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# Carotenoids, Color, and Ascorbic Acid Content of a Novel Frozen-Marketed Orange Juice

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A recently developed food, the so-called ultrafrozen orange juice (UFOJ), has been characterized in terms of carotenoid pigments, ascorbic acid, and color. The juice, obtained from Valencia late oranges, is frozen immediately after the squeezing of the oranges, which makes it a product showing good organoleptic and nutritional quality. In relation to the carotenoid profile, it was observed that the 5,6-epoxy carotenoids violaxanthin and antheraxanthin (specifically (9*Z*)-violaxanthin and (9*Z*)- or (9′*Z*)-antheraxanthin), were by far the major pigments and that dihydroxycarotenoids predominate over monohydroxycarotenoids. As far as color was concerned, it was seen that there were little differences among the juices analyzed. The hue of the samples, ranging from 77.19° to 80.15° and from 79.99° to 83.04° depending on the kind of instrumental measurement, and their chroma (ranging from 63.06 to 72.25 and from 44.40 to 58.38) revealed readily that the juice surveyed exhibited a deep orangeish coloration, the color coordinate best correlated with the total carotenoid content being *b**. The levels of ascorbic acid ranged from 332.64 to 441.44 mg/L, with an average content of 391.06 ± 28.86 mg/L.

**KEYWORDS: Ascorbic acid; carotenoids; CIELAB; color; ultrafrozen orange juice; Valencia oranges; vitamin C**

## INTRODUCTION

A large number of surveys conducted in the last years appear to reveal that diets rich in vegetables and fruits may be protective against certain human diseases, some of them, such as cancer or cardiovascular disease, being especially serious. These studies suggest that this protection may be due to the intake of antioxidants, so more attention is being paid to the assessment of potentially antioxidant species present in those sources, such as vitamin C, polyphenols, and carotenoids, among others (*1*−*5*). As a consequence of these facts, and despite that more sound in vivo studies in this field are still needed, consumption of high amounts of fruits and vegetables on a daily basis is being recommended (*6*, *7*).

Citrus products are known to be good sources of antioxidants species (*8*−*11*). For many years, the nutritional relevance of these foodstuffs resided almost exclusively in the fact that they were acknowledged as good sources of vitamin C (*12*), although a growing interest in them has arisen recently in relation to their content in carotenoids (*11*, *13*−*17*), some of which, such as the provitamin A ones α-carotene, *β*-carotene, and *β*-cryptoxanthin, as well as lutein and zeaxanthin, among others, are known to occur in human plasma and in other tissues (*18*−*21*). The citrus industry in general, and the orange juice industry in particular, has a great economic importance in Brazil, the United States, and the Mediterranean basin, Spain being the main producer in Europe (*8*, *15*, *22*, *23*). As far as the industrial processing is concerned, the usual practice for many years has been to obtain a concentrate from the juice, which was subsequently frozen in most cases and finally reconstituted with water of the appropriate quality to give the so-called orange juice from concentrate (OJFC), which still accounts for a large proportion of the orange juice market. Similarly, other kinds of orange juices are marketed after having been subjected to pasteurization treatments (*24*). In both cases, the juice undergoes thermal treatments that elongate its shelf life, but also lower notably its vitamin activity and deteriorate somehow its flavor, aroma, and color (*25*−*27*). Although these types of orange juices remain the most common, nowadays the consumers are demanding a more natural product, in consonance with their increasing concern about the wholesomeness of foodstuffs in general. Thus, at present, there is a renewed interest within the citrus industry in the development of innovative practices to meet the demand of orange juices of the highest quality, as a consequence of which a noticeable rise in the consumption of direct orange juices not subjected to thermal treatments has taken place in the last years (*24*, *26*, *28*). In response to these new trends, a further kind of orange juice, the so-called ultrafrozen orange juice (UFOJ), has been developed. The frozen orange juice analyzed in this survey is made out of oranges of the

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Valencia variety, acknowledged as the one of the highest quality and appreciated worldwide for having a deeper and more appealing coloration as a result of its higher content of carotenoid pigments relative to other varieties (*27*, *29*, *30*). The main novelty in the industrial processing of this juice lies in the fact that it is immediately frozen after the squeezing by using liquid nitrogen, which permits the juice to retain longer not only its appreciated organoleptic characteristics but also the levels of nutritionally important compounds. As a result of this industrial treatment that allows the preservation of the desirable characteristics of the product, UFOJ has been ranked the preferred of the different types of orange juices marketed in Spain in a wide variety of sensory trials (Bejines, personal communication). The goal of this study was to characterize this quality foodstuff in terms of ascorbic acid and individual carotenoids contents, with the aim of meeting the necessity of providing quantitative data on health-promoting substances in relation to the control of nutritional labels, the update of food databases, and the establishment of Dietary Reference Intakes (DRI). In this sense, carotenoids are becoming increasingly important (*31*, *32*) to the extent that they have been simultaneously determined alongside ascorbic acid in several recent studies (*11*, *33*−*37*). Furthermore, the color of the juice has been objectively assessed by Tristimulus colorimetry not only for the noteworthy deep orange color of the product and the fact that the color of citrus beverages influences greatly the consumers' choice (*38*−*41*) but also for the increasing interest that this property is arousing as a quality index in "carotenoid-containing" and other foods (*42*−*49*).

