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**Barbara Łata, Marzenna Przeradzka**
**Warsaw Agricultural University**
**Department of Pomology and Basic Research in Horticulture**
**Nowoursynowska 166, 02-787 Warsaw, POLAND**

# CHANGES OF ANTIOXIDANT CONTENT IN FRUIT PEEL AND FLESH OF SELECTED APPLE CULTIVARS DURING STORAGE

**ABSTRACT.** The aim of this preliminary research was to evaluate the level of glutathione, ascorbic acid and the activity of glutathione and ascorbate related enzymes (glutathione reductase and ascorbate peroxidase) in apple peel and flesh during cold storage. Four cultivars of apples: 'Jonagold', 'Šampion', 'Gloster' and 'Elise', were tested. Samples of apple tissues from cold storage were collected in October, November and December at 30-day intervals, frozen in liquid nitrogen and stored at –85 °C. The content of soluble reductants depended on cultivar, part of fruit and storage period. Total glutathione and ascorbate content in the peel was 2 and 6 times higher respectively, compared to their content in the flesh. Glutathione level increased in November and decreased in December both for the peel and flesh. Amounts of ascorbic acid in the flesh was nearly the same during storage while its concentration increased in the skin. The level of glutathione reductase activity generally increased during the tested period. Ascorbate

peroxidase activity did not significantly change between the tested cultivars and during storage.

**Key words:** apple, cultivar, glutathione, ascorbate, glutathione reductase, quality

**Abbreviations:** GSH – glutathione, ASC – ascorbate, GR – glutathione reductase, APX – ascorbate peroxidase

**INTRODUCTION.** Epidemiological studies have established that the consumption of fruits and vegetables is associated with a lowered risk of heart disease mortality, cancer and other degenerative diseases as well as ageing. Fruits and vegetables contain a significant level of biologically active components that prevent or reduce the level of free radicals in the body. Free radicals are very dangerous, because they may attack biomolecules, such as lipids, proteins, or nucleic acids. Ascorbate plays a key role in the destruction of active oxygen species, and glutathione is essential to the regeneration of ascorbate. Glutathione reductase and ascorbate peroxidase are involved in glutathione and ascorbate regeneration (Noctor and Foyer, 1998). If the reactive oxygen species are not removed, then the system tends to self-destruction in an autocatalytic fashion.

Among fruits, apples have huge antioxidant and anticancer properties. There are well known studies, which prove that a combination of plant chemicals, such as flavonoids and polyphenols known as “phytochemicals”, found in the flesh of apple and particularly in its skin, provides the fruit’s tremendous antioxidant and anticancer benefits (Eberhardt et al., 2000; Hermann, 2000).

Food producers should be interested in apple quality and in developing products with an increased content of health-protecting compounds (Van der Sluis et al., 2001). For this purpose it is important not only to know which antioxidants are present in raw materials and in what concentration, but also differences between cultivars, growing conditions (soil type, irrigation, fertilization), seasonal differences, harvest and storage conditions.

The aim of this study was to evaluate the antioxidant capacity of ascorbate-glutathione cycle in four cultivars of apples during cold storage.

**MATERIAL AND METHODS.** Research was carried out in 2000 in the Department of Pomology and Basic Research in Horticulture, Warsaw Agricultural University. Four cultivars of apple: 'Jonagold', 'Šampion', 'Gloster' and 'Elise', were tested in order to define the differences of glutathione and ascorbate content, activity of glutathione reductase and ascorbate peroxidase in apple peel and flesh during cold storage. Apples were harvested as follows: 'Elise' – 14 September; 'Jonagold', 'Šampion' – 22 September; 'Gloster' – 29 September, and immediately put into cold storage. All cultivars were kept at 2 °C and 95-97% RH. Samples of apple tissues were taken at the beginning of October, November and December, frozen in liquid nitrogen and stored at –85 °C until analysis.

Sample preparation: ten kilograms of apples were taken at random from cold storage. Ten medium size fruits were chosen and chemical analyses were performed separately for each of them. The flesh was cut by knife into thin pieces and a part of them from different sides of apple (about 20 g) was ground to a fine powder under liquid nitrogen. To determine the distribution of GSH, ASC and enzyme activity within an apple, it was peeled with a potato knife; a thin layer of the flesh remained adhered to the peel. Thus the peel can be considered as the epidermic zone of apples.

