

# Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage

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Received 11 July 2002; received in revised form 24 March 2003; accepted 24 March 2003

## Abstract

Citrus fruits of different species and cultivars, (“Red blush” grapefruit, “Palazzelli” mandarin-type fruit, “Minneola” tangelo and “Salustiana” and “Shamouti” orange) were minimally processed as segments or juices and cold-stored for up to 12 or 15 days, respectively. The flavanone glycosides, ascorbic acid (AA) content and antioxidant capacity were determined during storage. Minimal processing had almost no effect on the main chemical constituents, but ascorbic acid decreased significantly in “Minneola” and “Salustiana” segments, with values ranging from 1.63 to 5.10 mg per gram of dry matter, although only in the last samples taken. One of the three juices (Salustiana) also showed a decrease in AA content. The segments and juices showed different behaviour during storage with regard to the flavonoid content, which ranged from 0.77 mg to 8.32 mg/g dry matter in Palazzelli mandarin and Red blush grapefruit, respectively. A significant increase in total flavonoids (mainly hesperidin) was found in the segments, while the juices showed a diminution in flavonoid content. Antioxidant capacity increased significantly in “Red blush” grapefruit juices and “Salustiana” orange segments, decreased in “Salustiana” juices and “Minneola” tangelo segments and remained constant in the other samples. The antioxidant capacity, moreover, was clearly correlated ( $r=0.968$  and  $r=0.889$  in segments and juices, respectively) with the ascorbic acid content rather than with the presence of flavanone glycosides.

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*Keywords:* Citrus fruits; Minimal processing; Flavonoids; Ascorbic acid; DPPH

## 1. Introduction

Over the past 20 years there has been growing interest, on the part of the consumer, in minimally processed fruit and vegetables because of their freshness and convenience. Moreover, the benefits of fruit and vegetable consumption are now widely reported in the literature (Kaur & Kapoor, 2001; Scalbert & Williamson, 2000). Fruit and vegetables are a source of vitamin C and phenolic antioxidants, which are now associated with a low risk of developing degenerative diseases, such as cancer, diabetes, cardiovascular and neurological disease. However, operations such as peeling and cutting significantly reduce the shelf life of these products, since various metabolic processes are accelerated. Mechanical damage causes increased respiratory activity and the production of ethylene, with a consequent increase in

some biochemical effects, such as enzymatic discoloration and loss of aroma, firmness and nutritional qualities (Matto & Anderson, 1984; Rolle & Chism, 1987; Watada, Abe, & Yamauchi, 1990). Low temperature, during processing and storage, considerably slows biochemical activity and microbial proliferation (Rolle & Chism, 1987). Wounds on the fruit surface create optimal conditions for the breakdown of juice components and a rapid increase in microorganisms. To maintain good quality, therefore, the product must be processed and stored at temperatures no higher than 5 °C (Yildiz, 1994). At present, particularly in the countries of northern Europe, packaged vegetable products are sold over a wide area but prepared fruits have a more restricted distribution, because these products present more problems than vegetables due to their chemical-physiological composition.

Citrus fruits are important because of their nutritional and antioxidant properties. Besides ascorbic acid, this genus contains flavanone glycosides, such as hesperidin, narirutin and naringin, the most important phenols in

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the water-soluble fraction (Gil-Izquierdo, Gil, Ferreres & Tomas-Barberan, 2001). Furthermore, other compounds, such as the limonoids (triterpene derivatives), some flavones, such as sinensetin and nobiletin, and phenylpropanoids, such as the hydroxycinnamates, have high antioxidant potential and health-promoting capacity (Kaur & Kapoor, 2001). It is important to assess the effect of minimal processing on these compounds and their antioxidant capacity, which is known to be influenced by processing and storage (Gil-Izquierdo, Gil, Ferreres, & Tomas-Barberan, 2001).

Changes may occur in flavanone glycoside and ascorbic acid contents and antioxidant capacity, expressed as Trolox Equivalent Antioxidant Capacity (TEAC) of the water-soluble fraction of segments and juices derived from citrus fruits with minimum preparation.