## MATERIALS AND METHODS

**Orange Juice Samples.** The orange juice analyzed is obtained from Valencia late oranges in an appropriate stage of maturity, specifically when the ratio of the sugars to the titratable acidity is equal to or higher than 12. This variety is worldwide appreciated for the production of orange juice not only due to its pleasant sensory attributes, but also because it contains very few seeds and yields more juice than other sweet orange juice varieties. After having been mechanically extracted, the juice is countercurrently injected with nitrogen to remove air and prevent oxidative processes and microbial contaminations. Next, the content of pulp is adjusted and the juice is cooled to around 0 °C. Finally, the product is bottled in high-density propylethylene plastic containers (1.15 L total capacity, 50 mL headspace) with rectangular sides (6.7 × 26 cm) and passed through a freezing tunnel using liquid nitrogen as frigorigene fluid, with which a rapid freezing is achieved. The UFOJ so manufactured does not contain additives, is not enriched with vitamins or any other kind of permitted substance, and is to be kept at a temperature equal to or below −18 °C over storage, delivery, and retailing.

For this study, 16 samples of UFOJ provided by the firm Zumos Vitafresh (Almonte, Huelva, Spain) as representative juices of the 2003 season (May−August 2003) were analyzed. The juices were stored as recommended until their analysis in August of 2004, reflecting in such a way the quality of the product that is actually accessible to the consumer, because before its distribution, the juice is normally stored in appropriate freezer chambers in the factory for several months. Prior to their analysis, the samples were defrosted at room temperature.

**Color Measurement.** The reflectance measurements were performed by means of a CAS 140 B spectroradiometer (Instrument Systems, Munich, Germany) fitted with a Top 100 telescope optical probe (Instrument Systems, Munich, Germany), a Tamron zoom mod. SP 23A (Tamron USA, Inc., Commack, NY), and an external incandescent lamp as source of illumination. A plastic cuvette (475 × 350 × 10 mm) was used for the measurements. Blank measurements were made with the cuvette filled with distilled water and placed against a reference $BaSO_4$ pressed plate (USRS-99-010, Labsphere Inc. North Sutton, NH). As it is a common practice for translucent foods, the samples were measured against a white background (reference $BaSO_4$ pressed plate) and a black background (round-shaped plastic piece with homogeneous black color) because it has also been reported that sometimes the color parameters obtained by using one or another background are differently correlated with the visual perception of the color and other parameters (*40*, *46*).

The entire visible spectrum (380−770 nm) was recorded with a bandwidth of 1 nm, and the Illuminant D65 and the 10° Observer were taken as references such that the apparatus returned the color parameters of the uniform color space CIELAB (CIE, 1978). A more detailed explanation of the procedure for the color assessment can be found elsewhere (*40*). The color data obtained were averages of three measurements.

**Ascorbic Acid Analysis.** The assessment of ascorbic acid was performed by titration with the blue dye 2,6-dichlorophenolindophenol (*50*). The method is based on the reduction of the sodium salt of the dye by ascorbic acid, resulting in the formation of dehydroascorbic acid and a colorless derivative. The endpoint of the titration is indicated by the appearance of a persistent pinkish color in the solution being titrated.

*Sample Preparation.* Equal volumes (5 mL) of UFOJ samples and aqueous metaphosphoric acid (3% w/v) were blended and centrifuged for 5 min at 5000 rpm. The supernatants were recovered, and aliquots of 5 mL were taken from them for the titrations, for which they were further diluted with the metaphosphoric acid solution (*51*). The samples were analyzed in duplicate.

**Carotenoid Analysis.** *Pigment Extraction from Orange Juice and Saponification.* Ten-milliliter aliquots of the samples were gently mixed with 50 mL of the extracting solvent (hexane/methanol/acetone, 50:25:25, v/v/v, containing 0.1% butylated hydroxytoluene) and centrifuged for 10 min at 4000 rpm. Upon centrifugation, the upper colored layers containing the carotenoid pigments were recovered and washed with water (4 × 25 mL) to remove any trace of acetone. To obtain saponified carotenoids, the extracts were treated with 25 mL of ethanolic KOH (10% w/v) for 1 h under dim light and at room temperature, after which they were washed with water (4 × 25 mL) to remove any trace of base. The colored hexane extracts obtained were concentrated to dryness in a rotary evaporator at temperature below 35 °C and redissolved in 1 mL of a mixture of acetone:methanol (1:2, v/v, containing 0.1% butylated hydroxytoluene). The concentrated extracts were filtered through Millipore PVDF Millex filters (13 mm × 0.45 μm) (Bedford, MA) prior to their injection in the HPLC system. The analyses were performed in duplicate.

