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Provitamin A carotenoids and ascorbic acid contents of the different types of orange juices marketed in Spain

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Abstract

The vitamin C and provitamin A carotenoids contents of 25 commercially available Spanish orange juices were studied. Large differences in the levels of these compounds were found. On average, ultrafrozen orange juices (UFOJ) and orange juices from the ecological agriculture (OJFEA) showed the highest ascorbic acid contents (518 and 412 mg/l respectively) among the different kinds of orange juices studied. Some disagreement between the declared and the actual amounts of vitamin C were found. Provitamin A carotenoids were determined by means of the corresponding standards. The monohydroxycarotenoid accompanying β-cyptoxanthin in orange juices was identified as the non-provitamin A carotenoid, zeinoxanthin, on the basis of the methylation test with acidified methanol. Unusually high contents of β-carotene (>0.5 mg/l) were found in two samples, which could indicate that substantial amounts of the pigment were added to those juices. The mandarin juice analyzed showed the highest provitamin A activity (359.3 retinol activity equivalents/l) Among the orange juices surveyed, UFO proved to be the best source of provitamin A (78.5 retinol activity equivalents/l, on average). The lowest contents were found in orange juices from concentrate (OJFC) (22.4 retinol activity equivalents/l, on average, without considering the orange juices with unusual β-carotene contents).

Keywords: Ascorbic acid; C_{30}; Carotenoids; Orange juice; Provitamin A; Retinol activity equivalents (RAE); Vitamin A; Vitamin C

1. Introduction

Nowadays, it is commonly accepted that diets with high contents of fruits and vegetables are protective against several human diseases, some of which are especially serious, such as cardiovascular diseases and cancer. Owing to the fact that many studies seem to reveal that these protective effects may be a result of the intake of antioxidants, more attention is being focussed on potentially antioxidant substances, such as carotenoids and vitamin C (Mathews-Roth, 1991; Rock, Fada, Jacob, & Bowen, 1996; Halliwell, 1997; Fraser & Bramley, 2004; Meléndez-Martínez, Vicario, & Heredia, 2004). Despite the fact that there are no conclusive in vivo studies in this field yet, consumption of high amounts of fruits and vegetables on a daily basis is being recommended (Food & Nutrition Board, 2000).

Carotenoid pigments, apart from being responsible for the colour of a wide variety of foods and their likely protective role in human diseases, are important, from a nutritional point of view, due to the fact that some of them have provitamin A activity (Isler, 1971; Simpson & Chichester, 1981; Simpson, 1983). Although approximately 700 carotenoids have been reported (Britton, Liaaen-Jensen, & Pfander, 2004), only those with an unsubstituted β-ring with an 11-carbon polyene chain, have provitamin A activity. This structural requirement is satisfied by around 60 carotenoids (Rodriguez-Amaya, 2001). Vitamin A, that is, retinol (Fig. 1), can also be provided in the diet as other preformed forms (retinyl ester, retinal, 3-dehydroretinol, and retinoic acid) from foods of animal origin (liver, dairy products), or as provitamin A carotenoids, which are subsequently transformed into vitamin A (Rodriguez-Amaya, 1997).

L-Ascorbic acid is one of the most important organic acids in fruits and vegetables, in relation to the nutritional

