

Analytical, Nutritional and Clinical Methods

The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region

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Abstract

During the ripening of three apricot cultivars (“Keckemetska ruza”, “Madjarska najbolja” and “Velika rana”) grown in two different geographical region of Croatia the changes of polyphenols and carotenoids were determined by using high-performance liquid chromatography (HPLC) with UV–Vis photo diode array detection. The content of individual polyphenols during ripening was quite similar, whereas their amount differed significantly. Immature fruits showed the highest level of polyphenols, which decreased at semi-mature fruits and did not change remarkably in commercial mature fruits. Among polyphenols, flavan-3-ols, chlorogenic acid and quercetin-3-rutinoside were dominant in all ripening stages of all apricot cultivars. The quantity of polyphenols during apricot fruits ripening depended on cultivars, while the region of cultivation did not have remarkable influence on polyphenols amount. During ripening carotenoids increased significantly especially β -carotene which represented 70–85% of the total carotenoid content. Besides β -carotene, in all apricot cultivars γ -carotene was found. α -Carotene, zeaxanthin and lutein were found in cv. “Madjarska najbolja” and in cv. “Velika rana” α -carotene and lutein were determined. The region of cultivation and the cultivar were important factors, which influenced the carotenoid amount of apricot fruits, and this content was higher in cultivars grown in Mediterranean region.

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1. Introduction

Apricot fruits contain different level of phytochemicals such as vitamins, carotenoids and polyphenols, which contribute significantly to their taste, colour and nutritive values. There is a considerable interest in polyphenols and carotenoids because of their antioxidant properties and ability to alleviate chronic diseases (Gardner, White, McPhail, & Duthie, 2000; Rice-Evans, Miller, & Paganga, 1997; Vinson, Hao, Su, & Zubik, 1998). The carotenoids are the most widespread group of pigments in nature, and they are present in all photosynthetic organisms and are responsible for most of yellow to red colours of fruits and flowers (Bramley, 2003). Ripening of the fruits involves series of complex biochemical reactions which lead to production

of phenolic compounds, carotenoids and the formation of volatile compounds (Speirs & Brady, 1991). The differences in content and quantities of previously mentioned phytochemicals may occur but this depends on a number factors, sunlight, soils, season, region of cultivation, fruit variety, stages of maturity (Harris, 1977; Joshi, Chauhan, & Lal, 1991; Spanos & Wrolstad, 1990, 1992; Spanos, Wrolstad, & Heatherbell, 1990).

The apricot varieties contain different levels of polyphenols, which have been summarized by Macheix, Fleuriet, and Billot (1990). Chlorogenic acid (5-caffeoylquinic acid) is the dominant phenolic compound in apricots. The other phenolic compounds determined in apricots are: neochlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and their esters. (+)-Catechin and (–)-epicatechin are also determined in apricot fruits and their products (Arts, van de Putte, & Hollman, 2000; Dragovic-Uzelac, Delonga, Levaj, Djakovic, & Pospisil, 2005; Dragovic-Uzelac, Pospisil,

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Levaj, & Delonga, 2005; Garcia-Viguera, Zafrilla, & Tomas-Barberan, 1997; Herrmann, 1973; Radi, Mahrouz, & Jaouad, 1997; Rish & Herrmann, 1988). Flavonols in apricots occur mostly as glucosides and rutosides of quercetin and of kaempferol, however, quercetin 3-rutinoside (rutin) predominates (Dragovic-Uzelac, Delonga et al., 2005; Dragovic-Uzelac, Pospisil et al., 2005; Garcia-Viguera, Bridle, Ferreres, & Tomas-Barberan, 1994; Henning & Herrmann, 1980). Aesculetin and scopoletin have also been determined in lower amounts in some apricot cultivars (Fernandez de Simon, Perez-Ilzabre, & Hernandez, 1992; Macheix et al., 1990; Resche & Herrmann, 1981). Only few reports described the biochemical changes in apricot fruits at different ripening stages. Soluble and insoluble proteins were decreased during ripening apricot fruits, free amino acids varied according to the stages of maturity, while total and soluble carbohydrates increased (Sharaf, Ahmed, & El-Saadany, 1989). Differences in amounts of chlorogenic acid, kaempferol-3-rutinoside and quercetin-3-rutinoside were observed in 11 apricot fruits varieties in three stages of maturity (Garcia-Viguera et al., 1994).

Apricot fruits are regarded as a rich source of carotenoids, especially β -carotene which represents more than 50% of total carotenoid content (Radi et al., 1997; Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005). Besides β -carotene, apricot fruit and its products contain smaller amounts of α -carotene, γ -carotene, zeaxanthin and lutein (Fraser & Bramley, 2004). The data about carotenoid changes during apricot fruits ripening are not fully reported. In apricot fruits ripening is accompanied by enhanced biosynthesis of carotenoids (Katayama, Nakayama, Lee, & Chichester, 1971). The β -carotene level was determined to be significantly different, among varieties and among different regions within the same variety (Munzuroglu, Karatas, & Geckil, 2003). Research on other plant species indicated that significant changes in carotenoids amount occur according to the stage of maturity. During tomato fruit ripening there is a remarkable accumulation of carotenoids of which lycopene represents about 90% of the total (Fraser, Truesdale, Bird, Schuch, & Bramley, 1994). In acerola fruits (*Malpighia emarginata* D.C.) carotenoid content was higher in mature fruits (Lima et al., 2005).

There are not enough reports about influence of different geographical region of cultivation and ripening stages on polyphenol and carotenoid contents in apricot fruits. The objective of this work was to establish differences in content and amount of polyphenols and carotenoids in three apricot cultivars with depend on the ripening stages and geographical region of cultivation in Croatia.

2. Materials and methods

2.1. Standards and chemicals

Gallic, chlorogenic, and *p*-coumaric acid were obtained from Fluka (Neu-Ulm, Germany); (+)-catechin, (–)-epi-

catechin, quercetin-3-rutinoside, quercetin-3-glucoside, ferulic acid, α -carotene, β -carotene and lutein were obtained from Sigma (Deisenhofen, Germany); caffeic acid was obtained from Merck (Darmstadt, Germany). HPLC grade methanol, acetonitrile, acetone, hexane, acetic acid, 1,2-dichloroethane, *tert*butylhydroquinone (*t*BHQ) and butylhydroxytoluene (BHT) were obtained from Merck (Darmstadt, Germany).

