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The Influence of Short-Term Storage on the Content of Flavonoids and Vitamin C in Broccoli

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Summary

Postharvest handling of vegetable crops, including storage, may affect the level of bioactive compounds present in plants. Two-year study on the influence of temperature during short-term storage (6 and 24 h) at room temperature and cold-storage (one week) on the content of quercetin, kaempferol and vitamin C in broccoli heads were performed. General Linear Model (GLM) and cluster analysis (CA) were applied to evaluate the effect of time and temperature of storage on the content of tested compounds. It was found that quercetin and kaempferol were stable and no losses of these compounds were observed during storage at room temperature. Vitamin C content significantly decreased under these conditions, whereas cold-storage had no significant influence on the content of this compound in comparison to fresh broccoli. It was also found that the increase of room temperature of storage caused the increase in quercetin and kaempferol contents and higher losses of vitamin C. The drop of cold temperature of storage may also cause decrease in flavonoid content in broccoli. The results demonstrate the importance of storage temperature for the content of vitamin C and flavonoids in broccoli.

Key words. Antioxidants – *Brassica oleracea* var. *italica* – HPLC – storage

Introduction

Broccoli belongs to vegetables of high biological value due to the presence of health-promoting phytochemicals i.e. vitamin C and E, β-carotene, glucosinolates and polyphenols. The content of these compounds in broccoli is higher than in other cruciferous vegetables such as cabbage, cauliflower and Brussels sprouts (Moreno et al. 2006; Singh et al. 2007). High concentrations of antioxidants and glucosinolates in crucifers have significant influence on the anticarcinogenic properties of these vegetables (Fahey et al. 1997; Kurilich et al. 1999; Kris-Etherton et al. 2002; Podsędek 2007). The mechanism underlying the reduction of the risk of cancer by cruciferous vegetables is not clear, however, it is well known that antioxidants present in these vegetables have some contribution to this health effect (Plumb et al. 1997; Rice-Evans et al. 1997; Pietta 2000). The major flavonoids in broccoli are quercetin and kaempferol. They are known to be good free-radical scavengers and metal-chelators. Moreover, flavonoids are considered to be protective against cardiovascular disease by decreasing thrombocyte aggregation and strengthening walls of blood capillaries. In vitro studies confirmed their anti-inflammatory, anti-arthritis and anti-allergic activities (Prior and Cao 2000).

Vitamin C is considered the most important water-soluble antioxidant present in the extracellular and intracellular spaces in most biological systems where it can participate in redox reactions. The content of vitamin C in broccoli (*Brassica oleracea* var. *italica*) may vary from 348 to 1460 mg kg^{-1} of fresh weight (Favell 1998; Vallejo et al. 2002, 2003a, 2003b, 2003c). Based on available biochemical, clinical, and epidemiological studies, the current recommended daily acceptance (RDA) for ascorbic acid is suggested to be 100–120 mg per day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals (Naidu 2003). It means that 100–200 g of fresh broccoli may cover daily demand for this vitamin.

The content of flavonoids and vitamin C in plants depends especially on genotype (Kurilich et al. 1999; Vallejo et al. 2002; Horbowicz and Babik 2005a; Kaur et al. 2007; Sultana and Anwar 2008), plant age (Vallejo et al. 2003b), cultivation conditions (Vallejo et al. 2003b; Horbowicz and Babik 2005b; Gliszczyńska-Świgło et al. 2007), way of handling after harvest (Vallejo et al. 2003c; Winkler et al. 2007), period and method of storage (Leja et al. 2001; Starzyńska et al. 2003; Kevers et al. 2007) and also the method of preparation for consumption (Gębczyński and Lisiewska 2006; Gliszczyńska-Świgło et al. 2006; Migilio et al. 2008; Sikora et al. 2008).

