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ORIGINAL ARTICLE

Distribution of phenolic compounds and antioxidant capacity in apples tissues during ripening

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Abstract The effect of variety and ripening stage on the distribution of phenolic compounds and *in vitro* antioxidant capacity of Gala, Fuji Suprema and Eva apples were evaluated. Hydroxycinnamic acids, flavonoids, flavanols, flavonols, dihydrochalcones and antioxidant activity (FRAP and DPPH) were assessed in the epicarp, mesocarp and endocarp of three varieties at three ripening stages (unripe, ripe and senescent). The Fuji Suprema variety distinguished by its content of flavonols at senescent stage, while Eva variety distinguished by its content of dihydrochalcones (unripe stage) and anthocyanins (ripe stage). In general, phenolic acids and flavonoids decreased with ripening in the epicarp and endocarp. However, in the mesocarp, the effect of ripening was related with the apple variety. Hierarchical cluster analysis confirmed the influence of ripening in the apple tissue. The evolution of these compounds during ripening occurred irregularly and it was influenced by the variety.

Keywords *Malus domestica* Borkh · Eva · Gala · Fuji Suprema · Ripening · Phenolic profile

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Introduction

Apples (*Malus domestica* Borkh) contain significant quantities of phenolic compounds, which are responsible for various sensory attributes of fruits and their products, such as color, bitterness and astringency (Khanizadeh et al. 2008; Alberti et al. 2016). Furthermore, these compounds have antioxidant activity and are related to health benefits, such as reducing the risk of cardiovascular disease, lung cancer, asthma and diabetes (Babu et al. 2013; Yao et al. 2004; Tsao et al. 2005).

Phenolic compounds are products of the secondary metabolism of plants and they play an important role in the growth and reproduction of plants (by attracting pollinators) and providing protection against pathogens (antimicrobial activities). They can be synthesized in response to adverse conditions such as infection, injury, and UV irradiation (Ignat et al. 2011; Karaman et al. 2010; Duda-Chodak et al. 2011). These compounds are stored in vacuoles and cell walls after polymerization or conjugation with sugars or organic acids (Bidel et al. 2011). In the fruit, the phenolic compounds are formed during growth until the full maturity stage (Zhang et al. 2010) and they are distributed in various tissues and fractions (epicarp, mesocarp, endocarp and seeds). However, the compounds and their concentration may vary depending on the type of fruit and the variety (Le Bourvellec et al. 2015; Guo et al. 2013).

Studies have demonstrated the distribution of phenolic compounds in ripe apples (Kalinowska et al 2014; Tsao et al. 2003). Usually, epicarp is richer in phenolic compounds than the other apple tissues. Some groups of flavonoids are almost exclusively found in epicarp such as anthocyanins and flavonols (glycosides of quercetin). In other hand, monomeric and polymeric flavan-3-ols are the major phenolics of epicarp and mesocarp and can

represents about 60% of the total phenolic compounds in apples. Phloridzin (dihydrochalcone) that can be used as a biomarker of apple products, are distributed in epicarp and mesocarp, as well as the hydroxycinnamic acids.

However, knowledge about the distribution and evolution of phenolic compounds during ripening stages of apples can be helpful for consumers and the food industry in order to obtain high content of bioactive compounds. Thus, this study was intended to evaluate the effect of the variety and ripening stage on the distribution of phenolic compounds and the antioxidant capacity in apples.

Materials and methods

Materials

Apples from the Fuji Suprema variety were collected from the EPAGRI experimental station (Agricultural Research and Rural Extension of Santa Catarina), Caçador, Santa Catarina, Brazil (26°50′12″S, 50°58′23″O), while the Gala and Eva varieties were obtained from Boutin Agrícola, Porto Amazonas, Paraná, Brazil (25°32′08″S, 49°53′33″O). The fruits were collected (20 kg) in different cardinal points, at the top, middle and bottom from six trees at three ripening stages (unripe, ripe and senescent). The ripening index was determined by using the starch-iodine test (Blanpied and Silsby 1992). The iodine values for the fruits of all the varieties were 1 for unripe, 4–5 for ripe, and 8 for senescent.