2.2. Samples

Three apricot (*Prunus armeniaca* L.) cultivars known under local name “Keckemetska ruza”, “Madjarska najbolja” and “Velika rana” were harvested (during May, June and July 2004) in two geographical regions of Croatia (continental region: Baranja and Mediterranean region: Neretva valley). Raw apricot fruits, at three different stages of maturity (immature, IM; semi-mature, SM; and commercial mature, CM) were picked, covered with dark polyethylene foil, transported in laboratory and immediately frozen at -25°C until their analysis. The maturity of apricot fruit was determined according to firmness and skin colour. The penetrometer firmness ($F_{\text{pen.}}$) was measured by using hand-held penetrometer (Effegi, Italy) with a 8 mm diameter plunger. Three measurements were made on opposite side of fruits (10 apricot fruits) and results were expressed as average. Penetrometer measurements are reported in Newtons (N) with 10 N being approximately 1 kgf (standard industry units of kilogram-force). The skin colour was determined visually as follows: immature, green with small area of yellow colour; semi-mature, slightly yellow to orange colour; and commercial mature, intensive orange with small area of red colour. For analysis fruits were partially defrost, stone was removed, apricots (with skin) were cut into small pieces, and processing of purees were performed in the laboratory (batch size 1.5 kg) by using house blender (Mixy, Zepter International). Prepared purees immediately were used for determination of polyphenols and carotenoids.

2.3. Analysis of polyphenols

2.3.1. Extraction

The polyphenols in examined samples were extracted using a procedure described by Dragovic-Uzelac, Delonga et al. (2005, 2005) based on the method of Bengoechea et al. (1997). Each apricot fruit puree (50 g) was mixed with 50 mL methanol/HCl (100:1, v/v) which contained 2% *t*BHQ, in inert atmosphere (N_2) during 12 h at 35°C in dark. The extract was then centrifuged at 4000 rpm min^{-1} , and supernatant was evaporated to dryness under reduced pressure ($35\text{--}40^{\circ}\text{C}$). The residue was dissolved 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 sulphate, filtered through the Whatman No. 40 filter (Whatman International Ltd., Kent, England), and evaporated

to dryness under vacuum (35–40 °C). The residue was dissolved in 1 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 apparatus. Samples were extracted in triplicate.

2.3.2. HPLC analysis

Separation of polyphenols was performed by HPLC analysis, using a Varian ProStar System equipped with a ProStar Solvent Delivery Module 230, Injector Rheodyne 7125, ProStar 330 UV–Vis photo diode array detector. Chromatographic separations were performed on a Pinnacle II C-18 column (250 × 4.6 mm i.d., 5 µm) including Pinnacle C-18 guard column (10 × 4 mm i.d., 5 µm) (Restek, Bellefonte, USA). The content of solvents and used gradient elution conditions were previously described by Dragovic-Uzelac, Delonga et al. (2005, 2005). 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⁻¹; 40–45 min, from 30% A, 70% B to 20% A, 80% B with flow rate 1 mL min⁻¹; 45–55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 mL min⁻¹; 55–57 min, from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 mL min⁻¹; and 57–75 min 10% A, 90% B with flow rate 1.2 mL min⁻¹. Operating conditions were as follows: column temperature, 20 °C, injection volume, 20 µL, UV–Vis photo diode array detection at 278 nm.

Detection was performed with UV–Vis photo diode array detector by scanning spectra 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. Some phenolic compounds (procyanidin B1, procyanidin B2, procyanidin B3, quercetin-3-galactoside and kaempferol 3-rutinoside) were identified only by polarity and spectral data from literature. Procyanidins B1 and B3 were quantified as (+)-catechin and procyanidin B2 as (–)-epicatechin. Quercetin-3-galactoside was expressed as quercetin-3-glucoside and kaempferol-3-rutinoside was quantified as quercetin-3-rutinoside. Data presented are mean ± standard deviation.

2.3.3. Analytical quality control

The validation of method was previously described by Dragovic-Uzelac, Pospisil et al. (2005). Recoveries were measured by adding known amounts of each standard (2–25 mg L⁻¹) to apricot puree prior to extraction. In the calculation of final results, no correction for recovery was applied to the data. By analysing dilution series of pure standards solutions ranged from 0.05 to 2 mg L⁻¹, minimum detectable quantities were determined for the phenolics. The limit of detection was estimated using a signal to noise ratio of 4:0.08 mg L⁻¹ by *p*-coumaric acid, chlorogenic acid and (+)-catechin, 0.1 mg L⁻¹ by caffeic acid

and (–)-epicatechin and 0.15 mg L⁻¹ by ferulic acid and rutin.

2.4. Analysis of carotenoids

2.4.1. Extraction

The extraction method employed for examined samples were the method of Radi et al. (1997) modified as described in the following: apricot sample (10 g) was homogenized with acetone (2 × 20 mL) containing 0.1% BHT. Extract was filtered through Whatman No. 40 (Whatman International Ltd., Kent, England) by using Büchner funnel and vacuum. After filtration carotenoids were transferred to 30 mL hexane (containing 0.05% BHT). The hexane was washed with water (10 mL) three times to eliminate acetone. Hexan fraction was dried with anhydrous sodium sulphate, filtered through Whatman No. 40 (Whatman International Ltd., Kent, England), and evaporated to dryness under vacuum (25–30 °C). The residue was dissolved in 1 mL of mobile phase (acetonitrile:methanol: 1,2-dichloroethane, 60:35:5, v/v/v) which contained 0.1% BHT and filtered through 0.45 µm filter (Nylon Membranes, Supelco Inc., Bellefonte, PA, USA) before being injected (20 µL) into the HPLC apparatus. Samples were analyzed in triplicate.

2.4.2. HPLC analysis

The HPLC analysis was carried out on the same equipment as well as polyphenols. Samples (20 µL) containing carotenoids were injected onto Pinnacle C-18 column (250 × 4.6 mm i.d., 5 µm) protected by a guard column Pinnacle C-18 (10 × 4.6 mm i.d., 5 µm) (Restek, Bellefonte, USA) column. The separation of carotenoids was based on the procedure previously described by Pupin, Dennis, and Toledo (1999) with some modification. For analysis of carotenoids, isocratic elution at flow rate 1 mL min⁻¹ with acetonitrile:methanol: 1,2-dichloroethane in the ratio (60:35:5, v/v/v) which contained BHT (0.1%) and ammonium acetate (1%, w/v) was used. Operating conditions were as follows: column temperature, 25 °C, injection volume, 20 µL, UV–Vis diode array detection at 450 nm.

