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Flavonoids in Baby Spinach (*Spinacia oleracea* L.): Changes during Plant Growth and Storage

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The variation in flavonoid concentration and composition was investigated in baby spinach (*Spinacia oleracea* L.) cv. Emilia sown on three occasions, each harvested at three growth stages at 6-day intervals. After harvest, leaves were stored in polypropylene bags at 2 or 10 °C. Flavonoids were analyzed by reversed phase HPLC. Twelve flavonoid peaks were detected. The main flavonoid, making up on average 43% of the total flavonoid concentration, was identified as 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucuronide. Four other flavonoids each contributed 7−12% of the total flavonoid content. Total flavonoid content was relatively stable during normal retail storage conditions, although some of the individual flavonoid compounds showed considerable variation. The youngest plants had the highest flavonoid concentration, indicating that by harvesting the baby spinach a few days earlier than the current commercial stage of harvest, the flavonoid concentration in the product may be increased and the content of potentially health-promoting compounds enhanced.

KEYWORDS: Flavonoids; baby spinach; *Spinacia oleracea*; growth stage; sowing time; postharvest

INTRODUCTION

A diet rich in fruits and vegetables has a positive effect on human health, playing a role in the prevention of a number of chronic diseases. This protective effect has been attributed to a wide range of compounds present in fruits and vegetables, including those with antioxidant action, such as ascorbic acid, carotenoids, tocopherols, glutathione, phenolic acids, and flavonoids (*1*, *2*). Epidemiological research on flavonoids indicated that a high dietary intake is associated with a lower risk of coronary heart disease (*3*, *4*), dementia (*5*), and some forms of cancer (*6*). Consequently, the content of flavonoids and other phytochemicals in fruits and vegetables has been the subject of much research.

The content of flavonoids in vegetables and other plants varies with factors such as genotype, environmental growing conditions, growth stage, postharvest handling, and storage conditions (*7*−*9*). These factors affect both total flavonoid concentration and the composition of flavonoids in the plant. Environmental growing conditions generally vary considerably over the year, which may result in a fluctuating flavonoid content (*9*, *10*). Flavonoid concentration and composition may also change during plant growth (*11*−*14*). Furthermore, environmental conditions may affect the rate of plant growth and development, thereby influencing flavonoid content indirectly.

Spinach is a leafy vegetable that is provided fresh, frozen, or canned to the consumer. Spinach harvested after a shorter growth period than normal is called baby spinach and is marketed fresh in polypropylene bags. This is a relatively new product that has become increasingly popular in the past few years (*15*). The recommended storage temperature for fresh spinach is close to 0 °C (*16*), although the actual storage temperature is often higher (*17*). The differences in storage temperature and handling conditions between baby spinach and fresh spinach intended for cooking, on the one hand, and frozen or canned spinach, on the other, have a considerable impact on product shelf life and retention of nutrients and phytochemicals (*18*).

The postharvest stability of some antioxidant compounds in fresh leaves has previously been reported to differ between leaves of different age (*19*), possibly related to metabolic rates (*20*). Gil et al. (*21*) reported the flavonoid content of fresh-cut spinach to be rather stable during storage. However, we have found no comparisons of postharvest stability of flavonoids in leaves of different age in the literature.

The aim of this research was to investigate the quantitative and qualitative variation in flavonoids with sowing time and growth stage of baby spinach. In addition, the postharvest stability of baby spinach flavonoids was studied during 9 days of storage at different temperatures.

MATERIALS AND METHODS

Plant Material and Field Experiment Design. Spinach cv. Emilia was grown in a commercial production system in Öllöv, southern Sweden (56° 23′ N, 12° 43′ E). Seeds were sown on three occasions,

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Table 1. Temperature and Light Conditions during Field Growth of Baby Spinach at Different Sowing Times[a]

parameter	sowing time		
	Aug 2002	June 2003	July 2003
mean temperature (°C)	18.1	16.0	19.0
temperature range (°C)	4.4–27.4	7.3–24.2	10.2–27.6
mean photoperiod[b] (h:min)	15:10	19:44	16:58
photoperiod range (h:min)	13:59–16:23	19:37–19:53	15:44–18:12
mean global radiation ($W\ m^{-2}\ day^{-1}$)	4.2	5.1	5.0
total radiation ($W\ m^{-2}$)	125	159	153

[a] Values are from sowing until harvest at growth stage III. [b] Civil twilight to civil twilight.

