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Antioxidant properties of two apple cultivars during long-term storage

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Abstract

The antioxidant capacity was determined in the peel of two apple cultivars (Jonagold and S'ampion) stored for 120 days at 1 °C, either in the regular cold chamber or in CA (2% CO_2/2% O_2). During long-term cold storage as well as during an additional 7 day storage of fruits at 16 °C, total phenols, total antioxidant activity (TAA), and radical scavenging activity (RSA) increased considerably, irrespective of the storage conditions. A slight decrease in anthocyanins was observed in apples stored in air, while the CA treatment did not cause any significant changes. Increase of soluble peroxidase (POD) activity was much stronger in apples kept in air than in CA, while, after a subsequent 7 day storage at high temperature, a further increase in enzyme activity was observed in all treatments. The high activity of polyphenoloxidase (PPO), determined in freshly harvested fruits, dropped to low (Jonadgold) or undetectable (S'ampion) levels after cold storage, both in the regular chamber and in CA, and increased slightly after the high temperature treatment. In the stored fruits a marked ethylene evolution was found, especially when stored in air.

Keywords: Apple; Storage; Phenolics; Antioxidant activity

1. Introduction

The high participation of fruits and vegetables in the human diet, because of their ability to neutralize active oxygen species, hazardous for health, is of utmost importance. Plant tissue antioxidant capacity is closely associated with activity of "free radical scavenging enzymes" (superoxide dismutase, catalase, peroxidase) and with the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid (Bartosz, 1997).

Recently, antioxidant activity has been determined in many species of fruits, vegetables, herbs, cereals, sprouts and seeds (Kahkonen et al., 1999; Velioglu, Mazza, Gao, & Oomah, 1998). Especial attention is paid to fruits, as rich sources of phenolic compounds (Kalt, Forney, Martin, & Prior, 1999; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Wang & Lin, 2000). Among others, the antioxidant properties of apple polyphenols have been extensively examined (Ju & Bramlage, 1999; Lu & Foo, 2000; Robards et al., 1999). The apple phenolics, localized mainly in cortex and in skin, e.g. cinamic acid derivatives, flavonols and anthocyanins (Robards et al., 1999), are compounds with strong antioxidant activity (Lu & Foo, 2000).

The phenolic composition of fruits and, hence, their antioxidant properties may be modified by environmental and post-harvest factors, including storage and processing (Robards et al., 1999). The aim of the present research was to investigate the antioxidant properties of two apple cultivars, stored for 4 months at 0 °C in the regular storage chamber and in a controlled atmosphere. After cold storage, fruits were additionally kept for 1 week at 16 °C to simulate the commonly applied conditions. In the apple peel, total antioxidant activity (TAA), expressed as the percentage inhibition of linoleic acid peroxidation, and radical scavenging activity (DPPH-scavenging activity) were measured. Total phenolics and anthocyanins, as the main antioxidants, and activities of enzymes involved in oxidative reactions, such as polyphenoloxidase (PPO) and peroxidase (POD), were also determined. To evaluate the physiological maturity stage of the stored fruits, the level of endogenous ethylene was monitored.

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Nomenclature

AA	ascorbic acid
CA	controlled atmosphere
TAA	total antioxidant activity
RSA	radical scavenging activity
DPPH	α,α-diphenyl-β-picrazylhydrazyl radical
POD	peroxidase
PPO	polyphenoloxidase

2. Materials and methods

Apples of two cultivars (Jonagold and S'ampion) were harvested at the end of September 2000 and stored for 120 days at 0 °C, either under the regular storage conditions (85–90% RH) or in a controlled atmosphere (CA) chamber (2% CO_2, 2% O_2, 95% RH) until analysed. The fruit peel (mean sample of 16 fruits of each cultivar) was analysed just after harvesting, after cold storage, followed by 1 day storage at 16 °C and after an additional 7 days at 16 °C. Ethylene evolution was determined at the same times, in the whole fruits (mean sample of 16 fruits of each cultivar).

Total phenol content was measured by the colorimetric method with Folin's reagent (Swain & Hillis, 1959). Anthocyanins were detected according to the colorimetric method of Fuleki and Francis (1968). Total antioxidant activity (TAA) was estimated by measuring percentage inhibition of linoleic acid peroxidation, as described by Toivonen and Sweeney (1998). Radical scavenging activity (RSA) was determined by using DPPH as the stable radical and expressed as the percentage of its neutralization (Pekkarinen, Stockmann, Schwarz, Heinnonen, & Hopia, 1999). Polyphenoloxidase activity (PPO) was detected by the method of Siriphanich and Kader (1985), using chlorogenic acid as a substrate instead of caffeic acid. Soluble peroxidase activity was determined using the benzidine-ascorbate method (Lyr, 1975). Ethylene production was measured by a gas chromatograph, equipped with a flame-ionization detector.

All analyses were made in four replications and the results were statistically evaluated, using Duncan's multiple range test at $P = 0.05$.