Detection was performed with UV–Diode Array Detector by scanning from 360 to 550 nm. Identification of carotenoids was carried out by comparing retention times and spectral data with those of authentic standards. Quantitative determinations were carried out by using calibration curves of the standards. Zeaxantin and γ-carotene were identified by polarity and spectral data from literature. Zeaxantin was quantified as lutein and γ-carotene as β-carotene. Data presented are mean value ± standard deviation.

2.4.3. Analytical quality control

Recoveries for carotenoids were measured by adding known amounts of each standard (0.05–15 µg g⁻¹) to apricot puree prior to extraction. For lutein recovery was 86.56%, α-carotene 97.15% and for β-carotene 96.45%.

All carotenoids showed a linear response in the following ranges: 1–15 $\mu\text{g g}^{-1}$ ($n = 5$; $r = 0.974$) for lutein, 1.5–25 $\mu\text{g g}^{-1}$ ($n = 5$; $r = 0.988$) for α -carotene and 2.5–65 $\mu\text{g g}^{-1}$ ($n = 5$; $r = 0.986$) for β -carotene. In the calculation of final results, no correction for recovery was applied to the data. By analysing dilution series of pure standards solutions ranged from 0.01 to 2 $\mu\text{g g}^{-1}$, minimum detectable quantities were determined for the carotenoids. The following limits of detection was estimated using a signal to noise ratio of 4:0.07 $\mu\text{g g}^{-1}$ by lutein, and 0.055 $\mu\text{g g}^{-1}$ by α -carotene and 0.035 $\mu\text{g g}^{-1}$ by β -carotene.

3. Results and discussion

In all cases, the maturity of apricot fruit was determined on the day of harvesting (all cultivars were harvested at the same day in the Mediterranean region, and 1 day after in continental region), according to firmness and skin colour as described in Section 2. The skin colour was determined visually (data were not presented). Values for the apricot firmness ($F_{\text{pen.}}$) are presented in Table 1. The changes in firmness were associated with the stage of maturity. In immature (IM) apricot fruits, cultivar “Keckemetska ruza” (KR) the maximum firmness ($F_{\text{pen.}}$) was observed as 63.5 N and decreased to 24.5 N in semi-mature fruits (SM) and to 10.5 N in commercial mature (CM) fruits. In cultivar “Madjarska najbolja” (MN) $F_{\text{pen.}}$ ranged from 58 N in IM to 8.5 N in CM fruits and in cv. “Velika rana”(VR) from 61.5 N in IM to 6.75 N in CM fruits. The firmness of same cultivar harvested on different geographical region was similar. Polyphenol and carotenoid extracts obtained from apricot fruits harvested at three ripening stages (cultivars “KR”, “MN” and “VR”) and grown in two geographical regions in Croatia (Baranja and Neretva valley) were HPLC analysed. Detection of the separated compounds by using an UV–Vis photo diode array detector was done.

3.1. Changes of polyphenols during ripening

The polyphenols content of apricot cultivar “KR” harvested at three ripening stages (immature, IM; semi-mature, SM; and commercial mature, CM) grown in Ner-

etva valley is shown in Fig. 1. The analysed apricot cultivars contained a lot of phenolic compounds but no remarkable differences were determined in the content of polyphenols among examined apricot cultivars. During ripening the content of polyphenols did not change remarkably, but the differences exist in their amounts. Phenolic acids and their derivatives determined in all samples examined were: gallic acid, chlorogenic acid, neochlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid, which are partially in accordance with previously presented data obtained in cultivars “Madjarska najbolja”, “Velika rana” and “Ananas” harvested at commercial mature stage (Dragovic-Uzelac, Delonga et al., 2005; Dragovic-Uzelac, Pospisil et al., 2005). Among flavonoids in examined apricots the following flavonoids were found: (+)-catechin, (–)-epicatechin, quercetin-3-rutinoside and kaempferol-3-rutinoside in accordance with those found previously in different apricot cultivars (Dragovic-Uzelac, Delonga et al., 2005; Dragovic-Uzelac, Pospisil et al., 2005; Garcia-Viguera et al., 1994; Radi et al., 1997). Additionally, quercetin-3-glucoside, quercetin-3-galactoside, procyanidin B1, procyanidin B2 and procyanidin B3 in the same samples were also found. Quantitative differences in polyphenol amounts in apricot cultivars at three ripening stages have been observed, and the amounts of identified polyphenols are shown in Tables 2–4. The gallic acid amount in apricot fruits from all cultivars decreased during ripening and in cultivars “MN” and “VR” at commercial mature only in traces was determined. The changes of hydroxycinnamic acids (HCA) amount in apricot fruits from all cultivars showed the same trend during ripening, with the highest values at first ripening stage (immature) and the lowest values at commercial mature stage. The decrease of HCA amounts is a well-known phenomenon during ripening (Macheix et al., 1990). The major HCA derivative in all examined apricot fruits was chlorogenic acid and it was determined in the amount of 18.87 mg kg^{-1} (IM), 16.05 mg kg^{-1} (SM) and 14.69 mg kg^{-1} (CM) in apricot cultivar “KR” grown in Baranja. In the same cultivar grown in Neretva valley its amounts were higher. In apricot cultivar “MN” grown in Baranja, chlorogenic acid decreased from immature stage to semi-mature stage and it has not changed significantly at commercial mature

Table 1

Firmness determined in apricot cultivars “Keckemetska ruza” “Madjarska najbolja” and “Velika rana” harvested in three stages of maturity in two geographical region of Croatia^a

Cultivar	Geographical region	Stages of maturity		
		IM	SM	CM
Keckemetska ruza	Baranja	63.50 ± 0.75	24.50 ± 1.05	10.50 ± 0.55
	Neretva valley	61.25 ± 1.18	25.50 ± 0.75	11.25 ± 0.85
Madjarska najbolja	Baranja	56.50 ± 1.75	18.00 ± 0.86	8.50 ± 0.75
	Neretva valley	58.00 ± 0.87	20.25 ± 0.73	9.25 ± 0.67
Velika rana	Baranja	59.75 ± 1.25	20.75 ± 0.25	7.50 ± 0.15
	Neretva valley	61.50 ± 1.05	22.25 ± 0.55	6.75 ± 0.25

IM, immature; SM, semi-mature; and CM, commercial mature.

