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Antioxidative Capacity of Different Apple Cultivars after Long-time Storage

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Summary

The antioxidative capacity and vitamin C content of the apple cultivars 'Topaz', 'Bitterfelder', 'Fuji', 'Jonagold', 'Braeburn', and 'Bohnapfel' were examined in three consecutive years from 1999 to 2002 always immediately after harvest and after seven months of storage under air or controlled atmosphere (3 % carbon dioxide, 1 % oxygen) at 1 °C. Values for the antioxidative capacity stayed constant even after a long-time storage regardless of the cultivar and storage conditions. Loss in vitamin C during storage was detected by four of the six investigated cultivars except for 'Topaz' and 'Braeburn' apples. A high contribution of vitamin C to the antioxidative capacity could only be determined with the apple cultivars 'Topaz' and 'Braeburn'.

Introduction

In the body, the normal metabolism of oxygen in living cells, environmental pollutants, radiation, pesticides, various medications, and contaminated water cause the unavoidable production of oxygen-derived free radicals (hydroxyl, peroxyl, hydrogen peroxide, hypochlorous acid), which have been implicated in more than a hundred disease conditions in humans (MURCIA et al., 2001). Oxygen-derived free radicals, for example, are able to react with membrane lipids (KUNERT and DODGE, 1989; MÖRSEL, 1990; WINSTON, 1990), proteins, nucleic acids, and other substances (FOOTE et al., 1984; LARSON, 1988). These reactions result in destruction of numerous cell molecules and furthermore in reduction of important biological functions, and in the end the cell's death (SCANDALIOS, 1993). To reduce the negative effect of such reactions plants posses an antioxidative system. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (VELIOGLU et al., 1998). The group of antioxidants consists of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, phenolic compounds (tocopherols, flavonoids), carotinoids, and ascorbic acid. All these compounds can be found in fruit and vegetables. The consumption of fruit and vegetables has been associated with lower incidence and lower mortality rates of cancer in several human cohort and case-control studies for all common cancer sites (WANG et al., 1996).

Various authors did research on the antioxidative capacity of fruit and vegetables like strawberries, oranges, apples, tomatoes, broccoli flowers, cauliflower, and cabbage for example (WANG et al., 1996; CAO et al., 1996). Further investigations on antioxidants in high pressure treated fruit juice, e.g. orange juice, showed no significant differences in comparison to untreated juice even after storage of up to 21 days (FERNÁNDEZ GARCÍA et al., 2001).

The greater number of publications engaged in antioxidants of fruit and vegetables describe the antioxidant activity of fresh products (EBERHARDT et al., 2000). SCHMITZ-EIBERGER et al. (2003) determined the antioxidative capacity and ascorbic acid content of 31 different apple cultivars and found a large cultivar variation in the antioxidative capacity of fresh apples. Only little is known about the antioxidative capacity of stored apples (AWAD et al., 2003). Therefore, the purpose of our work was to determine the antioxidative capacity of six different apple cultivars after long-time storage under air or controlled atmosphere.

Material and methods

Apples of the cultivars 'Topaz', 'Bitterfelder', 'Fuji', 'Jonagold', 'Braeburn', and 'Bohnapfel' were stored under air and controlled atmosphere (3 % carbon dioxide, 1 % oxygen) at 1 °C for seven months from October to May. The apples were purchased from different regions in southern Germany. The antioxidative capacity of the fruit was determined three times: after harvest, in January, and at the end of the storage period in May. Antioxidative capacity was destinated according to the β-carotene bleaching method (CHEVOLLEAU et al., 1992) with 2-tert-butyl-4-methoxyphenol (BHA) as standard with slight modifications. For every determination juice of 12 apples was extracted and filtered through a 0.45 µm pore size syringe filter to get a random juice sample. 60 µl of the juice were adjusted to 2 ml with methanol and 100 µl of the solution were used for the determination of the antioxidative capacity. For a standard solution 10 mg BHA were mixed with 10 ml methanol and 20 µl of the solution were filled up to 2 ml with methanol. 100 µl of the standard solution were used in the test.

For a typical assay a β-carotene solution was prepared as follows: 3 mg of β-carotene were mixed with 10 ml chloroform, 5 ml of this β-carotene solution were added to a round-bottom flask containing 22 µl linoleic acid and 6 drops of Tween 40. Afterwards the chloroform was completely removed using a rotatory evaporater and 100 ml distilled water was added to the residue. 5 ml of the solution were filled into test-tubes containing either 100 µl methanol, 100 µl BHA or 100 µl apple test solution. The test-tubes were incubated in a waterbath at 50 °C for 30 minutes. The absorbance at 470 nm was monitored at the beginning and after 30 minutes of incubation on a spectrophotometer (Novaspec II, Pharmacia Biotec). All samples were assayed in triplicate. The change in absorbance per minute (v) was calculated with the following equation:

$$v = \frac{\log_{abs} 0 - \log_{abs} 30}{30}$$

v = change in absorbance per minute
$\log_{abs} 0$ = logarithm of the absorbance at the beginning of incubation
$\log_{abs} 30$ = logarithm of the absorbance after 30 minutes of incubation

The antioxidative capacity in µg equiv. BHA in 100 µl apple juice was calculated as follows:

$$\frac{vBHA}{vsample} \times \frac{100}{3} = \text{µg equiv. BHA/ 100 µl apple juice}$$

All above mentioned chemicals were obtained from Merck, Darmstadt. In addition to the antioxidative capacity the vitamin C content was

categorized using a determination kit from Boehringer, Ingelheim. The antioxidative capacity of the different apple cultivars after long-time storage under air and controlled atmosphere was monitored for three consecutive seasons (1999 – 2002) to consider seasonal influences on the antioxidative capacity of the apples.

