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Influences of Organic Fertilization, High Tunnel Environment, and Postharvest Storage on Phenolic Compounds in Lettuce

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Abstract. **As the largest group of phytochemicals, dietary phenolics play an important role in human health and disease prevention. Cultural practices have been shown to have the potential for affecting phenolic compounds in food crops. Spring and summer trials were conducted in 2003 to examine the effects of organic fertilization and high tunnel environments on phenolic constituents of lettuce (*Lactuca sativa* L.) cultivars Red Sails and Kalura. Effects of postharvest storage at 4 °C for 16 days on total phenolics of lettuce harvested from the summer trial were also evaluated. Total phenolics, excluding anthocyanins, were measured spectrophotometrically, and major phenolic constituents were identified and quantified by high-performance liquid chromatography. Chlorogenic acid and quercetin glycosides were found to be predominant in lettuce. 'Red Sails' consistently exhibited significantly higher phenolic concentrations than 'Kalura'. Organic (compost + fish emulsion) and conventional (N–P–K + $CaNO_3$) fertilization did not consistently differentially affect lettuce phenolics in our recently established organic and conventional plots. The high tunnel environment generally reduced phenolic levels in lettuce relative to the open field. However, differences between high tunnel and open field varied with cultivar and season. Effects of production factors on lettuce phenolics were maintained during cold storage. There was a substantial increase in total phenolics during storage, likely correlated with declining lettuce quality. Further studies are warranted to more fully assess the impact of cultivar and production management, including organic fertilization, on lettuce phenolics.**

The inverse relationship between dietary intake of fruit and vegetables and incidence of many chronic diseases has been primarily attributed to the health benefits of phytochemicals (Heber, 2004; Liu, 2003). Phenolics constitute the largest group of phytochemicals as a result of their great abundance in plant foods and consist chiefly of phenolic acids and flavonoids (Dillard and German, 2000; Manach et al., 2004). Dietary phenolic compounds significantly contribute to the antioxidant and antiproliferative activities of fruit and vegetables (Eberhardt et al., 2000; Liu, 2003).

Lettuce (*Lactuca sativa* L.) is among the top five most commonly consumed vegetables in the U.S. (Lucier and Jerardo, 2005). In lettuce, although phytochemical levels are relatively low on the basis of fresh weight, the high antioxidant capacity of lettuce phenolics, shown in scavenging peroxyl radicals, suggests the possibility of enhancing the potential health benefits of lettuce by increasing the levels of phenolic compounds (Caldwell, 2003). Phenolic composition and content of lettuce significantly vary among types, including loose leaf, oak leaf, iceberg, butterhead, and romaine, with iceberg lettuce tending to be the lowest (Caldwell, 2003; DuPont et al., 2000). Given that synthesis of phenolic compounds in plants is influenced by various abiotic and biotic stresses such as high ultraviolet irradiation, low temperature, nutrient deficiency, insect and pathogen attack (Dixon and Paiva, 1995), cultural practices are likely to play an important role in modifying levels of phenolics in lettuce.

Recent studies have attempted to examine the impact of organic production practices on phenolic content in fruit and vegetables. In comparisons of organically and conventionally grown apples (*Malus ×domestica*) (Weibel et al., 2000), peaches (*Purunus persica*) and pears (*Pyrus communis*) (Carbonaro et al., 2002), strawberries (*Fragaria ×ananassa*) (Asami et al., 2003), grapes (*Vitis vinifera*) (Malusà et al., 2004), Welsh onions (*Allium fistulosum*), green peppers (*Capsicum annuum*), spinach (*Spinacia oleracea*), and Chinese cabbage (*Brassica rapa nothovar*) (Ren et al., 2001), it was concluded that organic produce seemed to contain higher levels of polyphenols than did conventional produce. However, confounding production factors as well as drawbacks associated with experimental designs in most previous studies preclude definitive conclusions and indicate a need for further studies (Zhao et al., 2006). To develop a more complete understanding of the influence of organic production practices on phenolic compounds in fruit and vegetables, a wide range of crops grown under various conditions need to be tested. Few studies to date have investigated the influence of organic management on lettuce phenolics.

The environmental effects of protected production structures deserve further study as well. Among other influences, glazing and covering materials cause changes in the light that plants receive. In the case of a red loose leaf lettuce cultivar, exclusion of ambient solar ultraviolet B (280–320 nm) not only reduced the concentration of anthocyanins, but also led to a significant decrease of other presumptive flavonoids with absorbance at 270, 300, and 330 nm (Krizek et al., 1998). It has also been reported that lettuce grown in a polycarbonate greenhouse had lower content of flavonoids than that grown in the open field (Romani et al., 2002). The extent to which high tunnels, unheated polyethylene-covered greenhouse structures that are used in season-extending production for many horticultural crops (Wells, 1996), may affect phenolic content of lettuce has not been reported.

