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The effect of solar radiation on the flavonol content in broccoli inflorescence

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Abstract

The effect of solar radiation on the quercetin and kaempferol contents in the inflorescence of three broccoli cultivars ('Lord', 'Marathon' and 'Fiesta') was investigated from 1999 to 2001. Great differences in the contents of both flavonols, dependent on growing time and cultivar, were found. Quercetin and kaempferol contents varied from 14.3 to 81.0 mg kg^{-1} f.w. and from 35.9 to 213 mg kg^{-1} f.w., respectively. Inflorescences of the cultivar 'Lord' were characterised by the highest mean content of quercetin and those of cultivar 'Fiesta' of kaempferol. The contents of both flavonols were highly positively correlated with total solar radiation in the period from planting to the harvest of broccoli inflorescences.

Keywords: Broccoli; Flavonoids; Quercetin; Kaempferol; Growing season; Solar radiation

1. Introduction

Numerous epidemiological data have shown that a diet rich in fruits and vegetables can reduce the risk of coronary heart disease, stroke and certain forms of cancer (Arai et al., 2000; Herog, Hollman, & Katan, 1992a; Hollman & Katan, 1999; Knekt, Järvinen, Reunanen, & Maatela, 1996; Knekt et al., 1997). Many constituents of this type of food may contribute to its health-promoting properties. They include antioxidant vitamins (C and E), carotenoids, glucosinolates, selenium and other microelements as well as polyphenols, especially flavonoids. Antioxidant activity of natural antioxidants is one of the mechanisms involved in these chemoprotective effects (Middleton & Kandaswami, 1993; Prior & Cao, 2000a, 2000b). Cruciferous vegetables, especially broccoli, brussels sprouts, cauliflower and cabbages, are excellent dietary sources of antioxidant vitamins, glucosinolates and polyphenols (mainly flavonoids). The polyphenolic compounds may act as antioxidants or as agents in other mechanisms contributing to cardioprotective or anticarcinogenic effects.

The occurrence of at least five main flavonol glycosides (quercetin-3-*O*-sophoroside and kaempferol-3-*O*-sophoroside as the main flavonols and isoquercitrin, kaempferol-3-*O*-glucoside, and kaempferol diglucoside in less quantity) in broccoli florets has been reported (Price, Casuscelli, Colquhoun, & Rhodes, 1998; Vallejo, Thomas-Barberan & Garcia-Viguera, 2004).

A significant variation in the level of health-promoting constituents in broccoli was reported to be due to cultivar and variations in environmental and agronomic factors, such as water availability, sunlight access, soil composition and sulphur fertilization (Vallejo, Garcia-Viguera & Thomas-Barberan, 2003a; Vallejo, Thomas-Barberan & Garcia-Viguera, 2002, 2003b). However, information available on the influence of climatic and agronomic conditions on the level of these compounds is usually not complete and always it is restricted to one-year experiments.

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Repetition of the experiment in the next year might allow testing of the effect of various environmental conditions on the level of compounds under study. Also it could confirm or verify the results obtained and make them more reliable.

Therefore, a three-year study was undertaken in order to determine the effect of solar radiation (SR) on flavonol content in three cultivars of broccoli.

2. Materials and methods

2.1. Materials

Three cultivars of broccoli (*Brassica oleracea L.* var. *italica* Plenck), 'Marathon', 'Lord' and 'Fiesta', were grown in spring and autumn growing cycles in the Experimental Station "Marcelin" of the August Cieszkowski University of Agriculture in Poznań from 1999 to 2001. Each trial was established in randomised block design with four replications. Twenty-five transplants were field planted at the stage of four leaves in a spacing of 0.5×0.5 m. The experiment was established on the podzollic soil, of which the arable layer was loamy sand underlying sandy loam. Plants were grown in the same field in all growing seasons. The soil was tested before planting and 100 kg/ha of P_2O_5, 200–250 kg/ha of K_2O and 60 kg/ha of nitrogen were applied before soil preparation. Additionally, 150 kg/ha of nitrogen were 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 of each cultivar at optimum maturity were randomly selected in each plot from uniform-size plants, harvested and immediately transported to the laboratory where the edible parts were cut. Three samples from each inflorescence were taken, combined, weighed, frozen using liquid nitrogen and freeze-dried. Lyophilised samples were ground into a powder and stored under argon at −20 °C no longer than four months for further analysis on flavonoid content.

