Microbial Quality and Bioactive Constituents of Sweet Peppers from Sustainable Production Systems

ALICIA MARÍN,[†] MARÍA I. GIL,*,[†] PILAR FLORES,[‡] PILAR HELLÍN,[‡] AND MARÍA V. SELMA[†]

Research Group on Quality, Safety and Bioactivity of Plant Foods, Food Science and Technology Department, CEBAS-CSIC, and Quality and Food Guarantee Department, IMIDA

Integrated, organic, and soil-less production systems are the principal production practices that have emerged to encourage more sustainable agricultural practices and safer edible plants, reducing inputs of plaguicides, pesticides, and fertilizers. Sweet peppers grown commercially under integrated, organic, and soil-less production systems were compared to study the influence of these sustainable production systems on the microbial quality and bioactive constituents (vitamin C, individual and total carotenoids, hydroxycinnamic acids, and flavonoids). The antioxidant composition of peppers was analyzed at green and red maturity stages and at three harvest times (initial, middle, and late season). Irrigation water, manure, and soil were shown to be potential transmission sources of pathogens to the produce. Coliform counts of soil-less peppers were up to 2.9 log units lower than those of organic and integrated peppers. Soil-less green and red peppers showed maximum vitamin C contents of 52 and 80 mg $100\ g^{-1}$ fresh weight (fw), respectively, similar to those grown in the organic production system. Moreover, the highest content of total carotenoids was found in the soil-less red peppers, which reached a maximum of 148 mg $100\ g^{-1}$ fw, while slightly lower contents were found in integrated and organic red peppers. Hydroxycinnamic acids and flavonoids represented 15 and 85% of the total phenolic content, respectively. Total phenolic content, which ranged from 1.2 to 4.1 mg $100\ g^{-1}$ fw, was significantly affected by the harvest time but not by the production system assayed. Soil-less peppers showed similar or even higher concentrations of bioactive compounds (vitamin C, provitamin A, total carotenoid, hydroxycinnamic acids, and flavonoids) than peppers grown under organic and integrated practices. Therefore, in the commercial conditions studied, soil-less culture was a more suitable alternative than organic or integrated practices, because it improved the microbial safety of sweet peppers without detrimental effects on the bioactive compound content.

KEYWORDS: Antioxidant compounds; ascorbic acid; *Capsicum annuum* L.; carotenoids; flavonoids; soil-less; integrated; organic; phenolic compounds; provitamin A

INTRODUCTION

Fresh fruits and vegetables have become an essential part of the human diet because consumers perceive them as being healthy, tasty, and fresh. Sweet peppers have grown in popularity due to their health-promoting properties. These beneficial effects have been related to a high content of antioxidants and vitamins. Thus, fresh pepper is one of the vegetables that has a higher content of vitamin C (*1*) and provitamin A carotenoids (*2, 3*). The main carotenoid with provitamin A activity is β-carotene, although α-carotene and β-cryptoxanthin also confer provitamin A activity but to a lesser extent (*4*). Results of a previous study

have shown the beneficial effect of β-carotene obtained from natural sources in opposition to the high-dose supplements of this compound (*5*). These results support the importance of consumption of fruits and vegetables rich in carotenoids, such as fresh pepper, to obtain vitamin A for human needs. Fresh sweet peppers also have a significant level of flavonoids and phenolic acids (*3*). These phytochemicals as well as vitamins A, E, and C are responsible for the antioxidant activity, which has been strongly correlated to the prevention of cardiovascular disease, cancer, diabetes, and age-related disorders (*6−8*).

Some studies have evaluated the effect of crop production practices, for example, plant nutrition and water supply, on nutrients and antioxidant activity of pepper (*9, 10*). The conventional agriculture pepper farms utilize high-yield crop cultivars, chemical fertilizers, and pesticides. Crop science has searched for alternative production systems, and three principal

* To whom correspondence should be addressed. Tel: +34 968 396 315. Fax: +34 968 396 213. E-mail: migil@cebas.csic.es.
† CEBAS-CSIC.
‡ IMIDA.

areas have emerged as follows: integrated, organic, and soil-less cropping systems. In the integrated production system, the use of pesticides and chemicals is limited, and there is a rational application of biological, biotechnological, chemical treatments, culture practices, and plant selection. Organic production is a system of farming that promotes and enhances biodiversity, biological cycles, and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain, and enhance ecological harmony. The use of organic amendments or organic fertilizers, which are not produced by chemical synthesis, is one of the characteristics of organic farming practices (*11*). All of these products or organic materials have to be registered as ecological products. The main disadvantage of integrated and organic production systems is the lower crop yield. Furthermore, there is a potential risk of human pathogen contamination by organic and integrated culture practices when manure is not composted properly (*12*). Hydroponic or soil-less production systems could be more competitive alternatives than organic systems for pepper cultivation. Soil-less, in contrast to organic and integrated productions, allows crop culturing without soil fumigation and amendment by providing a system where the plant nutrient needs are met by mixing water-soluble nutrients with water. Very few studies have been performed to evaluate the microbial safety and bioactive constituents of soil-less vs organic peppers. The purpose of this study was to determine the influence of organic, integrated, and soil-less practices on the microbial quality and bioactive constituents such as vitamin C (ascorbic acid plus dehydroascorbic acid), individual and total carotenoids, hydroxycinnamic acids, and flavonoids of commercial sweet peppers at green and red maturity stages and at three harvest times (initial, middle, and late season).

