Industrial Orange Juice Debittering: Impact on Bioactive Compounds and Nutritional Value

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Abstract

The impact of an industrial debittering process (DP) on nutritional and bioactive compounds in orange juice (OJ) was studied. The DP was aimed at removing bitter components in OJ by physical adsorption in a resin. The levels of bioactive compounds (carotenoids, ascorbic acid and phenolics), total antioxidant activity and the colour in the fresh orange juices (non-debittered) and in the debittered counterparts were measured. The results demonstrated that the carotenoid contents were not significantly affected by the treatment. However, the debittered orange juices showed a reduction *($p<0.001$)* of 26% in ascorbic acid, 32% in hydroxycinnamic acids, 28% of flavones and 41% of flavanones in comparison with the non-treated juices. The antioxidant activity of the hydrophilic fraction (HF) was significantly higher *($p<0.05$)* in untreated juice than in debittered juices. Some colour parameters (L*, a* and h_{ab}) were also affected. Discriminant analysis revealed that the canonical function related to the levels of HF compounds allowed a 100% correct classifications of the different types of juices.

Keywords: carotenoids, colour, debittering; orange juice; phenolics

Abbreviations: debittering process (DP); orange juices (OJ); fresh orange juice (FOJ); debittered orange juice (DOJ); retinol activity equivalent (RAE); hydroxycinnamic acid derivatives (HCA); lipophilic fraction (LF); hydrophilic fraction (HF); hydrophilic antioxidant activity (HAA); Lipophilic antioxidant activity (LAA).

1. Introduction

Taste is one of the sensory quality attributes that, together with colour and flavour determines food selection. Bitter or astringent tastes tend to be rejected by the consumer, for that reason early season orange juice (OJ) or OJ from immature fruits must be subjected to an appropriate treatment to reduce bitterness.

Limonoids and flavonoids are the main groups of bitter compounds in citrus. Limonin, nomilin, and nomolinic acids are triterpene derivates compounds that occur gradually in certain varieties of citrus after juice processing giving a "delayed bitterness" (Puri, Marwaha, Kothari, & Kennedy, 1996). Limonin is the most representative compound in this group (Kimball & Kimball, 1991). Among flavonoids some are bitter while others are not, depending on the type of chain glycosides. The neohesperidose flavanones (rhamnosyl-α-1,2 glucose) (**Figure 1**), such as naringin, neohesperidin and neoeriocitrin are responsible for the bitter taste in grapefruit and bitter orange while rutinoside flavanones (ramnosyl-α-1,6 glucose) such as hesperidin, narirutin and didymin are tasteless (Horowitz, 1986). Naringin is found in the membranes and albedo of the fruit and is extracted into the juice, giving it an 'immediate' bitterness when their levels exceed 20 ppm (Fisher & Wheaton, 1976).

The bitterness level can be reduced by different technologies based on chemical, physical or microbiological processes. The current industrial technology for debittering is based on the adsorption of bitter compounds onto porous adsorbent resins (cellulose acetate or macroporous resin beads or cross-linked styrene divinylbenzene resins) (Shaw, Baines, Milnes, & Agmon, 2000). The effectiveness of different adsorbent resins to reduce the content of bitter compounds, mainly limonin have been assessed by several authors (Lee & Kim, 2003; Kola, Kaya, Duran, & Altan, 2010), but few have reported additional effects on other nutritional compounds like other flavonoids,

ascorbic acid or carotenoids. Some resins, like Dowex Optipore L285 have been reported to reduce the titratable acidity and increase the soluble solids content (Kola et al., 2010). Kimball and Norman (1990) reported that a commercial debittering system (Drow hydrophilic absorbent) reduced not only the bitter limonin contents by 71%, but also non-bitter flavonoids as hesperidin by 26%, in California navel orange juices.

The objective of the present work was to explore the impact of the industrial DP on bioactive compounds (carotenoids and phenolics) and on the nutritional value (provitamin A carotenoids and vitamin C) in orange juice. Additional parameters like colour and antioxidant activity were also evaluated.

2. Materials and Methods

2.1. Chemicals

Extraction solvents were analytical grade. Methanol, acetone and dichloromethane were purchased from Carlo-Erba (Milan, Italy). Analytic solvents were HPLC-grade, methanol, methyl-tert-butyl-ether and acetonitrile were procured from Merck (Darmstadt, Germany). Purified water was obtained from a NANOpure® DIamond™. (Barnsted Inc. Dubuque, IO). β-carotene, β-cryptoxantin and zeaxanthin were purchased from Sigma-Aldrich (Steinheim, Germany). Other carotenoids standards were either isolated from appropriate sources or semisynthesized in accordance to standard procedures as explained elsewhere (Meléndez-Martínez, Vicario, & Heredia, 2007). L-ascorbic acid was purchased from Panreac, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, narginin, naringenin, hesperidin and apigenin from Sigma-Aldrich (Steinheim, Germany), and neoericitrin and didimyn from Extrasynthese (Lyon-Nord, France).

2.2. Samples

Orange juice samples var. Salustiana were directly taken from the commercial orange juice production line at the firm "Zumos Pascual" (Palma del Río, Cordoba, Spain) at different times during the 2011 season. A basic flow scheme for this process is shown in **Figure 2**. The fresh orange juice is mechanically extracted with an FMC® in line Premium Juice Extractor (FMC Food Tech Citrus System, Lakeland, USA). The extracted juice is then conveyed to two finishing operations to separate juice sacs from the juice. The fresh industrial squeezed orange juice samples (FOJ) were taken at this stage.