^a Values are means ± SD ($n = 3$), and they are given as N.

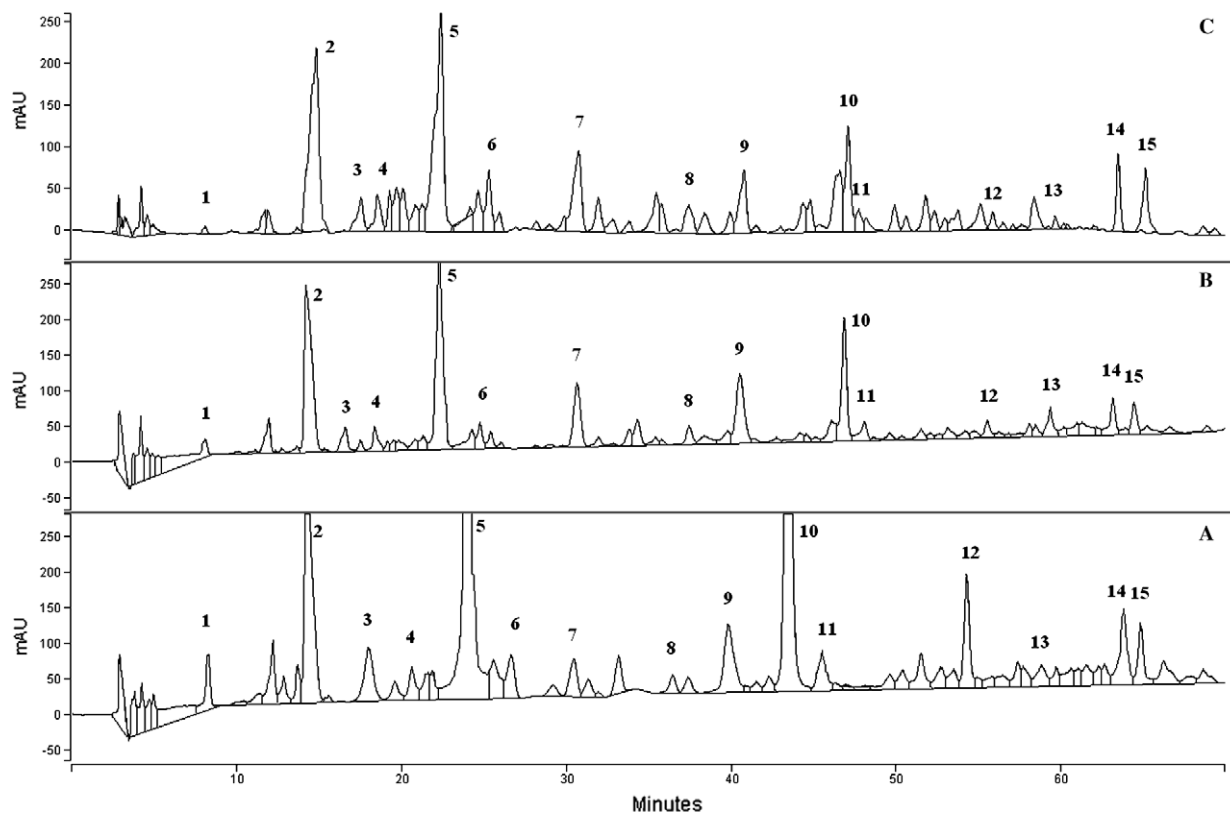


Fig. 1. The polyphenols content of apricot fruits cv. “Keckemetska ruza” at three ripening stages (IM, immature; SM, semi-mature; and CM, commercial mature) harvested in Neretva valley. Peaks identification: 1, gallic acid; 2, neochlorogenic acid; 3, procyanidin B1; 4, procyanidin B3; 5, chlorogenic acid; 6, caffeic acid; 7, (+)-catechin; 8, procyanidin B2; 9, *p*-coumaric acid; 10, (–)-epicatechin; 11, ferulic acid; 12, quercetin-3-galactoside; 13, quercetin-3-glucoside; 14, quercetin-3-rutinoside; and 15, kaempferol-3-rutinoside. HPLC conditions: column Pinnacle-II C18 (250 × 4.6 mm, ID 5 μm); mobile phases A, 3% acetic acid in water and B, water/acetonitrile/acetic acid (73:25:2, v/v/v). The elution gradient is given in Section 2. Detection at 278 nm.

Table 2
Polyphenols determined in apricot cultivar “Keckemetska ruza” harvested in three stages of maturity in two geographical region of Croatia^a

Polyphenols	Geographical region					
	Baranja			Neretva valley		
	IM	SM	CM	IM	SM	CM
Gallic acid	3.47 ± 0.28	2.35 ± 0.03	2.43 ± 0.23	2.70 ± 0.74	2.37 ± 0.52	0.61 ± 0.08
Chlorogenic acid	18.87 ± 0.33	16.05 ± 0.52	14.69 ± 0.98	23.84 ± 2.08	19.55 ± 1.94	16.71 ± 1.17
Neochlorogenic acid	13.04 ± 0.69	10.81 ± 1.01	11.90 ± 0.22	14.47 ± 1.63	10.79 ± 0.53	11.93 ± 1.87
Caffeic acid	7.83 ± 0.21	5.07 ± 0.17	2.84 ± 0.15	4.04 ± 0.20	2.39 ± 0.16	3.51 ± 0.25
<i>p</i> -Coumaric acid	10.47 ± 0.74	7.88 ± 0.74	5.09 ± 0.09	10.81 ± 0.36	8.79 ± 0.43	6.80 ± 0.41
Ferulic acid	2.51 ± 0.10	1.42 ± 0.13	1.03 ± 0.06	2.16 ± 0.05	1.56 ± 0.18	1.53 ± 0.11
(+)-Catechin	23.54 ± 0.77	17.54 ± 0.32	18.73 ± 1.01	18.14 ± 1.29	14.17 ± 0.71	9.20 ± 0.16
(–)-Epicatechin	56.10 ± 2.81	40.07 ± 0.23	27.48 ± 0.55	66.40 ± 0.75	46.22 ± 0.64	33.19 ± 2.36
Procyanidin B1	5.96 ± 0.38	4.54 ± 0.52	4.48 ± 0.48	16.13 ± 0.68	4.75 ± 0.16	5.76 ± 0.40
Procyanidin B2	3.79 ± 0.16	2.08 ± 0.14	2.63 ± 0.28	4.49 ± 0.16	2.43 ± 0.22	2.54 ± 0.29
Procyanidin B3	5.96 ± 1.05	4.55 ± 0.52	4.98 ± 0.23	3.87 ± 0.32	1.49 ± 0.16	1.86 ± 0.05
Quercetin-3-galactoside	7.25 ± 0.71	2.76 ± 1.01	5.38 ± 0.79	12.18 ± 1.57	5.05 ± 0.63	6.84 ± 1.03
Quercetin-3-glucoside	9.71 ± 1.20	13.11 ± 1.95	6.55 ± 0.86	28.49 ± 4.03	9.58 ± 1.29	7.03 ± 1.43
Quercetin-3-rutinoside	29.09 ± 0.51	18.55 ± 1.22	20.15 ± 0.83	17.73 ± 1.34	9.61 ± 0.36	12.55 ± 0.68
Kaempferol-3-rutinoside	20.11 ± 1.70	13.35 ± 1.71	12.81 ± 0.35	11.42 ± 1.56	9.91 ± 0.26	9.35 ± 0.17