## 2. Materials and methods

### 2.1. Fruits

The fruits were harvested in an experimental orchard at a commercial ripening stage.

### 2.2. Segments

“Shamouti” and “Salustiana” oranges (*Citrus sinensis* L. Osbeck), “Palazzelli” mandarin-type fruits (*Citrus reticulata* × *Citrus sinensis*) and “Minneola” tangelo fruits (*Citrus paradisi* × *Citrus reticulata*) were used. The mandarin-type and tangelo fruits were used only for segments as they are not usually processed as juices. Grapefruit segments were not as it was impossible to obtain undamaged segments by manual peeling. After harvesting, the fruits were selected, washed in sterile water (200 ppm NaOCl) and dried and peeled manually. Only undamaged segments were placed inside 250 ml volume polypropylene boxes (100 g per box), which were sealed with a 15 µm polyolefinic film. Sealing was done with a packaging device (98P-2596, ORVED, Venice, Italy), with the sealing bar operating at 155 °C. Table 1 lists permeability characteristics of the boxes and films. The boxes were immediately put into refrigerated cells at 4° C and left for 12 days. Samples of the contents of 12

boxes were taken at the beginning of storage and after 4, 8 and 12 days. Gas samples were taken daily to analyse the gas composition inside the boxes.

### 2.3. Juices

Fruits of “Shamouti” and Salustiana” oranges and “Red blush” grapefruits (*Citrus paradisi* Macf.) were used to prepare the juices. After the preliminary operations of selection, washing and drying, as described above, the fruits were cut crosswise and squeezed using a household electric juice extractor, and the juices put into 50 ml glass test tubes, leaving only 5 ml empty space at the top. The test tubes were hermetically sealed with screw lids and refrigerated at 4° C. The microbial load was <10<sup>2</sup> CFU/ml of juice. At harvesting and at 5, 10 and 15 days of storage, 12 samples of juice were taken for chemical analysis.

### 2.4. Chemical analysis and gas measurement

The juice samples were analysed to determine the following: pH, titratable acidity (% of citric acid), dry matter (d.m.) (%), total soluble solids (TSS) in °Brix, and ascorbic acid (mg/g d.m.) by the official method (AOAC, 1990). The juice was analysed after filtering through gauze and the segment homogenate was used for assay. To determine the glycosylated flavonoids, the juices of the whole fruit or the segment homogenate were centrifuged at 9000 rpm for 30 min at a temperature of 20 °C. The pellet was resuspended in 50 ml of water and centrifuged, under the same conditions, three more times. The three supernatants were added to the first supernatant and the total quantity recorded for exact calculation of the dilution factor. Ten ml of supernatant were then loaded into a Sep-Pak cartridge, activated with 5 mL MeOH and 10 mL 10% MeOH. The cartridge was washed with 15 mL 10% MeOH solution and the flavonoids eluted with 5 mL MeOH:DMSO solution (1:1) (Nogata, Ohta, Yoza, Berhow, & Hasegawa, 1994). The sample obtained was filtered through 0.22 µm cellulose acetate filters and analysed by HPLC (Mouly, Gaydou & Auffray, 1998; Merken & Beecher, 2000). A Hewlett Packard 1050 liquid chromatograph, coupled with a photodiode array detector (DAD) and a RP-18 Licrospher column (150 mm × 4.6 mm, 5 µm), was used. Operating conditions were the following: flow rate: 1 mL/min; phase A: H<sub>3</sub>PO<sub>4</sub> 0.01 M, phase B=MeOH; linear gradient: from 15% B to 75% B in 60 min; column temperature: 40° C; λ=280 nm. The compounds were quantified by calibration with the following standards: narirutin, hesperidin, didymin, naringin, neohesperidin and poncirin (Extrasynthese B.P. 62-69730, Genay, France).