*High-Performance Liquid Chromatography.* The HPLC analysis was carried out on an Agilent 1100 system consisting of a quaternary pump, a photodiode array detector, a column temperature control module, and an autosampler, which was set to draw 20 μL from the samples (Agilent, Palo Alto, CA). The pigments were separated on an YMC $C_{30}$ column (5 μm, 250 × 4.6 mm) (YMC, Wilmington, NC) kept at 17 °C. Methanol (MeOH), methyl-*tert*-butyl ether (MTBE), and water were used in the mobile phase. The linear gradient elution was the same as described elsewhere (*52*, *53*): 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 small proportions of butylated hydroxytoluene and triethylamine (0.1% and 0.05%, respectively) to protect the carotenoids over the HPLC analysis (*54*). The mobile phase was pumped at 1 mL/min, and the chromatograms were monitored at 430 nm.

*Identification of Carotenoids.* The identification of the great majority of the carotenoids detected was made by comparison of their chromatographic and UV/vis spectroscopic characteristics with those of standards either isolated from appropriate sources or semisynthesized in accordance to standard procedures (*55*−*57*). Thus, (9′*Z*)-neoxanthin, violaxanthin, and lutein were obtained from spinach leaves (*Spinacia oleracea* L.), β-cryptoxanthin and zeaxanthin from red peppers (*Capsicum annuum* L.), and α- and β-carotene from palm oil (*Elaeis guineensis* Jacq.). The antheraxanthin standard was semisynthesized by treating zeaxanthin with 3-chloroperoxybenzoic. Its purification was accomplished by thin-layer chromatography (TLC) on silica gel 60 $F_{254}$ plates (20 cm × 20 cm, 0.7 mm thick) (Merck, Darmstadt, Germany), using the mixture petroleum ether (65−95 °C)−acetone−diethylamine

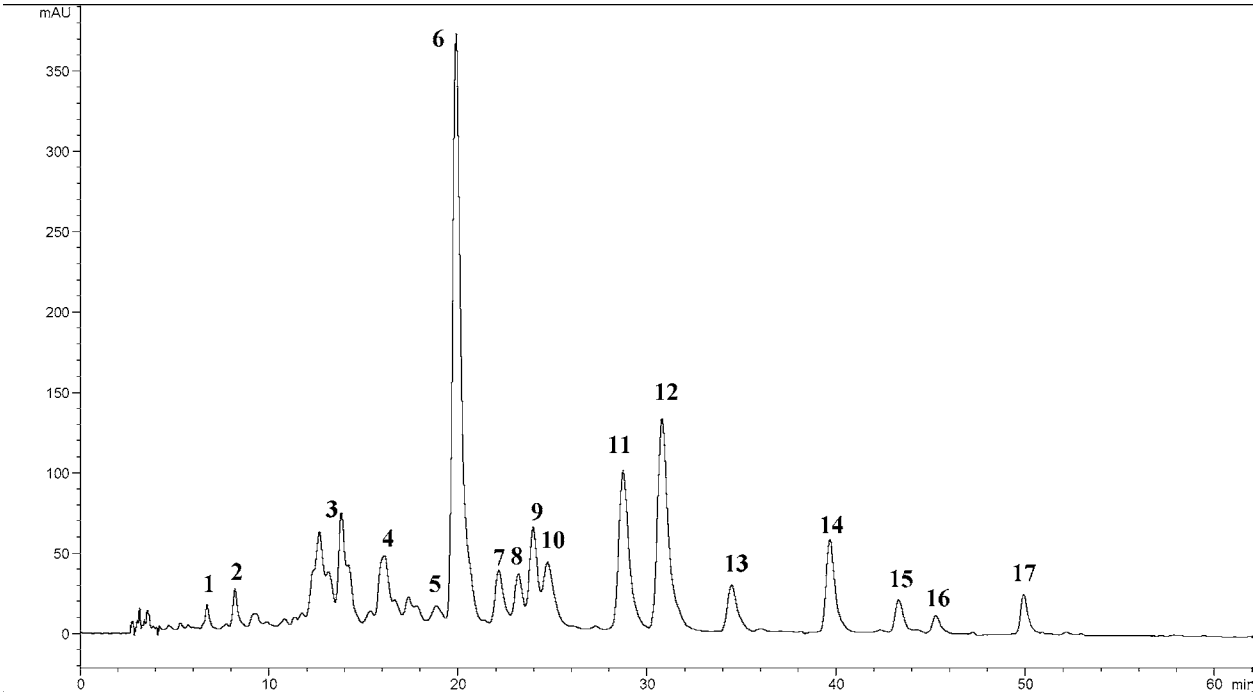


**Figure 1.** Chromatogram at 430 nm of a carotenoid extract from UFOJ. Peak identification in **Table 1**.

(10:4:1) (*56*) as mobile phase. The standards of 5,8-epoxycarotenoids were obtained from their 5,6-epoxy counterparts by treating the latter with a few drops of methanolic hydrochloric acid 0.1 M. Thus, luteoxanthin and auroxanthin were obtained from violaxanthin, mutatoxanthin from antheraxanthin, and neochrome from (9′*Z*)-neoxanthin.

For the identification of some other carotenoids, zeinoxanthin and different isomers of violaxanthin, antheraxanthin and ζ-carotene, appropriate fractions were isolated from the UFOJ itself as reported in other studies (*16*, *17*, *58*).