IM, immature; SM, semi-mature; and CM, commercial mature.

^a Values are means ± SD (*n* = 3), and they are given as mg kg⁻¹ of examined samples.

stage. The trend of chlorogenic acid amount changes during ripening of apricot cultivar “KR” and “VR” grown in Neretva valley was the same as well as in other cultivars. However, in the cultivar “VR” grown in Baranja its amount increased at semi-mature stage (22.56 mg kg⁻¹)

and slightly decreased at commercial mature stage (19.25 mg kg⁻¹). Generally, the amount of chlorogenic acid was the highest in immature fruits but the correlation between its amount and degree of maturity was not found. According to Garcia-Viguera et al. (1994) the quantity of

Table 3
Polyphenols determined in apricot cultivar “Madjarska najbolja” harvested in three stages of maturity in two geographical region of Croatia^a

Polyphenols	Geographical region					
	Baranja			Neretva valley		
	IM	SM	CM	IM	SM	CM
Gallic acid	2.95 ± 0.25	1.05 ± 0.14	tr	3.14 ± 0.36	2.57 ± 0.32	tr
Chlorogenic acid	24.70 ± 0.79	22.56 ± 0.98	22.85 ± 1.52	34.68 ± 1.76	28.25 ± 0.96	26.30 ± 1.86
Neochlorogenic acid	15.86 ± 1.55	13.93 ± 1.83	14.18 ± 1.69	16.82 ± 1.19	17.62 ± 2.05	17.23 ± 1.97
Caffeic acid	10.56 ± 0.23	9.61 ± 0.20	8.06 ± 0.81	14.87 ± 1.06	9.61 ± 0.18	8.04 ± 0.30
<i>p</i> -Coumaric acid	11.72 ± 0.81	9.73 ± 0.49	9.27 ± 0.38	12.14 ± 1.41	7.97 ± 0.78	3.24 ± 0.17
Ferulic acid	2.08 ± 0.11	0.71 ± 0.05	0.76 ± 0.06	2.16 ± 0.15	1.68 ± 0.03	1.39 ± 0.17
(+)-Catechin	30.12 ± 1.01	25.80 ± 0.89	23.08 ± 0.75	32.16 ± 1.06	23.24 ± 0.73	21.01 ± 0.11
(-)-Epicatechin	64.96 ± 3.46	56.72 ± 2.32	47.98 ± 0.82	49.05 ± 0.21	21.53 ± 1.26	20.16 ± 0.88
Procyanidin B1	8.16 ± 0.37	5.28 ± 0.17	4.08 ± 0.31	13.16 ± 1.41	10.24 ± 0.89	6.51 ± 0.29
Procyanidin B2	7.13 ± 0.77	4.82 ± 0.64	4.14 ± 0.17	7.29 ± 0.45	2.58 ± 0.03	2.84 ± 0.31
Procyanidin B3	13.17 ± 0.65	7.03 ± 0.35	7.95 ± 0.35	11.44 ± 1.42	5.31 ± 1.12	5.91 ± 0.41
Quercetin-3-galactoside	11.47 ± 1.92	6.19 ± 1.09	8.34 ± 0.93	13.96 ± 1.36	8.79 ± 1.62	9.03 ± 1.43
Quercetin-3-glucoside	14.38 ± 2.18	8.55 ± 0.61	12.88 ± 1.88	10.08 ± 1.47	7.64 ± 0.93	8.84 ± 1.31
Quercetin-3-rutinoside	18.67 ± 1.19	16.59 ± 0.52	15.49 ± 0.71	42.83 ± 1.62	31.94 ± 1.42	40.73 ± 1.40
Kaempferol-3-rutinoside	13.94 ± 0.92	12.05 ± 1.08	12.03 ± 1.06	25.86 ± 1.40	21.12 ± 0.21	24.17 ± 1.06

IM, immature; SM, semi-mature; and CM, commercial mature.

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

Table 4
Polyphenols determined in apricot cultivar “Velika rana” harvested in three stages of maturity in two geographical region of Croatia^a

