Eur Food Res Technol (2007) 225:255–260
DOI 10.1007/s00217-006-0412-z

ORIGINAL PAPER

Effects of organic farming on minerals contents and aroma composition of Clemenules mandarin juice

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Received: 23 January 2006 / Accepted: 8 June 2006 / Published online: 18 July 2006

Abstract Consumers demand organic products because they believe that the organic products are more flavorful and respectful to the environment and human health. The effects of organic farming on the minerals contents and aroma composition of Clemenules mandarin juices were studied. Minerals (Fe, Cu, Mn, Zn, Ca, Mg, K, and Na) were quantified using atomic absorption-emission spectroscopy, while volatile compounds were extracted using the dynamic headspace technique and were identified and quantified by GC–MS. In general, organic farming produced a mandarin juice with a higher quality than that produced by conventional agricultural practices. Higher concentrations of both minerals and positive volatile compounds were found in the organic juice, while the formation of off-flavors was higher in the conventional juice, although threshold values were not reached.

Keywords Citrus · Color · Dynamic headspace · GC–MS · Volatile compounds

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Introduction

Organic markets are thriving, as a growing number of consumers worldwide prefer organic products. Growth in the industry is fuelled in part by consumers' attitudes toward food production systems and product quality. With respect to product quality, surveys indicate that consumers consider organic foods to be more positive for the environment and human health and more flavorful than their conventionally grown counterparts [1].

Conventional, organic, and sustainable agriculture are the primary cultural practices used in the production of foods in Europe and the United States. The goal of each of these practices differs greatly with respect to crop yield, land and pesticide use, and environmental impact. Conventional agricultural practices utilize high-yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term sustainability associated with these practices [2].

Some studies have been performed to evaluate the impact of cultural practice (organic versus conventional production) on nutrients and antioxidant activity content in different fruit species [3, 4].

Citrus, like other fruits, vary in chemical composition, even within the same variety, depending on maturity, location of production, and agricultural practices, as well as on numerous environmental factors.

Commonly available citrus fruits have been analyzed for a considerable period of time in order to develop a citrus food composition table. Citrus juice contains minerals such as potassium, iron, copper, manganese, and zinc which are important for human health [5]. Other studies have shown that organic crops have higher levels of nutrients such as ascorbic acid and minerals than the conventional crops [6].

Citrus juices are widely consumed in many countries. Orange juice accounts for 60% of fruit juices and juice-based drinks consumed in western Europe [7].

Spain, the second largest producer of in world (1,779,800 tonnes in 2003), is the main supplier of mandarin in the international market, especially the northern European countries and the United States. The most grown and exported mandarin cultivars are Clementine [8]. Currently, the main two items elaborated with mandarin fruits are canned slices and juice, and they only represent about 7% of the total trade of this fruit [9].

Our objectives were to determine CIEL*a^*b^* color parameters, minerals contents, and volatile composition of mandarin juice from Clemenules mandarins cultivated under two different agricultural practices, organic and conventional.

Materials and methods

Fruit material

Mandarins were all grown in the same farm and under identical conditions of soil, irrigation, and illumination in eastern Spain (Librilla, Murcia). The rootstock was the same for both the mandarin trees, *Mandarino cleopatra*, and all selected trees were about 11 years old and free from diseases. Fruits were collected in winter (first week of December 2004). Fruits from these cultivars were selected on the basis of their diameter, pH, total soluble solids content (SSC, °Brix), and maturity index (total soluble content/titratable acidity, SSC/TA) (Table 1).

Mandarin cultivar Clemenules was studied on conventional and organic farming. Organic production means that no synthetic chemicals are used in the production of these fruit trees but only natural substances are used [1]. Farming of organic mandarin trees followed all rules established by the Board of Organic Agriculture of the Murcia Region [10]. A complete list of the materials used in both conventional and organic farming is included in Table 2.