Results

The average antioxidative capacity in October over a period of three consecutive seasons for the six inquired apple cultivars varied from 18.75 µg BHA equiv./100 µl apple juice in 'Fuji' apples to 41.46 µg BHA equiv./100 µl apple juice in apples of the cultivar 'Bitterfelder' (Fig. 1). Furthermore, Fig. 1 shows that the investigated apple cultivars possessed a lower antioxidative capacity in January and May in comparison to October after storage under air. The 'Bitterfelder' apples which had the highest antioxidative capacity in October showed the greatest loss but had still the second highest antioxidative capacity in May (28.09 µg BHA equiv./100 µl apple juice) following the cultivar 'Bohnapfel' with 30.87 µg BHA equiv./100 µl apple juice. The other four cultivars 'Topaz', 'Jonagold', 'Fuji', and 'Braeburn' showed no clear difference in their antioxidative capacity after storage under air (Fig. 1). It is also obvious though that the average antioxidative capacity in these four apple cultivars was lower over the three years' test period in comparison to the apples of the two old cultivars 'Bitterfelder' and 'Bohnapfel'.

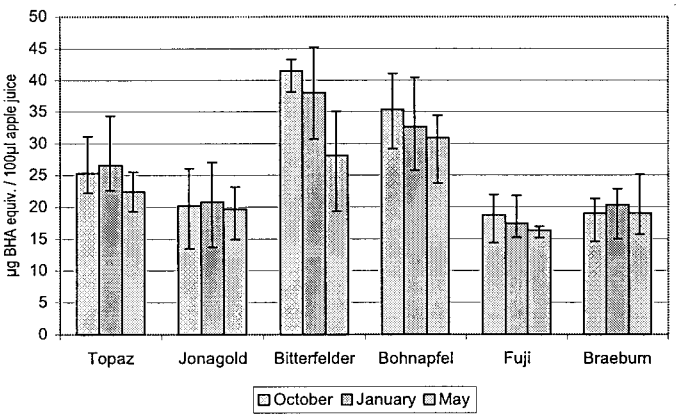

Fig. 1: Changes in antioxidative capacity of apples after 7 months storage time under air at 1 °C. The results are an average of a three years' test period.

The results in Fig. 1 show a good maintenance of the antioxidative capacity of air stored apples after a storage time of seven months. But it can also be seen by the marks for the highest and lowest result that there are strong seasonal differences in the antioxidative capacity of the apples, although the variations for certain storage periods are similar regardless of the apple cultivar.

To keep a good fruit quality of long-time stored apples for the consumer, apples are usually stored under controlled atmosphere (3 % carbon dioxide, 1 % oxygen) at 1 °C and not under air. For this reason, we investigated the changes in antioxidative capacity of the above mentioned different apple cultivars after seven months of storage under the conditions of controlled atmosphere. After a storage period of seven months the antioxidative capacity of stored apples was almost similar to the results of the apples stored under air. The two old cultivars 'Bitterfelder' and 'Bohnapfel' showed the highest antioxidative capacity at the beginning of storage in October and at the end in May. A distinction relative to the cultivars 'Topaz', 'Jonagold', 'Fuji', and 'Braeburn' between cold storage under air and controlled atmosphere storage couldn't be detected. The results are shown in Fig. 2.

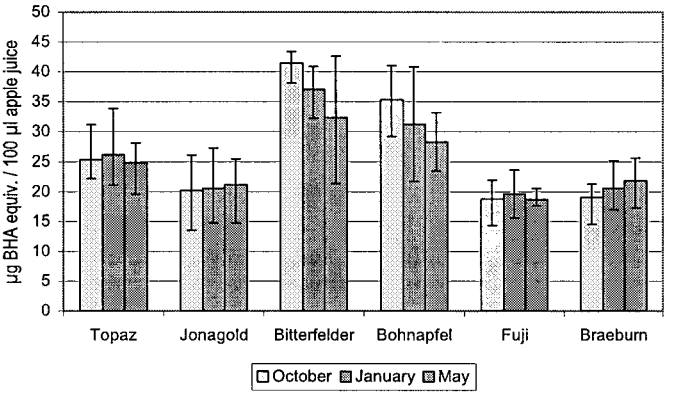

Fig. 2: Changes in antioxidative capacity of apples after 7 months of storage time under controlled atmosphere (3 % carbon dioxide, 1 % oxygen) at 1 °C. The results are an average of a three years' test period.