Postharvest storage also influences lettuce phenolics. Total phenolics in iceberg lettuce increased by 25.0% and 23.3% in outer and inner leaves, respectively, during 2 weeks storage at 4 °C (Zhang and Hamauzu, 2003). However, interactions among genotype, preharvest, and postharvest factors that may affect lettuce phenolics have not been well studied.

The aim of this study was to evaluate the effects of cultivar, organic fertilization, and high tunnel production on the phenolic constituents of lettuce. Individual phenolic compounds were analyzed to provide a comprehensive assessment of effects. Additionally,

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lettuce cultivars produced under organic and conventional systems in high tunnel and open field environments were examined for phenolic changes during postharvest storage.

Materials and Methods

Experimental setup. Two sequential lettuce trials were conducted at the Kansas State University Horticulture Research and Extension Center, Olathe, during the spring and summer of 2003. The soil type was Kennebec silt loam. Six high tunnels, 9.8 × 6.1 m, with 1.5-m sidewalls (Stuppy, North Kansas City, Mo.) and six adjacent 9.8 × 6.1-m field plots were used in both trials. The tunnels were covered with a single layer of 6-mil (0.153-mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, S.C.). End walls were open and sidewalls were rolled up throughout the spring and summer trials. Established in Spring 2002, the six high tunnel and field plots were divided into three groups (blocks), and the two high tunnels in each block were randomly assigned organic or conventional management treatments. Lettuce cultivars under investigation were grown in each plot. Drip and sprinkler irrigation were used during the early and later trials, respectively. High tunnels were covered with 39% white shadecloth (Pak Unlimited, Norcross, Ga.) in the summer trial.

Organic and conventional fertilization. Before each trial, soil samples were analyzed for macronutrients at the Kansas State University soils laboratory (Manhattan, Kan.), and preplant fertilization and fertigation were done based on Marr et al. (1998) to provide recommended rates of nitrogen to each plot. Organic fertilizers used were Hu-More 1N–0.4P–0.8K (composted cattle manure and alfalfa hay; Humalfa, Inc., Shattuck, Okla.) incorporated before planting followed by fertigation with fish emulsion 5N–0.4P–0.8K (Lilly Miller Brands, Clackamas, Ore.). Conventional plots received 13N–5.7P–10.8K preplant and fertigation with calcium nitrate 15.5N–0P–0K through the drip irrigation system. In the spring trial, all the plots received preplant fertilization at a rate of 48 kg·ha^{-1} total N. Plants were fertigated at 5-d intervals starting 3 weeks before harvest. Plots inside and outside received fertigation at a rate of 10 kg·ha^{-1} N and 3 kg·ha^{-1} N, respectively, at the first and second fertigation and at 5 kg·ha^{-1} N and 2 kg·ha^{-1} N, respectively, in the third fertigation. In the summer trial, high tunnel and open field plots received preplant fertilization at a rate of 17 kg·ha^{-1} N and 51 kg·ha^{-1} N, respectively. Plants in all the plots were fertigated through the drip irrigation system 10 d before harvest at a rate of 6 kg·ha^{-1} N.

Cultivars, planting, and sampling. Two lettuce cultivars (Johnny's Selected Seeds, Winslow, Maine), 'Kalura', a green romaine type, and 'Red Sails', a red loose leaf type, were selected for analysis of phenolics from trials of eight cultivars (manuscript in preparation for reporting elsewhere) that were planted in each trial as a continuation of 2002 cultivar trials. In both trials, transplants were produced in the greenhouse using 200-cell Speedling flats (Speedling Inc., Sun City, Fla.) with Jiffy mix (Jiffy Products of America, Norwalk, Ohio). In the spring trial, lettuce cultivars were seeded on 31 Mar., transplanted on 13 May, and harvested on 17 June. In the summer trial, seeds were sown on 29 May, and lettuce cultivars were transplanted on 8 July and harvested on 5 Aug. Plots consisted of 10 plants in single rows at a spacing of 20 cm within rows and 30 cm between rows. At harvest, three plants of each cultivar per plot were randomly selected for leaf sampling. The outermost leaves were removed and three leaves were taken from the next whorl of each plant to form a pooled sample of nine leaves per plot. Leaf samples were immediately frozen in liquid nitrogen and packed on ice in coolers for transport, within 2 h, to Kansas State University, Manhattan, Kan., where phytochemical analyses were conducted. Samples were stored at –20 °C for 1 week and then were freeze-dried and stored at –80 °C before extraction.

Postharvest storage. At harvest during the summer trial, three selected heads of each cultivar from each plot were placed in perforated plastic bags and transported to a walk-in cooler on campus for postharvest storage at 4 °C for 16 d. After storage, leaves were sampled and freeze-dried using the procedure described previously for freshly harvested lettuce.