Literature data on the changes in the content of flavonoids and vitamin C in broccoli upon storage are often contradictory and restricted to one-year study (Barth and Zhuang 1996; Leja et al. 2001; Starzyńska et al. 2003; Serrano et al. 2006; Kevers et al. 2007). Moreover, the influence of storage temperature on the content of health-promoting compounds in broccoli is not sufficiently discussed. Therefore, this study was undertaken to evaluate the effect of short-term storage for 6 and 24 h at room temperature and cold-storage for 1 week on the changes in the content of vitamin C and main flavonoids, i.e. quercetin and kaempferol in broccoli. The study was performed in two consecutive years to confirm or verify the results obtained.

Table 1. Thermal conditions during storage of broccoli heads in two-year study.

Year	Storage conditions	Temperature [°C]
I	6 h – room temperature	17.8
	24 h – room temperature	18.9
	1 week – cold-storage	3.8
II	6 h – room temperature	23.8
	24 h – room temperature	23.1
	1 week – cold-storage	1.5

Materials and Methods

Plant material

Experiments were carried out in two subsequent years at "Marcelin" Experimental Station of the Poznań University of Life Sciences. The studies comprised one cultivar of broccoli – 'Monterey'. Transplants were planted out in the field in the first decade of July and heads were harvested in September. The trial was established in randomised block design with four replications. Twenty-five transplants were planted at the stage of four leaves in a spacing of 0.5×0.5 m. The experiment was established on the podzolic soil, of which the arable layer was loamy sand underlying sandy loam. The soil was tested before planting and 100 kg ha^{-1} of P_2O_5, 200–250 kg ha^{-1} of K_2O and 60 kg ha^{-1} of nitrogen were applied before soil preparation. Additionally, nitrogen (150 kg ha^{-1}) was applied in three doses in the growing season. Plants were watered when the soil water potential had exceeded −0.04 MPa. Broccoli heads were harvested when they had maximum size and their bud diameter was 2 mm. Five inflorescences at harvest maturity were randomly selected in each plot from uniform-size plants, harvested and immediately transported to the laboratory (20 heads of broccoli). Ten heads were kept at room temperature and 55 % relative humidity (RH) for 6 and 24 h, while another five were left in a cold store (95 % RH) for 1 week. Storage temperatures are presented in Table 1. Subsamples of broccoli were taken and immediately analysed for vitamin C content. The rest of plant material was immersed in liquid nitrogen and freeze-dried. Liophilized samples were stored at −20 °C under nitrogen generally no longer than two weeks until analysis on flavonoid content. Before analyses, lyophilized samples were grounded into a powder.

Extraction and hydrolysis of flavonoid glycosides

Quercetin dihydrate, kaempferol and tert-butylhydroquinone (TBHQ) were obtained from Fluka (Buchs, Switzerland), thero-1,4-dimercapto-2,3-butandiol (DTT) from Aldrich (Steinheim, Germany) and ascorbic acid (vitamin C) from Merck (Darmstadt, Germany). HPLC grade solvents and analytical grade reagents were used for all purposes.

The content of flavonols present in broccoli, which occur as quercetin and kaempferol *O*-glycosides, was determined after their acid hydrolysis to quercetin and kaempferol. Extraction and acid hydrolysis of flavonol glycosides were performed as described in the literature with minor modifications (Hertog et al. 1992; Crozier et al. 1997). Extracts were prepared as follows: 40 mL of 62.5 % aqueous methanol, containing TBHQ (2 g L^{-1}), were added to 0.500 g of freeze-dried sample material and the mixture was sonicated for 20 min. Subsequently, 10 mL of 10 M HCl were added and everything was mixed carefully. The extraction solution thus obtained consisted of 2 M HCl in 50 % aqueous methanol (v/v). After refluxing at 90 °C for 2 h, the extract was allowed to cool and subsequently made up to 100 mL with methanol and sonicated for 5 min. All extracts were filtered through a 0.2 μm filter (Sartorius AG, Goettingen, Germany) before injection into the HPLC (Gliszczyńska-Świgło et al. 2007).