3. Results

Total phenol content increased significantly in apples stored at 0 °C, either in the regular cold chamber and CA (Jonagold) or in CA (S'ampion). The additional 7 day storage at 16 °C caused a further increase of phenolics in apples of the S'ampion cultivar, stored previously in a cold chamber, as well as in CA (Table 1). The level of phenol compounds determined in the peel of fruits of Jonagold was significantly higher than that of S'ampion.

Content of anthocyanins decreased in apples stored in the regular cold chamber while, in the case of CA treatment, their level did not change. After 7 days' storage at 16 °C, a further decrease was observed in fruits previously kept in the cold chamber; a slight reduction of anthocyanins in Jonagold apples stored in CA was also found. The level of anthocyanins was similar in both cultivars (Table 1).

The relatively low total antioxidant activity, observed in the freshly harvested apples, increased considerably after storage in the cold chamber and in CA. This high value of TAA was maintained during 7 days' storage at 16 °C. In apples of the S'ampion cultivar, previously stored in the cold chamber, a slight increase was noticed. In most cases total antioxidant activity detected in the peel of Jonagold apples was higher in comparison with S'ampion (Table 1).

High radical scavenging activity, observed after harvesting, rose significantly during cold storage, both in the cold chamber and in CA. The additional high temperature treatment caused a slight increase in RSA in Jonagold apples. The radical scavenging activity of S'ampion was higher than that of Jonagold (Table 1).

Storage at 0 °C, in the regular cold chamber, caused increase in activity of soluble peroxidase in both cultivars. A significant increase of the enzyme activity was also found in S'ampion apples stored in CA. Further increase in POD activity was noticed after an additional 7 day storage at 16 °C, except in the non-significant increase in apples of Jonagold, stored previously in the cold chamber. In apples of S'ampion cultivar, stored in the cold chamber, the activity of POD was much higher than in CA. Similarly, S'ampion apples, treated with high temperature, showed a higher level of enzyme activity when stored previously in the cold chamber in comparison with CA storage. The enzyme activity of S'ampion was significantly higher than in the case of Jonagold cultivar (Table 1).

High activity of polyphenoloxidase, observed in the freshly-harvested apples, decreased markedly after storage in the cold chamber, as well as in CA, reaching an undetectable level in the skin of S'ampion apples. The 7 day storage at 16 °C affected an increase in the enzyme activity; however, its level was much lower than the initial value. Activity of PPO in Jonagold apples was consideraby higher than in S'ampion (Table 1).

The low level of endogenous ethylene, observed in the freshly harvested apples, rose markedly after cold storage, especially in the case of the regular cold chamber. Further increase of C_2H_4 production was observed after 7 days' storage at high temperature in Jonagold apples kept both in the cold chamber and in CA and in S'ampion fruits in CA (Table 1).

Table 1
Influence of storage on various components of apples[a]

Components	Time of storage				
	0 time	120 days cold chamber	120 days CA chamber	Cold chamber + 7 days[b]	CA chamber + 7 days[c]
Total phenols (mg 100 g^{-1} f.w.)					
Jonagold	520 d	600 f	635 h	640 h	612 g
S'ampion	418 a	418 a	509 c	585 e	480 b
Anthocyanins (mg 100 g^{-1} f.w.)					
Jonagold	158 de	119 b	147 cd	103 a	138 c
S'ampion	160 e	138 c	156 de	122 b	152 de
Total antioxidant activity (%)					
Jonagold	26.0 a	66.2 de	61.5 de	62.5 de	68.3 e
S'ampion	29.0 a	43.2 b	46.9 bc	63.9 de	54.9 cd
Radical scavenging activity (%)					
Jonagold	53.8 a	72.2 c	70.6 c	83.8 e	77.8 d
S'ampion	61.7 b	88.4 f	88.3 f	88.3 f	91.1 f
Peroxidase activity (mg AA 100 g^{-1} f.w. s^{-1})					
Jonagold	2.10 a	5.08 bc	2.94 ab	7.19 cd	8.85 de
S'ampion	8.20 d	20.5 f	10.6 e	34.9 g	19.7 f
Polyphenoloxidase activity (U 100 g^{-1} f.w. min^{-1})					
Jonagold	62.1 g	14.2 de	13.50 d	17.1 e	24.3 f
S'ampion	15.4 de	0.00 a	0.00 a	7.31 c	2.99 b
Ethylene (nlC_2H_4 g^{-1} f.w. h^{-1})					
Jonagold	0.12 a	60.5 c	15.7 b	102.3 e	126.7 f
S'ampion	0.72 a	78.0 d	13.9 b	86.9 d	83.8 d

[a] Means followed by the same letters are not significantly different. f.w., fresh weight.
[b] Cold chamber (0 °C, air) + 7 days (16 °C, air).
[c] CA chamber (0°, 2%CO_2/2%O_2) + 7 days (16 °C, air).

