COLOR OF ORANGE JUICES IN RELATION TO THEIR CAROTENOID CONTENTS AS ASSESSED FROM DIFFERENT SPECTROSCOPIC DATA

Running title: Orange juice color as assessed from different spectra

A.J. Meléndez-Martínez[1], L. Gómez-Robledo[2], M. Melgosa[2], I.M. Vicario[1] and F.J. Heredia[1 *]

[1] *Food Colour & Quality Lab., Dept. Nutrition & Food Science. Universidad de Sevilla. Facultad de Farmacia, 41012 Sevilla, Spain (heredia@us.es).*

[2] *Department of Optics, Faculty of Sciences (Mecenas Building), University of Granada, 18071 Granada, Spain*

*Author to whom correspondence should be addressed

Francisco J. Heredia Mira

Food Colour & Quality Lab., Dept. Nutrition & Food Science. Universidad de Sevilla. Facultad de Farmacia, 41012 Sevilla, Spain

Telephone: ++34 9545 56761; Fax: ++ 34 9545 57017

e-mail: heredia@us.es

ABSTRACT

The color of orange juices influences the consumers' choice, hence the assessment of this attribute has been paid much importance for decades. The instrumental measurement of orange juices color can be harnessed for the rapid, quality-control oriented estimation of its carotenoids, which are health-promoting compounds. In this work we compared and analyzed different spectroscopic data that can be used for these purposes, like the reflectance spectra of orange juices and the absorption spectra of their carotenoid extracts. Additionally, we have revisited the Kubelka-Munk theory and have concluded that its parameters are suitable to assess the carotenoid content of the samples, but not as much as the CIELAB color coordinates. In this regard, we have observed that K/S is the Kubelka-Munk parameter better correlated with the carotenoid content of the orange juices surveyed (r = -0.84), although better correlations were observed when the CIELAB coordinate a* was considered (r = 0.86 and 0.88 for measurements with white and black background, respectively). However, in our opinion this fact should not lead to dismiss the usefulness of the Kubelka-Munk theory to estimate carotenoid contents, since its application can lead to obtain valuable information about the absorption and scattering properties of the samples.

KEYWORDS: Absorption spectra; carotenoids; color; Kubelka-Munk; orange juice; reflectance spectra.

INTRODUCTION

The color of orange juices (OJ) influences the consumers' preferences a great deal (Tepper, 1993). The hues of most OJ range from yellow to orange and are mainly due to their content in carotenoids. However, other oranges also accumulate anthocyanin pigments such that their juices exhibit a characteristic reddish color (Arena, Fallico, Maccarone, 2000, Kirca Cemeroglu, 2003, Meléndez-Martínez, Vicario, Heredia, 2005). Recently, two red-fleshed orange mutants that owe their reddish color mainly to the carotenoid lycopene and not to anthocyanins have also been described and studied (Lee, 2001, Liu et al., 2007, Alquezar, Rodrigo, Zacarías, 2008). Anyhow, the genotype is not the only factor determining the carotenoid pattern of oranges and therefore their color, since the climate of the area of production and the industrial processing, among others, are also related to the pigment content (Mouly, Gaydou, Lapierre, Corsetti, 1999, Meléndez-Martínez, Britton, Vicario, Heredia, 2008).

The citrus industry has been aware of the importance of the color of OJ since long ago, especially in the United States, where this attribute has been long used as a quality parameter for the commercial classification of the product. In relation to this it is important to mention that much research has been conducted in this country to standardize its assessment to the extent that many instruments have been evaluated, and that even a color number scale was developed for the rapid classification of the juices (Huggart Wenzel, 1954, Huggart, Barron, Wenzel, 1966, Hunter, 1967, Eagerman, 1978, Buslig Wagner Jr., 1984, Buslig Wagner Jr., 1985, Meléndez-Martínez et al., 2005). Anyway, in our opinion, internationally recommended color-specification systems, like CIELAB (CIE, 2004), must be always employed with

independence we consider OJ, virgin olive oils (Moyano, Melgosa, Alba, Hita, Heredia, 1999) or any other product.

Apart from its evident relationship with the perceived quality and the consumers' choices, with the derived economic repercussions, the study of the color of OJ is especially challenging for several reasons. On one hand, the carotenoid pattern of OJ is very complex and includes, among others, pigments with provitamin A activity (α-carotene, β-carotene and β-cryptoxanthin) and other biological functions or actions (besides the former, lutein, zeaxanthin and, in some genotypes, lycopene). This is important because the instrumental measurement of the color of OJ, apart from serving to assess this important quality attribute, could also be harnessed for the rapid, quality-control oriented estimation of these phytochemicals. In this sense, the application of tristimulus colorimetry in conjunction to multivariate statistical methods has proved a powerful tool that allows the determination of individual carotenoids (Meléndez-Martínez, Vicario, Heredia, 2003), and even hypothetical vitamin A activity (Meléndez-Martínez, Vicario, Heredia, 2007b) in OJ. In other studies carried out in our laboratory, multivariate statistical methods have been applied to gain insight into how the diverse carotenoids occurring in OJ "interplay" to produce a final color (Melendez-Martinez et al., 2010).

The instrumental measurement of the color of these products is also especially challenging because they are neither transparent nor opaque, but translucent. Whilst the color of transparent products can be readily ascertained from transmission measurements and that of opaque ones from reflection measurements, the behavior of light when reaches a translucent sample makes the instrumental measurement more complicate (Meléndez-Martínez et al., 2005). Over and above

this fact, the characteristic turbidity of OJ also plays an important role in its appearance, which is also an important factor to be considered when measuring its color (Rummens, 1970, Arena et al., 2000). The importance of the pulp particles in this regard is double, as they also contain the carotenoid pigments (Meléndez-Martínez, Vicario, Heredia, 2009).

Although the color definition of OJ continues attracting the interest of both scientists and the citrus industry in the first years of the 21st century (Arena et al., 2000, Lee Castle, 2001, Choi, Kim, Lee, 2002, Lee Coates, 2003, Meléndez-Martínez, Vicario, Heredia, 2004, Meléndez-Martínez, Britton, Vicario, Heredia, 2005, Pérez-López, Beltran, Serrano-Megías, Saura López, Carbonell-Barrachina, 2006, Cortes, Esteve, Frigola, 2008, Tiwari, Muthukumarappan, O' Donnell, Cullen, 2008), little attention has been paid to methodological aspects. In this paper we analyze different spectroscopic approaches that can be used to define the color of the juices with a view to determine their pigment content as the correlation between the color of several foodstuffs and their carotenoid content is raising much interest (Meléndez-Martínez et al., 2003, Humphries, Graham, Mares, 2004, Ruiz, Reich, Bureau, Renard, Audergon, 2008). In this regard, we discuss the absorbance spectra of OJ carotenoid extracts (which has been used for very long to quantitate them), the reflectance spectra of OJ (which have proved very useful to estimate carotenoid levels without having to extract them) and spectra derived from the application of the Kubelka-Munk theory, which has been used for translucent samples and whose application to the estimation of OJ carotenoid levels has not been assessed in detail.

Moreover, some observations regarding the use of black or white backgrounds for the color measurement of the juices are made.

MATERIAL AND METHODS

Samples

Seventy commercially available OJ were surveyed. The samples were divided in two main groups according to the industrial treatment undergone: ultrafrozen orange juices (UFOJ, juices not subjected to heat treatment that are stored and marketed at T < 18°C; , n = 26) and thermally-treated orange juices (TTOJ, n = 44). The UFOJ were supplied at different intervals by Zumos Vitafresh (Almonte, Huelva, Spain), whilst the TTOJ were purchased from several retailers in Seville at different times. All the samples were stored as recommended (the UFOJ at T < -18°C and the TTOJ at room temperature or 4°C) until their analysis. UFOJ were thawed at room temperature.