HPLC analysis of quercetin and kaempferol

HPLC analyses were performed using a Waters 600 high performance liquid chromatograph equipped with a Symmetry C_{18} column (150×3.9 mm, 5 μm, Waters, Millford, MA, USA) protected with a μBondapak C_{18} guard column. A mobile phase of acetonitrile (A) and water, adjusted to pH 2.5 with trifluoroacetic acid (B), was used according to the gradient described by Crozier et al. (1997): linear increase of A from 15 to 35 % within 20 min and the return to the initial conditions within next 10 min with the flow rate of 1 mL min^{-1}. The eluate was detected using a Waters 996 photodiode-array detector set at 370 nm. Quercetin and kaempferol were identified by comparing UV spectrum and retention times with those of standards. Quantification was done using the external standard method (Gliszczyńska-Świgło et al. 2007).

Extraction and determination of vitamin C

Extraction of vitamin C was performed as described previously (Gliszczyńska-Świgło et al. 2006) using the method of Kurilich et al. (1999) with small modification. Briefly, 0.500 g of broccoli was extracted (in triplicate) with 2 mL of 1 % meta-phosphoric acid in ultrasonic bath for 15 min. Then, additional 1 mL of 1 % meta-phosphoric acid was added, the mixture was sonicated for the next 15 min and made up to 5 mL with metaphosphoric acid. Samples were centrifuged at 600 g for 20 min. The supernatant (0.2 mL) and 5 % DTT (0.2 mL) were mixed and diluted to 2 mL with water.

The determination of vitamin C, using HPLC method, was performed as described previously (Gliszczyńska-Świgło et al. 2006). A Waters 600 high-performance liquid chromatograph (Waters) equipped with LiChrospher C18 (3.9×250 mm, 5 μm, Merck) fitted with the same guard column was applied. A gradient of mobile phase consisting of methanol (solvent A) and 5 mM KH_2PO_4, pH 2.65 (solvent B), was used according to the following gradient: linear increment starting with 5 % A to 22 % A in 6 min and the return to the initial conditions within next 9 min with the flow rate 0.8 $cm^3\ min^{-1}$. The eluate was detected using a Waters 996 photodiode-array detector set at 245 nm. Vitamin C was identified by comparing its ultraviolet absorption spectrum and retention time with that of standard. Quantification of vitamin C was done using the external standard method.

Statistics

Statistical analysis was done using a Statistica 9.0 (StatSoft, Inc. 2000) statistic programme. Data are presented as means ± SD of three separate determinations for each broccoli sample. All data were submitted to General Linear Model (GLM) procedures. Statistical analysis was performed for both years of the experiment taking into account different storage temperatures (room and cold storage) in each year (Table 1). A hierarchically nested ANOVA was chosen to analyze the influence of significant factors: time (A) and temperature (B) on the content of vitamin C and total flavonoids. For these selected factors (A and B), the model analyzed included effects for B nested within A, and A as the main effect.

Differences between fresh and stored broccoli samples were evaluated by Dunnett test with fresh broccoli as control sample. Moreover, multiple means comparison was carried out by Tukey's test. Both tests were performed at 5 % level of significance.

A cluster analysis (CA) was also done to classify samples of broccoli and confirm the results obtained by the GLM approach. A hierarchical classification was performed with Euclidean distance and the Ward's aggregation method. The results of CA were shown graphically using a tree diagram (dendrogram).

Pearson's correlation coefficients were also calculated to determine the possible association between the changes in flavonol and vitamin C contents and temperature of storage ($\alpha = 0.05$). All independent determinations of bioactive compounds were taken for regression analysis.

Results and Discussion

The results of the present study on the effect of short-term storage at room temperature and cold-storage for one week on the content of vitamin C and flavonoids in broccoli are presented in Table 2. On the basis of the results obtained, high differences were found in the contents of vitamin C, quercetin and kaempferol in fresh broccoli in the consecutive two years of cultivation. The quercetin and kaempferol contents were respectively 2.8 and 1.7 times higher, whereas the content of vitamin C was 2 times lower in broccoli samples from the second year comparing with first year of cultivation (Table 2).

Table 2. Vitamin C, quercetin, kaempferol and total flavonoid contents (mg kg^{-1} f.w.) in broccoli harvested in two consecutive years and stored at room (6 and 24 h) or cold temperatures (1 week).

