

Analytical, Nutritional and Clinical Methods

The study of phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity

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Received 1 January 2004; received in revised form 25 August 2004; accepted 5 September 2004

Abstract

The possibility of proving the authenticity of apricot nectars and jams has been investigated by using the phenol compound fingerprint. Phenol composition of the raw material (apricots and apples), their purees, apricot nectars and jams (with and without addition the definite shares of apple puree) prepared under laboratory conditions and commercial apricot nectars and jams has been determined by high performance liquid chromatography with the UV-Diode Array detection.

In addition to large number of various phenol compounds contained in an apples, characteristic marker compounds of the dihydrochalcone group (phloretin 2'-xylosylglucoside and phloretin 2'-glucoside), could be determined. By using the mentioned compounds, undeclared apple admixture in the apricot based products could be detected. The cheaper admixture of the apple puree ($\geq 10\%$) in the apricot nectars could be proved by using phloretin 2'-glucoside and phloretin 2'-xylosylglucoside, and only by using phloretin 2'-glucoside if apple puree was added in relatively low level about 5%. The undeclared admixture of apple puree in apricot jams could be detected by both mentioned dihydrochalcones if apple puree was added in level above 20%, and only by phloretin 2'-glucoside if apple puree was added in level about 10%.

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Keywords: Apricot nectars and jams; Phenolics; HPLC; Authenticity

1. Introduction

The apricot based products (nectars and jams) are highly appreciated on the market due to their specific taste, aroma and nutritive values, which particularly refers to nectars. The apricot as raw material belongs to costlier fruit sorts, and in many countries as well as in Croatia, it is a deficient raw material. Due to the mentioned reasons there is a possibility to fabricate apricot based products in order to meet market requirements and to make profit. The three most common types of

fraud concerning fruit nectars and jams include the addition of sugar, water and substitution of the named fruit by a cheaper variety (Fuchs & Koswig, 1997; Hammond, 1997).

The adulteration of fruit nectars and jams by the addition of other cheaper fruits is difficult to detect because sensory evaluation often fails in detecting the mentioned adulteration. To detect these frauds, increasingly sophisticated analytical methodologies, based on chemical markers, need to be developed. Successful methods include use of amino acids, hydroxy acids, pigments (carotenoids and anthocyanins), sugars (Dizy, Martin-Alvarez, Cabezudo, & Polo, 1992; Krueger, Krueger, & Maciel, 1992; Lo Voi, Impembo, Fasanaro,

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& Castaldo, 1995; Van Gorsel, Li, Kerbel, Smits, & Kader, 1992; Wallrauch & Faethe, 1988) and phenolic compounds (Andrade, Carvalho, Seabra, & Ferreira, 1998; Garcia-Viguera, Zafrilla, & Tomas-Barberan, 1997; Pan, Kilmartin, Smith, & Melton, 2002; Silva et al., 2000; Tomas-Lorente, Garcia-Viguera, Ferreres, & Tomas-Barberan, 1992).

The phenolic compounds, as secondary plant metabolites, are very suitable as chemotaxonomic markers and the composition of these compounds in fruits is characteristic to each species and variety (Harborne & Turner, 1984; Perez-Ilzabre, Hernandez, & Estrella, 1991). Quantitative differences may occur depending on fruit variety, stages of maturity and environmental growth, storage conditions (Joshi, Chauhan, & Lal, 1991; Spanos & Wrolstad, 1990, 1992; Spanos, Wrolstad, & Heatherbell, 1990) and on presence of the skin in fruit based products (Bengoechea et al., 1997; Garcia-Viguera, Bridle, Ferreres, & Tomas-Barberan, 1994). They have been successfully used for determination of adulteration of some fruit nectars and jams (Andrade et al., 1998; Fernandez de Simon, Perez-Ilzabre, & Hernandez, 1992; Silva et al., 2000; Tomas-Lorente et al., 1992).

The apricot varieties contain different levels of phenolic compounds, which have been summarized by Macheix, Fleuriot, and Billot (1990). The hydroxycinnamic acid (e.g. caffeic, *p*-coumaric and ferulic acids and their esters) are the most common compound (Herrmann, 1973; Radi, Mahrouz, & Jaouad, 1997). Chlorogenic acid (5'-caffeoylquinic acid) was dominant ester in apricots (Garcia-Viguera et al., 1997; Radi et al., 1997). The other phenolic compounds determined in apricots included presence of neochlorogenic acid (3'-coumaroylquinic acid), (+)-catechin and (–)-epicatechin (Arts, van de Putte, & Hollman, 2000; Radi et al., 1997; Rish & Herrmann, 1988). Flavonols in apricots occur mostly as glucosides and rutosides of quercetin and of kaempferol, and quercetin 3-rutinoside (rutin) was dominant (Garcia-Viguera et al., 1994; Henning & Herrmann, 1980). Coumarins, aesculetin and scopoletin have also been identified and quantified in smaller level, and they are characteristic for apricot fruits (Fernandez de Simon et al., 1992; Macheix et al., 1990; Resche & Herrmann, 1981).

The phenolic composition of apples is fairly well known (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998; Guyot, Doco, Souquet, Moutounet, & Drilleau, 1997; Lee & Wrolstad, 1988; Macheix et al., 1990; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994; Oleszek, Lee, Jaworski, & Price, 1988; Spanos & Wrolstad, 1990; Spanos et al., 1990; Tomas-Barberan, Garcia-Viguera, Nieto, Ferreres, & Tomas-Lorente, 1993; Tomas-Barberan & Clifford, 2000; Tomas-Lorente et al., 1992) and marker compounds existing only in apples are dihydrochalcones, phloretin 2'-xylosylglucoside and phloretin 2'-glucoside (phloridzin).