MATERIALS AND METHODS

Plant Material and Cultivation. Organic, integrated, and soil-less commercial farms were selected as representatives of the agricultural practices most commonly used in Spain. Sweet peppers (*Capsicum annuum* L. cv. Quito) were cultivated in commercial plastic greenhouses located in similar geographical and climatological areas of the southeast of Spain (Murcia; 38°1′N, 1°3′W). Eight greenhouses of each type of culture system (organic, integrated, and soil-less) were selected, as they used similar heat and management systems. Planting was carried out in November. For organic and integrated culture systems, ovine manure as an amendment was applied to soil before transplanting. Organic and integrated farms were managed attending the European Regulation EEC No. 2092/91 for organic production. For soil-less culture, coconut fiber as a substrate and a basic nutrient solution consisting of a modified Hoagland's solution were used. Thirty liter coconut bags with a density of 2.5 plants per m^2 were used. The macronutrient composition (mM) of the nutrient solution (EC 2.6 dS m^{-1}) used for irrigation was as follows: NO_3^-, 14.0; $H_2PO_4^-$, 1.5; SO_4^{2-}, 2.0; Ca^{2+}, 4.7; K^+, 4.8; and Mg^{2+}, 2.0. The micronutrient concentration (mM) was as follows: Fe, 1.80; Mn, 0.70; Zn, 0.12; B, 0.15; Cu, 0.07; and Mo, 0.05. During the summer months before transplanting, biofumigation and solarization were performed in one of the organic cultures. Biofumigation involved the uniform application of fresh horse manure at the rate of 4 kg m^2, mixing the organic matter with the top soil layer (10 cm). The soil was then covered with a plastic film, and the plastic greenhouse was closed from July to September (soil solarization). The microbial quality of peppers grown under biofumigated and nonbiofumigated organic practices was compared.

Pepper Sampling. Each greenhouse was visited during the spring of two consecutive years. For microbial analyses, three irrigation water samples, 10 soil samples, and 10 green pepper samples were collected randomly from different locations in each greenhouse at the end of the harvest period. Samples were transported in an ice chest with ice gel units to the laboratory, stored at 5 °C overnight, and processed the following day. For the antioxidant content analyses, peppers were harvested at two maturity stages named green (fully developed fruit just before the onset of maturation) and red ripe (completely red skin), respectively. During the initial (first harvest), middle, and late (last harvest) harvest periods, eight uniform fruits of each maturity stage (green and red) were selected per replicate and greenhouse. Three replicates were analyzed for each maturity stage and harvest time. Immediately after harvesting, fruits were transported to the laboratory (75 km) where peppers free of defects were selected. Peppers were hand cut into small pieces and separated into different batches depending on the analysis (vitamin C, carotenoids, and phenolic compounds). Samples were frozen and stored at −80 °C until analyses. Pepper peel was separated, freeze-dried, and extracted obtaining a more concentrated extract for phenolic compound identification.

Microbial Analysis. Twenty-five grams of soil amendment, soil, and pepper samples was homogenized in a 1:10 dilution of sterile 0.1% buffered peptone water (BPW, AES Laboratoire, Combourg, France) using sterile filter stomacher bags (Seward Limited, London, United Kingdom) and a stomacher (IUL Instrument, Barcelona, Spain) for 90 s and plated on appropriated media. Microbial quality of irrigation water samples was analyzed by filtering 100 mL of water through sterile 0.45 μm pore-size membrane filters (QA Life Sciences, Santiago, CA) and placing the filters on plates with appropriate media. Total aerobic mesophilic bacteria were enumerated by using plate count agar (PCA; Scharlau Chemie S.A., Barcelona, Spain) after incubation at 30 °C for 48 h. Total and fecal coliforms were isolated by using Chromocult agar (Oxoid, Basingstoke, Hampshire, United Kingdom) after incubation for 24 h at 37 °C. *Clostridium* spp. counts were carried out using sulfite-polymyxin-sulfadiazin-A (SPSS) agar (Scharlau Chemie S.A.) incubated at 37 °C for 24−36 h in anaerobic jars with an atmosphere generation system (Oxoid, Basingstoke). Suspected *Clostridium botulinum* and *Clostridium perfringens* colonies were confirmed using API 20 A strips (bioMérieux, Marcy L'Etoile, France). Colonies of *Listeria* spp. were enumerated on plates of *Listeria* selective agar (Oxford formulation, Oxoid, Basingstoke) incubated for 48 h at 37 °C. Samples were analyzed in duplicate, and microbial counts were expressed as log CFU g^{-1} or mL^{-1}.