Antioxidant capacity was assessed using the free radical DPPH (Bondet, Brand-Williams, & Berset,

Table 1  
Properties of the plastic materials used

Property	Plastic film	Plastic box
	Cryovac MY 15	
Water transmission rate	10 g day <sup>-1</sup> m <sup>-2</sup> at 38 °C and 100% ΔRH	–
O <sub>2</sub> permeability	3400 mL m <sup>-2</sup> day <sup>-1</sup> bar <sup>-1</sup>	37 mL m <sup>-2</sup> day <sup>-1</sup> bar <sup>-1</sup>
CO <sub>2</sub> permeability	8500 mL m <sup>-2</sup> day <sup>-1</sup> bar <sup>-1</sup>	192 mL m <sup>-2</sup> day <sup>-1</sup> bar <sup>-1</sup>

1997; De Ancos, Gonzales, & Cano, 2000). By this method, 50  $\mu\text{L}$  of centrifuged sample, after filtering and dilution (1:10), reacted for 15 min (until reaction reached plateau) in a cuvette containing 3 mL of a methanol solution of  $6 \times 10^{-5}$  M DPPH. The absorbance was transformed into % inhibition of the DPPH concentration (from a calibration curve) and expressed as TEAC (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993). The TEAC of 1 mM solution of ascorbic acid and of the six standards of flavanones found in the fruits was also measured, again using the free radical DPPH.

The internal atmosphere ( $\text{CO}_2$ ,  $\text{O}_2$  and  $\text{C}_2\text{H}_4$ ) was determined in a sample of 10 boxes of segments, using a combined infrared/paramagnetic detector for  $\text{CO}_2/\text{O}_2$  (Dansensor Combi Check, Milan) while ethylene was measured by gas chromatography using an FID detector (Piga, D'Aquino, Agabbio, & Continella, 1996).

### 2.5. Sensorial analysis

An informal panel of six untrained assessors evaluated the samples on the basis of degree of acceptability for taste, acidity, sweetness and aroma, using a scale ranging from 1 to 5, as follows: (1) taste (5, very good taste; 4, good; 3, acceptable; 2, poor; 1, very poor taste); (2) acidity: (5, very good; 4, good; 3, acceptable; 2, acid; 1, very acid); (3), sweetness (5, very sweet; 4, sweet; 3, acceptable; 2, poor; 1, very poor); and (4), aroma (5, very good; 4, good; 3, acceptable; 2, off-flavoured; 1, strongly off-flavoured). In all classifications we fixed the limit of acceptability at 3 points.

### 2.6. Statistical analysis

One-way ANOVA was performed for each cultivar and preparation to obtain a statistical assessment of the influence of storage period on chemical and antioxidant capacity evolution. The storage period was the variable considered. Means were separated, when necessary, by Duncan's multiple range test at 5 or 1% levels of significance.

## 3. Results and discussion

### 3.1. Changes in main chemical parameters of citrus segments and juices

The composition of the fruits at the beginning of the study is shown in Table 2. Differences in chemical composition of juices and segments at the start of the experiment can be seen in the varieties used, and can be attributed to genetic and physiological factors (Kefford & Chandler, 1970). Storage resulted in significant variations only in the chemical parameters of

Table 2  
Changes in main chemical parameters of citrus segments and juices during storage at 4 °C