Instrumental color measurement

For the color readings, the samples were placed in a plastic cuvette (47.5 × 35 × 10 mm) for reflection measurements. The measurements were carried out with subdued illumination to avoid possible light interferences. Moreover, the cuvettes were placed inside a cabin with grey walls. The reflectance visible spectra (380-770 nm, $\Delta\lambda$ = 1 nm) were recorded by means of a CAS 140 B spectroradiometer (Instrument Systems, Munich, Germany) fitted with a Top 100 telescope optical probe (Instrument Systems, Munich, Germany) and a Tamron zoom mod. SP 23A (Tamron USA, Inc., Commack, NY, USA). The zoom, to which the probe was attached, was held at a fixed distance of 50 cm in a straight line from the sample. For all the measurements the source of light was an external incandescent lamp providing a 45^{o} incident illumination with respect to the perpendicular to the cuvette. The apparatus was set to take three consecutive readings, so the color parameters

obtained were averages of three measurements. The instrument blank measurements were made with the cuvette filled with distilled water against a reference white pressed plate (SRS-99-010, Labsphere Inc. North Sutton, NH, USA). The OJ samples were measured against a white background (WB, the pressed white plate) and a black background (BB, a round plastic piece with homogeneous black color).

The illuminant D_{65} and the 10° Standard Observer were considered as references. The illuminant D_{65} is a standard illuminant defined by International Commission on Illumination and the 10° Standard Observer was considered to represent best average spectroscopic response of human observers

The color coordinates corresponding to the approximately uniform color space CIELAB (CIE, 1978) were obtained directly from the apparatus. Within this color space, two color coordinates, a^*_{10} and b^*_{10}, and a psychometric index of lightness, L^*_{10}, are defined. a^*_{10} takes positive values for reddish colors and negative values for the greenish ones, whereas b^*_{10} takes positive values for yellowish colors and negative values for the bluish ones. L^*_{10} is an estimation of the relative luminosity, and according to this parameter any given color can be regarded as equivalent to a member of a grey scale, between black ($L^*_{10} = 0$) and white ($L^*_{10} = 100$). From a^*_{10} and b^*_{10}, the psychometric parameters chroma ($C^*_{ab,10}$) and hue-angle ($h_{ab,10}$) are defined:

$$C^*_{ab,10} = [(a^*_{10})^2 + (b^*_{10})^2]^{1/2} \text{ (Eq. 1)}$$

$$h_{ab,10} = tan^{-1} (b^*_{10}/a^*_{10}) \text{ (Eq. 2)}$$

$C^*_{ab,10}$ (related to the quantitative attribute of colorfulness) allows to determine for each hue its degree of difference in comparison to a grey color with the same lightness, whilst hue-angle ($h_{ab,10}$) is the attribute according to which colors have been traditionally defined as reddish, greenish, etc.

Application of the Kubelka-Munk theory to the spectroscopic data

The theory can be briefly summarized into several main points (Calvo, 1993):

- The layer of sample can be divided into elementary layers with parallel sides to the total layer and identical optical properties.

- The elementary layer or sheet is defined as a sheet with parallel and infinite sides, so that the effect of the edges is eliminated. However its thickness is finite.

- The thickness of the elementary sheet is small to the total thickness of the sample, but large compared to the size of the particles.

- At any wavelength, the optical properties of the sample can be described by one scattering (S) and one absorption (K) coefficient. Such coefficients describe the amount of light scattered and absorbed when passing through the sample.

- There are an ascending flow and a descending flow of diffuse light. Each flow loses on its way through the sample an amount of light proportional to its energy and to K, due to absorption and another amount of light proportional to its energy and to S, owed to scattering. Likewise, each flow gains an amount of light proportional to the energy of the other one and to S, due to scattering.

Considering that i is the descending flow and j the ascending one. The following equations can be deduced:

$$dj = -(S + K)j\,dx + Si\,dx \qquad \text{(Eq. 3)}$$

$$-di = -(S + K)i\,dx + Sj\,dx \qquad \text{(Eq. 4)}$$

These equations, in turn, lead to the following formulas (Judd, Wyszecki, 1975, Wyszecki, Stiles, 1982, Hutchings, 1994):

$$a_\lambda = 1/2[R_\lambda + (R_{0\lambda} - R_\lambda + R_{g\lambda}/R_{0\lambda}R_{g\lambda})] = (S_\lambda + K_\lambda)/S_\lambda \qquad \text{(Eq. 5)}$$

$$b_\lambda = (a_\lambda - 1)^{1/2} \qquad \text{(Eq. 6)}$$

$$-S_\lambda = (b_\lambda X)^{-1} \, Arctgh\,[(1-a_\lambda R_{0\lambda})/b_\lambda R_{0\lambda}] \quad \text{(Eq. 7)}$$

$$K_\lambda = S_\lambda (a_\lambda - 1) \quad \text{(Eq. 8)}$$

where a_λ and b_λ are constants, $R_{g\lambda}$ is the spectral reflectance of the background, X is the sample thickness, R_λ and $R_{0\lambda}$ refers to the spectral reflectance of the sample with WB and BB, respectively, S_λ is the scattering coefficient, and K_λ the absorption coefficient.

Carotenoid analysis

Carotenoids were extracted and analyzed by HPLC according to the routine protocol followed in our laboratory, which is described in detail elsewhere (Meléndez-Martínez, Vicario, Heredia, 2007a). The total carotenoid content of the samples was calculated as the sum of the levels of the individual compounds.

The visible absorption spectra (380-770 nm) of the OJ carotenoid hexane extracts were recorded on an HP8452 UV/Vis diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA) with a wavelength accuracy of 2 nm. A 10 mm pathlength glass cell was used for the measurements.

Data analysis

The spectroscopic data were averages of three consecutive measurements. The color coordinates were automatically calculated by the software of the spectroradiometer. They and The Kubelka-Munk parameters were calculated according to the formulas indicated before. The statistical treatments of the data were carried out with the Statistica® v.6.0 software (StatSoft Inc., 2001).

RESULTS AND DISCUSSION

Spectroscopic information related to the color of orange juices

Typical reflectance visible spectra of UFOJ and TTOJ obtained placing the cuvette with the samples against a white and a black background (WB and BB, respectively) are represented in Figure 1. Overall, the shape of the spectra is very similar regardless of the type of the juice. The reflectance of the samples is lowest at around 450 nm and then increases sharply up to around 550 nm. From that wavelength onwards, the reflectance increases smoothly in the case of the measurements made with WB. When the BB is used, the reflectance between 550 nm and 770 nm diminishes and then increases smoothly or remain virtually constant, such that the reflectance values at around 550 nm and at 770 nm are very similar. Concerning the differences in the reflection spectra as a function of the type of juice, it was observed that, in most cases, the reflection of visible light was higher in the case of the thermally treated ones along the whole visible region. In the case of the spectra represented in Figure 1, it was observed that the reflection spectrum one of the thermally treated juices was very similar to that of the ultrafrozen one in the region 380-550 nm, although from 550 nm onwards its reflectance values were clearly higher. Typical absorption spectra of the carotenoid fraction of both UFOJ and TTOJ are represented in Figure 2. Exhibiting color ranging from yellow to orange, the carotenoids occurring in the OJ studied absorbed maximally in the region 400-500 nm. The different shapes of the represented spectra reflect the qualitative and quantitative differences in the carotenoid profiles of the UFOJ and TTOJ, since the visible absorption spectra of most carotenoids exhibit three absorption bands, the location of which depend on the number and arrangement of the conjugated double bonds in their molecules (Britton, 1995b, Meléndez-Martínez, Britton, Vicario, Heredia, 2007). The carotenoid content of the thermally treated juices analyzed was lower than those of the ultrafrozen orange juices, hence the higher absorbance

maxima of the latter. Apart from this, in Figure 2 it can be observed that the shape of the spectra of both kinds of juices are different, which reflects differences in their carotenoid profile. These differences are addressed in detail in other papers (Meléndez-Martínez, Britton, Vicario, Heredia, 2008, Melendez-Martinez et al., 2010).