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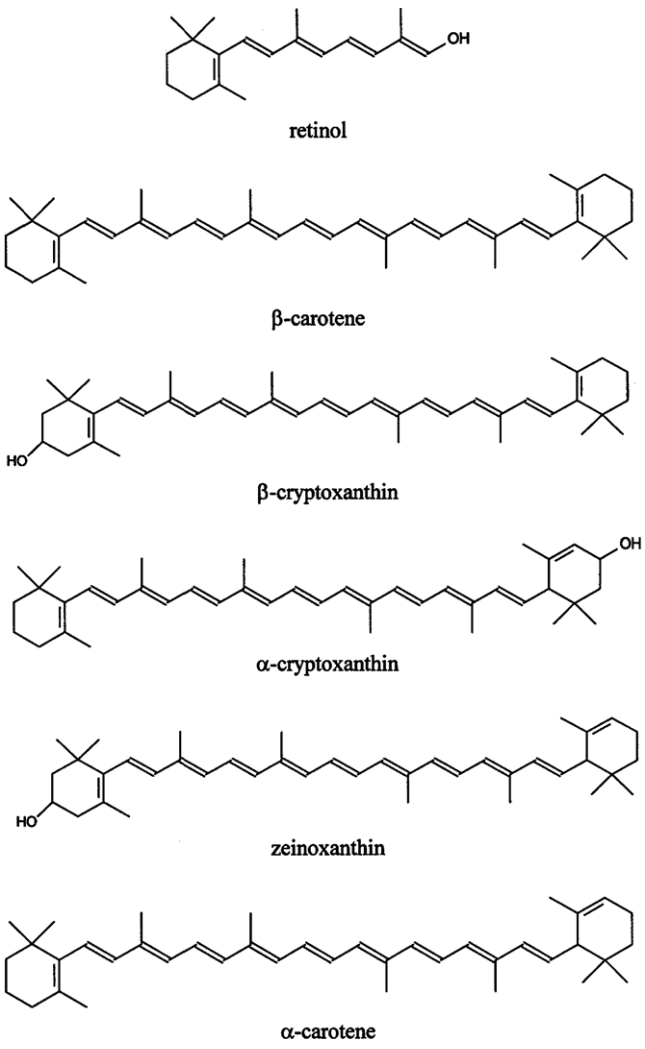


Fig. 1. Chemical structures of retinol, some provitamin A carotenoids and zeinoxanthin.

value of these foodstuffs. Its content has not only been used as a nutritional index, but also for evaluating processing effects, since it is highly unstable (Rock et al., 1996; Rojas-Hidalgo, 1998). Due to the fact that ascorbic acid is easily oxidized, many of its functions and activities are known to be based primarily on its properties as a reversible biological reductant. Aside from its antiscorbutic activity and its likely role in the serious human diseases mentioned above, ascorbic acid is involved in many other biological processes, such as the inhibition of enzymatic browning and the formation of nitrosamines, reduction of metallic ions and improvement of the stability and utilization of folic acid and vitamin E, among others (Rock et al., 1996; Rojas-Hidalgo, 1998).

Studies on the vitamin content of foods are of vital importance in relation to the control of nutritional labels, the update of food databases and the establishment of dietary reference intakes. In this sense, carotenoids are becoming increasingly important (Food & Nutrition Board, 2002; Dixon, Zimmerman, Kahle, & Subar, 2003). As a consequence of these and other needs, both vitamin C and carotenoids have been simultaneously determined in several recent studies (Assunçao & Mercadante, 2003; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003; Simonne, Simonne, Eitenmiller, Mills, & Green, 1997). Orange juice is probably the most globally accepted fruit juice and it is recognized worldwide as a good source of both ascorbic acid and provitamin A carotenoids (Nagy, 1980; Stewart, 1977). Due to these facts, this work is aimed at evaluating the contents of these vitamin compounds in the different types of orange juices marketed in Spain.

2. Materials and methods

2.1. Description of the samples

Twenty-four orange juices and one mandarin juice, corresponding to common brands retailed in Spain were analyzed. According to the information supplied on the labels, 12 of the samples studied were orange juices from concentrate (OJFC), nine (including the mandarin juice) were orange juices catalogued as juices from squeezed oranges (OJFSO), two were juices from ecological agriculture (OJFEA) and two were ultrafrozen orange juices (UFOJ).

Both OJFEA were labelled as orange juices from squeezed oranges, although, due to the peculiarities of this kind of agriculture, they were considered as a further kind of orange juice. Since there is only one brand that markets ultrafrozen orange juice, two samples from different batches were analyzed. The remaining samples were purchased in Seville, from supermarket firms established all over Spain.

OJFC are prepared by reconstituting concentrated orange juices, refrigerated or frozen, with water, which had been previously removed by means of concentration processes. This kind of orange juice is subjected to pasteurization, so its shelf-life is quite long and refrigerated storage is not normally necessary during retailing.