Liquid nitrogen (99%) was produced with StirLIN-1 (Stirling Cryogenics, Dwarka, New Delhi, India). Folin-Ciocalteau reagent, TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine), DPPH (2,2-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 5-caffeoylquinic acid, caffeic acid, gallic acid, *p*-coumaric acid, (+)-catechin, (–)-epicatechin, procyanidin B1, procyanidin B2, phloridzin, phloretin, quercetin, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside and quercetin-3-rutinoside were purchased from Sigma–Aldrich (St. Louis, MO., USA). Acetonitrile (99.9%), acetic acid (99.9%), methanol (99.9%) and acetone (99.5%) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Sodium nitrite and aluminum chloride were purchased from Vetec (Rio de Janeiro, RJ, Brazil) and Fluka (St. Louis, MO., USA), respectively.

Methods

Extraction of phenolic compounds

The tissues of fruit epicarp (1–2 mm outside of the fruit), mesocarp and endocarp (cylinder of the central part of the fruit with 20 mm diameter, without seeds) of each variety were mechanically removed and sprayed with an aqueous solution of cysteine (2.0 mmol/L) to inhibit oxidation reactions (Zardo et al. 2013). The samples were freeze-dried (LS 3000, São Paulo, SP, Brazil), and homogenized in a mortar. Two grams of the samples (epicarp, mesocarp and endocarp) were extracted with 30 mL of 85% methanol for 15 min at 28 °C (twice), followed by 65% acetone for 20 min at 10 °C (twice). The mixture was centrifuged (8160×*g*, 20 min at 4 °C) (Hitache Koki Co., Hitachi, Ibaraki, Japan), concentrated by evaporation under vacuum (40 °C, −600 mmHg) (Tecnal TE-211, Piracicaba, SP, Brazil), and freeze-dried. A solution (2 mL) of 2.5% acetic acid and methanol (3:1, v/v) was used to reconstitute the samples.

Analysis

Total phenolic compounds, flavonoids and flavanols

The content of phenolic compounds was determined by colorimetric analysis using the Folin–Ciocalteau method (Singleton and Rossi 1965). The results were expressed as milligrams of 5-caffeoylquinic acid equivalents per kilogram of the tissue of apple using a calibration curve (phenolic compounds content = 1470.4 × absorbance; $R^2 = 0.997$; $p < 0.001$). The total flavonoid content was determined according to the method described by Zhishen et al. (1999). The results were expressed as milligrams of catechin equivalents using a calibration curve (flavonoid concentration = 399.4 × absorbance; $R^2 = 0.999$; $p < 0.001$). The vanillin method was performed to determine the flavanols (Broadhurst and Jones 1978). The results were expressed in catechin equivalents (flavanols concentration = 351.4 × absorbance; $R^2 = 0.7$ $p < 0.001$).

HPLC analysis of phenolic compounds

The analysis of individual phenolic compounds was performed according to Alberti et al. (2014). The chromatographic system used was a 2695 Alliance (Waters, Milford, MA, USA), with photodiode array detector PDA 2998 (Waters), quaternary pump and auto sampler. Separation was performed on a Symmetry C_{18} column (4.6 × 150 mm, 3.5 µm; Waters) at 20 °C. The identification and quantification of the phenolic compounds was performed by comparison of the spectra and retention times of the standards. When commercial standards were not available, the quantification was performed from compounds belonging to the same class of phenolic compounds, as verified after the fractionation of the samples (Jaworski and Lee 1987; Alberti et al. 2016). The total peak area (obtained by HPLC analysis) was summed for each

phenolic class and quantified as the phloridzin, 5-caffeoylquinic acid and quercetin 3-β-D-glucoside equivalents for dihydrochalcones, hydroxycinnamic acids and flavonols, respectively.

Antioxidant capacity

The total antioxidant capacity of the extracts was determined using the ferric reduction antioxidant power (FRAP) method as described by Benzie and Strain (1996) and by DPPH according to the Brand-Williams et al. (1995) method. Measurements were performed using a microplate reader (Epoch microplate spectrophotometer, Synergy-BIOTEK, Winooski, VT, USA) and the results were expressed as Trolox equivalents per kilogram of apple (μmol TE /kg).

Statistical analysis

The data were presented as mean and pooled standard deviation (PSD). Hierarchical cluster analysis (HCA) was performed to assess the similarities between the fruit tissues (epicarp, mesocarp and endocarp) according to the phenolic composition and antioxidant capacity. Hartley's test was performed to check for homogeneity of variances, and one-way ANOVA and Fisher's LSD were performed to verify the differences between the groups. The p values below 0.05 were used to reject the null hypothesis. All the statistical analyses were performed using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA).

