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DETERMINATION ON VITAMIN C IN SEVERAL OLD ROMANIAN APPLE CULTIVARS BY HPLC DURING COLD STORAGE

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Abstract

High pressure liquid chromatographic (HPLC) methods were used for measurement of vitamin C and organic acid changes of forty old Romanian apple cultivars ("Prescurate","Gurguiate","Zori","Carla","Mohorat", "Trotuse", "Mere Tari","Mar Orbai" etc.) during cold storage. Harvested apples at the last stage of commercial ripeness were placed in perforated stored at 0°C temperature and 90-95% relative humidity for 60 days. Vitamin C content decreased in all cultivars but no significant differences were found in the most important of them from the beginning to the end of the storage. The highest share of total acids was contributed by citric acid. The high level of vitamin C was measured in the cultivars" Trotuse", "Ancuta","Wachsman Sammeling"and "Wachsman Amalie". Malic acid content of cultivars also decreased with storage time. Tartaric, oxalic and fumaric acid contents fluctuated during storage, but at the end of cold storage these organic acids had decreased in comparison to initial values.

Key words: *apple, acids, HPLC, organic ,storage, vitamin C.*

INTRODUCTION

The Apple (*Malus x malus*) is one of leading fruits which is being grown in temperate region of the world. Its beautiful appearance, crispy flesh, pleasant flavour and sweet taste attract the consumers and fetch high (Adisa, 1986)

Of the old genotypes known to exist in the Carpathian Basin, almost 200 local cultivars have been collected in the germoplasm collection over the last years.

Some of the cultivars found in the germoplasm collection could be use not only in breeding, but also in organic farming, so there is a great need for the re-evaluation of the old apple cultivars. Some of the old apple varieties have become well adapted to the soil and climatic conditions of the Carpathian Basin producing good yields and highly appreciated fruit quality (Mitre et al., 2009)

It is important to examine old apple cultivars because the pomological descriptions available for old cultivars are not uniform. Many collections of cultivar descriptions use several names for one cultivar, or the same name for several cultivars. Nowadays, in most cases the guidelines given by UPOV (Union Internationale pour la Protection des Obtentions Végétales) or CPVO (Community Plant Variety Office) are applied, as the brief descriptions and number codes provide sufficient information for cultivars to be differentiated. They have the advantage that they can be applied on an international scale and enable the results obtained by different research teams to be easily compared.

The nutritional value of apples is mainly due to the content of Vitamin C (Sanz et al., 1999). As antioxidant content is becoming an increasingly important parameter with respect to fruits and vegetables, it is of great interest to evaluate changes in the antioxidant status during fruit storage (Perez and Sanz, 2001).

During the postharvest period of strawberries, prompt cooling and providing proper temperature (0°C) and relative humidity (90-95%) are the most important factors to preventing the undesirable quality changes (Kader, 1990). The concentration of organic acids is an important factor influencing the organoleptic properties of fruits (Lee and Kader, 2000). Their changes during storage should be reduced. Besides good sensory features, consumers prefer apples because of their high content of vitamin C (Schöpplei et al., 2002).

Vitamin C, one of the most important nutritional quality factors in apples, has been found to prevent the formation of N-nitroso compounds, the cancer causing substances from nitrates and nitrites found in preserved meats and some drinking water (Du et al., 2009). Vitamin C has many biological activities (reducing carcinogenesis and cardiovascular diseases, stimulating the immune system) in the human body (Simon, 1992).

There are several methods to determine vitamin C content; however some of them need subjective evaluation and some are not practical (Agar, 1995). Several postharvest factors influence the vitamin C and organic acid contents of apple. Despite many investigations in the area of nutrition and postharvest changes of apples, knowledge about the determination of vitamin C and organic acids by using HPLC is inadequate. It has been indicated that ascorbic acid content of fruit should be measured by HPLC because ascorbic acid produces an oxidative-reduction reaction (Asami et al., 2003). The aim of this research was to measure the vitamin C (ascorbic acid) and organic acid contents of 20 old Romanian apples varieties by using High Performance Liquid Chromatography (HPLC) during cold storage period.