Vitamin C Analysis. Ten grams of fresh peppers was added to 10 mL of extraction medium (0.1 M citric acid, 0.05% w/v ethylenediaminetetraacetic acid disodium salt, 5% v/v methanol, and 4 mM NaF). The mixture was homogenized for 30 s, filtered, and centrifuged at 10500*g* for 5 min at 2−5 °C. The filtrate was flushed through an activated Sep-Pak C-18 cartridge (Waters, Milford, MA) and filtered through a 0.45 μm filter. Before the analysis of vitamin C by high-performance liquid chromatography (HPLC), dehydroascorbic acid was derivatized as described by Zapata and Dufour (*13*). The HPLC analysis was based on the method previously described (*3*). The content of vitamin C was calculated by adding ascorbic acid (AA) and dehydroascorbic acid (DHA) contents and results expressed as mg per 100 g fresh weight (fw). Standards of L-ascorbate, supplied by Sigma Chemical Co., and dehydroascorbate, from Aldrich Chemical Co., were used.

Carotenoid Analysis. Extraction and saponification protocols used were described by Minguez-Mosquera and Hornero-Méndez (*14*). HPLC analysis was based on the method of Marín et al. (*3*). β-Apo-8′-carotenal was used as internal standard. *trans*-β-Carotene and β-apo-8′-carotenal were purchased from Sigma Chemical Co. (St. Louis, MO). Neoxanthin, violaxanthin, and lutein were obtained from a saponified extract of mint (*Mentha piperita*) by thin-layer chromatography (TLC) (*15*). Standards of capsanthin and capsorubin were isolated from a saponified extract of red pepper (*14*). Once the pigments were purified, the concentration of each one was determined spectrophotometrically using the corresponding values of ε_0 (*16*). The results were expressed as mg per 100 g fw. Provitamin A was calculated on the basis of β-carotene and β-cryptoxanthin as (166.7 × mg of β-carotene) + (83.3 × mg of β-cryptoxanthin) per 100 g fw. The results were expressed as IU of provitamin A per 100 g fw.

Phenolic Compound Analysis. Freeze-dried sample (1 g) was homogeneized with 10 mL of methanol−water (7:3 v/v) using an Ultra-Turrax homogenizer. The extract was centrifuged (10500*g*) for 5 min, and the supernatant was filtered through 0.45 μm membrane filter
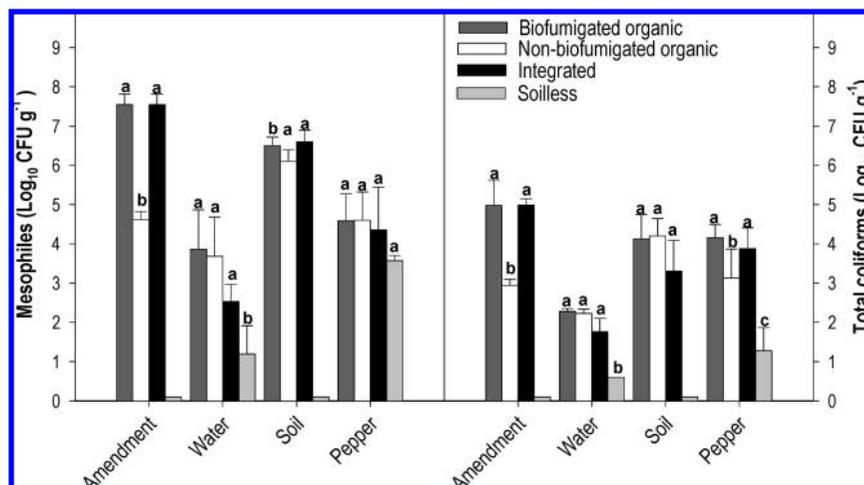
Figure 1. Total mesophilic bacteria and total coliforms in soil amendment, irrigation water, soil, and pepper samples in the biofumigated organic, nonbiofumigated organic, integrated, and soil-less culture systems. Error bars represent the standard deviation. Bars with different letters for the same samples are significantly different using Tukey's multiple range test (*P* = 0.05).

(Millex-HV 13MM, Millipore, Bedford, MA) and directly analyzed by HPLC. The results were expressed as mg per 100 g fw. Identification and quantification of phenolic compounds were carried out by means of their UV spectra, molecular weight, and their MS-MS fragments as previous described by Marín et al. (*3*). Whenever possible, the compounds were identified by chromatographic comparisons with markers previously isolated and identified in our research group. The measurement conditions were the same as those used in the analytical HPLC-diode array detector (DAD) explained above but using water with 0.1% formic acid (v/v) as the mobile phase. The HPLC system equipped with a DAD detector and mass detector in series consisted of a G1312A binary pump, a G1313A autosampler, a G1322A degasser, a G1315B photodiode array detector, and an ion trap mass spectrometer equipped with electrospray ionization and was operated in the negative ion mode controlled by software (v. 4.0.25) from Agilent Technologies (Waldbronn, Germany).

