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Determination of Vitamin C and Organic Acid Changes in Strawberry 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 two strawberry cultivars ('Dorit' and 'Selva') during cold storage. Harvested strawberries at the last stage of commercial ripeness were placed in perforated (8 perforations, 10 mm diameter) plastic boxes and stored at 0°C temperature and 90-95% relative humidity for 10 days. Vitamin C content decreased in both cultivars but no significant differences were found in 'Dorit' from the beginning to the end of the storage. The highest share of total acids was contributed by citric acid. It decreased with increase in storage time in both cultivars. 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.

Keywords: HPLC, *Fragaria vesca*, storage, vitamin C, organic acids

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) fruit has unique, highly desirable taste and flavor and is one of the most popular summer fruits. Consumers mainly purchase strawberry for an enjoyable eating experience (Ford *et al.*, 1997). The nutritional value of strawberries 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, 1993). Their changes during storage should be reduced. Besides good sensory features, consumers prefer strawberries because of their high content of vitamin C (Schöpplein *et al.*, 2002).

Vitamin C, one of the most important nutritional quality factors in strawberries, 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; Lee and Kader, 2000). In general, L-ascorbic acid (AA) constitutes 90% of the total vitamin C content of vegetables and fruits (Agar, 1995). Therefore, it is important to measure ascorbic acid (AA) in fruits and vegetables for vitamin C activity (Wills *et al.*, 1984). 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 strawberry. Despite many investigations in the area of nutrition and postharvest changes of strawberries, 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 'Dorit' and 'Selva' strawberry varieties grown in Isparta (Turkey) by using High Performance Liquid Chromatography (HPLC) during cold storage period.

Materials and methods

Sample preparation

Strawberry cultivars ('Dorit' and 'Selva') grown in the research and application center of Horticulture Department in Isparta located in Mediterranean region of Turkey 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 postharvest laboratory within 30 min. Strawberries 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. Strawberries were analysed at

2-3 days intervals (0, 2, 5, 7 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-6-AD), 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 C_{18} 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 discussion

Vitamin C (Ascorbic Acid)

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 strawberries during cold storage is shown in Fig. 1. Vitamin C levels decreased in both cultivars from the beginning to the end of the storage, but this reduction was not statistically significant ($P<0.05$) in 'Dorit'. 'Dorit' and 'Selva' showed an average content of vitamin C at harvest of 24.70 mg/100 g and 15.25 mg/100 g, and at the end of the storage these values decreased to 16.35 mg/100 g and 12.95 mg/100 g, respectively. Similar results were found by some researchers with storage of strawberries in air (2°C) (Perez *et al*., 1998; Sanz *et al*, 1999). On the other hand a slight increase was found in vitamin C content of strawberries stored in air (Perez and Sanz, 2001). Likewise, vitamin C content of 'Selva' increased at the 5th day of storage in comparison to initial value (Fig. 1). This can be due to continuous ripening process of fruits.

Vitamin C is quite unstable and thus it is also an indication of fruit freshness (Sanz *et al*., 1999). Vitamin C content of 'Dorit' was lower than 'Selva' at the end of storage (Fig. 1). 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.

Organic Acids

Citric acid is the most abundant organic acid in strawberry, followed by malic acid (Holcroft and Kader, 1999). Tartaric, oxalic and fumaric acids were present in the strawberry fruit in very small amounts (Sturm *et al*., 2003). Changes in citric, malic, tartaric, oxalic and fumaric acids during cold storage are shown in Fig. 2, 3, 4, 5 and 6, respectively.

Changes in all organic acids during cold storage were statistically significant for both 'Dorit' and 'Selva'. Citric acid was the dominant organic acid in both cultivars. Citric acid content of 'Selva' decreased with storage time, and was 5.27 mg/g at the end of storage. Its content in 'Dorit' changed from an initial value of 5.96 mg/g to 4.30 mg/g at the 10th day of storage. Similarly, some researchers determined a slight decreasing in citric acid content of strawberry at the 9th day of storage (Pelayo *et al*., 2003). While citric acid content of 'Dorit' fluctuated depending on storage time and reached a maximum value of 9.02 mg/g (at the 7th day of storage), 'Selva' showed a decrease in com-

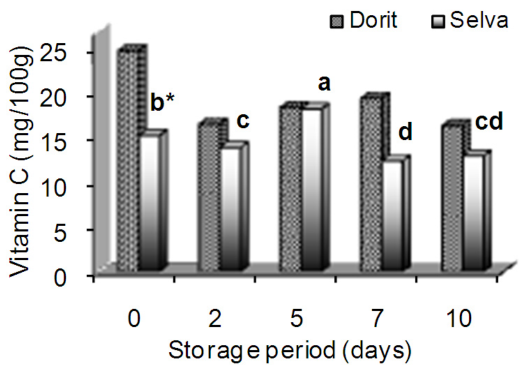

Fig. 1. Change of vitamin C content of strawberries during cold storage (mg/100g)

*Means with different letters are statistically significant at $P<0.05$

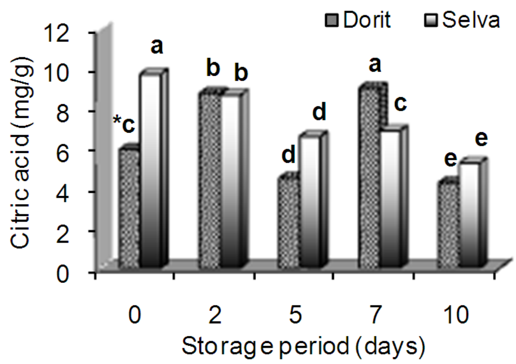

Fig. 2. Change of citric acid content of strawberries during cold storage (mg/g)