Sample extraction of phenolics. One gram of freeze-dried leaf samples was finely dispersed in 50 mL of 80% aqueous ethanol containing 20 ppm of 98% 2-naphthoic acid (Aldrich Chemical Co., Milwaukee, Wis.) as an internal standard and then refluxed at 90 ± 1 °C for 1 h. The mixture was centrifuged when cooled to room temperature. Twenty milliliters of the supernatant was evaporated to dryness using a rotary evaporator and then redissolved in 8 mL of water. Two milliliters of the water solution was subsequently purified by solid phase extraction (SPE) using a reverse-phase Accubond ODS C-18 column (Agilent Technologies, Stockport, Cheshire, U.K.) preconditioned with 2 mL of methanol and 2 mL of deionized water. After washing the column with 2 mL of deionized water, the phenolic compounds were eluted with 2 mL of methanol. As the anthocyanins were washed out with water, methanol extracts only contained phenolic acids, flavones, and flavonols.

Determination of total phenolics. Total phenolic concentration was analyzed spectrophotometrically using the Folin-Ciocalteau reagent following Singleton et al. (1999) with modifications. Briefly, the sample extract was neutralized with 50 µL of 2 M NaOH and diluted to exactly 5 mL with 5 mM phosphate buffer (pH 7.4); 2 mL of the diluted extract was transferred to a test tube followed by the addition of 200 µL of Folin-Ciocalteau reagent (Sigma-Aldrich, St. Louis, Mo.) and 400 µL of saturated Na_2CO_3 and allowed to stand for 3 min before incubation. After 60-min incubation in the dark at room temperature, the absorbance of the solution was measured at 725 nm. Quercetin (Sigma-Aldrich) was used as a standard, and results were expressed as milligrams of quercetin equivalents per gram dry weight.

High-performance liquid chromatography analysis of phenolics. Concentrations of selected phenolic acid and flavonoids were measured by high-performance liquid chromatography (HPLC). A 100-µL aliquot of the sample extract was injected into a Beckman "Gold Nouveau" HPLC system (Beckman Instruments, Fullerton, Calif.) equipped with an autosampler and a photodiode array detector. Separation of individual phenolic compounds was achieved on Alltima C-18 (250 × 4.6 mm, 4 mm i.d.) reverse-phase column (Alltech, Deerfield, Ill.) coupled to a guard column (Alltech). Elution was carried out using (A) 9 water : 1 acetic acid (by volume) and (B) HPLC-grade methanol as the mobile phase. The gradient used was as follows: start with 100% A, increase to 10% B in A within 5 min, maintain 10% B in A for 5 min, increase to 40% B in A within 20 min, and then to 70% B in A within 10 min. After maintaining at 70% B in A for 6 min, the solvent composition was returned to the initial condition (100% A) in 5 min, thus equilibrating the system for subsequent runs. The flow rate was 0.8 mL/min, and the chromatograms were recorded at 280 and 355 nm. Major peaks were identified by comparisons of the retention times and cochromatography with those of phenolic standards, chlorogenic acid (Sigma-Aldrich), quercetin-3-*O*-glucoside (Indofine Chemical Co., Inc., Hillsborough, N.J.), rutin (quercetin-3-*O*-rutinoside) (Acros Organics, Geel, Belgium), and luteolin-7-*O*-glucoside (Indofine Chemical Co., Inc.). In addition, fractions of some unknown peaks were collected and further characterized by mass spectrometry using a Bruker esquire 3000 plus MS system (Bruker Daltonics, Inc., Billerica, Mass.). Concentrations of chlorogenic acid, luteolin-7-*O*-glucoside, and rutin in samples were quantified by peak areas in the chromatograms using calibration curves obtained from external standards. Quantification of other quercetin glycosides was performed using quercetin as the standard. Results for all flavonoid conjugates were expressed as milligrams of aglycone per gram of dry weight.

Statistical analyses. For the experiments on preharvest factors, results were analyzed as a split–split plot design with environment (high tunnels versus open field) as the whole plot factor, fertilization (organic versus conventional) as the subplot factor, and cultivar as the subsubplot factor. Three blocks in each environment served as replications. For the investigation of postharvest storage, the design of repeated measures was applied to compare the results before and after storage. Analysis of variance was conducted using the SAS system for Windows (version. 9.1; Cary, N.C.), and the least significant difference test ($\alpha = 0.05$) was used for multiple comparisons.

Table 1. Analysis of variance of the effects of production environment, fertilizer source, and cultivar on total phenolic content of lettuce in spring and summer trials at Olathe, Kans., 2003.

