

SHORT COMMUNICATION

Effect of the Year and Storage on Ascorbic Acid Content and Total Polyphenol Content in Three Apple Varieties*

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Abstract

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Ascorbic acid (AA) and total polyphenol content (TP) are important plant antioxidants with many healthy effects, such as "scavengers of free radicals", they inhibit oxidation of low-density lipoproteins, lower cholesterol levels, decrease fragility of blood vessels and increase their permeability, decrease heart coronary risk, etc. One of important sources of these compounds in human nutrition are apples. In two years (1997 and 1998) ascorbic acid content (AA) and total polyphenol content (TP) were investigated in three apple varieties (Idared, Gloster and Ontario) cultivated under equal conditions on the experimental plot of the Czech University of Agriculture in Prague-Suchbát. AA content was determined by titration method with 2,6-dichlorophenolindophenol and TP content spectrophotometrically with Folin-Ciocalteu's reagent. In two years' trials AA and TP contents were estimated immediately after harvest in September and then monthly during storage for six months at +5°C. Statistically highly significant differences were determined in AA and TP contents during storage period – there was observed significant AA and TP decrease. The highest antioxidant content in both years was determined in early variety Ontario (in average 12.05 mg/kg AA, 2011.5 mg/kg TP), the lowest in semi-early variety Gloster (in average 5.45 mg/kg AA, 1738.0 mg/kg TP). In this context the variety Ontario is the most favourable. The highest decrease of AA content was in variety Ontario ($-30.05\%_{\text{rel}}$), the lowest in variety Gloster ($-22.93\%_{\text{rel}}$); in TP contents the differences were negligible (-27.14 and $-27.82\%_{\text{rel}}$). In spite of this decrease in Ontario variety both values after six months storage were the most favourable (8.45 mg/kg AA, 1466.5 mg/kg TP). Results were statistically processed by *F*-test and *t*-test. There were differences between the varieties and years of cultivation, but these differences were below the level of statistical significance showing only apparent tendencies. While varietal differences in a decrease in AA content during storage were greater (in the interval from $-22.93\%_{\text{rel}}$ in Gloster variety to $-30.05\%_{\text{rel}}$ in Ontario variety), decreases of TP contents during storage were nearly the same (about $-27\%_{\text{rel}}$ of original content).

Key words: apples; antioxidants; polyphenols; ascorbic acid; effect of variety; effect of year of cultivation; changes during storage

Apples constitute a significant source of the consumption of polyphenolic antioxidants and ascorbic acid (HERTOG *et al.* 1993; KNEKT *et al.* 1996). Their content is affected by many factors, e.g., HERTOOG *et al.* (1992) determined quercetin content in apples in the interval 21–72 mg per kg in relation to the variety, MAZZA & VELIOGLU (1992) found 95–100 mg/kg anthocyanins in Scugog cul-

tivar. KERMASHA *et al.* (1995) determined by HPLC total polyphenol content in apple juices in the interval 28.85 to 115.49 mg/l. FRAGNER *et al.* (1961) report the levels of ascorbic acid content in apples from 1.8 to 6.4 mg/100 g, in stewed apples 1.0 mg/100 g and dried apples 12.0 mg per 100 g. Ascorbic acid activity represents only a small part of total antioxidant activity of apples and apple juice

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(about 1%), meanwhile chlorogenic acid and glycosides of phloretin 32% and 11%, resp. (MILLER *et al.* 1995). Polyphenol content also depends on the quality of varieties, e.g., PIERZYNOWSKA-KORNIK *et al.* (1993) found the highest amounts of polyphenols in apple concentrates from early varieties. In red varieties during the maturation process it was possible to observe the synthesis of anthocyanidins, e.g., in Splendour variety – as high as 1 mg/g glycosides of cyanidin. Conversion of procyanidin monomers to oligomeric structures could also be observed during maturation (MAYR *et al.* 1995). MILLER *et al.* (1995) found that during storage of apple juice polyphenolic antioxidants were relatively stable, but ascorbic acid content decreased.

The aim of this work was to determine varietal differences in ascorbic acid content and total polyphenol content and their changes during storage.

MATERIAL AND METHODS

After the harvest in 1997 and 1998 ascorbic acid content (AA) and total polyphenol content (TP) were estimated in three apple varieties – Ontario, Gloster, and Idared. Apples of these varieties were cultivated in the same way and under the same conditions on the trial plot of the Czech University of Agriculture in Prague-Suchbát and harvested in September. Obtained results are average values of three parallel determinations and individual samples were obtained from four apples of two apple trees. Apples were stored in darkness at 5°C for six months.