To help identify geometrical isomers, ethanolic solutions of the standards were heated for half an hour at 80−100 °C and subsequently subjected to strong illumination overnight by means of a powerful lamp. The glassware containing these carotenoid solutions was gently blanketed with nitrogen to prevent oxidation.

*Quantitative Analysis.* The absolute concentrations of orange juice carotenoids were worked out by external calibration, performed in compliance with recommended guidelines (*59*, *60*) from calibration curves constructed with the corresponding standards.

The levels of zeinoxanthin were calculated out of the calibration curve of lutein, due to them having the same chromophore and therefore virtually identical spectra. When coelution occurred, quantification was made by considering the calibration curve of the major carotenoid in the mixture, which was determined by studying carefully the shape of the average spectrum in the mobile phase. The total contents of carotenoids were assessed as the sum of the content of the individual pigments.

## RESULTS AND DISCUSSION

**Carotenoid Analysis.** *Identification.* A typical chromatogram of the carotenoids present in UFOJ is depicted in **Figure 1**, their chemical structures being illustrated in **Figure 2**.

Peaks 1 and 2 corresponded to minor carotenoids, which were not identified due to the lack of appropriate standards. The absorption spectrum of peak 1 had sharp well-defined bands with maxima at 372, 392, and 414 nm, which may be indicative that the unidentified compound may have a chromophore with 7 or 8 conjugated double bonds. The spectrum of peak 2 showed equally well-defined absorption bands, although their maxima were located at longer wavelengths, specifically at 418, 440, and 468 nm. Both its shape and the location of the absorption maxima were very similar to those of one of the peaks of the mixture of the geometrical isomers of neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol), specifically the isomer identified as all-(*E*)-neoxanthin, although this eluted clearly later (13.09 min). Such mixture of geometrical isomers was obtained after the stereomutation of the (9*Z*)-neoxanthin standard isolated from spinach leaves by heating and illuminating as explained in the Materials and Methods.

The occurrence of several geometrical isomers of violaxanthin (5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-caroten-3,3′-diol) and antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol) in orange juice has been reported in other works (*16*, *61*−*63*). As a consequence of the scarce chromatographic resolution between the peaks corresponding to all-(*E*)-violaxanthin and its mono- and di-(*Z*)-isomers (*64*), they were considered altogether for quantitative purposes (peak 3). The (9*Z*)-isomer, the major carotenoid in the samples (*64*), eluted clearly apart from the others, although it was seen to coelute with all-(*E*)-antheraxanthin (peak 6) by injecting a combined extract of the violaxanthin and antheraxanthin isomers occurring in the ultrafrozen orange juice studied, which were isolated by thin-layer chromatography (TLC) from the juice as described elsewhere (*16*, *65*). As a result of the study of the average spectrum of the peak, which showed high fine structure, and, above all, of the isolation and study of the violaxanthin and antheraxanthin fractions isolated by TLC, it was checked that the amount of all-(*E*)-antheraxanthin in the peak was actually negligible in comparison to that of (9*Z*)-violaxanthin. This is a good illustration of the fact that, although $C_{30}$ stationary phases are necessary to gain a deeper knowledge of the carotenoid profile of any source, it is not exempt of drawbacks. Thus, the efficiency of this kind of column in the separation of carotenoids is such that it can sometimes lead to the coelution of some of them clearly differing in polarity, such as antheraxanthin and violaxanthin. In this regard, when $C_{30}$ columns are to be used for the analysis of materials with complex carotenoid profiles, it is recommendable to fractionate them into different fractions by other means (like thin-layer chromatography or column chro-