When comparing the spectra depicted in Figures 1 and 2 it can be readily seen that, obviously, the reflection of visible light by the juices is lowest where the carotenoids occurring in them absorb maximally. However, if we take a closer look to those figures some questions can be raised. Thus, for instance, the peaks and valleys that can be observed in the absorption spectra of the carotenoid fraction are not observed in the reflectance spectra of the juice samples. Over and above this, the absorption of the carotenoid extracts at 500 nm is rather negligible (Figure 2), which can lead to think that the reflection of the OJ at that wavelength should be high, which is not the case. Actually the reflection at that point is very close to the lowest (Figure 1). How can these differences be explained? In principle, it is sensible to think that such differences can be due, at least in part, to the fact that OJ is an aqueous suspension whilst the carotenoid extract is dissolved in hexane. Additionally, the carotenoids in OJ are located within the pulp particles, which also play an important role in the appearance, and therefore, in the spectra of the juices in relation to their turbidity, as mentioned earlier on. Put otherwise, in the case of the translucent OJ samples, apart from absorption, reflection and transmission phenomena there is also light scattering, which does not take place in the solutions of the carotenoid extracts in hexane. On the other hand, it is sensible to think that the fact that the absorbance is a logarithmic magnitude while the reflectance is not, is also related to the changes observed in the shape and sharpness of the spectra.

However, to fully understand the differences between the spectra of carotenoid extracts in organic solvents and those corresponding to biological structures containing these pigments it is interesting to come as far as the sub-cellular location of the pigments, that is, to the plastids. Due to their hydrophobicity, carotenoids can form aggregates in some media, which changes their physical properties (Britton, 1995a). In other words, whereas in hexane solutions of carotenoids there are single molecules of carotenoids, in their biological milieu they can form aggregates and crystals under some conditions, whose absorption spectra would differ from those of the monomer carotenoids. The aggregation of carotenoids and the derived color shifts are well-known. In the case of the so-called H-aggregates, the carotenoid molecules are disposed with their polyene chains parallel to each other, and a blue shift due to the hypsochromic (to shorter wavelengths) displacement of the absorption maxima can be observed. In the case of the J-aggregates, there is a head-to-tail association of the polyene chains, and a red shift due to a bathochromic (to longer wavelengths) displacement of the absorption maxima can be observed (Kön et al., 2008). In the literature there are many examples of typical spectra of monomer and aggregated carotenoids as well as of carotenoid-containing structures (Ruban, Horton, Young, 1993, Zsila, Deli, Simonyi, 2001, Fish, 2006, Kön et al., 2008), which serve to illustrate the shifts in the location of the absorption bands and the changes in the overall shape of the spectra. Thus, they could also serve to explain the differences between the shape of the reflection spectra of the biological materials and the sharp absorption spectra of their carotenoid fraction. As another example of the influence of aggregation states, it can be said that disturbed soil samples and soil aggregates show also significantly different colors

attributable to multiple causes (Sánchez-Marañón, Delgado, Delgado, Pérez, Melgosa, 1995, Sánchez-Marañón, Soriano, Melgosa, Delgado Delgado, 2004).

In our view, when it comes to measure the color of OJ or any other natural product with a view to correlate it with the pigment content, not only the physics and the mere pigment content but also other aspects related to their biological environment should be taken into account. For instance, in the case of OJ it is to be considered that it may also happen that not all the carotenoids are equally apparent within the pulp particles. In this regard it is important to note that OJ are complex sources of carotenoids with different chemical structures (Meléndez-Martínez, Britton, Vicario, Heredia, 2008) and that these are much related within their location and organization within the cell (Britton, 1995). In relation to this, we have reported recently that the pigments more related to the hue and the chroma of orange juices are not necessarily those occurring at the highest concentrations (Meléndez-Martínez et al., 2010).

The application of the Kubelka-Munk theory to orange juices revisited: assessing pigment contents from absorption / scattering data and CIELAB coordinates

As mentioned before, OJ are neither transparent nor opaque but translucent, so the instrumental measurement of its color is more challenging. In this kind of sample the instrumental measurement is largely dependent on both the thickness of the sample and the color of the background placed at its back. This fact can be overlooked by applying the Kubelka-Munk theory, which entails measuring the sample with two different backgrounds and obtaining constants that are independent of the background and the thickness of the sample.

The averaged values of all UFOJ and TTOJ for K_λ, S_λ and K_λ/S_λ are represented in Figure 3. The absorption coefficient K_λ is defined as "*...the rate of decrease of transmittance with thickness of a very thin layer...*" (the same meaning of the absorptivity in Beer law), whereas the scattering coefficient S_λ is defined as "*...rate of decrease of reflectance with thickness of a very thin layer...*" and gives us an idea of the amount of scattered light (Judd, Wyszecki, 1975) The K_λ/S_λ ratio is called the Kubelka–Munk index and is particularly useful to determine if the color measurements should be made by transmission or reflection. If Kubelka-Munk index is greater than 1, absorption is greater than scattering and transmission measurement is recommended, if Kubelka-Munk index is lower than 1 scattering is predominant and reflection measurement is recommended (Francis, 1987).

If we compare the typical reflection spectra of the OJ (Figure 1) with the spectral representation of S_λ (Figure 3) we can notice a great similarity, which may indicate that the reflection spectra of the samples is largely related to the scattering of light attributable to pigments. It is also interesting to note that the spectral representation of the Kubelka-Munk index (Figure 3) bears a great resemblance to the absorption spectra of the carotenoid extracts. Taking into consideration a work by Ronsholdt and McLean (Ronsholdt and McLean, 2001) in which different spectroscopic data were assessed for the quantification of carotenoids in rainbow trout, it can also be noticed that the representation of the K/S index bears clear similarities with the spectrum of astaxanthin, the major carotenoid in the fish muscle. In fact, the authors stated that the Kubelka-Munk transformation of the data removed much of the general spectral information of physical origin. In this sense, it is tempting to infer that this treatment of the data can be useful to obtain a good estimation of the carotenoid content of foods and other sources without having to

extract them, which offers many advantages (more efficient throughput of samples, little risk of pigment degradation, etc.).

The usefulness of the CIELAB color coordinates to estimate the individual or total carotenoid content of OJ has been demonstrated in some of our previous works (Meléndez-Martínez, Vicario, Heredia, 2003, Meléndez-Martínez, Vicario, Heredia, 2007). The average CIELAB color coordinates of the samples analyzed are displayed in Table 1 with their corresponding standard deviations, whilst the correlation coefficients between them and the total carotenoid content of the samples are shown in Table 2. All the correlations were significant at $p<0.05$, the highest values of linear correlation coefficient *r* (over 0.8) corresponding to a^*_{10} regardless of the background used for the measurements. The differences in the carotenoid levels and color coordinates within and between types of orange juices can be easily explained considering that it is well-known that the accumulation of secondary metabolites in general and of carotenoids in particular in plants depend on the genotype, climate and agronomic factors and type of processing and storage conditions, among others factors. Considering the averaged color data, it can be claimed that, overall, the TTOJ appear lighter and less vivid than the UFOJ.

