JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic Acids, Syringaldehyde, and Juglone in Fruits of Different Cultivars of *Juglans regia* L.

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Phenolic acids (chlorogenic, caffeic, *p*-coumaric, ferulic, sinapic, ellagic, and syringic acid) as well as syringaldehyde and juglone were identified in ripe fruits of 10 walnut cultivars: Adams, Cisco, Chandler, Franquette, Lara, Fernor, Fernette, Alsoszentivani 117 (A-117), Rasna, and Elit. Analyses were done using a high-performance liquid chromatograph equipped with a diode array detector. Significant differences in the contents of identified phenolics were observed among cultivars. Phenolics were determined separately in the kernel and in the thin skin of the walnut, termed the pellicle. Not only in the kernel but also in the pellicle did syringic acid, juglone, and ellagic acid predominate (average values of 33.83, 11.75, and 5.90 mg/100 g of kernel; and 1003.24, 317.90, and 128.98 mg/100 g of pellicle, respectively), and the contents of ferulic and sinapic acid (average values of 0.06 and 0.05 mg/100 g of kernel and 2.93 and 2.17 mg/100 g of pellicle, respectively) were the lowest in all cultivars. The highest differences in the sum of all identified phenolics were observed between Rasna and Fernette fruits; in Rasna there were >2-fold higher contents of identified phenolics in both kernel and pellicle. It was found that the walnut pellicle is the most important source of walnut phenolics. The ratio between the contents in pellicle and kernel varied by at least 14.8-fold for caffeic acid (cv. Adams) and by up to 752.0-fold for *p*-coumaric acid (cv. Elit).

KEYWORDS: ***Juglans regia* L.; walnuts; kernel; pellicle; phenolic compounds**

INTRODUCTION

The Persian or common walnut (*Juglans regia* L.) is a very important species of deciduous trees in the family Juglandaceae found primarily in temperate areas and cultivated commercially in the United States, western South America, Asia, and central and southern Europe. It is also a widely spread species in Slovenia (*1*, *2*).

An edible seed—the kernel—forms 42−60% of the weight of the mature nut. The kernel contains ∼60% oil, in which linoleic, oleic, and linolenic acid predominate, many essential amino acids, carbohydrates, vitamins, and minerals. Other parts of the tree (wood, bark, leaves, shells, and green walnuts) are also reported to be of general use in the timber, tilery, casting, and plastics industries, in textile fabrics for dyeing, and in the pharmaceutical and cosmetic industries (*1*).

The delicate, slightly astringent flavor of walnut fruits has been associated with the presence of phenolic compounds (*1*). Phenolic compounds are a wide group of plant natural products belonging to secondary metabolites known for their important role in plants as well as in food. One of the major groups of phenolics are phenolic acids, which include hydroxycinnamic acids and hydroxybenzoic acids. Besides phenolic acids there are other well-investigated phenolic groups, such as quinones, hydroxybenzaldehydes, flavonoids, and chalcones (*3*).

The phenolic content of fruits can be influenced by many environmental factors, as well as cultural practices. Among other factors also the genotype of different cultivars is of great importance. Many differences in phenolics contents among the cultivars have been noted in several fruit species, for example, apple, pear, plum, peach, and strawberry (*4*−*8*).

In walnut, inside the fruit shell a special protective tan-brown skin known as the pellicle surrounds the kernel. This thin cover is only 5% of the fruit weight, but it is naturally rich in antioxidant phenolic compounds that help protect the kernel against oxidation (rancidity) (*9*, *10*).

Phenolic compounds from walnut fruits have a positive influence on human health such as a decrease of coronary heart diseases, prevention of several kinds of cancer, and anti-inflammatory and antimutagenic activities (*10*). Besides, walnuts are reported to be one of the most enriched dietary plants in total antioxidants, ranked at the top of the scale just after dog rose and before pomegranates (*11*). Similarly, walnuts had the highest antioxidant activity among analyzed foods and drinks commonly consumed in Turkey (*12*). Walnut extracts, containing ellagic acid monomers, polymeric tannins, and other phenolics, effectively inhibited human plasma and low-density lipoprotein (LDL) oxidation in vitro (*10*). In vivo studies

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10.1021/jf050721n CCC: $30.25
Published on Web 07/13/2005