Table 2 Materials used in both conventional and organic farming

Compound	Farming: Conventional	Organic
Fertilizers	Ammonium nitrate, calcium nitrate, ammonium sulfate, phosphoric acid, diammonium phosphate, potassium sulfate, potassium nitrate, potassium chloride	Manure, compost, and fulvic and humic acids
Foliar fertilizers	Potassium phosphate, magnesium nitrate, urea, mixture of oligoelements	Algae extracts, aminoacids
Herbicides	Bromacil, diuron, diquat, fluroxypyr, glyphosate, norflurazon, paraquat, sulfosate, simazine	None; weeds are removed by mechanical methods
Pesticides	Malathion, dicofol, methidathion, clopidol	Neem oil, pheromone traps

Sample preparation

Mandarins were collected and processed the same day, 9 December. The weights of fruits processed were 15,000 and 5,000 kg of conventional and organic Clemenules, respectively. Mandarin juices were processed in a commercial plant (Murcia, Spain). The juices were obtained by using a Premium Juice Extractor (FMC Corporation, Florida, USA) [11]. This type of machinery leads to a juice with a low content of essential oils [12].

Freshly squeezed juices were treated in an Alfa Laval plate heat exchanger (Alfa Laval Iberia S.A., Madrid, Spain) for 20 s at a temperature of 98 °C. After this heat treatment, the juice was first transferred to a precooler, which cooled the juice down to 30 °C using forced air, and then to a cooler, which finally took the temperature down to 2 °C. Heat-treated juices were stored in aseptic metallic deposits of 50,000 L at a temperature of 4 °C until the juice was ready for the market. Finally, juices were packaged in brick cartons, made of aluminum foil and polyethylene, and stored under refrigeration conditions (4 °C).

Table 1 Main Properties of Clemenules mandarins at collection time

Cultivar	Weight (g)	Diameter (mm)	Vitamin C (mg L^{-1})	SSC[a] (°Brix)	TA (% citric acid)	Maturity index (SSC/TA)
Organic Clemenules	82.1 a[b]	83.5 a	514.2 a	12.5 a	0.94 a	13.3 a
Conventional Clemenules	80.1 a	82.4 a	483.3 a	12.2 a	0.89 a	13.6 a

[a]SSC, soluble solid content; TA, Titratable acidity; maturity index is the ratio between the solid soluble content and titratable acidity.

[b]Mandarin juice cultivars with the same letters were not significantly different at $p<0.005$ for the attribute evaluated (Tukey multiple range test).

Physico-chemical analyses

SSC, expressed in °Brix, was determined using a portable refractometer Comecta, S.A., model C3 (Barcelona, Spain).

Titratable acidity (TA), expressed as percent citric acid, was determined in 10 mL of juice by titration to pH 8.2 ± 0.05 with a 0.1 N NaOH solution.

The maturity index (MI) was calculated for each mix and expressed as the percentage of the ratio between the SSC and titratable acidity.

Vitamin C (reduced ascorbic acid) was measured following the AOAC Official Method 985.33 [13]. Ascorbic acid is estimated by titration with colored oxidation–reduction indicator, 2,6-dichloroindophenol. EDTA is added as a chelating agent to remove Fe and Cu interferences.

All physico-chemical analyses were analyzed in 20 fruits of each cultivar; results provided are the mean of one replicate of 20 fruits.

Color measurement

Color determinations were made at 25 ± 1 °C, using a Hunterlab Colorflex® (Hunterlab, Reston, Virginia). This spectrophotometer uses an illuminant D_{65} and a 10° observer as references. A sample cup for reflectance measurements was used (5.9 cm internal diameter × 3.8 cm height) with a path length of light of 10 mm. Blank measurements were made with the cup filled with distilled water against a reference white background [14].

Color data are provided as CIEL*a^*b^* coordinates, which define the color in a three-dimensional space [15].

Minerals contents

A multi-place digestion block, Selecta Block Digest 20 (Barcelona, Spain), was used for sample mineralization. Fifteen milliliters of juice were treated with 5 mL of 65% (w/v) HNO_3 in Pyrex tubes, placed in the digestion block, and heated at 60 °C for 60 min and at 130 °C for 120 min [16]. Solutions were left to cool to room temperature, transferred to a volumetric flask, and then diluted to a final volume of 25 mL with ultrahigh-purity deionized water.

Determination of Ca, Mg, K, Na, Cu, Fe, Mn, and Zn in previously-mineralized samples was performed with a Unicam Solaar 969 atomic absorption spectrometer (Unicam Limited, Cambridge, UK).

Instruments were calibrated using certified standards. In each analytical batch, at least two reagents blanks, one international reference material (CRM) and one spike were included to assess the precision and accuracy of the chemical analysis. The certified material selected for the current experiment was GBW07603 (bush, branches, and leaves); this material was provided by LGC Deselaers S.L. (Barcelona, Spain) and produced by the Institute of Geophysical and Geochemical Exploration of China (GBW07603). Analyses were run in five replicates.