Due to the interesting similarities found between the reflectance spectra of carotenoid-containing samples and some of the spectra obtained considering the Kubelka-Munk theory commented before, it appeared interesting to assess the usefulness of the Kubelka-Munk parameters to assess the carotenoid content of the juices analyzed in this study. The results of this assessment are shown in Figure 4, where the linear correlation coefficient *r* between the Kubelka-Munk parameters (at 500nm, wavelength where K_λ/S_λ is approximately 1) and the carotenoid content of

each OJ is shown. When comparing the values of *r* with the ones displayed in Table 2, it can be readily concluded that they are similar but not higher than the highest found with CIELAB color parameters. This seems to be indicative that the Kubelka-Munk parameters (above all *K/S*, which was found to be better correlated to the pigment content than *K* and *S* considered individually) can be used to estimate the carotenoid content of OJ, although they are not as suitable as the CIELAB colorimetric coordinates. Apart from these observations it has to be considered that the application of the Kubelka-Munk theory requires two measurements (with WB and BB), a more complex mathematical treatment and involves more error sources than those linked to a CIELAB colorimetric measurement. It is interesting to note that there are large differences in the correlations between the K/S index and the carotenoid levels as a function of the juices considered (r = 0.44 and 0.75 for UFOJ and TTOJ, respectively). This could reflect marked differences in the absorption and scattering of light between UFOJ and TTOJ, which could in turn be related to the characteristics of their pulp particles, these being interesting aspects to be addressed in future research.

Despite the Kubelka-Munk theory has been widely applied to foods, paints, biological material, etc. (Huang, Francis, Clydesdale, 1970, Hetherington Mac Dougall, 1992, Calvo, 1993, Yang, Celmer, Koutcher, Alfano, 2000, Berns Mohammadi, 2007) for several purposes, in the case of orange-based drinks and other foodstuffs sometimes it does not lead to higher correlations with other parameterss as compared to other methodologies (Rummens, 1970, Gullett, Francis, Clydesdale, 1972). In this regard, as we reviewed some years ago (Meléndez-Martínez, Vicario, Heredia, 2005), some authors reported that the use of the color or spectroscopic information obtained with only one background could be more

valuable for some purposes (Little, 1964, Durán, Rodrigo, Alcedo, 1976, Lafuente, Gasque, Nieto, Izquierdo, 1979). Considering the spectra represented in Figure 1 the main differences, in spectroscopic terms, derived from the use of the WB and the BB can be readily inferred. Thus, it is evident that the spectra of the juices are largely very similar within the interval of wavelengths from 380 nm to ca. 550 nm, the region of the electromagnetic spectrum where the OJ carotenoids absorb maximally. From ca. 550 onwards, the reflectances of the samples measured with the BB are much lower. This is logical since the light that reaches the BB is not reflected, which can occur when the WB is used. From our previews studies on the matter some other conclusions derived from the use of either background can be drawn. For instance, we have reported that the use of a black background led to a better discernment of OJ dilutions both visually and instrumentally (Meléndez-Martínez, Vicario, Heredia, 2004). On the other hand, the use of either background did not affect much the correlations between individual carotenoids and the CIELAB color coordinates (Meléndez-Martínez, Vicario, Heredia, 2003). In the case of the correlation between those color coordinates and the retinol activity equivalents (parameter used to assess the potential vitamin A activity of a product) of OJ, it was concluded that the use of the BB led to slightly better values of *r*. At this point, it is pertinent to stress that the actual vitamin A activity of the juices depend on many factors (human genotype, food matrix, carotenoid isomeric form, etc.) and that therefore, we are very far to use color data to accurately estimate it. However, since the objective measurement of color has proved useful to determine the hypothetical vitamin A activity, its applicability in food labelling appears very promising (Meléndez-Martínez, Vicario, Heredia, 2007).

CONCLUDING REMARKS

Despite the color of OJ continues attracting much attention from scientists and the industry, few studies have dealt with methodological aspects in the last years. In this paper we have commented the features of different spectra that can be used to evaluate the color of these products and of the pigments accounting for it. Furthermore we have applied the Kubelka-Munk theory to the spectroscopic data to ascertain whether K_λ, S_λ or K_λ/S_λ can lead to better correlations with the carotenoid content of the samples as compared to those obtained with the color coordinates of the CIELAB space. In this sense, we have concluded that *K/S* is the Kubelka-Munk parameter better correlated with the carotenoid content of orange juices subjected to different processing conditions, although it does not involve a better estimation of the carotenoid content. In conclusion, it can be stated that, apart from the traditional quantification of total carotenoid contents by means of the absorption spectra of their extracts, it is also possible to use the color coordinates (like a*) computed from the reflectance spectra of the product for that purpose. This has the advantage that the carotenoids do not have to be extracted, so the assessment is more rapid and risks of degradation and/or formation of artifacts are minimized. Additionally, good correlations ($r > 0.8$ in absolute values) were observed between the K/S index and the total carotenoid levels, which indicate that this Kubelka-Munk parameter also seems useful for the quantification of OJ carotenoids. Although the correlation of this parameter with the total carotenoid level was slightly lower as compared to a* ($r > 0.85$), the applicability of this theory for that purpose should not be dismissed since it offers the possibility to obtain valuable data about the absorption and scattering properties of the samples.

ACKNOWLEDGMENTS

Research project FIS2007-64266, Ministerio de Educación y Ciencia (España), with European Regional Development Fund (ERDF) support.

REFERENCES

Alquezar, B., Rodrigo, M. J., Zacarias, L. (2008). Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry*, *69*(10), 1997-2007.

Arena, E., Fallico, B., Maccarone, E. (2000). Influence of carotenoids and pulps on the color modification of blood orange juice. *Journal of Food Science*, *65(3)*, 458-460.

Berns, R. S.,& Mohammadi, M. (2007). Single-constant simplification of Kubelka-Munk turbid-media theory for paint systems - A review. *Color Research and Application*, *32*(3), 201-207.

Britton, G. (1995a). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, *9*, 1551-1558.

Britton, G. (1995b). UV/Visible Spectroscopy. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1B: Spectroscopy*, (pp. 13-62) Basel, Switzerland: Birkhäuser.

Buslig, B. S.,& Wagner Jr., C. J. (1984). General purpose tristimulus colorimeters for color grading orange juice. *Proc.Fla.State Hort.Soc.*, *97*, 74-76.

Buslig, B. S.,& Wagner Jr., C. J. (1985). Instrumental measurement of orange juice color. *Food Technology, 39(9)*, 95-97.

461 Calvo, C. (1993). La medida del color en alimentos translúcidos: teoria de Kubelka-
462 Munk. *Rev.Esp.Cienc.Tecnol.Aliment., 33 (6)*, 597-605.

463 Choi, M. H., Kim, G. H., Lee, H. S. (2002). Effects of ascorbic acid retention on
464 juice color and pigment stability in blood orange (*Citrus sinensis*) juice
465 during refrigerated storage. *Food Research International, 35*, 753-759.

466 CIE (1978). *Recommendations on Uniform Color Spaces, Color-Difference*
467 *Equations, Psychometric Color Terms, CIE Publication No. 15 (E-1.3.1)*
468 *1971, Supplement 2*. Vienna: CIE Central Bureau.

469 CIE (2004). *CIE Publication 15:2004. Colorimetry. 3rd Edition*. Vienna: CIE
470 Central Bureau.

471 Cortes, C., Esteve, M. J., Frigola, A. (2008). Color of orange juice treated by High
472 Intensity Pulsed Electric Fields during refrigerated storage and comparison
473 with pasteurized juice. *Food Control*, *19*(2), 151-158.

474 Durán, L., Rodrigo, M., Alcedo, M. J. (1976). Medida del color y de la turbiedad en
475 zumo de naranja. *Rev.Agroquim.Tecnol.Aliment.*, *16(1)*, 98-105.