Polyphenols	Geographical region					
	Baranja			Neretva valley		
	IM	SM	CM	IM	SM	CM
Gallic acid	1.59 ± 0.31	0.85 ± 0.14	tr	2.03 ± 0.43	1.17 ± 0.21	tr
Chlorogenic acid	21.33 ± 1.89	22.56 ± 2.05	19.25 ± 1.27	24.88 ± 1.97	21.35 ± 1.09	16.93 ± 1.15
Neochlorogenic acid	11.56 ± 1.15	13.23 ± 0.98	12.15 ± 1.16	13.99 ± 1.25	16.08 ± 1.22	15.74 ± 1.11
Caffeic acid	12.15 ± 0.95	14.05 ± 1.25	10.94 ± 1.08	16.77 ± 1.26	13.60 ± 1.27	14.25 ± 0.85
<i>p</i> -Coumaric acid	17.05 ± 0.99	16.33 ± 1.04	12.85 ± 0.87	14.49 ± 1.54	12.78 ± 1.09	13.02 ± 1.05
Ferulic acid	3.11 ± 0.32	2.75 ± 0.22	1.54 ± 0.12	6.09 ± 0.75	4.97 ± 0.55	4.28 ± 0.56
(+)-Catechin	38.95 ± 2.07	31.87 ± 1.88	33.02 ± 1.99	44.05 ± 2.55	38.98 ± 2.05	36.99 ± 1.02
(-)-Epicatechin	48.91 ± 3.15	46.25 ± 2.71	45.28 ± 2.85	59.54 ± 3.27	51.96 ± 2.68	52.18 ± 3.25
Procyanidin B1	6.22 ± 1.03	6.08 ± 0.77	4.87 ± 0.97	10.19 ± 1.22	9.17 ± 0.85	8.55 ± 1.02
Procyanidin B2	5.13 ± 0.71	4.58 ± 0.94	3.74 ± 0.52	6.98 ± 0.75	4.99 ± 1.11	4.41 ± 0.75
Procyanidin B3	10.85 ± 1.46	9.15 ± 1.23	8.12 ± 0.85	12.31 ± 1.52	11.17 ± 1.07	11.27 ± 1.15
Quercetin-3-galactoside	5.47 ± 1.92	4.75 ± 0.82	7.01 ± 0.99	6.68 ± 0.62	5.87 ± 0.53	6.83 ± 0.74
Quercetin-3-glucoside	8.12 ± 1.02	6.98 ± 0.75	9.17 ± 0.68	14.25 ± 1.15	12.44 ± 1.38	15.08 ± 1.47
Quercetin-3-rutinoside	23.01 ± 1.45	20.97 ± 2.46	21.67 ± 2.17	29.51 ± 1.97	25.23 ± 2.04	26.86 ± 2.32
Kaempferol-3-rutinoside	13.96 ± 1.27	11.85 ± 1.54	12.24 ± 0.76	17.95 ± 1.04	17.28 ± 1.15	14.09 ± 1.42

IM, immature; SM, semi-mature; and CM, commercial mature.

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

chlorogenic acid in apricot fruits varied depending on maturity stages but the correlation between the degree of maturity and its amount was not observed. Neochlorogenic acid was present in remarkable amounts in all samples. It was the highest at immature stage in apricot cultivar “KR” grown in both geographical region and “MN” grown in Baranja, and it varied during ripening. In apricot cultivars “KR” and “MN” its amount slightly decreased at semi-mature stage and again increased at commercial mature stage. Furthermore, its amount did not change remarkably during the ripening of apricot cultivar “MN” grown in Neretva valley and apricot cultivar “VR” grown in both geographical regions. The existing of neochlorogenic acid in apricot fruits was confirmed by other authors

(Radi et al., 1997). The other HCA identified in apricot fruits (caffeic, *p*-coumaric and ferulic acid) decreased remarkably during ripening. A decrease of mentioned phenolic acids was different in each apricot cultivar. In apricot cultivar “KR” grown in Baranja caffeic and ferulic acids decreased about 60%, while *p*-coumaric acid decreased 50% at commercial mature stage. In apricot fruits grown in Neretva valley the decrease of mentioned phenolic acids during ripening ranged from 13% (caffeic acid) to 37% (*p*-coumaric acid). In apricot fruits cultivar “MN” grown in Baranja harvested at commercial mature stage, the amounts of caffeic acid (8.06 mg kg⁻¹) and *p*-coumaric acid (9.27 mg kg⁻¹) were about 20% and ferulic acid (0.76 mg kg⁻¹) about 60% lower than in immature fruits

(10.56 mg kg⁻¹, 11.27 mg kg⁻¹ and 2.08 mg kg⁻¹). In the same cultivar grown in Neretva valley caffeic acid decreased 46%, ferulic acid 36%, while *p*-coumaric acid decreased 73%. The changes of caffeic and *p*-coumaric acids in apricot cultivar “VR” grown in both geographical regions were the lowest and these phenolic acids decreased about 10% and 24%. In the same apricot cultivar grown in Baranja ferulic acid decreased 50% and in those grown in Neretva valley 30%. The data about changes of HCA during ripening are not fully reported, while the amounts of HCA mentioned above in apricot fruits harvested at commercial mature stage are in accordance with previously presented data by Dragovic-Uzelac, Delonga et al. (2005, 2005).

Flavan-3-ol monomers (catechin and epicatechin) were the major phenolic compounds in immature apricot fruits and they declined remarkably during fruit ripening. In apricot cultivar “KR” grown in Baranja (+)-catechin decreased 30% from immature stage to the commercial mature stage, while at the same cultivar grown in Neretva valley (Mediterranean region) (+)-catechin decreased 50%. A decrease of (+)-catechin in cultivar “MN” grown in Baranja was 23% and in those grown in Neretva valley 35%, while in cultivar “VR”, grown in both geographical region, (+)-catechin decreased 15%. The (–)-epicatechin amount in apricot fruits grown in both climate region decreased from the highest values at immature stage (ranged from 48.91 in cv. “VR” grown in Baranja to 66.40 mg kg⁻¹ in cv. “KR” grown in Neretva valley) to the lowest at commercial mature stage (ranged from 20.16 in cv. “MN” grown in Neretva valley) to 52.18 mg kg⁻¹ in cv. “VR” grown in the same region. The other authors also confirmed high amount of (–)-epicatechin in apricot fruits (Arts et al., 2000; Dragovic-Uzelac, Delonga et al., 2005, 2005; Garcia-Viguera et al., 1997; Radi et al., 1997). In cultivars “KR” and “MN” the decrease was about 50%, while in cultivar “VR” it was only about 10%. Much of the decline in procyanidins occurred between immature and semi-mature fruits, whereas their amounts were not remarkable different at semi-mature and commercial ripening stages. In apricot cultivars “KR” and “MN” their amounts almost were slightly increased at commercial mature stage. Quercetin-3-galactoside and quercetin-3-rutinoside were presented in higher amounts at the beginning (IM) and at the end (CM) of ripening for all apricot cultivars. However, at semi-mature stage quercetin-3-galactoside decreased about 50% and at the end of ripening a slight increase was observed. At the beginning of ripening the amount of quercetin-3-glucoside ranged from 18.67 mg kg⁻¹ in apricot cultivar “KR” grown in Baranja to 42.83 mg kg⁻¹ in apricot cultivar “MN” grown in Neretva valley. At semi-mature stage it decreased between 10% and 20% in cultivars “MN” and “VR” and about 40% in cultivar “KR”, while its amount slightly increased at commercial mature stage. The quantities of flavonoids in apricot fruits varied according to ripening stages and it was not possible to establish a correlation between flavonoid con-

tent and the ripening stages (Garcia-Viguera et al., 1994). From a geographical point of view, there were not great variations in the levels of flavonoids of apricots cultivars grown in continental region (Baranja) and those grown in Mediterranean region (Neretva valley). The qualitative and quantitative differences of phenolic compounds could be due to the genetic characteristics of the examined cultivars. In particular, the amounts of polyphenols and their different trends during the ripening are probably due to the different extent by which the biosynthetic pathways of these compounds are affected during the ripening (Macheix et al., 1990).