Statistical Analysis. Analysis of variance (ANOVA), followed by Tukey's test with a significance level of $P \leq 0.05$, was carried out on these data using SPSS (Windows 2000, Statistical Analysis).

RESULTS AND DISCUSSION

Influence of Pepper Production Systems on Microbial Quality. Mesophilic microorganisms were evaluated as an estimation of total viable populations. Moreover, total and fecal coliforms were tested to evaluate the hygiene level in the preplant amendment, water, soil, and pepper samples. The total mesophilic bacteria and coliform counts of preplant amendment samples from the different production systems were quite variable (**Figure 1**). Preplant amendment used on either the biofumigated organic or the integrated cultures reached total mesophilic bacteria and coliform counts of 7.5 and 5.0 log CFU g^{-1}, respectively (**Figure 1**). In contrast, preplant amendment used on the nonbiofumigated organic culture contained much lower total mesophilic bacteria and coliform counts (4.6 and 2.9 log CFU g^{-1}, respectively) than that used in biofumigated production systems (**Figure 1**). The total and fecal coliform counts showed that preplant amendment had poor general hygiene, especially those used in biofumigated organic and integrated pepper cultures analyzed.

The irrigation water samples of the tested farms showed nonsignificant differences on the mesophilic and total coliform counts except for the soil-less system where count levels were up to 1.7 log units lower than the others (**Figure 1**). Despite the differences in microbial quality of preplant amendments, the soil of organic and integrated production systems showed

similar counts of mesophilic bacteria and coliforms (**Figure 1**). Results of the soil samples suggested that the coliform levels found in irrigation water could counterbalance the microbial loads present in the amendment samples. However, in the pepper fruits, the total coliforms were significantly different among culture systems (**Figure 1**). In fact, coliform counts of pepper samples from the soil-less culture were up to 2.9 log units lower than those from the other cultures (**Figure 1**). In addition, pepper samples from the nonbiofumigated organic culture contained coliform counts up to 1.1 log units lower than those from the biofumigated culture (**Figure 1**). Taking into account that amendments, irrigation water and soil are the main contamination sources of crops (*17, 18*), the lower coliform counts found in soil-less peppers were the consequence of a reduction in the microbial levels of the risk sources, decreasing the microbial risk of soil-less peppers. In contrast, pepper samples from the different culture systems showed no significant differences on the total mesophilic bacteria counts (**Figure 1**). Therefore, this microbial group was not a good indicator for the evaluation of the microbial quality derived from different agricultural practices. Comparable conclusions were shown in a previous study where the total microbial load of potatoes was similar, independently of the microbial counts obtained from soil amendments and irrigation water (*19*).

The enumeration of fecal coliforms from irrigation water, soil, and pepper samples was, in most of the cases, lower than the detection limit (<1 log CFU g^{-1} or mL^{-1}). Only irrigation water samples from nonbiofumigated organic system and soil samples from biofumigated organic crop were fecal coliform positive having counts of 2.2 and 1.4 log CFU per mL or g, respectively. This indicated that although biofumigation and soil solarization used in the tested biofumigated organic farms can be useful for eliminating plant pathogens and minimize pests, it can contaminate the soil with fecal coliforms that can be potential human pathogens. This could be avoided using, for biofumigation, organic matter of nonanimal origin. In addition, preplant amendments contained fecal coliform levels of 2.6 log CFU g^{-1}. The survival of this bacterial group in soil amendments could result in an added risk factor for the potential contamination of the fruits. For this reason, manure-processing methods are an active area of research to minimize the level of pathogens (*17*).

Listeria spp., a soil resident associated with decomposed organic matter (*20, 21*), was present in all preplant amendment

Table 1. Listeria spp. and *C. botulinum* Counts of Green Sweet Peppers Cultivated Under Biofumigated Organic, Nonbiofumigated Organic, Integrated, and Soil-less Production Systems[a]

samples	microorganism	biofumigated organic	nonbiofumigated organic	integrated	soil-less
amendment	*Listeria* spp.	2.6 (0.1)	2.7 (0.1)	3.9 (0.1)	NA
	C. botulinum	<1	<1	<1	NA
irrigation water	*Listeria* spp.	<1	<1	<1	<1
	C. botulinum	<1	<1	<1	<1
soil	*Listeria* spp.	4.9 (0.5)	4.5 (0.7)	4.3 (0.3)	NA
	C. botulinum	<1	<1	<1	NA
pepper	*Listeria* spp.	<1	<1	<1	<1
	C. botulinum	<1	<1	<1	<1

[a] Means are expressed as log CFU g^{-1} or mL^{-1}, and standard deviations are in parentheses. NA, not analyzed.

samples in levels of 2.6–3.9 log CFU g^{-1} (**Table 1**). A good correspondence between the positive detection of *Listeria* in the preplant amendment and in the soil was observed for all of the soil samples from the different cultivation systems. However, soil samples contained higher levels of *Listeria* than the preplant amendment, which ranged from 4.3 to 4.9 log CFU g^{-1}. This suggested that factors other than amendments could affect the soil contamination. In irrigation water and pepper samples, *Listeria* spp. was lower than the detection limit (<1 log CFU g^{-1} or mL^{-1}). *C. botulinum* was not detected in any of the preplant amendment, water, soil, and pepper samples (**Table 1**). Therefore, *C. botulinum* was not a risk factor of the studied culture systems despite being one of the greatest concerns in relation to fresh produce (*22*).