Aug 18, 2002, June 8, 2003, and July 27, 2003. Temperature and radiation conditions are given in **Table 1**. For each sowing date, leaves were harvested at three growth stages, I, II, and III, at 6-day intervals, starting 17−18 days after sowing for growth stage I. Stage II corresponded to the normal harvest stage in commercial production of baby spinach. The stages were thus defined by time from sowing to harvest, and the spinach at growth stage I was harvested 6 days prior to the expected normal harvest (stage II). The design of the field trial was completely randomized, with four plots for each of the three growth stages. The spinach was cut manually using sharp knives, at a petiole length of approximately three-fourths of the leaf blade length. Harvested leaves were placed under black plastic and transferred to dark cool rooms at a temperature of ∼4 °C within 1 h after harvest.

Postharvest Treatments. After a few hours of cooling, 12 subsamples from each plot were placed in unperforated oriented polypropylene (OPP) bags, using an industrial packing machine. Each bag contained ∼100 g and was stored for 5 or 9 days in a climate chamber at 10 or 2 °C (only 2003), simulating practical commercial conditions. Thus, three bags per plot were subjected to each storage regime ($n = 4$). To determine the fresh weight loss during storage, the OPP bags including leaves were weighed before storage, and the leaves were weighed after storage. After storage, the three subsamples were combined to one sample within each plot and storage regimen.

Sample Preparation and Extraction. On the harvest day or after storage, the spinach was frozen at −80 °C. An aliquot of the material from each plot and treatment was freeze-dried, ground into a fine powder, and stored in a desiccator until extraction. Samples were extracted in triplicate under dim green light. Each sample, consisting of 60 mg of freeze-dried material, was extracted with 5 mL of 40% methanol. The samples were shaken for 20 h at 4 °C and 150 rpm and then centrifuged at 10000*g* at 4 °C for 10 min. Extracts were stored at −80 °C until analysis by HPLC.

HPLC Analysis of Flavonoids. Flavonoids were analyzed by reversed phase HPLC on an Agilent 1100 series system (Agilent Technologies, Waldbronn, Germany). The mobile phase was a gradient of (A) water/methanol/formic acid (69:30:1 v/v) and (B) methanol. The gradient was as follows: 0−18 min, 15−45% eluent B; 18−23 min 45−100% B; 23−27 min 100% B. Flow rate was 0.7 mL/min. The flavonoids were separated using a Phenomenex Luna Phenyl-hexyl column (250 × 4.6 mm, 5 μm), with a security guard column, Phenomenex C_{18} ODS (4 × 3.0 mm). The absorbance was recorded at 340 nm using a diode array detector (Waters, Milford, MA). Sample injection volume was 10 μL. Flavonoid content was quantified using an external spiraeoside standard (Extrasynthese, Lyon, France).

LC-MS Analysis. Flavonoids were identified by LC-MS/MS. Identification of each compound was done by combining UV spectra and data from MS, MS/MS, and retention times. The HPLC analyses were carried out using an Alliance 2695 system and a diode array detector 996 (Waters). The MS instrument was a Quattro LC triple quadrupole (Micromass, Cheshire, U.K.), equipped with a Z-spray API source using the ESI inlet. The molecular weights of the glycosylated flavonoids, separated by HPLC, were established by MS scan (negative mode, *m*/*z* 100−1000, cone = 30 V), and the molecular weights of the matching aglycones by MS/MS (collision energy = 23−35 V). The aglycones, produced by in-source fragmentation, were further subjected to MS/MS for identification purposes (*22*).

Statistical Analysis. The mean of the three laboratory replicates within each plot and treatment was calculated before statistical analyses. Data were statistically evaluated using analysis of variance (GLM). Tukey's test was used for post-hoc comparisons. All statistical analyses were performed using the statistical software MINITAB 14 (Minitab Inc., State College, PA). A significance level of 5% was used.