Fruits	Days	pH	Titrateable acidity (% citric acid)	TSS (° Brix)	Dry matter (%)
Shamouti orange (segments)	0	3.33b	1.28a	11.55a	12.58a
	4	3.31b	1.28a	11.45a	12.15a
	8	3.49a	1.20a	11.75a	12.53a
	12	3.53a	1.16a	11.50a	12.20a
Significance		*	ns	ns	ns
Shamouti orange (juices)	0	3.34a	1.21a	12.25a	13.10a
	5	3.37a	1.23a	12.10a	13.24a
	10	3.41a	1.21a	12.35a	13.30a
	15	3.43a	1.32a	12.30a	13.25a
Significance		ns	ns	ns	ns
Palazzelli mandarin (segments)	0	3.45a	0.88a	14.30b	15.07a
	4	3.45a	0.91a	14.70a	15.63a
	8	3.47a	0.91a	15.00a	15.60a
	12	3.49a	0.93a	15.15a	15.60a
Significance		ns	ns	**	ns
Red blush grapefruit (juices)	0	3.23a	1.42a	10.15a	10.66a
	5	3.24a	1.44a	10.25a	10.61a
	10	3.23a	1.43a	10.20a	10.67a
	15	3.26a	1.38a	10.10a	10.59a
Significance		ns	ns	ns	ns
Minneola tangelo (segments)	0	3.41b	1.55a	13.45a	14.25a
	4	3.51a	1.59a	13.40a	13.91a
	8	3.41b	1.49a	13.20b	13.85a
	12	3.46b	1.45a	13.10b	13.44a
Significance		ns	ns	*	ns
Salustiana orange (segments)	0	3.79a	0.94a	13.15a	13.84a
	4	3.79a	0.92ab	13.05a	13.70a
	8	3.93a	0.83c	13.10a	13.65a
	12	3.94a	0.86bc	13.10a	13.67a
Significance		ns	*	ns	ns
Salustiana orange (juices)	0	3.90a	0.91a	12.80a	13.14a
	5	4.06a	0.97a	12.70a	13.29a
	10	4.08a	0.95a	12.90a	13.07a
	15	4.07a	0.95a	12.65a	13.19a
Significance		ns	ns	ns	ns

Means in each column within each product with the same letter do not differ significantly according to Duncan's multiple range test at  $P < 0.01$  or  $P < 0.05$ . ns, not significant.

\*  $P$  significant at 1% level; \*\*  $P$  significant at 5% level.

the segments. We observed significant changes of TSS in "Palazzelli" mandarin and "Minneola" tangelos, of pH in "Shamouti" oranges and "Minneola" tangelos, and of acidity in "Salustiana" oranges. These changes, although significant, did not affect fruit sensorial properties (taste, sweetness, acidity and aroma), as revealed by an informal tasting in the laboratory (data not shown).

Table 3  
Evolution of flavonoid and ascorbic acid contents in citrus segments during storage at 4 °C

Fruits	Days	Flavonoids <sup>a</sup> (mg/g of dry matter)							Ascorbic acid (mg/g dry matter)
		Narir.	Hesp.	Didym.	Naring.	Neohesp.	Poncir.	Totals	
Shamouti orange	0	0.588b	0.887b	0.066b	–	–	–	1.54b	5.10a
	4	0.593b	2.28a	0.077b	–	–	–	2.95a	5.14a
	8	0.558b	2.28a	0.078b	–	–	–	2.91a	5.00a
	12	0.782b	2.24a	0.100a	–	–	–	3.12a	5.02a
Significance		**	**	**				**	ns
Palazzelli mandarin	0	0.269a	0.489b	0.017c	–	–	–	0.775b	2.38a
	4	0.212a	1.11a	0.022b	–	–	–	1.34a	2.23a
	8	0.243a	1.16a	0.028a	–	–	–	1.43a	2.26a
	12	0.243a	1.04a	0.028a	–	–	–	1.31a	2.29a
Significance		ns	**	**				**	*
Minneola Tangelo	0	0.521c	1.32c	0.105c	–	–	–	1.95d	1.63a
	4	0.630b	1.42bc	0.135a	–	–	–	2.18c	1.56a
	8	0.546c	2.25a	0.101c	–	–	–	2.89a	1.57a
	12	0.743a	1.54b	0.121b	–	–	–	2.40b	1.35b
Significance		**	**	**				**	**
Salustiana orange	0	0.828b	2.06a	0.146a	–	–	–	3.03a	4.53a
	4	0.727b	1.95ab	0.086c	–	–	–	2.76b	4.46a
	8	0.620c	1.86b	0.096c	–	–	–	2.57c	4.43a
	12	1.89a	1.36c	0.120b	–	–	–	2.56c	4.02b
Significance		**	**	**				**	**

Means in each column within each product with the same letter do not differ significantly according to Duncan's multiple range test at  $P < 0.01$  or  $P < 0.05$ . ns, not significant.

