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Influence of Cold Storage on Quality of Croatian Traditional Apple Cultivars

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Abstract

The aim of this work was to investigate the influence of cold storage at 2 °C for 180 days on water content, total acids, reducing and total sugar, antioxidant activity, polyphenol oxidase activity, and polyphenol profile of three Croatian traditional apple cultivars ('Kanadska Reneta', 'Ilzer Rosenapfel', 'Wagener'). A significant variation in polyphenol profile of apple cultivars under investigation was noted. The most represented polyphenols in all apple cultivars were procyanidin B2 followed by chlorogenic acid, epigallocatechin, epicatechin, and phloridzin. After storage, the content of procyanidin B2 and chlorogenic acid was higher, while content of epigallocatechin after storage was lower in all investigated apple cultivars. Water content before storage ranged between 79.17 and 80.76 %, and after storage between 75.62 and 78.56 %. Total acids, polyphenol oxidase activity, and flavonoids had decreased during storage, while reducing and total sugar content was higher after storage. Moreover, after storage, cultivar 'Kanadska Reneta' had the highest antioxidant activity measured by both ABTS and DPPH methods, 1.65 and 0.70 mmol trolox kg^{-1}, respectively. A significant variation in polyphenol profile of apple cultivars under investigation was noted. The most represented polyphenols in all apple cultivars were procyanidin B2, followed by chlorogenic acid, epigallocatechin, epicatechin, and phloridzin. After storage, the content of procyanidin B2 and chlorogenic acid was higher, while content of epigallocatechin after storage was lower in all investigated apple cultivars. Overall, it can be concluded that investigated apple cultivars, after storage, preserved most of the antioxidants and functional properties.

Keywords

Kanadska Reneta, Ilzer Rosenapfel, Wagener, polyphenol profile, antioxidant activity

1 Introduction

The focus on fresh fruits has increased in recent years for their nutritional and health benefits. Apples are largely consumed as fresh fruit, but they can also be processed under different products, and therefore constitute a main ingredient in the human diet. Like in other fruits and vegetables, polyphenols are the main ingredients that are considered to have a positive impact on health. It was confirmed that the regular use of apples in a diet contributes significantly to the intake of polyphenols. The consumption of apples has been linked to the prevention of degenerative diseases; reduction in the risk of lung cancer, asthma, type-2 diabetes, thrombotic stroke, ischemic heart disease, and anti-proliferative activities have been attributed to apple consumption.[1]

The maintenance of quality after harvest is an important topic for fresh horticulture crops. Apples are harvested generally in the late summer and autumn, but are available to consumers the entire year. Cold storage is the basis for maintaining fruit quality over extended periods. Lowering the storage temperature lowers the rate of deterioration (*i.e.,* quality is maintained and shelf life increased), and can delay the onset of ripening.[2] Several factors, such as environmental conditions, cultivars, cultural practices, time of harvest, and postharvest conditions, determine the quality of these commodities. However, it is well established that the cultivar plays a major role in controlling the composition of biochemical compounds in apples.[3]

Fruits undergo several changes during harvesting, transportation, and postharvest storage, which affect the nutritional compounds and enzymes involved in the metabolism of those compounds. The changes during prolonged storage periods are related to the taste, nutritional quality, and shelf life of the product. Since every commodity shows different responses through storage, it is difficult to preserve the nutritional quality of all fruits with a single technology.[4] Such changes may not always result in a reduction in the health-promoting compounds. The concentration of phytochemicals and antioxidant activity in some fruits and vegetables were actually enhanced by postharvest storage and processing parameters.[5] Considering polyphenols, they 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 an increase in TPC (first 60 days) and a decrease after 100 days.[6–8] In contrast, some researchers found that epicatechin, quercetin glyco-

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sides, and procyanidins in Granny Smith apples generally decreased during storage.[9] The majority of researchers reported that no change occurred in the concentrations of simple phenols (mainly chlorogenic acid), flavonoids, and anthocyanin during storage.[10–12]

The aim of this work was to investigate the influence of cold storage at 2 °C for 180 days on polyphenol profile, water content, total acids, reducing and total sugars, antioxidant activity, and polyphenol oxidase activity of three Croatian traditional apple cultivars ('Kanadska Reneta', 'Ilzer Rosenapfel', 'Wagener').