Influence of Pepper Production Systems on Vitamin C Content. The vitamin C content of peppers grown under organic, integrated, and soil-less production systems is shown in **Figure 2**. Ascorbic acid was present in a higher content than dehydroascorbic acid in both green and red maturity stages, with a ratio of 94:6 and 97:3 in green and red peppers, respectively. The content of vitamin C in cv. Quito was in the same range as that shown in previous studies for sweet pepper cvs. Vergasa and Almuden (*3, 23*). In the present study, the vitamin C content was significantly affected by the agricultural production systems (*P* < 0.001). The highest content of vitamin C was observed in organic and soil-less cultivated peppers, while the lowest content was detected in integrated grown peppers for both maturity stages and along the harvest season (**Figure 2**). Organic red peppers showed the highest vitamin C content (93.7 mg 100 g^{-1} fw), whereas integrated green peppers had the lowest value (22.9 mg 100 g^{-1} fw). The highest content of vitamin C in organic peppers could be a protection response to biotic stress as plants synthesize it to facilitate resistance to oxidative reactions associated with biotic and abiotic stresses (*24, 25*). Green and red peppers grown in soil-less culture system contained a maximum vitamin C content of 51.7 and 80.64 mg 100 g^{-1} fw, respectively.

The effect of maturity stage (green and red) and the effect of harvest time on vitamin C content were also evaluated. In general, an increase in the ascorbic acid content was observed during maturation (*P* < 0.001) showing higher vitamin C content in red peppers than in green ones (**Figure 2**). This result is in agreement with other previous studies (*3, 25, 26*). The content of vitamin C was also affected by harvest time (*P* < 0.001) (**Figure 2**). In green peppers, the vitamin C content was reduced from the first harvest to the last one, in either organic or soil-less cultivated fruits (**Figure 2A**). In contrast, in red peppers, the vitamin C content increased from the first harvest to the following ones either in organic or soil-less fruits (**Figure 2B**). The amount and intensity of light could explain the increase in vitamin C content of red peppers from the last harvest as compared to the first one. These results are in agreement with previous studies that reported the light intensity and doses during the growing season have a definite influence on ascorbic acid synthesis (*25, 26*). Only in the integrated production system did the vitamin C content remain stable during the harvest season (**Figure 2A,B**). Therefore, other preharvest factors different from light intensity could have influenced on ascorbic acid synthesis (*25*).

Influence of Pepper Production Systems on Carotenoid Content. Lutein was the major carotenoid detected in the green stage of cv. Quito although in other previous study of peppers cv. Vergasa under conventional practices, the predominant carotenoid in the green state was β-carotene (*3*). Xanthophylls such as capsorubin, capsanthin, antheraxanthin, mutatoxanthin, cucurbitaxanthin, and zeaxanthin were only present when peppers reached the red stage (**Table 2**), and the predominant carotenoid was capsanthin, which was significantly higher in soil-less cultivated peppers as compared to organic and integrated ones (**Table 2**). Levels of other carotenoid pigments were also significantly influenced (*P* < 0.001) by the production system (**Table 2**). In fact, the content of most individual pigments, including β-carotene, capsorubin violaxanthin, and zeaxanthin, was higher in soil-
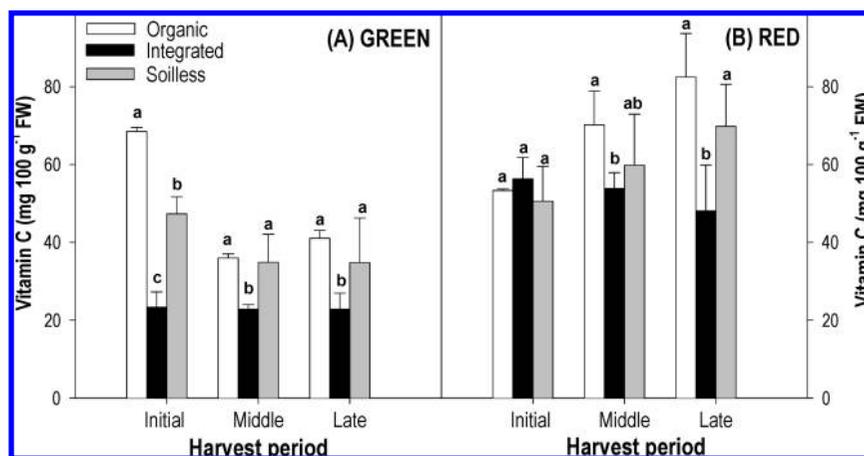
Figure 2. Vitamin C content of green and red peppers cultivated under organic, integrated, and soil-less production systems at three harvest periods (initial, middle, and late). Bars with different letters for the same harvest period are significantly different using Tukey's multiple range test (*P* = 0.05).