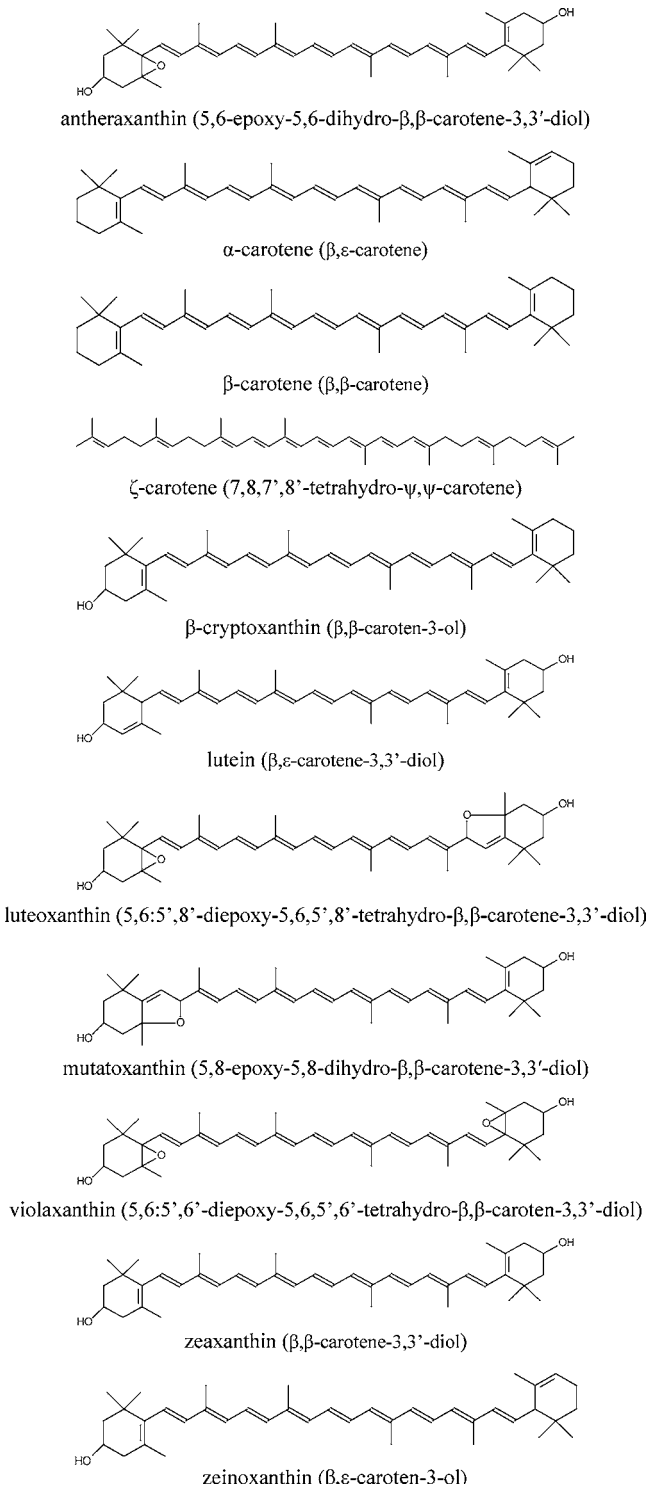


**Figure 2.** Chemical structures of the major carotenoids detected in UFOJ.

matography) and study their chromatographic behavior in such a stationary phase for a more accurate identification.

Aside from all-(*E*)-antheraxanthin, other geometrical isomers of antheraxanthin were detected in the juice (peaks 4, 5, and 12), the (9*Z*)- or (9′*Z*)-isomer (peak 12) being the major one (*16*). Because of the inherent acidity of the juice, which promotes 5,6-epoxy to 5,8-epoxy rearrangements, isomers of the 5,8-epoxyderivatives of violaxanthin and antheraxanthin, luteoxanthin (5,6:5′,8′-diepoxy-5,6,5′,8′-tetrahydro-*β*,*β*-carotene-3,3′-diol, peaks 4 and 7) and mutatoxanthin (5,8-epoxy-5,8-dihydro-*β*,*β*-carotene-3,3′-diol, peaks 8 and 10), were found at detectable levels, unlike auroxanthin (5,8:5′,8′-diepoxy-5,8,5′,8′-tetrahydro-*β*,*β*-carotene-3,3′-diol), the 5,8:5,8-diepoxy derivative of violaxanthin.

Zeinoxanthin (*β*,*ϵ*-caroten-3-ol), which had been wrongly identified in orange juice as α-cryptoxanthin (*β*,*ϵ*-caroten-3′-ol) in a number of works, has been recently correctly identified by means of the methylation test with acidified methanol and by comparison with appropriate standards (*17*, *65*). As for peak 15, it was identified as a *Z*-isomer of ζ-carotene (7,8,7′,8′-tetrahydro-*ψ*,*ψ*-carotene) with the help of a mixture of geometrical isomers of this pigment obtained from UFOJ after TLC on silica. Such identification was made by comparing the absorption maxima and fine structure (%III/II) of its spectrum in the mobile phase (380, 400, and 424 nm and 58.5%, respectively) with those of the isomer identified as all-(*E*)-ζ-carotene (382, 402, and 426 nm and 93.6%) (*58*). Lutein (*β*,*ϵ*-carotene-3,3′-diol), zeaxanthin (*β*,*β*-carotene-3,3′-diol), *β*-cryptoxanthin (*β*,*β*-caroten-3-ol), α-carotene (*β*,*ϵ*-carotene), and *β*-carotene (*β*,*β*-carotene) were readily identified by comparison of their chromatographic and spectroscopic features with those of their corresponding standards.

*Quantitative Analysis.* The accuracy of the quantitative determination of carotenoids relies on the accuracy of the tabulated absorption coefficients used for working out the concentration of the standard solutions, which can be found in a number of sources (*66*, *67*). In any case, it is important to keep in mind that the quantification of these compounds always involves a certain degree of inaccuracy, which is partly due to the fact that the absorption coefficients themselves are difficult to determine (*68*). On the other hand, there is still a lack of appropriate coefficients for different geometrical isomers of the same carotenoid (*69*), such that the estimation of the levels of *Z*-isomers through the absorption coefficients of their all-*E*-counterpart is another source of inaccuracy that it is unavoidable in most cases. Thus, due to the quantitative analysis of carotenoids being rather inaccurate all along, special care is to be taken to avoid other sources of error, such as the quantification of carotenoids differing greatly in their chromophores with the same calibration curve, usually obtained for *β*-carotene, which has been a usual practice in the quantification of orange juice carotenoids in past years. This fact is to be taken into consideration when establishing comparisons, as well as the different factors that do affect the carotenoid profile (climatic factors, variety, industrial processing, etc.) (*60*), to avoid drawing wrong conclusions.