The first pre-treatment in the DP is a centrifugation (Centrifuge, GEA Westfalia Group, Munich, Germany) to remove the excess of pulp (up to 1-3%). Subsequently, the juice is subjected to the DP which consists in passing the juice through a column, packed with a neutral resin with highly specific adsorption, to selectively remove the bitter juice components. The polymeric resin Lewatit VPOC 1064 MD PH (Bayer, Germany) was the absorbent used in this industry. This is a macroporous hydrophobic adsorbent resin without functional groups, based on a cross linked polystyrene in the form of porous beads of uniform size (0.44–0.54 mm). The specific area and pore volume of the resin were 800 m^2/g and 1.2 cm^3/g, respectively. After passing through the resin, the juice was reconstituted by adding pulp to the desired level. The debittered industrial OJ samples (DOJ) were taken at this stage. 27 samples of FOJ and DOJ counterparts were taken at 9 different dates (3 samples/ day). All analyses were done in triplicate.

2.3. Colour Measurement

The reflectance spectra were obtained by means of a CAS 140 B spectroradiometer (Instrument Systems, Germany) fitted with a Top 100 telescope optical probe, a Tamron zoom mod. SP 23A (Tamron USA, Inc., Commack, NY), and an external light source a white light 150W-metal-halide lamp Phillips MHN-TD Pro (12900 lumen, 4200 K

colour temperature) as source of illumination. Blank measurements were made with distilled water against a white background. The spectroradiometer was set to take three consecutive measurements of each sample, and colour coordinates were calculated as an average of the three replicates. The entire visible spectrum (380-770 nm) was recorded at 1 nm of bandwidth. From the spectra, the colour coordinates of the uniform colour space CIELAB (L*, a*, b*, h_{ab}, and C_{ab}*) were calculated by IS-SpecWin Software, considering the Illuminant D65 and the 10° Observer as references, (CIE, 1978). The colour differences (ΔE^*_{ab}) between two points in the CIELAB space are defined as the Euclidean distance between their locations in the three-dimensional space defined by L*, a*, and b*. This was calculated using the formula:

$$\Delta E^*_{ab} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \qquad (1)$$

where ΔL*, Δa* and Δb* are differences between the orange juice colour of FOJ and DOJ.

2.4. Carotenoid Analysis

The extraction and analyses of carotenoids were carried out according to the method described by Stinco et al. (2012). The identification of carotenoids 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 as explained elsewhere (Meléndez-Martínez et al., 2007). The carotenoid content of orange juice was worked out by external calibration performed in compliance with recommended guidelines (Rodríguez-Amaya, 2001) from calibration curves constructed with the corresponding standards, as explained elsewhere (Meléndez-Martínez et al., 2007). The total content was assessed as the sum of the content of individual pigments.

The vitamin A activity of the OJ samples was expressed in terms of retinol activity equivalents (RAE) (Food and Nutrition Board, 2002). The following formula was used for obtaining the RAE value and the results were referred to 1 L of OJ:

$$RAE = \frac{\mu g\ \beta\ carotene}{12} + \frac{\mu g\ \beta\ cryptoxanthin + \mu g\ \alpha\ carotene}{24} \quad (2)$$

2.5. Analysis of Ascorbic acid

The ascorbic acid was determined by HPLC with isocratic elution (Oruña-Concha, González-Castro, López-Hernández, & Simal-Lozano, 1998). Five hundred-µL aliquots of the OJ were gently mixed with 500 µL of 10 % metaphosphoric acid and centrifuged at 18000 g for 5 min. Eventually, the supernatant was filtered through a 0.45 µm pore size membrane filters before injection (Ross, 1994). An HPLC-DAD analysis was carried out on an Agilent 1200 system (Agilent, Palo Alto, CA) using a C18 column (2.5 µm, 10 cm x 4.6 mm) (Análisis Vínicos, Ciudad Real, Spain) kept at 20 ºC. The mobile phase was 0.02 M orthophosphoric acid and the isocratic flow was set at a rate of 1 mL/min. The chromatograms were monitored at 254 nm and the injection volume was 20 µL. Ascorbic acid peaks were identified by comparison of their retention times and spectra with those of the standard and the concentrations were worked out by external calibration. The results were expressed as milligrams of ascorbic acid per 100 mL of juice.

2.6. Analysis of Phenolic compounds

All individual phenolics were analyzed by ultra-high performance liquid chromatography (UPLC) with direct injection of the sample. Samples were centrifuged at 18000 g for 15 min at 4 ºC and subsequently filtered through a 0.45-µm pore size membrane filter before injection. The UPLC analyses were carried out on an Agilent 1260 system equipped with a diode-array detector, which was set to scan from 200 to

770 nm. Open lab ChemStation software was used and the chromatograms were monitored at 280, 320 and 370 nm. A C18 Poroshell 120 column (2.7 μm, 5 cm x 4.6 mm), (Agilent, Palo Alto, CA) kept at 25 ºC was used as stationary phase, and the injection volume was set at 20 μL. The mobile phase was pumped at 1.5 mL/min and consisted of two solvents: solvent A, water/formic acid (99:1; v/v) and solvent B, acetonitrile. The linear gradient elution was 0 min, 100% A; 5 min, 95% A + 5% B; 20 min, 50% A + 50% B; 22 min, 100% A; 25 min, 100% A.

The identification of phenolics compounds was carried out by considering chemical standards and mass spectra data using an Agilent 1100 (Agilent Technologies) system with a diode array detector (DAD). A Waters Spherisorb S3 ODS-2 C18, 3 μm (4.6 mm × 150mm) column thermostated at 25°C was used. The methodology described by Rodríguez-Pulido et al. (2012) was followed. The mass spectrometer (MS) was connected to the HPLC system via the DAD cell outlet. MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst 5.1 software. The quantification was carried out by external calibration considering the following wavelengths: 320 nm for hydroxycinnamic acids and flavones and 280 nm for flavanones. The results were expressed in mg/L of orange juice, as mean ± standard deviation.