Squeezed (not from concentrate) orange juice can be marketed after being subjected to pasteurization or just as freshly-squeezed orange juices (Farnworth, Lagacé, Couture, Yaylayan, & Stewart, 2001; Pupin, Dennis, & Toledo, 1999). Its shelf-life is shorter than that corresponding to OJFC.

Orange juices from ecological agriculture are those obtained according to the principles of this kind of agriculture, such as the avoidance of the use of synthetically-compounded fertilizers, pesticides and growth regulators. Rejection of these substances is based on their potential adverse impacts on soil organisms, wildlife, livestock and human health.

Ultrafrozen orange juice is a recently developed type of orange juice. In the industry, the juice is cooled and subsequently frozen by means of liquid nitrogen. The commercial samples must be kept at (at least) −18 °C. Ascorbic acid is not externally added to this kind of juice.

Recommendations regarding the storage of the juices were followed up to the analysis of the samples. These

recommendations are summarised in Table 1 because they are related to the industrial processing of the juices. Ultra-frozen orange juices were thawed at room temperature.

2.2. Analysis of ascorbic acid

2.2.1. 2,6-Dichlorophenolindophenol titration

The titration method is based on the reduction of the sodium salt of the blue dye 2,6-dichlorophenolindophenol by ascorbic acid, resulting in the formation of a colourless derivative and dehydroascorbic acid. The endpoint of titration is indicated by the persistence of the pink colour of the solution (AOAC International, 1995).

2.2.2. Sample preparation

Equal volumes (5 ml) of orange juice and metaphosphoric acid as an aqueous solution (3% w/v) were mixed and subsequently centrifuged at 5000 rpm for 5 min. Five-millilitre aliquots of the supernatant were recovered and further diluted with the metaphosphoric acid solution before titration, according to a previous study (Meléndez-Martínez, Rodríguez, Bejines-Mejías, & Heredia, 2001). Samples were analyzed in duplicate.

2.3. Analysis of provitamin A carotenoids

2.3.1. High-performance liquid chromatography

HPLC analyses were carried out by means of a Hewlett–Packard 1100 system, consisting of a quaternary pump, a photodiode array detector and a column temperature control module (Hewlett-Packard, Palo Alto, CA, USA). A 20 μl loop and a C_{30} YMC column (5 mm, 250 × 4.6 mm) (Wilmington, NC, USA) were used. The column was kept at 17 °C and the flow rate was 1 ml/min. The diode array detector was set at 450 nm. The gradient elution was the same described elsewhere (Mouly, Gaydou, Lapierre, & Corsetti, 1999): 0 min: 90% MeOH + 5% MTBE + 5% water; 12 min: 95% MeOH + 5% MTBE; 25 min: 89% MeOH + 11% MTBE; 40 min: 75% MeOH + 25% MTBE; 60 min: 50% MeOH + 50% MTBE; 62 min: 90% MeOH + 5% MTBE + 5% water. MeOH and MTBE contained a small proportion of BHT (0.1%) and triethylamine (0.05%) in order to protect the carotenoids during the chromatographic analysis (Hart & Scott, 1995).

2.3.2. Sample preparation

Ten-millilitre aliquots of UFOJ or 25 ml aliquots of the remaining types of orange juices were gently mixed with 50 ml of the extracting solvent (methanol/acetone/hexane, 25:25:50, v/v/v, containing 0.1% butylated hydroxytoluene (BHT)) and the resulting mixture subsequently centrifuged for 10 min at 4000 rpm. The top layer of hexane, containing the carotenoid pigments, was recovered and washed four times with water to remove any trace of acetone. Saponification was carried out by adding 25 ml of ethanolic KOH (10% w/v). After 1 h, the reaction was stopped by adding aqueous NaCl (10%) to remove the alkali. The carotenoid extract was washed three more times. The

Table 1
Storage conditions, ascorbic acid contents and retinol activity equivalents for provitamin A carotenoids of the orange juices analyzed