RESULTS AND DISCUSSION

The total flavonoid content of baby spinach was found to be 13−23 mg/g of dry weight in the fresh leaves. Twelve flavonoid peaks were detected (**Figure 1**): peak 1, patuletin-3-glucosyl-(1−6)[apiosyl(1−2)]-glucoside, *m*/*z* 787; peak 2, spinacetin-3-glucosyl-(1−6)[apiosyl(1−2)]-glucoside, *m*/*z* 801, including a patuletin glycoside, tentatively identified as peak 1 plus coumaric acid, *m*/*z* 933; peak 3, patuletin-3-gentiobioside, *m*/*z* 655, including a patuletin glycoside, tentatively identified as peak 1 plus coumaric acid, *m*/*z* 933; peak 4, patuletin-3-(2″-feroylglucosyl)(1−6)[apiosyl(1−2)]-glucoside, *m*/*z* 963; peak 5, spinacetin-3-(2″-feroylglucosyl)(1−6)[apiosyl(1−2)]-glucoside, *m*/*z* 977; peak 6, spinacetin-3-gentiobioside, *m*/*z* 669, including a compound probably consisting of patuletin with gentiobioside and rhamnoside, *m*/*z* 801; peak 7, a patuletin-3-gentiobioside substituted with feroyl, *m*/*z* 831; peak 8, a compound tentatively identified as patuletin with gentiobioside and rhamnoside, *m*/*z* 801; peak 9, spinacetin-3-(2″-feroylglucosyl)(1−6)-glucoside, *m*/*z* 845; peak 10, spinatoside-4′-glucuronide, *m*/*z* 521; peak 11, 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide, *m*/*z* 519; and peak 12, 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone-4′-glucuronide, *m*/*z* 533. Some major peaks were not identified (e.g., $t_R \sim 13.7$ ∼17.1 min, **Figure 1**), but as estimated from their UV and mass spectra, they were not believed to be flavonoids. The identified flavonoids corresponded in large part to those reported by Ferreres et al. (*23*).

The major flavonoid compound, 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide (peak 11), made up on average 43% of the total flavonoid content (**Table 2**). Patuletin-3-glucosyl-(1−6)[apiosyl(1−2)]-glucoside (peak 1), patuletin-3-(2″-feroylglucosyl)(1−6)[apiosyl(1−2)]-glucoside (peak 4), spinatoside-4′-glucuronide (peak 10), and patuletin-3-gentiobioside including a patuletin glycoside (unseparated, peak 3) each made up 7−12% of the total flavonoid content when averaged between treatments. The other flavonoids were minor compounds, each contributing ≤5%. It has been proposed that the position and degree of hydroxylation is fundamental to the antioxidant activity of flavonoids, especially the ortho-dihydroxylation of the B ring, the carbonyl at position 4, and the free hydroxyl group at positions 3 and/or 5 in the C and A rings, respectively (*24*). According to this structure−activity relationship, the 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide (peak 11) and the patuletin glycosides (peaks 1, 3, 4, and 7) would all exhibit high antioxidant activity, whereas the spinacetin glycosides (peaks 2, 5, 6, and 9) and spinatoside-4′-glucuronide (peak 10) would have comparably lower, but still relatively high, antioxidant activity. In addition, the physiological activity of the individual flavonoids may also be determined by their ability to affect enzyme activity (*25*) and their bioavailability, which has been proposed to vary widely (*26*). However, no data are available for these parameters with regard to individual flavonoids in spinach.

The flavonoid content of fresh baby spinach found in this study corresponded to a concentration of 0.6−1.5 g/kg of fresh weight, which is in accordance with what has previously been reported in spinach (*21*). A screening study of 28 vegetables