Certified values for Ca (%), Mg (%), K (%), Na (mg kg^{-1}), Cu (mg kg^{-1}), Fe (mg kg^{-1}), Mn (mg kg^{-1}), and Zn (mg kg^{-1}) were 1.81 ± 0.07, 0.65 ± 0.03, 1.38 ± 0.04, 200 ± 10, 274 ± 10, 9.3 ± 0.5, 45 ± 2, and 37 ± 1, respectively, while the measured values for the same elements were 1.80 ± 0.05, 0.64 ± 0.02, 1.40 ± 0.02, 204 ± 9, 275 ± 11, 9.3 ± 0.3, 46 ± 3, and 37 ± 2, respectively.

Volatile compounds extraction and quantification

For the extraction of volatile compounds from the mandarin juices, a modification of the dynamic headspace technique was used [17, 18]. Analyses were run for three replicates. Each mandarin juice sample (200 mL) was placed in an Erlenmeyer vessel hermetically closed by a plastic tap. This extraction step was carried out at room temperature (22 ± 2 °C) and the samples were continuously agitated using magnetic stirrers. Chromatographic nitrogen (Carburos Metálicos, Barcelona, Spain) was passed through at a flow rate of 200 mL min^{-1} for 24 h and the effluent was passed through an ORBO-32 tube (Supelco, Bellefonte, PA, USA) containing activated charcoal. The volatile compounds were recovered from the adsorbent material with 1 mL of carbon disulfide, CS_2, (Supelco). The solid-CS_2 phases were treated by ultrasonic waves for 5 min. Phases were separated by centrifugation for 5 min at 5,000 rpm at 0 °C, and the supernatant was manually collected using a Pasteur pipette. Cinnamaldehyde was used as an internal standard.

The isolation, identification, and quantification of the volatile compounds were performed on a Shimadzu gas chromatograph GC-17A coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A (Shimadzu Corporation, Kyoto, Japan). The GC-MS system was equipped with a Supelcowax-10 column (Supelco, Kyoto, Japan; 60 m × 0.25 mm × 0.25 μm film thickness). Analyses were carried out using helium as a carrier gas at a flow rate of 0.8 mL min^{-1} in a split ratio of 1:20 and the following temperature program: initial temperature 100 °C; from 100 to 170 °C at 2 °C min^{-1}; from 170 °C to 230 °C at 10 °C min^{-1}, and held at 230 °C for 5 min. Injector and detector were held at 250 °C. The mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra in the range of 40–450 m/z was used. In all analyses, a volume of 1 μL was injected.

The GC-MS apparatus was linked to a PC running software (LabSolutions, GCMSsolution version 1.01; Shimadzu Corporation, Kyoto, Japan). Identification of the compounds was confirmed by the comparison of collected mass spectra with those of the authenticated reference standards and spectra in the Wiley 229 mass spectra library [19]. A comparison of the compound's experimental retention index with that of the authentic reference standards was carried out.

The quantification of compounds identified in this study was performed using MS response factors from the standard calibration curves. Four multiple standards with different concentrations of the 12 volatile compounds identified were prepared and used for quantification purposes. All standards were purchased from Sigma-Aldrich (Milwaukee, WI).

Volatile compounds analyzed were selected as being important compounds in citrus juice flavor [20–24].

Statistical analysis

Instrumental color measurements and minerals contents were run in five replications, while volatile compounds quantification was run in triplicate. All data were subjected to analysis of variance (ANOVA) and the Tukey's least significant difference multi-comparison test to determine significant differences among mandarin juices. Significance of differences was represented as $p \leq 0.001$. The statistical analyses were done using SPSS 12.0 (SPSS Science, Chicago) and figures using Sigma Plot 8.0 (SPSS Science, Chicago).

Results and discussion

The main reason for selecting Clemenules conventional and organic farming as the only mandarin cultivar to compare is that Clemenules are the most industrially processed mandarin cultivars for juice production in Spain. Besides, Clemenules mandarins are appreciated worldwide due to their deep orange color [14].

No significant differences in the quality parameters of the conventional and organic mandarins were found initially in the mandarins at the collection time (Table 1). The main properties of the mandarins selected for this experiment were 81.1 g, 83.0 mm diameter, 498.8 mg vitamin C L^{-1}, and maturity index 13.5.

