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EFFECT OF VARIETY, GROWING SEASON AND STORAGE ON POLYPHENOL PROFILE AND ANTIOXIDANT ACTIVITY OF APPLE PEELS

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Summary

Identification and quantification of the major polyphenols, and antioxidant activity (AOA), in the peel of apple varieties, Granny Smith (GS) and Gold Rush (GR), during storage and over two growing seasons (2011 and 2012) were examined.GR had higher amount of flavan-3-ols, dihydrochalcones, and flavonols compared to GS. Of all polyphenolic groups, flavonols were the most influenced by the climacteric conditions during the growing season. The higher amount of phenolic acids was detected in 2012 for the both apple varieties. Dihydrochalcones were influenced more by the variety than by the climacteric conditions during growing season. Changes in polyphenol content (TPC) and AOA during storage depended on the variety. Samples of apple peel powder, after storage, preserved the most of the antioxidants and functional properties, suggesting that apple peel powder may be used in a various food products to add phytochemicals and promote good health.

Keywords: Apple by-product, Phenolics, Freeze-drying, Antioxidant activity

Introduction

Recently, a general consensus has been achieved to sustain the hypothesis that the specific intake of foods and beverages, containing relatively high concentrations of phytochemicals such as flavonoids, may play a meaningful role in reducing cardiovascular disease (CVD) risk through an improvement in vascular function and a modulation of inflammation (Habauzi and Morand, 2012): Apples are generally considered "healthy food" with the existing saying "An apple a day keeps the doctor away". Like in other fruits and vegetables, polyphenols are the main ingredients that are considered to have a positive impact on health (Boyer and Liu, 2004; Xiuzhenet al.,2007; Hyson, 2011):The phytochemical composition of apples varies greatly between different varieties of apples, and it was found that there are also small changes in phytochemicals during the maturation and ripening of the fruit (Kondo et al.,2002; McGhieet al.,2005; Wojdyło et al.,2008):Ceymannet al.(2012) conducted study on 104 European apple varieties for 12 polyphenols by UHPLC–MS. This study is one of the more comprehensive evaluations of the polyphenol content and profile of different apple varieties so far. They identified two main classes of apples based on their polyphenol profile: those rich in flavan-3-ols and those rich in phenolic acids. From this and other studies it could be concluded that polyphenol profile of apples are highly variety dependent (Łata and Tomala, 2007; Łata, 2007; Matthes and Schmitz-Eiberger, 2008; Wojdyło et al., 2008; Neveuet al., 2010; Ceymannet al., 2012): The concentration of polyphenols is influenced by the environmental factors and by the geographic region, storage, and growing season. Various authors reported seasonal effect on antioxidant capacity and polyphenols (Matthes and Schmitz-Eiberger, 2008; Mainlaet al., 2011; Keverset al., 2011):Strackeet al., (2009) reported growing season variations in the antioxidant capacity and the polyphenol content up to 20%. In conflict to this van Der Sluiset al. (2001) and Guyotet al. (2003) observed little or no seasonal effect on antioxidant capacity and polyphenol concentration in apple varieties when they compared the results of different growing seasons. However, at the present time, the influence of pre-harvest factors on polyphenol profiles has only been investigated in relation to growing season, and the impact of the other pre-harvest factors on polyphenol profile remains unknown.

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Polyphenols seem to be stable during storage. Regarding apple peel, it was reported that phenolic metabolism in apple peel is relatively stable, and the health benefits of phenolics in apple peel should be maintained during long-term storage, while some studies reported increase of TPC (first 60 days) and decrease after 100 days (Lattanzioet al.,2001; van der Sluiset al.,2001; Golding et al.,2001; Napolitano et al.,2004): In contrast, some researchers found that epicatechin, quercetin glycosides and procyanidins in GS apples generally decreased during storage (Pirettiet al.,1994): The majority of researchers reported that no change occurred in the concentrations of simple phenols (mainly chlorogenic acid), flavonoids and anthocyanin during storage (Perez-Ilzarbeet al.,1997; Awad and de Jager, 2000; Golding et al.,2001):

In 2000, it was estimated that 2.7 million deaths (4.9%) and 26.7 million disability adjusted life years (DALYs; 1.8%) were attributable to low fruit and vegetable intake globally. Manny diseases could be prevented by increasing dietary intake of fruits and vegetables to the minimum recommended daily intakes established by the WHO (Lock et al.,2004): One way of increasing the intake of phytochemicals from fruit and vegetable is restoration and enrichment of food products with by-products obtained from fruits and vegetables processing industry.

