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Phenolic Profiles of Raw Apricots, Pumpkins, and Their Purees in the Evaluation of Apricot Nectar and Jam Authenticity

Verica Dragovic-Uzelac,* Karmela Delonga, Branka Levaj, Senka Djakovic, and Jasna Pospisil

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, Zagreb 10000, Croatia

The possibility of proving the undeclared addition of pumpkin puree in apricot nectars and jams has been investigated by using the phenol compound fingerprint and sensory evaluation. The cheaper pumpkin admixtures in apricot nectars and jams could not be detected by the sensory evaluation, particularly if present in quantities of <15%. The lower admixtures of pumpkin puree in apricot nectars and jams could be detected by the presence of syringic acid, a phenolic compound characteristic of the investigated pumpkins (*Cucurbita pepo* cv. Gleisdorff and Table Gold, *Cucurbita maxima* cv. Turkinja, and *Cucurbita moschata* cv. Argenta). Syringic acid was isolated from pumpkin puree and determined by using HPLC with diode array detection. By using the phenolic profile, undeclared pumpkin admixture (\geq 5%) in the apricot nectars and jams could be proven.

KEYWORDS: Authenticity; apricot; pumpkin; phenolics; syringic acid; HPLC

INTRODUCTION

Apricot as raw material belongs to costlier fruit sorts, and in some countries as well as in Croatia, it is a deficient raw material. As fruits are the costliest ingredients in nectars and jams, potentially the most lucrative method of adulteration is their partial substitution by cheaper ingredients such as alternative fruits, vegetables, or sugars (1-3). To protect the consumer, as well as avoid unfair competition, it is important to be able to determine and check the composition of foods.

The adulteration of fruit nectars and jams by the addition of other cheaper fruits is difficult to detect because sensory evaluation often fails to reveal the mentioned frauds. Successful methods to detect these frauds include the use of amino acids, hydroxy acids, pigments (carotenoids and anthocyanins), sugars (4-7), and phenolic compounds (8-12).

Phenolic compounds are very suitable as chemotaxonomic markers, and certain of them are characteristic to some species or varieties (13), whereas quantitative differences may occur depending on fruit variety, stages of maturity, storage conditions (14-17), and the presence of the peel in fruit-based products (18, 19). For certain fruits characteristic phenolic compounds have been successfully used for the determination of adulteration of fruit nectars and jams with cheaper fruits (8, 10, 11, 20, 21).

Apricot varieties contain many phenolic compounds present in different concentrations. The hydroxycinnamic acid derivatives identified in apricot are caffeic, *p*-coumaric, and ferulic acids and their esters (22-24). Other phenolic compounds determined in apricots are neochlorogenic acid (3-caffeoylquinic

* Corresponding author (telephone + 385 1 4605 036; fax +385 1 4836 083; e-mail vdragov@pbf.hr).

acid), (+)-catechin, and (-)-epicatechin (24-26), but chlorogenic acid (5-caffeoylquinic acid) is the dominant ester in apricots (9, 24). Flavonols in apricot occur mostly as glucosides and rutinosides of quercetin and kaempferol, and quercetin 3-rutinoside (rutin) is dominant (18, 27). Coumarins such as aesculetin and scopoletin have also been identified and quantified in small amounts, and they were defined as chracteristic for apricot fruits (20, 28).

Little is known about the phenolic compounds of pumpkin varieties *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*. Small amounts of vanillic acid, *p*-coumaric acid, and sinapic acid were found in pumpkin peel (29). Flavone luteolin was found in pumpkin puree at the concentration of 16.3 mg kg⁻¹ (30). These are the only data available in the literature on the phenolic acids and flavonoids of pumpkins.

Pumpkins are among the cheapest fruits, and they are widespread, because they can grow under different climate conditions. The investigated cultivars of pumpkins have texture and color similar to those of apricot and, consequently, the apricot nectars and jams can be partially adulterated with pumpkin puree.

Therefore, the aim of this paper was to determine undeclared admixtures of pumpkin puree in apricot nectars and jams by using phenolic compound fingerprint and sensory evaluation. The phenolic profiles (qualitative and quantitative) of raw apricots, raw pumpkins, and their purees were determined, and specific phenolics in pumpkin puree were established. Another purpose was the identification of specific phenolic compounds isolated from pumpkin purees as well as the determination of their stability during processing.

MATERIALS AND METHODS

Standards and Reagents. Chlorogenic, *p*-coumaric, and syringic acid were obtained from Fluka (Neu-Ulm, Germany); (+)-catechin, (-)-epicatechin, rutin, and ferulic acid were obtained from Sigma (Deisenhofen, Germany); caffeic acid was obtained from Merck (Darmstadt, Germany). HPLC grade methanol, acetonitrile, *tert*-butylhydroquinone, and acetic acid were also obtained from Merck.