Table 2. Carotenoid Pigment Composition of Green and Red Sweet Peppers Cv. Quito Cultivated under Organic, Integrated, and Soil-less Production Systems[a]

carotenoid pigments	green			red		
	organic	integrated	soil-less	organic	integrated	soil-less
neoxanthin	1.8 a	1.9 a	1.3 b			
lutein	6.5 a	6.0 a	4.1 b			
violaxanthin	1.1 a	1.2 a	0.8 b	4.8 b	4.0 b	7.1 a
β-cryptoxanthin				2.6 a	3.1 a	3.4 a
β-carotene	1.5 a	2.2 a	1.6 a	3.6 b	6.7 a	8.0 a
capsorubin				3.8 b	4.8 b	7.6 a
capsanthin 5,6-epoxide				4.4 a	3.3 b	4.9 a
capsanthin				49.2 b	51.3 b	61.8 a
antheraxanthin				4.4 a	2.9 b	4.6 a
cis-capsanthin				3.8 b	3.7 b	4.7 a
mutatoxanthin				4.9 a	3.6 b	5.6 a
cucurbitaxanthin A				8.1 a	6.7 b	8.8 a
zeaxanthin				3.4 b	5.9 a	6.7 a
total carotenoids	10.9 a	11.3 a	7.8 b	93.3 b	96.0 ab	123.2 a

[a] Means are in mg 100 g^{-1} fw. For the same maturity stage, rows with different letters are significantly different using Tukey's multiple range test ($P = 0.05$).

less peppers rather than in organic ones (**Table 2**). Total carotenoid content of peppers, expressed as the sum of the individual carotenoids, is shown in **Figure 3**. The carotenoid content increased with maturation in accordance with previous studies (*3, 27*). Red peppers had 6–12 times higher content of carotenoids than green fruits (**Figure 3A,B**). In green peppers, the content of total carotenoids was not affected by the agricultural practices at any harvest time (**Figure 3A**). However, in the red stage, the carotenoid content was significantly influenced by the culture system

assayed ($P < 0.001$) at the initial and middle harvest season. The highest content in total carotenoids was found in red peppers cultivated under soil-less conditions, which reached a maximum of 148 mg 100 g^{-1} fw (**Figure 3B**). A slightly lower content was found in red peppers grown in integrated and organic culture systems, which reached maximum concentrations of carotenoids of 116.4 and 96.4 mg 100 g^{-1} fw, respectively. A previous study showed that as response to biotic stress, the cytosolic isoprenoid pathways are channeled toward the synthesis of the capsidiol phytoalexin at the expense of the carotenogenic pathways (*28*). Therefore, the lower carotenoid content in organic and integrated red peppers could be the consequence of their higher exposure to biotic stress as compared to soil-less pepper plants, which can trigger the phytoalexin synthesis. In addition, carotenoid content was lower than that reported in a previous study where organic and integrated red pepper cv. Almuden contained 323 and 249 mg 100 g^{-1} fw, respectively (*29*). This confirms that carotenoid content can be influenced by factors such as genetics, irrigation, and salinity (*4, 30*). The total carotenoid content was slightly reduced in the course of the harvest season. As a result, in the last harvest, red peppers cultivated under organic, integrated, and soil-less culture systems had similar carotenoid contents (70.9, 85.2, and 83.0 mg per 100 g fw, respectively).

As was expected, similar results to carotenoid content in green and red peppers were obtained from the provitamin A analysis (**Figure 3**). The mean value of provitamin A content in green peppers was 278 IU 100 g^{-1} fw without significant differences depending on agricultural practices (organic, integrated, and soil-less culture systems) along the harvest season (**Figure 3C**). In contrast, provitamin A accumulation in red pepper was affected
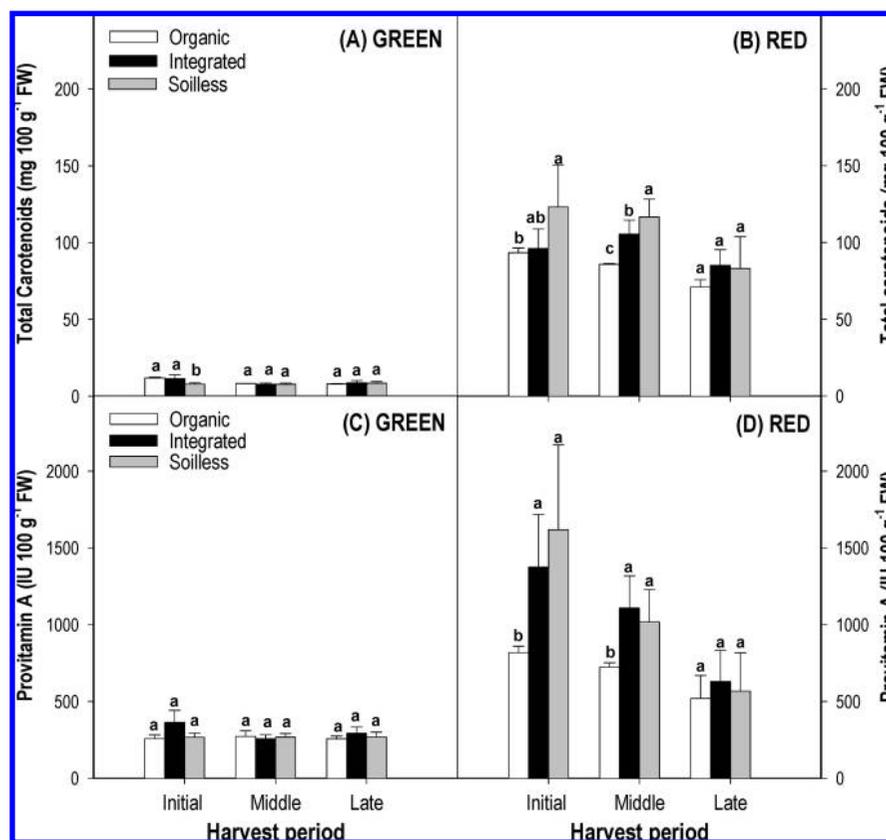
Figure 3. Total carotenoids and provitamin A of green and red peppers cultivated under organic, integrated, and soil-less production systems at three harvest periods (initial, middle, and late). Bars with different letters for the same harvest period are significantly different using Tukey's multiple range test ($P = 0.05$).