Because there is an increasing demand of consumers for apricot products, we tried to study the phenolic profiles (qualitative and quantitative) of apricot and apple purees to determine if they could be used as a tool for the searching apple puree in authentication of apricot nectars and jams. Due to the mentioned reasons, the aim of our study was to determine the lowest level of apple puree admixture, which could be proved by characteristic marker compounds of dihydrochalcone group. To study the mentioned profiles, used methodology was modified to separate, identify and quantify several classes of phenolic compounds (phenolic acids, flavan-3-ols, dihydrochalcones, flavonol glycosides, procyanidins and related compounds).

2. Materials and methods

2.1. Standards and chemicals

Chlorogenic and *p*-coumaric acid were obtained from Fluka (Neu-Ulm, Germany); (+)-catechin, (–)-epicatechin, rutin, phloridzin 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 obtained from Merck (Darmstadt, Germany).

2.2. Samples

For experiments raw apricots cultivars “Ananas”, “Madjarska najbolja” and “Velika rana” grown in Nova Gradiska; “Madjarska najbolja” grown in Opuzen and raw apple cultivar “Idared” grown in Velika Ludina were used. After preparing fruits, (removing stone-apricots and removing core-apples) they were cut into small pieces, blanched in water vapor (apricots 5 min, apples 8 min) and homogenized in house blender (Mixy, Zepter International). Investigation of phenolic compounds was performed in all apricot cultivars, while for preparing apricot nectars and jams only the cultivar “Ananas” was used. Prepared apricot and apple purees were blended in proportions 95:5, 90:10 and 80:20 (m/m) respectively, and from those samples nectars and jams were prepared. Nectar and jam were also prepared from apricot puree without apple puree addition. Fruit nectars contained 40% and jams contained 7% of fruit in total dry matter. Total dry matter in fruit nectars was 12% and in jams 68%. All nectars and jams were stored at 4 °C until their analysis. Four different commercial apricot nectars (1, 2, 3, 4), one apricot/apple cocktail (5) and five apricot jams (1, 2, 3, 4, 5) were excluded from the local market and also studied.

2.3. Analysis of phenolic compounds

2.3.1. Phenolics extraction

The phenolic compounds in investigated samples were extracted using a modification of the procedure described by Bengoechea et al. (1997). Each sample (50 g of fresh fruits, fruit purees or jams; 100 mL of apricot nectars evaporated under vacuum to 50 mL before extraction) were mixed with 50 mL methanol/HCl (100:1, v/v) which contained 2% *tert*-butylhydroquinone, in inert atmosphere (N₂) during 12 h at 35 °C in dark. The extract was then centrifuged at 4000 rpm/min, and 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 the Whatman-40 filter (Whatman International Ltd., Kent, England), and evaporated to dryness under vacuum (35–40 °C). The residue was redissolved in 5 mL of methanol/water (50:50, v/v) and filtered through 0.45 µm filter (Nylon Membranes, Supelco, Inc. Bellefonte, PA, USA) before injected (20 µL) into the HPLC aperture. Samples were analyzed in triplicate.

2.3.2. HPLC analysis of phenolic compounds

Separation of phenolics were performed by HPLC analysis, using a Varian LC star System equipped with a Star Solvent Delivery System 9010, Injector Rheodyne 7125, Polychrom 9065 (UV-Diode Array Detector). Chromatographic separation were performed on a Nucleosil C-18 column (250 × 4.6 mm i.d., 5 µm) including Nucleosil C-18 guard column (10 × 4.6 mm i.d., 5 µm) (Supelco, Inc. Bellefonte, PA, USA). The composition of solvents and used gradient elution conditions were described previously by Bengoechea et al. (1997) with some modification.

For gradient elution mobile phase A contained 3% acetic acid in water; solution B contained mixture of 3% acetic acid, 25% acetonitrile and 72% water. The following gradient was used: 0–40 min, from 100% A to 30% A, 70% B with flow rate 1 mL/min; 40–45 min, from 30%A, 70% B to 20% A, 80% B with flow rate 1 mL/min; 45–55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 ml/min; 55–57 min, from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 mL/min; 57–75 min 10% A, 90% B with flow rate 1.2 mL/min. Operating conditions were as follows: column temperature, 20 °C, injection volume, 20 µl, UV-Diode Array detection at 278 nm.

Detection was performed with 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. Quantitative determinations were carried out

using calibration curves of the standards. Phloretin 2'-xylosylglucoside was quantified as phloridzin. Some phenolic compounds (procyanidin B2, unidentified procyanidins, and kaempferol 3-rutinoside) were identified only by polarity and spectral data from literature and they were not determined quantitatively, except kaempferol 3-rutinoside which was quantified as quercetin 3-rutinoside. Data presented are mean ± standard deviation.

3. Results and discussion

For determining the undeclared addition of apple puree in apricot nectars and jams, the phenolic compounds determined by HPLC with UV-Diode Array detection were used. Phenolic compounds in all investigated samples were determined by the extraction method and gradient conditions previously described by Bengoechea et al. (1997), which were modified in part of extraction and chromatographic analysis. For the extraction of majority of structurally different phenolic compounds present in apricots and apples, a mild solution of methanol with the presence of *tert*-butylhydroquinone (TBHQ) as antioxidant was used. The extraction was conducted in the atmosphere of inert gas nitrogen at 35 °C, through the time of 12 h in the dark space. By using described method of extraction, in investigated samples phloretin 2'-xylosylglucoside, phloretin 2'-glucoside, quercetin 3-rutinoside and kaempferol 3-rutinoside were extracted, which were not extracted in the same samples with method presented by Bengoechea et al. (1997). By using the described gradient in Section 2, better separation of phenolic compounds in the retention time from $R_t = 30$ min through $R_t = 40$ min, and from $R_t = 57$ min through $R_t = 70$ min was achieved.

3.1. Characterization of phenolic compounds in investigated samples

A characteristic HPLC chromatograms of phenolic compounds in raw apricots cultivar “Ananas” and apples cultivar “Idared” are shown in Fig. 1. Eight compounds were identified on chromatograms, matching retention data and spectral characteristic of the corresponding peaks of those of external standards.