Year	Storage	Vitamin C	Quercetin	Kaempferol	Total flavonoids
I	Fresh	791.5 (± 48.3)	48.7 (± 3.3)	128.9 (± 10.3)	177.6 (± 12.8)
	6 h	690.2* (± 35.9)	47.4 (± 1.7)	103.9 (± 8.7)	151.3 (± 9.2)
	24 h	586.6* (± 28.2)	49.3 (± 0.3)	124.0 (± 22.1)	173.2 (± 22.0)
	1 week	803.9 (± 26.5)	57.8* (± 1.8)	127.3 (± 1.5)	185.1 (± 3.2)
II	Fresh	393.4 (± 6.0)	136.5 (± 7.8)	221.9 (± 19.9)	358.4 (± 22.5)
	6 h	332.0* (± 7.5)	160.8* (± 4.4)	264.4* (± 4.5)	428.8* (± 10.3)
	24 h	207.7* (± 18.0)	160.4* (± 0.1)	251.7* (± 3.8)	409.6* (± 8.0)
	1 week	378.8 (± 10.2)	126.3 (± 12.3)	177.9* (± 11.3)	304.2* (± 23.6)

* Significantly different (Dunnett test; $P < 0.05$) from the respective control sample (fresh broccoli)

These results are in agreement with those reported by Vallejo et al. (2003b) and Gliszczyńska-Świgło et al. (2007) who showed that the content of phenolic compounds in the same broccoli cultivar harvested in different seasons is variable due to different weather conditions. It was also proved in our studies. In the second year of the experiment, much higher temperatures during 30 days before harvest and at the harvest were observed (Table 3). Schonhof et al. (2007) also reported a strong impact of thermal conditions on the vitamin C content during the period of broccoli growth. In their study, plants cultivated in temperature conditions ranging from 15 to 20 °C were found to contain 38 % less vitamin C in comparison with plants growing at the temperature of 7–12 °C. In contrast, Vallejo et al. (2003b) observed that cultivars grown under late season condition (temperature 14.9–25.9 °C) in comparison to early season (temperature 7.8–18.8 °C) showed higher vitamin C content. Kaur et al. (2007) found that ascorbic acid content in broccoli genotypes grown under Indian conditions did not differ with the year of harvest.

The content of vitamin C in broccoli (*Brassica oleracea* var. *italica*) may vary from 348 to 1460 mg kg^{-1} of f.w. (Favell 1998; Vallejo et al. 2002, 2003a, 2003b, 2003c). In the present study, the vitamin C content in fresh broccoli heads of cultivar 'Monterey' ranged from 393.4 to 791.5 mg kg^{-1} of f.w. (Table 2). These levels are comparable to the ranges reported in literature.

The content of flavonoids in broccoli depends strongly on cultivar (Hertog et al. 1992; Prior and Cao 2000; Vallejo et al. 2002; Naidu 2003). However, Borowski et al. (2008) reported also high variability within the same variety cultivated in the same agricultural and climatic conditions. Moreover, several studies, including the present one, demonstrated that kaempferol is predominant flavonoid in broccoli, followed by quercetin (Vallejo et al. 2002; Horbowicz and Babik 2005a, 2005b). In the present two-year study, the kaempferol content in fresh broccoli heads was also found to be significantly higher than quercetin. The above data are in line with our previous results (Gliszczyńska-Świgło et al. 2007) showing 2–3 times higher content of kaempferol than quercetin in three cultivars of broccoli cultivated in different seasons of three consecutive years. Similar results were reported by Koh et al. (2009) who compared eight planting dates of broccoli and only once they found opposite result. In contrast, Bahorun et al. (2004) reported five times higher content of quercetin than kaempferol in broccoli.

Table 3. Temperatures in the period of 30 days before harvest and at the harvest in two-year study.