Soil-less, Organic, and Integrated Pepper Production Systems

J. Agric. Food Chem., Vol. 56, No. 23, 2008   **11339**

Table 3. Phenolic Composition of Green and Red Sweet Peppers Cv. Quito Cultivated under Organic, Integrated, and Soil-less Production Systems[a]

phenolic compounds	green			red		
	organic	integrated	soil-less	organic	integrated	soil-less
total hydroxicinnamic acids	0.29 a	0.29 a	0.21 b	0.19 b	0.26 a	0.24 ab
flavonoids						
1	0.91 b	1.24 a	0.83 b	0.46 a	0.48 a	0.81 a
2	0.22 b	0.41 a	0.26 b	0.21 a	0.22 a	0.32 a
3	0.62 a	0.59 a	0.61 a	0.30 a	0.37 a	0.31 ab
other flavonoids	0.18 b	0.12 b	0.27 a	0.28 b	0.44 a	0.19 c
total flavonoids	1.93 ns	2.12 ns	1.97 ns a	1.25 ns	1.51 ns	1.63 ns
total phenolic compounds	2.22 ns	2.41 ns	2.18 ns	1.44 ns	1.77 ns	1.87 ns

[a] Means are in mg 100 g^{-1} fw. For the same maturity stage, rows with different letters are significantly different using Tukey's multiple range test ($P = 0.05$); ns, not significantly different. Key: **1**, *m/z* 447 quercetin 3-*O*-rhamnoside; **2**, *m/z* 783 luteolin-7-(2-apiosyl-4-glucosyl-6-acetyl)glucoside; and **3**, *m/z* 621 luteolin-7-(2-apiosyl-6-acetyl)glucoside.

by the agricultural practices ($P < 0.001$), harvest time ($P < 0.001$), and also by the combination of both ($P < 0.01$). In the first harvest, integrated and soil-less red peppers contained a provitamin A mean value of 1652 IU 100 g^{-1} fw, significantly higher than that shown in organic peppers, which contained 818 IU 100 g^{-1} fw (**Figure 3D**). As was shown in carotenoid analyses of red fruits, provitamin A content was reduced during the harvest season, and this reduction was more marked in integrated and soil-less fruit than in organic one. Consequently, fruits collected at the end of the season had a similar provitamin A content without significant differences derived from the culture systems (**Figure 3D**).

Influence of Pepper Production Systems on Phenolic Content. The polyphenol profile of the pepper extracts consisted of a combination of hydroxycinnamic acids and flavonoids. Three main compounds were tentatively identified as quercentin-3-*O*-rhamnoside (*m/z* 447), luteolin-7-(2-apiosyl-4-glucosyl-6-acetyl) glucoside (*m/z* 783), and luteolin-7-(2-apiosyl-6-acetyl)glucoside (*m/z* 621). In trace concentrations, other minor flavonoid compounds were shown in the MS fragmentation, which corresponded to *C*-glycosides and *O*-glycosides as described in a previous study conducted in our laboratory (*3*). The content of individual and total phenolic compounds in green and red peppers cultivated in organic, integrated, and soil-less systems was very similar, and only slight variations were observed among the different production systems. **Table 3** shows the phenolic composition of green and red peppers corresponding to the mean values of the three harvest times. Hydroxycinnamic acids and flavonoids represented 15 and 85%, respectively, of the total phenolic content. Green and red peppers contained 0.26 and 0.23 mg of hydroxycinnamic acids per 100 g fw, respectively. These concentrations were in the same range than those found in cv. Vergasa (*3*). As occurred with total hydroxycinnamic acids, the content of other individual phenolic compounds was slightly affected by the agricultural practices, and similar concentrations were found during the harvest season (**Table 3**). Total phenolic content, which ranged from 1.45 to 2.41 mg 100 g^{-1} fw, was not affected by the culture system assayed in both green and red maturity stages (**Table 3**).