Results and Discussion

The content of phenolic compounds was different between the analyzed varieties. Eva had the lowest levels of phenolic compounds, however, the highest level of phenolics were found in both Gala and Fuji Suprema depending on the type of tissues and the ripening stage (Table 1). Other studies have reported the effect of the variety on the concentration of phenolics in apples (Guo et al. 2013; Huber and Rupasinghe 2009), mainly between apples for fresh consumption and industrial apples; the latter usually have higher concentrations of phenols and are classified as astringent and/or bitter (Sanoner et al. 1999; Tsao et al. 2005).

The epicarp of the apples showed higher concentration of phenolic compounds (up to 11 times) followed by the endocarp and mesocarp (Table 1). The same occurred for total flavonoids and, consequently, for the antioxidant capacity. Flavanols were found mostly in the epicarp of fruits; they include catechin, epicatechin and procyanidins (Table 2). Catechin was identified in the three ripening stages only in the Gala variety, where a reduction in senescence (about 50% in epicarp and 33% in mesocarp) occurred. For the Eva and Fuji Suprema apples, catechin was only found in the epicarp of senescent and ripe fruits, respectively. Epicatechin was only found in the epicarp of Gala and Eva apples, and a reduction concomitant with ripening (27 and 63%, respectively) was observed. In the Fuji Suprema variety, the reduction in epicatechin was less significant (7%), and an increase of 400% in the content of this compound in the mesocarp was observed (Table 2). Procyanidin B2 showed the same behavior as epicatechin in relation to the varieties; however, procyanidin B1 was only quantified in the epicarp of senescent fruits, especially in the Eva apples, with a content of 400 mg/kg. The procyanidin B1 was only found in senescent fruits, possibly due to the disruption of the interflavan linkages of the procyanidins at a higher degree of polymerization due to the ripening process (Alonso-Salces et al. 2004). In addition to the high antioxidant activity, procyanidins are related to the sensory characteristics of fruits, such as bitterness and astringency. The higher the degree of polymerization of procyanidins the greater its contribution to an astringent taste (Vidal et al. 2003).

Flavonols were found almost exclusively in the epicarp of the fruits, and are related to the adaptation process, which exists in fruit in order to avoid damage caused by UV-B irradiation (Khanizadeh et al. 2008; Solovchenko and Merzlyak 2008). The Fuji Suprema apples had a high content of total flavonols, especially glycosides of quercetin. In this variety, the total flavonols and quercetin-3-rutinoside content increased with ripening; however, the levels of quercetin 3-β-D-glucoside, quercetin-3-D-galactoside and quercetin 3-O-rhamnoside decreased. In Gala and Eva varieties, the quercetin glycosides, as well as the total flavonols content, increased from the unripe to the ripe stages and decreased with senescence.

There was a reduction in the concentration of the dihydrochalcones in the epicarp and endocarp of the three varieties in line with the senescence of the fruits. However, an increase was observed in the mesocarp of the Fuji Suprema and Eva varieties. Phloridzin, which is a dihydrochalcone, is found in large quantities only in apples (Gosch et al. 2010). This compound is related to the prevention of diseases such as diabetes (Masumoto et al. 2009) and neurological diseases, as well as being used as an additive in foods and beverages (Guyot et al. 2007). The Eva variety had high levels of phloridzin in the epicarp, which decreased (7%) with ripening. In the Gala and Fuji Suprema varieties, this compound was predominant in the endocarp in the unripe and ripe stages. In the senescent fruits, due to the reduction in the levels in the endocarp (70 and 48%, respectively), the highest concentration was in the epicarp (Table 2).

Table 1 Phenolic classes (mg/kg) and antioxidant capacity (μmol TE/kg) of apple (epicarp, mesocarp e endocarp) at different ripening stages