<sup>a</sup> Narirutin (Narir.), Hesperidin (Hesp.), Didymin (Didym.), Naringin (Naring.), Neohesperidin (Neohesp.) and Poncirin (Poncir.).

\*  $P$  significant at 1% level; \*\*  $P$  significant at 5% level.

Table 4  
Changes of flavonoids and ascorbic acid content in citrus juices during storage at 4 °C

Fruits	Days	Flavonoids <sup>a</sup> (mg/g of dry matter)							Ascorbic acid (mg/g dry matter)
		Narir.	Hesp.	Didym.	Naring.	Neohesp.	Poncir.	Totals	
Shamouti orange	0	0.749a	1.03a	0.085a	–	–	–	1.86a	5.50a
	5	0.642bc	0.480b	0.046b	–	–	–	1.17b	5.26a
	10	0.669b	0.464b	0.046b	–	–	–	1.18b	5.47a
	15	0.599c	0.431b	0.041b	–	–	–	1.09b	5.51a
Significance		*	**	**				**	ns
Salustiana orange	0	0.855a	2.33a	0.163a	–	–	–	3.35a	4.27a
	5	0.682b	0.538b	0.058b	–	–	–	1.28b	4.25a
	10	0.696b	0.494b	0.049b	–	–	–	1.24b	3.93b
	15	0.682b	0.454b	0.045b	–	–	–	1.18b	3.71c
Significance		*	**	**				**	**
Red blush grapefruit	0	2.25b	0.242a	0.096b	5.24a	0.240a	0.271a	8.32bc	4.22a
	5	2.52a	0.248a	0.110a	5.93a	0.250a	0.311a	9.37a	4.22a
	10	2.12b	0.223b	0.095b	4.99a	0.236a	0.272a	7.92c	4.22a
	15	1.90c	0.206c	0.096b	4.60a	0.204a	0.271a	7.27d	4.30a
Significance		*	*	**	ns	ns	ns	*	ns

Means in each column within each product with the same letter do not differ significantly according to Duncan's multiple range test at  $P < 0.01$  or  $P < 0.05$ . ns, not significant.

<sup>a</sup> Narirutin (Narir.), Hesperidin (Hesp.), Didymin (Didym.), Naringin (Naring.), Neohesperidin (Neohesp.) and Poncirin (Poncir.).

\*  $P$  significant at 1% level; \*\*  $P$  significant at 5% level.

### 3.2. Changes of flavonoids, ascorbic acid and antioxidant capacity in citrus segments and juices

Variations in the amounts of flavanone glycosides, ascorbic acid and antioxidant capacity are shown in Tables 3, 4 and 6. The segments showed a significant increase in total flavonoids, mainly hesperidin, during storage. Anomalous behaviour was observed in the “Salustiana” orange which, from the first samples, showed a significant decrease in total flavonoid content, although narirutin content increased.

A decrease in the amount of single flavonoids, and therefore in the total flavonoid content, was found in the orange juices. Grapefruit juices showed significant differences only for narirutin, hesperidin and total content (Table 4). The results on the citrus fruit segments and juices are in agreement with those reported by other authors for minimally processed vegetables (Peiser, Lopez-Galvez, Cantwell, & Saltveit, 1998; Rolle & Chism, 1987; Supri, Persons, & Ross, 1998). Some authors have demonstrated that wounds and exposure to ethylene stimulate PAL activity with consequent further production of the main phenolic compounds and synthesis of new polyphenolic substances (Ke & Saltveit, 1989; Tomas-Barberan, Loaiza-Velarde, Bonfanti, & Saltveit, 1997). In our case, the preliminary preparation (although not as drastic as that of other products) and the high ethylene concentration inside the boxes (Table 5) could have caused the marked increase in flavonoid content. This increase was not observed in the “Salustiana” orange segments.