Effects	Spring	Summer
Environment (high tunnel vs. open field)	*	NS
Fertilizer (organic vs. conventional)	NS	NS
Environment × fertilizer	NS	NS
Cultivar	***	***
Environment × cultivar	NS	NS
Fertilizer × cultivar	NS	NS
Environment × fertilizer × cultivar	NS	*

NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 2. Total phenolic concentrations[z] (mean ± SE) in lettuce cultivars grown under organic (ORG) and conventional (CON) fertilization in high tunnel and open field environments during spring and summer at Olathe, Kans., 2003.

	High tunnel				Open field			
	Kalura		Red Sails		Kalura		Red Sails	
Trial	ORG	CON	ORG	CON	ORG	CON	ORG	CON
Spring	6.2 ± 2.1	4.6 ± 2.1	9.2 ± 2.1	9.4 ± 2.1	6.2 ± 2.1	6.6 ± 2.1	12.4 ± 2.1	19.8 ± 2.1
Summer	3.2 ± 1.7	4.0 ± 1.7	10.1 ± 1.7	5.4 ± 1.7	5.0 ± 1.7	4.2 ± 1.7	11.0 ± 1.7	14.3 ± 1.7

[z]Milligrams quercetin equivalents/gram dry weight.

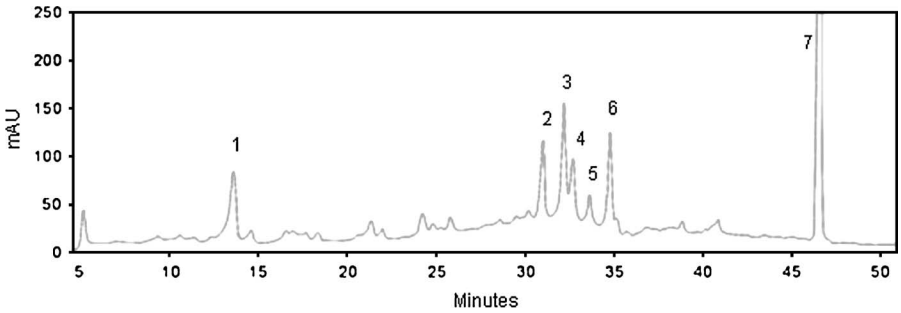

Fig. 1. High-performance liquid chromatogram of lettuce extract ('Kalura') at 280 nm: 1) chlorogenic acid; 2) quercetin-3-*O*-glucuronide; 3) quercetin-3-*O*-glucoside; 4) luteolin-7-*O*-glucoside; 5) rutin; 6) quercetin-3-*O*-malonylglucoside; and 7) internal standard (2-naphthoic acid).

Results

Total phenolic content. In both trials, the total phenolic content of 'Red Sails' was significantly higher than 'Kalura' (Tables 1 and 2). In contrast to the highly significant cultivar effect, the effects of production environment, fertilization, and interactions among factors were inconsistent (Table 1). Only during the spring trial were total phenolic levels significantly increased in open field production compared with high tunnels (Tables 1 and 2). During the summer trial, the only significant effect other than cultivar was the three-way interaction of environment, fertilizer, and cultivar as a result of a significant elevation of total phenolics in 'Red Sails' in the open field under conventional fertilization, but not in other cultivar and fertilizer treatment combinations (Tables 1 and 2).

Individual phenolic compounds. Individual phenolics were analyzed to examine further the effects of genotype and production conditions in lettuce. Quercetin glycosides were found to be the dominant flavonoids in both 'Red Sails' and 'Kalura'. Chlorogenic acid and the five flavonoid conjugates quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, luteolin-7-*O*-glucoside, rutin (quercetin-3-*O*-rutinoside), and quercetin-3-*O*-malonylglucoside were found in all lettuce samples (Fig. 1). As with total phenolics, cultivar was the predominant factor affecting the major phenolic compounds in lettuce (Table 3). 'Red Sails' contained significantly higher concentrations of all individual phenolic compounds than 'Kalura' in both trials, except for luteolin-7-*O*-glucoside in the spring trial (Tables 3, 4 and 5).

Effects of fertilizer and production environment varied with season and with phenolic compound investigated. In the spring trial, both lettuce cultivars grown in high tunnels had significantly reduced concentrations of chlorogenic acid, quercetin-3-*O*-glucoside, and rutin. For quercetin-3-*O*-malonylglucoside, there was a cultivar × environment interaction with levels only significantly reduced in 'Red Sails' grown in high tunnels (Tables 3 and 4). In the spring trial, fertilizer regime had a significant effect on chlorogenic acid, which was higher under conventional fertilization (Tables 3 and 5). There were also significant environment × fertilizer interactions for chlorogenic acid and quercetin-3-*O*-malonylglucoside, largely as a result of significantly higher levels of these compounds under conventional fertilization but only in the open field (Tables 3 and 6). In the summer trial, there were no effects of fertilizer. Quercetin-3-*O*-glucoside was the only compound found to be significantly affected by production environment with higher levels in lettuce from open field than from high tunnels (Tables 3 and 4).