2.7. Assessment of the in vitro antioxidant activity of lipophilic and hydrophilic extracts by the TEAC (Trolox Equivalent Antioxidant Capacity Assay) method

The method used is based on the capture of the radical cation ABTS+ generated in the reaction medium, compared to an standard antioxidant (Re et al., 1999). The 2,2'-Azinobis(3-ethylbenzothiazoline- 6-sulfonic acid) radical cation (ABTS+) was produced by reacting an ABTS aqueous solution (7 mM) with potassium persulfate

(2.45 mM final concentration). The mixture stood in the dark at room temperature for 12–16 h before use. The ABTS+ solution was diluted with ethanol (in the case of the lipophilic fractions) and PBS (in the case of the hydrophilic fraction) to an absorbance of 0.7 at 734 nm (30º). Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as a standard for comparison of the scavenging capacity.

Lipophilic TEAC Assay. One mL of the ABTS radical solution was added to the cuvette and the absorbance was measured at time 0. Subsequently, 5μL and 15 μL of the same extracts used to determine carotenoids by HPLC were added to the cuvette.

Hydrophilic TEAC Assay. One mL of the ABTS radical solution was added to the cuvette and the absorbance was measured at time 0. Subsequently, 10 μL and 25 μL of the supernatant (obtained by centrifugation at 5000 rpm for 15 min at 4ºC) were added.

In both cases the mixtures were stirred and incubated at 30°C. After 6 minutes, the absorbance was measured at 734 nm on a HP-8453 spectrophotometer equipped with temperature controller. The dose-response curve for Trolox consisted of plotting the absorbance at 734 nm as a percentage of the absorbance of the uninhibited radical cation (blank) and was based on triplicate analysis. The Trolox equivalent antioxidant activity (TEAC) was calculated by dividing the gradient of the curve of the sample and the gradient of the standard Trolox curve, taking into account the dilution used. The antioxidant activities of the lipophilic and hydrophilic fractions were expressed in millimols of Trolox per L of orange juice.

2.8. Statistical Analysis

The statistical design consisted of one-way repeated measures analysis of variance (ANOVA). Two factors were considered: the process with two levels (fresh and debittered) and the outcome variables, including the bioactive compounds, the antioxidant activity, the physicochemical and the colorimetric variables. The 27 cases

(orange juice samples) were used as random factor, for the purpose of establishing differences between process stages (fresh and debittered). Statistically significant differences *($p<0.05$)* were determined using the Turkey multiple comparison procedure. Pattern recognition techniques, such as stepwise Linear Discriminant Analysis (SLDA), were applied on experiment standardized data to distinguish between different types of orange juices. All the statistical analyses were performed with Statistica v.8.0 software (StatSoft, 2007).

3. Results and Discussion

3.1. Effect of the DP over the lipophilic fraction: carotenoids and colour

Table 1 shows the mean levels of the carotenoids quantified in the fresh (FOJ) and debittered orange juices (DOJ), their vitamin A activity expressed as retinol activity equivalent (RAE), as well as the colourimetric parameters.

The predominant carotenoids were 5,6-epoxycarotenoids (violaxanthin, antheraxanthin and geometrical isomers), followed by 5,8-epoxycarotenoids (luteoxanthin and mutatoxanthin), β-cryptoxanthin, zeaxanthin, lutein, zeinoxanthin, α-carotene and β-carotene. The establishment of meaningful comparisons with respect to the carotenoid profiles of other OJ is difficult as these depend on several factors such as the variety (Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, & Amiot, 2005), the agronomic factors, (Dhuique-Mayer, Fanciullino, Dubois, & Ollitrault, 2009) and the technology applied (thermal pasteurization, microwave heating and pulsed electric and high pressure) (Lee & Coates, 2003; Fratianni, Cinquanta, & Panfili, 2010; Plaza et al., 2011). However, it was noticed that the OJ samples from the Salustiana variety analyzed in this study had lower levels of β-carotene (0.17 mg/L) and lutein (0.57 mg/L) in comparison to other varieties. These results are in accordance with those reported by Dhuique et al. (2005) (0.11 and 0.60 mg/L, respectively) in the same

variety. Concerning the effect of the debittering treatment, the results showed that FOJ and DOJ were not significantly different in relation to the carotenoid contents and the RAE values. This is in accordance with the fact that only 1% to 3% of the pulp is passed through the debittering column, as shown in **Figure 1,** and that the majority of the carotenoids are present in the pulp.

In relation to the colour, the DP decreased the lightness and hue *($p<0.05$)*. Neither the chroma nor the b* values were significantly affected and only the a* value was increased by 23%. In summary, the DOJ were more reddish and darker than the FOJ. Few studies have evaluated the effect of the DP on the colour of citrus juices. In one of them, Lee and Kim (2003) reported that debittered red grapefruit juice exhibited less chroma and more lightness than the control samples. The total colour difference (ΔE^*_{ab}) values between FOJ and DOJ ranged from 0.87 to 5.88 CIELAB units, (mean value· ΔE^*_{ab}= 3.28 ± 1.64 CIELAB units). The mean value of ΔE^*_{ab} was close to the visual discrimination threshold (ΔE^*_{ab} >3), indicating that the colour changes could be slightly appreciable visually (Melgosa, Pérez, Yebra, Huertas, & Hita, 2001).

In summary, our results indicated that the industrial debittering using the polymeric resin Lewatit VPOC 1064 did not affect the carotenoid content nor the theoretical vitamin A activity (calculated as RAE value). Although some colourimetric parameters were affected (L*, h_{ab} and a*), the colour change was not significant enough to have consequences on the consumer acceptability.

3.2. Effect of the DP on the hydrophilic fraction: Ascorbic acid and phenolic compounds

The mean ascorbic acid content of FOJ was 61.79 mg/ 100 mL, which was in agreement with the results reported in juices from the same variety by other authors (Dhuique-Mayer et al., 2005; Rapisarda, Bianco, Pannuzzo, & Timpanaro, 2008).