In the present work, the levels of carotenoids were obtained with the corresponding "all-*E*-standard" wherever possible. When this was not possible, a standard of another carotenoid with the same chromophore was used for quantification, as the molar absorption coefficient is theoretically a characteristic of the chromophore (*69*).

The total content of carotenoids ranged between 17.21 and 29.36 mg/L. It was clearly observed that xanthophylls predominated over carotenes (**Table 1**) and that the 5,6-epoxy carotenoids violaxanthin and antheraxanthin, in this order, were the major carotenoids. The levels of the mixture of (9*Z*)-violaxanthin and all-(*E*)-antheraxanthin (peak 6), comprised almost exclusively of the former and ranging from 4.52 to 9.08 mg/L, were worthy of note. The next major carotenoids were also isomers of these 5,6-epoxycarotenoids, specifically the mixture of geometrical isomers of violaxanthin (peak 3), with an average content of 3.38 mg/L, and (9*Z*)- or (9′*Z*)-antheraxanthin (peak 12), with an average content of 2.59 mg/L. The 5,8-epoxy derivatives of violaxanthin and antheraxanthin, luteoxanthin and

**Table 1.** Chromatographic and Spectroscopic Features of the Orange Juice Carotenoids Detected at 430 nm

| peak | $r_t$[a] (min) | identification | $\lambda_{max}$ (nm) | mean (range)[b] |
|---|---|---|---|---|
| 1 | 6.71 | unidentified | 372, 392, 414 | not quantified |
| 2 | 8.19 | unidentified | 418, 440, 468 | not quantified |
| 3 | 12.65–14.18 | all-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers | | 3.38 (2.10–4.44) |
| 4 | 16.10 | luteoxanthin + (*Z*)-antheraxanthin isomer | | 1.22 (0.80–1.64) |
| 5 | 18.85 | (*Z*)-antheraxanthin isomer | 330, 432, 458 | 0.26 (0.15–0.37) |
| 6 | 19.88 | (9*Z*)-violaxanthin + antheraxanthin | 326, 412, 436, 464 | 6.81 (4.52–9.08) |
| 7 | 22.14 | (*Z*)-luteoxanthin isomer | 396, 416, 442 | 0.66 (0.40–0.90) |
| 8 | 23.17 | mutatoxanthin epimer | 426, 452 | 0.61 (0.40–0.87) |
| 9 | 23.96 | lutein | 424, 444, 472 | 0.93 (0.68–1.32) |
| 10 | 24.72 | mutatoxanthin epimer | 426, 452 | 1.20 (0.78–1.80) |
| 11 | 28.72 | zeaxanthin | 450, 474 | 2.00 (1.57–3.14) |
| 12 | 30.77 | (9*Z*)- or (9′*Z*)-antheraxanthin | 332, 440, 468 | 2.59 (1.81–3.63) |
| 13 | 34.63 | zeinoxanthin | 424, 444, 472 | 0.50 (0.36–0.65) |
| 14 | 39.65 | $\beta$-cryptoxanthin | 452, 476 | 1.19 (0.79–1.60) |
| 15 | 43.29 | (*Z*)-$\zeta$-carotene isomer | 380, 400, 424 | 0.40 (0.29–0.55) |
| 16 | 45.26 | $\alpha$-carotene | 424, 446, 472 | 0.15 (0.10–0.22) |
| 17 | 49.92 | $\beta$-carotene | 452, 476 | 0.36 (0.26–0.55) |

[a] Retention time. [b] Average of the 16 samples in mg/L.

mutatoxanthin, respectively, probably formed out of the acidity of the juice, were also detected.

As for the remaining xanthophylls, it was observed that the dihydroxycarotenoids zeaxanthin and lutein, with average contents of 2.00 and 0.93 mg/L, respectively, predominate over the monohydroxycarotenoids, in such a way that the mean concentrations of $\beta$-cryptoxanthin and zeinoxanthin, 1.19 and 0.50 mg/L, respectively, were approximately one-half of those of zeaxanthin and lutein, respectively. Taking into consideration both fractions separately, it was seen that the levels of zeaxanthin roughly doubled those of lutein, which was basically in agreement with the findings of other authors on samples from diverse orange juices (*11*), although it contrasted with the data reported on other Spanish Valencia and navel orange juices where the levels of lutein were reportedly higher or similar to those of zeaxantin (*70*, *71*). As for the levels of $\beta$-cryptoxanthin, it was found that they were 2−3-fold higher relative to zeinoxanthin, which agreed well with the data drawn from other recent studies, although in some of them zeinoxanthin was tentatively identified as the provitamin A carotenoid $\alpha$-cryptoxanthin (*65*, *70*, *71*).