Sample	Type	Storage conditions	Ascorbic acid content (mg/L)	RAE/L orange juice[a]
1	UFOJ	⩽−18 °C	546	66.3
2	UFOJ	⩽−18 °C	490	90.8
3	OJFC	In fridge	227	19.7
4	OJFC	In fridge	433	27.3
5	OJFC	In fridge	196	20.0
6	OJFC	In fridge	333	43.5
7	OJFC	In fridge	374	40.9
8	OJFC	Room temperature	461	14.8
9	OJFC	Room temperature	511	11
10	OJFC	Room temperature	324	16.4
11	OJFC	Room temperature	634	9.69
12	OJFC	Room temperature	307	20.2
13	OJFC	Room temperature	200	64.5
14	OJFC	Room temperature	284	94.8
15	OJFSO[b]	In fridge	350	359
16	OJFSO	In fridge	247	52.7
17	OJFSO	In fridge	308	71.3
18	OJFSO	In fridge	292	89
19	OJFSO	In fridge	197	23.0
20	OJFSO	In fridge	440	37.7
21	OJFSO	In fridge	276	52.7
22	OJFSO	Room temperature	584	32.2
23	OJFSO	Room temperature	502	20.4
24	OJFEA[c]	Room temperature	382	41.3
25	OJFEA[c]	Room temperature	442	63.0

[a] RAE referred to 1 litre of orange juice.
[b] Mandarin juice.
[c] Both OJFEA were labelled as orange juices from squeezed oranges.

hexane extract was concentrated to dryness in a rotary evaporator at a temperature below 35 °C and the carotenoids were re-dissolved in 1 millilitre of a mixture acetone:methanol (1:2, v/v, containing 0.1% BHT). Prior to injection in the HPLC system, the extract was filtered through Millipore PVDF Millex© filters (13 mm × 0.45 μm) (Bedford, MA, USA). Samples were analyzed in duplicate.

2.3.3. Identification of provitamin A carotenoids

The identification of β-cryptoxanthin, α-carotene and β-carotene (structures in Fig. 1) was done by comparison of their spectroscopic and chromatographic features with those of authentic standards.

β-cryptoxanthin was isolated from a saponified extract of red peppers (*Capsicum annuum* L.). TLC separation of the pigments was performed on plates of silica gel 60 GF_{254} (20 × 20 cm) (Merck, Darmstadt, Germany), using light petroleum ether (bp 65–95 °C)–acetone–diethylamine (10:4:1) as solvent system (Mínguez-Mosquera & Hornero-Méndez, 1993; Mínguez-Mosquera, 1997).

α-Carotene and β-carotene were isolated from palm oil. For this purpose, 10 ml of oil was dissolved in ethanol and aqueous KOH (1 g/ml) was added. Saponification was done overnight under nitrogen. The pigments were transferred to petroleum ether by adding distilled water and stirring carefully. The emulsion formed was broken by adding ethanol. The petroleum ether phase was washed three more times with water and evaporated to dryness in a rotary evaporator at a temperature below 35 °C. The dried extract was re-dissolved in a small volume of petroleum ether and chromatographed in a silica column with petroleum ether to obtain the fraction containing carotenes. This fraction was further chromatographed in an alumina column (activity grade I) with petroleum ether–diethyl ether (96:4) and subsequently with petroleum ether to obtain α-carotene and β-carotene, respectively. Purity of the standards, assessed by HPLC, was over 95%.

The monohydroxycarotenoid accompanying β-cryptoxanthin in orange juices, which has been tentatively identified as the provitamin A carotenoid α-cryptoxanthin (Fig. 1) in some studies (Rouseff, Raley, & Hofsommer, 1996; Mouly et al., 1999; Lee, Castle, & Coates, 2001; Meléndez-Martínez, Vicario, & Heredia, 2003), was identified as the non-provitamin A carotenoid zeinoxanthin (Fig. 1) on the basis of the results of the methylation test with acidified methanol (Meléndez-Martínez, Britton, Vicario, & Heredia, 2005).

2.3.4. Quantitative analysis

Absolute concentrations of the provitamin A carotenoids were obtained by external calibration, according to recommended guidelines (Kimura & Rodriguez-Amaya, 1999). For this purpose, four solutions of each standard were prepared, taking successively greater aliquots of the stock solutions. The concentrations of these solutions were determined spectrophotometrically using the following values of $A^{1\%}_{1\,cm}$: 2460 (at 450 nm in hexane) for β-cryptoxanthin, 2710 (at 445 nm in hexane) for α-carotene, and 2620 (at 450 nm in acetone) for β-carotene (Mínguez-Mosquera, 1997; Rodriguez-Amaya, 2001).