The main phenolic acids and their derivatives identified in all investigated apricot cultivars were hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic acid) and chlorogenic acid (5'-caffeoylquinic acid). The same phenolic acids and their derivatives were also identified in raw apples, except ferulic acid, which was identified only in raw apricots. Among the hydroxycinnamic acid derivatives, chlorogenic acid was the major phenolic compound in both fruits. Flavonoids determined in both

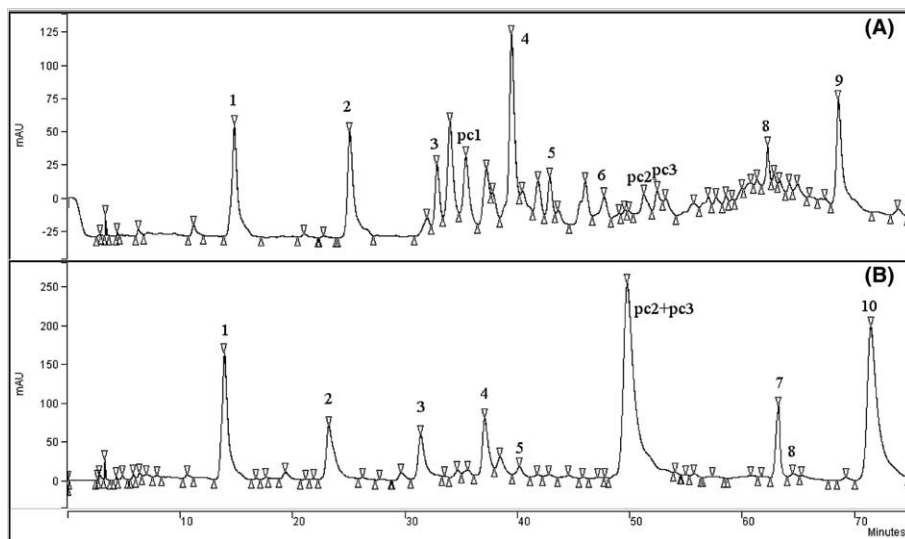


Fig. 1. High-performance liquid chromatograms of phenolic compounds in raw apricots cultivar “Ananas” (A); raw apples cultivar “Idared” (B). Chromatographic conditions: column: Nucleosil C-18, (250 × 4.6 mm i.d., 5 μm); mobile phase A, 3% acetic acid in water; mobile phase B, water/acetonitrile/acetic acid (73:25:2, v/v/v). The gradient elution are given in Section 2. Volume injected, 20 μL; equilibration time, 10 min; UV detection $\lambda_{\max} = 278$ nm. Peaks identification: 1, chlorogenic acid; 2, caffeic acid; 3, (+)-catechin; 4, *p*-coumaric acid; 5, (–)-epicatechin; 6, ferulic acid; 7, phloretin 2'-xylosylglucoside; 8, rutin; 9, kaempferol 3-rutinoside; 10, phloridzin; pc1, (+)-procyanidin B2; pc2, pc3, unidentified procyanidins.

fruits (apricots and apples) were: quercetin 3-rutinoside (rutin), (+)-catechin, (–)-epicatechin and procyanidin B2. In raw apricots a distinctive peak at $R_t = 68.55$ min was tentatively identified as kaempferol 3-rutinoside. It exhibited a kaempferol spectrum, and its elution order corresponded to kaempferol 3-rutinoside, which is present in apricot and its products (Radi et al., 1997). In raw apple a distinctive peak at $R_t = 58.23$ min (peak 7) was tentatively identified as phloretin 2'-xylosylglucoside by comparing its spectral data with those of phloretin 2'-glucoside (Fig. 2) and the elution order with those of phloretin 2'-xylosylglucoside found in different variety of apples (Guyot et al., 1997; Hernandez, Ausin, Bartolome, Estrella, & Gomez-Coroves, 1997; Suarez, Picinelli, Moreno, & Mangas,

1998; Tomas-Barberan & Clifford, 2000; Tomas-Lorente et al., 1992). Comparing retention time, UV spectra and polarity with those of (+)-catechin and (–)-epicatechin peak pc1 was identified as procyanidin B2. Peak pc1 had UV spectra (Fig. 3) similar to the spectrum of (+)-catechin or (–)-epicatechin, with absorption maximums at 228.4 and 276.3 nm. Retention time of the pc1 peak ($R_t = 35.38$ min) is different from the retention times of (+)-catechin ($R_t = 32.04$ min) and of (–)-epicatechin ($R_t = 41.99$ min), and judging by polarity and elution order, peak pc1 could present procyanidin B2. By binding flavan-3-ol monomers, what arises are polymeric procyanidins of B group, whose UV spectra very much resemble to those of flavan-3-ol, but their retention time differ due to their different polarity (Bartolome

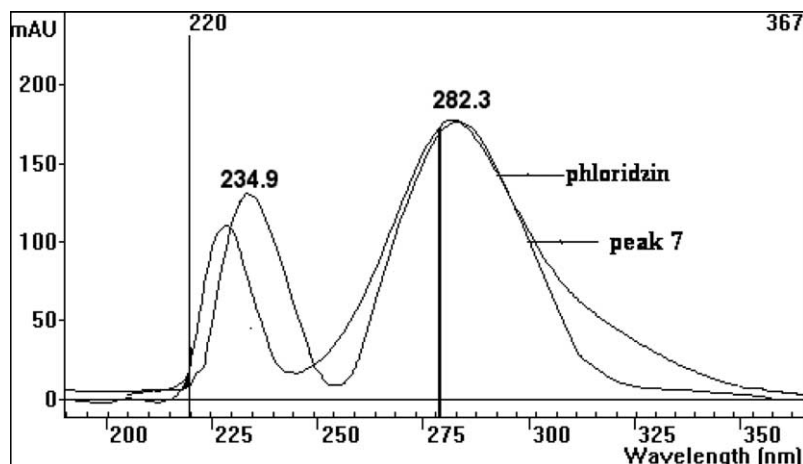


Fig. 2. Comparison of UV spectra of peak 7 separated in extract of apple and apple puree with UV spectra of phloridzin.