The levels of colored carotenes (the colorless ones, phytoene and phytofluene, were not determined) were very low, and, considering individual compounds, it was observed that the levels of $\beta$-carotene and the *Z*-isomer of $\zeta$-carotene were similar and clearly higher than those of $\alpha$-carotene (**Table 1**). In this sense, it has been pointed out that the amounts of these hydrocarbon carotenoids, although increasing over the ripening of the oranges, remain at low levels, the lycopene-containing red navel Cara Cara oranges being an exception (*72*).

**Color Assessment.** Typical reflection spectra obtained with white and black backgrounds from an UFOJ sample are shown in **Figure 3**. Regardless of the background used for the measurements, it was seen that the reflectance was minimum at around 450 nm, due to the absorption of light by the carotenoid pigments present in the juice. The two spectra were virtually identical in the interval 380−525 nm, although from this wavelength on it was observed that the reflection of light was clearly higher when the white background was used, as expected.

Information on the color of the juices analyzed, expressed in terms of the color coordinates of the uniform color space CIELAB (*73*), is summarized in **Table 2**. In general, not large

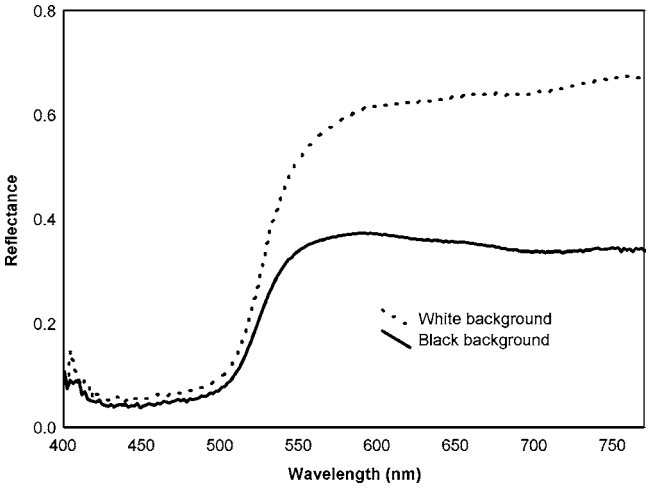


**Figure 3.** Typical reflection spectra of an UFOJ.

differences were observed, which indicated that the product was quite homogeneous as far as color was concerned. Thus, the differences between the maximum and the minimum values of $L^*$ and $a^*$ were below CIELAB 6 units, being slightly higher for $b^*$ and $C_{ab}^*$ (around 9 units for measurements with white background and 14 units for those made with the black background). As it has been already reported in other papers (*40*, *74*), hue values were slightly higher when the black background was used, although this angular coordinate was indeed the least affected quantitatively by the type of background used for the measurements. As for the remaining coordinates linked to the chromaticity, $a^*$, $b^*$, and $C_{ab}^*$, it was observed that their values were lower when the black background was used for the measurement, which was more noticeable for the color coordinate $a^*$. In any case, the hue values obtained (ranging from 77.19° to 80.15° when the white background was used and from 79.99° to 83.04° when the measurements were made against the black background) agreed well with orangeish colors, whereas the chroma ($C_{ab}^*$) of the samples revealed that they showed a deep color.

The location of the samples within the plane $a^*b^*$ is depicted in **Figure 4**, in which it can be observed that all of them are located in the first quadrant (positive values of $a^*$ and $b^*$) regardless of the background used for the measurements. The scattering of the samples within the plane was slightly higher

**Table 2.** Summary of the Color Coordinates of the UFOJ Samples Analyzed

| | $L^*$ | $a^*$ | $b^*$ | $C_{ab}^*$ | $h_{ab}$[a] |
|---|---|---|---|---|---|
| | | White Background | | | |
| mean[b] ± SD | 73.59 ± 1.40 | 13.71 ± 1.10 | 66.80 ± 2.58 | 68.20 ± 2.66 | 78.40 ± 0.74 |
| range | 71.42–76.77 | 11.78–15.99 | 61.84–70.51 | 63.06–72.25 | 77.19–80.15 |
| | | Black Background | | | |
| mean[b] ± SD | 60.22 ± 1.58 | 7.84 ± 1.07 | 52.77 ± 3.63 | 53.36 ± 3.68 | 81.55 ± 0.94 |
| range | 58.28–63.96 | 5.90–9.91 | 44.01–57.95 | 44.40–58.38 | 79.99–83.04 |

[a] Sexagesimal degrees. [b] Average of the 16 samples.