MATERIALS AND METHODS

Forty old apples cultivars (Prescurate, Gurguiate, Viesti, Rosii Stetin, Zori, Carla, Mohorat, Gustav Durabil, Wachsman Sammeling, Wachsman Amelie, Ancuta, Ardelean, Trotuse,etc.) grown in the research and application center of Horticulture Department in Bucharest located in south of Romania region were harvested at the last stage of commercial ripeness (red colour with a surface area of > 75-80%). Harvested fruits in the early morning were transported to the post harvest laboratory within 30 min. Apples cultivars were sorted to eliminate fruits with defects including overripe or too small fruit. Fruits were selected randomly and placed in perforated (8 perforations with 10 mm diameter on each box) plastic boxes (capacity: 750 g) for each replicates. Four replicates were used per treatment. Packaged fruits were stored at 0°C temperature and 90-95% relative humidity for 10 days. Apples fruits cultivars were analyzed at 5 days intervals (0, 5, and 10 days of storage) during cold storage.

Vitamin C (Ascorbic acid) and Organic Acid Analysis

The HPLC analysis was carried out to determine the vitamin C and organic acids on a Shimadzu class LC VP HPLC system with class LC-VP software, a pump (LC-6AD), and a UV-VIS detector (SPD-10AV VP). The columns used were YMC Pack-ODS (250 mm x 4.6 mm I.D., 5 μm) for organic acids and SGE (250 mm x 4.6 mm I.D., 5 μm) for vitamin C. The mobile phases were water adjusted to pH 2.2 with trifluoroacetic acid (organic acids) and to pH 3 with phosphoric acid (vitamin C). Separation was carried out by isocratic elution with a flow rate of 0.4 ml min-1 and column temperature was ambient. The UV detector was set at 210 nm and 254 nm, respectively. Quantitation was based on the peak area measurement.

Sample (10 g) was extracted in 10 ml water adjusted to pH 1.5 with trifluoroacetic acid for organic acids and with 10 ml phosphoric acid-water (2%, v/v) for vitamin C. The extracts were filtered through filter paper. Then, 1.5 ml buffer (0.01 M KH_2PO_4, pH 8.0) was added to 1.5 ml sample extract. From this, 1.5 ml (organic acids) and 1 ml (vitamin C) of these mixtures were loaded on to C18 cartridges. After loading, 3 ml water adjusted to pH 1.5 with trifluoroacetic acid for organic acids and 2 ml phosphoric acid-water (2%, v/v) for vitamin C were passed through the cartridges. For HPLC, 20 μl of the eluents were injected.

RESULTS AND DISCUSSIONS

Generally, fruits and vegetables show a gradual decrease in vitamin C content as the storage temperature or duration increases (Adisa, 1986). In this research, change in vitamin C of apples during cold storage is shown in Figure 1. Vitamin C levels decreased for most of cultivars from the beginning to the end of the storage, but this reduction was not statistically significant ($P<0.05$) in 'Trotuse' and 'Ancuta' showed an average content of vitamin C at harvest of 35.32 mg/100 g and 31.42 mg/100 g, and at the end of the storage these values decreased to 14.94 mg/100 g and 13.42 mg/100 g, respectively. (Figure 1) On the other hand a slight increase was found in vitamin C content of apples stored in air (Perez and Sanz, 2001).

Likewise, vitamin C content of ‘Gurguiate, Mohorat si Rosii de Stetin, Muntenesc cu coada scurta’ decreases drastically at the 15th day of storage in comparison to initial value (Figure 2). This can be due to continuous ripening process of fruits.

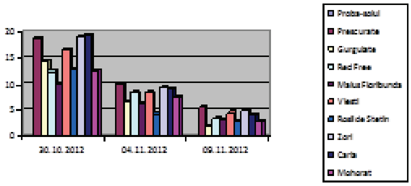

Figure 1. Vitamin C of old apples cultivars during cold storage.