The ascorbate concentration was analysed colorimetrically and by HPLC technique (Anderson et al., 1992). The sum of the reduced and oxidized forms of glutathione was determined using a kinetic assay in which catalytic amounts of GSH, GSSG and glutathione reductase brought about the continuous reduction of 5,5'-dithiobis (2-nitrobenzoic acid) [DTNB] by NADPH. The formation of 5-thio-2-nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm. All values were expressed as GSH equivalents (Akerboom and Sies, 1981). Enzymatic activities were carried out at 25 °C in a total volume of 1 ml. Activity of glutathione reductase [GR; EC 1.6.4.2] was determined by

decrease in absorbance at 340 nm as NADPH was oxidized according to the method described by Foyer and Halliwell (1976). Ascorbate peroxidase [APX; EC 1.11.1.11] activity was calculated from decrease in absorbance at 290 nm as ascorbate was oxidized (Nakano and Asada, 1987). Blank rates in the absence of extract were determined for each test system.

The results were elaborated by an analysis of variance. Significance of differences between means were evaluated using the Tukey`s test at P=0.05.

**RESULTS AND DISCUSSION.** In the light of recent research studies the efficiency of plant antioxidative system, caused by molecular hydrophilic and lypophylic antioxidants and the enzyme activity connected with them, seems to be a very important factor that decides about the plant tolerance toward stress and in case of vegetables and fruits also about their biological values. It was proved that antioxidants responsible for plant protection against the oxidative stress and free radicals play similar functions in the human body, with one difference, they need to be taken with daily diet. That is also why natural and fresh food seams to be a proposition for all consuments that will not bring any doubts.

The content of soluble reductants depended on cultivar, part of fruit and storage period. The significant differences in the level of total GSH between all tested cultivars were found (Tab. 1). The lowest concentration of GSH was observed in 'Jonagold' and the highest in 'Šampion': 28.6 and 53.6 nmol $g^{-1}$ f.w., respectively. Glutathione content significantly increased in November in comparison to October, then decreased in December. The highest content of ASC was noted in 'Šampion' (Tab. 2). It was significantly higher in comparison to the rest of tested cultivars. The ascorbate content was nearly the same in October and November, and contrary to GSH significantly increased in December. As suggested by Noctor et al. (1998), glutathione accumulation is found to compensate for the decreases in the capacity of other antioxidants. Curry (1997) reported that in 'Red Delicious' fruits the level of antioxidants increased 2- to 10-fold after

2 months of cold storage while in ‘Grany Smith’ the increase was 10-fold. In both cultivars, antioxidant content after 4 or 6 months of storage was lower than after 2 months. The reduction of both ascorbic acid and polyphenols during 6 months of storage has also been observed by Lachman et al. (2000ab).

Table 1. Content of GSH [nmol $g^{-1}$ f.w.] in four cultivars of apple during cold storage

| Month (B) | Cultivar (A) | | | | Mean |
|---|---|---|---|---|---|
| | ‘Jonagold’ | ‘Gloster’ | ‘Elise’ | ‘Šampion’ | |
| X | 24.4 | 41.8 | 54.6 | 45.4 | 41.5 |
| XI | 28.6 | 40.0 | 43.7 | 79.8 | 48.0 |
| XII | 32.7 | 31.6 | 45.8 | 35.5 | 36.4 |
| Mean | 28.6 | 37.8 | 48.0 | 53.6 | X |

$LSD_{(A)}$ = 5.1 $LSD_{(B)}$ = 4.0 $LSD_{(AxB)}$ = 8.7 $LSD_{(BxA)}$ = 8.0

Table 2. Content of ASC [µg $g^{-1}$ f.w.] in four cultivars of apple during cold storage

| Month (B) | Cultivar (A) | | | | Mean |
|---|---|---|---|---|---|
| | ‘Jonagold’ | ‘Gloster’ | ‘Elise’ | ‘Šampion’ | |
| X | 142.0 | 154.7 | 164.9 | 266.9 | 182.1 |
| XI | 226.3 | 101.2 | 86.6 | 340.5 | 188.7 |
| XII | 297.3 | 123.2 | 163.3 | 336.9 | 230.2 |
| Mean | 221.9 | 126.4 | 138.3 | 314.8 | X |

$LSD_{(A)}$ = 27.9 $LSD_{(B)}$ = 21.9 $LSD_{(AxB)}$ = 48.3 $LSD_{(BxA)}$ = 43.90

Changes in antioxidant content during storage depended on the cultivar. The greatest fluctuation in antioxidant capacity appeared in ‘Šampion’ (GSH content and GR activity) and in ‘Jonagold’ (ASC content). However, generally the content and activity of antioxidants in December remained at the level of October or increased. Hence these compounds were probably at sufficient levels to be effective during such a period of storage. These results may also suggest that

the protection from oxidative damage runs in different ways in selected genotypes. The plant can achieve such protection either by possessing a high endogenous level of non-enzymatic and enzymatic antioxidants or/and by the quick induction of synthesis of those compounds.

