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Antioxidant ability of broccoli flower buds during short-term storage

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Abstract

Some metabolic changes in broccoli heads stored under commonly-applied conditions were investigated. Freshly harvested broccoli of Lord cultivar were stored at 20°C and at 5°C for 3 and 7 days, respectively, either non-packaged or packaged in polymeric film samples. Short-term storage at room temperature induced accumulation of total phenols, especially in non-packaged broccoli. With low-temperature treatment, phenol content rose only after 7 day storage of non-packaged heads. Both low temperature and application of polymeric foil stopped losses of ascorbic acid. Total antioxidant activity increased considerably during storage in all treatments. Changes of fatty acids were manifested as a slight decrease in saturated fatty acids in cold storage and increase of polyunsaturated fatty acids in most treatments. Metabolism of fatty acids did not correspond to thiobarbituric acid-reactive substances (products of lipid peroxidation).

1. Introduction

Broccoli (*Brassica oleracea* var. Italica) has recently become more popular as a component of human diet of high nutritional value. The post-harvest senescence of this perishable vegetable can, however, lower its quality. Apart from yellowing of broccoli heads, caused by chlorophyll degradation (King & Morris, 1994), protein decomposition (Zhuang, Hildebrand & Barth, 1995, 1997) usually accompanied by considerable increase in free amino-acid level (King & Morris 1994; Leja, Wojciechowska, Mareczek & Kunicki, 1997) has also been observed. In short-term stored broccoli, the distinct reduction in soluble sugar, as well as ascorbic acid content, were reported (Pogson & Morris, 1997; Ye & Chen, 1995). According to Zhuang et al. (1995, 1997) post-harvest senescence of broccoli, expressed by the chlorophyll losses, is correlated with lipid peroxidation, leading to cell-membrane disintegration.

The aim of the present study was to investigate some metabolic changes in broccoli flower buds during short-term storage of broccoli heads under commonly-applied conditions. The levels of antioxidant compounds, such as ascorbic acid and total phenolics, as well as antioxidant activity were measured . To estimate the degree of plant deterioration, the fatty acid composition of the protein-lipid membrane, and the products of lipid peroxidation, were also determined.

2. Materials and methods

Broccoli of the Lord cultivar was grown in 1999 in the spring and autumn growing cycles, in the Agricultural University experimental station in the Kraków area. The broccoli heads were harvested in June (spring experiment) and in October (autumn experiment).

The freshly-harvested heads were analyzed immediately (0 time); the others were taken for the storage experiment. The samples (six heads in each sample) were packaged in the commercial polymeric film and stored either at 20°C for 3 days, or at 5°C for 7 days in darkness, at high relative humidity. The non-packaged broccoli was stored under the same conditions.

The samples were taken for analysis after 1 and 3 days (room temperature) and after 1, 3 and 7 days (low temperature) of storage. Total phenols and ascorbic acid level were measured in the edible parts of broccoli; for total antioxidative activity, lipid composition and TBS (malondialdehyde and monoaldehydes), the flower buds were used.

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Nomenclature

TBS	thiobarbituric acid reactive substances
TAA	total antioxidant activity
SA	saturated fatty acids
PUFA	polyunsaturated fatty acids
MDA	malondialdehyde
MA	monoaldehydes

Total phenols were estimated according to the photometric method with Folin's reagent (Swain & Hillis, 1959). Ascorbic acid content was measured using the iodate-titration method described by Samotus, Leja and Ścigalski (1982). Total antioxidant activity was determined by the measurement of inhibition of linoleic acid peroxidation, given by Toivonen and Sweeney (1998), modified by application of 80% methanol as an extraction solvent, instead of buffer. Fatty acid composition was determined according to the method presented by Zhuang, Barth and Hildebrand (1994); the methylated fatty acids were measured by gas chromatography using a Supelcowax, 3 m×53 mm, column. The products of fatty acid decomposition, such as malondialdehyde and monoaldehydes were estimated by reaction with thiobarbituric acid (Zhuang et al., 1997).

For each determination, four replications were made. The results obtained were statistically evaluated, using Duncan's test, for significance at $P < 0.05$.

The results of the spring and autumn experiments were similar, so only those of the autumn growing cycle are presented.

3. Results and discussion

Application of commercial polymeric film delays the senescence process by lowering of O_2 and elevating CO_2 level. According to Zhuang et al. (1994), oxygen and carbon dioxide concentrations, measured inside the polymeric film package of broccoli, stored at 5°C, were above 10% and about 7–7.5%, respectively. Hence, storage conditions applied in the present study (low temperature and polymeric film) favoured the senescence inhibition, while storage of non-packaged samples, particularly at room temperature, induced it.

Special attention was paid to post-harvest metabolism of substances considered as antioxidants against active oxygen species (AOS), such as ascorbic acid and soluble phenolics. According to recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Vinson, Yong, Xuchui & Zubik, 1998; Velioglu, Mazza, Gao & Oomah, 1998). In the edible parts of broccoli, 75% of the phenolic fraction consists of antioxidant agents (Plumb, Price, Rhodes & Williamson, 1997). In the present investigations, a considerable increase in total phenol level was observed during short-term storage of broccoli at room temperature, but this was less distinct in packaged heads. In the case of low temperature treatment, the phenol content rose after 7 day storage and in non-packaged samples only (Table 1). Accumulation of phenolic compounds, as an index of post-harvest senescence, was observed during short-term storage of lettuce at 20°C and 5°C (Leja, Rożek & Myczkowski, 1994). However, application of an elevated CO_2 level (15%) to lettuce stored for 6 days at 0°C suppressed increase in phenolics (Siriphanich & Kader, 1995).