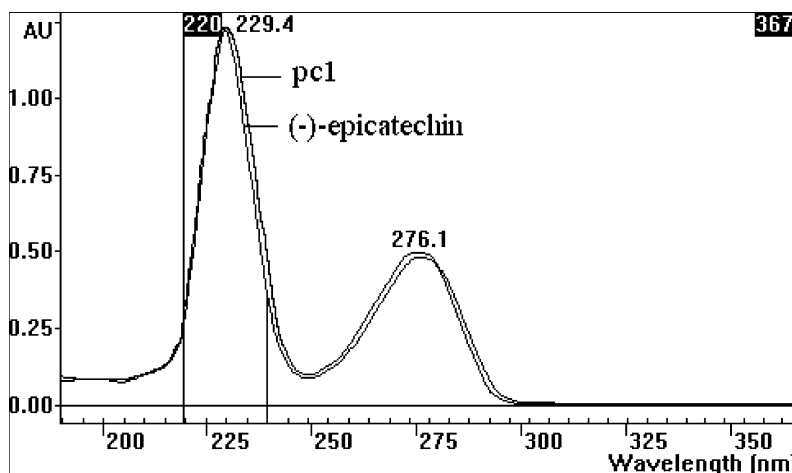


Fig. 3. Comparison of UV spectra of peak pc1 separated in extract of apricot and apricot puree, with UV spectra of (–)-epicatechin.

et al., 1996; Macheix et al., 1990). The elution order and presence of procyanidin B2 in apricots and apricot based products have also been reviewed by several authors (Bartolome et al., 1993, 1996; Merken & Beecher, 2000). Peaks pc2 ($R_t = 51.39$ min) and pc3 ($R_t = 53.67$ min) were assigned as unidentified procyanidins. The results of phenolic composition of raw apricots, apricot purees, raw apples and apple puree are in a good agreement with previously reported data for apricots and apricot based products (Dragovic-Uzelac, Delonga, & Pospisil, 2002; Garcia-Viguera et al., 1994; Henning & Herrmann, 1980; Radi et al., 1997) and for apples and apple purees (Dragovic-Uzelac et al., 2002; Guyot et al., 1997; Hernandez et al., 1997; Tomas-Barberan & Clifford, 2000).

One notable and remarkable difference between investigated apricot fruits and apples was that phloretin 2'-glucoside and phloretin 2'-xylosylglucoside were not found in three common apricot cultivars ("Ananas", "Madjarska najbolja" and "Velika rana") grown in Croatia. Dihydrochalcones (phloretin 2'-glucoside and phloretin 2'-xylosylglucoside) were identified only in apples. Garcia-Viguera et al. (1994) investigated the phenolic compounds of 11 different cultivars of Spanish apricots, and the phloretin 2'-glucoside and phloretin 2'-xylosylglucoside were not found. Furthermore, in nine apricot cultivars grown in France, chlorogenic and neochlorogenic acid, (–)-epicatechin, (+)-catechin and rutin were the main phenolic compounds, while the other phenolic compounds were not found (Radi et al., 1997). The data presented by other authors also confirmed absence of dihydrochalcones in apricot fruits (Fernandez de Simon et al., 1992; Henning & Herrmann, 1980; Macheix et al., 1990; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Tomas-Lorente et al., 1992). The same phenolic compounds which have been determined in raw fruits, have also been identified in apricot and apple purees, but in lower levels.

The levels of identified phenolic compounds in raw apricots, raw apple and in their purees prepared in laboratory conditions are shown in Tables 1 and 2. The results showed that differences in composition of phenolic compounds between investigated apricot cultivars did not exist, while their levels were remarkable different. The level of chlorogenic acid was similar in all apricot cultivars and ranged from 21.76 mg kg^{-1} in cultivar "Ananas" to 28.01 mg kg^{-1} in cultivar "Madjarska najbolja" grown in Nova Gradiska. The levels of caffeic, *p*-coumaric and ferulic acid, and especially (+)-catechin, (–)-epicatechin and kaempferol 3-rutinoside were remarkable higher in apricots cultivar "Ananas". The high levels of flavan-3-ols in apricot fruits have been reported previously (Arts et al., 2000; Rish & Herrmann, 1988). The levels of caffeic, *p*-coumaric and ferulic acid, (+)-catechin, (–)-epicatechin and kaempferol 3-rutinoside in other apricot cultivars were between 30% and 50% lower than in cultivar "Ananas". Raw apricots and apricot purees were characterized by high level of rutin and by presence of kaempferol 3-rutinoside, in agreement with previous studies (Garcia-Viguera et al., 1994; Radi et al., 1997). Additionally, kaempferol 3-rutinoside was not found in apples. From a geographical point of view, there were not great variations in the levels of phenolic compounds of apricots cultivar "Madjarska najbolja" grown in Nova Gradiska and those grown in Opuzen.

Compared with apricots cultivars studied here, raw apples cultivar "Idared" had higher level of chlorogenic acid (39.78 mg kg^{-1}), contained phloretin 2'-xylosylglucoside and phloretin 2'-glucoside, which were not found in apricots. Furthermore, the levels of caffeic and *p*-coumaric acid in raw apples were similar as well in apricots cultivar "Madjarska najbolja" from both geographical region. Among the flavan-3-ols, (–)-epicatechin was the most common compound and it was determined in the level of 50.39 mg kg^{-1} . Raw apple

Table 1

The levels of identified phenolic compounds in raw apricots cultivar “Madjarska najbolja” grown in different geographical regions (I and II), cultivar “Velika rana” and in their purees^a