The debittering treatment decreased *(p<0.001)* the ascorbic acid content by 27% in relation to FOJ. Similarly, Lee et al. (2003) reported a loss 26 % in grapefruit juices by the DP with a XAD-16 resin. Other published data (Kola et al., 2010) on Washington navel orange juices debittered using ion exchange resins (Dowex Optipore L285) and adsorbent resins (XAD-16HP) reported a 22% and 17% of ascorbic acid reduction, respectively. A 9% *(p<0.001)* of reduction was reported by Kimball and Norman (1990) in Drow hydrophilic absorbent at industrial scale.

Table 2 shows the mean levels of phenolic compounds determined by UPLC in FOJ and DOJ. A total of 24 phenolics were identified, which can be classified into three major categories: hydroxycinnamic acid derivatives (HCA) (caffeic, ferulic, *p*-coumaric, sinapic acids and derivates), flavones (apigenin and derivate) and flavanones (hesperidin, narirutin, naringin, neoeriocitrin and didymin). Such categories represented 9%, 8% and 82%, respectively, of the total quantified phenols in orange juices, which is in accordance with previous studies in sweet orange juices, regardless of the variety (Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007). The content of total phenolics compounds calculated as the sum of total hydroxycinnamic acids, flavones and flavanones determined by UPLC, were 536.95 and 326.75 mg//L, in FOJ and DOJ respectively. In general, it can be observed that the DP significantly *(p<0.001)* affected the phenol contents, decreasing in average by 39 % in DOJ.

Considering the phenolic compounds by groups, the mean content of HCA (caffeic, *p*-coumaric, ferulic and sinapic acids and nine derivates) was 51.26 mg/l. Ferulic acid was the main one (24.89 mg/L), followed by caffeic acid (9.29 mg/L), sinapic acid (8.60 mg/L) and *p*-coumaric acid (8.49 mg/L), in accordance with data previously reported by Kebelek et al. (2009) and Rapisarda et al. (2008; 1998) in other varieties.

As it can be observed in **Table 2**, the levels of hydroxycinnamic acids (as the sum of the content of individual compounds) decreased by 32% in DOJ *vs* FOJ *($p<0.001$)* (**Figure 3**). Specifically, reductions by 36% for sinapic acid, 33% for ferulic, 30% for caffeic and 27% for *p*-coumaric acids were found. The lowest decrease was observed in caffeic acid dimer, 18%.

Two flavones, vicenin-2 (apigenin 6,8-C-diglucoside) and a derivative of apigenin, were detected at a higher level (43.73 mg/L) than previously reported by Gil Izquierdo, Gil and Ferreres (2002). After the DP, the amount of total flavones and of each individual compound decreased significantly *($p<0.05$)*. Vicenin-2 (with an initial content of 32.61 mg / L) decreased by 36% while the apigenin derivate (11.11 mg/L) was only reduced by 3% (**Figure 3**).

The major flavonoids in citrus species are flavanones, which can be present in the glycoside or aglycone forms (Tripoli, Guardia, Giammanco, Majo, & Giammanco, 2007). As it can be observed in **Table 2**, the flavanone contents of FOJ were clearly higher than those of other HCA and flavones (representing 80% of total polyphenols).

Six flavanones, namely narirutin (naringenin 7-O-rutinoside), hesperidin (hesperetin 7-O-rutinoside), didymin (isosakuranetin 7-O-rutinoside), neoeriocitrin (eriodictyol 7-O-neohesperidoside), naringin (naringenin 7-O-neohesperidoside) and derivates (as narirutin- hexose) were identified in the juices. Hesperidin was the most abundant flavanone in FOJ, accounting for 80% of the total content. These results were in agreement with previous findings (Gattuso et al., 2007; Tomás-Barberán & Clifford, 2000; Dhuique-Mayer et al., 2005). The second most abundant flavanone was naritutin, at a concentration of 23.35 mg/L, lower than that previously reported in this variety by other authors (Dhuique-Mayer et al., 2009). Other minor flavanones were neoeriocitrin and didymin at mean concentrations of 7.33 and 4.99 mg/L, respectively. The industrial

DP reduced significantly *($p<0.05$)* the levels of these compounds. The total content of flavanones decreased from 442.29 to 260.34 mg/L *($p<0.001$).* The highest reduction was observed in a naringin derivate (60%; *$p<0.001$*), followed by a hesperidin derivate (55%; *$p<0.001$*), as shown in **Figure 3**. The comparison of these results with those reported in the literature are not straightforward whatsoever due to the marked differences in the type of resin and the debittering treatments, as well as in the type of juice analyzed. However, some similarities were found. For instance, our results were in accordance with those reported by Kranz, Alder and Kunz (2011), who found a 51% reduction in the naringin content when debittering grapefruit juices in a laboratory-scale downflow column packed with XAD-7HP. Similarly, Lee et al. (2003) reported that, when debittering red grapefruit juice concentrate in a pilot plant in a XAD-16 adsorption column, more than 78% of the bitterness, based on the naringin content, was removed. Moreover, some of the non-bitter flavonoids, such as narirutin and hesperidin, were also nearly completely removed.

In agreement with data reported by Kimball and Norman (1990) hesperidin underwent the lowest reduction (39%, *$p<0.001$*).

3.3. Effect of the DP on the antioxidant activity of the lipophilic (LF) and hydrophilic fractions (HF)

The total antioxidant activity of orange juice is due to both the HF (containing ascorbic acid and phenolic compounds) and the LF (containing mainly carotenoids). The predominant antioxidant activity of both fractions is believed to be radical scavenging, through inhibiting free radical reaction by donating electrons or hydrogen atoms (Tripoli et al., 2007). For this reason, the antioxidant capacities of both fractions were measured as free radical-scavenging capacity using the TEAC method.