During apple juice, sauce and canned apple manufacture, the antioxidant - rich peels of apples are discarded. It is known that apples and especially their skins have high concentration of phenolic compounds, dietary fiber, and minerals and may assist in the prevention of chronic diseases (Wolf et al., 2003; Biedrzyckaet al., 2008; Denis et al., 2013): Some authors found that on average 46% of the polyphenolics in the whole apples were in the skin (McGhieet al., 2005): It is well known that freeze–drying is superior process of dehydration. Freeze-drying utilizes the principle that under high vacuum, frozen water is directly removed by sublimation of ice without passing through intermediate liquid stage, those providing product with no damage, little or no loss in sensory qualities, and a porous honeycomb structure (Sethiet al.,2007):The aim of this work was to investigate how rich is the freeze-dried apple skin in polyphenols in order to be used in a various food products.

Materials and methods

Chemicals

Folin-Ciocalteu reagent was purchased from Kemika (Zagreb, Croatia), 2,6-dichlorophenol indophenols, phloretin, catechin, epicatechin, rutin, quercitin, chlorogenic acid, caffeic acid from Sigma Chemical Co. (St. Louis, USA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), 2,2-diphenyl-1-picrylhydrazyl, procyanidin B2 from Fluka (St. Louis, USA) phlorizin from Aldricht (St. Louis, USA), methanol (HPLC gradient grade), and o-phosphoric acid (85%) from Panareac (Barcelona, Spain):

Apple varieties used for experiment

Actual apple varieties, Granny Smith (GS), and Gold Rush (GR) were harvested from the Agricultural Institute (Osijek, Croatia) in 2011 and 2012. Both apple varieties were stored in the commercial storage facility under the normal atmosphere at 2-6 oC before they were purchased for study. Physical and chemical analysis was carried on the whole apples (flesh and peel): Total polyphenol content (TPC) and antioxidant activity (AOA) of the apple peels were evaluated immediately after harvesting and after freeze–drying, and after 1 and 6 months of storage.

Physical and chemical analysis

Apples were held at room temperatures for cca 1 h before preparing for analysis. Before the apple fruits were disintegrated (Braun Multiquick Professional 600 Watt Turbo) for the analysis the core was removed. Content of soluble solids of apples were measured with table Abbe refractometer and is given in Brix (°Brix): Acids were measured by titration with 0.1 M NaOH and phenolphthalein as an indicator and given in g/100 g, as malic acid. Reducing and total sugar content was determined by Luff Schoorl's method (Eganet al., 1981), and vitamin C (L-ascorbic acid) by volumetric method - titration with di-

chlorophenol indophenol (DCPIP):

Freezing and freeze – drying

Before freeze-drying apple peels were firstly disintegrated and then placed in a plastic bags and frozen at -18 °C for 12 h before freeze-drying in laboratory freeze-dryer (Christ Freeze Dryer, Gamma 2-20, Germany): Drying conditions were as follows: freezing temperature: -55 °C; the temperature of sublimation: -35 °C to 0 °C; and the vacuum level: 0.220 mbar. The temperature of isothermal desorption varied from 0 °C to 22 °C under the vacuum of 0.060 mbar. Freeze-drying lasted about 48 h until the total content of solids was 94-98%.

Determination of total polyphenol content

The extraction of polyphenols from prepared apple peels was carried out with acidified methanol (1 g apple purée in 10 mL acidified methanol): The samples were held at the ambient temperature for 1 h. After 1 h mixture was filtered through pleated filter paper. The extracts were used for the determination of TPC and AOA. Extraction of phenolics for identification of phenolics were performed as follows: phenolics were extracted from freeze-dried samples (250 mg) using 80% aqueous methanol (5 mL): The mixture was sonicated for 15 min and centrifuged at the room temperature for 15 min. After extraction samples were filtered through 0.45 μm poly(tetrafluoroethylene) syringe-tip filter and extracts were used for HPLC analysis.
The TPC was determined by the modified colorimetric Folin-Ciocalteu method (Ough and Amerine, 1988): 0.2 mL of apple extract and 1.8 mL of deionizer water were added to a 23 mL test tube. 10 mL of Folin-Ciocalteu reagent (1:10) was added to the solution, and finally 8 mL of 7.5% of sodium carbonate (Na2CO3) solution was transferred into the test tubes. The color was developed in 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK): The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as g of gallic acid per kg of sample equivalents (g GAE/kg) of sample.