2 Experimental

2.1 Plant material

The traditional apple cultivars 'Kanadska Reneta', Ilzer Rosenapfel' and 'Wagener' were collected at Šašinovec (45°85'00.3"N, 16°17'75.2"E). Cultivars were grafted on semi-dwarf rootstocks MM 106, and trees were planted at a distance of about 5 m between rows and 4 m within. All studied apple cultivars were authenticated by a pomologist. Traditional apple cultivars were stored in a cold chamber at 2 °C for 180 days. Extraction of bioactive polyphenolic compounds was performed by Ultrasound (Elma, Elmasonic P 70 H). Average sample was prepared from 10 apples of each cultivar, which were previously lyophilised (Christ, Osterode am Harz, Germany) and pulverised. The 250 mg of average sample was mixed with extraction solvent (80 % MeOH in water). Ultrasound-assisted extraction (UAE) was performed in an ultrasonic bath at 35 kHz for 15 min in 20 ml test tubes. Each extraction was performed in triplicate.

'Kanadska Reneta' 'Ilzer Rosenapfel' 'Wagener'

Fig. 1 – Traditional apple cultivars used in the experiment
Slika 1 – Tradicionalne sorte jabuka upotrijebljene u eksperimentu

2.2 Determination of physicochemical composition

Physicochemical composition of selected apples encompassed the determination of water content in a vacuum dryer at 65 °C and 60 mbar to constant weight, determination of soluble dry matter with table Abbe refractometer and is given in Brix (°Brix), pH value with a pH meter (Mettler Tolledo Columbus, Ohio, SAD). 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.

2.3 Determination of antioxidant activity

The 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) scavenging activity ABTS assay followed the method of *Arnao et al.*[13] with some modifications. An amount of 3.2 ml of ABTS solution was added to 0.2 ml of the apple extract. After an hour and 35 min, the absorbance was measured at 734 nm. The results were expressed as mmol trolox equivalents/kg of sample. Additional dilution was needed if the measured ABTS value was above the linear range of the standard curve.

For 1,1-diphenyl-2-picryl-hydrazil (DPPH•) scavenging activity assay, 0.2 ml of the apple extract was diluted with methanol (2 ml), and 1 ml of DPPH ethanol solution (0.5 mM) was added. After 15 min, the absorbance was measured at 517 nm.[14] The results were expressed as mmol trolox equivalents/kg of sample. Additional dilution was needed if the measured DPPH value was above the linear range of the standard curve.

2.4 Determination of polyphenol oxidase activity

Polyphenol oxidase (PPO) activity was determined by measuring the initial rate of quinone formation, as indicated by an increase in the absorbance units (AUs) at 420 nm according to *Sikora et al.*[15]

2.4 Determination of polyphenols

The total polyphenol content in the traditional apple were measured by using Folin-Ciocalteu method with several modifications. The procedure can be found in our previous work.[16] The absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, Stone, UK). For each sample, the measurements were performed in triplicates, and the average value was interpolated on a gallic acid calibration curve and expressed as g kg^{-1}. Monomeric anthocyanins were determined using the method described by *Giusti et al.*[17] Total monomeric anthocyanins were expressed as cyanidin-3-glucoside, and the obtained values were expressed as mg kg^{-1}.

The total flavonoid content was determined according to *Makris et al.*[18] Briefly, 0.5 ml of extract was mixed with 4 ml distilled water, then 0.3 ml 5 % $NaNO_2$ was added and allowed to react for 5 min. Following this, 0.3 ml 10 % $AlCl_3$ was added, and the mixture allowed to react for a further 5 min. At the end, 2 ml 1 M Na_2CO_3 and 2.4 ml distilled water were added to the reaction mixture, and the absorbance at 510 nm was read against a blank. For each sample, the measurements were performed in triplicates, and values were interpolated on calibration curve using catechin as a standard and expressed as g catechin equivalents *per* kg of apple (g CE/kg).