The hydrophilic antioxidant activity (HAA) values for FOJ and DOJ, were 6.15± 1.20 and 4.58 ± 0.96 mM Trolox/L juices, respectively. These results are in agreement with those reported by Proteggente, Saija, De Pasquale and Rice-Evans (2003) for orange juices of different varieties (5.32, 4.79 and 3.49 mM Trolox/L for Ovale, Navel and Valencia respectively)

HAA was significantly decreased by 27% *($p<0.001$)*, which is in accordance with the reduction of the compounds in this fraction (previously reported in section 3.2). On the contrary, the lipophilic antioxidant activities (LAA) determined (0.025 and 0.024 mM Trolox/L juices for FOJ and DOJ, respectively) were not affected by the industrial DP. This was a foreseeable result, since the DP did not affect the carotenoid content, as previously discussed.

The values of HAA and LAA can reflect the contribution of the different antioxidants to the total antioxidant activity of the OJ. In order to make an assessment of the contribution of each compound to the LAA and HAA, multiple regressions were carried out. The coefficient obtained when the set of individual phenolic compound and ascorbic acid content were considered as predictors and all the samples were included, was 0.96 *($p<0.001$)*. Similarly, there was a positive and significant correlation between carotenoids and colourimetric parameter (L*, a*, b*) and the LAA, assessed by the ABTS method (r = 0.86, *$p<0.001$*).

3.4. Multivariate analysis

To ascertain whether it was possible to discriminate between FOJ and DOJ by considering the compounds present in the LF (carotenoids, colour parameters and antioxidant activity) or in the HF (ascorbic acid, phenolics compound and antioxidant activity) two discriminant analyses were carried out. The criterion for the selection of

variables was the Wilks' lambda, which maximizes the ratio of variance between groups to that within groups.

Taking into account the compounds in the LF a mathematical model was proposed based on five variables, namely, a*, (9*Z*)-violaxanthin + antheraxanthin, luteoxanthin + (*Z*)-antheraxanthin, L* and β-cryptoxanthin (**Table 3**). It classified correctly 91% of the cases *($p<0.001$)*. Canonical function was mainly related to the variables a*, luteoxanthin + (Z)-antheraxanthin, (positive sign) and (9*Z*)-violaxanthin + antheraxanthin, L* and β-cryptoxanthin (negative sign). **Figure 4** shows the distribution of the juices onto the space defined by the canonical functions.

The second discriminant analysis was performed on the compounds of the HF. Forward stepwise analysis was applied and 17 variables were chosen as shown in **Table 4.** The canonical function was mainly related to the levels of ferulic acid dimer, *p*-coumaric acid d_3 and narirutin (with positive coefficients) and ferulic acid d_2, *p*-coumaric d_1 and vicenin-2 (with negative coefficients). This model allowed a 100% correct classification as it can be observed in **Figure 5**. These results confirm the observations previously discussed in relation to the higher impact of the DP on the HF rather than on the LF.

4. Conclusion

In conclusion, the DP decreased significantly the ascorbic acid content *($p<0.001$)* and the phenolic compounds *($p<0.001$)*, consequently, the debittered juices showed a significantly lower antioxidant activity than the fresh juices. However this process, did not affect the lipophilic compounds. These results suggest that debittering decreases the nutritional quality concerning the vitamin C and phenolic compounds, but not the provitamin A.

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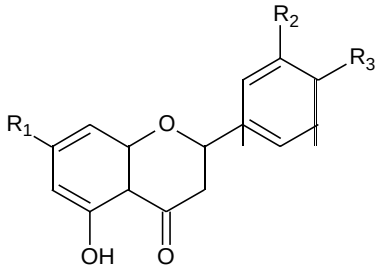
* Key references: These are the most original articles and book chapter on the effects of the debittering process on citrus juices which contributed to new knowledge in this field.

1 Figure 1

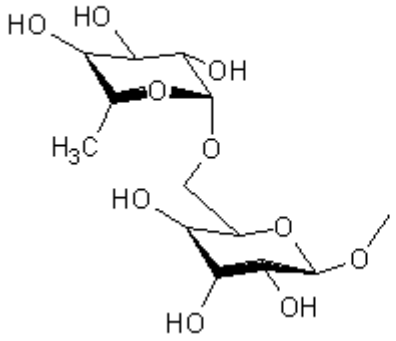
Flavanone-O-glycosides



Compound				
Name	**R_1**	**R_2**	**R_3**	**Taste**
Didymin	O-Ru[a]	H	OMe	tasteless
Hesperidin	O-Ru[a]	OH	OMe	tasteless
Narirutin	O-Ru[a]	H	OH	tasteless
Naringin	O-Nh[b]	H	OH	bitter
Neohesperidin	O-Nh[b]	OH	OMe	bitter
Neoeriocitrin	O-Nh[b]	OH	OH	bitter