Change of total phenolics during postharvest storage. 'Red Sails' maintained a higher phenolic content than 'Kalura' at the end of 16-d storage at 4 °C (Fig. 2A). Total phenolic concentrations increased during storage of both lettuce cultivars from organically and conventionally managed high tunnel and open field plots. Phenolic levels in 'Kalura' increased during storage, but did not differ between high tunnel and open field (Fig. 3A), whereas 'Red Sails' from open field plots went into and came out of storage with significantly higher phenolic content than that from high tunnels (Fig. 3B). There were no differences between total phenolic levels resulting from organic or conventional fertilizers either before or after storage (Fig. 2B).

Discussion

Excluding anthocyanins, the phenolic compounds reported here probably reflect

Table 3. Analysis of variance of the effects of production environment, fertilizer source, and cultivar on levels of individual phenolic compounds in lettuce grown in the spring and summer at Olathe, Kans., in 2003.[z]

Effects	ChlgA		Q-3-gluA		Q-3-glu		L-7-glu		Rutin		Q-3-Mglu	
Environment (high tunnel vs. open field)	*	NS	NS	NS	**	***	NS	NS	*	NS	**	NS
Fertilizer (organic vs. conventional)	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Environment × fertilizer	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Cultivar	**	***	*	**	*	***	NS	*	***	**	***	**
Environment × cultivar	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Fertilizer × cultivar	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Environment × fertilizer × cultivar	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]The two columns for each variable represent results from sequential trials.

ChlgA, chlorogenic acid; Q-3-gluA, quercetin-3-*O*-glucuronide; Q-3-glu, quercetin-3-*O*-glucoside; L-7-glu, luteolin-7-*O*-glucoside; Q-3-Mglu, quercetin-3-*O*-malonylglucoside.

NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 4. Concentrations[z] (mean ± SE) of chlorogenic acid and flavonoids identified in lettuce cultivars grown in high tunnel and open field environments during spring and summer trials 2003.

		Kalura		Red Sails	
Season	Compound	High tunnel	Open field	High tunnel	Open field
Spring	ChlgA	0.6 ± 0.2	2.0 ± 0.9	3.5 ± 1.3	7.9 ± 0.9
	Q-3-gluA	1.0 ± 0.4	2.7 ± 1.0	3.3 ± 1.0	4.1 ± 0.6
	Q-3-glu	0.6 ± 0.1	1.9 ± 0.4	1.3 ± 0.6	3.8 ± 0.5
	L-7-glu	0.4 ± 0.1	0.9 ± 0.4	0.9 ± 0.3	1.3 ± 0.1
	Rutin	0.3 ± 0.1	0.6 ± 0.2	2.3 ± 0.8	4.6 ± 0.3
	Q-3-Mglu	0.6 ± 0.1	1.3 ± 0.4	2.3 ± 0.7	5.6 ± 0.6
Summer	ChlgA	0.6 ± 0.1	1.3 ± 0.5	5.8 ± 1.4	5.7 ± 1.2
	Q-3-gluA	0.5 ± 0.1	0.8 ± 0.2	2.2 ± 0.4	1.9 ± 0.4
	Q-3-glu	0.4 ± 0.0	2.1 ± 0.4	2.1 ± 0.4	4.8 ± 0.5
	L-7-glu	0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.9 ± 0.2
	Rutin	0.1 ± 0.0	0.5 ± 0.2	1.6 ± 0.1	2.7 ± 0.6
	Q-3-Mglu	0.5 ± 0.1	1.1 ± 0.2	2.6 ± 0.1	4.4 ± 0.8

[z]Units for chlorogenic acid were milligrams/gram dry weight; units for flavonoid glycosides were milligrams/gram dry weight expressed as corresponding aglycones.
ChlgA, chlorogenic acid; Q-3-gluA, quercetin-3-*O*-glucuronide; Q-3-glu, quercetin-3-*O*-glucoside; L-7-glu, luteolin-7-*O*-glucoside; Q-3-Mglu, quercetin-3-*O*-malonylglucoside.

a fairly complete picture of major phenolics present in lettuce. Chlorogenic acid and quercetin glycosides have been previously identified as the predominant phenolic acid and flavonoids in lettuce (Caldwell, 2003; DuPont et al., 2000; Ferreres et al., 1997). Levels of aglycone flavonoids in lettuce were extremely low in relation to the flavonoid conjugates (Young et al., 2005). Our study focused on genetic and environmental influences on these major lettuce phenolics. Conjugation of flavonoids typically takes place after biosynthesis of flavonoid aglycones in plants (Strack, 1997), and although aglycones possess higher antioxidant capacity than glycosides (Heim et al., 2002), lettuce phenolics are known to have high antioxidant activity (Caldwell, 2003).