Limits of detection (LOD) were calculated from the equation of the calibration curves according to the formula $LOD = a + 2s_{y/x}$, where a is the intercept and $s_{y/x}$ the standard deviation of the calibration curve (Miller & Miller, 1993). Ranges of concentrations injected, fitting equations, coefficients of correlation and LOD are summarised in Table 2.

3. Results and discussion

3.1. Ascorbic acid content

Table 1 shows the ascorbic acid contents and the RAE/l of the samples analyzed in this study.

Titration with 2,6-dichlorophenolindophenol is a rapid, easy and cheap method, so it has been widely used for the determination of ascorbic acid, not only in foodstuffs, but also in pharmaceutical products, sometimes as a reference method for assessing the validity of new ones (Ijeri, Jaiswal, & Srivastava, 2001; Meléndez-Martínez et al., 2001; Piga, Agabbio, Gambella, & Nicoli, 2002; Rapisarda, Pannuzzo, Romano, & Russo, 2003). The main drawback of the method is that it cannot detect dehydroascorbic acid, which can be converted back to ascorbic acid (Rojas-Hidalgo, 1998). However, several studies in which both ascorbic acid and dehydroascorbic acid were assessed in orange juices revealed that the latter accounts for a small percentage of the vitamin C activity of the samples (around 5%) (Nisperos-Carriedo, Buslig, & Shaw, 1992; Behrens & Madére, 1994; Sánchez-Moreno et al., 2003). Experiments accomplished in our laboratory showed that centrifugation of the mixture orange juice-metaphosphoric acid, followed by further dilution with the acid solution led to virtually null errors between duplicate analyses (Meléndez-Martínez et al., 2001; Meléndez, Bejines, Vicario, & Heredia, 2004).

Table 2
Ranges of concentrations injected, wavelength of detection (λ), fitting equations, coefficients of correlation (r) and limits of detection (LOD)

Pigment	Range[a]	λ[b]	Fitting equation	r	LOD[a]
β-Cryptoxanthin	2.42–24.19	450	$y = 0.417 + 0.005x$	0.9991	0.064
α-Carotene	1.58–5.91	450	$y = -0.141 + 0.005x$	0.9992	0.043
β-Carotene	1.33–48.09	450	$y = -0.101 + 0.004x$	0.9999	0.131

[a] In mg/l.
[b] In nm.

Ascorbic acid contents of the orange juices studied ranged from 196 to 634 mg/l, with an average content of 374 mg/l. Such a wide range could be easily understood taking into account all the factors that can affect the levels of this nutrient, which, in addition, make any attempt to try to assertively explain these substantial differences useless. The vitamin C content of oranges depends on many factors, such as variety, mineral composition of the soil, stage of maturity, and climatic factors (Nagy, 1980). In relation to this, it is well-known that thermal processing of orange juice in the industry has a negative effect, not only on the levels of ascorbic acid, but also on other physical and chemical parameters (Farnworth et al., 2001; Nagy, 1980; Nagy, Chen, & Shaw, 1993). However, some interesting data were found in this study, which are useful for achieving an overall impression of the vitamin C content of the commercially available orange juices in Spain at a definite period.

The levels of vitamin C in the UFOJ studied (546 and 490 mg/l) were clearly higher than the general mean (374 mg/l), which may be due in part to the different industrial processing, with a complete absence of concentration processes, and to the storage conditions. In relation to this, it has been demonstrated, in frozen orange juices, that the content of ascorbic acid declined noticeably in both pasteurized and unpasteurized orange juices stored at a temperature of −18 °C or lower over a long term (Lee & Coates, 1999; Farnworth et al., 2001).