The most well-known natural antioxidant is probably vitamin C. But the majority of the antioxidant activity of fruit and vegetables may be from compounds such as polyphenols and not vitamin C (WANG et al., 1996; LU and FOO, 2000; MURCIA et al., 2001; SCHMITZ-EIBERGER et al., 2003). WANG et al. (1996) calculated the contribution of vitamin C to the total antioxidative capacity of fruit of less than 15 %. Fig. 1 and 2 show a relative constant antioxidative capacity of apples during a storage time of seven months. It is also known that the vitamin C content in apples during storage can drop by 50 %. For this reason, in this study the vitamin C content of stored apples was determined to demonstrate the role of vitamin C for the antioxidative capacity of apples during storage.

Results in Fig. 3 demonstrate four apple cultivars, 'Jonagold', 'Bitterfelder', 'Bohnapfel', and 'Fuji', respectively, showing a significant lower vitamin C content at the beginning of storage under controlled atmosphere compared to their antioxidative capacity. Similar values for antioxidative capacity and vitamin C content could only be evaluated for 'Topaz' and 'Braeburn' apples in October before storage. After a storage period of seven months all six apple cultivars possessed almost the same antioxidative capacity in consideration of the highest and lowest measured values. In the same time the vitamin C content of five apple cultivars dropped to values ap-

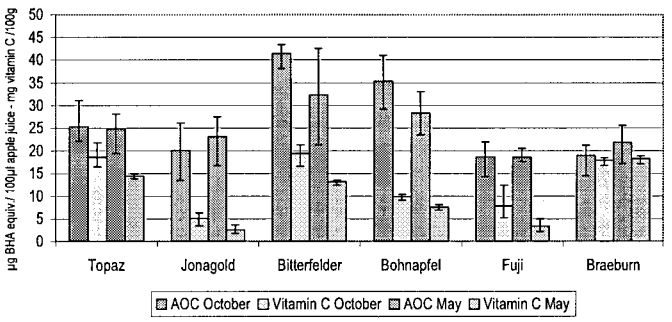

Fig. 3: Antioxidative capacity and vitamin C content of different apple cultivars at the beginning and the end of a seven months' storage period under controlled atmosphere (3 % carbon dioxide, 1 % oxygen) at 1 °C. AOC = antioxidative capacity. Bars show the mean value of a three years' test period.

proximately 50 % of the content in October, e.g. 'Jonagold' and 'Fuji'. In the available study a low contribution of vitamin C of about 15 % to the antioxidative capacity as calculated by WANG et al. (1996) could only be detected for the apple cultivars 'Jonagold' and 'Fuji'. A remarkable result could be determined for the cultivar 'Braeburn'. The vitamin C content in 'Braeburn' apples did not change during storage under controlled atmosphere for seven months. Furthermore, Fig. 3 shows clearly that vitamin C is the main antioxidant in 'Braeburn' apples in contrast to the other investigated apple cultivars.

Discussion

Fruit and vegetables have to be found to posses a possible protective effect against cancer relating to their antioxidative capacity. The purpose of most of the publications was to determine the antioxidative capacity of fresh fruit and vegetables immediately after harvest. The objective of this study was to investigate the stability of the antioxidative capacity of different apple cultivars during storage under air and controlled atmosphere at 1 °C. The results clearly show a very good maintenance of the antioxidative capacity during a storage period of seven months regardless of the cultivar and storage conditions (Fig. 1 and 2). With regard to the apple cultivar the result of the old apple cultivars 'Bitterfelder' and 'Bohnapfel' was remarkable. Both cultivars showed the highest antioxidative capacity of all in this study examined apples. This result for the apple 'Bohnapfel' agrees with the results of SCHMITZ-EIBERGER et al. (2003). On the other hand the vitamin C content could be determined in the range of the other investigated apple cultivars. This result makes obvious that the contribution of vitamin C to the antioxidative capacity can be 50 % or less depending on the apple cultivar. A higher proportion of vitamin C in the antioxidative capacity could only detected for the apples of the cultivars 'Topaz' and 'Braeburn'.

Summarising the results presented in this work, it can be assumed that the healthy properties of apples depending on the antioxidative capacity are not reduced during long-term storage under air or controlled atmosphere. In comparison to the antioxidative capacity the vitamin C content of the different apple cultivars was clearly reduced during storage except for the apple cultivar 'Braeburn'. For this cultivar Fig. 3 also shows a very good preservation of the vitamin C content during storage and, therefore, also long-time stored 'Braeburn' apples could serve as a good vitamin C source in human nutrition. Furthermore, our results are similar to other publications, e.g. WANG et al. (1996), which found little contribution of vitamin C to the antioxidative capacity except the apple cultivars 'Topaz' and 'Braeburn', or AWAD et al. (2003) who found a similar antioxidative capacity in 'Jonagold' and 'Elstar' apples after a storage period of 8 months regardless to the storage conditions.

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