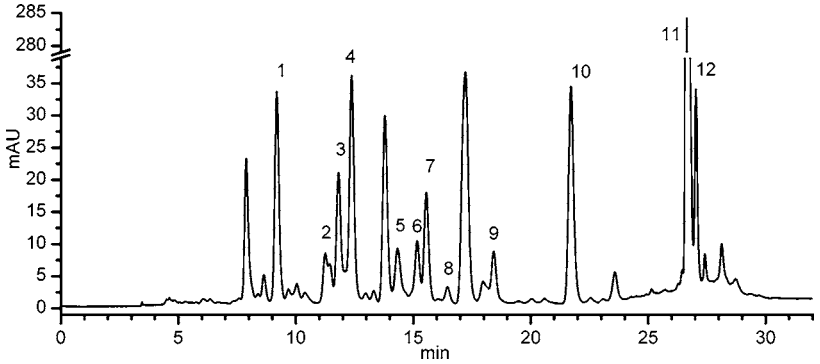

Figure 1. HPLC chromatogram of baby spinach flavonoids at 340 nm. Flavonoids peaks: 1, patuletin-3-glucosyl-(1–6)[apiosyl(1–2)]-glucoside; 2, spinacetin-3-glucosyl-(1–6)[apiosyl(1–2)]-glucoside including a tentatively identified patuletin glycoside; 3, patuletin-3-gentiobioside including a tentatively identified patuletin glycoside; 4, patuletin-3-(2″-feroylglucosyl)(1–6)[apiosyl(1–2)]-glucoside; 5, spinacetin-3-(2″-feroylglucosyl)(1–6)[apiosyl(1–2)]-glucoside; 6, spinacetin-3-gentiobioside including a compound probably of patuletin with gentiobioside and rhamnoside; 7, a patuletin-3-gentiobioside substituted with feroyl; 8, probably a compound of patuletin with gentiobioside and rhamnoside; 9, spinacetin-3-(2″-feroylglucosyl)(1–6)-glucoside; 10, spinatoside-4′-glucuronide; 11, 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide; 12, 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone-4′-glucuronide.

Table 2. Flavonoid Content (Milligrams per Gram of Dry Weight) in Baby Spinach Cv. Emilia Sown at Different Times, Harvested at Growth Stage I, II, or III, and Stored at Different Times and Temperatures[a]

