Betoret Valls, ME.; Sentandreu Vicente, E.; Betoret Valls, N.; Fito Maupoey, P. (2012). Homogenization pressures applied to citrus juice manufacturing. Functional properties and application. Journal of Food Engineering. 111(1):28-33. doi:10.1016/j.jfoodeng.2012.01.035.

Effects of pressure homogenization on particle size and the functional properties of citrus juices.

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A B S T R A C T

Homogenization is useful in the citrus industry to improve the quality of citrus juices. The aim of this work was to study the effect of the homogenization pressures 0, 5, 10, 15, 20, 25 and 30 MPa on the particle size distribution, colour, cloudiness, and flavonoid content of fresh citrus juices to determine the ideal conditions of juices to be used in the development of functional fresh fruit. The results showed that homogenization pressure affected the particle size distribution and colour of the citrus juices, which made it possible to define different sample groups on the basis of the applied pressure. In fresh juice the contents of the flavonoids were not affected by homogenization pressure but after five months stored juice the content of the flavonoid hesperidin was affected.

Keywords: Flavonoids, Particle size, Cloudiness, Color, Citrus juice, Homogenization

1. Introduction.

The beneficial effects of citrus fruit consumption on human health have long been known. The antioxidant and antiradical properties of some of the compounds in fruit, particularly in the juice of the fruit, are responsible for these effects.

Flavonoids are the most studied compounds in juice known to have antioxidant and antiradical activities. The activity of flavonoids has been shown in vitro (Bocco et al., 1998; Rapisarda et al., 2000; Burda and Oleszek, 2001) and in vivo. It is known that flavonoids have important health-related properties, such as antimicrobial (Bylka et al., 2004), anticarcinogenic (Benavente-Garcia et al., 1997; Kohno et al., 2001), antiaggregative (Middleton et al., 2000) properties and are known to protect against cardiovascular diseases (Hollman et al., 1996). Moreover, it is important to note that no adverse physiological reactions to the consumption of flavonoids have been described.

Narirutin, hesperidin, and didymin belong to the flavanona glycosides group and are the most abundant flavonoids in mandarin orange juice. They have antioxidant activity and appear to influence lipid metabolism. Different studies have shown that hesperidin can inhibit chemically induced breast cancer (So et al., 1996), bladder cancer (Yang et al., 1997), and colon cancer (Tanaka et al., 1997; Miyagi et al., 2000) in laboratory animals.

The characteristics of mandarin orange juice mentioned above make it suitable as a medium to develop fresh and functional foods via vacuum impregnation (Fito et al., 2000).

It is important to consider that the cloudiness of citrus juice is an indicator of quality. Citrus juice clouds contain cellular organelles and membranes, chromatophores, oil droplets, flavonoid crystals, and cell wall fragments such as pectin, cellulose, and hemicellulose (Baker and Cameron, 1999).

Orange cloud particles range in size from 400 to 5000 nm; particles smaller than 2000 nm are the most stable (Buslig and Carter, 1974). Thus, an easy way to increase the cloudiness of citrus juices is to reduce the size of the cloud particles. Homogenization is a process that involves applying pressure to liquids to fragment the solid particles and oil droplets into smaller particles. Thus, homogenization is useful in the citrus industry for increasing the yield of citrus juices (Lortkipanidze et al., 1972) and for improving some quality factors of citrus juices, such as viscosity (Crandall and Davis, 1991), colour (Lee and Coates, 2004), cloudiness (Baker, 1977), and the stability of suspended solids (Carle et al., 1998). Homogenization converts the sensible pulp to background pulp (Kupper et al., 1987), which modifies the flavour of the juice (Marsh et al., 2006).

Homogenization can also be applied like pre-treatment in other processes where the particle size affects the efficiency and yield of the process, and to the quality factors of the final products (membrane processes, vacuum impregnation). For to have impregnated food with functional value added, mandarin juice has to include the most part of the pulp, because of some functional components are there (Garau et al., 2007). Pulp of citrus juices has big size of particles that can be inconvenient for to introduce the juice inside of the fruit extracellular spaces. Reducing particle size by homogenization pressures and centrifugating after it for to eliminate biggest particles can help to introduce more quantity of juice and therefore functional components inside of fruit structure by vacuum impregnation. References about the quantitative effect of the homogenization on the particle size in citrus juices have not been found, but it is possible that different homogenization conditions cause different distribution of cloud particle size, affecting physical properties of treated juices.