Individual polyphenols were determined by high-performance liquid chromatography (HPLC) which was performed on the Shimadzu HPLC instrument, equipped with the SIL-10 AF autosampler, the LC-20AD pump and the SPD-M20A PDA detector. LabSolution Life (Release 5.52) controlled the system. The mobile phase consisted of A

(water containing 1 % formic acid) to B (methanol containing 1 % formic acid). The 10 µl of sample was injected in duplicate onto the column kept at 50 °C, and the flow rate of mobile phase was 0.8 ml min^{-1}. The UV-Vis absorption spectra of the standards, as well as the samples, were recorded in the range of 190 to 600 nm. Determined were five flavanols, four flavonols, five phenolic acids, two dihydrochalcones, and three anthocyanins by the comparison of their retention time and UV-Vis spectra to those of pure standards and detected at 280, 320 and 360, and 520 nm. The concentration of polyphenols was expressed as µg g^{-1}. Table 1 presents the results of physicochemical composition of traditional apple cultivars, and Table 2 polyphenol the profile of traditional apple cultivars during storage.

3 Results and discussion

The results of physicochemical composition of traditional apple cultivars during storage are shown in Table 1. The investigated apple cultivars showed to have a water content between 75.62 and 80.76 %. During storage, the water content is reduced, which is expected and well documented in previous research.[19] Total soluble solids were in the range 15.80–19.40 %, and increased during storage due to water loss. The obtained results regarding the total acids indicated a decrease in total acids during storage in all investigated apple cultivars. The content of reducing and total sugars had increased during storage, which was confirmed by the results of total soluble solids, and the values before and after storage were in the range of the results obtained by *Kim et al.*[20] The obtained content of total polyphenols, flavonoids, and anthocyanins (Table 1) was in the range of those reported by other authors.[21–24] The content of total polyphenols and flavonoids before and after storage was the highest in cultivar 'Kandska Reneta' (604.62 and 600.13 g kg^{-1} and 374.89 and 183.91 g kg^{-1}, respectively). The cultivar 'Ilzer Rosenapfel' had the highest total anthocyanin content both before and after storage (10.77 mg kg^{-1} and 2.50 mg kg^{-1}, respectively). In some cases, the antioxidant activity increased after storage; however, the higher antioxidant activity could be a consequence of the oxidation of polyphenols.[25,26] On the other hand, the content of PPO activity decreased in all samples after storage.

The results of individual polyphenols obtained by HPLC are shown in Table 2. The most dominant individual polyphenols in investigated apple cultivars were chlorogenic acid, epigallocatechin, phloridzin, and procyanidin B2. The results suggested that the amount of individual polyphenols had decreased during storage (idaein chloride, gallic acid, epigallocatechin, caffeic acid, procyanidin A2, catechin, phloridzin and epicatechin), while the proportion of some polyphenols increased (*trans*-ferulic acid, chlorogenic acid, *p*-coumaric acid, myricetin, quercetin-3-rutinoside, quercetin-3-D-glucoside, procyanidin B1 and procyanidin B2). The greatest decrease was recorded in the 'Kandska Reneta', where the content of epigallocatechin decreased from 1186.69 to 524.13 µg g^{-1}. The greatest increase was recorded for chlorogenic acid, procyanidin B1 and B2. As for phloridzin, its value decreased during cold storage from 580.41 to 465.31 µg g^{-1} in the apple cultivar 'Kanadska Reneta', while in cultivars 'Ilzer Rosenapfel' and 'Wagener' its value increased (217.96–249.20 µg g^{-1} and 325.89–384.33 µg g^{-1}, respectively) (Table 2).