Year	Mean temperature [°C]			
	30 days before harvest		At harvest	
	Maximal	Minimal	Daily	Daily
I	19.4	11.4	15.1	10.9
II	27.1	14.8	20.8	20.7

Many studies indicate also the effect of storage on the level of flavonoids and vitamin C in broccoli (Barth and Zhuang 1996; Leja et al. 2001; Starzyńska et al. 2003; Serrano et al. 2006; Kevers et al. 2007; Winkler et al. 2007), however the results are not consistent. In our study, vitamin C content was found to decrease significantly after storage of broccoli heads for 6 and 24 h both at the temperature of 18–19 °C (first year) and at 23–24 °C (second year), whereas no losses of vitamin C were observed during cold-storage of broccoli in comparison to fresh broccoli (Table 2). In contrast to our results, Leja et al. (2001) reported 15 % and 25 % increase in the content of vitamin C in broccoli stored for 1 day at 20 °C and 7 days at 5 °C, respectively. This increase in vitamin C content was explained as a result of harvesting stress and low temperature treatment. Opposite effect was reported by Barth and Zhuang (1996) who found 80 % decrease in vitamin C content in broccoli stored for 6 days at 5 °C as well as by Serrano et al. (2006) who reported about 12 % decrease for broccoli stored for 5 days at 1 °C.

Quercetin content after 6 and 24 h storage of broccoli at the temperature of 18–19 °C was not significantly changed with comparison to fresh broccoli, whereas when the storage temperature was elevated to 23–24 °C (second year), quercetin content increased by 17.8 and 17.5 %, respectively (Table 2). After one week of cold-storage at the temperature of 3.8 °C (first year), quercetin content was found to increase by 18.7 %, whereas storage at 1.5 °C (second year) resulted in 7.5 % decrease of the content of this compound. Similar results were obtained for kaempferol. After 6 and 24 h storage at the temperature of 18–19 °C, the content of kaempferol was not significantly changed with comparison to fresh broccoli, whereas storage of broccoli heads at the temperature of 23–24 °C caused the increase in kaempferol content. After 6 h storage, the content of kaempferol increased by 19.1 % and after 24 h by 13.4 %. Cold-storage for 1 week had no effect on kaempferol content (first year) or caused almost 20 %-decrease (second year) in the content of this compound. The results for total flavonoids are similar to those obtained for kaempferol: no significant effect of short-term storage at room temperature or in cold for one week (first year) or increase in the content of flavonoids in broccoli stored at room temperature and decrease for stored in the cold (second year). It is known that after harvest the metabolic changes in plants still continue resulting in a decrease or an increase in the content of some bioactive compounds. The losses of sugars, organic acids and proteins in broccoli within the first 6 h of harvest were observed, followed by an increase in the free amino acid pools (Jahangir et al. 2009). Hagen et al. (2009) observed an increase in the kaempferol content during curly kale cold storage, whereas quercetin content

has remained unchanged. Substantial increase in phenolics was observed in red and white onions upon storage at fluctuating ambient temperature (Rodrigues et al. 2010) and in broccoli, tomato, plum, and black grape, whereas in some other fruits and vegetable it decreased (Kevers et al. 2007). The direction of changes is difficult to predict due to many factors, which may influence the metabolism of plants after harvest.

The increase in quercetin and kaempferol contents found in this study after short storage of broccoli heads at the temperature of 23–24 °C (second year) are in line with the results obtained by Starzyńska et al. (2003) who reported a distinct increase in the content of flavonoids in broccoli stored at 20 °C for 1 day. In the study of Winkler et al. (2007), small increase in the content of flavonoids in broccoli stored either at 1 °C or 4 °C for 7 days was observed thus they concluded that these temperatures and time of storage do not have a deleterious effect on the level of flavonoids in broccoli.

The GLM approach was applied to draw out reliable conclusions from the two-year study on the influence of time and temperature of storage on the content of vitamin C and flavonoids in broccoli. Analysis of hierarchically nested ANOVA was carried out to assess the attributes, which differ significantly broccoli samples. The results of ANOVA for vitamin C and flavonoid contents are summarised in Table 4. For vitamin C, both effects were found to be highly significant: (1) there is significant influence of storage temperature and (2) at room temperature, there is significant influence of storage time on the vitamin C content in broccoli (Table 2 and 4). In contrast to vitamin C, for flavonoids, there is no significant influence of nested storage time on their content. Moreover, this effect has small contribution to overall variance of model (F = 2.29, p = 0.26). However, there is statistically significant influence of storage temperature. The differences between the content of tested compounds in stored and fresh broccoli were calculated for all independent determinations performed in each year of the study and these differences were correlated with temperatures of storage. A significant correlation coefficients between the changes in quercetin (R = 0.56; p = 0.016), kaempferol (R = 0.63; p = 0.006) and vitamin C (R = 0.65; p = 0.003) contents and storage temperature were found indicating that the increase in the storage temperature may affect the content of flavonoids and vitamin C in broccoli.