Antioxidant activity determination

2,2′-Azino-bis-3ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS) scavenging activity
ABTS assay followed the method of Arnaoet al.(2001) with some modifications. The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured ABTS value was over the linear range of the standard curve.
1, 1-Diphenyl-2-picryl-hydrazil (DPPH•) scavenging activity
For DPPH assay 0.2 mL of the apple extract was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min the absorbance was measured at 517 nm (Brand-Williams et al.,1995): The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve.

Identification of phenolics

The analytical HPLC system employed consisted of a Varian LC system (USA) equipped with a ProStar 230 solvent delivery module, and ProStar 330 PDA Detector. Phenolic compounds separation was done in an OmniSpher C18 column (250 x 4.6 mm inner diameter, 5 μm, Varian, USA protected with guard column (ChromSep 1 cm x 3 mm, Varian, USA): The data were collected and analyzed on IBM computing system equipped with Star Chromatography Workstation software (version 5.52): The same solvents and gradient elution program were used in determination of phenolic acids and flavonols. Solvent A was 0.1% phosphoric acid and solvent B was 100% HPLC grade methanol. The elution conditions were as follows: 0-30 min from 5% B to 80% B; 30-33 min 80% B; 33-35 min from 80%, B to 5% B; with flow rate=0.8 mL/min (Jakobeket al.,2007): Phenolic standards were used to generate characteristic UV – vis spectra and calibration curves. Individual phenolics in the sam-

ple were tentatively identified by comparison of their UV – vis spectra and retention times with spiked input of polyphenolic standard. Three replicated HPLC analyses were performed for each sample.

Statistical analysis

All measurements were done in triplicate and data were expressed as mean ± standard deviation. The experimental data were subjected to an one-way analysis of variance (ANOVA) and Fisher's LSD were calculated to detect significant difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program MS Excel (Microsoft Office 2007 Professional):

Results and discussion

Effect of variety

Apples, and especially their skins, contain the polyphenol groups (flavonols, flavan-3-ols, phenolic acids, dihydrochalcones) of which the main compounds are: epicatechin, procyanidin B2, phloretin xyloglucoside, phloridzin, and chlorogenic acid. GS and GR apple peel contained an additional six quercetin glycoside (glucoside-galactoside, rhamnoside, xyloside, arabinoside, and rutinoside): Differences in phenolic profile between GS and GR apple peel were only in levels of certain phenolic compounds. Apple variety GS contained the lower sum of polyphenols determined by HPLC than GR.

Table 1. The physicochemical parameters of GS and GR apple varieties

	Granny Smith		Gold Rush	
Parameters	2011	2012	2011	2012
The average fruit weight (g)	206.83 ± 11.2	168.51 ± 17.5	192.74 ± 12.6	107.95 ± 9.3
Hardness (kg/cm2)	9.9 ± 0.170	9.64 ± 0.090	10.33 ± 0.123	10.02 ± 0.206
Moisture (%)	83.76 ± 0.104	81.49 ± 0.111	82.84 ± 0.034	82.89 ± 0.014
Soluble solids (° brix)	12.03 ± 0.150	14.63 ± 0.060	15.00 ± 0.000	14.13 ± 0.120
L-ascorbic acid (mg/100 g)	5.47 ± 0.240	7.80 ± 0.201	8.42 ± 0.450	4.75 ± 0.000
Acids (g/100 g of malic acid)	0.48 ± 0.010	0.63 ± 0.000	0.51 ± 0.040	0.38 ± 0.019
pH	3.33 ± 0.010	3.31 ± 0.014	3.72 ± 0.02	3.80 ± 0.016
Sugars				
Reducing	6.45 ± 0.24	7.25 ± 0.034	6.21 ± 0.12	7.35 ± 0.018
Total	9.19 ± 0.180	9.86 ± 0.028	8.08 ± 0.14	9.27 ± 0.072
TPC (g EGA/kg)				
Peel	3.11 ± 0.138	2.88 ± 0.062	3.53 ± 0.059	3.57 ± 0.072
Peel + Flesh	1.06 ± 0.034	1.29 ± 0.068	1.07 ± 0.024	1.14 ± 0.007
Flesh	0.76 ± 0.015	0.90 ± 0.046	0.97 ± 0.049	1.05 ± 0.019
AOA				
ABTS (mmol TE/100ml)				
Peel	47.89 ± 0.07	49.28 ± 1.127	54.08 ± 0.686	54.83 ± 0.259
Peel + Flesh	7.00 ± 0.242	7.35 ± 1.068	11.99 ± 0.484	12.78 ± 0.880
Flesh	4.26 ± 0.348	3.35 ± 0.816	9.06 ± 0.461	8.92 ± 0.432
DPPH (mmol TE/100ml)				
Peel	3.89 ± 0.299	4.03 ± 0.116	4.73 ± 0.064	4.61 ± 0.095
Peel + Flesh	1.69 ± 0.226	2.19 ± 0.052	1.79 ± 0.045	3.04 ± 0.070
Flesh	1.62 ± 0.008	2.04 ± 0.122	1.77 ± 0.048	2.66 ± 0.094