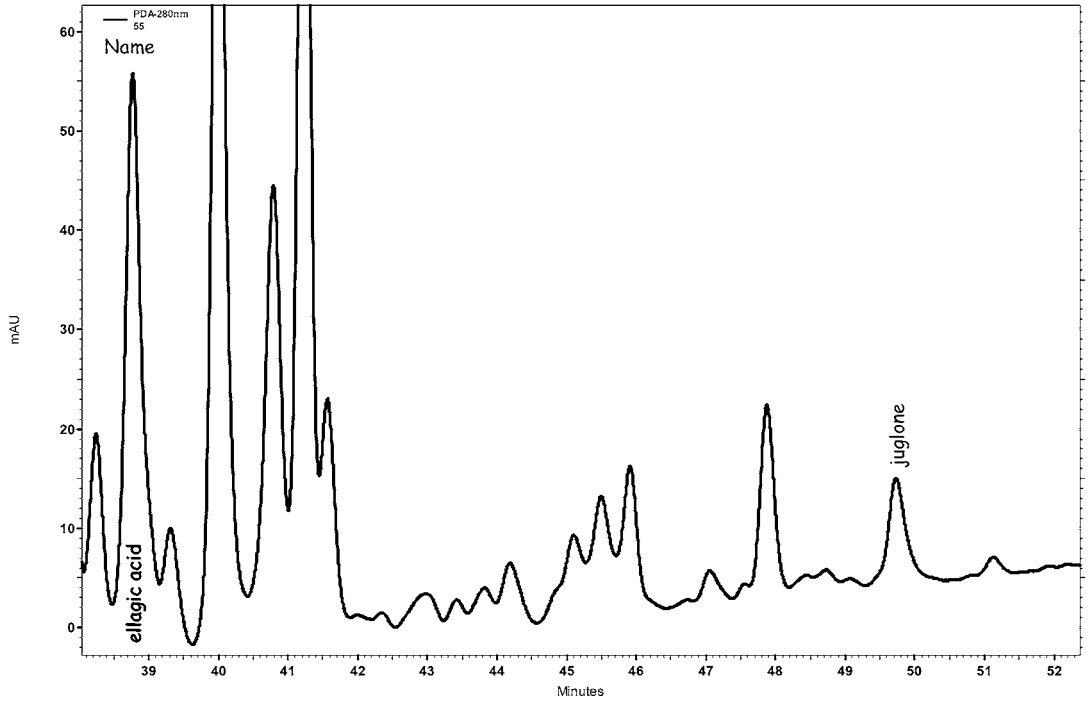

Figure 1. HPLC chromatogram of the walnut fruits of the cultivar A-117 recorded at 280 nm.