Mean content of ascorbic add in OJFC (357 mg/l) was slightly lower than the general average (374 mg/l). The orange juices with the lowest (196 mg/l) and the highest contents (634 mg/l) of the vitamin belonged to this group. Information regarding the vitamin C content of the juices was available in seven of them. In four cases, the actual amounts (432, 332, 373 and 633 mg/l); were considerably higher than those stated on the label (200, 200, 200 and 400 mg/l, respectively). The opposite was observed in the remaining three cases, the real concentrations being 227, 196, and 324 mg/l and the amounts declared 330, 330 and 400 mg/l, respectively. That is, these latter juices might not meet the intake of vitamin C expected by the consumers according to the nutritional information provided.

The average content of vitamin C found in the OJFSO analyzed (355 mg/l) was also slightly lower than the general average (374 mg/l). The range of vitamin levels was slightly narrower (197–584 mg/l) than that reported in OJFC (196–634.9 mg/l). In those juices in which nutritional information was provided by the manufacturers, actual vitamin C contents of two of them were higher (350 and 276 mg/l) than those stated on the label (300 and 240 mg/l, respectively). The opposite was observed in three cases, the actual levels being 307, 292 and 197 mg/l and those provided by the manufacturers 330, 400 and 240 mg/l respectively. In relation to these findings, it would be important to consider that consumers should also be aware of the stability of this nutrient throughout storage, distribution and retailing, since its levels can decrease dramatically. Losses of ascorbic acid ranging between 29% and 41% were found in different commercial fruit juices stored in closed containers at room temperature for 4 months. Significantly different decreased rates of vitamin C were also reported in the same study for long-life commercial orange juices and fresh orange juices stored in the refrigerator over one month (60% and 7%, respectively). Curiously, losses in the same juices under the same conditions, but stored in open containers, were only slightly higher (67% and 13%, respectively) (Kabasakalis, Siopidou, & Moshatou, 2000). Weekly losses of around 18.6% for control juices and 10.7% for fortified juices have recently been reported in pasteurized blood orange juices stored at 4.5 °C over a 7-week period, which may suggest that the total amount of ascorbic acid is an important factor in relation to its own stability (Choi, Kim, & Lee, 2002).

In the case of OJFEA, the vitamin C contents found (382 and 442 mg/l) were higher than the general mean (374 mg/l). Actual vitamin C contents were, in both cases, considerably higher than those stated on the label (300 mg/l).

The fact that, in some cases, the actual levels of vitamin C were higher than those declared, might indicate that ascorbic acid is added in excess to some samples in order to compensate for losses during retailing, or that the declared content is underestimated with the same purpose.

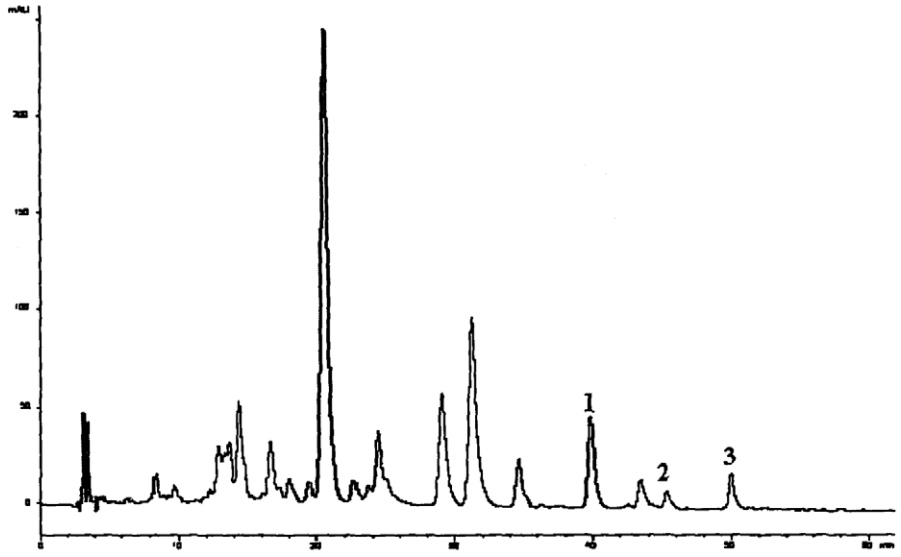
3.2. Provitamin A carotenoids content

A typical chromatogram of the carotenoids occurring in orange juices is shown in Fig. 2. Chromatographic and spectroscopic data of the provitamin A carotenoids are summarised in Table 3.