Epicatechin with 8.6 – 14.1 mg/100 g and procyanidin B2 with 4.3 – 8.0 mg/100 g contributed the most to the total flavan-3-ol content. The phenolic acids were dominated by chlorogenic acid with <lod-13.6 mg/100 g. Dihydrochalcones (phloridzin and phloretin-xyloglucoside) were found in both varieties, but amount of dihydrochalcones in GS were relatively lower compared to those in GR (0.3 – 1.4 mg/100 g and 0.4 – 1.3 mg/100 g, respectively): Flavonols were present in the range of 6.6 to 14.5 mg/100 g, among which rutin accounted for approximately half of the flavonols (4.0 - 5.6 mg/100 g): Comparing the polyphenol patterns of GS and GR, it can be seen that the total levels of flavan-3-ols and flavonols are similar in GS (2012) and GR (2011), but both varieties had slightly higher amounts of flavan-3-ols than flavonols. In samples GS (2011) and GR (2012) dominant polyphenol group was flavan-3-ols. Dihydrochalcones and phenolic acids contribute with proportions of approximately 4% and 20%, respectively to the polyphenol pattern of both analyzed varieties (Table 2): Summing up these polyphenol groups, analyzed by HPLC (Table 2), the amounts were between 27 and 50 mg/100 g FM (depending on growing season) which is lower than the TPC determined by the Folin-Ciocalteu method with 288 to 357 mg gallic acid equivalents/100 g (Table 1): This comparison between these two methods showed that the latter was several times higher than the sum of polyphenols calculated from HPLC data. This trend was found in both apple varieties. The difference can be explained by poor specificity of the Folin-Ciocalteu assay, which is known to measure additional components present in the extract (Singleton et al.,1999):Vrhovseket al.(2004) excluded these substances by an additional cleanup step on a C-18 cartridge. The consequence of this additional clean up step is that the TPC reported by Vrhovseket al.(2004) are lower than reported by other studies. For example, Sanoneret al.(1999) reported 128 mg epicatechin equivalents/100 g in the cortex area of Golden Delicious using epicatechin as a reference, compared to 86 mg catechin equivalents/100 g in the same variety reported by Vrhovseket al.(2004): GR had also higher AOA (Table 1): Higher AOA is associated with higher levels of procyanidin B2 and epicatechin, which is in accordance to results reported by Tsaoet al. (2005) for procyanidin B2 and epicatechin, which were the major contributors to the AOA of apple. Besides, GS apple fruits were bigger in size, with higher acid content, but of similar hardness and moisture content as GR apples.

Table 2. Polyphenol profile of apple peels of GS and GR apple varieties (mg/100g fw) in two apple seasons.