Pérez-López et al. [14] studied the effect of the mandarin cultivar on the color coordinates of mandarin juices and concluded that juice from Clemenules mandarins, under conventional agricultural conditions, presented the higher values of lightness, L^*, coordinates green–red, a^*, and blue–yellow, b^*, and Chroma, C^*, of the 11 mandarin cultivars under study (Oronules, Marisol, Hernandina, Orogrande, Clementpons, Satsuma owari, Fortuna, Ellendale, Nova, Ortanique, and Clemenules). The values of L^*, a^*, b^*, and C^* for the Clemenules juice and the mean for the other 10 cultivars were 53.02 ± 0.02, 8.57 ± 0.10, 29.40 ± 0.07, 30.63 ± 0.09, and 50.68 ± 0.06, 7.81 ± 0.06, 28.41 ± 0.14, and 29.46 ± 0.15, respectively.

Results from the current experiment are in agreement with those reported previously by Pérez-López et al. [14], with mean values of L^*, a^*, b^*, and C^* for both Clemenules juices, traditional and organic, being 53.14 ± 0.10, 8.89 ± 0.03, 29.67 ± 0.05, and 30.97 ± 0.07, respectively (Table 3). These results proved that Clemenules mandarins provide a juice with a high intensity of orange color, independent of the type of agricultural practices.

Table 3 Effects of organic farming on color coordinates of Clemenules mandarin juices

Cultivar	L^*	a^*	b^*	C^*	h_{ab}
Organic Clemenules	53.26 a[a]	9.21 a	29.93 a	31.31 a	72.89 a
Conventional Clemenules	53.02 a	8.57 a	29.40 a	30.63 a	73.75 a

[a]Mandarin juice cultivars with the same letters were not significantly different at $p<0.005$ for the attribute evaluated (Tukey multiple range test).

Organic farming had a significant effect on the content of all analyzed elements, macro-nutrients (Ca, Mg, and K) and micro-nutrients (Fe, Cu, Mn, and Zn). In general, all nutrients contents were higher in the juice from the organic mandarins (Table 4).

The concentrations of almost all nutrients analyzed in this study, except Mn, fall within the ranges reported in the literature for mandarin juices from different geographical areas, such as Florida, California, México, and Brazil [21], and within the ranges suggested by the guidelines of AIJN [25].

A complete understanding of flavor requires an investigation of the reactants and dynamics of the flavor reaction. For example, *d*-limonene contributes very little to the aroma of the mandarin juice, even though it is the organic volatile compound with the highest concentrations. The contribution of chemical compounds to food odor and flavor is best understood when their perception threshold is known. The major limitation in this approach is that it requires the use of published threshold values, mostly established in water or in air [18]. However, Plotto et al. [26] published the thresholds for key aroma compounds of orange juice determined in a deodorized orange juice matrix (Table 5). In this study, for instance, the odor threshold of *d*-limonene, 13.7 mg L^{-1}, was much higher than that of other compounds such as linalool (0.11 mg L^{-1}), myrcene (0.77 mg L^{-1}), α-pinene (1.65 mg L^{-1}), and γ-terpinene (3.26 mg L^{-1}). The high odor threshold of *d*-limonene is the main reason for its low contribution to the final aroma of the mandarin juice.

Initially, total concentrations of volatile compounds of the fresh organic and conventional Clemenules juices were 83.1 ± 2.1 and 61.1 ± 1.8 mg L^{-1}, respectively (Table 5). A thermal treatment of 98 °C for 20 s was applied to juices and they were packed and stored at 4 °C for 60 days. At the end of this storage time, the total concentrations of volatile compounds were 50.8 ± 1.7 and 32.1 ± 1.4 mg L^{-1} for organic

Table 4 Effects of organic farming on minerals contents of Clemenules mandarin juices

Cultivar	Minerals (mg L^{-1}) Ca	Mg	K	Na	Fe	Cu	Mn	Zn
Organic Clemenules	43.80 a[a]	133.0 a	1584 a	6.54 a	0.58 a	0.35 a	0.16 a	0.44 a
Conventional Clemenules	39.80 b	120.0 b	1416 b	4.42 b	0.48 b	0.23 b	0.12 b	0.27 b

[a]Mandarin juice cultivars with the same letters were not significantly different at $p<0.005$ for the attribute evaluated (Tukey multiple range test).