*Means with different letters are statistically significant at $P<0.05$

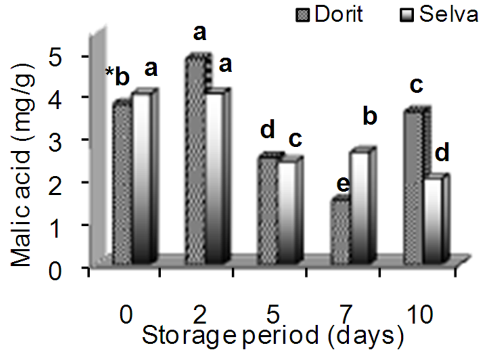

Fig. 3. Change of malic acid content of strawberries during cold storage (mg/g)

*Means with different letters are statistically significant at P<0.05

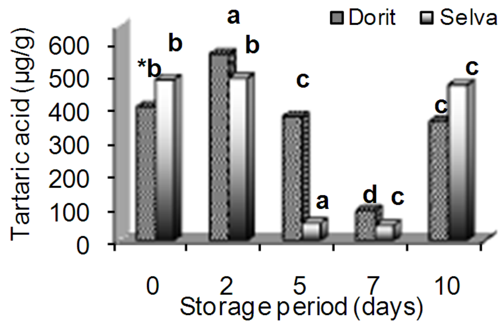

Fig. 4. Change of tartaric acid contents of strawberries during cold storage (µg/g)

*Means with different letters are statistically significant at P<0.05

parison to its initial value (Fig. 2). Similar fluctuations in citric acid content of strawberry in cold storage was found by some researchers (Pelayo *et al*., 2003).

Malic acid contents showed a fluctuation in both 'Dorit' and 'Selva' during cold storage, decreasing with storage time from initial values of 3.78 mg/g and 4.03 mg/g to 3.61 mg/g and 2.03 mg/g at the end of storage, respectively (Fig. 3). Similar results were found by some researchers (Sanz *et al.*, 1999; Perez and Sanz, 2001; Pelayo *et al.*, 2003) . Malic and citric acid levels could give an indication of degree of ripeness (Sanz *et al*., 1999). It was reported that, as with sucrose, malic and citric acid contents decreased during strawberry ripening (Reyes *et al*., 1982).

No clear changing was observed in the tartaric acid contents of the cultivars. Strawberries showed average tartaric acid content at harvest of 403 µg/g ('Dorit') and 485 µg/g ('Selva'), and by the end of the storage these values had decreased to 359 µg/g and 469 µg/g, respectively (Fig. 4).

A fluctuation was seen in oxalic and fumaric acid contents of the cultivars. Oxalic acid values were 143.41µg/g ('Dorit') and 147.49µg/g ('Selva') at harvest date and had decreased to 70.00 µg/g and 137.77 µg/g, respectively at the end of storage. Oxalic acid content of 'Selva' decreased in the first 5 days of storage and then increased to initial levels, but it had decreased sharply in 'Dorit' within the first 2 days and almost remained constant for the rest of

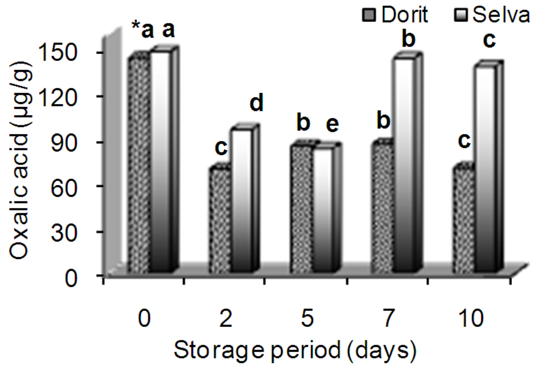

Fig. 5. Change of oxalic acid content of strawberries during cold storage (µg/g)

*Means with different letters are statistically significant at P<0.05

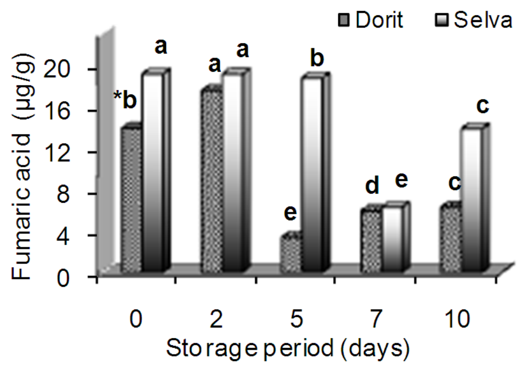

Fig.6 . Change of fumaric acid content of strawberries during cold storage (µg/g)

*Means with different letters are statistically significant at P<0.05

the storage time (Fig. 5). Fumaric acid content of 'Dorit' and 'Selva' changed from initial values of 13.85 µg/g and 19.05 µg/g to 6.30 µg/g and 13.80 µg/g, respectively (Fig. 6).

The content of total acids, comprising of the sum of individual acids, decreased with the storage time, and this was primarily due to the decline in citric acid content. In this study, the decreasing in organic acid content of strawberries could be dependent on continued ripening during the storage (Sturm *et al*., 2003).

Conclusions

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

Malic acid content of the cultivars also decreased within the storage period. Citric and malic acids were present in the strawberry fruit in significant amounts; therefore they could influence the flavour of the fruits. Thus, measurement of these acids during cold storage is important. Tartaric, oxalic and fumaric acid content of 'Dorit' and 'Selva' exhibited slight fluctuations during the storage period, but at the end of the storage, these organic acids had decreased 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 strawberries during storage.

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