		Granny Smith		Gold Rush	
Polyphenols		2011	2012	2011	2012
Procyanidin B2		4.25 ± 0.024^{c}	4.31 ± 0.081^{c}	5.68 ± 0.032^{b}	7.97 ± 0.062^{a}
Phloridzin		0.50 ± 0.163^{c}	0.26 ± 0.016^{d}	1.28 ± 0.016^{a}	1.38 ± 0.081^{b}
(-)Epicatchin		8.86 ± 0.260^{c}	8.60 ± 0.1.65^{c}	9.60 ± 0.098^{b}	14.13 ± 0.227^{a}
Phloretin xyloglucoside		0.37 ± 0.054^{c}	0.37 ± 0.022^{c}	1.31 ± 0.013^{a}	1.02 ± 0.217^{b}
Chlorogenic acid		1.56 ± 0.193^{c}	10.24 ± 0.010^{b}	<lod	13.63 ± 0.044^{a}
Caffeic acid		1.24 ± 0.035^{a}	0.18 ± 0.001^{c}	<lod	0.18 ± 0.004^{b}
Quercetin* glycoside	23.517	0.70 ± 0.038^{b}	<lod	2.51 ± 0.029^{a}	<lod
	24.280	0.89 ± 0.025^{c}	1.72 ± 0.034^{a}	0.82 ± 0.005^{d}	1.48 ± 0.050^{b}
	24.605	1.07 ± 0.034^{b}	0.91 ± 0.019^{c}	3.29 ± 0.035^{a}	<lod
	24.848	<lod**	2.34 ± 0.036^{b}	2.73 ± 0.018^{a}	1.76 ± 0.058^{c}
	25.605	<lod	1.44 ± 0.008^{b}	0.37 ± 0.003^{c}	2.52 ± 0.088^{a}
Rutin		3.96 ± 0.322d	5.47 ± 0.089^{b}	4.78 ± 0.338^{c}	5.54 ± 0.020^{a}

**Quercetin glycoside retention times.*

***<lod: below limit of detection (<0.15 mg/100 g). Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at p ≤ 0.05, (ANOVA, Fisher's LSD).*

Effect of growing season

All apples are collected from the orchard of the Agricultural institute Osijek near Osijek (45° 32.041', 18° 45.121'): Harvest dates, localization, tree cultivation technique were the same in both growing seasons. Weather conditions are presented in Fig. 1.

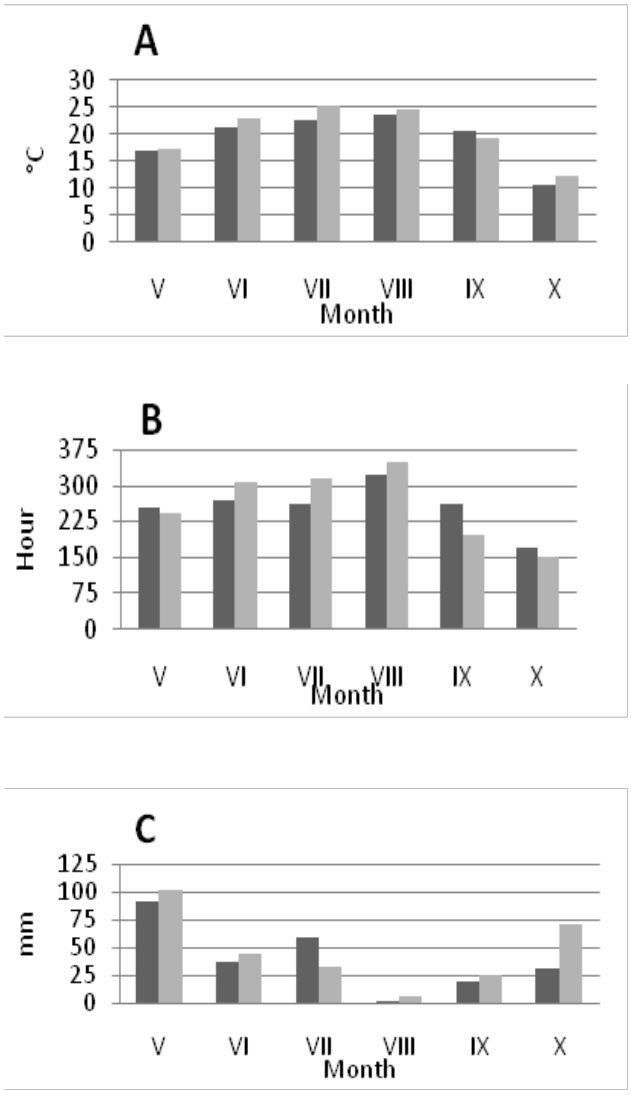

Fig. 1. The weather conditions for growing season 2011 and 2012. A - Mean monthly air temperature (°C); B – Sunshine (h); C –Precipitation (mm) in growing season.