Quercetin dihydrate, kaempferol and tert-butylhydroquinone (TBHQ) were obtained from Fluka (Buchs, Switzerland). Acetonitrile and methanol were of HPLC grade.

2.2. Measurement of solar radiation

Weather conditions were recorded hourly by data loggers, Hobo H08-007-02 (Onset Computer Corporation, USA). Throughout the growth period total incident solar radiation was measured by the Pyranometer type 6005 (Theodor Friedrichs GmbH and Co., Germany) at 3 m above ground level.

2.3. Extraction and hydrolysis of flavonoid glycosides

Concentrations of flavonols present in broccoli, which occur as quercetin and kaempferol *O*-glycosides, were 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 (Crozier, Lean, McDonald, & Black, 1997; Herog, Hollman, & Venema, 1992b). Extracts were prepared as follows: 40 ml of 62.5% aqueous methanol, containing TBHQ (2 g/l), 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 column.

2.4. Analytical quality control

In each series of analyses, different concentrations of standards of quercetin and kaempferol were included, to check possible changes in the analytical system applied. The relative standard deviation (RSD) value of the standard curve slopes generated during a whole project were not higher than 3.6% for quercetin and 2.8% for kaempferol, indicating good repeatability of the analytical system used. Moreover, a control sample of lyophilised broccoli was prepared and stored at −20 °C under argon. At the start of the project, the quercetin and kaempferol contents in the control sample were determined. Each series of analyses included the control sample analysed in triplicate. The low variation in flavonol content of the control sample over the period of the whole project (RSD = 3.9% for quercetin content, 2.9% for kaempferol content, and 2.5% for total flavonol content; $n = 9$) demonstrated the absence of a significant long-term variability of flavonol analysis in the laboratory. The results obtained also indicated that the stability of flavonols in lyophilised broccoli samples stored at −20 °C was adequate over a period of almost three years and that the differences between samples were not due to instability of flavonols in broccoli samples.

2.5. HPLC analysis of quercetin and kaempferol

HPLC analyses were performed in 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 Nova Pak C_{18} guard column. A mobile phase of acetonitrile and water, adjusted to pH 2.5 with trifluoroacetic acid (TFA), was used according to the gradient described by Crozier et al. (1997). 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.

2.6. Statistical analysis of data

Statistical analysis was done using a Statistica 5.5 (Statsoft 2000) statistic programme. Data are presented as means ± SD of three determinations. They were statistically evaluated using ANOVA/MANOVA and Duncan's multiple range test. Differences were considered to be significant at $P < 0.05$. Pearson's correlation coefficients between flavonol content and solar radiation (SR) were also determined. Effects of both total SR and cultivar on flavonol content were assessed using multiple correlation.

3. Results and discussion

Three cultivars of broccoli, 'Marathon', 'Lord' and 'Fiesta', grown under uniform conditions and harvested in different seasons over three years (1999–2001), were examined for the concentrations of quercetin and kaempferol (Table 1). Great differences were found in quercetin and kaempferol contents, depending on cultivar and time of broccoli inflorescence formation. Quercetin and kaempferol contents varied from 14.3 to 81.0 mg kg^{-1} f.w. and from 35.9 to 213 mg kg^{-1} f.w., respectively. Quercetin content was generally higher in inflorescences of cultivar 'Lord' than in other cultivars, whereas kaempferol content was higher in those of cultivar 'Fiesta'. Total quercetin and kaempferol contents ranged from 57.0 to 273 mg kg^{-1} f.w. The results obtained are in agreement with literature data (Herog et al., 1992a; Vallejo et al., 2003a, 2003b).