476 Eagerman, B. A. (1978). Orange juice color measurement using general purpose
477 tristimulus colorimeters. *Journal of Food Science*, *43*, 428-430.

478 Fish, W. W. (2006). Interaction of Sodium Dodecyl Sulfate with Watermelon
479 Chromoplasts and Examination of the Organization of Lycopene within the
480 Chromoplasts. *Journal of Agricultural and Food Chemistry, 54*(21), 8294-
481 8300.

Francis, F. J. (1987). Food colorimetry: Measurement and interpretation. In R. Jowitt, F. Escher, M. Kent, B. McKenna, & M. Roques (Eds.), *Physical properties of foods-2.*, (pp. -) London: Elsevier Appl. Sci.

Gullett, E. A., Francis, F. J., Clydesdale, F. M. (1972). Colorimetry of foods: orange juice. *Journal of Food Science, 37*, 389-393.

Hetherington, M. J.,& Mac Dougall, D. B. (1992). Optical properties and appearance characteristics of tomato fruit (Lycopersicon esculetum). *Journal of the Science of Food and Agriculture, 59 (4)*, 537-543.

Huang, I.-L., Francis, F. J., Clydesdale, F. M. (1970). Colorymetry of foods. 2. Color measurement of squash puree using the Kubelka-Munk concept. *Journal of Food Science*, *35*, 315-317.

Huggart, R. L., Barron, R. W., Wenzel, F. W. (1966). Evolution of the Hunter Citrus Colorimeter for measuring the color of orange juice. *Food Technology, 20*, 109-111.

Huggart, R. L.,& Wenzel, F. W. (1954). Measurement and control of color of orange concentrate. *Proc.Fla.State Hort.Soc.*, *67*(210), 216-.

Humphries, J. M., Graham, R. D., Mares, D. J. (2004). Application of reflectance color measurement to the estimation of carotene and lutein content in wheat and triticale. *Journal of Cereal Science*, *40*, 151-159.

Hunter, R. S. (1967). Development of the Citrus Colorimeter. *Food Technology*, *21*, 906-911

503 Hutchings, J. B. (1994). *Food color and appearance*. Glasgow: Blackie Academic
504 Professional.

505 Judd, D. B., Wyszecki, G. (1975). *Color in business, science and industry, 3rd ed.*
506 New York: John Wiley Sons.

507 Kirca, A., Cemeroglu, B. (2003). Degradation kinetics of anthocyanins in blood
508 orange juice and concentrate. *Food Chemistry, 81*, 583-587.

509 Kön, S., Kolbe, H., Korger, M., Köpsel, C., Mayer, B., Auweter, H., Lüddecke, E.,
510 Bettermann, H., Martin, H.-D. (2008). Aggregation and interface behaviour
511 of carotenoids. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.),
512 *Carotenoids. Volume 4: Natural functions*, (pp. 53-98) Basel, Switzerland:
513 Birkhäuser.

514 Lafuente, B., Gasque, F., Nieto, P., Izquierdo, L. (1979). Nota. Métodos para obtener
515 mezclas de zumos de naranja de color preestablecido.
516 *Rev.Agroquim.Tecnol.Aliment.*, *19(4)*, 549-553.

517 Lee, H. S.,& Coates, G. A. (2003). Effect of thermal pasteurization on Valencia
518 orange juice color and pigments. *Lebensmittel-Wissenschaft und*
519 *Technnologie-Food Science and Technology*, *36*, 153-156.

520 Lee, H. S. (2001). Characterization of carotenoids in juice of red navel orange (Cara
521 Cara). *Journal of Agricultural and Food Chemistry*, *49*, 2563-2568.

522 Lee, H. S.,& Castle, W. S. (2001). Seasonal changes of carotenoid pigments and
523 color in Hamlin, Earlygold, and Budd Blood orange juices. *Journal of*
524 *Agricultural and Food Chemistry*, *49*, 877-882.

525 Little, A. C. (1964). Color Measurement of Translucent Food Samples. *Journal of*
526 *Food Science*, *29*, 782-789.

527 Liu, Q., Xu, J., Liu, Y., Zhao, X., Deng, X., Guo, L., Gu, J. (2007). A novel bud
528 mutation that confers abnormal patterns of lycopene accumulation in sweet
529 orange fruit (Citrus sinensis L. Osbeck). *Journal of Experimental Botany*,
530 *58*(15-16), 4161-4171.

531 Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2003). Application of
532 Tristimulus Colorimetry to estimate the carotenoids content in ultrafrozen
533 orange juices. *Journal of Agricultural and Food Chemistry*, *51*(25), 7266-
534 7270.

535 Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2004). Correlation between
536 visual and instrumental color measurements of orange juice dilutions. Effect
537 of the background. *Food Quality and Preference*, *16*, 471-478.

538 Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2005). Instrumental
539 measurement of orange juice color: A review. *Journal of the Science of Food*
540 *and Agriculture*, *85*, 894-901.

541 Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., Heredia, F. J. (2005). Color
542 and carotenoid profile of Spanish Valencia late ultrafrozen orange juices.
543 *Food Research International*, *38*, 931-936.

544 Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., Heredia, F. J. (2007).
545 Relationship between the color and the chemical structure of carotenoid
546 pigments. *Food Chemistry*, *101*, 1145-1150.

Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2007a). Carotenoids, color and ascorbic acid content of a novel frozen-marketed orange juice. *Journal of Agricultural and Food Chemistry*, *55*(4), 1347-1355.

Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2007b). Rapid assessment of vitamin A activity through objective color measurements for the quality control of orange juices with diverse carotenoid profiles. *Journal of Agricultural and Food Chemistry*, *55*(8), 2808-2815.

Melendez-Martinez, A. J., Britton, G., Vicario, I. M., Heredia, F. J. (2008). The complex carotenoid pattern of orange juices from concentrate. *Food Chemistry*, *109*(3), 546-553.

Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. (2009). Effect of ascorbic acid on deterioration of carotenoids and color in ultrafrozen orange juice. *Journal of Food Composition and Analysis*, *22*(4), 295-302.

Melendez-Martinez, A.J., Escudero-Gilete, M.L., Vicario-Romero, I.M., Heredia, F.J., 2010. Study of the influence of carotenoid structure and individual carotenoids in the qualitative and quantitative attributes of orange juice colour. *Food Research International, 43* ,1289 -1296.

Mouly, P. P., Gaydou, E. M., Lapierre, L., Corsetti, J. (1999). Differentiation of several geographical origins in single-strength Valencia orange juices using quantitative comparison of carotenoid profiles. *Journal of Agricultural and Food Chemistry*, *47*, 4038-4045.

568 Moyano, M. J., Melgosa, M., Alba, J., Hita, E., Heredia, F. J. (1999). Reliability of
569 the bromthymol blue method for color in virgin olive oils. *Journal of the*
570 *American Oil Chemists' Society*, *76*, 687-692.

571 Pérez-López, A. J., Beltran, F., Serrano-Megías, M., Saura López, D., Carbonell-
572 Barrachina, Á. A. (2006). Changes in orange juice color by addition of
573 mandarin juice. *European Food Research and Technology*, *222*, 516-520.

574 Ronsholdt , B., McLean, E., 2001. Determination of total carotenoid content in
575 rainbow trout muscle by multivariate calibration of VIS reflectance spectra.
576 *Journal of Food Composition and Analysis 14*, 345-357.

577 Ruban, A. V., Horton, P., Young, A. J. (1993). Aggregation of higher plant
578 xanthophylls: Differences in absorption spectra and in the dependency on
579 solvent polarity. *Journal of Photochemistry and Photobiology B: Biology*,
580 *21*(2-3), 229-234.