Table 1 – Physicochemical composition of traditional apple cultivars during storage
Tablica 1 – Fizikalno-kemijski sastav tradicionalnih sorti jabuka tijekom skladištenja

	Kandadska Reneta		Ilzer Rosenapfel		Wagener	
	0 months	6 months	0 months	6 months	0 months	6 months
water content / %	80.76 ± 0.02	78.24 ± 0.83	79.17 ± 0.12	78.56 ± 0.30	79.61 ± 0.09	75.62 ± 0.32
total soluble solids content / %	15.80 ± 0.00	19.4 ± 0.00	15.70 ± 0.00	17.9 ± 0.00	16.40 ± 0.00	19.4 ± 0.00
pH	3.16	3.42	3.25	3.75	3.04	3.59
total acids / g/100 ml	0.13 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.07 ± 0.00	0.21 ± 0.00	0.09 ± 0.00
natural sugars / g/100 g	10.68 ± 0.02	12.71 ± 0.00	7.43 ± 0.11	10.55 ± 0.00	7.80 ± 0.10	11.29 ± 0.02
total sugars / g/100 g	15.25 ± 0.00	16.51 ± 0.00	12.38 ± 1.28	16.51 ± 0.00	12.86 ± 0.21	16.98 ± 0.03
total polyphenols / g kg^{-1}	604.62 ± 8.01	600.13 ± 23.35	299.49 ± 4.53	430.90 ± 18.26	146.92 ± 12.69	210.38 ± 5.09
flavonoids / g CE kg^{-1}	374.89 ± 1.51	183.91 ± 1.64	166.36 ± 2.05	99.79 ± 3.76	70.19 ± 1.61	38.32 ± 0.00
antocyanins / mg kg^{-1}	0.00 ± 0.00	0.00 ± 0.00	10.77 ± 0.00	2.505 ± 0.00	0.75 ± 0.00	0.00 ± 0.00
DPPH	0.56 ± 0.00	0.70 ± 0.00	0.39 ± 0.03	0.64 ± 0.02	0.21 ± 0.03	0.31 ± 0.01
ABTS	1.79 ± 0.01	1.65 ± 0.05	0.89 ± 0.02	1.23 ± 0.03	0.31 ± 0.00	0.50 ± 0.06
PPO activity / %	339	66	283	240	190	77

Table 2 – Polyphenol profile of traditional apple cultivars during storage [μg g^{-1}]
Tablica 2 – Polifenolni profil tradicionalnih sorti jabuka tijekom skladištenja [μg g^{-1}]

	Kandadska Reneta		Ilzer Rosenapfel		Wagener	
Polyphenols	0 months	6 months	0 months	6 months	0 months	6 months
Idaein chloride	0.00 ± 0.00	0.00 ± 0.00	112.95 ± 1.15	95.04 ± 3.51	0.00 ± 0.00	0.00 ± 0.00
Gallic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
Trans-ferulic acid	0.65 ± 0.04	1.22 ± 0.44	0.40 ± 0.01	0.49 ± 0.03	0.37 ± 0.13	0.57 ± 0.21
Chlorogenic acid	2234.35 ± 56.29	2937.83 ± 12.91	601.23 ± 4.66	928.23 ± 2.55	653.64 ± 4.72	1052.72 ± 12.92
Epigalocatechin	1186.69 ± 97.34	524.13 ± 41.93	143.37 ± 15.98	106.87 ± 4.24	0.00 ± 0.00	0.00 ± 0.00
Caffeic acid	5.72 ± 0.13	4.73 ± 0.02	2.06 ± 0.01	3.36 ± 0.01	0.88 ± 0.02	0.35 ± 0.13
Procyanidin A2	41.30 ± 9.51	13.09 ± 7.32	1.39 ± 1.01	6.90 ± 0.84	1.06 ± 0.12	0.58 ± 0.07
Catechin	206.86 ± 8.59	99.25 ± 8.33	91.92 ± 2.35	76.59 ± 0.98	9.24 ± 1.03	4.56 ± 0.19
p-coumaric acid	0.89 ± 0.04	1.40 ± 0.02	0.29 ± 0.01	0.46 ± 0.02	0.27 ± 0.16	0.32 ± 0.02
Phloridzin	580.41 ± 97.44	465.31 ± 4.18	217.96 ± 1.03	249.20 ± 0.81	325.89 ± 2.83	384.33 ± 1.50
Epicatechin	791.7 ± 29.2	771.92 ± 6.93	581.5 ± 16.96	771.95 ± 5.29	23.26 ± 1.14	20.48 ± 0.21
Myricetin	16.06 ± 0.32	44.54 ± 0.14	20.74 ± 0.71	29.07 ± 0.12	11.46 ± 0.07	32.88 ± 0.06
Quercetin-3-rutinoside	50.34 ± 1.19	193.14 ± 0.69	129.26 ± 0.90	90.95 ± 0.54	64.85 ± 0.33	93.19 ± 0.43
Quercetin-3-D-glucoside	14.4 ± 0.43	42.58 ± 0.91	38.46 ± 0.25	36.37 ± 0.55	21.75 ± 0.22	29.87 ± 0.52
Procyanidin B1	0.00 ± 0.00	109.37 ± 22.97	71.67 ± 10.36	154.50 ± 7.17	0.00 ± 0.00	131.69 ± 24.02
Oenin chloride	0.00 ± 0.00	0.00 ± 0.00	1.85 ± 0.01	1.27 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Quercetin	0.16 ± 0.02	0.20 ± 0.04	0.23 ± 0.01	0.15 ± 0.02	0.22 ± 0.05	0.37 ± 0.08
Procyanidin B2	2590.49 ± 114.9	2571.75 ± 20.13	1588.1 ± 63.18	2239.99 ± 55.66	81.87 ± 4.02	84.78 ± 3.58
Pelargonidine-3-glucoside	0.00 ± 0.00	0.00 ± 0.00	5.11 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