Samples. Samples of raw apricots, cv. Ananas and Madjarska Najbolja, grown in Nova Gradiska; raw pumpkins, C. pepo cv. Gleisdorff, grown in Slavonija, and cv. Table Gold, grown in Slavonija and Medjimurje, C. maxima cv. Turkinja, grown in Slavonija and Medjimurje, and C. moschata cv. Argenta, grown in Slavonija, were used. The apricot fruits were harvested during June 2003, and the pumpkins were harvested between September 15 and November 10, 2003. After harvesting, raw apricots and pumpkins were transported to the laboratory and immediately processed into puree. Edible parts of fruits (stones were removed from apricots; peel and core were removed from pumpkins) were cut into small pieces, blanched in water vapor (apricots, 5 min; pumpkins, 8 min), and homogenized in house blender (Mixy, Zepter International). Prepared purees were stored at -20 °C (apricot pure for ~ 3 months, pumpkin pure for ~ 7 days) until nectars and jams were prepared. Prepared pumpkin purees were sensory analyzed (see Sensory Analysis), and those which had neutral smell and taste (Gleisdorff and Turkinja) were chosen for further investigation. Prepared apricot and pumpkin purees were blended in proportions of 95:5, 90:10, and 85:15, respectively, and nectars and jams were prepared from those samples. Nectars and jams were also prepared from apricot purees without pumpkin puree addition. Processing of nectars was performed in the laboratory (batch size = 5.5 kg), by mixing a definite amount of fruit puree (each fruit nectar contained 40% of dry matter of fruit in total dry matter, total dry matter in fruit nectars was 12%) with sucrose and water in a closed system. The mixture was heated at 85 °C to 12% of total dry matter (~10 min) and kept under that temperature for 5 min. After that, nectars were poured into heated glass bottles (100 mL) and closed. The apricot jams were prepared by cooking a definite amount of fruit puree (batch size = 5.5kg) with sucrose until 68% of total dry matter was attained. Each jam contained 8% of dry matter of fruit in total dry matter. All nectars and jams were stored at 4 °C until their analysis.

Phenolics Extraction. The extraction method employed for the investigated samples was the method of Bengoechea et al. (19) as modified by Dragovic-Uzelac et al. (21), described as follows. Each sample (50 g of fresh fruits, fruit purees, or jams; 100 mL of apricot nectars evaporated under reduce pressure to 50 mL before extraction) was weighed into a 250 mL Erlenmeyer flask and mixed with 50 mL of methanol/HCl (100:1, v/v) containing 2% tert-butylhydroquinone. The sample was bubbled with nitrogen for 1-2 min, after which the flask was sealed tightly. Extraction was carried out in a shaking water bath at 35 °C, in the dark for 12 h. The extract was then cooled and centrifuged at 4000 rpm, and the supernatant was evaporated to dryness under reduced pressure (35-40 °C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30-40 min with anhydrous sodium sulfate, filtered through a Whatman no. 40 filter (Whatman International Ltd., Kent, U.K.), and evaporated to drynesss under reduced pressure (35-40 °C). The residue was redissolved in 5 mL of methanol/water (50:50, v/v) and filtered through a 0.45 µm membrane filter (Nylon Membranes, Supelco, Bellefonte, PA) before it was injected (20 μ L) into the HPLC apparatus. The sugars and pectins present in jams were partially removed by using the abovedescribed method of extraction. Because they did not interfere during chromatographic analysis, the same extraction method for jams as well as for nectars was applied.

HPLC Analysis of Phenolic Compounds. The analytical HPLC system employed consisted of a Varian LC Star system (Palo Alto, CA) equipped with a Star solvent delivery system 9010, a Rheodyne 7125 injector, and a Polychrom 9065 UV diode array detector. Phenolic compounds separation was done in a Nucleosil C-18 column (250 \times 4.6 mm i.d., 5 μ m) with a Nucleosil C-18 guard column (10 \times 4.6 mm i.d., 5 μ m) (Supelco, Inc.). Gradient elution was employed with a

mobile phase A consisting of 3% of acetic acid in water and mobile phase B consisting of 3% of acetic acid, 25% of acetonitrile, and 72% of water as follows: 0-40 min, from 100% A to 30% A, with flow rate = 1 mL/min; 40-45 min, from 30% A to 20% A, with flow rate = 1.2 mL/min; 45-55 min, from 20% A to 15% A, with flow rate = 1.2 mL/min; 55-57 min, from 15% A to 10% A with flow rate = 1.2 mL/min; 57-75 min 10% A with flow rate = 1.2 mL/min; 00 conditions were as follows: column temperature, 20 °C; injection volumes, 20 μ L of the standards and sample extracts; UV diode array detection at 278 nm.