Our study showed that overall, genotype was the predominant factor influencing lettuce phenolic levels. During both spring and summer trials, and under each production condition, the red loose leaf lettuce 'Red Sails' contained higher concentrations of phenolic compounds than the romaine lettuce 'Kalura'. Although two cultivars are insufficient for generalizing about differences among lettuce types, our results were in line with previous reports indicating higher phenolics in leaf lettuce than in romaine lettuce cultivars (Caldwell, 2003; DuPont et al., 2000). If anthocyanins had been considered in our analysis of individual phenolic compounds, the phenolic content in 'Red Sails' would have been even higher because red lettuce cultivars contain cyanidin glycosides (anthocyanins), which are generally absent from or present in only trace amounts in green cultivars (DuPont et al., 2000; Kleinhenz et al., 2003). In addition to anthocyanins, red leaf lettuce has been reported to contain elevated levels of other phenolics relative to green leaf lettuce (DuPont et al., 2000). Further studies involving a range of colors and types of lettuce would help to identify cultivars richest in phenolics and to clarify relationships among lettuce types, leaf colors, and phenolics.

The impact of protected environment outweighed that of fertilizer on lettuce phenolics in our study. Total phenolics and some of the individual phenolics were significantly lower under high tunnel production in the spring trial. This was perhaps the result of reduced light in the high tunnels, because the clear polyethylene film decreased intensity of photosynthetically active (*PAR*) radiation light and largely blocked ultraviolet radiation. It has been demonstrated that both solar ultraviolet A (320–400) and ultraviolet B (280–320 nm) can induce anthocyanins in red leaf lettuce, whereas ultraviolet B is important for induction of other flavonoids (Krizek et al., 1998). Shading reduced anthocyanin concentrations in red lettuce (Kleinhenz et al., 2003). 'Red Sails' grown in the open field had a more pronounced red color in the spring. In the summer trial, open field production only induced higher total phenolics in 'Red Sails' under conventional fertilization, indicating a complicated relationship among cultivar, fertilizer source, and environment with respect to lettuce phenolics. However, these results were not reflected in the analysis of individual phenolic compounds. The only significance found in the summer trials was the increase of quercetin-3-*O*-glucoside in open field production across cultivar and fertilizer treatments. Whereas the light intensity in shaded high tunnels was only half of that in the open field during summer (data not shown), it was surprising to observe fewer environmental effects than in the spring trial. Phenolics synthesized in response to high light intensity have been suggested to play a role in the dissipation of photochemical energy (Grace and Logan, 2000). It is possible that daily sprinkler irrigation (used to provide cooling as well as irrigation) in our summer trial reduced the light intensity that lettuce received diminishing phenolic content in both high tunnels and open fields. According to Lester (2006), irrigation method can influence ascorbic acid content in vegetables by altering light intensity. It is also likely that higher average temperatures during the summer trial (27.4 °C in summer versus 18.4 °C in spring) differentially affected phenolic metabolism of the two crops. Temperature, in addition to light and genotype, has been shown to influence anthocyanin levels in lettuce (Kleinhenz et al., 2003) and other crops (Shvarts et al., 1997). Relationships among temperature, light, and lettuce phenolics may warrant further study. Additional studies should also include assessment of phenolics in lettuce during fall and winter seasons.

Table 5. Concentrations[z] (mean ± SE) of chlorogenic acid and flavonoids identified in lettuce cultivars grown under organic and conventional fertilizer sources during spring and summer trials 2003.

		Kalura		Red Sails	
Season	Compound	Organic	Conventional	Organic	Conventional
Spring	ChlgA	0.9 ± 0.2	1.7 ± 0.9	3.6 ± 1.3	7.8 ± 1.0
	Q-3-gluA	1.8 ± 0.5	1.9 ± 0.9	2.6 ± 0.7	4.8 ± 0.9
	Q-3-glu	1.2 ± 0.3	1.4 ± 0.3	2.3 ± 0.7	2.8 ± 0.4
	L-7-glu	0.6 ± 0.1	0.7 ± 0.4	1.0 ± 0.3	1.2 ± 0.2
	Rutin	0.4 ± 0.1	0.5 ± 0.2	2.7 ± 0.6	4.3 ± 0.6
	Q-3-Mglu	0.8 ± 0.2	1.0 ± 0.4	3.2 ± 0.7	4.7 ± 0.6
Summer	ChlgA	1.1 ± 0.4	0.8 ± 0.2	6.1 ± 1.5	5.5 ± 1.1
	Q-3-gluA	0.7 ± 0.2	0.6 ± 0.2	2.1 ± 0.5	2.0 ± 0.3
	Q-3-glu	1.3 ± 0.1	1.2 ± 0.4	3.2 ± 0.5	3.7 ± 0.4
	L-7-glu	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.2
	Rutin	0.4 ± 0.2	0.2 ± 0.1	1.9 ± 0.5	2.3 ± 0.4
	Q-3-Mglu	1.0 ± 0.2	0.7 ± 0.2	3.6 ± 0.8	3.5 ± 0.1