Determination of total polyphenol content (TP): A modified method after LACHMAN *et al.* (1997) with Folin-Ciocalteu's phenol reagent (Fluka Chemie AG) was used. Apples were homogenised in the shortest time. For the determination 10 g apple mass were weighed into 100 ml flask and the flask was filled with 80% ethanol to the mark in the shortest time. After thorough agitation and homogenisation the solution was filtered and for the determination 5 ml aliquots were pipetted into 50 ml flasks.

The TP content was determined in centrifugates after addition of Folin-Ciocalteu's phenol reagent (2.5 ml) and 20% Na₂CO₃ solution (7.5 ml) spectrophotometrically on the Spekol 11 spectrophotometer at $\lambda = 765$ nm against blank. The absorbancy of formed blue colour was measured after 2 hours standing after centrifugation and TP content was expressed as gallic acid.

Determination of ascorbic acid content (AA): Ascorbic acid content was determined by the titration method

with 2,6-dichlorophenolindophenol according to DAVÍDEK *et al.* (1977). 2 g apple mass were homogenised with 3 ml 3% HCl, the homogenate was then transferred into 25 ml flask, filled with this acid solution to the mark, agitated and centrifuged. For determination 4 ml aliquots were pipetted and titration with 0.001 mol/l 2,6-dichlorophenolindophenol (DCHPIP) solution was performed under intensive agitation until weak pink colour appeared.

RESULTS AND DISCUSSION

In three apple varieties (Ontario and Idared – early, Gloster – semi-early) AA and TP contents were estimated in 1997 and 1998 after harvest and during storage period of 6 months (from September to February) at 5°C. AA and TP values were related to fresh matter.

It was found that the results of titration determination of AA with 2,6-dichlorophenolindophenol corresponded with the results obtained polarographically on the EKO TRIBO Polaro-Sensors polarograph. TP determination with Folin-Ciocalteu's phenol reagent was equally sensitive and reproducible with relative standard deviation $\pm 1.96\%$ _{rel.}

In both years there existed varietal differences. The lowest AA content was determined in Gloster variety (4.01 mg per kg in 1997/98 and 4.30 mg/kg in 1998/99) and the highest in Ontario variety (11.8 mg/kg in 1997/98 and 12.3 mg per kg in 1998/99). These obtained results are in accordance with data of FRAGNER (1991) who characterises apple AA content as 1.8–6.4 mg/100 g. USDA Database Reference (1998) gives average AA content 5–7 mg/100 g for apples with skin, without skin 4.4 mg/100 g and for apple juice 0.9–1.2 mg/100 g.

During storage there was an apparent and significant decrease of AA content in all investigated varieties (Table 1). In both investigated years a decrease of AA content was statistically significant. It represents a decrease approximately by 20–40%_{rel.} of original amounts. Relatively lowest decrease was in Gloster variety in both investigated seasons (from the original level 5.3 to 4.1 mg/kg and from 5.6 to 4.3 mg/kg) and in Idared variety in the 1998/99 season (from the original level 6.3 to 5.2 mg/kg). The highest decrease was determined in Ontario variety (from 11.8 to 7.3 mg/kg in the 1997/98 and from 12.3 to 9.6 mg/kg in the 1998/99).

TP content varied in the interval 1231.5–2165 mg/kg (Table 2). There was a statistically significant TP decrease during the storage process. In both seasons the highest TP content was found in Idared variety (in 1997/98 the original

Table 1. Ascorbic acid content (AA) in three apple varieties during storage [mg/kg]

Variety	1997/98						1998/99					
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Idared	6.7	6.3	6.0	5.8	5.4	4.3	6.3	6.1	6.0	5.9	5.6	5.2
Gloster	5.3	5.1	4.9	4.7	4.3	4.1	5.6	4.9	4.9	4.7	4.6	4.3
Ontario	11.8	10.5	10.3	9.5	8.5	7.3	12.3	11.8	11.6	11.3	10.3	9.6

Table 2. Total polyphenol content (TP) in three apple varieties during storage [mg/kg]

Variety	1997/98						1998/99					
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Idaret	2165	2032	1893	1765	1585	1470	1811	1730	1673	1556	1478	1405
Gloster	1787	1692	1658	1397	1357	1232	1689	1580	1479	1321	1348	1274
Ontario	1999	1795	1633	1602	1432	1324	2024	1959	1833	1723	1674	1609

content after harvest 2165 mg/kg decreased to 1450 mg per kg after 6 months storage, in the season 1998/99 the original content after harvest 2046 mg/kg decreased to 1405 mg/kg). In both investigated seasons the lowest TP content was determined in Gloster variety (1787 mg/kg in the 1997/98 and 1689 mg/kg in the 1998/99).