581 Ruiz, D., Reich, M., Bureau, S., Renard, C. M. G. C., Audergon, J. M. (2008).
582 Application of Reflectance Colorimeter Measurements and Infrared
583 Spectroscopy Methods to Rapid and Nondestructive Evaluation of
584 Carotenoids Content in Apricot (Prunus armeniaca L.). *Journal of*
585 *Agricultural and Food Chemistry*, *56*(13), 4916-4922.

586 Rummens, F. H. A. (1970). Color measurement of strongly scattering media, with
587 particular reference of orange-juice beverages. *Journal of Agricultural and*
588 *Food Chemistry*, *18*(3), 371-376.

589 Sánchez-Marañón, M., Delgado, G., Delgado, R., Pérez, M., Melgosa, M. (1995).
590 Spectroradiometric and visual color measurements of disturbed and
591 undisturbed soil samples. *Soil Science, 160*, 291-303.

592 Sánchez-Marañón, M., Soriano, M., Melgosa, M., Delgado G., Delgado R. (2004).
593 Quantifying the effects of aggregation, particle-size and components on the
594 color of Mediterranean soils. *European Journal of Soil Science, 55*, 551-565.

595 StatSoft Inc. Statistica 6.0 for Windows Computer Program Manual, Tulsa, OK,
596 2001.

597 Tepper, B. J. (1993). Effects of a slight color variation on consumer acceptance of
598 orange juice. *Journal of Sensory Studies*, *8*, 145-154.

599 Tiwari, B. K., Muthukumarappan, K., O' Donnell, C. P., Cullen, P. J. (2008).
600 Modelling color degradation of orange juice by ozone treatment using
601 response surface methodology. *Journal of Food Engineering*, *88*(4), 553-560.

602 Wyszecki, G., Stiles, W. S. (1982). *Color Science. Concepts and Methods.*
603 *Quantitative Data and Formulae*. New York: John Wiley Sons, Inc.

604 Yang, Y., Celmer, E. J., Koutcher, J. A., Alfano, R. R. (2000). DNA and protein
605 changes in tissues probed by Kubelka-Munk spectral function. *Proceedings*
606 *of SPIE - The International Society for Optical Engineering, 3917*, 150-153.

607 Zsila, F., Deli, J., Simonyi, M. (2001). Color and chirality: Carotenoid self-
608 assemblies in flower petals. *Planta, 213*(6), 937-942.

609

610
611

FIGURE CAPTIONS

Figure 1. Reflectance visible spectra of an ultrafrozen (UFOJ) and two thermally-treated (TTOJ) orange juice samples obtained by spectroradiometry with white background (solid lines) and black background (dotted lines)

Figure 2. Absorption visible spectra of the carotenoid fraction of an UFOJ and two TTOJ in hexane

Figure 3. Spectroscopic representation of the Kubelka-Munk parameters of an UFOJ and two TTOJ

Figure 4. Representation of the levels of carotenoids of the samples *vs* the Kubelka-Munk parameters

Table 1

Table 1. Average total carotenoid content and CIELAB color coordinates of the samples analyzed and standard deviations (SD)

ULTRAFROZEN ORANGE JUICES (n = 26)					
White background					
	L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
Mean±SD	73.41±1.29	13.94±1.60	68.92±3.76	70.32±3.94	78.59±0.87
Black background					
	L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
Mean±SD	61.13±2.42	8.15±1.24	55.75±3.75	56.35±3.80	81.69±1.04
Total carotenoid content (mean±SD): 24.08±2.59 mg/l					
THERMALLY TREATED ORANGE JUICES (n = 44)					
White background					
	L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
Mean±SD	76.79±2.43	8.32±3.08	63.38±7.49	63.97±7.72	82.62±2.12
Black background					
	L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
Mean±SD	64.80±3.30	3.45±2.55	50.52±7.40	50.68±7.53	86.24±2.30
Total carotenoid content (mean±SD): 6.34±4.56 mg/l					

Table 2

Table 2. Linear correlation coefficients between the total carotenoid content and the CIELAB color coordinates as a function of the background used for the measurements. All the correlations were significant ($p < 0.05$)

UFOJ AND TTOJ (n = 70)				
White background				
L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
-0.67	0.86	0.67	0.70	-0.82
Black background				
L^*_{10}	a^*_{10}	b^*_{10}	$C^*_{ab,10}$	$h_{ab,10}$
-0.49	0.88	0.62	0.64	-0.86

Figure 1

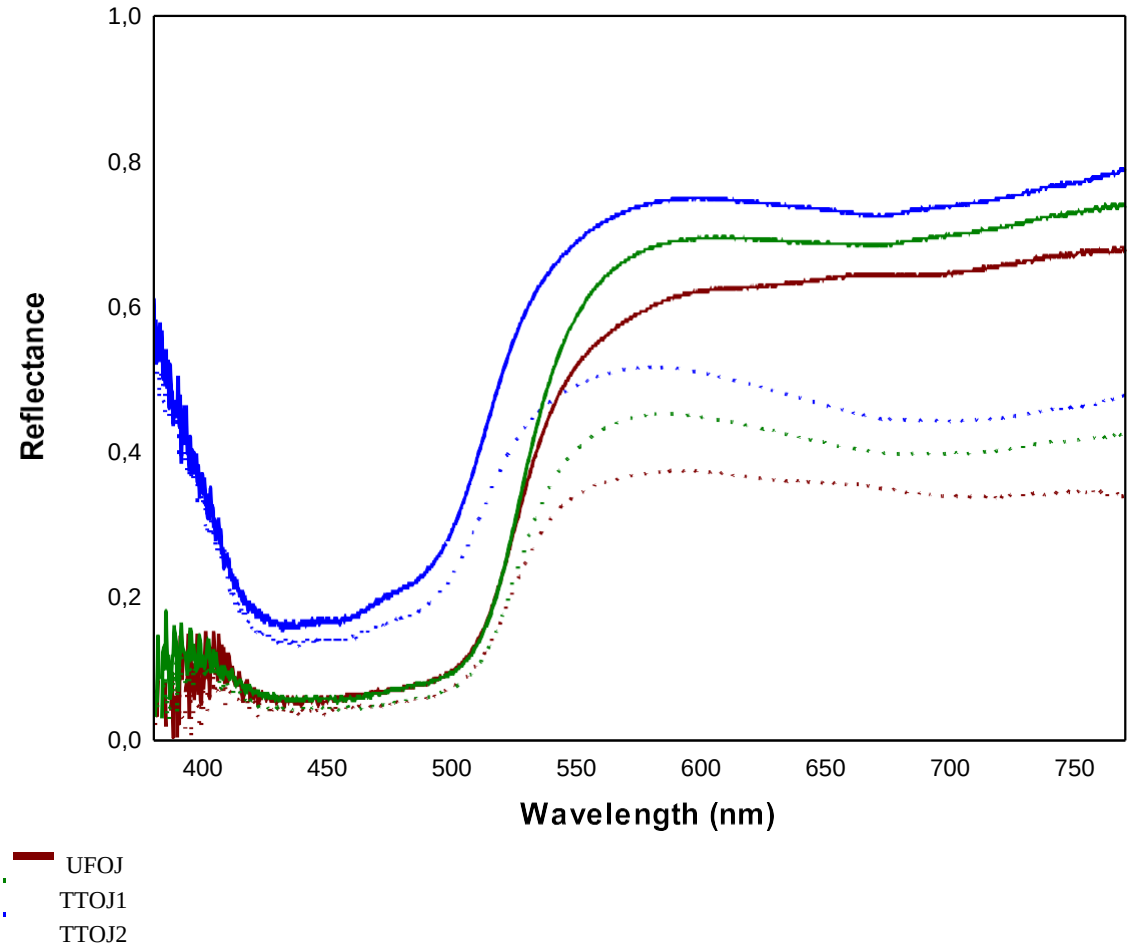


Figure 1. Reflectance visible spectra of an ultrafrozen (UFOJ) and two thermally-treated (TTOJ) orange juice samples obtained by spectroradiometry with white background (solid lines) and black background (dotted lines)

