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Full Length Research Paper

Ascorbic acid, β-carotene, total phenolic compound and microbiological quality of organic and conventional citrus and strawberry grown in Egypt

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A two year study at Alexandria University compared ascorbic acid, β-carotene, total phenolic compound, nitrite content and microbiological quality of orange and strawberry fruits grown under organic and conventional management techniques to see if producers concerns are valid. Organically grown oranges and strawberries had lower titratable acidity (TA); whereas, there was no significant difference in total soluble solid (TSS) of oranges and strawberries between two production systems. Higher ascorbic acid and β-carotene content was found in organically grown oranges and strawberries, compared to conventionally grown ones. Total phenol content (TPC) was significantly higher in conventional oranges compared to its organic production. Conversely, TPC was significantly higher in organic strawberries than the conventional ones. Comparative analyses of the microbial counts of organic and conventional oranges and strawberries fruit showed that Escherichia coli (E. coli) were not detected in any sample. However, conventional oranges and strawberries fruits have greater counts of yeasts and mold than organic ones. Nitrites were detected in all samples. The nitrites levels from organic production varied between 0.13 and 0.16 mg/kg fresh weight (FW), whereas those from conventional production ranged from 0.20 to 0.25 mg/kg FW. Our results show that the ascorbic acid, βcarotene, TPC, TA, nitrite content and biological quality were dependent on the agricultural production system, while for TSS%, this dependency was not pronounced.

Key words: Ascorbic, β -carotene, organic, orange, strawberry, yeasts and molds.

INTRODUCTION

Organic food is tasty, healthy and safe. Sales figures and popularity of organic food have been continuously rising for years. The Egyptian organic agriculture movement was started in the 1990s by SEKEM, a non-governmental organization which applied organic techniques. In Egypt, more than 95% of organic products are exported (IFOAM and FiBL, 2006). Egypt has one of the biggest organic sectors of all African nations and likewise one of the

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Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution License 4.0</u> International License biggest domestic markets, though it is still on the whole quite small. Egypt grows a total of 24, 548 ha in organic agriculture, 0.72% of its total agricultural land. Main organic crops include fresh vegetables, tropical fruits, dried fruits, cotton, herbs and spices, medicinal plants and cereals (IFOAM and FiBL 2006). A private investor is currently seeking to certify over thirty million hectares of desert to be reclaimed for organic farming (Hashem, 2006).

Orange and strawberry are two from the most important fruits production, consumption and export in Egypt. Egypt is among the top four citrus producers in the Mediterranean Basin. Oranges are the main citrus fruit grown in Egypt, accounting for about 60% of total citrus production. Egypt is the sixth largest orange producer and the second biggest exporter (FAOSTAT, 2009). According to Food and Agriculture Organization (FAO) of the United Nations, in 2010, Egypt production of strawberries increased more than 3 times from 70,000 to 240,000 tons, and Egypt becomes the seventh largest strawberry producer in the world. Moreover, the identification of oranges and strawberries with high nutritive value represent a useful approach to select those fruits with better health-promoting properties.

Organic oranges and strawberries achieved higher prices than conventional ones, because these products are often linked to sew up the environment, better quality (taste, storage) and most people believe that they are healthier. Moreover, research results on the effects of organic and conventional production on guality sometimes are contradictory. In terms of quality, some studies report better taste, higher vitamin C contents and higher levels of other quality related compounds for organically grown products (Mitchell et al., 2007; Caris-Veyrat et al., 2004), whereas, several other studies have found the opposite or no differences in quality characteristics between organically and conventionally grown fruits and vegetables (Caris-Veyrat et al., 2004). In addition, based on the fact that organically grown fruit and vegetables often rely on manure as fertilizer, these products are perceived to pose a greater risk for foodborne disease (Salmonella spp., Listeria monocytogenes and Escherichia coli) than conventional crops (Johannessen et al., 2004). However, there have been very few scientist reports that have conducted microbiological analysis of organic fruit and vegetables.