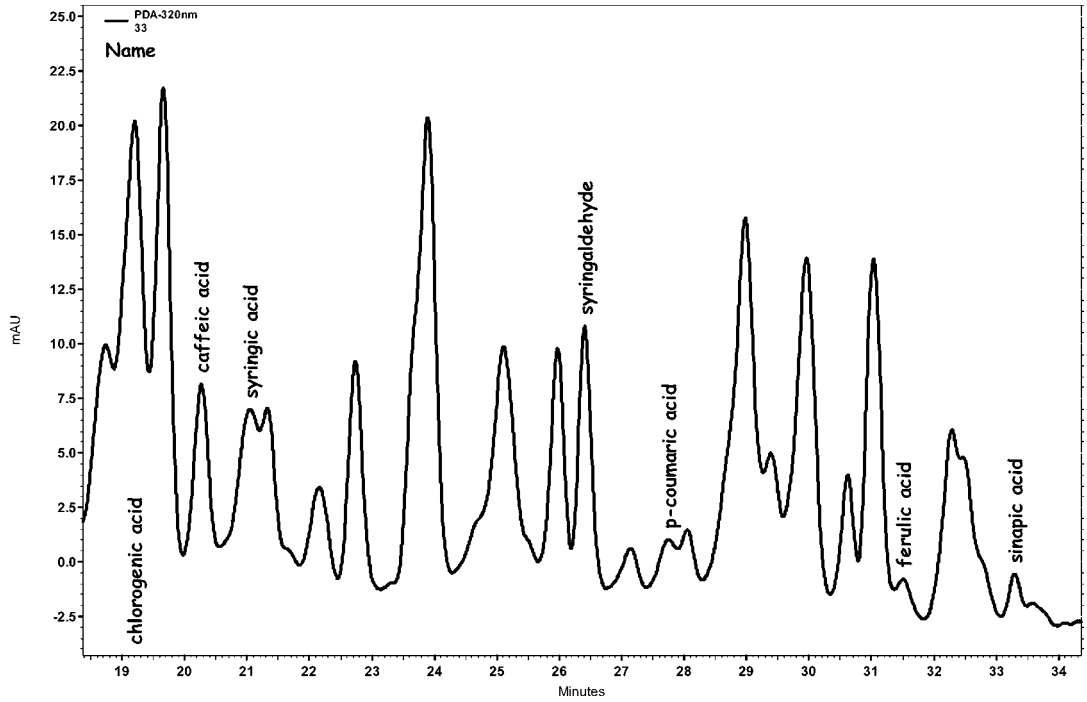

Figure 2. HPLC chromatogram of the walnut fruits of the cultivar Fernor recorded at 320 nm.

reported that daily consumption of walnuts significantly lowered total and LDL cholesterol not only in normal young men (*13*) but also in men and women with polygenic hypercholesterolemia (*14*). Already Kris-Etherton et al. (*15*) have mentioned ellagic acid and flavonoids in walnuts to have potential serum cholesterol-modulating effects. Juglone, known for its antimicrobial effect, decreased the incidence of tumors of the small intestine in rats (*16*).

Individual phenolics contents in edible walnut fruits of different cultivars have been poorly investigated, although some authors have reported on walnut phenolics (*1*, *9*, *10*, *17*). More has been done on leaves (*18*, *19*), shoots (*20*, *21*), and green walnut husks (*22*, *23*) and recently on a product from green fruits—walnut liqueur (*23*, *24*). However, quantitative data are often missing. The main focus of this study is to present the contents of identified phenolics of 10 different cultivars in walnut kernel and pellicle separately.

MATERIALS AND METHODS

Chemicals. The following standards prepared in methanol were used for the determination and quantification of phenolic compounds. Chlorogenic acid (5-caffeoylquinic acid), sinapic acid, and ellagic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) was from Merck KgaA (Darmstadt, Germany); and caffeic acid, *p*-coumaric acid, and ferulic acid were from Fluka Chemie GmbH (Buchs, Switzerland). Syringal-

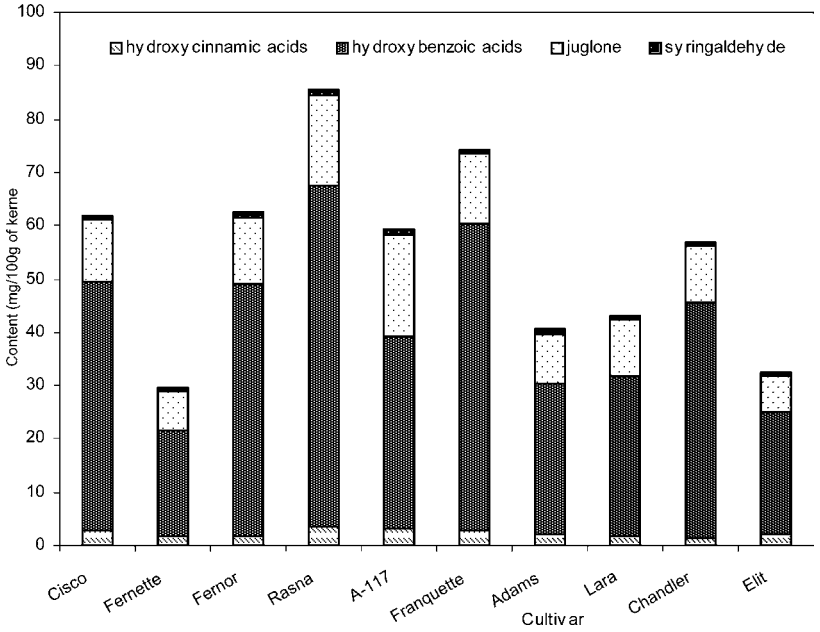

Figure 3. Identified phenolics in walnut kernel (mg/100 g of kernel) classified in groups of hydroxycinnamic (chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapic acid) and hydroxybenzoic acids (syringic and ellagic acid), as well as juglone and syringaldehyde.