4 Conclusion

From the obtained results, it may be concluded that Croatian traditional apple cultivars could be successfully cold stored for at least 6 months. The physicochemical composition of traditional apple cultivars under cold storage was expected and in accordance with previous reports. In addition, this research has shown that the high content of polyphenols in apple samples and their antioxidant potential can be preserved under cold storage conditions, meaning that the fruit can greatly contribute to a diet rich in antioxidants.

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SAŽETAK

Utjecaj skladištenja kontroliranom atmosferom na kvalitetu hrvatskih tradicionalnih sorti jabuke

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Cilj ovog rada bio je istražiti utjecaj skladištenja kontroliranom atmosferom na 2 °C tijekom 180 dana na polifenolni profil, sadržaj vode, ukupne kiseline, reducirajuće i ukupne šećere, antioksidacijsku aktivnost i aktivnost polifenol oksidaze triju hrvatskih tradicionalnih sorti jabuke (´Kanadska Reneta', 'Ilzer Rosenapfel', 'Wagener'). Uočena je značajna varijacija u profilu polifenola ispitivanih sorti jabuke. Najzastupljeniji polifenoli kod svih sorti jabuke bili su procijanidin B2, zatim klorogenska kiselina, epigalokatehin, epikatehin i floridzin. Nakon skladištenja sadržaj procijanidina B2 i klorogenske kiseline bio je veći, dok je sadržaj epigalokatehina nakon skladištenja bio manji kod svih ispitivanih sorti jabuke. Sadržaj vode prije skladištenja kretao se između 79,17 i 80,76 %, a nakon skladištenja između 75,62 i 78,56 %. Tijekom skladištenja došlo je do smanjenja ukupnih kiselina, aktivnosti polifenol oksidaze i udjela flavonoida, dok je sadržaj ukupnih šećera bio veći nakon skladištenja. Štoviše, sorta 'Kanadska Reneta' nakon skladištenja imala je najveću antioksidacijsku aktivnost mjerenu ABTS i DPPH metodom, 1,65 odnosno 0,70 mmol trolox/kg^{-1}. Uočena je značajna varijacija u polifenolnom profilu ispitivanih sorti jabuke. Najzastupljeniji polifenoli kod svih sorti jabuke bili su procijanidin B2, zatim klorogenska kiselina, epigalokatehin, epikatehin i floridzin. Nakon skladištenja sadržaj procijanidina B2 i klorogenske kiseline bio je veći, dok je sadržaj epigalokatehina nakon skladištenja bio niži kod svih ispitivanih sorti jabuke. Sveukupno se može zaključiti da su ispitivane sorte jabuka nakon skladištenja sačuvale najveći dio antioksidativnih i funkcionalnih svojstava.

Ključne riječi
Kanadska Reneta, Ilzer Rosenapfel, Wagener, polifenolni profil, antioksidacijska aktivnost

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