According to data from 1960th to 2012, in both growing season, the mean air temperature was above average except for September (2011) 10.7 °C, average is 11.2 °C. During June, July and August (2011, 2012) the weather was fairly dry 1.7 - 90.46% of the average precipitation for these three months fell. Only in May (2011, 2012) and September (2012), the precipitation was more than average for this period. Sufficient water supply may result with higher fruit weight; in arid years the fruit weight may be reduced including a lower dilution of the polyphenols in fruits, in contrast to a high fruit weight with a greater dilution. Sunlight hours in both growing seasons were longer than the average; however duration was slightly higher in 2012. Jackson and Lombard (1993) reported that high content of polyphenols is induced during ripening at mean air temperatures between 9° and 29°C or high radiation level. This report is consistent with our results since the higher polyphenol content was measured in 2012 compared to 2011. From these results we can also see that depending on growing season the level of polyphenol classes, flavan-3-ols and flavonols was inversely proportional. GS had higher amount of flavan-3-ols in 2011 and lower amount of flavonols compared to 2012, where we observed higher amount of flavonols and lower amount of flavan-3-ols. We have noticed opposite effect for GR where we found higher amount of flavonols and lower amount of flavan-3-ols compared to 2012, where we observed a higher amount of flavan-3-ols and lower amount of flavonols. Flavonols were the more influenced by the growing season. The extent of sun exposure could be a reason for those findings, because flavonols are located in the skin of the apple and their production is induced by sunlight (Awadet al.,2001): Additionally, the elevated light level provides more energy for carbon assimilation and thus more carbon resources for biosynthesis of polyphenols (Treutter, 2010): The highest amount of phenolic acids was detected in 2012 for both varieties. Polyphenol profiles remained fairly constant over the two years for both varieties, but amount of dihydrochalcones were influenced more strongly by the variety than by the growing year. That is in agreement with Guyotet al.(2003) who concluded an overall stability of polyphenol composition from one year to another, but also found significant year-to-year variations in the levels of individual polyphenol compounds, comparable to our own results. The significant difference between apples, in two growing seasons, was in the fruit size. Mean fruit

weight varied from 206.83 ± 11.2 g to 168.51 ± 17.5 g per fruit for GS, and 192.74 ± 12.6 g to 107.95 ± 9.3 g per fruit for GR in 2011 and 2012, respectively. The level of soluble solids, L-ascorbic acid and acids was higher in 2012 for GS, but in contrast to these levels they were lower for GR in 2012 (Table 1):Considering sugars both varieties had higher amount of reducing and total sugars in 2012.

Effect of storage

Freeze-dried apple peel was stored (180 days) in hermetically closed glass dish, and kept at room temperature. The TPC of GS was increased during the storage, while TPC of GR was decreased. Same trend was observed with AOA.

Table 3. Total phenol content and antioxidative activity of apple peel powder of GS and GR apple variety.

Apple variety		Granny Smith		Gold Rush	
Growing season		2011	2012	2011	2012
TPC (g EGA/kg)	0 day	1.79 ± 0.040	2.30 ± 0.106	3.19 ± 0.080	2.76 ± 0.087
	30 day	2.04 ± 0.030	2.49 ± 0.060	2.91 ± 0.050	2.40 ± 0.056
	180 day	2.06 ± 0.020	2.67 ± 0.121	2.77 ± 0.060	2.47 ± 0.261
ABTS (mmol TE/100ml)	0 day	10.74 ± 0.640	26.73 ± 0.766	46.00 ± 0.170	36.29 ± 0.216
	30 day	31.79 ± 0.360	29.99 ± 0.998	31.50 ± 0.050	34.41 ± 0.727
	180 day	30.79 ± 0.450	28.80 ± 0.116	29.89 ± 0.160	25.18 ± 0.369
DPPH (mmol TE/100ml)	0 day	2.51 ± 0.150	1.49 ± 0.029	2.37 ± 0.040	1.85 ± 0.058
	30 day	2.78 ± 0.080	2.88 ± 0.086	1.30 ± 0.005	2.71 ± 0.066
	180 day	1.79 ± 0.050	1.27 ± 0.133	0.52 ± 0.040	0.64 ± 0.044

An increase of TPC during storage was also reported by Mareczeket al. (2000); Awad and de Jager (2000); Lattanzioet al.(2001): After storage the levels of phloridzin, (-) epicatechin and phloretin xyloglucoside of GS peel remained approximately at the same levels. However, there was significant increase of procyanidin B2 and quercetin glycosides. After the storage, flavan-3-ols, dihydrochalcones and flavonols, in GS peel, were present in the range of 19.7 – 20.6 mg/100 g; 1.1 – 1.3 mg/100 g; 11.5 – 15.7 mg/100 g in 2011 and 2012, respectively. This increase also increased AOA of GS peel (Table 3): Procyanidin B2, quercetin and its glycosides are

Table 4. Polyphenol profile of apple peel powder of GS and GR apple varieties (mg/100g fw) after storage.

