid and undemanding for instrumentation. As it is obvious from the above-mentioned correlation, it can serve for a rapid evaluation of differences in the amounts of the sum of ferulic and coumaric acids contained in oat samples. This finding can be utilised in purchasing and choosing oats as raw materials for the production of foods with health-beneficial effects on human organism.

CONCLUSION

All 21 investigated oat genotypes were grown in one locality (Vígľaš-Pstruša) in the same year (2003), which minimises the influence of natural and climatic conditions on the biosynthesis of phenolic acids and proves that considerable differences in the contents of ferulic and coumaric acids as well as in the total contents of phenolic compounds are given by the genetic outfit of oats. A larger amount of ferulic acid than that of coumaric acid was found in chaffless oat genotypes with a low content of both acids (up to 50 mg/100 g grain), while in chaffy oats with the contents of both ferulic and coumaric acids above 200 mg/100 g grain coumaric acid occurs in relatively higher amounts (Table 3). Among the Slovak genotypes, the average content of ferulic and coumaric acids was detected in the cultivar Zvolen and an insignificant content of these acids was found in the cultivar Detvan.

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