The mean content of total quercetin and kaempferol varied from 98.2 mg kg^{-1} f.w. for the cultivar 'Marathon' to 104 mg kg^{-1} f.w. for 'Fiesta' when harvested in September and October of 2000 and 2001 (Table 2). It was much higher and exceeded 200 mg kg^{-1} f.w. for each of three cultivars when harvested in July (Table 1). The same relationships were found in the case of quercetin and kaempferol contents.

There were significant differences in total quercetin and kaempferol content in broccoli harvested in the year 2000 and 2001 (Table 2). Mean content of total quercetin and kaempferol in broccoli inflorescences harvested in September 2000 was about 40% lower than that in 2001. In contrast, broccoli inflorescences harvested in October contained more flavonols in 2000 than in 2001.

Table 1
Quercetin and kaempferol content in three broccoli cultivars harvested in different seasons of 1999–2001 and total solar radiation (SR) in the period from planting to the harvest of broccoli

Year	Month of harvest	Cultivar	Flavonol content [mg kg^{-1}]			SR [MJ m^{-2}]
			Quercetin	Kaempferol	quercetin + kaempferol	
1999	July	Fiesta	52.9 ± 5.4	213 ± 7.5	266 ± 12.8	1449
2000	September	Marathon	21.1 ± 2.2	35.9 ± 1.7	57.0 ± 2.2	910
		Lord	27.7 ± 1.5	41.8 ± 0.7	69.5 ± 2.4	
		Fiesta	14.3 ± 0.7	45.0 ± 2.2	59.3 ± 3.1	
	October	Marathon	45.7 ± 1.4	117 ± 3.0	162 ± 4.0	1190
		Lord	42.9 ± 0.6	119 ± 3.9	162 ± 3.7	
		Fiesta	33.3 ± 1.1	98.4 ± 2.9	132 ± 4.0	
2001	July	Marathon	62.5 ± 2.4	140 ± 8.1	203 ± 10.5	1348
		Lord	81.0 ± 0.5	132 ± 6.6	213 ± 6.1	
		Fiesta	72.4 ± 0.8	201 ± 5.4	273 ± 6.2	
	September	Marathon	26.2 ± 0.6	70.2 ± 0.9	96.4 ± 0.3	1078
		Lord	28.1 ± 2.1	58.4 ± 1.7	86.4 ± 1.6	
		Fiesta	26.7 ± 2.1	94.4 ± 4.2	121 ± 6.2	
	October	Marathon	21.7 ± 1.5	55.1 ± 1.7	76.7 ± 0.2	1008
		Lord	23.4 ± 0.7	59.1 ± 5.1	82.5 ± 5.8	
		Fiesta	21.2 ± 1.4	82.2 ± 0.3	103 ± 1.7	

Table 2
Total quercetin and kaempferol contents in three broccoli cultivars in 2000–2001

Cultivar	Total quercetin and kaempferol contents in mg kg^{-1} of fresh edible part						
	September			October			Mean** 2000–2001
	2000	2001	Mean	2000	2001	Mean	
Fiesta	59.3	121	90.2	132	103	118	104 a
Lord	69.4	86.4	77.9	162	82.5	122	100 b
Marathon	57.0	96.4	76.7	162	76.7	120	98.2 b
Mean*	61.9 d	101 b	81.6	152 a	87.5 c	120	

* Means with the same letters in the row are not significantly different at $P < 0.05$.
** Means with the same letters in the column are not significantly different at $P < 0.05$.