Cv.	Ripening stage	Tissue	TPC[A]	Phenolic classes						Antioxidant capacity	
				HCA[B]	Flavonoids	Flavanols	Flavonols	DHC[C]	Anthocyanins	FRAP	DPPH
Eva	Unripe	Epicarp	2252.71^{h}	47.05^{q}	1673.71^{h}	700.70^{h}	279.58^{i}	576.22^{a}	0.00	4593.81^{l}	5425.10^{i}
		Mesocarp	232.79^{x}	12.58^{y}	102.03^{t}	13.12^{y}	5.39^{l}	6.47^{v}	0.00	895.78^{y}	805.63^{v}
		Endocarp	675.69^{r}	73.77^{o}	416.58^{n}	128.51^{q}	0.00	187.89^{e}	0.00	1783.69^{v}	2044.65^{r}
	Ripe	Epicarp	2880.93^{f}	28.34^{v}	1794.47^{g}	970.89^{g}	388.48^{f}	364.00^{c}	114.83^{a}	7555.46^{g}	6684.55^{f}
		Mesocarp	644.28^{r}	24.19^{w}	313.94^{q}	95.09^{t}	9.64^{k}	13.94^{s}	0.00	2245.74^{s}	1608.13^{t}
		Endocarp	595.89^{s}	71.67^{o}	330.58^{q}	78.58^{u}	0.00	84.14^{n}	0.00	521.64$^{\alpha}$	2168.04^{q}
	Senescent	Epicarp	1960.41^{i}	33.59^{u}	1588.63^{i}	571.29^{i}	351.79^{h}	531.51^{b}	92.94^{b}	4633.27^{k}	5460.28^{i}
		Mesocarp	390.68^{v}	17.31^{x}	182.27^{r}	16.37^{x}	9.32^{k}	18.59^{r}	0.00	612.95^{z}	1034.92^{u}
		Endocarp	723.03^{q}	77.75^{n}	319.52^{q}	42.16^{v}	0.00	45.86^{q}	0.00	2094.02^{t}	3177.85^{n}
Gala	Unripe	Epicarp	4440.80^{c}	170.30^{d}	3068.71^{d}	1430.40^{e}	477.68^{e}	212.45^{d}	11.13^{h}	16,021.03^{b}	8266.02^{c}
		Mesocarp	1069.32^{k}	135.98^{f}	848.04^{j}	337.92^{k}	0.00	18.58^{r}	0.00	3383.03^{n}	3231.06^{m}
		Endocarp	1540.59^{j}	268.22^{a}	729.58 ^{k}	443.89^{j}	0.00	177.30^{f}	0.00	6374.95^{h}	5646.35^{h}
	Ripe	Epicarp	4648.80^{b}	158.59^{e}	3893.57^{b}	1942.91^{b}	645.64^{d}	152.96^{j}	51.68^{d}	14,263.08^{c}	7267.95^{e}
		Mesocarp	600.57^{s}	110.04^{h}	446.15^{m}	193.53^{o}	0.00	17.23^{r}	0.00	2321.73^{r}	2887.32^{o}
		Endocarp	1149.71^{k}	240.44^{b}	383.37^{p}	218.64^{l}	0.00	158.71^{h}	0.00	5130.94^{i}	4399.81^{j}
	Senescent	Epicarp	3287.95^{e}	102.28^{j}	3117.28^{c}	1823.60^{c}	353.92^{g}	154.71^{i}	55.70^{c}	11,430.95^{d}	7548.54^{d}
		Mesocarp	759.36^{p}	80.00^{m}	330.21^{q}	201.38^{n}	0.00	11.46^{t}	0.00	2467.45^{q}	2889.28^{o}
		Endocarp	513.89^{t}	105.03^{i}	392.67^{op}	108.67^{s}	0.00	68.19^{p}	0.00	1191.05^{w}	1801.12^{s}
Fuji Suprema	Unripe	Epicarp	5194.08^{a}	157.39^{e}	4742.57^{a}	2670.57^{a}	750.37^{c}	159.00^{h}	33.55^{g}	18,882.17^{a}	9034.43^{a}
		Mesocarp	453.85^{u}	54.06^{p}	151.72^{s}	26.53^{w}	0.00	5.34^{u}	0.00	1149.52^{x}	1600.57^{t}
		Endocarp	978.84^{m}	203.84^{c}	459.72^{m}	166.81^{p}	0.00	170.65^{g}	0.00	3301.79^{o}	3966.63^{k}
	Ripe	Epicarp	2538.97^{g}	96.65^{k}	2452.56^{f}	1173.27^{f}	810.32^{b}	80.97^{o}	36.16^{f}	8677.43^{f}	6538.05^{g}
		Mesocarp	672.55^{r}	54.55^{p}	319.71^{q}	108.69^{s}	0.00	13.32^{s}	0.00	2002.22^{u}	2452.36^{p}
		Endocarp	909.46^{n}	121.20^{g}	601.64^{l}	200.63^{n}	0.00	93.63^{l}	0.00	5055.57^{j}	2848.30^{o}
	Senescent	Epicarp	3919.90^{d}	112.03^{h}	3008.86^{e}	1526.51^{d}	1071.92^{a}	113.06^{k}	40.98^{e}	10,324.17^{e}	8396.32^{b}
		Mesocarp	804.87^{o}	84.28^{l}	456.63^{m}	210.47^{m}	26.42^{j}	19.38^{r}	0.00	2688.97^{p}	3461.68^{l}
		Endocarp	1035.62^{l}	123.45^{g}	410.39^{no}	118.40^{r}	0.00	93.07^{m}	0.00	3549.57^{m}	3470.80^{l}
PSD[1]			1445,67	65,11	1294.13	708.04	292.51	146.65	23.47	486.27	2418.48
p (Hartley)[2]			1,0	1,0	0.71	1.0	1.0	0.79	0.70	1.0	1.0
p (ANOVA)[3]			<0,001	<0,001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values expressed as mean (n = 3) in wet basis