Table 4. Hierarchically nested ANOVA F-values.

Compound	Temp. A	Time(temp.) B(A)
Vitamin C	292.34	27.88
Flavonoids (quercetin, kaempferol, total flavonoids)	199.12	2.29^{ns}

^{ns} not significant (P > 0.05)

The above conclusions can be confirmed by hierarchical classification in which two clusters were separated (Fig. 1). As shown in Fig. 1, the first cluster includes fresh (A1) and cold-stored (A4) broccoli, whereas the second cluster includes samples stored at room temperature for 6 (A2) and 24 (A3) hours. Analysis of variance performed has shown that the main criterion determining the adherence of the sample to the cluster was vitamin C content. First cluster including fresh and cold-stored broccoli cha-

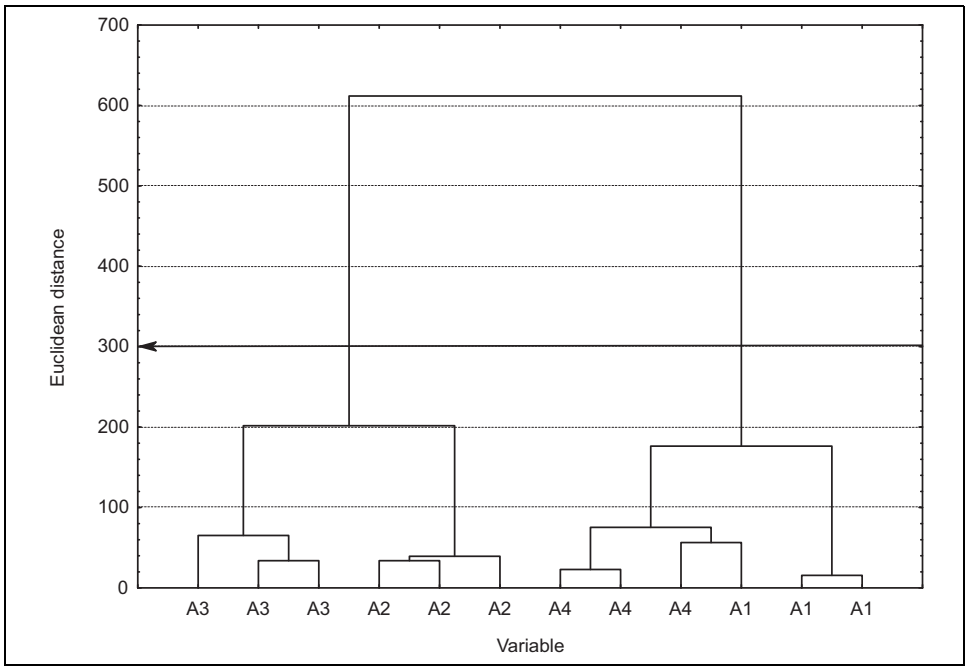


Fig. 1. Dendrogram obtained by cluster analysis of broccoli samples. A1 – three independent determinations of vitamin C and flavonoids in fresh broccoli; A2, A3 – in broccoli stored for 6 and 24 h at room temperature, respectively; A4 – in broccoli stored in the cold for 1 week.

racterizes significantly higher content of vitamin C as compared with second cluster.

Altogether, the results of the present study indicate that the temperature of storage affects vitamin C and flavonoid contents in broccoli. In contrast to available literature data they explicitly showed that short-term storage of broccoli at room temperature causes significant losses of vitamin C, whereas cold-storage for 1 week has no significant influence on the changes in the content of this compound with comparison to fresh broccoli. Flavonoids present in broccoli (quercetin and kaempferol) proved to be stable compounds and no losses were observed during short-term storage at room temperature. However, it was also found that the increase of room temperature of storage causes the increase in quercetin and kaempferol contents and higher losses of vitamin C. The drop of cold temperature of broccoli storage may also cause decrease in flavonoid content.