The results of many experiments clearly demonstrated that antioxidant content depends on the cultivar, harvest time and length of storage (Curry, 1997; Lachman et al., 2000ab; Van der Sluis et al., 2001). Subsequently a high cellular level of antioxidant has also been shown to correlate with the plant adaptation to extremes of temperature, water deficit stress, xenobiotic and other environmental stress factors. Hence the level and activity of antioxidant system(s) may be a convenient marker of resistance. Lachman et al. (2000a) reported that the content of anthocyanins and chalcones is positively correlated with the resistance of apple trees to low and variable temperatures. Van der Sluis et al. (2001) reported that of the four tested cultivars, 'Jonagold' apples possessed both the highest antioxidant activity and flavonoid concentration.

The level of glutathione reductase activity generally increased during the tested period and the highest was exhibited by 'Šampion' (Tab. 3). Ascorbate proxidase activity did not significantly change between the tested cultivars and during storage (Tab. 4). The lowest activity of APX was obtained for 'Jonagold', but simultaneously this cultivar had a high concentration of ascorbate. Changes in antioxidant metabolism are a general feature of the plant response to different environmental conditions. Larrigaudiere et al. (2001) reported that immediately after storage the level of $H_2O_2$ sharply increased for both cold storage and controlled atmosphere. The highest amounts of $H_2O_2$ were found after 4 days. Ascorbate and glutathione content declined up to 8 days and then started to increase. At low temperature, the $H_2O_2$ concentration in the peel of 'Red Delicious' apples gradually increased to a maximum in November (ZhiGuo et al., 1994). A major function of GSH and ASC in the protection against oxidative stress is the removal of hydrogen peroxide. Additionally, an efficient recycling of glutathione is ensured by glutathione reductase activity.

Table 3. Activity of GR [U $g^{-1}$ f.w.] in four cultivars of apple during cold storage

| Month (B) | Cultivar (A) | | | | Mean |
|---|---|---|---|---|---|
| | 'Jonagold' | 'Gloster' | 'Elise' | 'Šampion' | |
| X | 0.18 | 0.08 | 0.24 | 0.17 | 0.17 |
| XI | 0.21 | 0.25 | 0.11 | 0.42 | 0.24 |
| XII | 0.20 | 0.28 | 0.15 | 0.48 | 0.28 |
| Mean | 0.20 | 0.20 | 0.17 | 0.35 | X |

$LSD_{(A)}$ = 0.09 $LSD_{(B)}$ = 0.07 $LSD_{(AxB)}$ = 0,16 $LSD_{(BxA)}$ = 0.14

Table 4. Activity of APX [U $g^{-1}$ f.w.] in four cultivars of apple during cold storage

| Month (B) | Cultivar (A) | | | | Mean |
|---|---|---|---|---|---|
| | 'Jonagold' | 'Gloster' | 'Elise' | 'Šampion' | |
| X | 2.34 | 3.44 | 2.57 | 4.01 | 4.01 |
| XI | 2.10 | 3.13 | 3.37 | 3.16 | 3.16 |
| XII | 1.99 | 3.65 | 3.45 | 3.36 | 3.36 |
| Mean | 2.14 | 3.40 | 3.13 | 3.51 | X |

Flesh and peel significantly differed in GSH and ASC content as well as in GR and APX activity (Tab. 5). Glutathione and ascorbate content in the peel was about 2 and 6 times higher, respectively in comparison to the flesh and the difference was stable during storage in all tested cultivars. The skin is an important barrier to protect fruits against unfavourable environmental factors (e.g. elevated thermal and UV radiation) and in view of the human diet it is a rich source of antioxidants. The results obtained by ZhiGuo et al. (1996) suggest that the concentration of simple phenols in fruit peel at harvest affects tissue browning during scald development, and that anthocyanins may play a protective antioxidant role in this respect.