Compounds	“Madjarska najbolja”/ I ^b		“Madjarska najbolja”/ II ^b		“Velika rana” ^b	
	Raw	Puree	Raw	Puree	Raw	Puree
Chlorogenic acid	28.01 ± 0.27	26.85 ± 2.31	27.20 ± 0.17	25.27 ± 1.85	23.15 ± 1.25	20.85 ± 1.10
Caffeic acid	8.11 ± 0.15	7.90 ± 0.35	9.05 ± 0.11	7.55 ± 0.40	15.19 ± 1.12	12.23 ± 0.75
(+)-Catechin	38.20 ± 0.29	15.78 ± 0.67	32.85 ± 0.30	12.50 ± 1.05	42.73 ± 2.05	15.20 ± 0.80
<i>p</i> -Coumaric acid	11.39 ± 0.25	10.58 ± 0.73	13.27 ± 0.30	11.07 ± 0.95	15.70 ± 1.30	10.15 ± 1.05
(-)-Epicatechin	41.94 ± 1.51	26.37 ± 0.27	37.22 ± 1.25	21.15 ± 1.14	56.05 ± 3.95	29.85 ± 2.45
Ferulic acid	2.96 ± 0.17	1.98 ± 0.19	3.12 ± 0.25	1.58 ± 0.25	5.11 ± 0.72	2.19 ± 0.35
Quercetin 3-rutinoside	21.46 ± 0.42	19.85 ± 0.61	19.96 ± 0.87	18.21 ± 1.12	19.82 ± 1.25	15.17 ± 1.20
Kaempferol 3-rutinoside	11.21 ± 0.39	9.18 ± 0.26	10.42 ± 0.45	9.25 ± 1.05	14.27 ± 1.17	11.27 ± 1.15

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

^b Apricot cultivars: “Madjarska najbolja”/ I and “Velika rana” – grown in Nova Gradiska; “Madjarska najbolja”/ II – grown in Opuzen.

Table 2

The levels of identified phenolic compounds in raw apricots cultivar “Ananas”, raw apples cultivar “Idared” and in their purees^a

Compounds	“Ananas” ^b		“Idared” ^b	
	Raw	Puree	Raw	Puree
Chlorogenic acid	21.76 ± 0.16	20.97 ± 1.05	39.78 ± 0.70	26.19 ± 1.15
Caffeic acid	27.24 ± 0.85	14.12 ± 1.05	13.02 ± 0.65	11.40 ± 0.55
(+)-Catechin	66.20 ± 1.45	20.26 ± 0.95	21.70 ± 1.12	20.26 ± 1.29
<i>p</i> -Coumaric acid	21.89 ± 0.95	15.50 ± 0.68	9.53 ± 0.70	7.84 ± 0.80
(-)-Epicatechin	82.89 ± 2.15	45.70 ± 2.15	50.39 ± 3.11	45.70 ± 1.65
Ferulic acid	7.01 ± 0.15	2.01 ± 0.10	–	–
Phloretin 2'-xylosylglucoside	–	–	35.54 ± 1.17	32.18 ± 1.25
Quercetin 3-rutinoside	26.36 ± 1.12	21.81 ± 1.17	4.56 ± 0.50	3.21 ± 0.15
Kaempferol 3-rutinoside	28.12 ± 1.22	22.05 ± 1.56	–	–
Phloretin 2'-glucoside	–	–	63.73 ± 3.15	55.54 ± 2.27

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

^b Apricots cultivar “Ananas” grown in Nova Gradiska; apples cultivar “Idared” grown in Velika Ludina.

cultivar “Idared” contained lower level of (+)-catechin than investigated apricot varieties. The levels of flavan-3-ols are in accordance with data presented in literature (Arts et al., 2000). During processing of raw fruits into their purees flavan-3-ols were unstable. In apricot purees the level of (+)-catechin was about 70% lower and (-)-epicatechin about 45% lower than in raw fruits, while in apple puree those compounds were more stable. The other phenolic compounds determined in raw apricots and apple were relatively stable during processing into their purees.

Among various phenolic compounds identified in apples and apple puree, the major compounds were from dihydrochalcones group (phloretin 2'-glucoside and phloretin 2'-xylosylglucoside). They were present in raw apples in the levels of 63.73 and 35.54 mg kg^{-1} , and in apple puree in the levels of 55.54 and 22.18 mg kg^{-1} . The manufacture of raw apple into apple puree had influence on decreasing these phenolic compounds, in accordance with those reported previously (Hernandez et al., 1997; Tomas-Barberan & Clifford, 2000). After blanching, the level of phloretin 2'-glucoside was 13% and of phloretin 2'-xylosylglucoside 38% lower in apple puree than in raw apples. Since dihydrochalcones

are characteristic phenolic compounds of raw apples, since they are not found in apricot fruits, and since they are present in high levels and they are relatively stable during processing of fresh fruit into puree, they were chosen as markers in proving the undeclared admixture of apple puree in the apricot based products.

The possibility of proving the undeclared addition of apple puree in apricot nectars or jams has been investigated by using the phenol compound fingerprint. Since it was confirmed that investigated apricot cultivars did not contain phloretin 2'-glucoside and phloretin 2'-xylosylglucoside and since apricot cultivar “Ananas” was the best according to sensory evaluation, it was chosen for preparing the apricot nectars and jams. The composition of phenolic compounds in apricot nectar without addition of apple puree and apricot nectar with 10% addition of apple puree are shown in Fig. 4. The major phenolic compounds were identified and quantified (Table 3). In all apricot nectars, without or with addition of apple puree, quite similar phenolic compounds as in raw apricot and apricot puree were identified. In apricot nectars with addition of 5%, 10% and 20% of apple puree, the dihydrochalcones were identified in remarkable levels. In apricot nectars with addition of 5%, 10% and