Segments of Minneola and Salustiana cultivars showed a significant decrease in ascorbic acid (AA) only in the last sample taken. A lower AA content was found in Salustiana juices from the third sampling. These results are in agreement with previous data obtained in our laboratory for other citrus fruit varieties (Piga, Gambella, Agabbio, & Nicoli, 2002) and with other reports in the literature. Trifirò, Gherardi, and Calza (1995) describe a maximum decrease of 8% in AA in fresh juices of blood oranges stored at 3 °C, although these juices were pasteurised and stored for longer than the fruits in the present study (30 days). The methods we used, including the type of packaging, the low temperature during storage and the reduced space left at the top of the test tubes, helped to maintain AA content fairly constant.

Table 6 shows the changes in antioxidant capacity, expressed as TEAC. Different behaviour can be seen between segments and juices. There was a slight decrease in TEAC values of some segments, either throughout the test or in the first 8 days of storage (as in the “Palazzelli” mandarin and the “Shamouti” orange), and a more significant decrease in segments of Minneola tangelos. On the other hand, “Salustiana” segments showed a marked increase but only at the end of shelf life. TEAC values of the fresh juices increased slightly in

the case of “Shamouti” oranges but decreased in “Salustiana” oranges, while a significant increase was observed in “Red blush” grapefruit. The correlation between TEAC values and AA content of citrus fruit segments showed the fairly high value of  $r = 0.968$ , again confirming that AA is the main antioxidant in fruits of the *Citrus* genus. The level decreased slightly ( $r = 0.889$ ) in the juices (Arena, Fallico, & Maccarone, 2001; Gardner, White, McPhail, & Duthie, 2000). Values were much lower when TEAC values were correlated with total flavonoid content (data not shown).

In each sample, the contribution of the total flavonoids to antioxidant activity, which was calculated by multiplying the concentration of each compound in the sample by its TEAC value, was much lower than the contribution of ascorbic acid (Table 6). TEAC values obtained by a 1 mM standard solution were the following: 1.11 for ascorbic acid, 0.01 for naringin and narirutin, 0.07 for hesperidin and 0.04 for neohesperidin, while the value was 0 for poncirin and didymin. This could be due to the low antiradical activity of the flavanones because of their chemical structure (Burda & Oleszek, 2001). Furthermore, TEAC values for the

Table 5  
Evolution of CO<sub>2</sub>, O<sub>2</sub> and C<sub>2</sub>H<sub>4</sub> in-package concentrations of minimally processed citrus segments during 12 days of storage at 4 °C

Fruits	Sampling (day)	CO <sub>2</sub> (%)	O <sub>2</sub> (%)	C <sub>2</sub> H <sub>4</sub> (mg/L)
Shamouti orange	1	2.39	18.4	0.05
	2	6.06	15.7	0.43
	4	5.59	16.2	0.77
	6	4.65	17.2	0.89
	8	3.49	18.6	1.24
	11	1.85	19.2	1.36
	12	1.63	19.3	1.32
Palazzelli mandarin	1	3.63	17.0	0.15
	2	8.30	11.7	1.12
	4	8.28	11.8	1.08
	6	7.03	13.9	1.28
	8	4.70	17.0	1.47
	11	3.01	18.0	2.18
	12	3.56	17.5	1.79
Minneola tangelo	1	5.98	15.9	0.20
	2	5.51	15.4	0.41
	4	4.63	17.1	0.58
	6	3.40	18.1	0.88
	8	2.06	19.3	1.19
	11	1.18	19.9	1.41
	12	1.03	20.0	1.50
Salustiana orange	1	1.27	20.2	0.04
	2	0.95	20.0	0.03
	4	0.79	20.2	0.05
	6	0.56	20.4	0.05
	8	0.69	20.4	0.16
	11	0.47	20.6	0.14
	12	0.52	20.3	0.11



Table 6  
Changes of calculated<sup>a</sup> and measured TEAC of minimally processed segments and juices of different *Citrus* species during cold storage