[a] O- Rutinose; [b] O- Neohesperidose

Rutinose



Neohesperidose

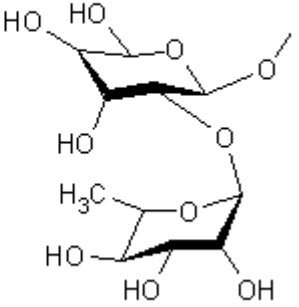
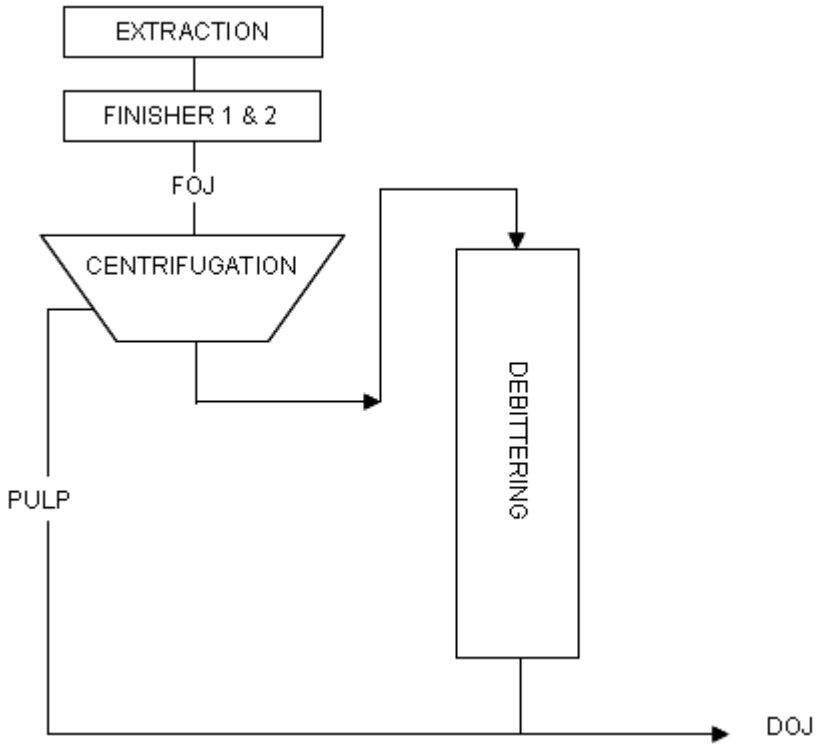