In order to explain the reason for the differences in flavonol content in broccoli, an attempt was made to determine the correlations between weather conditions in different growing seasons and flavonol content. It is known that light is one of the most important factors controlling flavonoid synthesis in plants. Light is necessary, for example, for full development of anthocyanin colour in apples, and strawberry, and in most garden flowers. It is also reported that flavonol synthesis in foliage and pea plants is light-dependent (Harborne, 1967). However, there are no literature reports of a quantitative relationship between total solar radiation and flavonoid content in plants. In our investigation, the total solar radiation in the periods from planting to harvest varied from 910 to 1449 MJ m^{-2} (Table 1). The radiation for broccoli harvested in July was much higher than for broccoli harvested in September and October. Figs. 1 and 2 present solar radiation plotted against quercetin and kaempferol contents in broccoli harvested from 1999 to 2001. The reliable correlations obtained ($r = 0.88$ and $r = 0.94$ for quercetin and kaempferol contents, respectively) indicate that the content of flavonols in broccoli quantitatively depends on solar radiation.

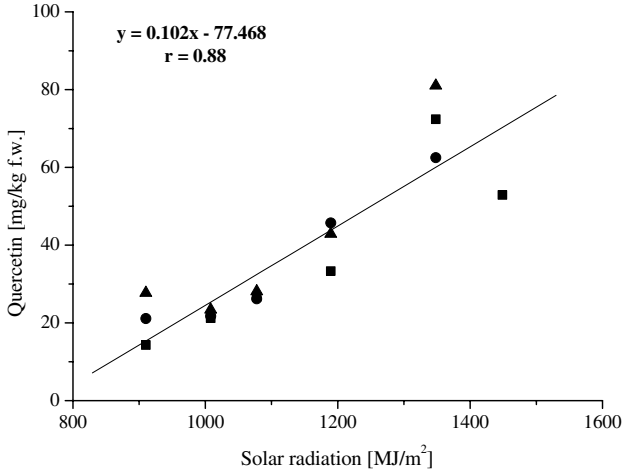

Fig. 1. Correlation between total solar radiation during the growing period and quercetin content in broccoli inflorescence (Fiesta (■), Marathon (●) and Lord (▲)).

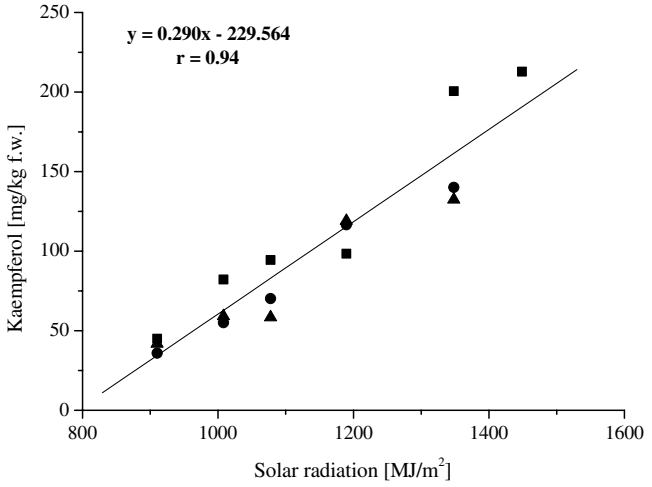

Fig. 2. Correlation between total solar radiation during the growing period and kaempferol content in broccoli inflorescence (Fiesta (■), Marathon (●) and Lord (▲)).

Although the mean flavonol content in 2000–2001 in broccoli inflorescence of cultivar 'Fiesta' is statistically significantly higher than those of cultivars 'Lord' and 'Marathon' (Table 2), these differences do not allow unequivocal conclusions about the cultivar effect. Multiple correlation between flavonol contents and SR/cultivars confirmed that only total SR in the period from planting to the harvest has a significant effect on flavonol content in tested broccoli (results not shown).

Altogether, the results presented in this paper indicate that the contents of quercetin and kaempferol in broccoli inflorescence are highly positively correlated with the solar radiation in the period from planting to harvest. Variability in flavonol content among broccoli grown in the seasons with different solar radiation suggests that potential beneficial health effects of broccoli inflorescence, as a source of flavonoids as natural antioxidants, are dependent, not only on the cultivar, as reported in the literature (Vallejo et al., 2002, 2003a, 2003b), but also on the sunlight access in the growing period.

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