Fruits	Days	TEAC A.A. × conc A.A. (mM)	TEAC Flav. × conc. Flav. (mM)	Sum of calculated TEAC <sup>a</sup>	Measured TEAC
Shamouti orange (segments)	0	4.05	0.014	4.06	4.50a
	4	3.94	0.033	3.97	4.29a
	8	3.95	0.034	3.98	4.36a
	12	3.86	0.033	3.89	4.34a
Significance					ns
Palazzelli Mandarin (segments)	0	2.26	0.009	2.27	2.74a
	4	2.20	0.020	2.22	2.59bc
	8	2.13	0.021	2.15	2.57c
	12	2.25	0.019	2.27	2.73ab
Significance					*
Shamouti orange (juices)	0	4.43	0.017	4.45	4.69a
	5	4.39	0.009	4.40	4.86a
	10	4.43	0.008	4.44	4.81a
	15	4.39	0.008	4.40	4.80a
Significance					ns
Minneola tangelo Mandarin (segments)	0	1.47	0.023	1.49	2.35a
	4	1.33	0.024	1.35	1.81b
	8	1.37	0.037	1.40	1.73b
	12	1.15	0.025	1.17	1.58b
Significance					**
Salustiana orange (segments)	0	3.97	0.035	4.00	4.10b
	4	3.68	0.031	3.71	3.83b
	8	3.52	0.029	3.55	3.81b
	12	3.46	0.024	3.49	4.51a
Significance					**
Salustiana orange (juices)	0	3.54	0.037	3.58	4.12a
	5	3.56	0.010	3.57	3.92b
	10	3.24	0.009	3.25	3.68c
	15	3.08	0.008	3.09	3.52d
Significance					**
Red blush grapefruit (juices)	0	2.83	0.018	2.85	3.47b
	5	2.82	0.020	2.84	4.29a
	10	2.84	0.017	2.86	4.26a
	15	2.82	0.015	2.83	4.26a
Significance					**

Means in each column within each product with the same letter do not differ significantly according to Duncan's multiple range test at  $P < 0.01$  or  $P < 0.05$ . ns, not significant.

<sup>a</sup> Sum of AA and flavonoids TEAC calculation was made by multiplying the concentration of each compound by its TEAC value.

\*  $P$  significant at 1% level; \*\*  $P$  significant at 5% level.

flavanones, obtained by using the radical DPPH, were lower than those obtained with the radical ABTS (Gardner, White, McPhail, & Duthie, 2000). It is likely that DPPH, despite being recommended as an easy, accurate method for measuring antioxidant activity of fruit and vegetable juices, is less sensitive to hydrophilic antioxidants (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000). It is noteworthy that the TEAC measured was always higher than that calculated. This difference may be due to other phenolic compounds that were not determined. A possible explanation for the marked increase in TEAC in grapefruit juices could be

(a) the higher antioxidant potential of the polyphenols in intermediate stages of oxidation (Cheigh, Um, & Lee, 1995) or (b) the neof ormation of hydroxycinnamic acids due to PAL and C4H activity. However, this is temporary because, at a more advanced stage of oxidation, the molecules gradually lose this property, and there is a drastic reduction in antioxidant capacity.

#### 4. Conclusion

The results of this study indicate that the main chemical parameters and AA content of the species and varieties of fruits tested are not always affected by minimal processing and then only after lengthy storage. Flavonoid changes differ on the basis of the kind of preparation. Flavonoids decrease in juices and increase in segments, with the exception of the Salustiana orange. These data demonstrate, therefore, that segments retain their nutritional and health-giving properties, which actually increase in some cases after minimal processing. However, further studies are needed to explain the mechanisms of flavonoid increase.

#### References

- AOAC. (1990). In: Horwitz, E. (Ed.), *Official methods of analysis*. AOAC International.
- Arena, E., Fallico, B., & Maccarone, E. (2001). Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. *Food Chemistry*, 74, 423–427.
- Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanism of antioxidant activity using the DPPH• free radical method. *Lebensmittel-Wissenschaft und Technologie*, 30, 609–615.
- Burda, S., & Oleszek, W. (2001). Antioxidant and Antiradical activities of flavanoids. *Journal of Agriculture and Food Chemistry*, 49, 2774–2779.
- Cheigh, H. S., Um, S. H., & Lee, C. Y. (1995). Antioxidant characteristics of melanin-related products from enzymatic browning reaction to catechin in a model system. ACS Symposium Series 600. *American Chemical Society*, 200–208.
- De Ancos, B., Gonzales, E. M., & Cano, M. P. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agriculture and Food Chemistry*, 48, 4565–4570.
- Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contribution of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, 68, 471–474.
- Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agriculture and Food Chemistry*, 48, 4581–4589.
- Gil-Izquierdo, A., Gil, M. I., Ferreres, F., & Tomas-Barberan, F. (2001). In vitro availability of flavanoids and other phenolics in orange juice. *Journal of Agriculture and Food Chemistry*, 49, 1035–1041.
- Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables—the millennium's health. *International Journal of Food Science and Technology*, 36, 703–725.