Figure 2



4 **Figure 3**

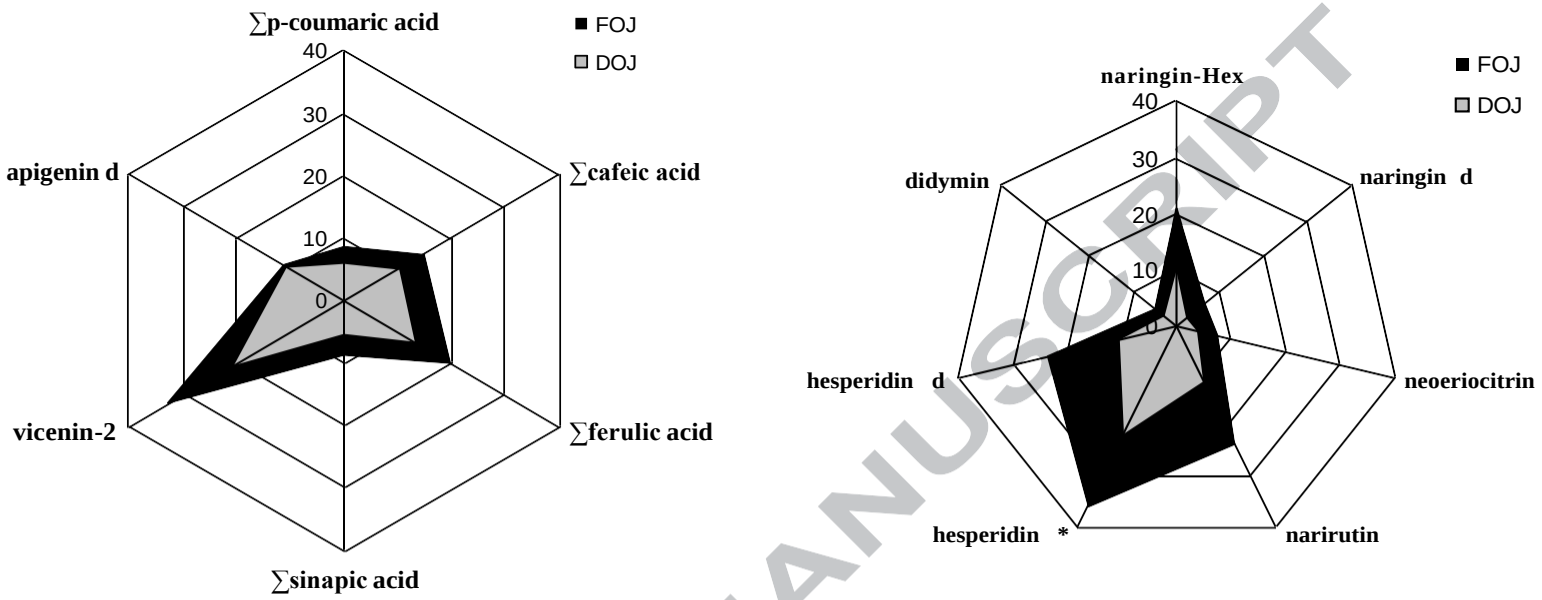


Figure 4

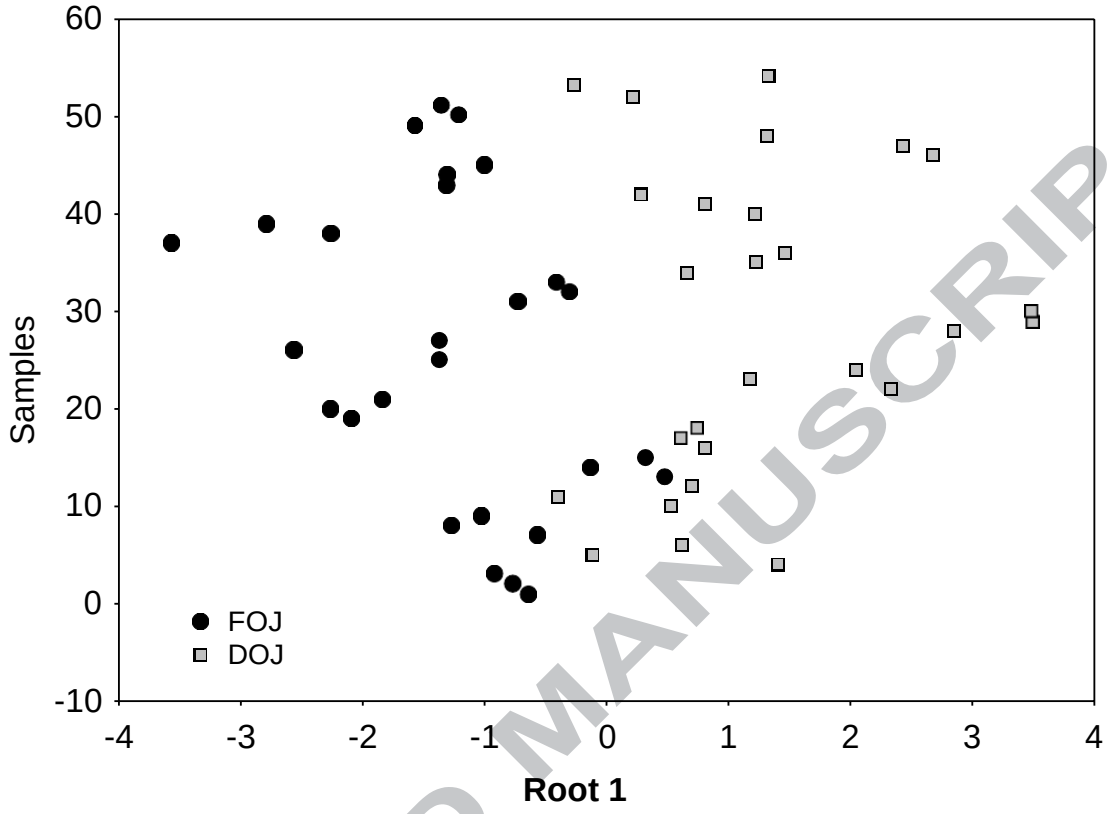


Figure 5

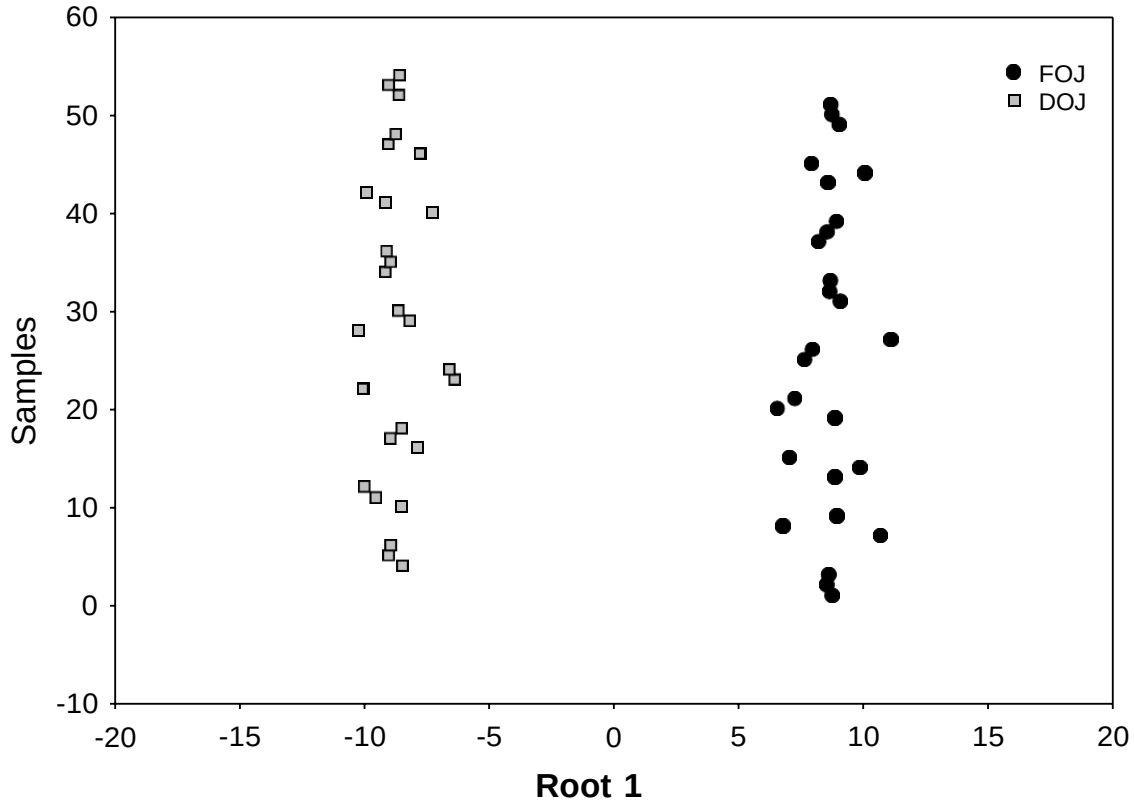


Table 1 Colourimetric parameters, Carotenoids levels (mg/L) and Retinol Activity Equivalents (RAE) for the different orange juices analyzed (FOJ: Fresh Industrial Squeezed and DOJ: Debittered Industrial Squeezed).