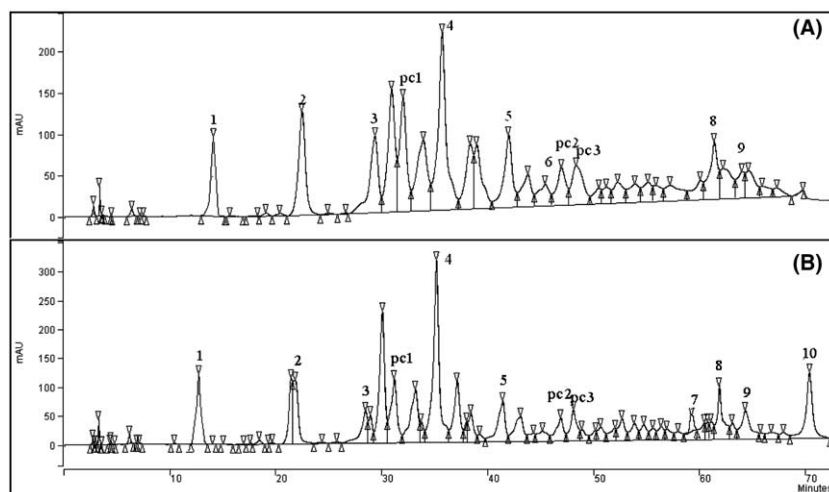


Fig. 4. High-performance liquid chromatograms of phenolic compounds in apricot nectars produced from apricot puree of cultivar “Ananas” and apple puree of cultivar “Idared”: 100% of apricot puree (A); apricot/apple puree (90:10, m/m) (B). Chromatographic conditions as on Fig. 1. Peaks identification: 1, chlorogenic acid; 2, caffeic acid; 3, (+)-catechin; 4, *p*-coumaric acid; 5, (–)-epicatechin; 6, ferulic acid; 7, phloretin 2'-xylosylglucoside; 8, rutin; 9, kaempferol 3-rutinoside; 10, phloridzin; pc1, (+)-procyanidin B2; pc2, pc3, unidentified procyanidins.

Table 3

The levels of identified phenolic compounds in apricot nectars^a

Compounds	Apricot nectars ^b			
	A	B	C	D
Chlorogenic acid	6.84 ± 0.08	6.54 ± 0.42	7.53 ± 0.17	7.80 ± 0.15
Caffeic acid	3.96 ± 0.05	2.07 ± 0.01	2.73 ± 0.02	2.39 ± 0.01
(+)-Catechin	6.68 ± 0.16	4.04 ± 0.05	6.96 ± 0.15	6.30 ± 0.17
<i>p</i> -Coumaric acid	5.94 ± 0.15	5.90 ± 0.05	3.33 ± 0.08	4.86 ± 0.09
(–)-Epicatechin	12.29 ± 0.94	4.18 ± 0.07	9.55 ± 0.10	11.18 ± 0.11
Ferulic acid	0.40 ± 0.01	–	–	–
Phloretin 2'-xylosylglucoside	–	–	1.28 ± 0.01	2.52 ± 0.01
Quercetin 3-rutinoside	8.13 ± 0.16	4.77 ± 0.06	4.40 ± 0.07	7.43 ± 0.03
Kaempferol 3-rutinoside	2.07 ± 0.05	1.15 ± 0.05	–	1.35 ± 0.01
Phloretin 2'-glucoside	–	1.05 ± 0.02	2.10 ± 0.04	4.19 ± 0.07

^a Values are means ± SD ($n = 3$), and they are given as mgL^{-1} of investigated nectars.

^b Apricot nectars produced from apricot puree of cultivar “Ananas” and apple puree of cultivar “Idared” blended in proportions as follows: A – 100% of apricot puree; B – apricot/apple puree (95:5, m/m); C – apricot/apple puree (90:10, m/m); D – apricot/apple puree (80:20, m/m).

20% apple puree phloretin 2'-glucoside was determined in the levels of 1.05, 2.10 and 4.19 mgL^{-1} . In apricot nectars with addition of 10% and 20% apple puree phloretin 2'-xylosylglucoside was determined in the levels of 1.28 and 2.52 mgL^{-1} , while in apricot nectar with addition 5% of apple puree was not determined. Concerning the share of fruit (40%) in total dry matter of apricot nectars (12%), it was proven that the dihydrochalcones were stable during processing of apricot nectars from puree. The presence of apple puree in apricot nectars could be proved through phloretin 2'-xylosylglucoside and phloretin 2'-glucoside if the admixture of apple puree is higher than 10%. If the admixture of apple puree is 5%, than the apple puree presence in apricot nectar could be proved only through phloretin 2'-glucoside. During processing apricot nectars from apricot purees with or without definite share of apple puree

kaempferol 3-rutinoside was the most unstable, and it decreased about 80%. Compared with levels of dihydrochalcones in apricot nectars, caffeic acid, *p*-coumaric acid, (+)-catechin and (–)-epicatechin were also decreased in remarkable amounts (20–60%). Chlorogenic acid was relatively stable during processing apricot nectars from fruit purees.

The composition and the levels of identified phenolic compounds in apricot jam without addition of apple puree and apricot jams with definite addition of apple puree are shown in Table 4. The composition of phenolic compounds in all apricot jams was quite similar as in apricot puree or in apricot nectars. However, separation, identification and quantification of phenolic compounds in apricot jams were more difficult than in apricot nectars, because in jams high levels of sugars and pectins were present, which agrees with previous

Table 4
The levels of identified phenolic compounds in apricot jams^a

Compounds	Apricot jams ^b			
	A	B	C	D
Chlorogenic acid	6.53 ± 0.95	5.72 ± 1.15	5.82 ± 1.74	7.25 ± 1.82
Caffeic acid	3.16 ± 0.75	1.54 ± 0.08	2.56 ± 0.09	2.28 ± 0.09
(+)-Catechin	6.67 ± 0.11	5.59 ± 0.08	6.59 ± 0.95	6.82 ± 0.84
<i>p</i> -Coumaric acid	3.06 ± 0.01	1.72 ± 0.07	2.43 ± 0.05	2.56 ± 0.05
(-)-Epicatechin	13.70 ± 0.07	0.36 ± 0.01	9.45 ± 0.17	8.32 ± 0.19
Ferulic acid	0.60 ± 0.01	–	–	–
Phloretin 2'-xylosylglucoside	–	–	tr ^c	0.21 ± 0.01
Quercetin 3-rutinoside	5.40 ± 0.15	2.91 ± 0.02	4.53 ± 0.07	3.89 ± 0.08
Kaempferol 3-rutinoside	1.15 ± 0.21	0.95 ± 0.01	–	–
Phloretin 2'-glucoside	–	–	0.28 ± 0.02	0.43 ± 0.01

^a Values are means ± SD (*n* = 3), and they are given as mgkg⁻¹ of investigated jams.