People often buy organic fruits and vegetables because they consider organic fruits and vegetables to be more beneficial to both human health and environment, and with better flavor than conventional or integrated counterparts (Lester et al., 2007). Here we evaluated if there are significant differences in orange and strawberry fruit quality from commercial organic and conventional agroecosystems in Egypt. There is a scarcity of data on orange and strawberry fruit quality under organic farming system. The aim of the present study was to compare phytochemical characteristics, nitrite content and microbiological quality of Valencia orange and Festival strawberry produced under organic and conventional farming systems in Egypt in order to be able to address consumer's considerations.

MATERIALS AND METHODS

Sampling

A total of 100 orange and strawberry samples (50 organic and 50 conventional) were purchased in Carrefour hypermarket in the city of Alexandria, located in the south region of Egypt. The samples included: orange (Citrus Sinensis L., cv. Washington navel) and strawberry (Fragaria vesca L., var. Festival). Organic oranges and strawberries were produced from organic agriculture company located in Nobaria region. The organic fruits had a certificate issued by Demeter and/or Bio-Suisse certification. All samples were taken to laboratory in sterile plastic bags and they were processed immediately. Some samples were used for analysis of ascorbic acid, total soluble solid (TSS %) and titratable acidity on the same day and were therefore stored at room temperature. The other samples were stored in refrigerator at approximately 4°C until tested on the next day for β -carotene and total phenol content. Three replicates per treatment (each replicates contained 10 fruits of oranges and strawberries) were washed under running water and the non-edible parts (orange peel and leaves of strawberry) were removed. Oranges were hand squeezed and strawberries were cut and homogenized. Orange juice and strawberries homogenate were utilized for the ascorbic acid, TSS and acidity determination.

Determination of total soluble solids (TSS) and titratable acidity (TA)

A portion of fresh orange juice and a small fraction of strawberries homogenate were centrifugated at 4000 g for 5 min and the supernatant was analyzed for TSS and TA. The percentage of total soluble solids (TSS) was measured by a hand refractometer (ATC-1E, Atago, Japan), and acidity as tartaric acid (TA) was determined by titration with 0.1 N NaOH according to AOAC (1995).

Extraction and high performance liquid chromatography (HPLC) analysis of ascorbic acid and β -carotene

Ascorbic acid was extracted according to modified method described by Abdulnabi et al. (1997). Homogenized fresh sample (10 g) of strawberries and another sample of 5 ml of orange juice were extracted with a 5 ml solution of (0.3 M) meta-phosphoric acid and (1.4 M) acetic acid. The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract and then injected directly into Shimadzu HPLC (model CR4A, Japan).

β-carotene was extracted according to the slight modified method of Tee et al. (1996). A sample of strawberries (10 g) and another sample of 5 ml of orange juice were homogenized with 40 ml of 99.8% ethanol and 10 ml of 100% potassium hydroxide for 3 min by a blender. The mixture was heated for 30 min then cooled to room temperature. Exactly 50 ml of n-hexane was added to the texture and shaken strongly for a few seconds and the upper layer (hexan extract) allowed to separate and then removed. The aqueous layer was re-extracted twice with 50 ml of n-hexane in each time. The extract was filtered through anhydrous sodium sulphate to remove any water residue and exposure to reduced pressure at 45°C to

Farming system	Ascorbic acid (mg/100 g)	β-Carotene (IU/100 g)	Total phenolic content (mg/100 g)	TSS (%)	Acidity (%)
2012					
Conventional	55.57 ±0.57 ^z b	543.67 ±1.52b	165.28 ±0.34a	9.98 ±0.12a	1.40 ±0.05a
Organic	77.62 ±1.18a	582.77 ±1.45a	126.20 ±0.57b	10.00 ±0.15a	0.90 ±0.12b
2013					
Conventional	55.71 ±0.50 ^z b	542.89 ±2.04b	164.65 ±0.68a	10.40±0.12a	1.42±0.03a
Organic	78.43 ±1.12a	587.46 ±1.70a	121.29 ±2.10b	10.46±0.03a	0.90±0.12b

Table 1. Mean concentration of ascorbic acid, β -carotene, total phenolic content, TSS and acidity in orange fruits produced by organic and conventional farming in 2012 and 2013 seasons.