Table 5. Distribution of GSH, ASC and enzyme activity in fruit peel and flesh

| Compound | Part of a fruit | |
|---|---|---|
| | flesh | peel |
| GSH [nmol $g^{-1}$ f.w.] | 28.5a | 55.5b |
| ASC [μg $g^{-1}$ f.w.] | 56.6a | 344.1b |
| GR [U $g^{-1}$ f.w.] | 0.10a | 0.36b |
| APX [U $g^{-1}$ f.w.] | 2.20a | 3.89b |

Eberhardt et al. (2000) reported that the total antioxidant activity of a 1 g sample of apple with skin was 83.3 TOSC (µmol vit. C equivalent) and without skin 46.07. It was also suggested that phytochemicals in apples other than ascorbic acid seem to significantly enhance their antioxidant properties and capacity to inhibit the proliferation of tumour cells in vitro. There were considerable differences in the content of flavonoids and phenolic compounds, e.g. extract from 'Red Delicious' contained 290.2 and 219.8 mg phenolics, and 142.7 and 97.6 mg flavonoids per 100 g of apples with and without skin, respectively. It was concluded that natural antioxidant from fresh fruits could be more effective than a dietary supplement.

Glutathione and ascorbate likewise phenolic compounds primarily appeared in the peel of all tested cultivars. However their content between genotypes significantly differed. Changes in antioxidant metabolism of apple may be perceived as oxidative stress during cold storage, but antioxidant content and enzyme activity were probably high enough to protect fruits against such damage during the short time of cold storage.

## CONCLUSIONS

1. Content of tested antioxidants depended on cultivar, part of fruit and time of storage.
2. The highest content of glutathione and ascorbate was found in 'Šampion' apples.
3. Changes in glutathione-ascorbate cycle during storage run in different ways in tested genotypes of apples.
4. Natural antioxidants in fresh apples are concentrated in the peel that is important for keeping their quality and for consumption benefits.

## REFERENCES

Akerboom T.P.M., Sies H. 1981. Assay of glutathione, glutathione disulphide, and glutathione mixed disulfides in biological samples. METH. ENZYMOL. 77: 373-382.

Anderson J.V., Chevone B.I., Hess J.L. 1992. Seasonal variation in the antioxidant system of eastern white pine needles. PLANT PHYSIOL. 98: 501-508.

Curry E.A. 1997. Effect of postharvest storage on apple nutritional status using antioxidant as a model. Proc. “Health functional fruit and vegetables”, Lexington, Kentucky, 8 October, 1996. HORT. TECHNOL. 7: 240-243.

Eberhardt M.V., Lee Ch.Y., Liu R.H. 2000. Nutrition: Antioxidant activity of fresh apples. NATURE 405: 903-904.

Foyer C.H., Halliwell B. 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. PLANTA 133: 21-25.

Hermann M.E. 2000. An apple a day.....oder die gesundheitliche Bedeutung des Apfels. ERWEBSOBSTBAU 42: 113-117.

Lachman J., Orsak M., Pivec V. 2000a. Antioxidant complex of bioflavonoids and ascorbic acid in apples (*Malus pumila* Mill.). CZECH J. FOOD SCI. 18: 153-158.

Lachman J., Orsak M., Pivec V., Kucera J. 2000b. Effect of the year and storage on ascorbic acid content and total polyphenol content in three apple varieties. CZECH J. FOOD SCI. 18: 71-74.

Larrigaudiere C., Lentheric I., Pinto E., Vendrell M. 2001. Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in conference pears. J. PLANT PHYSIOL. 158: 1015-1022.

Nakano Y., Asada K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts, its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. PLANT CELL PHYSIOL. 28: 131-140.

Noctor G., Foyer C. 1998. Ascorbate and glutathione: Keeping active oxygen under control. ANNU. REV. PHYSIOL PLANT MOL. BIOL. 49: 249-279.

Noctor G., Arisi A.C.M., Jouanin L., Kunert K.J., Rennenberg H., Foyer C. H. 1998. Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. EXP. BOT. 49: 623-647.

Van der Sluis A., Dekker M., de Jager A., Jongen W.M.F. 2001. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J. AGRIC. FOOD CHEM. 49: 3606-3613.

ZhiGuo J., YongBing Y., ChengLian L., HongYi D., RunJin L. 1994. Effect of low temperature on $H_2O_2$ during storage of apples. J. FRUIT SCI. 11: 10-13.

ZhiGuo J., YongBing Y., ChengLian L., ShuMin Z., MingXiang W. 1996. Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility. POSTHARVEST BIOL. TECHNOL. 8: 83-93.