Figure 2

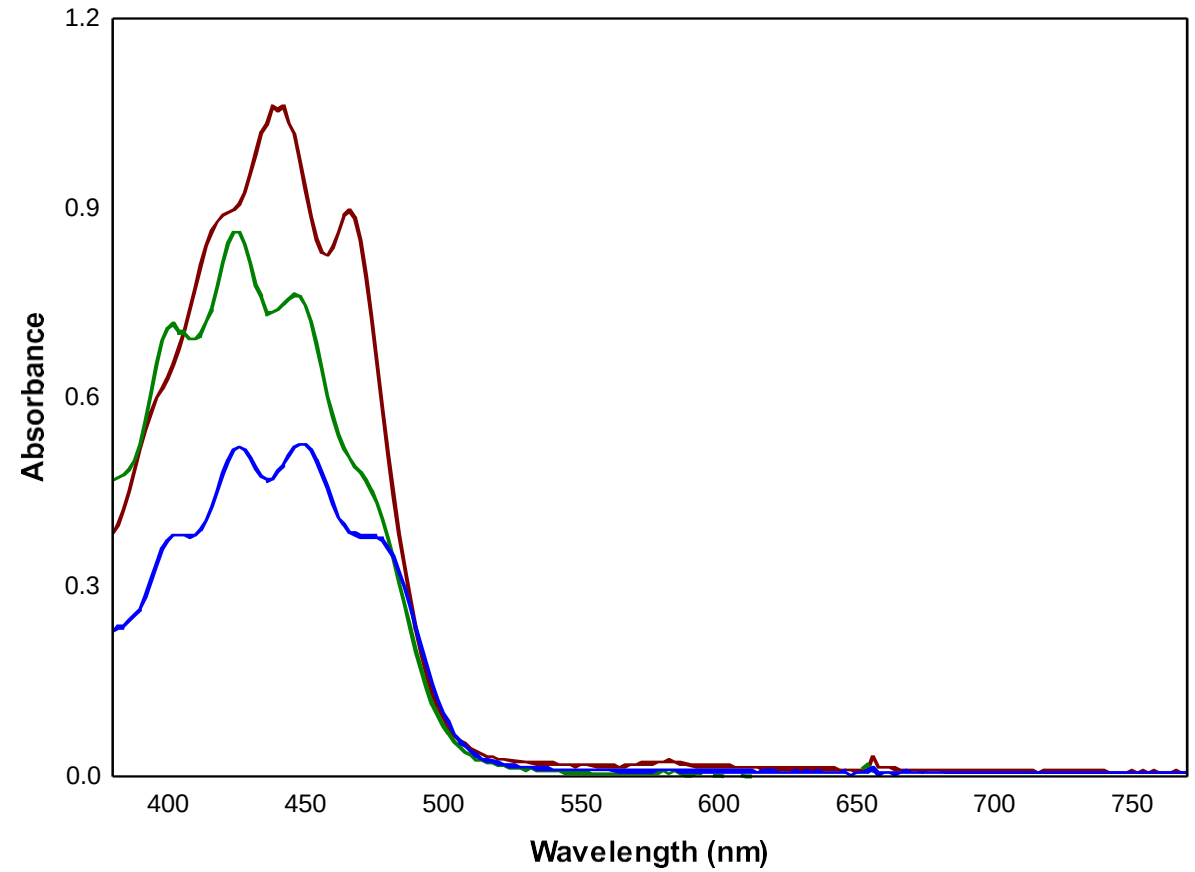
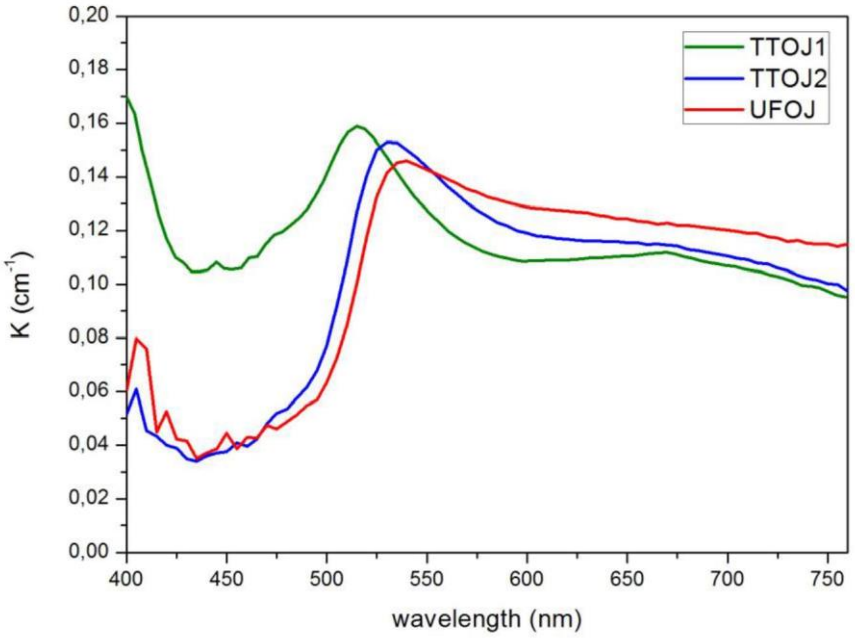
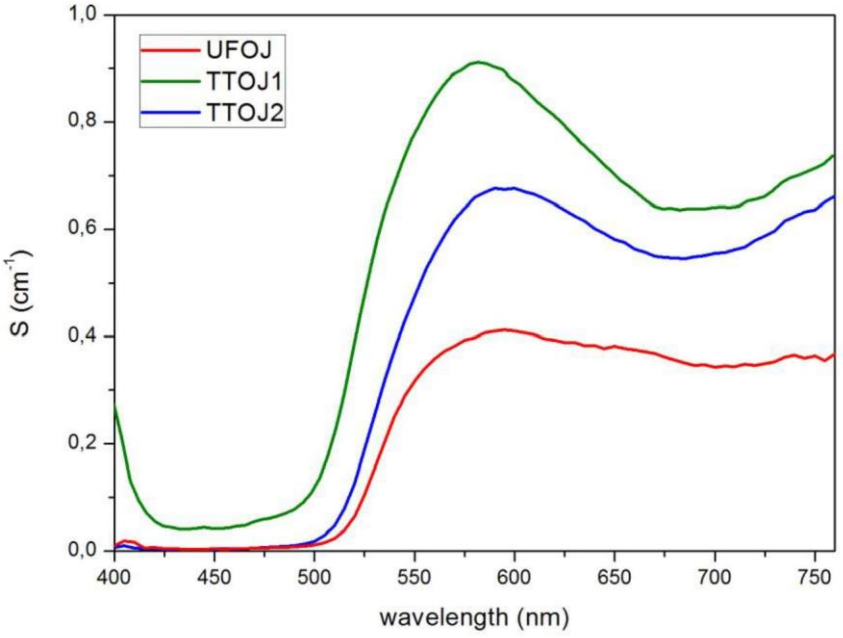


Figure 2. Absorption visible spectra of the carotenoid fraction of an ultrafrozen orange juice (UFOJ) and two thermally treated orange juices (TTOJ) in hexane