Carotenoid contents	**FOJ (n=27)**	**DOJ (n=27)**
violaxanthin isomers	2.62 ± 0.99	2.93 ± 1.30
luteoxanthin + (*Z*)-antheraxanthin	1.33 ± 0.45	1.61 ± 0.69
(9*Z*)-violaxanthin + antheraxanthin	5.70 ± 2.19	6.76 ± 1.31
(*Z*)-luteoxanthin	0.61 ± 0.22	0.71 ± 0.30
mutatoxanthin	0.70 ± 0.19	0.82 ± 0.28
lutein	0.57 ± 0.15	0.59 ± 0.19
mutatoxanthin	1.29 ± 0.38	1.45 ± 0.50
zeaxanthin	0.55 ± 0.18	0.59 ± 0.20
antheraxanthin	1.18 ± 0.46	1.34 ± 0.69
zeinoxanthin	0.31± 0.07	0.32 ± 0.09
β-cryptoxanthin	1.69 ± 0.66	1.82 ± 0.69
(Z)-ξ-carotene isomer	not quantified	not quantified
α-carotene	0.19 ± 0.04	0.21 ± 0.06
β–carotene	0.17 ± 0.06	0.17 ± 0.07
∑**Total Carotenoids**	16.91 ± 5.70	18.58 ± 7.40
RAE	92.43 ± 32.72	98.99 ± 34.56

Colourimetric parameters	**FOJ**	**DOJ**
L*	74.61 ± 1.10	74.01 ± 0.66*
a*	7.45 ± 0.63	9.19 ± 1.55***
b*	76.60 ± 5.76	77.40 ± 4.68
C^*_{ab}	76.97 ± 5.71	77.95 ± 4.74
h_{ab}	84.40 ± 0.76	83.24 ± 1.01***

Different superscripts within the same row indicate statistically significant differences * *p<0.05*, ** *p < 0.01* and *** *p<0.001*

Table 2 Phenolic compounds levels (mg/L) on the different orange juices analyzed (FOJ: Fresh Industrial Squeezed and DOJ: Debittered Industrial Squeezed).

Phenolic Compouds	FOJ (n=27)	DOJ (n=27)
p-coumaric acid-d_1	1.80 ± 0.43	1.31 ± 0.46***
caffeic acid dimer	0.50 ±0.09	0.41 ± 0.09***
p-coumaric acid-d_2	1.44 ± 0.33	1.07 ± 0.37***
ferulic acid-d_1	0.87 ± 0.22	0.67 ± 0.26**
ferulic acid dimer	1.90 ± 0.37	1.40 ± 0.50***
p-coumaric acid-d_3	2.32 ± 0.60	1.68 ± 0.64***
ferulic acid-d_2	4.94 ± 0.97	3.54 ± 1.43***
caffeic acid-d_1	0.98 ± 0.23	0.73 ± 0.24***
p-coumaric acid dimer	2.93 ± 0.79	2.17 ± 0.89**
sinapic acid-d	3.70 ± 0.76	2.52 ± 1.14***
ferulic acid-d_3	5.44 ± 0.99	4.04 ± 1.62***
caffeic acid	7.81± 2.17	5.09 ± 1.71***
ferulic acid-d4	2.31 ± 0.59	1.61 ± 0.67***
ferulic acid hexose	9.58 ± 1.69	5.97 ± 2.31***
sinapic acid hexose	4.90 ± 0.70	3.00 ± 0.96***
∑Hydroxycinnamic acid	51.02 ± 9.19	34.81 ± 12.86***
apigenin-d	11.11 ± 0.69	10.76 ± 0.37*
vicenin-2	32.61 ± 6.35	20.87 ± 4.45***
∑Flavones	43.72 ± 6.56	31.63 ± 4.71***
narirutin hexose	20.83 ± 5.36	10.72 ± 5.43***
neoeriocitrin	7.33 ± 1.63	3.95 ± 1.45***
hesperidin-d	23.55 ± 10.92	10.50 ± 5.96***
narirutin	23.35 ± 6.78	11.05 ± 4.20***
hesperidin	355.92± 72.38	218.65 ± 66.60***
didymin	4.99 ± 1.71	2.95 ± 0.88***
naringin-d	6.32 ± 2.58	2.51 ± 1.59***
∑Flavanones	442.30 ± 84.44	260.34 ± 81.52***
∑Total Phenols	536.95 ± 97.06	326.75 ± 94.91***

Different superscripts within the same row indicate statistically significant differences

* $p<0.05$, ** $p < 0.01$ and *** $p<0.001$

Table29 Summary of the forward stepwise discriminant analysis on the compounds the lipophilic fraction, antioxidant activity and colour

Step	Variable	F-value	Standardized coefficients
			Root 1
1	a^*	19.99	0.93
2	(9Z)-violaxanthin + antheraxanthin	8.16	-1.52
3	luteoxanthin + (Z)-antheraxanthin	7.03	1.47
4	L^*	7.61	-0.61
5	β-cryptoxanthin	5.02	-0.73
	p-level		*<0.001*
	Eigenvalue		1.62

Table 30 Summary of the forward stepwise discriminant analysis on the compounds the hydrophilic fraction and antioxidant activity.

Step	Variable	F-value	Standardized coefficients
			Root 1
1	ascorbic acid	52.86	3.21
2	sinapic acid hexose	6.12	4.16
3	ferulic acid dimer	17.51	25.95
4	apigenin-d	17.85	-2.32
5	narirutin hexose	5.42	3.35
6	HAA	36.88	2.49
7	narirutin	44.19	10.68
8	didymin	9.97	-2.96
9	caffeic acid	27.35	-5.58
10	ferulic acid-d_2	42.25	-22.16
11	*p*-coumaric acid d_1	16.71	-14.97
12	vicenin-2	14.97	-7.51
13	naringin d	23.21	3.51
14	hesperidin-d	19.91	-4.46
15	*p*-coumaric acid-d_3	4.24	11.34
16	ferulic acid-d_1	9.73	-3.19
17	ferulic acid-d_3	4.54	-4.60
	p-level		*<0.001*
	Eigenvalue		78.18

Highlights

- Industrial debittering was carried out on a polymeric resin Lewatit VPOC 1064.
- The process affected the nutritional quality of the debittered juices
- The debittering process reduced the Vitamin C and bioactive flavonoids levels
- Debittering did not affect the provitamin A carotenoid level
- The antioxidant activity was reduced by the debittering process.