The aim of this work was to study the effect of homogenization pressure on the particle size distribution, colour, cloudiness, and flavonoid content of fresh citrus juices to determine the ideal conditions of juices to be used in the development of functional fresh fruit.

2. Materials and methods.

2.1 Sample preparation.

Ortanique fruit, a hybrid of tangerine and sweet orange (Citrus sinensis x Citrus reticulata), and Salustiana oranges (C. sinensis) were harvested in an orchard

located in Vilamarxant (Valencia), Spain, and used immediately for juices preparation. Two different juices were prepared, one from Ortanique fruits and another from Salustiana fruits. The fruits were washed by immersing them in tap water, drained, and squeezed in an industrial extractor with finger cups (Exzel, Luzzysa; El Puig, Valencia, Spain). Raw juice coming for each variety was homogenized with a Manton-Gaulin pilot homogenizer (model 15M8TBA) at different pressures, centrifuged with a Westfalia centrifuge (model SAOH 205), pasteurized at 63 ºC for 15 s, collected in sterile jars, and quickly frozen at -18 ºC until analyzed. The preparation of the juices was carried out according to the patent PO/2007/042593 titled ‘‘Method of obtaining refrigerated pasteurized citrus juices” (Izquierdo et al., 2007).

2.2. Brix, acidity, and essential oils.

Total soluble solids were measured as Brix with a digital refractometer (Pal-1; Atago Co., Ltd., Tokyo, Japan). Total titratable acidity was assessed by titration with 0.1 N NaOH and expressed as the percentage of citric acid. Recoverable essential oils were determined by bromate titration, according to the Scott Method (Scott and Veldhuis, 1966). The values provided are the average of three replicates.

2.3. Suspended pulp and cloudiness.

To measure the suspended pulp, a graduated centrifuge tube with a conical bottom was filled with 10 ml of juice. The sample was centrifuged with Eppendorf Refrigerated Multipurpose centrifuge (model 5804R) for 10 min at 365 g at 27 ºC and then the pulp volume was read (FMC FoodTech, 2005). The supernatants were collected, and their transmittance was analyzed for cloudiness at 650 nm with a UV/Visible spectrophotometer (Ultrospec 3300 pro; Amersham Bioscience, Piscataway, NJ, USA.). The values provided are the average of three replicates.

2.4. Particle size.

Particle size was determined by using Malvern Mastersizer equipment (Model 2000; Malvern Instruments Limited, Worcestershire, U.K.) with a short-wavelength blue light source in conjunction with forward and backscatter detection to enhance sizing performance in the range 0.02–2000 μm. The values 1.73 and 1.33 were used for the refractive indices of cloud and dispersed phase, respectively, and 0.1 was used for the absorption index of cloud particles (Corredig et al., 2001). To measure the sizes of the particles the equipment is using laser diffraction technique and because of this the particle size distribution obtained was based on volume, i.e., the particle size interval obtained represented the total volume (%) of all particles with a diameter included in this interval in relation to the total volume of all particles in the distribution. In the case of monomodal distributions, the

particle size was described by the volume-weighted mean diameter (D[4,3]) (Eq.(1)) and the diameter corresponding at the maximum value of the peak (D_{peak})

$$D[4,3] = \sum_i n_i d_i^4 \Big/ \sum_i n_i d_i^3 \quad (1)$$

where n_i is the number of particles of diameter d_i. The values provided are the average of at least three replicates.

2.5. Colour.

Colour was measured with a Hunter colorimeter (Labscan II model controlled by a computer) following the indications described by Bayarri et al. (2001). Samples were contained in optical glass cells 3.8 cm high and 6 cm in diameter. The results were provided in a CIELAB system for illuminant D65 and a 10º angle of vision. The registered parameters were as follows: L* (brightness), a* (red-green component), b* (yellow-blue component), h_{ab} (hue, attribute related to the differences in absorbance at different wavelengths and considered the qualitative attribute of colour) (Eq. (2)), and C_{ab} (chrome, quantitative attribute of colourfulness) (Eq. (3)). The global colour difference (ΔE) was calculated by using equation 4. The values provided are the average of three replicates.

$$h_{ab}^* = \arctan(b^*/a^*) \quad (2)$$

$$C_{ab}^* = [(a^*)^2 + (b^*)^2]^{\frac{1}{2}} \quad (3)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}} \quad (4)$$