[A] *TPC* Total Phenolic compounds, [B] *HCA* hydroxycinnamic acids, [C] *DHC* dihydrochalcones, [D] *PSD* pooled standard deviation, [E] probability values obtained by Hartley test (*F* max) for homogeneity of variances, [F] probability values obtained by One-way ANOVA

Different letters in the same column represent statistical different results according to the Fischer LSD test ($p \leq 0.05$)

Table 2 Phenolic composition (mg/kg) of apple (epicarp, mesocarp e endocarp) at different ripening stages

Cv.	Ripening stage	Tissue	Phenolic compounds									
			CQA	PLZ	EPI	CAT	PB1	PB2	QGA	QGL	QRH	QRU
Eva	Unripe	Epicarp	0.0	375.6^{a}	191.2^{g}	0.0	0.0	140.2^{e}	12.9^{g}	10.3^{i}	38.5^{g}	1.4^{g}
		Mesocarp	0.0	4.5^{s}	0.0	0.0	3.5^{e}	2.9^{l}	0.0	0.0	0.0	0.0
		Endocarp	27.3^{l}	130.8^{d}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Ripe	Epicarp	0.0	276.0^{c}	0.0	0.0	0.0	0.0	90.4^{d}	59.9^{e}	68.8^{e}	23.2^{b}
		Mesocarp	3.6^{r}	10.5^{p}	0.5^{m}	0.0	1.9^{f}	0.0	0.7^{i}	0.0	1.1^{f}	0.0
		Endocarp	22.4^{m}	60.7^{j}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Senescent	Epicarp	5.5^{q}	348.4^{b}	70.5^{h}	6.7^{g}	401.0^{a}	57.8^{f}	65.1^{f}	42.3^{h}	35.0^{h}	8.7^{d}
		Mesocarp	4.0^{qr}	7.0^{q}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Endocarp	3.8^{r}	32.5^{n}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gala	Unripe	Epicarp	65.5^{e}	81.3 ^{h}	327.1^{a}	38.5^{a}	0.0	248.7^{a}	82.8^{e}	51.9^{f}	107.6^{c}	7.1^{e}
		Mesocarp	48.1^{h}	7.4^{q}	10.2^{k}	15.8^{e}	0.0	29.2^{j}	0.0	0.0	0.0	0.0
		Endocarp	90.9^{a}	112.3^{f}	0.0	0.0	0.0	36.8^{i}	0.0	0.0	0.0	0.0
	Ripe	Epicarp	78.6^{c}	57.3^{k}	268.6^{b}	29.7^{b}	0.0	222.4^{c}	122.4^{b}	88.1^{b}	114.5^{b}	16.8^{c}
		Mesocarp	34.4^{k}	5.3^{r}	0.0	10.6^{f}	0.0	0.0	0.0	0.0	0.0	0.0
		Endocarp	84.3^{b}	98.9^{g}	0.0	0.0	0.0	30.8^{i}	0.0	0.0	0.0	0.0
	Senescent	Epicarp	63.2^{e}	57.3^{k}	236.9^{e}	19.4^{d}	69.8^{c}	200.1^{d}	66.1^{f}	44.0^{g}	59.9^{f}	5.4^{f}
		Mesocarp	18.9^{n}	3.6^{t}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Endocarp	38.2^{j}	35.6^{m}	13.5^{j}	0.0	0.0	25.9^{j}	0.0	0.0	0.0	0.0
Fuji Suprema	Unripe	Epicarp	69.3^{d}	119.8^{e}	261.6^{c}	0.0	0.0	247.3^{a}	137.7^{a}	92.2^{a}	144.9^{a}	16.9^{c}
		Mesocarp	3.6^{r}	4.1^{s}	4.5^{l}	0.0	0.0	12.4^{k}	0.0	0.0	0.0	0.0
		Endocarp	44.9^{i}	129.7^{d}	14.7^{j}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Ripe	Epicarp	83.5^{b}	55.4^{l}	226.2^{f}	23.1^{c}	0.0	201.4^{d}	124.3^{b}	79.1^{c}	103.5^{d}	15.1^{c}
		Mesocarp	11.0^{p}	9.3^{p}	13.3^{j}	0.0	0.0	35.7^{i}	0.0	0.0	0.0	0.0
		Endocarp	58.7^{f}	82.9^{h}	0.0	0.0	0.0	41.3^{h}	0.0	0.0	0.0	0.0
	Senescent	Epicarp	85.4^{b}	98.1^{g}	243.5^{d}	0.0	161.8^{b}	240.0^{b}	118.9^{c}	64.0^{d}	68.2^{e}	25.0^{a}
		Mesocarp	15.2^{o}	12.3^{o}	18.5^{i}	0.0	0.0	37.9^{i}	4.9^{h}	1.5^{j}	6.1^{i}	0.0
		Endocarp	52.3^{g}	69.4^{i}	0.0	2.7^{h}	11.0^{d}	45.7^{g}	0.0	0.0	0.0	0.0
PSD[A]			33.2	98.7	110.0	10.4	89.0	90.1	48.2	31.3	43.9	9.7
p (Hartley)[B]			1.0	0.8	0.9	1.0	1.00	0.80	1.00	1.00	1.00	1.00
p (ANOVA)[C]			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CQA 5-caffeoylquinic acid, *PLZ* phloridzin, *EPI* epicatechin, *CAT* catechin, *PB1* procyanidin B1, *PB2* procyanidin B2, *QGA* quercetin-3-D-galactoside, *QGL* quercetin-3-β-D-glucoside, *QRH* quercetin-3-*o*-rhamnoside, *QRU* quercetin-3-rutinoside, [A] *PSD* pooled standard deviation, [B] probability values obtained by Hartley test (F max) for homogeneity of variances, [C] probability values obtained by one-way ANOVA