Detection was performed with a UV diode array detector by scanning from 210 to 360 nm. Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards. Identified phenolic compounds were quantified using the external standard method, and quantification was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol to yield 2–30 mg L⁻¹ (syringic and ferulic acid), 5–50 mg L⁻¹ (chlorogenic acid and catechins), 5–30 mg L⁻¹ (caffeic and *p*-coumaric acid), and 2–20 mg L⁻¹ rutin. Procyanidin B₂ and kaempferol 3-rutinoside were identified only by polarity and spectral data from the literature, and they were not determined quantitatively except for kaempferol 3-rutinoside, which was quantified as quercetin 3-rutinoside. The samples were prepared and analyzed in triplicate. Data presented are mean \pm standard deviation (SD).

Analytical Quality Control. Recoveries were measured by adding known amounts of each standard $(2-25 \text{ mg L}^{-1})$ to apricot nectars or jams prior to extraction. In the calculation of final results, no correction for recovery was applied to the data. By analyzing dilution series of pure standard solutions ranging from 0.05 to 2 mg L⁻¹, minimum detectable quantities were determined for the phenolics.

Sensory Analysis. Five trained judges were selected for sensory evaluation of pumpkin purees, according to degree of acceptability for taste (maximum score = 7), smell (maximum score = 5), color (maximum score = 4), and texture (maximum score = 4). According to total score the limit of acceptability for pumpkin purees was 18.

A panel of 15 untrained assessors (simulating ordinary consumers) evaluated the nectars according to degree of acceptability for taste (maximum score = 8), smell (maximum score = 4), color (maximum score = 4), and homogeneity (maximum score = 4) and evaluated the jams for taste (maximum score = 6), smell (maximum score = 4), color (maximum score = 4), and texture (maximum score = 6). Maximum total score for nectars and jams was 20. The limit of acceptability according to total score was 17.5.

Statistical Analysis. An analysis of variance (ANOVA) was performed for each nectar or jam to obtain a statistical assessment of the influence of adding 5, 10, and 15% of pumpkin puree to apricot nectars or jams on sensory characteristics (taste, smell, color, and homogeneity or texture). Means were separated when necessary by Duncan's multiple-range test (P < 0.05; P < 0.01).

RESULTS AND DISCUSSION

Pumpkin purees investigated in this study were sensory evaluated according to degree of acceptability (results are not presented). According to total score, pumpkin purees obtained from *C. pepo* cv. Gleisdorff and *C. maxima* cv. Turkinja, were more acceptable than the other cultivars investigated. They had neutral smell and taste, whereas other pumpkin purees had more intense smell and taste. Due to the mentioned reason, only Gleisdorff and Turkinja pumpkins were chosen for addition to the prepared apricot nectars and jams. Therefore, the undeclared addition of pumpkin puree in apricot nectars and jams was proven by a parallel experiment with two different cultivars of apricot (Ananas and Madjarska Najbolja) and two different pumpkins (*C. pepo* cv. Gleisdorff and *C. maxima* cv. Turkinja) using phenolic compounds fingerprint and sensory evaluation.

Phenolic Compounds. After extraction, recoveries of single phenolic compounds were tested. A known amount of each standard was added to apricot nectars or jams, after which they



Figure 1. HPLC chromatograms of the phenolic compound extracts of raw apricot cv. Madjarska Najbolja (A) and raw pumpkin *C. maxima* cv. Turkinja grown in Slavonija (B). Peak identification: 1, chlorogenic acid; 2, caffeic acid; 3, (+)-catechin; pc1, procyanidin B2; 4, *p*-coumaric acid; 5, syringic acid; 6, (–)-epicatechin; 7, ferulic acid; 8, quercetin 3-rutinoside; 9, kaempferol 3-rutinoside.

were extracted as previously described under Materials and Methods. The recoveries were good or tolerable (71–94%) for most of the phenolic compounds studied in this work. For syringic acid, recoveries were 91% in nectars and 87% in jams. All phenolic compounds showed a linear response within the range studied of 2–50 mg L⁻¹ (r = 0.985-0.999). The following limits of detection were estimated using a signal-to-noise ratio of 4: 0.08 mg L⁻¹ by *p*-coumaric acid, chlorogenic acid, syringic acid, and (+)-catechin; 0.1 mg L⁻¹ by caffeic acid and (-)-epicatechin; and 0.15 mg L⁻¹ by ferulic acid and rutin.