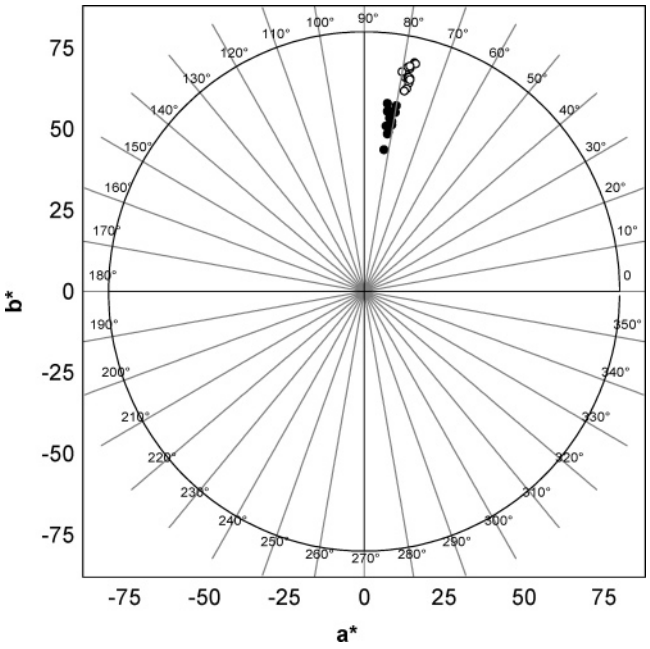


**Figure 4.** Location of the samples within the plane $a^*b^*$: ○ white background; ● black background.

**Table 3.** Simple Regression Coefficients ($r$) between the Total Carotenoid Contents and the Color Coordinates[a]

| | $L^*$ | $a^*$ | $b^*$ | $C_{ab}^*$ | $h_{ab}$ |
|---|---|---|---|---|---|
| white background | –0.41 | 0.43 | *0.86* | *0.85* | –0.01 |
| black background | –0.16 | *0.51* | *0.77* | *0.77* | –0.14 |

[a] Coefficients in italics are significant at $p < 0.05$.

when the black background was used, due to a higher variability in $b^*$, although considering measurements made with the same background, all of the samples were very grouped, due to them differing very little in color.

The simple regression coefficients ($r$) obtained when the correlation between the total carotenoid content and the different color coordinates were studied are summarized in **Table 3**. The results of such analyses revealed that the total amount of carotenoids in the orange juice samples was quite well correlated with $b^*$ and $C_{ab}^*$ (the latter being regarded the quantitative attribute of colorfulness), above all when the color readings obtained with the white background were considered. The poor correlations between the carotenoids content and hue (−0.01 and −0.14 for measurements with white and black background, respectively) were noteworthy, as such a parameter is considered the qualitative attribute of colorfulness.

**Ascorbic Acid Content.** The titrimetric method chosen for the analysis of ascorbic acid has been widely applied to foods and other samples (*37*, *75*, *76*) due to the fact that it is an easy, rapid, and cheap method that does not require any complex instrumentation and therefore can be used in any laboratory. The main drawback of the method resides in the fact that it cannot detect dehydroascorbic acid, species that can be converted back into ascorbic acid and therefore is considered to exhibit vitamin C activity (*2*). Nonetheless, it is a useful method for the assessment of the vitamin C activity of orange juices, as several studies in which both species, ascorbic and dehydroascorbic acids, were quantified in such source showed that the latter accounts only for some 5% on average or even less of the vitamin C activity of the samples (*11*, *70*, *77*, *78*).

The levels of ascorbic acid among the samples surveyed ranged from 332.64 to 441.44 mg/L, which indicated that the content was rather homogeneous, above all considering that the ascorbic acid content of the oranges in general depends upon a wide variety of factors, such as the chemical characteristics of the soil, the climate of the area of production, the amount of light received, etc. (*12*). These factors make somewhat difficult the establishment of meaningful comparisons among different types of juices, above all, among orange juices obtained from different orange varieties or by means of different industrial processing. The average ascorbic acid content of the samples analyzed was 391.06 ± 28.86 mg/L, being higher than that corresponding to other set of UFOJ from the 1999 season (360.85 mg/L) (*79*). In either case, a 250-mL glass of UFOJ would meet the DRI of both males and females (90 and 75 mg/day, respectively) (*6*), despite the fact that this kind of orange juice is not enriched with such nutrient. This mean value was also higher than those corresponding to other kinds of orange juices analyzed in our laboratory for other survey, orange juices from concentrate (357.00 mg/L) and orange juices from squeezed oranges (355.06 mg/L), although lower than those corresponding to the OJFC and orange juices from the ecological agriculture analyzed for the same study (with average ascorbic acid levels of 518.02 and 412.26 mg/L, respectively) (*37*). As far as the establishment of comparisons with other orange juices is concerned, it must be borne in mind that the original ascorbic acid levels of the UFOJ analyzed were higher, as losses of ascorbic acid do occur even over long-term frozen storage (*24*, *26*). This means that the ultrafrozen orange juice studied is a very good natural source of vitamin C even 1 year after its production. Coincidentally, it was found that the average ascorbic acid content of the juices analyzed in the present study was very similar to those of Spanish Valencia orange juices analyzed after several days of storage at 4 °C (406.36 mg/L for juices not subjected to any treatment, 389.79 mg/L for juices subjected to high pressure) (*70*), and to those of frozen unpasteurized orange juices also from Florida (406 mg/L) (*24*). Likewise, its average vitamin C activity was very similar to

that of other Valencia orange juices produced in Australia (406 mg/L), although lower than those of navel orange juices analyzed in the same study (498 mg/L) (*80*) and others produced in the Mediterranean area from different orange varieties, ranging from 386.2 to 620.0 mg/L (*11*, *81*).

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