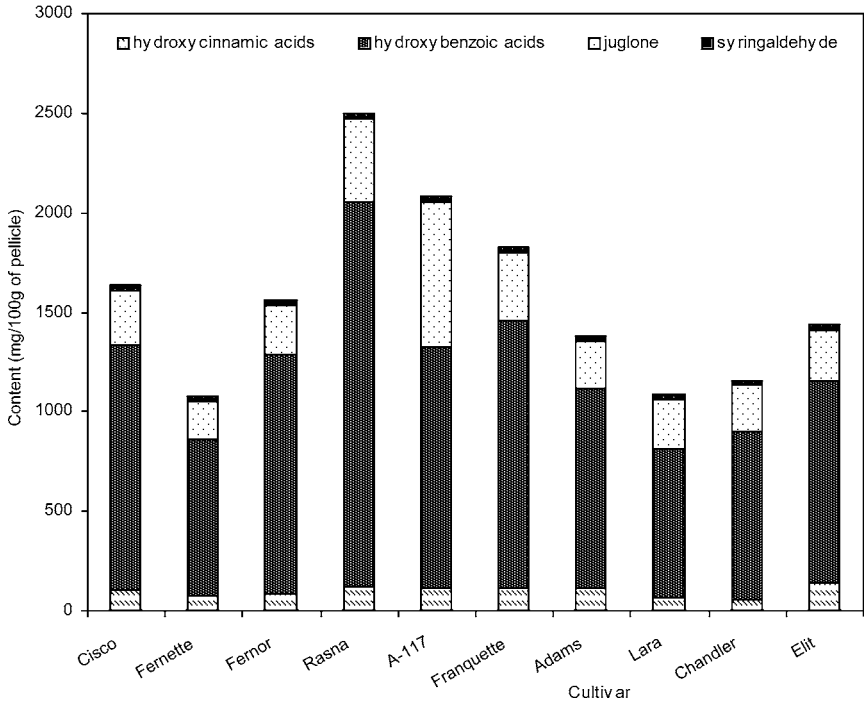

Figure 4. Identified phenolics in walnut pellicle (mg/100 g of pellicle) classified in groups of hydroxycinnamic (chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapic acid) and hydroxybenzoic acids (syringic and ellagic acid), as well as juglone and syringaldehyde.

dehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) and juglone (5-hydroxy-1,4-naphthoquinone) were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Methanol, used as an extraction solvent, and acetonitrile, used as an elutant in the HPLC system, were purchased from Merck KgaA and were of HPLC grade. Butylated hydroxytoluene [2,6-di-*tert*-butyl-4-methylphenol (BHT)], which was used as an antioxidative agent in the extraction solution, was obtained from Sigma Chemical Co. Water used for sample preparation, solutions, and analyses was bidistilled and purified in a Milli-Q water purification system from Millipore (Bedford, MA).

Plant Material. Ten different walnut cultivars, Adams, Cisco, Chandler, Franquette, Lara, Fernor, Fernette, Alsoszentivani 117 (A-117), Rasna, and Elit, were used. Ripe and dried (in forced-air dryers at 30−35 °C until 8% moisture content was achieved) healthy walnut fruits were obtained from the experimental orchard of the Biotechnical Faculty (Maribor, Slovenia) from the year 2004. They were growing under the same agricultural, geographical, and climatic conditions. Unshelled walnuts were kept in the storeroom at 4 °C until sample preparations.

Sample Preparation. Five replications for each cultivar were done. Randomly, five fruits for each replication were selected. The same walnuts were used for the phenolics analyses of pellicle and kernel. Each nut was shelled and divided into fourths; then from the same nut two fourths were carefully peeled to obtain pellicle and the other two fourths were left unpeeled (kernel).

The samples were cut into thin slices and then ground in a mortar to a fine texture, and precisely 150 mg of each sample was weighed into a test tube and extracted once with 10 mL of a methanol containing 1% of BHT in an ultrasonic bath for 40 min to obtain phenolic