Phenolic extracts obtained from the raw fruits, their purees, nectars, and jams were HPLC analyzed using a UV photodiode array detector to record the UV spectra of the separated phenolic compounds. The phenolic compositions of raw apricot cv. Madjarska Najbolja and raw pumpkin variety C. maxima cv. Turkinja grown in Slavonija are shown in Figure 1. The analyzed raw apricots of both cultivars contained many phenolic compounds, whereas raw pumpkins of C. pepo and C. maxima contained three and C. moschata four phenolic compounds. The differences in the composition of phenolic compounds among apricot cultivars Ananas and Madjarska Najbolja were not evaluated. Raw apricots of cultivars Ananas and Madjarska Najbolja (Figure 1A) were characterized by the presence of chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, (+)catechin, (-)-epicatechin, procyanidin B₂, quercetin 3-rutinoside, and kaempferol 3-rutinoside. Some of these results are in accordance with those found for other apricot cultivars (18, 24, 31). The investigated pumpkins C. pepo (cv. Gleisdorff and Table Gold), C. maxima (cv. Turkinja), and C. moschata (cv. Argenta) had a similar composition of phenolic compounds. One notable and remarkable difference between the investigated apricot fruits and pumpkins was that syringic acid was found only in pumpkins. In the investigated apricot cultivars grown in Croatia syringic acid was not observed. These data are in accordance with those found for apricot cultivars (Ananas, Madjarska Najbolja, and Velika Rana) grown in two different geographical regions of Croatia (Nova Gradiska and Opuzen),

 Table 1. Concentrations of Identified Phenolic Compounds in Raw

 Apricots Cv. Ananas (A) and Madjarska Najbolja (B) and in Their

 Purees^a

| | | raw apricots and | d apricot purees | |
|---|--|--|---|--|
| compound | apricot A | apricot B | puree A | puree B |
| chlorogenic acid caffeic acid (+)-catechin <i>p</i> -coumaric acid (-)-epicatechin ferulic acid quercetin 3- rutinoside kaempferol 3- rutinoside | $\begin{array}{c} 21.76 \pm 0.16 \\ 27.24 \pm 0.85 \\ 66.20 \pm 1.45 \\ 21.89 \pm 0.95 \\ 82.89 \pm 2.15 \\ 7.01 \pm 0.15 \\ 26.36 \pm 1.12 \\ 28.12 \pm 1.22 \end{array}$ | $\begin{array}{c} 28.01 \pm 0.27 \\ 8.11 \pm 0.15 \\ 38.20 \pm 0.29 \\ 11.39 \pm 0.25 \\ 41.94 \pm 1.51 \\ 2.96 \pm 0.17 \\ 21.46 \pm 0.42 \\ \end{array}$ | $\begin{array}{c} 20.97 \pm 0.70 \\ 14.12 \pm 0.65 \\ 20.26 \pm 1.12 \\ 15.50 \pm 0.70 \\ 45.70 \pm 3.11 \\ 2.01 \pm 0.10 \\ 21.81 \pm 0.50 \\ \end{array}$ | $\begin{array}{c} 26.85 \pm 2.31 \\ 7.90 \pm 0.35 \\ 15.78 \pm 0.67 \\ 10.58 \pm 0.73 \\ 26.37 \pm 0.27 \\ 1.98 \pm 0.19 \\ 19.85 \pm 0.61 \\ 9.18 \pm 0.26 \end{array}$ |

 a Values are means \pm SD (n = 3), and they are given as mg kg^{-1} of investigated samples.

which did not contain syringic acid (21). Data presented by other authors also confirmed the absence of syringic acid in apricot cultivars (18, 23, 24, 26). The main phenolic compounds observed in all pumpkin cultivars were chlorogenic acid and peak 5 ($t_R = 39.42$ min) identified as syringic acid. Caffeic acid was determined in traces in pumpkin cultivars Table Gold and Gleisdorff and in small amounts in cultivars Turkinja and Argenta. Only in pumpkin variety *C. moschata* cv. Argenta was a small amount of *p*-coumaric acid determined.

The phenolic compounds that have been found in raw fruits have also been identified in their purees but in lower concentrations. The amounts of identified phenolic compounds in raw fruits and their purees are shown in **Tables 1** and **2**. Flavan-3-ols were found in the highest concentrations in raw apricots of both cultivars, especially (–)-epicatechin (in Ananas, 82.89 mg kg⁻¹; and in Madjarska Najbolja, 41.94 mg kg⁻¹). The amounts obtained for raw apricot cv. Ananas were higher than those reported in the literature, whereas in cv. Madjarska Najbolja they were in accordance with the literature (26). During processing of raw apricots into their purees the concentration

Table 2. Concentrations of Identified Phenolic Compounds in Raw Pumpkins *C. pepo* Cv. Gleisdorff and Table Gold, *C. maxima* Cv. Turkinja, and *C. moschata* Cv. Argenta and in Their Purees^a