4. Discussion

The observed increase of total phenols, especially in apples stored in the regular cold chamber, was in most cases accompanied by a decrease of anthocyanins. The present results are different from those reported by Mazza and Miniati (1993) who observed a relatively stable level of apple anthocyanins in fruits stored at 2 °C and a decrease in a low O_2 and high CO_2 atmosphere. Decrease in anthocyanins, in apples stored for 7 days at high temperature, does not correspond to the findings of Kalt et al. (1999); according to them, a short-term storage (8 days) of small fruits (raspberry and strawberry) at high temperatures (10, 20 and 30 °C) strongly affected accumulation of phenolics and anthocyanins.

According to our results of ethylene evolution, apples of both cultivars were harvested at the pre-climacteric stage. Long-term storage strongly induced production of C_2H_4, especially in apples stored in the regular cold chamber. Short-term high temperature treatment intensified ethylene evolution. The significant increase in total phenolics, observed during cold storage and during additional storage at 16 °C, could be due to ethylene action. This hormone stimulates activity of phenylalanine ammonia lyase, a key enzyme in biosynthesis of phenolic compounds and accumulation of phenolic constituents (Hwang, Myoung-Won, & Young-Hee, 1994; Ritenour, Ahrens, & Saltveit, 1995). On the other hand, a very low level of polyphenoloxidase activity, found in the stored apples, might have reduced oxidation of phenolic substrates to quinones, and, hence, their content determined after storage was higher than in freshly harvested fruits, where PPO activity was high.

According to many authors, antioxidant activity of fruits results mainly from phenolics, particularly flavonoids. Kalt et al. (1999) found a strong correlation between antioxidant capacity and total phenols ($r = 0.83$), as well as anthocyanin ($r = 0.90$) contents, in four species of berries. Wang and Lin (2000) found a linear correlation between total phenolic content and ORAC activity for fruits and leaves of blackberry, raspberry and strawberry; in ripe berries the level of anthocyanins corresponded with antioxidant activity. Ju and Bramlage (1999) observed strong antioxidant activity of free phenolics obtained from apple cuticle, especially of quercetin, when measured in a linolate

emulsion (oil-in-water) model system. Lu and Foo (2000), who investigated antioxidant properties of apple pomace, both in beta-carotene/linoleic acid and a DPPH-scavenging system, reported high effectivness of all the examined phenolics. These authors observed particularly high RSA of quercetin glycosides, procyanidins and chlorogenic acid, considerably exceeding those of ascorbic acid and tocopherol.

The interdependence between phenolics and antioxidative properties of apples seems, however, to be questionable. Kahkonnen et al. (1999) did not find any correlation between the high antioxidant activity and relatively low level of phenol constituents in apple extracts. Similarly, in the preliminary investigations of the present authors (Mareczek, Leja, & Ben, 2000) the high content of total phenols in the peel of Golden Reinders and Gala Must fruits did not correspond to the low antioxidant activity, expressed as inhibition af linoleic acid peroxidation.

In the present study, antioxidant capacity of fruit peel, determined either as inhibition of linoleic acid peroxidation or DPPH-scavenging activity, increased significantly after cold storage, irrespective of the storage conditions, and was accompanied by an increase in the total phenol content but not in anthocyanins Additional storage at 16 °C caused slight further increase in TAA of S'ampion apples as well as in RSA, of Jonagold fruits stored previously in the regular cold chamber; increase of total phenols was observed in some cases.

In general, changes of TAA in stored apples were more distnct than those of RSA. This phenomenon was probably due to the composition of phenols synthesized in the peel (Ju & Bramlage, 1999; Lu & Foo, 2000). In a linoleate-emulsion system, lipophilic phenolics were more active than hydrophilic ones (Ju & Bramlage, 1999), and the order of antioxidant capacity of phenolics obtained from apple pomace was slightly different, in the case of a beta carotene/linoleic acid system, from a DPPH-scavenging one (Lu & Foo 2000). The separation of apple phenolics seems to be necessary in further investigations.

Especial attention should be paid to peroxidase activity, as an enzyme involved in neutralisation of active oxygen species (Bartosz, 1997). Considerable increase in POD activity in the peel of apples stored at low temperature might have been caused by ethylene action. According to Gaspar, Penel, Thorpe, and Greppin (1982), peroxidase activity is stimulated by C_2H_4 evolution. In apples stored in CA, this increase was either non-significant (Jonagold) or less intensive than in the regular cold chamber (S'ampion).

The reduced concentration of oxygen inhibits activity of POD (Ke & Saltveit, 1989). However, in lettuce stored in a controlled atmosphere for 3 weeks, a marked increase in soluble peroxidase activity was noticed (Leja, Mareczek, & Rożek, 1996).

The results obtained in the present study showed a distinct effect of long term storage at low temperature and of an additional short-term treatment with high temperature, on phenolic accumulation and antioxidant capacity of apple peel, irrespective of atmosphere composition (air or CA). Only increase in soluble peroxidase activity was less in CA than in the regular cold chamber conditions. The high antioxidant capacity of apple peel, observed in fruits stored for 120 days, may be regarded as evidence of their high nutritional quality.

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