When evaluating the content of hydroxycinnamic acids in organic, integrated, and soil-less cultivated peppers along the season in both green and red stages, a very similar content was observed, although slight differences were observed at a particular harvest time. In the initial harvest, the hydroxycin-
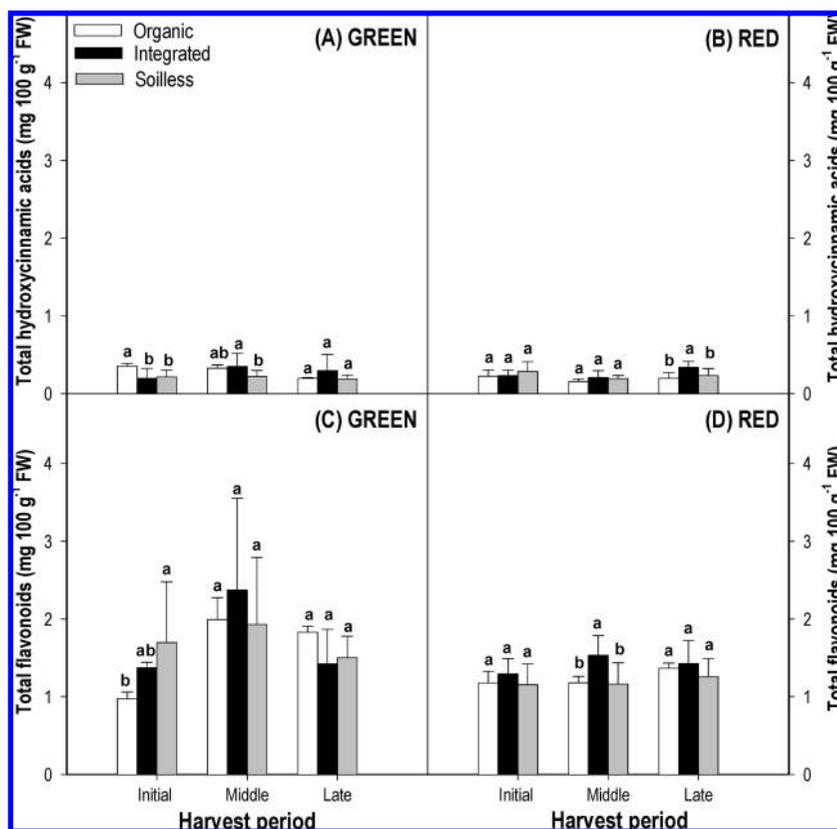
Figure 4. Total hydroxycinnamic acids and total flavonoids of green and red peppers cultivated under organic, integrated, and soil-less production systems at three harvest periods (initial, middle, and late). Bars with different letters for the same harvest period are significantly different using Tukey's multiple range test ($P = 0.05$).

namic acid content of organic green peppers was 1.7 times higher than that detected for integrated and soil-less peppers (**Figure 4A**), while in the last harvest, integrated peppers showed a higher content as compared with the other production systems (**Figure 4B**). The content of total flavonoids was generally 6.8 and 5.8 times higher than hydroxycinnamic acids in green and red peppers, respectively, and almost no differences were observed among peppers from the different production systems (**Figure 4C,D**). The mean contents of the flavonoids quercentin-3-*O*-rhamnoside, luteolin-7-(2-apiosyl-4-glucosyl-6-acetyl) glucoside, and luteolin-7-(2-apiosyl-6-acetyl) glucoside were 1.0, 0.3, and 0.6 mg 100 g^{-1} fw, respectively. These concentrations were in the same range than those previously described for cv. Vergasa (*3*).

From this study in greenhouse conditions, we conclude that although peppers are aerial fruits, irrigation water, manure, and soil used in crops are potential transmission sources of human pathogen. Therefore, cultures should be tested regularly to ensure that amendments, irrigation water, soil, and produce are of acceptable quality. Soil-less culture was a safer alternative than those with soil (biofumigated organic, nonbiofumigated organic, and integrated cultures in the employed conditions) as the absence of organic soil amendments contributes to a reduction in the numbers of potential human pathogens and an improvement of the general hygiene of peppers. Moreover, a soil-less system allows an absence of competing weeds, soil-borne pests, and toxic residues. Finally, soil-less peppers have shown similar or even higher concentrations of antioxidant compounds (vitamin C, provitamin A, total carotenoid, hydroxycinnamic acids, and flavonoids) than organic peppers. Therefore, soil-less culture was shown to be a more suitable alternative than organic and integrated systems for improving the microbial safety of sweet peppers without detrimental effects on their nutritional content.

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Soil-less, Organic, and Integrated Pepper Production Systems

J. Agric. Food Chem., Vol. 56, No. 23, 2008   **11341**

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