| pumpkin | | chlorogenic acid | caffeic acid | p-coumaric acid | syringic acid |
|----------------------------|--------------|---|---|---|---|
| Gleisdorff/I ^b | raw puree | $\begin{array}{c} 19.42 \pm 0.66 \\ 18.49 \pm 0.48 \end{array}$ | tr ^c tr | | $\begin{array}{c} 21.17 \pm 0.34 \\ 20.16 \pm 0.90 \end{array}$ |
| Table Gold/I ^b | raw puree | $\begin{array}{c} 15.59 \pm 1.25 \\ 13.41 \pm 1.07 \end{array}$ | tr | | $\begin{array}{c} 21.15 \pm 1.05 \\ 18.38 \pm 0.95 \end{array}$ |
| Table Gold/II ^d | raw puree | $\begin{array}{c} 20.57 \pm 1.25 \\ 17.05 \pm 1.22 \end{array}$ | tr | | 24.01 ± 1.87 22.11 ± 1.90 |
| Turkinja/I ^b | raw puree | $\begin{array}{c} 17.37 \pm 0.61 \\ 15.60 \pm 0.51 \end{array}$ | $\begin{array}{c} 1.21 \pm 0.13 \\ 1.15 \pm 0.03 \end{array}$ | | $\begin{array}{c} 19.56 \pm 0.92 \\ 18.67 \pm 0.38 \end{array}$ |
| Turkinja/II ^d | raw puree | $\begin{array}{c} 23.05 \pm 2.03 \\ 21.34 \pm 1.43 \end{array}$ | $\begin{array}{c} 1.05 \pm 0.52 \\ 0.69 \pm 0.10 \end{array}$ | | $\begin{array}{c} 20.54 \pm 1.54 \\ 18.75 \pm 1.48 \end{array}$ |
| Argenta/I ^b | raw puree | $\begin{array}{c} 21.15 \pm 1.75 \\ 19.67 \pm 1.22 \end{array}$ | $\begin{array}{c} 2.33 \pm 0.75 \\ 1.15 \pm 0.21 \end{array}$ | $\begin{array}{c} 1.27 \pm 0.31 \\ 1.05 \pm 0.15 \end{array}$ | $\begin{array}{c} 27.13 \pm 1.95 \\ 25.09 \pm 1.27 \end{array}$ |

^a Values are means \pm SD (n = 3), and they are given as mg kg⁻¹ of investigated samples. ^b I, pumpkins grown in Slavonija. ^c Traces (<0.1 mg kg⁻¹). ^d II, pumpkins grown in Medjimurje.

of (+)-catechin decreased by \sim 65% and that of (-)-epicatechin by \sim 40%. The amounts of other phenolic compounds have also decreased during the processing of apricot puree, but they were more stable than flavan-3-ols.

Raw pumpkins and pumpkin purees contained high concentrations of chlorogenic acid, ranging from 15.59 mg kg⁻¹ in cv. Table Gold to 23.05 mg kg⁻¹ in cv. Turkinja grown in Medjimurje. Syringic acid was found in high concentrations in raw pumpkins of all cultivars, especially in cv. Argenta (27.13 mg kg⁻¹), whereas caffeic acid was found in low concentration or in traces. During processing of raw pumpkins into pumpkin purees, syringic acid was decreased by $\sim 5-6\%$ in all investigated varieties. Winter pumpkins investigated in this work are hand harvested 3-4 months (from September to November) after planting in different geographical regions of Croatia. The quality, chemical composition, and phenolic composition depend on cultivar. From a geographical point of view, there were variations in the concentration of chlorogenic acid in pumpkins of the same cultivars grown in different geographical regions, whereas regional differences were not remarkably influenced by the level of syringic acid in pumpkins. Four striking characteristics of syringic acid are remarkable in the phenolic profile of pumpkins: it was found in relative high concentrations (ranging from 19.56 to 27.13 mg kg⁻¹), there were no great variations depending on region of cultivation, it was stable during the processing of raw pumpkins into their puree, and it was not found in raw apricots or their purees. Those statements are in accordance with the criteria for authenticity marker peaks (33).

The possibility of proving the undeclared admixture of pumkin puree in apricot nectars or jams using syringic acid as marker has been investigated by using apricot cultivars Ananas and Madjarska Najbolja and pumpkin cultivars Gleisdorff and Turkinja. The composition of phenolic compounds in apricot (cv. Madjarska Najbolja) nectars without and with 10% addition of pumpkin (cv. Turkinja) puree is shown in **Figure 2**. The amounts of identified phenolic compounds in apricot nectars and jams are shown in **Tables 3** and **4**. The phenolics of apricot nectars or jams were similar to raw apricot and its puree, but they were, obviously, present in lower amounts. In all of the analyzed apricot nectars and jams remarkable amounts of chlorogenic, caffeic, and *p*-coumaric acid were found, whereas ferulic acid was present only in apricot nectar and jam produced from apricot puree of cv. Ananas, which is due to the lower

amount of ferulic acid in these raw apricots and their purees. In addition, apricot nectars and jams also contained significant amounts of (+)-catechin, quercetin 3-rutinoside, and especially (-)-epicatechin, which was the main flavonoid of all the investigated nectars and jams. Syringic acid was determined in remarkable amounts in apricot nectars and jams that contained 10 and 15% of added pumpkin puree and in detectable amount in those that contained 5% of added pumpkin puree. Considering the share of dry matter of fruit (40% in nectars and 8% in jams) in total dry matter of apricot nectars or jams, it was proven that the syringic acid was stable during nectar or jam processing. Addition of 5% of pumpkin puree in apricot nectars and jams may be not profitable, but it was the lowest detectable amount. Addition of 10 and 15% of pumpkin puree could be profitable because the prices of apricot purees are remarkably higher compared to the prices of pumpkin purees.