Table 1. Phenolics Content[a] of Walnut Kernel in Different Cultivars

cultivar	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid	syringic acid	ellagic acid	juglone	syring-aldehyde	sum of identified phenolics
Cisco	2.57 ± 0.25 c	0.13 ± 0.01 ab	0.11 ± 0.01 abc	0.05 ± 0.00 abc	0.03 ± 0.00 ab	40.01 ± 3.13 b	6.70 ± 0.60 c	11.58 ± 0.67 bc	0.76 ± 0.04 bc	61.93 ± 4.43 cd
Fernette	1.39 ± 0.12 a	0.20 ± 0.02 bc	0.05 ± 0.00 a	0.05 ± 0.00 ab	0.03 ± 0.00 ab	16.57 ± 2.61 a	3.26 ± 0.18 a	7.27 ± 0.92 a	1.03 ± 0.11 de	29.85 ± 3.77 a
Fernor	1.25 ± 0.16 a	0.11 ± 0.01 a	0.14 ± 0.04 bc	0.05 ± 0.00 abc	0.05 ± 0.01 bc	43.40 ± 6.01 b	4.17 ± 0.35 ab	12.32 ± 1.04 bc	0.95 ± 0.09 cd	62.44 ± 7.52 cd
Rasna	2.44 ± 0.08 bc	0.54 ± 0.04 f	0.13 ± 0.01 bc	0.11 ± 0.01 e	0.14 ± 0.02 d	57.45 ± 2.96 c	6.59 ± 0.53 c	17.02 ± 1.49 d	1.16 ± 0.08 e	85.59 ± 4.47 e
A-117	2.41 ± 0.13 bc	0.40 ± 0.04 e	0.18 ± 0.01 c	0.09 ± 0.01 e	0.06 ± 0.01 c	26.29 ± 1.63 a	9.77 ± 0.75 d	19.16 ± 1.37 d	1.04 ± 0.06 de	59.42 ± 3.62 cd
Franquette	2.28 ± 0.19 bc	0.17 ± 0.02 abc	0.29 ± 0.06 d	0.08 ± 0.01 d	0.05 ± 0.00 bc	48.78 ± 7.22 bc	8.87 ± 0.91 d	12.88 ± 1.06 c	0.88 ± 0.05 bcd	74.27 ± 8.57 de
Adams	1.46 ± 0.13 a	0.29 ± 0.03 d	0.13 ± 0.02 bc	0.06 ± 0.01 bcd	0.04 ± 0.00 bc	22.83 ± 4.64 a	5.75 ± 0.60 bc	9.19 ± 1.04 ab	0.86 ± 0.05 bcd	40.61 ± 6.00 a
Lara	1.28 ± 0.18 a	0.22 ± 0.01 cd	0.11 ± 0.01 abc	0.06 ± 0.01 cd	0.05 ± 0.00 bc	25.59 ± 2.73 a	4.53 ± 0.31 ab	10.57 ± 0.99 bc	0.72 ± 0.06 b	43.13 ± 4.05 ab
Chandler	1.06 ± 0.05 a	0.12 ± 0.01 ab	0.15 ± 0.01 bc	0.04 ± 0.00 a	0.03 ± 0.00 ab	39.77 ± 1.49 b	4.30 ± 0.26 ab	10.55 ± 0.97 bc	0.84 ± 0.03 bcd	56.86 ± 2.54 bc
Elit	1.96 ± 0.20 b	0.17 ± 0.03 abc	0.07 ± 0.01 ab	0.05 ± 0.01 abc	0.02 ± 0.01 a	17.60 ± 2.41 a	5.09 ± 0.56 bc	6.93 ± 0.60 a	0.49 ± 0.05 a	32.39 ± 3.40 a

[a] Data are expressed as mean ± standard error (SE) in mg/100 g of kernel. [b] Data followed by different letters within each column are significantly different according to Duncan's multiple-range test at $P < 0.05$.