Comparison of average values obtained in both years (Table 3) suggests that the highest AA and TP contents were recorded in the early Ontario variety (12.05 mg/kg AA and 2011.5 mg/kg TP) and the lowest in semi-early Gloster variety (5.45 mg/kg AA and 1738 mg/kg TP). These values correspond with an average relative decrease of these compounds during 6 months storage, when the highest AA decrease was in Ontario variety (30.05%_{rel}) and the lowest in Gloster variety (–22.93%_{rel}). Decreases

of TP amounts were very similar (–27.82%_{rel} in Gloster variety and –27.14%_{rel} in Ontario variety). In spite of the high decrease of AA of Ontario variety is the richest source of ascorbic acid and TP with antioxidant effects even after storage for 6 months (8.45 mg/kg AA and 1466.5 mg/kg TP). The lowest amounts of these compounds were determined in Gloster variety (4.20 mg/kg AA and 1253.0 mg per kg TP).

Varietal and year's dependence and changes in AA and TP contents were evaluated statistically by *F*-test and *t*-test. Statistically significant was a decrease of AA and TP contents during storage. Varietal and year's differences have only approximated to the level of statistical significance. Statistical evaluation of AA and TP determination with ANOVA software tool is shown in Table 4.

Table 3. Average ascorbic acid content (AA) and total polyphenol content (TP) in apples in 1997/98 and 1998/99 seasons and their content and decrease after storage for 6 months

Variety	Average content [mg/kg]			Variance of AA during storage	Average TP content after 6 months storage [mg/kg]	Variance of TP during storage	Decrease after six months storage [% _{rel}]	
	of AA	of TP	of AA after 6 months storage				of AA	of TP
Idared	6.50	1988.0	4.75	0.220334	1437.5	612.4787	–26.64	–27.26
Gloster	5.45	1738.0	4.20	0.200567	1253.0	361.2110	–22.93	–27.82
Ontario	12.05	2011.5	8.45	1.938334	1466.5	416.4234	–30.05	–27.14

Table 4. Statistical evaluation of ascorbic acid (AA) and total polyphenol (TP) determination

Source of variability		SS	Difference	MS	<i>F</i>	<i>P</i> -value	<i>F</i> _{crit.}
TP 1997/98	among data selections	2718.143	2	1359.072	2.294292	0.135102	3.682317
	all data selections	8885.562	15	592.3708			
	total	11603.71	17				
TP 1998/99	among data selections	2473.941	2	1236.971	3.699393	0.049431	3.682317
	all data selections	5015.568	15	334.3712			
	total	7489.509	17				
AA 1997/98	among data selections	79.02333	2	39.51167	39.15492	1.11E-06	3.682317
	all data selections	15.13667	15	1.009111			
	total	94.16	17				
AA 1998/99	among data selections	126.4133	2	63.20667	111.9142	9.66E-10	3.682317
	all data selections	8.471667	15	0.564778			
	total	134.885	17				

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Souhrn

LACHMAN J., ORSÁK M., PIVEC V., KUČERA J. (2000): Vliv ročníku a skladování na obsah askorbové kyseliny u tří odrůd jablek. *Czech J. Food Sci.*, **18**: 71–74.

Askorbová kyselina (AK) a celkové polyfenoly (CP) jsou významnými antioxidanty jablek. U tří vybraných odrůd jablek (Idared, Gloster, Ontario) byl sledován obsah AK (titrační metodou s 2,6-dichlorfenolindofenolem) a CP (spektrofotometrickou metodou s Folin-Ciocalteuovým činidlem) u vzorků vypěstovaných za stejných podmínek na pokusném pozemku ČZU Praha-Suchdol v letech 1997 a 1998 po sklizni a v průběhu šestiměsíčního skladování při teplotě +5 °C. Během skladování docházelo ke statisticky významnému poklesu obsahu AK a CP. Byly zjištěny značné rozdíly mezi odrůdami i mezi ročníky, které však nedosáhly meze statistické průkaznosti. Nejvyšších hodnot AK a CP dosahovala v obou letech raná odrůda Ontario (12,05 a 2 011,5 mg/kg) a nejnižších středně raná odrůda Gloster (5,45 a 1 738 mg/kg). Přes největší pokles obsahu AK u odrůdy Ontario po šestiměsíčním skladování (–30,05 %_{rel}) a nejmenší pokles u odrůdy Gloster (–22,93 %_{rel}), zůstává odrůda Ontario z hlediska obsahu antioxidantů typu AK a CP jejich nejcennějším zdrojem a má pozitivní vliv na zdraví člověka.

Klíčová slova: jablka; antioxidanty; polyfenoly; askorbová kyselina; vliv odrůdy; vliv ročníku; změny při skladování

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