Fruit-based products (juices, nectars, jams, fillings, and jellies) constitute an important sector of the European food industry, where a great deal of time and energy has been spent in building the Code of Pratice to help maintain quality standards. Within the European Union (EU) umbrella agency, the Association of the Industry of Juices and Nectars (AIJN) from fruits and vegetables have come together to establish reference guidelines by which the quality and authenticity of fruit juices can be judged. Fruit-based products are covered by Council Directive 93/77/EEC for fruit juices and nectars (34) and by Council Directive 79/693/EEC for fruit jams, jellies, and marmalades (35). Values that largely reflected commercial standards which have existed for a number of years in some European countries (France, Germany, The Netherlands, etc.) are the German Juice Industry guide values, known as the RSK (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen) values (36). Most EU countries have accepted the mentioned legislation (AIJN, RSK), and even countries outside the EU such as Croatia are using them. However, as the defrauding is becoming more sophisticated by using the methods presented in the mentioned legislative documents, it is often not easy to detect different types of adulteration, especially the undeclared addition of cheaper fruits. Due to the mentioned reason and in order to protect the consumer and prevent unfair competition, there is a need to develop sophisticated techniques for proving authenticity. The phenol compound fingerprint presented in this work could be a contribution to detecting undeclared cheaper material (pumpkin puree) in fruit nectars and jams.



Figure 2. HPLC chromatograms of the phenolic compound extracts of apricot (cv. Madjarska Najbolja) nectar (A) and apricot nectar with 10% addition of pumpkin (*C. maxima* cv. Turkinja) puree (B). Peak identification: 1, chlorogenic acid; 2, caffeic acid; 3, (+)-catechin; pc1, procyanidin B2; 4, *p*-coumaric acid; 5, syringic acid; 6, (–)-epicatechin; 7, ferulic acid; 8, quercetin 3-rutinoside; 9, kaempferol 3-rutinoside.

| Table 3. Concentrations of Identified Phenolic Compounds in Apricot Ne |
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| | apricot nectar ^b | | | | | | | |
|-------------------------|-----------------------------|-----------------------------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|
| compound | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 |
| chlorogenic acid | 6.84 ± 0.08 | 7.65 ± 0.15 | 8.58 ± 0.22 | 8.55 ± 0.30 | 7.67 ± 0.95 | 7.55 ± 0.86 | 7.57 ± 1.05 | 6.68 ± 0.78 |
| caffeic acid | 3.96 ± 0.05 | 3.21 ± 0.05 | 3.70 ± 0.10 | 3.73 ± 0.15 | 3.21 ± 0.75 | 3.05 ± 0.66 | 2.98 ± 0.25 | 2.48 ± 0.05 |
| (+)-catechin | 6.68 ± 0.16 | 7.85 ± 0.10 | 7.30 ± 0.50 | 7.05 ± 0.25 | 2.26 ± 0.11 | 2.15 ± 0.15 | 1.95 ± 0.03 | 1.83 ± 0.11 |
| p-coumaric acid | 5.94 ± 0.15 | 4.55 ± 0.05 | 3.42 ± 0.15 | 3.65 ± 0.11 | 4.50 ± 0.01 | 4.22 ± 0.05 | 4.31 ± 0.12 | 3.92 ± 0.02 |
| syringic acid | | 0.52 ± 0.01 | 1.05 ± 0.05 | 1.45 ± 0.15 | | 0.39 ± 0.01 | 0.88 ± 0.05 | 1.30 ± 0.05 |
| (-)-epicatechin | 12.29 ± 0.94 | 10.25 ± 0.25 | 8.90 ± 0.27 | 10.18 ± 0.35 | 6.04 ± 0.07 | 5.37 ± 0.25 | 4.12 ± 0.22 | 3.53 ± 0.10 |
| ferulic acid | 0.40 ± 0.01 | tr ^c | | | | | | |
| quercetin 3-rutinoside | 8.13 ± 0.16 | 7.22 ± 0.20 | 6.12 ± 0.25 | 5.52 ± 0.15 | 3.59 ± 0.15 | 3.63 ± 0.30 | 3.44 ± 0.25 | 1.34 ± 0.02 |
| kaempferol 3-rutinoside | 2.07 ± 0.05 | $\textbf{2.10} \pm \textbf{0.15}$ | 1.85 ± 0.05 | 1.89 ± 0.05 | 1.15 ± 0.21 | 1.20 ± 0.05 | 1.12 ± 0.02 | 1.07 ± 0.07 |