Table 2. Phenolics Content[a] of Walnut Pellicle in Different Cultivars

cultivar	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid	syringic acid	ellagic acid	juglone	syring-aldehyde	sum of identified phenolics
Cisco	55.31 ± 3.39 cd	3.74 ± 0.29 b	38.64 ± 3.95 bc	2.13 ± 0.14 bc	1.41 ± 0.17 ab	1103.73 ± 79.77 d	128.71 ± 6.73 c	279.04 ± 15.74 b	22.65 ± 2.22 ab	1635.36 ± 104.73 cd
Fernette	47.73 ± 4.15 bc	7.81 ± 0.58 c	17.73 ± 1.82 a	1.52 ± 0.12 ab	2.15 ± 0.23 cd	720.16 ± 46.26 a	60.66 ± 6.28 a	190.47 ± 7.47 a	29.58 ± 2.28 cde	1077.80 ± 56.02 a
Fernor	30.98 ± 1.58 a	1.76 ± 0.26 a	43.96 ± 4.07 c	1.69 ± 0.08 ab	2.58 ± 0.15 de	1115.25 ± 87.83 d	89.34 ± 3.80 b	251.07 ± 10.25 b	27.28 ± 1.02 cd	1563.92 ± 102.34 bc
Rasna	61.75 ± 2.04 d	10.82 ± 0.84 d	39.91 ± 2.26 bc	3.50 ± 0.16 d	4.00 ± 0.30 f	1810.90 ± 74.50 e	124.46 ± 6.05 c	414.35 ± 16.61 d	30.77 ± 0.68 de	2500.45 ± 95.49 f
A-117	58.66 ± 3.42 d	8.25 ± 0.25 c	43.18 ± 3.02 c	3.47 ± 0.12 d	1.87 ± 0.13 abc	945.04 ± 39.27 c	266.19 ± 10.98 e	727.48 ± 35.47 e	31.54 ± 1.33 de	2085.69 ± 80.20 e
Franquette	46.23 ± 2.51 b	3.13 ± 0.45 ab	55.53 ± 4.41 d	5.97 ± 0.56 f	2.75 ± 0.25 e	1146.67 ± 24.81 d	200.08 ± 3.08 d	340.56 ± 17.05 c	26.16 ± 1.31 bc	1827.07 ± 29.35 d
Adams	40.44 ± 1.63 b	4.35 ± 0.66 b	57.53 ± 2.46 d	5.15 ± 0.28 e	2.35 ± 0.14 cde	889.89 ± 18.40 bc	118.25 ± 1.86 c	236.45 ± 8.72 ab	31.16 ± 0.77 e	1386.26 ± 25.24 b
Lara	26.28 ± 1.36 a	3.58 ± 0.23 b	35.34 ± 2.10 bc	2.15 ± 0.15 bc	1.37 ± 0.08 ab	650.38 ± 34.02 a	94.03 ± 1.97 b	250.63 ± 7.68 b	25.53 ± 1.25 bc	1089.30 ± 43.22 a
Chandler	23.84 ± 1.30 a	1.84 ± 0.17 a	32.38 ± 1.44 b	1.13 ± 0.09 a	1.31 ± 0.06 a	758.97 ± 29.72 ab	78.36 ± 2.29 b	233.57 ± 10.40 ab	21.08 ± 0.66 a	1152.48 ± 40.55 a
Elit	76.25 ± 4.61 e	3.36 ± 0.50 b	53.75 ± 3.54 d	2.60 ± 0.20 c	1.92 ± 0.21 bc	891.41 ± 47.91 bc	129.73 ± 3.19 c	255.36 ± 8.64 b	27.65 ± 1.00 cde	1442.02 ± 53.19 bc

[a] Data are expressed as mean ± SE in mg/100 g of pellicle. [b] Data followed by different letters within each column are significantly different according to Duncan's multiple-range test at $P < 0.05$.

Table 3. Relationship between the Content of Individual Phenolic Compounds in the Walnut Pellicle and Walnut Kernel in Different Cultivars (Content of Phenolics in Pellicle/Content of Phenolics in Kernel)

cultivar	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid	syringic acid	ellagic acid	juglone	syring-aldehyde
Cisco	21.5	29.2	357.9	43.5	43.9	27.6	19.2	24.1	29.8
Fernette	34.3	38.2	351.2	33.3	77.4	43.5	18.6	26.2	28.8
Fernor	24.7	16.4	310.8	31.9	51.9	25.7	21.4	20.4	28.8
Rasna	25.3	19.9	308.2	33.0	28.7	31.5	18.9	24.3	26.4
A-117	24.3	20.7	234.7	37.5	29.3	35.9	27.3	38.0	30.2
Franquette	20.3	18.2	192.3	79.6	57.7	23.5	22.6	26.5	29.7
Adams	27.8	14.8	450.7	85.2	55.6	39.0	20.6	25.7	36.3
Lara	20.5	16.0	318.7	33.3	29.9	25.4	20.8	23.7	35.6
Chandler	22.6	14.9	219.3	30.1	37.9	19.1	18.2	22.1	25.0
Elit	38.9	20.0	752.0	49.2	92.9	50.6	25.5	36.8	56.2

compounds according to the method of Stampar et al. (*23*) with minor modifications. The extracted samples were centrifuged at 13000*g* (Eppendorf centrifuge 5810 R, Hamburg, Germany) for 7 min at 10 °C, and the supernatant was concentrated to dryness by a rotary evaporator system under vacuum, keeping the bath temperature under 40 °C (Rotavapor R-114 and Vacobox B-171, Büchi, Flawil, Switzerland). The dried extracts were dissolved in 2 mL of methanol and filtered through a polyamide Chromafil filter with a 45 μm pore diameter (Macherey-Nagel, Düren, Germany) into a vial and stored at −20 °C until used for HPLC analyses of phenolics contents. All samples were prepared within 2 months of collection.