The results obtained in this study indicate the importance of not only time but also temperature of storage for the content of vitamin C and flavonoids in broccoli. It can be important for both suppliers and consumers of this vegetable.

References

Bahorun, T., A. Luximon-Ramma, A. Crozier and O.I. Aruoma 2004: Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric. **84**, 1553–1561.

Barth, M. and H. Zhuang 1996: Packaging design affects antioxidant vitamin retention and quality of broccoli florets during postharvest storage. Postharv. Biol. Technol. **9**, 141–150.

Borowski, J., A. Szajdek, E.J. Borowska, E. Ciska and H. Zieliński 2008: Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.). Eur. Food Res. Technol. **226**, 459–465.

Crozier, A., E.J.L. Michael, M.S. McDonald and Ch. Black 1997: Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. J. Agric. Food Chem. **45**, 590–595.

Fahey, J.W., Y. Zhang and P. Talalay 1997: Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Nat. Acad. Sci. USA **94**, 10367–10372.

Favell, D.J. 1998: A comparison of the vitamin C content of fresh and frozen vegetables. Food Chem. **62**, 59–64.

Gębczyński, P. and Z. Lisiewska 2006: Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. Innov. Food Sci. Emerg. Technol. **7**, 239–245.

Gliszczyńska-Świgło, A., E. Ciska, K. Pawlak-Lemańska, J. Chmielewski, T. Borkowski and B. Tyrakowska 2006: The changes in the contents of health-promoting compounds and antioxidant activity of broccoli upon domestic processing. Food Addit. Contam. **23**, 1088–1098.

Gliszczyńska-Świgło, A., A. Kałużewicz, K. Lemańska, M. Knaflewski and B. Tyrakowska 2007: The effect of solar radiation on the flavonol content in broccoli inflorescence. Food Chem. **100**, 241–245.

Hagen, S.F., G.I.A. Borge, K.A. Solhaug and G.B. Bengtsson 2009: Effect of cold storage and harvest date on bioactive compounds in curly kale (*Brassica oleracea* L. var. *acephala*). Postharv. Biol. Technol. **51**, 36–42.

Hertog, M.G.L., P.C.H. Hollman and M.B. Katan 1992: Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. J. Agric. Food Chem. **40**, 2379–2383.

Horbowicz, M. and I. Babik 2005a: Sulforaphane and flavonoid contents in chosen broccoli cultivars. Veget. Crops Res. Bull. **62**, 127–138.

Horbowicz, M. and I. Babik 2005b: Effect of potassium sulfate fertilization on content of some bioactive constituents in broccoli cv. Marathon F_1. Veget. Crops Res. Bull. **63**, 109–116.

Jahangir, M., I.B. Abdel-Farid, H.K. Kim, Y.H. Choi and R. Verpoorte 2009: Healthy and unhealthy plants: The effect of stress on the metabolism of Brassicaceae. Environ. Experiment. Bot. **67**, 23–33.

Kaur, Ch., K. Kumar, D. Anil and H.C. Kapoor 2007: Variations in antioxidant activity in broccoli (*Brassica oleracea* L.) cultivars. J. Food Biochem. **31**, 621–638.

Kevers, C., M. Falkowski, J. Tabart, J.O. Defraigne, J. Dommes and J. Pincemail 2007: Evolution of antioxidant capacity during storage of selected fruits and vegetables. J. Agric. Food Chem. **55**, 8596–8603.

Koh, E., K.M.S. Wimalasiri, A.W. Chassy and A.E. Mitchell 2009: Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli. J. Food Comp. Anal. **22**, 637–643.

Kris-Etherton, P.M., K.D. Hecker, A. Bonanome, S.M Coval, A.E Binkoski, K.F. Holpert, A.E. Griel and T.D. Etherton 2002: Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. **113**, 71–88.