^{*a*} Values are means \pm SD (*n* = 3), and they are given as mg L⁻¹ of investigated nectars. ^{*b*} A1–A4, apricot nectars produced from apricot puree cv. Ananas and pumpkin puree *C. pepo* cv. Gleisdorff; B1–B4, apricot nectars produced from apricot puree cv. Madjarska Najbolja and pumpkin puree *C. maxima* cv. Turkinja blended in proportions as follows: A1/B1, 100% apricot puree; A2/B2, apricot/pumpkin puree (95:5, m/m); A3/B3, apricot/pumpkin puree (90:10, m/m); A4/B4, apricot/pumpkin puree (85:15, m/m). ^{*c*} Traces (<0.15 mg L⁻¹).

| | Table 4. | Concentrations | of Identified | Phenolic Com | pounds in A | pricot Jams |
|--|----------|----------------|---------------|--------------|-------------|-------------|
|--|----------|----------------|---------------|--------------|-------------|-------------|

| | apricot jam ⁶ | | | | | | | |
|-------------------------|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| compound | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 |
| chlorogenic acid | 6.53 ± 0.95 | 5.98 ± 0.25 | 4.91 ± 0.20 | 4.06 ± 0.25 | 4.84 ± 0.29 | 4.83 ± 0.33 | 5.01 ± 0.25 | 5.15 ± 0.55 |
| caffeic acid | 3.16 ± 0.75 | 1.54 ± 0.05 | 1.57 ± 0.05 | 1.51 ± 0.10 | 5.14 ± 0.35 | 4.97 ± 0.56 | 5.14 ± 0.64 | 3.97 ± 0.25 |
| (+)-catechin | 6.67 ± 0.11 | 6.46 ± 0.20 | 6.35 ± 0.15 | 6.10 ± 0.32 | 1.63 ± 0.03 | 1.55 ± 0.05 | 1.47 ± 0.05 | 1.43 ± 0.05 |
| p-coumaric acid | 3.06 ± 0.01 | 2.37 ± 0.05 | 1.87 ± 0.10 | 1.82 ± 0.05 | 5.33 ± 0.39 | 4.86 ± 0.25 | 4.42 ± 0.65 | 4.33 ± 0.30 |
| syringic acid | | 0.45 ± 0.03 | 1.01 ± 0.01 | 1.22 ± 0.01 | | 0.30 ± 0.02 | 0.65 ± 0.02 | 1.25 ± 0.02 |
| (-)-epicatechin | 13.70 ± 0.07 | 9.57 ± 0.25 | 9.29 ± 0.30 | 7.15 ± 0.25 | 7.15 ± 1.07 | 6.57 ± 0.75 | 6.52 ± 0.49 | 5.21 ± 0.47 |
| ferulic acid | 0.60 ± 0.01 | 0.30 ± 0.05 | tr ^c | | | | | |
| quercetin 3-rutinoside | 5.40 ± 0.15 | 4.86 ± 0.15 | 4.04 ± 0.25 | 4.59 ± 0.15 | 6.86 ± 0.98 | 6.15 ± 0.86 | 6.22 ± 0.39 | 6.03 ± 0.75 |
| kaempferol 3-rutinoside | 1.15 ± 0.21 | 1.21 ± 0.03 | 1.05 ± 0.22 | 0.98 ± 0.05 | 2.64 ± 0.22 | 2.66 ± 0.33 | 2.55 ± 0.20 | 2.07 ± 0.10 |

^a Values are means \pm SD (n = 3), and they are given as mg kg⁻¹ of investigated jams. ^b A1–A4, apricot jams produced from apricot puree cv. Ananas and pumpkin puree *C. pepo* cv. Gleisdorff; B1–B4, apricot jams produced from apricot puree cv. Madjarska Najbolja and pumpkin puree *C. maxima* cv. Turkinja blended in proportions as follows: A1/B1, 100% apricot puree; A2/B2, apricot/pumpkin puree (95:5, m/m); A3/B3, apricot/pumpkin puree (90:10, m/m); A4/B4, apricot/pumpkin puree (85:15, m/m). ^c Traces (<0.15 mg L⁻¹).

Sensory Evaluation. The possibility of proving the undeclared addition of pumpkin puree in apricot nectars and jams has also been investigated by using sensory evaluation. For sensory evaluation untrained assessors were selected, because of the plan to examine the possibility that ordinary consumers can detect the undeclared addition of pumpkin puree in apricot nectars and jams. The aim was also to determine the level of pumpkin puree that could be detected by sensory evaluation.