		Granny Smith		Gold Rush	
Polyphenols		2011	2012	2011	2012
Procyanidin B2		4.72 ± 0.098^{c}	4.59 ± 0.048^{c}	5.93 ± 0.204^{b}	8.22 ± 0.275^{a}
Phloridzin		0.46 ± 0.011^{d}	0.78 ± 0.044^{c}	1.23 ± 0.147^{b}	1.36 ± 0.115^{a}
(-)Epicatchin		8.88 ± 0.030^{b}	9.06 ± 0.054^{b}	9.58 ± 0.108^{b}	14.35 ± 0.149^{a}
Phloretin xyloglucoside		0.62 ± 0.051^{c}	1.09 ± 0.022^{a}	0.89 ± 0.036^{b}	0.91 ± 0.086^{b}
Chlorogenic acid		<lod*	<lod	<lod	4.11 ± 0.020^{a}
Caffeic acid		<lod	<lod	<lod	<lod
Quercetin* glycoside	23.517	<lod	<lod	<lod	<lod
	24.280	1.82 ± 0.002^{bc}	3.03 ± 0.018^{a}	1.72 ± 0.010^{c}	1.95 ± 0.004^{b}
	24.605	1.17 ± 0.063^{a}	1.16 ± 0.008^{a}	<lod	<lod
	24.848	2.77 ± 0.048^{b}	4.48 ± 0.028^{a}	1.84 ± 0.016^{d}	2.27 ± 0.006^{c}
	25.605	2.30 ± 0.004^{c}	2.66 ± 0.018^{b}	2.36 ± 0.022^{c}	3.37 ± 0.013^{a}
Rutin		3.61 ± 0.046^{b}	4.39 ± 0.1.88^{a}	3.36 ± 0.008^{c}	4.43 ± 0.143^{a}

*Quercetin glycoside retention times.

**<lod: below limit of detection (<0.15 mg/100 g).Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD).

considered to be a strong antioxidant due to their ability to scavenge free radicals. The increase of AOA can also be explained by the presence of polyphenols with an intermediate oxidation state which can exhibit higher radical scavenging activity than the non-oxidized ones. Storage also influenced the level of dihydrochalcones and phenolic acids of GR, in both experimental years, while the level of flavonols was lower in 2012. However, levels of flavan-3-ols were higher after storage. Lattanzioet al.(2001), also, reported that after 60 days of storage the concentration of total phenolics in the skin of Golden Delicious apples increased. After the storage, flavan-3-ols, dihydrochalcones and flavonols, in GR peel, were present in the range of 18.5 – 22.6 mg/100 g; 2.1 – 2.3 mg/100 g; 9.3 – 12.0 mg/100 g in 2011 and 2012, respectively. Phenolic acids were the most susceptible to changes during storage. After the storage there was a complete loss of phenolic acids, in GS peel, only in the case of GR harvested in (2012) the loss was approximately 70%.

Conclusion

Differences in phenolic profile between compared apple varieties were only in levels of phenolic compounds. GR had higher amount of flavan-3-ols, dihydrochalcones, and flavonols compared to GS. Of all the polyphenol groups, flavonols were more influenced by the growing year. The extent of sun exposure, during growing period, could be a reason, because flavonols are located in the skin of the apple and their production is induced by sunlight. For phenolic acids the differences between the growing seasons were significant and the highest amounts were detected for both varieties harvested in 2012. Dihydrochalcones were influenced more by the variety than by the growing season. During storage changes in TPC and AOA depended on the apple variety. The greatest difference was observed in levels of procyanidin B2 and flavonols. Apple peel powder, stored for 1-6 months, preserved the most of antioxidants and functional properties, suggesting that apple peel powder may be used in a various food products to add phytochemicals and promote good health.

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