Analytical Methods. To determine the phenolics an HPLC analysis was performed using a Surveyor HPLC system (Thermo Finnigan, San Jose, CA) and a diode array detector scanning the spectra of wavelength in the range from 220 to 360 nm. The system was controlled using the ChromQuest 4.0 chromatography workstation software system. Separations were carried out using a Chromsep HPLC column SS (250 × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 × 3 mm) from Chrompack (Middelburg, The Netherlands), maintained at 25 °C. The flow rate was 1 mL/min. The injection volume was 20 μL. The chromatographic conditions were as previously described by Schieber et al. (*25*) with minor modifications. Two percent acetic acid was prepared in bidistilled water as solvent A and 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1) as solvent B. The gradient used for the phenolics analysis was as follows: from 90% A to 45% A (50 min), from 45% A to 0% A (10 min), from 0% A to 90% A (5 min). The total run time was 65 min, and 15 min of equilibration treatment (90% A) between each analysis was performed.

Identification and Quantification of Phenolics. Phenolic compounds investigated and determined in fruits were chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, syringic acid, syringaldehyde, and juglone. The individual phenolic compounds were identified by comparing their UV−vis spectra with those obtained from standards in combination with retention times as well as by the addition of standard solutions. Quantification was achieved according to the concentrations of a corresponding external standard. Absorbance was monitored at 280 nm for ellagic acid and juglone (**Figure 1**) and at 320 nm for hydroxycinnamic acids, as well for syringic acid and syringaldehyde **(Figure 2**).

Statistical Analysis. Data are presented as means of five replications ± standard error (SE) (milligrams per 100 g of walnut kernel or pellicle, separately). The one-way analysis of variance (ANOVA) to test the significance of the observed differences was performed using the Statgraphics plus 4.0 program (Manugistics, Inc., Rockville, MD). The differences in the contents of phenolic compounds among cultivars were compared by Duncan's multiple-range test. P values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid (hydroxycinnamic acids) and ellagic acid and syringic acid (hydroxybenzoic acids) as well as syringaldehyde (hydroxybenzoic aldehyde) and juglone (quinone) were identified in walnut kernel and pellicle (**Figures 3** and **4**). Already Prasad (*1*) has reported that walnut kernels contained syringic, caffeic, ferulic, vanillic, gallic, protocatechuic, and phenylacetic acid in very small quantities ranging from 2 to 20 μg/100 g of kernels.

Cultivar variations in phenolics contents were observed in our study, and many statistically significant differences (Duncan's test, $P < 0.05$) were found among them in walnut kernels (**Table 1**), as well as in walnut pellicles (**Table 2**).

In Fernette fruits the lowest contents of *p*-coumaric acid and ellagic acid were found, whereas Chandler fruits were the poorest in ferulic acid in both kernel and pellicle. A-117 fruits had the highest ellagic acid and juglone contents in pellicle. In A-117 and Franquette kernels had the highest ellagic acid contents, and in A-117 and Rasna kernels had the highest juglone contents. In kernel as well in pellicle, Rasna fruits exhibited not only the highest amounts of syringic, caffeic, and sinapic acid among the studied cultivars but also the highest sum of all identified phenolics.

The analyses show that the pellicle is a much better source of phenolic compounds than the kernel (**Table 3**); the pellicle values were higher than in kernel in all cases. Although the pellicle contributes to the fruit weight only ∼5%, the phenolics contents of pellicle were so high in comparison with kernel that the results must be taken into consideration. Relationships between pellicle and kernel individual phenolic compounds varied from the lowest ratio of 14.8 for caffeic acid (cv. Adams) to the highest ratio of 752.0 for *p*-coumaric acid (cv. Elit). Among the most abundant phenolic compounds the walnut pellicle contained on average 32.7-fold higher contents of syringic acid, 26.8-fold higher contents of juglone, and 21.3-fold higher contents of ellagic acid than the walnut kernel.