Different letters in the same column represent statistical different results according to the Fischer LSD test ($p \leq 0.05$)

Anthocyanins were found exclusively in the epicarp of the fruits and their behavior in relation to maturation varied according to the variety. Ripe Eva apples showed the highest content of anthocyanins with a reduction in senescence. This was also observed in the Gala variety, although this compound was identified in the unripe fruit. In the Fuji Suprema apples, the highest content was found in the unripe samples (Table 1), probably because this variety displayed a red color from the fruit growth phase (Zielinski et al. 2014).

Unlike flavonoids, hydroxycinnamic acids were found predominantly in the endocarp of the fruit. In the Gala and Fuji Suprema varieties, the phenolic acid content decreased with ripening, while in the Eva variety it remained stable from the unripe to ripe stages and then increased with senescence. 5-caffeoylquinic acid, which is the main phenolic acid in apples (Tsao et al. 2005), was found in the highest concentrations in the endocarp of the unripe and ripe Eva and Gala varieties. At senescence, the levels decreased (86 and 58%, respectively) in the endocarp, and thus there were higher concentrations of 5-caffeoylquinic acid in the epicarp. In the Fuji Suprema variety, hydroxycinnamic acid was mainly present in the epicarp and its contents, as well as in other tissues; the levels increased with the ripening of the fruit (Table 2).