One day of storage at room temperature caused significant increase in ascorbic acid content, followed by decrease, observed in non-packaged samples. Broccoli, stored at 5°C, reacted by increase in ascorbic acid level, irrespective of the packaging. A slight decrease in this compound was found after 7 day cold storage in the packaged broccoli heads (Table 1). Rożek, Leja, Myczkowski and Mareczek (1994) observed similar changes of ascorbic acid in stored lettuce; the increase in this compound was probably due to the stress of harvesting and low temperature treatment. Application of polymeric film stopped the ascorbic acid losses at room temperature, while a considerable reduction in vitamin C level was found in the leaves of lettuce stored in a controlled atmosphere (Adamicki, 1989; Leja, Mareczek & Rożek, 1996).

Total antioxidant activity, determined in the flower buds of stored broccoli, showed a marked increase which was less rapid in the case of packaged samples. According to investigations of Toivonen and Sweeney (1998) in flower buds of broccoli, stored for 4 days at 13°C, only a slight increase in total antioxidant activity was noticed, accompanied by rise of activities of "free radical scavaging enzymes" such as superoxide dismutase, peroxidase and catalase. Total antioxidant activity, determined by these authors in two broccoli cultivars, was very high in the freshly harvested heads (80%) which, during short-term storage, increased to 90% (Toivonen & Sweeney). Broccoli of Lord cultivar, examined in the present study, had relatively low initial TAA (4.8%), however, this value increased distinctly (to 60–70%) during storage, especially with a 7 day low temperature treatment (Table 1). The elevated total antioxidant activity in brocoli stored at 20°C might have been due to phenolic antioxidant agents. The phenol accumulation was accompanied by an increase in TAA; however, in the case of cold storage when total phenolics were accumulated only after 7 day storage in non-packaged samples, this dependence was not evident (Table 1). According to Velioglu et al. (1998) who examined 28 plant products, in many cases the high antioxidant activity was not correlated with the phenol content; probably other factors played major roles as antioxidants.

Table 1
Influence of packaging and temperature on various components of broccoli

Components	Days of storage[a]					
	0 time	1 day		3 days		7 days
		20°C	5°C	20°C	5°C	5°C
Total phenols (mg·100^{-1} f.w.)[b]						
Non-packaged	56.2c	76.0f	57.9c	78.9g	57.8c	71.2e
Packaged		65.22d	53.25b	73.18e	50.47a	56.08c
Ascorbic acid (mg·100^{-1} f.w.)						
Non-packaged	60.1c	69.3f	68.3ef	44.7a	71.0g	75.1h
Packaged		71.13g	67.61e	61.60d	68.05ef	58.37b
Total antioxidant activity (%)						
Non-packaged	4.8a	30.1c	26.6c	41.8d	30.0c	69.5f
Packaged		11.7ab	14.4b	30.5c	23.7c	59.4e
Saturated fatty acids (%)						
Non-packaged	36.1d	35.4d	28.3bc	38.6d	28.2bc	25.1ab
Packaged		24.6ab	30.8c	30.5c	27.0abc	23.5a
Polyunsaturated fatty acids (%)						
Non-packaged	55.5ab	61.7bc	70.0e	54.2a	59.7abc	70.3e
Packaged		70.27e	69.20de	63.28cd	63.24cd	69.87e
Malondialdehyde ($\mu M \cdot g^{-1}$ f.w.)						
Non-packaged	13.8d	13.0cd	12.7bcd	17.9e	13.6d	10.8b
Packaged		11.0bc	11.2bc	14.4d	13.0d	7.07a
Monodialdehydes ($\mu M \cdot g^{-1}$ f.w.)						
Non-packaged	239f	179cde	197.6e	189.7de	163bcd	207e
Packaged		153abc	182de	132a	138ab	125a

[a] Means followed by the same letters are not significantly different.
[b] f.w., fresh weight

Zhuang et al. (1997) reported, that 85% of total fatty acids in broccoli flower buds consisted of phospholipids and glycolipids of the cell membranes, so the changes in their level reflects the degree of post-harvest deterioration. Zhuang et al. (1994) observed, in non-packaged broccoli stored for 96 h at 5°C, lowering of PUFA and increased level of them in MAP, while the contents of saturated fatty acids decreased both in non-packaged samples and in MAP. In the present study, a slight decrease in saturated fatty acids at 5°C was found, both in packaged and in non-packaged samples (Table 1), caused mainly by reduction of C 18:0.

The PUFA level rose in the packaged broccoli heads stored at 20°C and at low temperature, both in packaged and non-packaged ones (Table 1). This increase was caused by accumulation of C 18:2 and C 18:3 fatty acids.

Zhuang et al. (1997) noticed a decrease in polyunsaturated fatty acids in broccoli stored at 13 and 23°C, which was associated with increased content of MDA (malondialdehyde) and MA (monoaldehydes) and with an elevated activity of lipoxygenase. Changes in fatty acid composition reported in the present investigations did not correspond to the content of the thiobarbituric acid-reactive substances (MDA and MA), being the products of lipid peroxidation. In flower buds of broccoli stored at room temperature, no significant changes of MDA were observed, except for its increase caused by 3 day storage at 20°C. The decrease in this compound was found after 7 day storage at 5°C, particularly in the packaged samples (Table 1). In most treatments the level of MA was reduced, especially in the packaged broccoli heads (Table 1).

In general, the post-harvest deterioration of short-term stored broccoli was not advanced enough to be presented by the breakdown of protein-lipid membranes and by the increase of TBA-reacting substances. Better indices of the senescence process, affected by temperature and by the modified atmosphere treatments, seemed to be phenolics and total antioxidant activity. These parameters also characterise the defence ability of the plant against active oxygen species.

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