^b Apricot jams produced from apricot puree of cultivar "Ananas" and apple puree of cultivar "Idared" blended in proportions as follows: A – 100% of apricot puree; B – apricot/apple puree (95:5, m/m); C – apricot/apple puree (90:10, m/m); D – apricot/apple puree (80:20, m/m).

^c tr, traces (<0.1 mgL⁻¹).

studies (Garcia-Viguera et al., 1997; Tomas-Barberan et al., 1993). Phloretin 2'-xylosylglucoside was determined only in apricot jam with 20% addition of apple puree in the level of 0.17 mgkg⁻¹, while phloretin 2'-glucoside was determined in jams with 10% and 20% addition of apple puree in the levels of 1.78 and 2.43 mgkg⁻¹. Due to the mentioned reasons the adulteration of apricot jams with undeclared admixture of apple puree could be detected through the phloretin 2'-glucoside but only if addition of apple puree was higher than 10%, and through phloretin 2'-xylosylglucoside if addition of apple puree was higher than 20%.

Also what was examined was the possibility of using the phenolic compounds, when proving the authenticity of commercial apricot nectars and jams from the market. The composition of phenolic compounds in the commercial products (Fig. 5) was similar as in raw apricots (Fig. 1A). The levels of phenolic compounds identi-

fied in commercial apricot nectars are shown in Table 5. The analysed apricot nectars differ remarkable in the levels of phenolic compounds, and generally the levels of phenolic acids in investigated apricot nectars were higher than the levels of flavonoids in the same samples. Chlorogenic acid was the main phenolic acid determined in all commercial apricot nectars, in agreement with the findings of Garcia-Viguera et al. (1994). The caffeic and *p*-coumaric acid were also present in all investigated nectars, while ferulic acid was present only in apricot nectar 5. Among the flavan-3-ols, (+)-catechin was identified in three out of five investigated apricot nectars and (-)-epicatechin was identified only in two. Concerning the flavonols, quercetin 3-rutinoside was determined in all commercial apricot nectars in the levels ranged from 0.8 to 2.71 mgkg⁻¹, and that levels are lower than those presented by Garcia-Viguera et al. (1994). The only one apricot nectar (nectar 1) contained kaempferol 3-rutino-

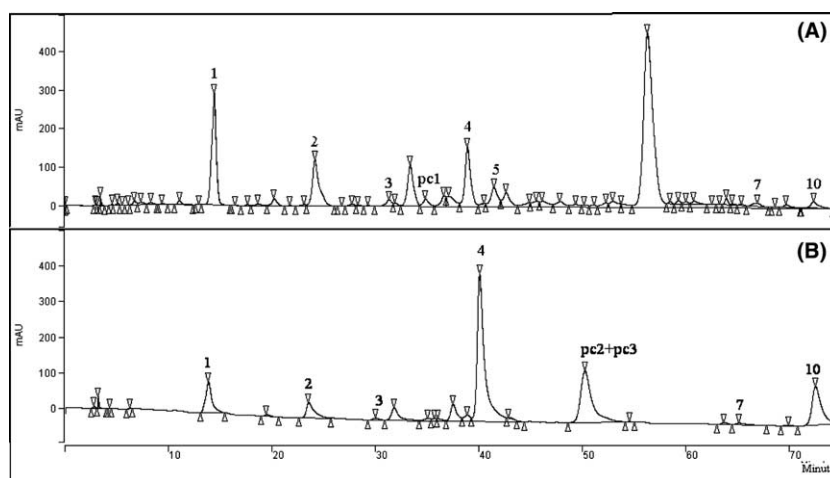


Fig. 5. High-performance liquid chromatograms of phenolic compounds in commercial apricot jams: jam 1 (A); jam 3 (B). Chromatographic conditions as on Fig. 1. Peaks identification: 1, chlorogenic acid; 2, caffeic acid; 3, (+)-catechin; 4, *p*-coumaric acid; 5, (-)-epicatechin; 6, ferulic acid; 7, phloretin 2'-xylosylglucoside; 8, rutin; 9, kaempferol 3-rutinoside; 10, phloridzin; pc1, (+)-procyanidin B2; pc2, pc3, unidentified procyanidins.

Table 5
The levels of identified phenolic compounds in commercial apricot nectars^a

Compounds	Commercial apricot nectars ^b				
	1	2	3	4	5
Chlorogenic acid	10.26 ± 1.76	12.75 ± 1.54	5.16 ± 0.35	8.86 ± 1.11	16.44 ± 1.82
Caffeic acid	2.35 ± 0.07	2.10 ± 0.05	0.65 ± 0.01	2.56 ± 0.07	0.80 ± 0.03
(+)-Catechin	2.91 ± 0.05	0.13 ± 0.01	–	2.83 ± 0.09	–
<i>p</i> -Coumaric acid	6.99 ± 0.31	2.55 ± 0.19	5.96 ± 0.07	4.44 ± 0.09	3.12 ± 0.31
(–)-Epicatechin	9.79 ± 1.05	1.51 ± 0.03	–	–	–
Ferulic acid	–	–	–	–	0.30 ± 0.05
Phloretin 2'-xylosylglucoside	–	–	–	–	–
Quercetin 3-rutinoside	2.71 ± 0.08	1.22 ± 0.07	0.80 ± 0.01	0.90 ± 0.02	1.57 ± 0.05
Kaempferol 3-rutinoside	6.95 ± 0.98	–	–	–	–
Phloretin 2'-glucoside	–	–	–	–	1.79 ± 0.04

tr, traces (<0.1 mgL⁻¹).