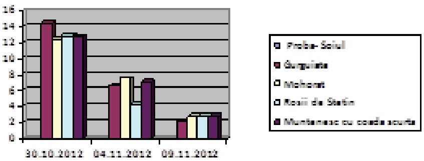

Figure 2. Vitamin C content of varieties ‘Gurguiate, Mohorat si Rosii de Stetin, Muntenesc cu coada scurta’

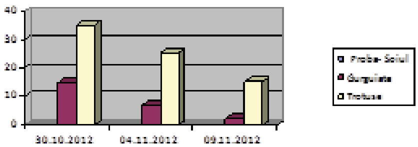

Figure 3. Vitamin C content of varieties ‘Gurguiate’ and ‘Trotuse’ at the end of stor¬age.

Vitamin C is quite unstable and thus it is also an indication of fruit freshness (Holcroft and Kader, 1999). Vitamin C content of ‘Gurguiate’ was lower than ‘Trotuse’ at the end of storage (Figure 3). It can be concluded that the change in vitamin C content is cultivar dependent. Therefore, this characteristic of cultivars should be taken into consideration in choosing a cultivar for storage. The cultivars which lose less vitamin C during storage could be preferred.

Table 1. Results concerning the vitamin C in old Romanian apples varieties 0, 5, and 10 days of storage

Nr. Crt.	Cultivar	30.10.2012 Mg/100 g.p.p.	04.11.2012 Mg/100 g.p.p.	09.11.2012 Mg/100 g.p.p.
1.	Prescurate	18.59	10.11	5.59
2.	Gurguiate	14.52	6.51	2.04
3.	Red Free	12.45	8.24	3.45
4.	Malus Floribunda	10.04	6.22	3.04
5.	Viesti	16.54	8.45	4.52
6.	Rosii de Stetin	12.82	4.22	2.82
7.	Zori	18.86	9.15	4.86
8.	Carla	19.15	8.98	4.17
9.	Mohorat	12.49	7.52	2.79
10.	Gustav Durabil	14.57	9.36	4.69
11.	Wachsman Sammeling	24.34	16.3	8.34
12.	Wachsman Amalie	28.55	19.51	10.55
13.	Ancuta	31.42	25.61	13.42
14.	Ardelean	17.98	8.96	4.43
15.	Trotuse	35.32	25.36	14.94
16.	Rosii de Geoagiu	25.86	13.51	4.86
17.	Knis	12.91	9.36	3.91
18.	Nobile de Geoagiu	21.78	12.36	5.78
19.	Favoritul lui Polocsay	12.87	8.89	3.87
20.	Mere tari	14.73	8.52	4.73
21.	Malus Teifera	15.28	7.69	5.28
22.	Pokomache	15.01	8.61	5.01
23.	Satmaresti	16.50	9.63	5.50
24.	Cormose	18.83	12.31	4.83
25.	Calugaresc	16.96	12.36	5.26
26.	Anisovska	15.93	8.96	3.93
27.	Marut alb	14.36	8.21	4.36
28.	Seghese	15.12	9.65	5.12
29.	Poinic	19.91	14.31	5.91
30.	Mar Muntenesc	15.23	9.63	4.23
31.	Smeurii	12.27	7.25	3.27
32.	Muntenesc cu coada scurta	12.73	6.94	2.73
33.	Rosior calugaresc	15.68	8.51	3.68
34.	Rosior romanesc	19.32	12.35	5.32
35.	Mar Orbai	18.49	13.21	6.67
36.	Verzi de Radaseni	18.57	11.11	4.57
37.	Fara nume	24.42	21.32	9.42
38.	Dulci de Radaseni	22.50	16.63	10.50
39.	Cernenko	19.65	12.67	4.76
40.	Rosu de Cluj	23.11	19.20	13.11

CONCLUSIONS

The accurate analysis of vitamin C and organic acids of apples by HPLC enables us to observe the quality changes during postharvest period. Vitamin C and organic acid content of the majority of cultivars changed as a function of storage time. Both cultivars had lost vitamin C contents at the end of the storage, but 'Trotuse' showed a greater decrease than 'Gurguiate'.

These results showed that changes in vitamin C contents of apples are cultivar dependent. On the other hand, the consumer should take into consideration that the loss of vitamin C increases with storage time. During storage, the highest share of total acids was exhibited by citric acid. This acid decreased by 10 days of cold storage in comparison to initial values.

In future investigations, we propose that the objective analytical determination of these critical components should be coupled with subjective evaluation by a taste panel to provide useful and meaningful information about quality changes of apples during the storage.

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