- Ke, D., & Saltveit, M. E. (1989a). Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiologia Plantarum*, *76*, 412–418.
- Kefford, J. F., & Chandler, B. V. (1970). General composition of citrus fruits. In Kefford, J. F., Chandler, B. V. (Eds.), *The chemical constitution of citrus fruits* (pp. 5–22). Academic Press, Orlando, FA.
- Matto, A. K., & Anderson, J. D. (1984). Wound-induced increase in 1-aminocyclopropane-1-carboxylic synthase activity: regulatory aspects and membrane association of the enzyme. In Y. Fuchs, & E. Chalutz (Eds.), *Ethylene: biochemical, physiological and applied aspects* (pp. 139–147). The Hague: Martinus Nijhoff/Dr. W. Junk.
- Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high-performance liquid chromatography: a review. *Journal of Agriculture and Food Chemistry*, *48*, 577–599.
- Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, *84*, 407–412.
- Mouly, P., Gaydou, E. M., & Auffray, A. (1998). Simultaneous separation of flavanone glycosides and polymethoxylated flavones in citrus juices using liquid chromatography. *Journal of Chromatography A*, *800*, 171–179.
- Nogata, Y., Ohta, H., Yoza, K., Berhow, M., & Hasegawa, S. (1994). High-performance liquid chromatographic determination of naturally occurring flavonoids in Citrus with a photodiode-array detector. *Journal of Chromatography A*, *667*, 59–66.
- Peiser, G., López-Gálvez, G., Cantwell, M., & Saltveit, M. E. (1998). Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. *Postharvest Biology & Technology*, *14*, 171–177.
- Piga, A., D'Aquino, S., Agabbio, M., & Continella, G. (1996). Effect of packaging and coating on fruit quality changes of loquat during three cold storage regimes. *Advances in Horticultural Science*, *3*, 120–125.
- Piga, A., Gambella, F., Agabbio, M., & Nicoli, M. C. (2002). Retention of antioxidant activity in minimally processed mandarin and satsuma fruits. *Lebensmittel-Wissenschaft und Technologie*, *35*, 344–347.
- Rolle, R. S., & Chism, G. W. III (1987). Physiological consequences of minimally processed fruits and vegetables. *Journal of Food Quality*, *10*, 157–177.
- Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, 2073S-2085S.
- Supri, J. L., Persons, K., & Ross, E. (1998). Degradation of flavonoids in orange and grapefruit juices. *IFT'S Annual Meeting*, June 20–24.
- Tomas-Barberan, F. A., Loaiza-Velarde, J., Bonfanti, A., & Saltveit, M. E. (1997). Early wound- and ethyl-induced changes in phenylpropanoid metabolism in harvested lettuce. *Journal of American Society for Horticultural Science*, *122*, 399–404.
- Trifirò, A., Gherardi, S., & Calza, M. (1995). Effects of storage time and temperature on the quality of fresh juices from pigmented oranges. *Industria delle Conserve*, *70*(3), 243–251.
- Watada, A. E., Abe, K., & Yamauchi, N. (1990). Physiological activities of partially processed fruits and vegetables. *Food Technology*, *XX*, 116, 118, 120–122.
- Yildiz, F. (1994). Initial preparation, handling and distribution of minimally processed refrigerated (MPR) fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruit and vegetables* (pp. 15–65). Orlando, FL, USA: Chapman and Hall.