Figure 3


TTOJ1
TTOJ2
UFOJ
K (cm-1)
wavelength (nm)
0,00
0,02
0,04
0,06
0,08
0,10
0,12
0,14
0,16
0,18
0,20
400
450
500
550
600
650
700
750

1


UFOJ
TTOJ1
TTOJ2
S (cm-1)
wavelength (nm)
0,0
0,2
0,4
0,6
0,8
1,0
400
450
500
550
600
650
700
750

2

3

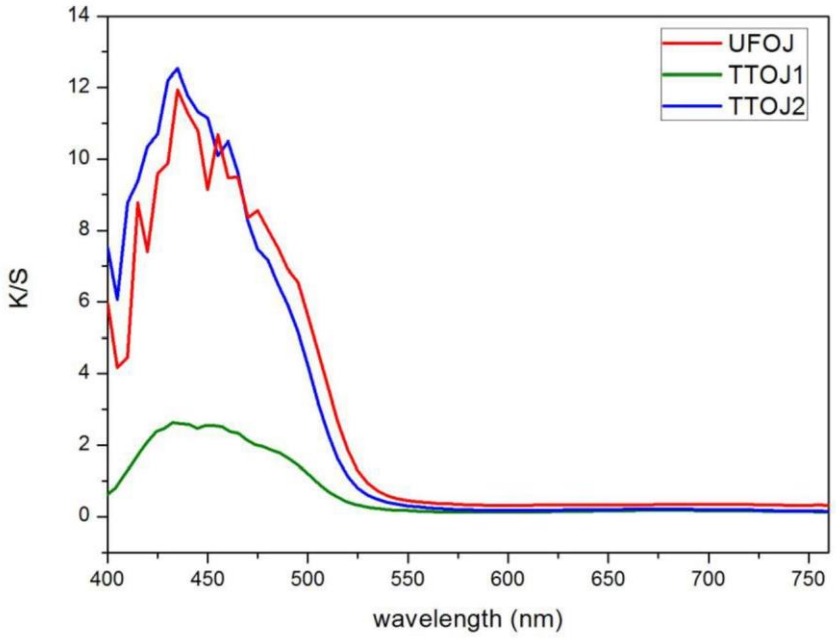
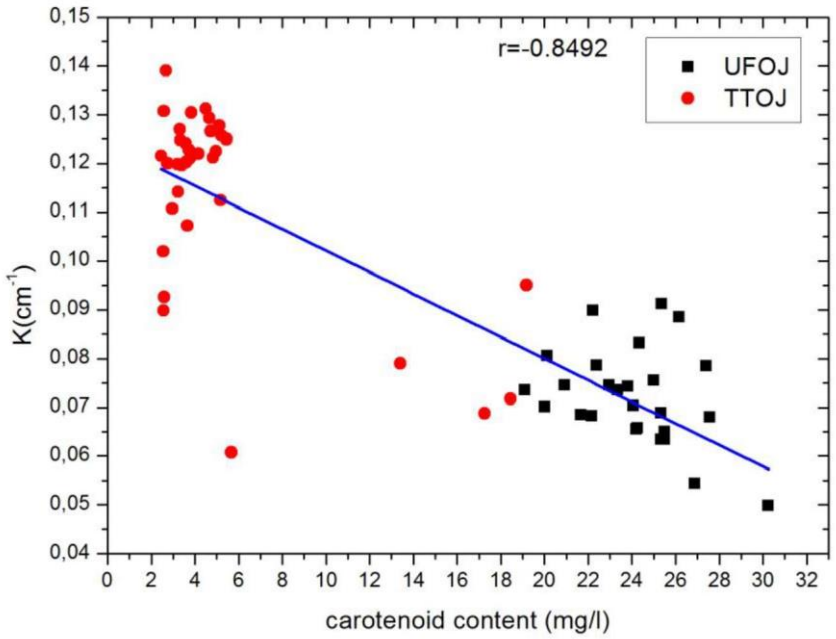
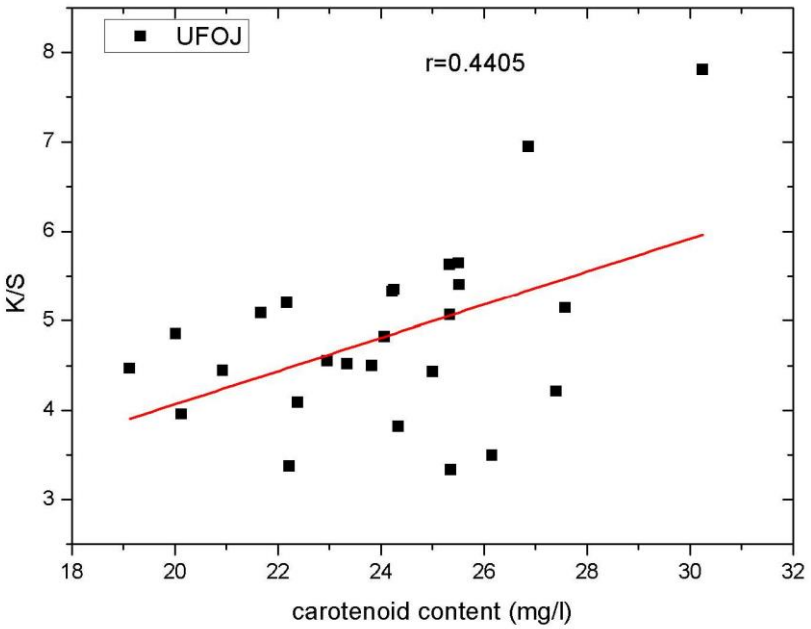


Figure 3. Spectroscopic representation of the Kubelka-Munk parameters of an ultrafrozen orange juice (UFOJ) and two thermally treated orange juices (TTOJ)

Figure 4


r=-0.8492
UFOJ
TTOJ
K(cm-1)
carotenoid content (mg/l)


UFOJ
r=0.4405
K/S
carotenoid content (mg/l)

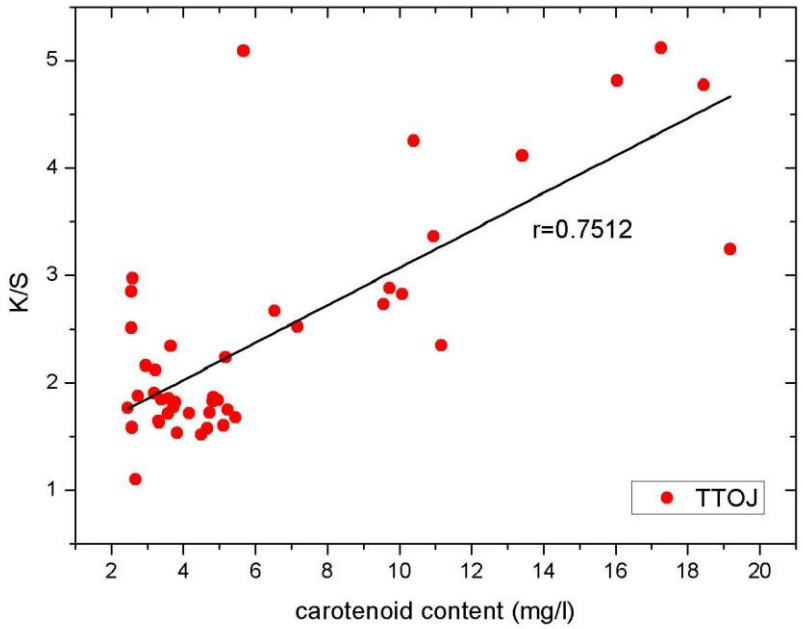


Figure 4. Representation of the levels of carotenoids of the samples (UFOJ n= 26, TTOJ n = 44) *vs* the Kubelka-Munk parameters, linear fits and simple regression coefficients