3.2. Changes of carotenoids during ripening

The profiles of carotenoids obtained in typical HPLC runs in apricot fruits cv. “MN” harvested in Mediterranean region (Neretva valley) at three ripening stages are shown in Fig. 2. Five carotenoids were identified on chromatograms, α -carotene, β -carotene, γ -carotene, zeaxanthin and lutein. The carotenoid amounts in apricot fruits at three ripening stages, harvested in two geographical regions of Croatia (Baranja and Neretva valley) are shown in Table 5. Carotenoid content was different among the cultivars studied and also among the geographical regions. In apricot fruits of all cultivars the predominant pigment β -carotene accumulated rapidly during ripening. Besides β -carotene, γ -carotene was also determined in all examined samples. α -Carotene and zeaxanthin were determined only in cultivar “MN” grown in both geographical regions. Lutein was determined in apricot cultivars “MN” and “VR”. In nine apricot cultivars grown in France β -carotene was the major carotenoid, while the α -carotene, zeaxanthin and lutein were not found (Radi et al., 1997). The same authors found γ -carotene in four out of nine cultivars and lycopene only in two cultivars. The data presented by other authors also confirmed the high amount of β -carotene in apricot fruits (Amotz & Fishler, 1998; Munzuroglu et al., 2003; Zeb & Mehmood, 2004) and also the presence of α -carotene, zeaxanthin and lutein in lower amounts (Fraser & Bramley, 2004). In the apricot cultivar “KR” grown in continental region (Baranja) and Mediterranean region (Neretva valley) the β -carotene amount increased from 54.3 μ g/100 g and 75.06 μ g/100 g at the immature stage to 585.4 μ g/100 g and 795.5 μ g/100 g, respectively, at the commercial mature stage. In the same cultivar, γ -carotene was determined in the amounts of 17.66 μ g/100 g and 20.45 μ g/100 g at the immature stage and in the amounts 97.49 μ g/100 g and 125.9 μ g/100 g at the commercial mature stage (Table 5). From the immature stage to the commercial mature stage, the β -carotene amount in the cultivar “MN” grown in Baranja and Neretva valley increased from 176.7 μ g/100 g and 203.1 μ g/100 g to 1075 μ g/100 g and 1376 μ g/100 g. In the same cultivar “MN”, γ -carotene and lutein were determined in the amounts of 225.6 μ g/100 g and 131.3 μ g/100 g and 343.2 and 188.1 μ g/100 g. Compared to the amounts of β -caro-

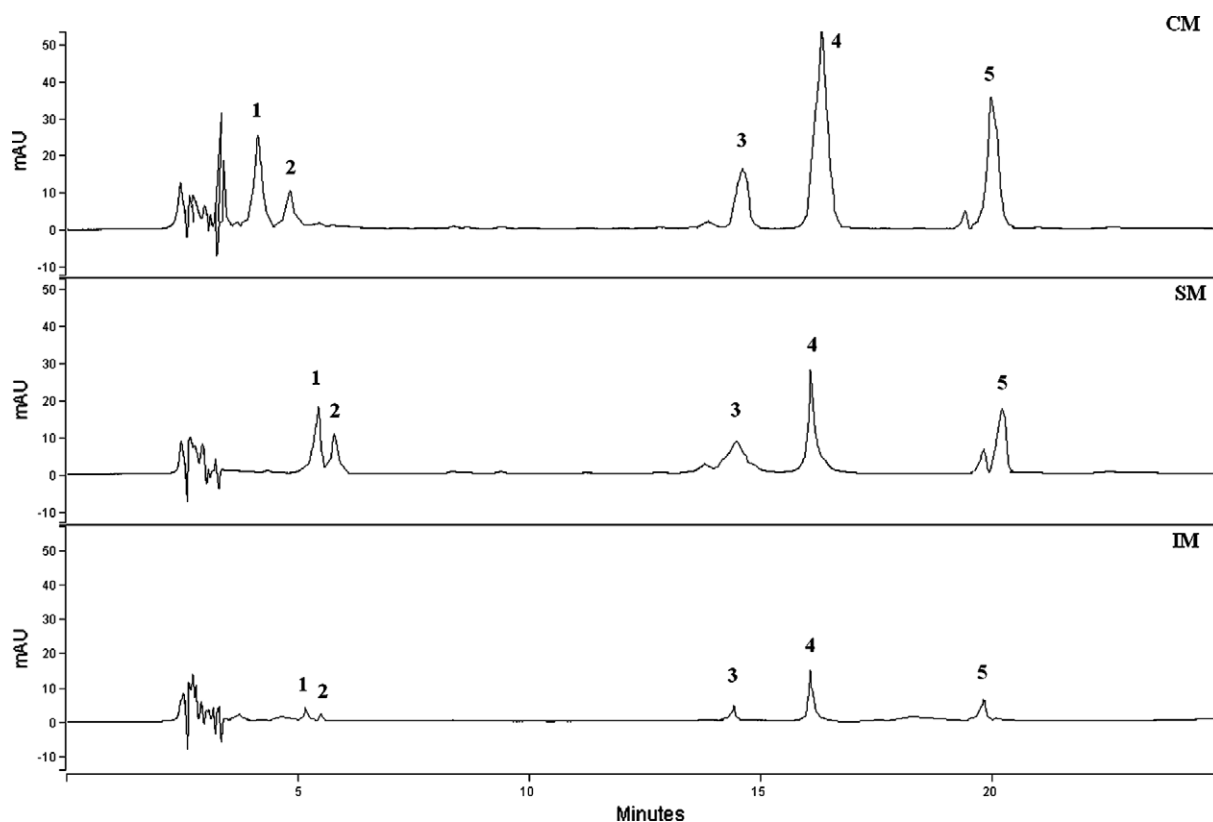


Fig. 2. The carotenoids content of apricot fruits cv. “Madjarska najbolja” at three ripening stages (IM, immature; SM, semi-mature; and CM, commercial mature) harvested in Neretva valley. Peaks identification: 1, lutein; 2, zeaxantin; 3, α -caroten; 4, β -caroten; and 5, γ -caroten. HPLC conditions: column Pinnacle-II C18 (250 \times 4.6 mm, ID 5 μ m); mobile phase acetonitrile/methanol/1,2-dichloroethane (60:35:5, v/v/v) with 0.1% BHT; isocratic elution; flow rate 1 mL min⁻¹; detection at 450 nm.