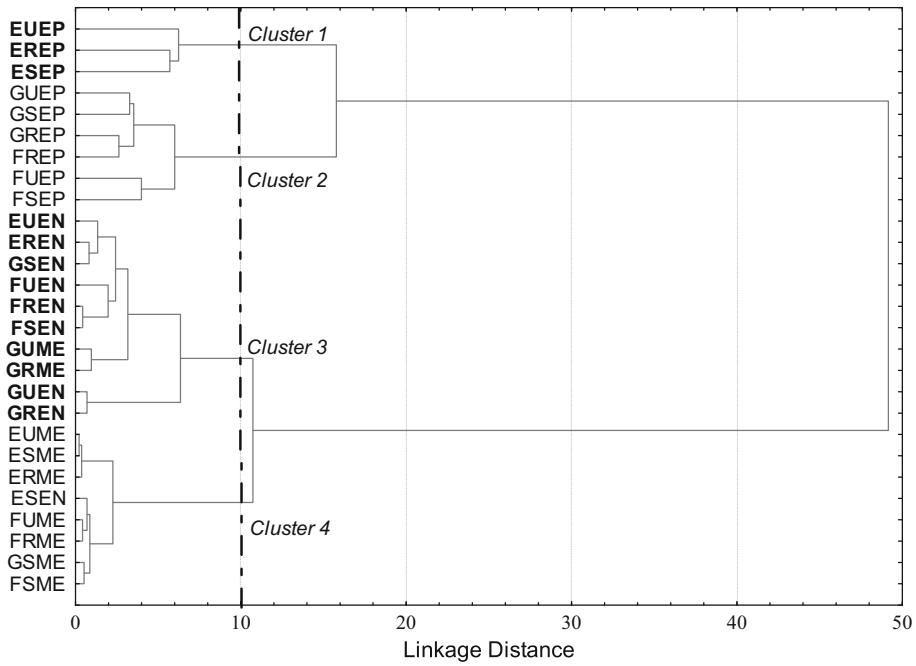

Fig. 1 Dendrogram for apple tissues at different ripening stages obtained from hierarchical cluster analysis. *EUEP* Epicarp of unripe Eva apple, *EREP* epicarp of ripe Eva apple, *ESEP* epicarp of senescent Eva apple, *GUEP* epicarp of unripe Gala apple, *GREP* epicarp of ripe Gala apple, *GSEP* epicarp of senescent Gala apple, *FUEP* epicarp of unripe Fuji Suprema apple, *FREP* epicarp of ripe Fuji Suprema apple, *FSEP* epicarp of senescent Fuji Suprema apple, *EUEN* endocarp of unripe Eva apple, *EREN* endocarp of ripe Eva apple, *ESEN* endocarp of senescent Eva apple, *GUEN* endocarp of unripe Gala apple, *GREN* endocarp of ripe Gala apple, *GSEN* endocarp of senescent Gala apple, *FUEN* endocarp of unripe Fuji Suprema apple, *FREN* endocarp of ripe Fuji Suprema apple, *FSEN* endocarp of senescent Fuji Suprema apple, *EUME* mesocarp of unripe Eva apple, *ERME* mesocarp of ripe Eva apple, *ESME* mesocarp of senescent Eva apple, *GUME* mesocarp of unripe Gala apple, *GRME* mesocarp of ripe Gala apple, *GSME* mesocarp of senescent Gala apple, *FUME* mesocarp of unripe Fuji Suprema apple, *FRME* mesocarp of ripe Fuji Suprema apple, *FSME* mesocarp of senescent Fuji Suprema apple

The antioxidant capacity are strong correlated with total flavonoids (FRAP: $r = 0.95$; DPPH, $r = 0.91$) of apple tissues. The highest positive correlation of epicatechin (FRAP: $r = 0.87$; DPPH, $r = 0.84$), procyanidin B2 (FRAP: $r = 0.89$; DPPH, $r = 0.86$) and quercetin-3-*o*-rhamnoside (FRAP: $r = 0.92$; DPPH, $r = 0.87$) suggests that they are major contributors to the antioxidant activity of the apple epicarp. According Firuzi et al. (2005), the *o*-dihydroxy structure in the B ring, the 2,3-double bond and the 3-hydroxy group in the C ring, contribute to antioxidant activity. Although phloridzin was found in all apple tissues at all ripening stages, there was weak correlation of DPPH ($r = 0.42$) and no significant correlation ($p > 0.05$) with FRAP method, as previously reported by Tsao et al. (2005). Differences in antioxidant activity results occurs due to the different mechanisms of FRAP and DPPH assays. DPPH is a stable free radical that when mixed with the antioxidant compound that can transfer a hydrogen atom or eletrons, is converted in its reduced form. On the other hand, FRAP is characterized by electron transfer ability, that result in the reduction of Fe (III) to Fe (II) by antioxidants (APAK et al. 2016).

Hierarchical cluster analysis (HCA) was applied to analyze all the data simultaneously and to evaluate the distribution of phenolic compounds in the apples, as well as the influence of variety and the ripening stages. Consequently, it was possible to separate the samples into four clusters, as shown in Fig. 1.

Cluster 1 consisted of the samples of Eva epicarp at the three analyzed ripening stages. The higher content of procyanidin B1, phloridzin, total dihydrochalcones and anthocyanins distinguished Cluster 1 from Cluster 2, which was formed by the epicarp of the Gala and Fuji Suprema varieties (Table 3).