^a Values are means ± SD (*n* = 3), and they are given as mgL⁻¹ of investigated nectars.

^b 1–4 – apricot nectars, 5 – apricot/apple cocktail of different producers.

side in the level of 6.95 mgkg⁻¹, which was higher compared to level of kaempferol 3-rutinoside in previously studied apricot nectars (Garcia-Viguera et al., 1994). None of the apricot nectars contained the dihydrochalcones phloretin 2'-glucoside and phloretin 2'-xylosylglucoside, considered the chemical markers of apples. However, they were determined in apricot/apple cocktail. In general, the levels of major phenolic compounds separated and identified in commercial apricot nectars were lower than in apricot nectars produced in laboratory conditions.

The levels of phenolic compounds identified in commercial apricot jams are shown in Table 6. The composition of phenolic compounds in apricot jams was similar as well in apricot nectars, apricot purees or in raw apricots. Chlorogenic acid was the main phenolic compound in investigated commercial apricot jams, and ranged from 7.60 mgkg⁻¹ in jam 3 to 29.26 mgkg⁻¹ in jam 2. Caffeic and *p*-coumaric acid were present in remarkable lower level than chlorogenic acid. None of the apricot jams contained ferulic acid. Among the flav-

ononoids, flavan-3-ols ((+)-catechin and (–)-epicatechin) and flavonol glycosides (quercetin 3-rutinoside and kaempferol 3-rutinoside) were the most common compounds. Apricot jams contained (+)-catechin in the level ranged from 1.54 mgkg⁻¹ in jam 5 to 4.93 mgkg⁻¹ in jam 1, and (–)-epicatechin in the level ranged from 2.12 mgkg⁻¹ in jam 3 to 5.70 mgkg⁻¹ in jam 1. These results are in accordance with those determined in apricot jam from Netherlands (brand name “Albert Heijn”) (Arts et al., 2000). In jam 4 (–)-epicatechin was not found. The quercetin 3-rutinoside was found in all apricot jams, while kaempferol 3-rutinoside was found only in jam 5 in the levels of 4.22 mgkg⁻¹. Apricot jam 5 had the highest level of quercetin 3-rutinoside (9.7 mgkg⁻¹), which is in agreement with the findings of Garcia-Viguera et al. (1994). The levels of quercetin 3-rutinoside in other commercial apricot jams were remarkable lower than in jam 5. In two out of five apricot jams (1 and 3) phloretin 2'-glucoside was identified and in one of them also the trace of phloretin 2'-xylosylglucoside. The levels of phloretin 2'-glucoside in

Table 6
The levels of identified phenolic compounds in commercial apricot jams^a

Compounds	Commercial apricot jams ^b				
	1	2	3	4	5
Chlorogenic acid	16.61 ± 1.19	29.26 ± 1.03	7.60 ± 0.07	27.76 ± 1.15	13.94 ± 0.18
Caffeic acid	7.22 ± 0.23	3.40 ± 0.03	3.84 ± 0.04	4.86 ± 0.07	8.90 ± 0.91
(+)-Catechin	4.93 ± 0.16	1.96 ± 0.02	2.26 ± 0.02	4.13 ± 0.03	1.54 ± 0.01
<i>p</i> -Coumaric acid	5.41 ± 0.23	3.54 ± 0.04	2.93 ± 0.03	5.23 ± 0.21	8.29 ± 0.81
(–)-Epicatechin	5.70 ± 0.15	3.33 ± 0.09	2.12 ± 0.01	–	2.26 ± 0.01
Ferulic acid	–	–	–	–	–
Phloretin 2'-xylosylglucoside	–	–	tr ^c	–	–
Quercetin 3-rutinoside	1.26 ± 0.02	1.73 ± 0.03	0.86 ± 0.01	1.98 ± 0.07	9.70 ± 1.09
Kaempferol 3-rutinoside	–	–	–	–	4.22 ± 0.51
Phloretin 2'-glucoside	0.47 ± 0.01	–	1.18 ± 0.13	–	–

^a Values are means ± SD (*n* = 3), and they are given as mgkg⁻¹ of investigated jams.

^b 1–5 apricot jams of different producers.

^c tr, traces (<0.1 mgL⁻¹).

apricot jams 1 and 3 were 0.47 and 1.18 mg kg⁻¹. The phloretin 2'-xylosylglucoside was not determined in commercial apricot jams, except in apricot jams 3 in traces (<0.1 mg L⁻¹). The levels of identified phenolic compounds in commercial apricot jams were similar as in apricot jams produced in laboratory. The presence of mentioned dihydrochalcones in commercial apricot jams suggests adulteration with apples.

Upon the total score number of sensory evaluation (carried out by means of 15 group members), the difference between apricot nectars and jams and those prepared with addition of apple puree was not found. Sensory analysis of single characteristics (colour, taste, smell) did not show differences between apricot nectars or jams without addition of apple puree and apricot nectars or jams adulterated with 5 and 10% apple puree. However, addition of apple puree of 20% was detected in adulterated apricot nectars through to the lightest colour and foreign taste, and in adulterated jams through to the foreign smell and taste. (The results are not presented.)

4. Conclusions

The results showed that the HPLC profile of phenolic compounds could be used as "fingerprint" in detecting qualitative and quantitative differences in apricot purees, nectars and jams and in detecting authenticity of those products. The addition of apple puree to the apricot puree during processing of commercial nectars and jams could be easily detected by the presence of phloretin 2'-glucoside and phloretin 2'-xylosylglucoside, which are the compounds characteristic for apples. The lower admixtures of apple puree (≥5%) in apricot nectars and (≥10%) in apricot jams were proved by presence of phloretin 2'-glucoside. Phloretin 2'-xylosylglucoside was used as marker in detecting adulteration of apricot nectars, which contained ≥10% of apple puree, and apricot jams, which contained minimum 20% of apple puree. HPLC/DAD methodology for the separation of phenolic compounds may be used for the detection of the authenticity of apricot purees as semi-products and the authenticity of commercial apricot nectars and jams.

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