[z]Units for chlorogenic acid were milligrams/gram dry weight; units for flavonoid glycosides were milligrams/gram dry weight expressed as corresponding aglycones.
ChlgA, chlorogenic acid; Q-3-gluA, quercetin-3-*O*-glucuronide; Q-3-glu, quercetin-3-*O*-glucoside; L-7-glu, luteoli-7-*O*-glucoside; Q-3-Mglu, quercetin-3-*O*-malonylglucoside.

Fertilizer source had few significant effects on lettuce phenolics in our study with the most significant effects occurring in the spring trial when higher concentrations of chlorogenic acid and quercetin-3-*O*-malonylglucoside were induced in conventionally fertilized open field plots. Our results did not support the hypothesis of higher phytochemical content in organically grown crops as a result of differential availability of nutrients (Lundegardh and Martenson, 2003; Malusà et al., 2004; Toor et al., 2006; Veberic et al., 2005). However, it should be noted that this was only the second year of production on our organic and conventional production plots at Olathe, Kans., and that these plots were on a rich silt loam soil with high levels of organic matter (>3.0%). Very likely, high levels of mineralization in all plots provided sufficient nutrients for crop growth and contributed to relatively uniform conditions in organic and conventional plots. Fertilizer application rate has the potential to

Table 6. Concentrations[z] (mean ± SE) of chlorogenic acid and flavonoids identified in organically and conventionally grown lettuce from high tunnel and open field during spring and summer trials 2003.

		High tunnel		Open field	
Season	Compound	Organic	Conventional	Organic	Conventional
Spring	ChlgA	2.4 ± 1.3	1.6 ± 0.5	2.0 ± 0.4	7.9 ± 0.2
	Q-3-gluA	2.0 ± 0.6	2.3 ± 0.9	2.4 ± 0.6	4.4 ± 1.0
	Q-3-glu	1.1 ± 0.5	0.8 ± 0.3	2.4 ± 0.5	3.4 ± 0.4
	L-7-glu	0.9 ± 0.3	0.5 ± 0.2	0.8 ± 0.1	1.5 ± 0.4
	Rutin	1.4 ± 0.6	1.3 ± 0.6	1.7 ± 0.3	3.6 ± 0.3
	Q-3-Mglu	1.6 ± 0.6	1.2 ± 0.5	2.4 ± 0.5	4.5 ± 0.6
Summer	ChlgA	4.2 ± 1.1	2.3 ± 0.9	3.0 ± 1.1	3.9 ± 0.8
	Q-3-gluA	1.6 ± 0.4	1.1 ± 0.2	1.2 ± 0.4	1.6 ± 0.3
	Q-3-glu	1.4 ± 0.2	1.1 ± 0.3	3.1 ± 0.5	3.8 ± 0.5
	L-7-glu	0.4 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	0.7 ± 0.2
	Rutin	1.1 ± 0.0	0.6 ± 0.1	1.3 ± 0.5	1.9 ± 0.4
	Q-3-Mglu	2.0 ± 0.1	1.2 ± 0.1	2.6 ± 0.8	3.0 ± 0.2

[z]Units for chlorogenic acid were milligrams/gram dry weight; units for flavonoid glycosides were milligrams/gram dry weight expressed as corresponding aglycones.
ChlgA, chlorogenic acid; Q-3-gluA, quercetin-3-*O*-glucuronide; Q-3-glu, quercetin-3-*O*-glucoside; L-7-glu, luteoli-7-*O*-glucoside; Q-3-Mglu, quercetin-3-*O*-malonylglucoside.

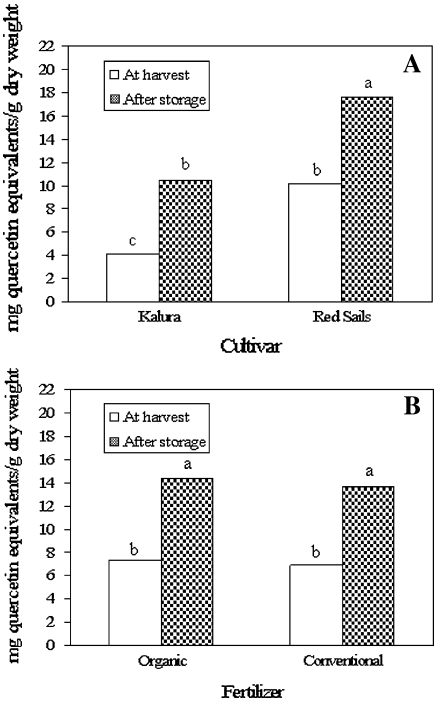

Fig. 2. Changes in total phenolics in romaine lettuce cultivar Kalura and loose leaf lettuce cultivar Red Sails (**A**) and in these lettuce cultivars grown organically or conventionally (**B**) after storage at 4 °C for 16 d. Differences among treatment combinations indicated by different letters are significant at the 0.05 level.