Kurilich, A.C., G.J. Tsau, A. Brown, L. Howard, B.P. Klein, E.H. Jeffery, M. Kushad, M.A. Wallig and J.A. Juvik 1999: Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. J. Agric. Food Chem. **47**, 1576–1581.

Leja, M., A. Mareczek, A. Starzyńska and S. Rożek 2001: Antioxidant ability of broccoli flower buds during short-term storage. Food Chem. **72**, 219–222.

Migilio, C., E. Chiavaro, A. Visconti, V. Fogliano and N. Pellegrini 2008: Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. J. Agric. Food Chem. **56**, 139–147.

Moreno, D.A., M. Carvajal, C. López-Berenguer and C. García-Viguera 2006: Chemical and biological characterisation of nutraceutical compounds of broccoli. J. Pharmac. Biomed. Anal. **41**, 1508–1522.

Naidu, K.A. 2003: Vitamin C in human health and disease is still a mystery? An overview. J. Nutr. **2**, 1–16.

Pietta, P. 2000: Flavonoids as antioxidants. J. Nat. Prod. **63**, 1035–1042.

Plumb, G.W., K.R. Price, M.J.C. Modes and G. Wiliamson 1997: Antioxidant properties of the major polyphenolic compounds in broccoli. Free Radic. Res. **27**, 429–435.

Podsędek, A. 2007: Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT Food Sci. Technol. **40**, 1–11.

Prior, R.L. and G. Cao 2000: Flavonoids: diet and health relationships. Nutr. Clin. Care **3**, 279–288.

Rice-Evans, C.A., N. Miller and G. Paganga 1997: Antioxidant properties of phenolic compounds. Trends Plant Sci. **2**, 152–159.

Rodrigues, A.S, M.R. Pérez-Gregorio, M.S. García-Falcón, J. Simal-Gándara and D.P.F. Almeida 2010: Effect of post-harvest practices on flavonoid content of red and white onion cultivars. Food Control **21**, 878–884.

Schonhof, I., H.P. Kläring, A. Krumbein, W. Claußen and M. Schreiner 2007: Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agric. Ecosys. Environ. **119**, 103–111.

Serrano, M., D. Martinez-Romero, F. Guillén, S. Castillo and D. Valero 2006: Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Postharv. Biol. Technol. **39**, 61–68.

Sikora, E., E. Cieślik, T. Leszczyńska, A. Filipiak-Florkiewicz and P.M. Pisulewski 2008: The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem. **107**, 55–59.

Singh, J., A.K. Upadhyay, K. Prasad, A. Bahadur and M. Rai 2007: Variability of carotenes, vitamin C, E and phenolics in *Brassica* vegetables. J. Food Comp. Anal. **20**, 106–112.

Starzyńska, A., M. Leja and A. Mareczek 2003: Physiological changes in the antioxidant system of broccoli flower buds senescing during short-term storage, related to temperature and packaging. Plant Sci. **165**, 1387–1395.

Sultana, B. and F. Anwar 2008: Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem. **108**, 879–884.

Vallejo, F., F.A. Tomás-Barberán and C. García-Viguera 2002: Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. J. Sci. Food Agric. **82**, 1293–1297.

Vallejo, F., C. García-Viguera and F.A. Tomás-Barberán 2003a: Changes in broccoli (*Brassica oleracea* L. var. *italica*) health-promoting compounds with inflorescence development. J. Agric. Food Chem. **51**, 3776–3782.

Vallejo, F., F.A. Tomás-Barberán and C. García-Viguera 2003b: Effect of climatic and sulphur fertilization conditions, on the phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars. Eur. Food Res. Technol. **216**, 395–401.

Vallejo, F., F.A. Tomás-Barberán and C. García-Viguera 2003c: Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. J. Agric. Food Chem. **51**, 3029–3034.

Winkler, S., J. Faragher, P. Franz, M. Imsic and R. Jones 2007: Glucoraphanin and flavonoid levels remain stable during simulated transport and marketing of broccoli (*Brassica oleracea* var. *italica*) heads. Postharv. Biol. Technol. **43**, 89–94.

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