Means in a column followed by a different letter differ significantly at P = 0.05 by L.S.D test. ^zMeans ± SE.

Table 2. Mean concentration of ascorbic acid, β -carotene, total phenolic content, TSS and acidity in strawberry fruits produced by organic and conventional farming in 2012and 2013 seasons.

Farming system	Ascorbic acid (mg/100 g)	β-Carotene (IU/100 g)	Total phenolic content (mg/100 g)	TSS (%)	Acidity (%)
2012					
Conventional	31.12 ±0.32 ^z b	351.55 ±1.02b	7.65 ±0.18b	6.78 ±0.05a	0.39 ±0.02a
Organic	51.27 ±1.01a	407.21 ±0.29a	16.05 ±0.36a	6.80 ±0.05a	0.23 ±0.01b
2013					
Conventional	33.75 ± 0.61 ^z b	351.57 ±2.50b	8.24 ±0.18b	6.78 ±0.05a	0.38 ±0.02a
Organic	51.27 ±1.01a	413.50 ±2.59a	17.04 ±0.12a	6.80 ±0.05a	0.22 ±0.01b

Means in a column followed by a different letter differ significantly at P = 0.05 by L.S.D test. ^zMeans ± SE.

remove hexane residue then injected directly into a shimadzu HPLC.

HPLC analysis of ascorbic acid and β -carotene were performed on LC- 6A equipment consisting of LC- 6AD pumps, an in-line degasser, a CTO-6A column oven, a SCL-6A system controller, a SPD 6Avp, a photo diode array detector, a refractive index detector and operated by LC solution software (Shimadzu, Japan).

Determination of total phenol content

Homogenized fresh sample (0.5 g) of strawberries and another sample of 5 ml of orange juice were extracted with a 5 ml 75% (v/v) ethanol under periodical stirring at 45°C (Roussos et al., 2009). After centrifugation (4000 x g for 10 min), a quantity of 0.5 ml Folin-Denis reagent was added to 1 ml of the alcoholic extract and after 5 min, 7 ml saturated sodium carbonate solution was added, shaken and left for 0.5 h. Optical density was measured at 750 nm and total phenols were calculated from a standard curve of tannic acid. These data were expressed as the mg tannic acid equivalents per gram of fresh weight basis according to Slinkard and Singteton (1977).

Determination of nitrite

The nitrite contents in the oranges and strawberries were determined by a spectrophotometric method on foodstuff and water. The nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1- naphthyl)-ethylenediamine dihydrochloride to

form a highly colored azo dye that is measured at 540 nm (Merino, 2009). Three replicates were analyzed; nitrite levels were expressed as mg/kg fresh weight (FW).

Microbiological analysis

Fruit were kept in refrigerator for no longer than 24 h prior to analysis. Each fruit was transferred to an individual, sterile plastic bag using gloves with 30 ml of 0.1% buffered peptone water (BPW). The sterile bags were hand- rubbed for a minute to remove surface microorganisms (Parish et al., 2001). Number of colony (yeasts and molds) was counted by methods (Beuchat and Cousin, 2001; Morton, 2001). *E. coli* were counted by the fast Petrifilm TM method (Kornacki and Johnson, 2001), and the results were reported as colony forming units per gram (CFU/g).

RESULTS AND DISCUSSION

There was no significant difference in TSS content between organically grown oranges and strawberries (Tables 1 and 2). Compared with conventional production system, higher TSS content was reported in oranges, lemons and mandarin grown under organic production system (Duarte et al., 2010). Consistent with our results, no significant differences in TSS percentage were found for citrus and strawberries fruits between organic and conventional systems (Nunes et al., 2010; Camin et al., 2011 and Roussos, 2011).