As mentioned for vitamin C, comparison between the carotenoid contents of the different juices is difficult, due to the numerous factors affecting carotenoid composition, such as climate, variety and stage of maturity (Stewart, 1977), among others (Lee & Castle, 2001) Table 4.

With respect to the industrial processing of orange juice, there is controversy about to the effect of pasteurization on the provitamin A carotenoid content. In a recent study (Lee & Coates, 2003), a significant decrease in the total amount of carotenoids has been reported after pasteurization at 90 °C for 30 s. Important losses of violaxanthin and antheraxanthin were observed, although the content of provitamin A carotenoids did not change considerably. In contrast, losses of provitamin A carotenoids (around 36%) were found in orange juices pasteurized at 80 °C for 2 min (Lessin, Catignani, & Schwartz, 1997). Studies on the effect of storage conditions on the carotenoid content of orange juices are scarce, although losses of 6.6% and 2.8% for control and fortified orange juices, respectively, have been recently reported over a 7-week refrigerated storage (Choi et al., 2002; Lessin et al., 1997).

β-Cryptoxanthin, which has been considered the main source of provitamin A in orange juices for some time (Stewart, 1977), was detected in all the samples analyzed. Except in two cases (samples 13 and 14), it was the major



Peak identification: 1. β-cryptoxanthin; 2. α-carotene; 3. β-carotene

Fig. 2. Typical chromatogram of ultrafrozen orange juice carotenoids plotted at 450 nm.

provitamin A carotenoid in absolute terms. The unusual high β-carotene contents of those samples, not only in relation to β-cryptoxanthin but also compared to the remaining carotenoids, could indicate that external β-carotene was added. The extremely high content of β-cryptoxanth in (7.52 mg/l) in the mandarin juice is noteworthy. Indeed, mandarin is considered to be the citrus fruit with the deepest colour, which is mainly attributed to its high content of β-cryptoxanthin (Farin, Ikan, & Gross, 1983).

α-Carotene was not clearly detected in some samples and was, in general, the provitamin A carotenoid occurring in the lowest proportion.

With respect to the occurrence of *cis* isomers of provitamin A carotenoids, small amounts were found in some samples.

The retinol activity equivalents (RAE) of the samples analyzed, referred to one litre of orange juice, are shown in Table 1. The bioavailability of carotenoids is influenced by many factors, such as amount, food matrix, age, existence of certain diseases or parasite infestation, intake of fat, vitamin E and fibre, protein and zinc status (Yeun & Russell, 2002; Food & Nutrition Board, 2002). According to this, it is clear that it is difficult to accurately determine the retinol activity equivalents (RAE) of any food, as has been recently discussed (Scott & Rodriguez-Amaya, 2000). In this study, calculations were performed, considering new guidelines (Food & Nutrition Board, 2000; Food & Nutrition Board, 2002), according to the following formula:

$$\text{RAE} = (\mu\text{g } \beta\text{-carotene}/12) + (\mu\text{g } \alpha\text{-carotene} + \mu\text{g } \beta\text{-cryptoxanthin}/24)$$

Table 3
Chromatographic and spectroscopic data of the provitamin A carotenoids

Peak	Carotenoid	Retention time (min)	Absorption maxima (nm)
1	β-Cryptoxanthin	39.90	452, 478
2	α-Carotene	45.39	424, 446, 474
3	β-Carotene	50.01	452, 478

Table 4
Provitamin A carotenoid contents (mg/l) in the 25 commercial orange juices analyzed