The determination of phenols in peel (skin) and in seed has gained increasing importance with the recognition that these parts are often a source of various phenolic compounds in much higher concentration than the flesh. Higher phenolics contents in peel were also noted in fruit species other than walnuts, among which the most investigated are apples and pears (*4*, *6*, *26*).

Syringic acid was the most abundant phenolic of the analyzed walnut kernel and pellicle (average of all cultivars was 33.83 mg/100 g in kernel and 1003.24 mg/100 g in pellicle) followed by juglone (11.75 and 317.90 mg/100 g in kernel and pellicle, respectively) (**Tables 1** and **2)**. Green walnut husks are a much better source of juglone. Radix et al. (*27*) and Stampar et al. (*23*) reported juglone contents of green walnut husks harvested in June of up to 2200 mg/100 g of dry weight in Franquette and up to 1404 mg/100 g of dry weight in Elit. Prasad and Gülz

(*28*) reported juglone concentrated in the epicuticular wax of walnut fruit, constituting up to an enormous 29.8% of the wax.

The average ellagic acid content of all cultivars was 5.90 mg/100 g in kernel and 128.98 mg/100 g in pellicle. As early as 1956 ellagic acid in walnut pellicle was identified, but it was not quantitatively estimated (*9*). Mahoney and Molyneux (*29*) reported that ellagic acid contributes to pellicle in *Juglans regia* species up to 15.9% of dry weight and in *Juglans nigra* species only up to 2.6% of dry weight.

The average content of chlorogenic acid was 1.81 mg/100 g in kernel and 46.75 mg/100 g in pellicle. Chandler, Lara, and Fernor pellicles and Chandler, Lara, Fernor, Fernette, and Adams kernels had the lowest contents of chlorogenic acid.

The average contents of syringaldehyde were 0.87 and 27.34 mg/100 g in the kernel and pellicle and those of caffeic acid 0.24 and 4.86 mg/100 g in the kernel and pellicle, respectively. Rasna kernel and pellicle significantly differed from other cultivars in the contents of caffeic acid. The content of caffeic acid was close to that measured in the green walnut husks harvested in June (1.45 mg/100 g of dry weight) (*23*).

Together with chlorogenic, caffeic, and ferulic acid, *p*-coumaric acid was found to be among the most abundant phenolic acids in fruits of temperate zone (*26*). In our study the average content of *p*-coumaric acid was 0.14 mg/100 g in the kernel and 41.80 mg/100 g in the pellicle. The content of ferulic acid was lower, with mean values of 0.06 and 2.93 mg/100 g in the kernel and pellicle, respectively.

Among identified phenolic compounds the lowest values were observed for sinapic acid (average values of 0.05 and 2.17 mg/100 g of kernel and pellicle, respectively). The contents of ferulic and sinapic acid in pellicle were in a similar range as in green walnut husks harvested in August, in which the content of ferulic acid was 0.99 mg/100 g and that of sinapic acid 1.92 mg/100 g of dry weight (*23*).

Phenolic compounds in walnut fruits have an important antioxidative role in protecting fatty acids from oxidation (*9*). The studied compounds identified in our experiment can share a part of that role. As well, many health-beneficial effects of walnut phenolics have also been described (*10*, *15*−*17*, *30*). Much higher contents of phenolics, especially of syringic acid, juglone, and ellagic acid, were observed in the pellicle. In pellicle as well as in kernel the lowest contents of ferulic acid and sinapic acid were found among the identified phenolics, but Rice-Evans et al. (*31*) found that sinapic acid was the one with higher antioxidative activity, among other phenolic acids (ferulic, caffeic, *p*-coumaric, syringic, and vanillic acid). Moreover, the study showed differences in the sum of all identified phenolics between the cultivars. The highest differences were observed between Rasna and Fernette fruits; in Rasna there were >2-fold higher contents of identified phenolics in both kernel (the sum was 85.59 mg/100 g) and pellicle (the sum was 2500.45 mg/100 g). Gunduc and El (*12*) reported total phenolics content of up to 7052 mg/kg of walnut kernel. The cultivars with higher phenolic contents (individual or sum) may have influence on human health; however, this should be a topic of other investigations. Also, sensory evaluations of fruits with the aim to compare sensory attributes and phenolics contents of walnut fruits should be carried out.

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Received for review March 30, 2005. Revised manuscript received June 13, 2005. Accepted June 17, 2005. This work is part of the program Horticulture P4-0013-0481, granted by the Slovenian Ministry of Higher Education, Science and Technology.

JF050721N