Organically grown oranges and strawberries had lower TA than conventionally grown oranges and strawberries (Tables 1 and 2). This is in contrast to what was observed by Koneru (2013) who found that compared with conventional farming, higher TA content was reported in organically grown peaches. In agreement with our results, organic 'Washington Navel' oranges showed lower TA than organic ones (Candir et al., 2013). Based on previous reports, there is a slight difference on the organic acid concentration in the juice according to farming system. This could be attributed to the difference in fertilization system.

The data in Tables 1 and 2 clearly indicates that organically grown oranges and strawberries had higher ascorbic acid content than conventionally grown oranges and strawberries during both seasons. For example, in 2012 season, conventionally and organically grown oranges contained 55.57 and 77.62 mg/100 g, respectively. The corresponding values for strawberries were 31.12 and 51.27 mg/100 g, respectively. Many investigators also reported this increase in ascorbic acid content such as Duarte et al. (2010) on 'Valencia late' and 'Baia' oranges, Lester et al. (2007) on 'Rio Red' grapefruit, Asami et al. (2005) on Northwest Totem strawberry variety and (Jin et al., 2011) on Earliglow and Allstar strawberries. They concluded that a significantly higher ascorbic acid concentration for organically grown versus conventionally grown citrus and strawberries. A review in 2006 reported that organic foods had higher amounts of antioxidant (ascorbic acid) and lower levels of pesticide residues, nitrates and heavy metals contaminations than conventionally grown crops. Through that, organic crops had higher nutritional value and lower risk of causing disease due to contamination (Gyorene et al., 2006). Moreover, Duarte et al. (2012) demonstrated that the higher ascorbic acid content in citrus fruit juice from organic production system depend on species and cultivar.

According to previous studies, the possible interprettation for this finding is that nitrogen fertilizers under high rates seems to decrease the concentration of ascorbic acid content in fruits and vegetables (Lee and Kader, 2000) Besides Lee and Kader (2000) reported that the use of agrochemicals and pesticides may affect the nutritional quality of fruits and vegetables.

A greater β -carotene content in oranges and strawberries from organic compared to conventional farming systems was found (Tables 1 and 2). Roussos (2011) reported that organic management increased carotenoid concentration significantly compared to integrated farming system. The accumulation of carotenoid under organic farming system could be attributed to fertilization strategy. According to Gross (1987), soil fertilization is one of the factors that affects the biosynthesis of carotenoids in fruits. A common explanation for reported differences in phytochemicals between organic and conventional produce is that organic systems are more stressful than conventional systems due to the limited and restricted use of pesticides in organic systems, thus allowing for greater incidence of biotic stresses (Tarozzi et al., 2006).

Among the TPC detected in the orange juice, significant differences were observed, where the juice of conventional produced fruits exhibited higher values (Tables 1 and 2). In contrast, the results indicated that there were significantly lower concentrations of TP in conventionally grown strawberry than the organically grown one. Jin et al. (2011) indicated that the TPC was significantly higher in organically cultivated strawberries than in conventionally cultivated strawberries. Biosynthesis of phenolic compounds in plants is strongly affected by the cultivator techniques, environmental conditions and the fertilizers used. Häkkinen and Törrönen (2000) reported that, of three strawberry cultivars tested by sampling from organic and conventional farms that increased, phenolic compounds only occurred in one cultivar under organic conditions, possibly due to pathogen attack. It has previously been reported that the phenol concentration is influenced by level of available nitrogen (Brandt and Molgaard, 2001). Increase in phenolic compounds is related to the defense role they play in plants under stressed conditions (Dixon and Paiva, 1995). In the absence of pesticides, plants could contain higher levels of antioxidant components as a result of enhanced synthesis of active phytochemicals produced in defense against biotic and abiotic stress (Tarozzi et al., 2006).

The microbiological quality of organic and conventional oranges and strawberries was determined by analysis of yeasts and molds and *E. coli* (Table 3). *E. coli*. (0.0 MPN/100 g) was not detected in oranges and strawberries fruits under organic and conventional production system.