Sample	Type	β-Cryptoxanthin	α-Carotene	β-Carotene
1	UFOJ	1.02 ± 0.06	0.14 ± 0.01	0.21 ± 0.02
2	UFOJ	1.33 ± 0.07	0.19 ± 0.01	0.33 ± 0.02
3	OJFC	0.33 ± 0.02	0.03 ± 0.00	0.06 ± 0.00
4	OJFC	0.53 ± 0.02	0.03 ± 0.00	0.05 ± 0.00
5	OJFC	0.34 ± 0.07	0.03 ± 0.01	0.06 ± 0.01
6	OJFC	0.85 ± 0.11	0.03 ± 0.00	0.08 ± 0.01
7	OJFC	0.82 ± 0.05	0.02 ± 0.00	0.07 ± 0.01
8	OJFC	0.24 ± 0.01	0.02 ± 0.00	0.05 ± 0.00
9	OJFC	0.27 ± 0.00	n.d.	n.d.
10	OJFC	0.25 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
11	OJFC	0.20 ± 0.00	0.03 ± 0.00	n.d.
12	OJFC	0.33 ± 0.01	0.03 ± 0.00	0.06 ± 0.01
13	OJFC	0.16 ± 0.01	0.31 ± 0.01	0.54 ± 0.01
14	OJFC	0.31 ± 0.01	n.d.	0.98 ± 0.02
15	OJFSO[a]	7.52 ± 0.02	n.d.	0.55 ± 0.04
16	OJFSO	1.01 ± 0.06	0.06 ± 0.00	0.10 ± 0.01
17	OJFSO[b]	1.21 ± 0.02	0.12 ± 0.00	0.19 ± 0.01
18	OJFSO	1.39 ± 0.18	0.19 ± 0.05	0.28 ± 0.04
19	OJFSO	0.32 ± 0.01	0.05 ± 0.00	0.09 ± 0.00
20	OJFSO	0.73 ± 0.02	0.05 ± 0.00	0.06 ± 0.01
21	OJFSO	1.05 ± 0.06	0.04 ± 0.00	0.09 ± 0.00
22	OJFSO	0.77 ± 0.09	n.d.	n.d.
23	OJFSO	0.49 ± 0.04	n.d.	n.d.
24	OJFEA[b]	0.99 ± 0.06	n.d.	n.d.
25	OJFEA[b]	1.51 ± 0.05	n.d.	n.d.

[a] Mandarin juice.
[b] Both OJFEA were labelled as orange juices from squeezed oranges.

With the exception of the two orange juices containing unusual high levels of β-carotene, β-cryptoxanthin was the main source of provitamin A in the juice. The RAE/l content of the mandarin juice (359) stood out from the remainder to the extent that it was four times higher than that of the second most important juice in terms of provitamin A activity (sample 2, RAE = 90.8). RAE content of the juices surveyed ranged from 9.69 to 359 and the average content was 55.3.

Average RAE contents of UFOJ, OJFC, OJFSO and OJFEA were 78.5, 31.9, 82.0 and 52.2, respectively. Leaving out the juices containing unusually levels of β-carotene as well as the mandarin juice, RAE contents of the OJFC and OJFSO studied would range from 9.69 to 43.5 and from 20.4 to 89.1, respectively. Average contents would be 22.4 for OJFC and 47.4 for OJFSO. Taking into account these data, it can be observed that, in general terms, the UFOJ showed the highest provitamin A activity. Average RAE content of this kind of juice was over 1.5 times higher than that of OJFSO and 3.5 times higher than that of OJFC, which proved to be the type of orange juice with the lowest levels of provitamin A carotenoids. Average RAE content in OJFEA (52.2) was higher than those corresponding to OJFSO and OJFC but still quite low in comparison to that of UFOJ.

To sum up, it has been seen that, in general terms, the least common types of orange juices, OJFEA and, above all, UFOJ, seemed to be the best sources of ascorbic acid among the different kinds of orange juices considered. The comparison between the declared and the actual amounts of vitamin C revealed the existence of disagreements in most cases. Problems related to accuracy in quality control are easily understandable, although special care should be taken, as the content of such important nutrients is very often dramatically overestimated. In order to avoid supplying misleading information to consumers, further data ought to be provided by the industry, such as loss rates over storage. In addition, maybe some strategies applied in quality control, concerning the frequency of samples collection and the study of loss rates upon storage, should be carefully revised.

With respect to the provitamin A carotenoid contents of the samples surveyed, considering strictly orange juices, UFOJ proved to be the best source of provitamin A. Average RAE of this kind of juice was four times higher than that of OJFC.

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