Table 5 Effects of organic farming on the volatile compounds generated from Clemenules mandarin juice

		Concentration (mg L^{-1})			
		Organic		Conventional	
Chemical compound	Odor threshold (mg L^{-1})	Day 0	Day 60	Day 0	Day 60
α-Pinene	1.65[a]	0.35 a[b,c]	0.17 b	0.20 b	0.02 c
β-Pinene	37.2	0.12 a	0.05 b	0.08 b	n.d
Myrcene	0.77	1.71 a	0.92 b	1.03 c	0.41 d
α-Terpinene		0.11 a	0.03 b	0.01 b	n.d
d-Limonene	13.7	77.2 a	48.4 b	59.2 c	31.2 d
Sabinene		1.21 a	0.72 b	0.18 c	0.07 d
γ-Terpinene	3.26	0.86 a	0.21 b	0.05 c	0.01 d
p-Cymene		0.55 a	0.16 b	0.11 c	0.02 d
α-Terpineol	25.9	n.d	0.03 a	n.d	0.12 a
Valencene		0.63 a	0.33 b	0.07 c	0.01 d
Terpinen-4-ol		n.d	0.02 a	0.03 b	0.12 a
Linalool	0.11	0.37 a	0.11 b	0.17 c	0.08 d
Total		83.11	50.82	61.13	32.06

[a]Odor threshold in deodorized orange juice matrix [26].

[b]Mandarin juice cultivars with the same letters, within the same row, were not significantly different at $p<0.005$ for the studied compound (Tukey multiple range test)

[c] n.d., not detected (<0.01 mg L^{-1}).

and conventional mandarin juices, respectively. Therefore, a higher reduction of volatile compounds was experienced by the conventional juice, 47.6%, compared to the organic juice, 38.5%.

In general, organic Clemenules juice presented, initially, significantly higher concentrations of some most of the volatile compounds in mandarin juice [20–24], e.g., α-pinene, myrcene, *d*-limonene, *p*-cymene, and linalool, than conventional Clemenules juice (Table 5).

Pérez-López et al. [18] reported formation of off-flavor components, α-terpineol and terpinen-4-ol, and simultaneous decomposition of *d*-limonene and linalool. No α-terpineol and terpinen-4-ol were initially found in the organic juice, showing the high quality of the freshly squeezed juice studied (Table 5); however, 0.03 mg L^{-1} of terpinen-4-ol were found in the conventional juice. It is important to remark that the concentrations of these two off-flavor compounds were below their perception thresholds in this type of matrix.

Thermal and catalytic decompositions of food components occur during heat treatment and storage of juices. In this study, a good example of a thermally induced alteration appeared to be the thermal reaction by which *d*-limonene, linalool, and/or α-pinene transform into compounds such as α-terpineol and terpinen-4-ol [18]. Linalool also undergoes the same reaction and it is far more reactive than *d*-limonene. In this way, most of the α-terpineol formed was probably derived from linalool, although mandarin juices contain much less linalool than *d*-limonene.

d-Limonene concentrations decreased from 77.2 ± 0.9 and 59.2 ± 1.4 to 48.4 ± 1.2 and 31.2 ± 1.5 mg L^{-1} in organic and conventional Clemenules juices, respectively. These decreases in *d*-limonene corresponded with increases in the concentrations of α-terpineol from traces levels initially in both mandarin juices to 0.03 ± 0.01 and 0.12 ± 0.01 mg L^{-1} in organic and conventional Clemenules juices, respectively, at the end of the storage period. A pattern similar to that already described for α-terpineol was found for terpinen-4-ol; its concentrations increased from traces level and 0.03 to 0.02 ± 0.01 and 0.12 ± 0.01 mg L^{-1} in organic and conventional Clemenules juices, respectively.

In summary, Clemenules mandarins produced freshly squeezed juices with a very intense orange color. The organic juice contained higher mineral contents and volatile compounds than the conventional juice. Besides, the formation of off-flavors compounds was lower in the organic juice compared to the conventional one, even though their perception thresholds were not reached. Besides, the concentrations of linalool and myrcene (compounds considered as markers of high-quality juices [24]) decreased below their threshold values, at the end of the storage period, only in conventional juices. Therefore, organic farming had a positive effect on the quality and stability of mandarin juices.

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