Table 5

Carotenoids determined in apricot cultivars “Keckemetska ruza” “Madjarska najbolja” and “Velika rana” harvested in three stages of maturity in two geographical region of Croatia^a

Cultivar	Carotenoids	Geographical region					
		Baranja			Neretva valley		
		IM	SM	CM	IM	SM	CM
Keckemetska ruza	β -Carotene	54.35 \pm 2.05	235.40 \pm 12.25	585.4 \pm 20.08	75.06 \pm 8.76	418.7 \pm 19.87	795.5 \pm 25.17
	γ -Carotene	17.66 \pm 1.25	33.38 \pm 2.53	97.49 \pm 5.99	20.45 \pm 1.27	64.42 \pm 6.09	125.9 \pm 9.12
Madjarska najbolja	Lutein	18.47 \pm 1.82	71.70 \pm 5.77	131.3 \pm 3.45	35.13 \pm 2.85	96.84 \pm 6.87	188.11 \pm 7.82
	Zeaxantin	5.92 \pm 0.45	18.62 \pm 0.55	25.43 \pm 1.96	11.94 \pm 0.56	31.07 \pm 1.95	38.96 \pm 2.07
	α -Carotene	nd	14.81 \pm 0.56	22.31 \pm 0.92	12.64 \pm 0.45	32.35 \pm 1.08	43.67 \pm 2.15
	β -Carotene	176.69 \pm 9.95	622.98 \pm 10.11	1074.99 \pm 5.47	203.01 \pm 2.97	750.50 \pm 5.18	1374.95 \pm 13.85
	γ -Carotene	43.66 \pm 1.55	85.59 \pm 1.95	225.60 \pm 4.59	63.15 \pm 2.08	157.61 \pm 4.55	343.20 \pm 10.05
Velika rana	Lutein	10.18 \pm 0.33	75.29 \pm 1.08	123.44 \pm 2.82	21.39 \pm 1.25	88.18 \pm 5.87	131.35 \pm 4.55
	Zeaxantin	nd	nd	nd	nd	nd	nd
	α -Carotene	nd	nd	nd	nd	nd	9.67 \pm 0.15
	β -Carotene	107.57 \pm 2.55	454.06 \pm 5.87	828.50 \pm 10.15	154.46 \pm 2.76	585.69 \pm 4.85	948.33 \pm 7.88
	γ -Carotene	13.39 \pm 0.54	44.65 \pm 1.25	113.19 \pm 5.07	20.47 \pm 0.87	69.68 \pm 1.15	163.32 \pm 4.25

IM, immature; SM, semi-mature; CM, commercial mature; nd, not detected.

^a Values are means \pm SD ($n = 3$), and they are given as μ g 100 g⁻¹ of examined samples.

tene, γ -carotene and lutein, zeaxantin and α -carotene were determined in insignificant amounts (Table 5). In the apricot cultivar “VR” grown in the same region as cultivars “KR” and “MN”, β -carotene increased from 107.57 μ g/

100 g and 154.46 μ g/100 g to 828.5 μ g/100 g and 948.33 μ g/100 g. Lutein and γ -carotene were determined in similar amount (Baranja: 123.44 μ g/100 g and 113.19 μ g/100 g; Neretva valley: 151.35 μ g/100 g and

163.32 $\mu\text{g}/100\text{ g}$) within the same region. One study found the accumulation of β -carotene from trace amount in the immature fruit to about 22 $\mu\text{g}/\text{g}$ in the ripe stage (Katayama et al., 1971). As it was reported before, the amount of β -carotene, zeaxanthin, lutein, neoxanthin and biosynthetically related compounds increased in examined ripe fruit (Römer et al., 2000). In most carotenoid containing fruits ripening is accompanied by enhanced carotenoid biosynthesis, such as in apricot (Katayama et al., 1971), mango (Mercadante & Rodriguez-Amaya, 1993), and papaya (Wilberg & Rodriguez-Amaya, 1995). However, the regulation of carotenoid biosynthesis is poorly understood. During apricot fruit ripening there is a massive deposition of carotenoids of which β -carotene represents some 70% in cultivar “MN” and 85% in cultivar “KR” of total carotenoids. According to Radi et al. (1997) β -carotene represents more than 50% of total carotenoid content. β -Carotene accumulation in apricot fruits probably occurs as a result of up regulating the expression of a fruit ripening-enhanced phytoene synthase (*Psy-1*). It is well known that phytoene synthase catalyses the first step in the formation of carotenoids (Karvouni et al., 1995; Kuntz et al., 1992; Schledz et al., 1996). Furthermore, *Psy-1* exhibits the highest flux control coefficient among the enzymes of the pathway and it poses the greatest control over flux through the pathway. Consequently, it has been the principal target for amplification with the objective of increasing the lycopene and β -carotene content in fruit (Fraser & Bramley, 2004; Fray et al., 1995). Generally, the amounts of all carotenoids were higher in apricot fruits grown in Mediterranean region (Neretva valley) than in those grown in continental region (Baranja).

4. Conclusions

The qualitative differences of polyphenols in examined apricot cultivars during ripening were not observed. However, the quantity of polyphenols during apricot fruits ripening depended on cultivars but there were not great variations in the amount of polyphenols in apricots grown in different regions. The highest amount of polyphenols was determined in immature apricots, decreasing to semi-mature and commercial mature stages of ripening, whereas the changes in content of some polyphenols in semi-mature and commercial mature stages were not uniform.

The quantitative and qualitative differences between carotenoids in apricot fruits during ripening were observed which depend on cultivar and geographical region of cultivation. Generally the carotenoids amount was higher in cultivars grown in Mediterranean region than in those cultivated in continental region, especially in fruits at commercial mature stages. Carotene analysis showed increasing of all separated carotenoids in apricot fruits during ripening, particularly β -carotene content, which was about 10-fold higher in commercial mature fruits than in immature fruits.

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