Cluster 3 contained the samples of endocarp of the three varieties and the mesocarp of unripe and ripe Gala, while Cluster 4 consisted of the mesocarp of the three varieties and the endocarp of senescent Eva apples. The levels of 5-caffeoylquinic acid, hydroxycinnamic acid, phloridzin and total dihydrochalcones, which were mainly located in the endocarp, differentiated these two groups (Table 3). The presence of the samples of Gala mesocarp in the cluster formed mainly by fruit endocarp, and the endocarp of senescent Eva in the cluster formed by mesocarps shows

Table 3 Phenolic compounds (mg/kg) of the clusters of apple tissues according to HCA

Phenolic compounds	Cluster 1 (n = 3)	Cluster 2 (n = 6)	Cluster 3 (n = 10)	Cluster 4 (n = 8)	PSD[A]	p (ANOVA)[B]
5-Caffeoylquinic acid	1.8[c]	74.3[a]	50.2[b]	7.5[c]	21.7	<0.001
Total hydroxycinnamic acid	36.3[b]	132.9[a]	145.4[a]	50.6[b]	65.7	<0.001
Catechin	2.2[b]	18.4[a]	2.9[b]	0.0[b]	8.5	<0.01
Epicatechin	87.2[b]	260.7[a]	3.8[c]	4.6[c]	81.4	<0.001
Procyanidin B1	133.7[a]	38.6[ab]	1.1[b]	0.7[b]	82.4	0.05
Procyanidin B2	66.0[b]	226.6[a]	21.0[c]	11.1[c]	91.2	<0.001
Total Flavanols	747.6[b]	1761.2[a]	199.5[c]	89.2[c]	719.1	<0.001
Quercetin-3-D-galactoside	56.1[b]	108.7[a]	0.0[c]	0.7[c]	38.9	<0.001
Quercetin-3-β-D-glucoside	37.5[b]	59.9[a]	0.0[c]	0.2[c]	31.7	<0.001
Quercetin-3-*o*-rhamnoside	47.4[b]	99.8[a]	0.0[c]	0.9[c]	34.5	<0.001
Quercetin-3-rutinoside	11.1[a]	14.4[a]	0.0[b]	0.0[b]	4.4	<0.001
Total Flavonols	339.9[b]	685.0[a]	0.0[c]	6.3[c]	310.0	<0.001
Phloridzin	333.3[a]	78.2[b]	73.3[b]	10.5[c]	70.0	<0.001
Total Dihydrochalcones	490.6[a]	145.5[b]	106.9[b]	16.8[c]	148.3	<0.001
Total Anthocyanins	69.2[a]	38.2[b]	0.0[c]	0.0[c]	0.0	<0.001
Total Flavonoids	1685.6[b]	3380.6[a]	501.9[c]	272.0[c]	1310.6	<0.001
Total Phenolic compounds	2364.7[b]	4005.1[a]	907.0[c]	585.2[c]	1464.0	<0.001

[A] *PSD* Pooled standard deviation, [B] probability values obtained by One-way ANOVA

Different letters in the same line represent statistical different results according to the Fischer LSD test ($p \leq 0.05$)

the influence of the ripening stage in the content of phenolic compounds in apples.

The epicarp, mesocarp and endocarp differed qualitatively and quantitatively in terms of their phenolic composition. The epicarp had a higher content of phenolic compounds in all the analyzed classes (approximately 55%), except for phenolic acids, which were concentrated in the endocarp and mesocarp (around 42%). According to Zardo et al. (2013), the epicarp and mesocarp correspond to 7–10% and 73–83% of the weight of apples, respectively. However, compounds such as flavonols and anthocyanins are only present in the epicarp, indicating that consumption of apple skin is beneficial from a functional point of view.

Conclusion

The distribution of phenolic compounds and antioxidant capacity in apple depending on the variety, type of tissues and the ripening stage. The Fuji Suprema variety had the highest content of flavonols, while epicarp of Eva had the highest content of dihydrochalcones and anthocyanins (at ripe and senescent stage). The epicarp had a higher content of phenolic compounds in all the analyzed classes, except for phenolic acids, which were concentrated in the endocarp and mesocarp. The mesocarp contained lower content of phenols, however, if the percentage that corresponded to the fruit is analyzed, it provided greater quantity in the intake. Phloridzin was found in higher amounts in the endocarp of unripe fruits that decreased with ripening and consequently, in the senescent apples, the epicarp showed higher contents. In general, phenolic acids and flavonoids decrease with ripening in the epicarp and endocarp. However, in the mesocarp, the effect of the ripening is related with the apple variety. We would like to emphasize that the apple, in many countries it is one of the main sources of phenolic compounds. Therefore, future studies associated the distribution of phenols during the ripening process must be performed in others varieties in order to obtain an utilization with a higher functionality.

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