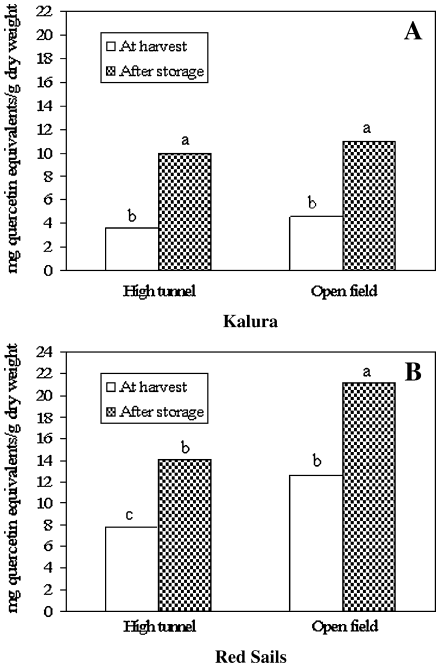

Fig. 3. Change in total phenolics in romaine lettuce cultivar Kalura (**A**) and loose leaf lettuce cultivar Red Sails (**B**) grown in high tunnel and open field environments after storage at 4 °C for 16 d. Differences among treatment combinations indicated by different letters are significant at the 0.05 level.

be important with respect to flavonoid content in leafy crops because nitrogen deficiency induced flavonol accumulation in the leaves of tomato (*Lycopersicon esculentum*) plants (Stewart et al., 2001). Levels of flavonoids and phenolic acids in barley (Hordeum vulgare) leaves decreased with increasing rates of animal manure applied (Nørbaek et al., 2003). The potential impact of fertilization under organic production extends beyond nutrient provision per se, because organically managed soil may possess higher microbial activity (Mäder et al., 2002), and soil microbial activity may vary with different organic inputs (Tu et al., 2006). It has been shown that soil management can play a role in manipulating crop phytochemicals (Lombardi-Boccia et al., 2004). Further studies involving different organic fertility amendments and fertilizer levels in organic and conventional plots at Olathe and at sites with distinct soils are warranted.

In our trial, lettuce phenolic contents increased during storage relative to content at harvest, probably indicating an active phenolic metabolism during storage. Elevated levels of total phenolics in 'Kalura' and 'Red Sails' after storage were consistent with results reported for iceberg lettuce by Zhang et al. (2000) in which increases in phenolic content and phenylalanine ammonia-lyase (PAL) activity were observed in the folded middle leaves of iceberg lettuce during 16-d storage at 4 °C. We did not measure changes in individual flavonoids during storage, but loss of flavonol glycosides was reported in lettuce stored at 1 °C for 7 d (DuPont et al., 2000), so significant changes in relative concentration of individual phenolics may occur during storage. Wounding has been shown to elevate phenolic levels in midribs of minimally processed lettuce throughout cold storage (Cantos et al., 2001; Castañer et al., 1999; Ferreres et al., 1997). Despite the health implication of phenolic compounds, their role as substrates for enzymatic browning and the resultant effect on sensory attributes of fresh produce should be recognized (Tomás-Barberán and Espín, 2001). Taste and flavor quality of lettuce is best at harvest and declines during storage. Stem browning (butt discoloration) of harvested head lettuce is a concern of quality loss during postharvest storage (Tomás-Barberán et al., 1997). Because PAL activity can increase in response to pathogen infection, accumulation of phenolics as a result of microbial infection was also possible in our study owing to reduced visual quality of lettuce by the end of storage. Our finding of similar levels of total phenolics in organically and conventionally grown lettuce after storage was in agreement with a cold storage study of Tarozzi et al. (2004) in which total phenolic concentrations either decreased in apples with skin or remained stable in apples without skin regardless of production method. Further studies may help to clarify the relationship of freshness and nutritional quality of lettuce during postharvest storage.

In conclusion, the profound cultivar effect needs to be considered for manipulation of lettuce phenolics for health benefits. As a result of reduced *PAR* light and ultraviolet radiation, high tunnel environments might decrease lettuce phenolic content as compared with the open field, but the magnitude may vary with cultivar and season. The role of organic fertilization on lettuce phenolics warrants further investigation. Different types and amounts of organic inputs should be investigated in longer-term studies with multiple seasons and locations. If effects of cultural practices are evident, those preharvest factors deserve further study during postharvest storage of lettuce.

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