Table 5. Sensory Attribute Scores of Apricot Nectars

| sensory | | | | apricot | nectara | | | |
|--|---------------------------------------|---------------------------------------|---------------------------------------|--|---------------------------------------|---------------------------------------|---------------------------------------|--|
| attribute | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 |
| taste ^b smell ^b color homogeneity total score ^b | 6.85 3.80 3.35 3.50 17.50 | 6.95 3.75 3.35 3.70 17.75 | 6.50 3.65 4.00 3.50 17.65 | 5.17° 2.67° 3.85 3.55 15.24° | 7.65 3.90 3.75 3.80 19.10 | 7.55 3.85 3.85 3.75 19.00 | 7.35 3.55 4.00 4.00 18.90 | 5.80 ^c 2.55 ^b 4.00 3.65 16.00 ^c |

^a A, apricot nectars produced from apricot puree cv. Ananas and pumpkin puree *C. pepo* cv. Gleisdorff; B, apricot nectars produced from apricot puree cv. Madjarska Najbolja and pumpkin puree *C. maxima* cv. Turkinja blended in proportions as follows: A1/B1, 100% apricot puree; A2/B2, apricot/pumpkin puree (95:5, m/m); A3/B3, apricot/pumpkin puree (90:10, m/m); A4/B4, apricot/pumpkin puree (85:15, m/m). ^b *P* significant at 1 and 5% levels. ^c Values in the same row differ significantly according to Duncan's multiple-range test at *P* < 0.01 or *P* < 0.05.

Table 6. Sensory Attribute Scores of Apricot Jams

| sensory | | | | aprico | ot jam ^a | | | |
|--------------------------|-------|-------|-------|--------------------|---------------------|-------|-------|--------|
| attribute | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 |
| taste ^b | 5.75 | 5.95 | 5.55 | 4.83 ^c | 6.00 | 5.75 | 5.80 | 4.17 |
| smell ^b | 3.85 | 3.75 | 3.65 | 3.33 ^c | 3.90 | 3.85 | 3.55 | 2.504 |
| color | 3.83 | 3.50 | 4.00 | 3.67 | 4.00 | 3.75 | 4.00 | 4.00 |
| texture | 5.65 | 5.70 | 5.50 | 5.55 | 5.35 | 5.75 | 6.00 | 5.35 |
| total score ^b | 19.08 | 18.90 | 18.70 | 17.38 ^c | 19.25 | 19.10 | 19.35 | 16.024 |

^a A, apricot jams produced from apricot puree cv. Ananas and pumpkin puree *C. pepo* cv. Gleisdorff; B, apricot jams produced from apricot puree cv. Madjarska Najbolja and pumpkin puree *C. maxima* cv. Turkinja blended in proportions as follows: A1/B1, 100% apricot puree; A2/B2, apricot/pumpkin puree (95:5, m/m); A3/B3, apricot/pumpkin puree (90:10, m/m); A4/B4, apricot/pumpkin puree (85:15, m/m). ^b *P* significant at 1 and 5% levels. ^c Values in the same row differ significantly according to Duncan's multiple-range test at *P* < 0.01 or *P* < 0.05.

The results of sensory evaluation of apricot nectars and jams with or without definite shares of pumpkin puree admixture are shown in Tables 5 and 6. Apricot nectars produced from apricot cv. Ananas with 5 and 10% pumpkin puree admixture (cv. Gleisdorff) had higher total scores than apricot nectars without the addition of pumpkin puree. Apricot nectars produced from cv. Madjarska Najbolja and apricot nectars with 5 and 10% pumpkin puree admixture (cv. Turkinja) did not differ significantly in total score. Only the nectars or jams with 15% of pumpkin puree admixture had lower total scores (15.24, 16, 17.38, and 16.02) than was the limit of acceptability (17.5). The addition of lower proportions ($\leq 10\%$) of pumpkin puree into apricot nectars or jams did not influence single sensory characteristics such as taste, smell, color, and homogeneity/ texture, whereas the higher proportions ($\geq 15\%$) significantly influenced the mentioned characteristics, especially taste and smell. These results were confirmed by analysis of variance (ANOVA) at P < 0.01 and P < 0.05 and by Duncan's test. The ANOVA of the sensory evaluation data also confirmed that the addition of pumpkin purees (cv. Gleisdorff and Turkinja) did not affect significantly the color and homogeneity/texture of apricot nectars and jams.

Conclusion. Raw apricots and apricot purees differ significantly from raw pumpkins and pumpkin purees in their compositions of phenolic compounds. The raw pumpkins *C. pepo* cv. Gleisdorff and Table Gold, *C. maxima* cv. Turkinja, and *C. moschata* cv. Argenta contain syringic acid. This phenolic acid was found in relatively high concentration in all cultivars of pumpkin, it was stable during the processing of raw pumpkins into their purees, and it was not detected in raw apricots. The region of cultivation seems not to be an important determinative factor affecting the syringic acid level in the investigated

pumpkin cultivars. Consequently, syringic acid can be used as a suitable marker for the detection of adulteration of apricot nectars or jams with the investigated pumpkin cultivars. The sensory evaluation failed to detect low pumpkin admixtures in apricot nectars and jams ($\leq 10\%$); however, such adulteration could be detected by phenolic compound fingerprint.

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