		Aug 2002 sowing			June 2003 sowing			July 2003 sowing		
	storage	I	II	III	I	II	III	I	II	III
total flavonoids	at harvest	21.2 ± 2.2	18.4 ± 1.2	20.6 ± 0.6	19.2 ± 0.9	14.9 ± 0.8	16.2 ± 1.3	21.8 ± 0.6	16.5 ± 0.8	15.7 ± 1.4
	5 days, 10 °C	21.6 ± 0.7	19.0 ± 0.9	23.0 ± 1.0	22.0 ± 1.0	14.5 ± 1.4	14.3 ± 0.9		16.6 ± 2.0	16.2 ± 1.8
	9 days, 10 °C	22.7 ± 0.9	17.6 ± 0.7	20.4 ± 2.7	22.8 ± 0.8	12.6 ± 2.0	12.8 ± 2.0	23.4 ± 0.9	16.7 ± 0.7	12.7 ± 1.4
	9 days, 2 °C					16.2 ± 1.0	17.7 ± 1.6		17.1 ± 2.8	16.8 ± 1.9
patuletin-3-glucosyl-(1–6)[apiosyl(1–2)]-glucoside (peak 1)	at harvest	2.22 ± 0.29	1.86 ± 0.13	2.43 ± 0.12	1.95 ± 0.08	1.69 ± 0.13	1.75 ± 0.11	2.86 ± 0.07	2.06 ± 0.14	1.98 ± 0.18
	5 days, 10 °C	1.84 ± 0.10	1.60 ± 0.10	2.39 ± 0.24	1.56 ± 0.04	1.07 ± 0.15	1.16 ± 0.12		1.58 ± 0.31	1.57 ± 0.37
	9 days, 10 °C	1.68 ± 0.15	1.19 ± 0.06	1.83 ± 0.45	1.08 ± 0.16	0.79 ± 0.10	0.68 ± 0.27	2.21 ± 0.22	1.25 ± 0.09	0.73 ± 0.28
	9 days, 2 °C					1.56 ± 0.11	1.65 ± 0.20		1.85 ± 0.37	1.96 ± 0.32
patuletin-3-gentiobioside including another patuletin glycoside (peak 3)	at harvest	2.02 ± 0.32	1.45 ± 0.17	2.55 ± 0.13	1.48 ± 0.07	1.20 ± 0.05	1.64 ± 0.10	1.83 ± 0.07	1.20 ± 0.08	1.60 ± 0.16
	5 days, 10 °C	1.80 ± 0.15	1.24 ± 1.3	2.48 ± 0.29	1.41 ± 0.08	0.70 ± 0.09	0.94 ± 0.11		0.87 ± 0.13	1.13 ± 0.31
	9 days, 10 °C	1.81 ± 0.18	1.05 ± 0.07	1.85 ± 0.47	1.31 ± 0.17	0.56 ± 0.05	0.56 ± 0.28	1.59 ± 0.17	0.72 ± 0.08	0.52 ± 0.19
	9 days, 2 °C					1.08 ± 0.08	1.47 ± 0.21		1.07 ± 0.22	1.53 ± 0.29
patuletin-3-(2″-feroylglucosyl)-(1–6)[apiosyl(1–2)]-glucoside (peak 4)	at harvest	1.47 ± 0.15	1.57 ± 0.11	1.57 ± 0.06	1.14 ± 0.08	1.08 ± 0.08	1.31 ± 0.14	1.86 ± 0.06	1.27 ± 0.08	1.14 ± 0.13
	5 days, 10 °C	2.03 ± 0.07	1.97 ± 0.18	2.04 ± 0.04	2.06 ± 0.13	1.17 ± 0.15	1.18 ± 0.11		1.66 ± 0.26	1.37 ± 0.23
	9 days, 10 °C	2.45 ± 0.10	1.83 ± 0.07	1.86 ± 0.25	2.44 ± 0.23	1.32 ± 0.05	1.03 ± 0.25	2.94 ± 0.14	1.82 ± 0.10	0.91 ± 0.26
	9 days, 2 °C					1.38 ± 0.10	1.60 ± 0.14		1.59 ± 0.30	1.40 ± 0.18
spinatoside-4′-glucuronide (peak 10)	at harvest	2.27 ± 0.30	2.01 ± 0.15	1.84 ± 0.05	2.65 ± 0.09	1.82 ± 0.09	1.48 ± 0.13	2.62 ± 0.11	2.19 ± 0.23	1.83 ± 0.04
	5 days, 10 °C	2.25 ± 0.07	1.95 ± 0.14	2.08 ± 0.07	2.95 ± 0.12	1.95 ± 0.12	1.39 ± 0.07		2.30 ± 0.22	1.91 ± 0.10
	9 days, 10 °C	2.28 ± 0.19	1.84 ± 0.05	1.90 ± 0.13	3.02 ± 0.06	1.64 ± 0.35	1.34 ± 0.15	2.70 ± 0.10	2.34 ± 0.17	1.76 ± 0.07
	9 days, 2 °C					2.07 ± 0.10	1.56 ± 0.15		2.28 ± 0.39	1.91 ± 0.18
5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxy-flavone-4′-glucuronide (peak 11)	at harvest	8.22 ± 0.65	7.53 ± 0.59	6.74 ± 0.27	8.94 ± 0.47	6.52 ± 0.54	6.61 ± 0.56	8.72 ± 0.27	6.98 ± 0.23	6.35 ± 0.55
	5 days, 10 °C	8.29 ± 0.26	7.94 ± 0.23	7.65 ± 0.05	10.15 ± 0.49	7.10 ± 0.74	6.49 ± 0.44		7.35 ± 0.90	7.17 ± 0.72
	9 days, 10 °C	8.92 ± 0.15	7.72 ± 0.47	7.15 ± 0.51	11.01 ± 0.17	6.20 ± 1.34	6.56 ± 0.66	9.79 ± 0.17	7.56 ± 0.47	6.56 ± 0.42
	9 days, 2 °C					7.16 ± 0.71	7.49 ± 0.50		7.32 ± 0.99	6.64 ± 0.80
minor flavonoids[b]	at harvest	5.01 ± 0.49	4.01 ± 0.20	5.44 ± 0.20	3.06 ± 0.20	2.59 ± 0.09	3.43 ± 0.27	3.88 ± 0.14	2.78 ± 0.19	2.80 ± 0.40
	5 days, 10 °C	5.36 ± 0.27	4.29 ± 0.20	6.40 ± 0.32	3.86 ± 0.21	2.50 ± 0.23	3.13 ± 0.21		2.86 ± 0.29	3.06 ± 0.39
	9 days, 10 °C	5.52 ± 0.17	3.99 ± 0.15	5.79 ± 0.91	3.95 ± 0.38	2.11 ± 0.23	2.67 ± 0.61	4.12 ± 0.22	3.04 ± 0.15	2.24 ± 0.28
	9 days, 2 °C					2.99 ± 0.15	3.90 ± 0.42		2.95 ± 0.54	3.31 ± 0.38

[a] Values are mean ± standard deviation of four field plots, each determined as the mean of three replicates. Flavonoids expressed as spiraeoside equivalents. [b] Spinacetin-3-glucosyl-(1–6)[apiosyl(1–2)]-glucoside, a compound tentatively identified as patuletin glycoside; spinacetin-3-(2″feroylglucosyl)(1–6)[apiosyl(1–2)]-glucoside and spinacetin-3-gentiobioside, two compounds tentatively identified as patuletin + gentiobioside + rhamnoside; patuletin-3-gentiobioside substituted with a feroyl; spinacetin-3-(2″feroylglucosyl)(1–6)-glucoside; and 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone-4′-glucuronide.