In oranges, yeasts and molds were present in smaller accounts $(1.0 \times 10^2 \text{ CFU/g})$ under organic production system whereas conventional oranges presented higher counts (4.0x10² cfu/gm) than organic ones. Conventional samples of strawberries presented higher yeasts and molds counts than organic samples. In strawberries, yeasts and molds count ranged from 11x10 to 14x10 CFU/g under organic production system, whereas the count was from 60×10^4 to 77×10^4 CFU/g under conventional production system. These results contradict those of Maffei et al. (2013) who reported that E. coli was found in organic and conventional vegetables and higher microbial count of organic vegetables compared with conventional ones. Although yeasts and molds are associated with food spoilage, the mycotoxins produced by molds may be dangerous to health (Maffei et al., 2013). Mycotoxins caused many diseases, including carcinogen and immunosuppressive effects (Kovacs, 2004). Many other studies would be necessary to confirm these

Table 3. Yeasts and molds and *Escherichia coli* in orange and strawberry fruits produced by organic and conventional farming in 2012 and 2013 seasons.

_	Yeasts and molds (cfu/g)				Escherichia coli			
Sample	Organic		Conventional		Organic		Conventional	
	2012	2013	2012	2013	2012	2013	2012	2013
Citrus	1.0x10 ²	0.0x10	2.0x10 ²	4.0x10 ²	0.00	0.00	0.00	0.00
Strawberry	11.0x10	14.0x10	60x10 ⁴	77 x10 ⁴	0.00	0.00	0.00	0.00

 Table 4. Mean concentrations of nitrates in orange and strawberry fruits produced by organic and conventional farming in 2012 and 2013 seasons.

		Nitrates	s (mg/l)		
Sample	Org	anic	Conventional		
	2012	2013	2012	2013	
Citrus	0.20 ±0.01 ^z a	0.20 ±0.01 ^z a	0.14 ±0.01b	0.13 ±0.01b	
Strawberry	0.25 ±0.01a	0.24 ±0.01a	0.16 ±0.02b	0.15 ±0.01b	

Means in a column followed by a different letter differ significantly at P = 0.05 by L.S.D test. ^zMeans \pm SE.

observations. In addition, handling condition especially in Egypt should be considered, since they may impact the microbial profile of organic and conventional fruits.

To our knowledge, this is the first report that compares the nitrite level in oranges and strawberries fruit in organic and conventional production systems under Eqypt conditions. The results obtained for nitrite levels are shown in Table 4. The results show a considerable significant variation in the average levels of nitrite contents between the two production systems. The average levels of nitrites were higher in conventional oranges and strawberries fruits. The nitrites levels from organic production varied between 0.13 and 0.16 mg/kg fresh weight (FW), whereas those from conventional production ranged from 0.20 to 0.25 mg/kg FW. Similar tendency was found by Gonzalez et al. (2010) and Aires et al. (2012): they reported significant differences in the average levels of nitrate contents from organic and conventional produce. The limits detected for nitrite in our samples are within the legal limits (0-1 mg/kg FW for orange and strawberry) recommended by European Union regulations (1995); thus, from the point of view of nitrites, this type of fruits are safe.

From this study, we can conclude that farm management techniques can affect the overall quality of orange and strawberry fruit. Organically grown oranges and strawberries had higher ascorbic acid and β -carotene content and lower titratable acidity than conventionally grown ones. Total soluble solid (TSS) of oranges and strawberries were not affected by the production systems. Total phenol content (TPC) was significantly higher in conventional oranges compared to its organic production. Conversely, TPC was significantly higher in

organic strawberries than the conventional ones. Conventional oranges and strawberries fruits have greater counts of yeasts and mold than organic ones. The average levels of nitrites were lower in organic oranges and strawberries fruits. Organic fruits and vegetables seem to become popular because of the concerns over environmental contamination and health benefits. However, it is important to analyze a wide variety of fruit and vegetables to elucidate the possible benefits of the consumption of organic foods as part of a whole diet.

Conflict of interests

The author(s) have not declared any conflict of interests.

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