and 9 fruits classified spinach as a low-flavonoid vegetable (*27*). However, contrary to many other vegetables, spinach lacks or contains very low levels of the flavonoids quercetin, kaempferol, myricetin, luteolin, and apigenin, which are usually analyzed in screening studies. The total flavonoid content found in this study and by Gil et al. (*21*) would position spinach among the very-high-flavonoid vegetables. Previous research on flavonoids in spinach has identified rather rare flavonoid glycosides that are not commonly found in other vegetables (*21*, *23*, *28*−*32*). A few screening studies have reported that spinach contains quercetin, luteolin, kaempferol, and myricetin (*33*, *34*). However, those studies identified compounds only by comparison of retention times with known standards at a single wavelength. No comparisons were made of absorption spectra or mass spectra, and it is therefore possible that the identification of the individual flavonoids was incorrect.

In the present study, the younger baby spinach leaves (growth stage I) had the highest concentration of total flavonoids. The concentration was significantly lower at the later stages (II and III) in June and July. Thus, the changes in total flavonoid content during leaf growth differed somewhat among sowing times. Similar results have been found in *Rosmarinus officinalis*, in which flavonoid concentration peaked in young leaves (*12*). In *Cistus laurifolius* leaves, however, the flavonoid glycoside

content was relatively low in the young leaves and accumulated during leaf development (*11*). Flavonoid content of cereal leaves has been observed to decrease as the leaves approach senescence (*13*, *35*). The spinach leaves in the present study did not show any signs of senescence at harvest. Baby spinach is harvested after a shorter growth period than normal spinach, and even when harvested at growth stage III the baby spinach leaves were younger than those of normal spinach.

Dissimilar patterns were found among the individual flavonoid compounds. The content of patuletin-3-gentiobioside including a patuletin glycoside (unseparated, peak 3) was high at stage I, decreased to stage II, and then increased again to stage III (**Table 2**). Spinatoside-4′-glucuronide (peak 10) and 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide (peak 11) contents decreased significantly from stage I to stage III at all sowing times. In addition, in 2003, the spinatoside-4′-glucuronide (peak 10) content decreased significantly from each growth stage to the next, and the 5,3′,4′-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4′-glucuronide (peak 11) content was significantly higher at stage I than at the other two stages. Hence, although the same flavonoid compounds were found at all growth stages and sowing times, the relative amounts of the individual flavonoids changed. As bioavailability and antioxidant activity differ among individual flavonoid compounds (*24*, *26*, *36*), an alteration of the flavonoid composition may affect the flavonoid bioactivity and potential health benefits of the vegetable in the diet, even though the total flavonoid concentration may be the same. Changing leaf flavonoid composition during the ontogenic cycle has previously been observed in several plants (*14*), but to our knowledge not yet in leafy vegetables (*11*, *12*, *37*).

To discern between effects of leaf growth and season progression, the study on changes during leaf growth was repeated three times during the field season, at increasing, close to maximum, and decreasing day length, respectively. Under natural conditions in the field, seasonal changes in light and temperature conditions unavoidably occur concurrently as the leaves develop. By repeating the leaf growth study several times, the effects of season progression and changing environmental conditions may be distinguished from the effects of leaf growth and development.

In the present study, sowing time had some effect on flavonoid concentration and composition. Total flavonoid content was highest in August and lowest in June, when comparisons were made at growth stage II (current commercial stage of harvest, **Figure 2**). Total flavonoid content varied up to 31% among sowing times when comparisons were made within each growth stage. The individual flavonoids varied up to 91% (**Table 2**), which is little compared to the reported 91-fold variation in the isorhamnetin-3-rutinoside content of *Diplotaxis tenuifolia* between spring (March) and summer (September) harvesting (*10*). The field season for cv. Emilia spinach in southern Sweden is from May to September, and differences might have been greater if the whole growing season had been monitored in this investigation. Annual fluctuations in radiation, temperature, and other environmental parameters, as well as time of season, are likely to affect these changes. Howard et al. (*9*) found a higher total phenolic content in spinach harvested in spring than in fall. However, the spinach they harvested in spring was overwintered. Thus, plant age was different between the two harvests, a fact that may also have had an effect on the phenolic content, again showing the difficulty of distinguishing between seasonal variation and ontogenic changes.

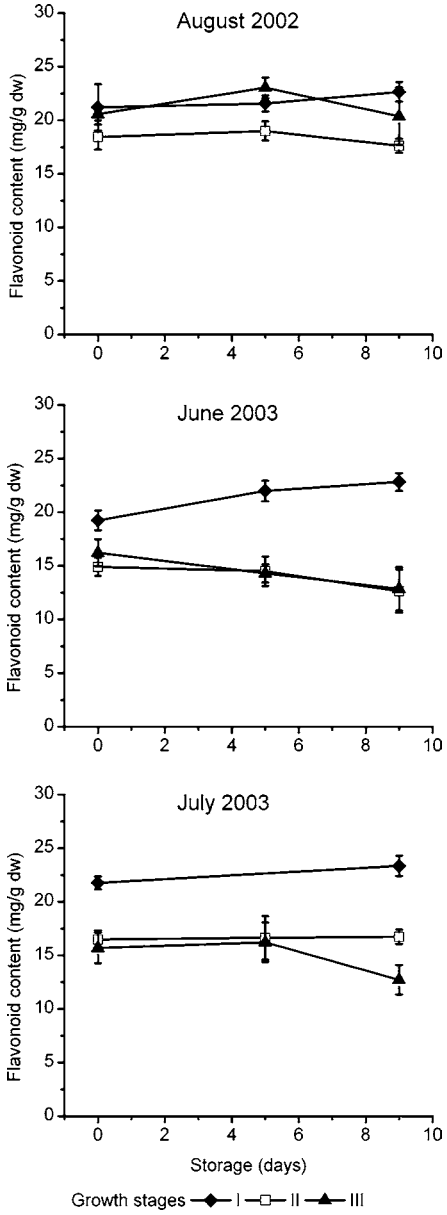

Figure 2. Flavonoid content in baby spinach sown in August 2002, June 2003, or July 2003, harvested at different growth stages and stored at 10 °C in unperforated polypropylene bags. Flavonoid content is expressed as milligrams of spiraeoside equivalents per gram of dry weight, $n = 4$.

Total flavonoid content changed somewhat during storage, but the pattern varied among treatments (**Figure 2**; **Table 2**). There was an increase during storage at growth stage I, although not significant in August. In some cases (stages II and III in June and stage III in July) a decreasing tendency was observed during storage at 10 °C, but this was only statistically significant at stage III. During the 2 °C storage, however, total flavonoid content increased slightly, although not significantly.

The rather stable total flavonoid content during storage is in accordance with what has previously been reported in spinach (*21*). The flavonoid content of spinach also appears to be relatively stable compared to that of some other leafy vegetables. Nine of 11 lettuce and endive varieties studied by DuPont et al. (*8*) showed a decreased flavonoid glycoside content during storage at 1 °C for 7 days; one was not significantly affected, and one showed an increase.

Some of the individual flavonoid compounds changed considerably during storage at 10 °C for 9 days. The contents

of patuletin-3-glucosyl-(1−6)[apiosyl(1−2)]-glucoside (peak 1) and patuletin-3-gentiobioside (peak 3) decreased significantly, whereas most of the other flavonoids remained rather stable or showed inconsistent responses to storage. The content of patuletin-3-[2″feroylglucosyl)(1−6)[apiosyl(1−2)]-glucoside (peak 4) increased during storage at stages I and II, the increase being more marked at stage I. Changes were smaller at 2 °C than at 10 °C. Although we found no case of individual flavonoids disappearing altogether during storage, the changes in individual flavonoids found in this study are similar to those found by DuPont et al. (*8*), who observed that some doubled in content and others disappeared.

The flavonoid content is one of the many factors that determine the quality of vegetables. Other parameters that may be influential include other bioactive constituents, visual appearance, and microbial infestation. We have previously investigated the variation in vitamin C and carotenoid contents as well as visual quality during baby spinach growth (*19*). Those results indicated that the nutritional and visual quality can be increased by harvesting a few days earlier than what is normal commercial practice today. However, the lower yield at the early harvest (*19*) should also be taken into consideration. This might possibly be compensated for by different cultivation practices, such as additional harvests per season, but further developmental work is needed considering both practical and economic aspects. We also believe that the improved nutritional and visual quality might give the product an added value that would make up for the lower yield at an earlier harvest. Although the health effects of the individual flavonoids in spinach have not been elucidated, the improvements of visual quality and nutritional content in the previous investigation, together with indications of positive health effects of flavonoids (*38*), lead to the recommendation of an earlier harvest of baby spinach.

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