2.6. Flavonoids.

The content of the flavonoids narirutin, hesperidin, and didymin was determined using high-performance liquid chromatography (HPLC). An Alliance 2996 chromatograph (Waters) was used with a diode array detector (DAD) and filled with a C18 reversed-phase column (250 x 4.6 mm and 5 μm; Luna II Phenomenex). For the extraction of flavonoids, juice samples were centrifuged for 15 min at 1400 rpm at 4 ºC using a Meldifriger SL centrifuge. The supernatant was filtered using a Whatman nº1 filter; 30 ml of the filtered samples was passed through a Sep-Pack C18 cartridge, and the cartridge was cleaned with 5 ml of water and eluted with 5 ml of water:acetonitrile in proporcion (4:6, v/v). Two hundred fifty milliliters of cumaric acid (1 μg/μl) was added to the eluate. The resulting sample was filtered using an Albet nylon membrane filter with a pore diameter of 0.45 μm. Finally, a 1 ml aliquot was collected, and 5 μl was injected into the HPLC system. A solution of water:tetrahydrofuran (solvent A) and a solution of acetonitrile:tetrahydrofuran (solvent B) were used for the mobile phase. The HPLC system was operated in gradient at a flow rate of 1 ml/min.

Flavonoids were detected at a wavelength of 280 nm. The values provided are the average of three replicates.

2.7. Statistical analysis.

All the values provided are the average of at least three replicates and to determine the significant differences of the results an Analysis of Variance test was carried out (One-way ANOVA or Multifactor ANOVA) with confidence level of 95% ($p < 0.05$) using the program STATGRAPHICS PLUS v.5.1.

3. Results and discussion.

Fresh juice samples were characterized by measuring the following: contents of soluble solids, essential oils, and suspended pulp; acidity; maturity index; and pH. The results are shown in Table 1.

Table 1. Characteristics of fresh citrus juices (mean ± standard deviation).

	Ortanique	Salustiana
Brix	12.9 ± 0.1	12.7 ± 0.1
Acidity	1.28 ± 0.56	1.10 ± 0.75
Maturity index (Brix/Acidity)	10.08 ± 0.32	12.12 ± 0.51
Essential oils (%)	0.045 ± 0.003	0.044 ± 0.004
Suspended pulp (%)	25.0 ± 1.3	16.5 ± 1.0
pH	3.42 ± 0.02	3.50 ± 0.03

The results of the cloudiness and suspended pulp determinations are shown in Table 2.

Table 2. Effect of homogenization pressure on the suspended pulp and cloudiness of Ortanique and Salustiana juices (mean ± standard desviation.

		Homogenization pressure						
		0 MPa	**5 MPa**	**10 MPa**	**15 MPa**	**20 MPa**	**25 MPa**	**30 MPa**
Ortanique	Pulp separated (%) by centrifugation at 365 *g* for 10 min	25.0±1.3	20.3±1.0	18.2±1.2	15.5±0.5	14.5±1.0	16.5±1.5	16.0±0.8
	Transmittance (%) of supernatant separated by centrifugation at 365 *g* for 10 min	1.17±0.03	0.56±0.02	0.39±0.02	0.35±0.03	0.35±0.02	0.28±0.02	0.21±0.02
	Pulp separated (%) by centrifugation at 3000 *g* for 10 min	11.2±0.5	10.0±0.5	9.2±0.8	9.0±0.5	8.5±0.5	9.2±0.8	9.0±0.5
	Transmittance (%) of supernatant separated by centrifugation at 3000 *g* for 10 min	7.30±0.04	1.59±0.03	0.67±0.03	0.67±0.03	0.70±0.04	0.55±0.03	0.36±0.03
Salustiana	Pulp separated (%) by centrifugation at 365 *g* for 10 min	16.5	-	-	11.5	-	-	9.5
	Transmittance (%) of supernatant separated by centrifugation at 365 *g* for 10 min	3.54±0.13	-	-	0.23±0.02	-	-	0.13±0.01

The precipitated volume and supernatant transmittance in samples centrifuged at 365 g decreased as the homogenization pressure increased. When the pressure increased from 0 to 15 MPa, the changes in both variables were higher than when the pressure increased from 15 to 30 MPa.

The low values obtained in transmittance show the high level cloudiness and suspended pulp in juice that increased when the homogenization pressure increased.

The particle size distribution in Ortanique juice samples not homogenized or homogenized at 5, 10, 15, 20, 25, and 30 MPa and in Salustiana juice not homogenized or homogenized at 15 and 30 MPa is shown in Figs. 1 and 2, respectively. Particle size showed a distribution between 0.5 and 1000 μm, with irregular peaks between 100 and 1000 μm and with a natural evolution to smaller sizes when the homogenization pressure increased.

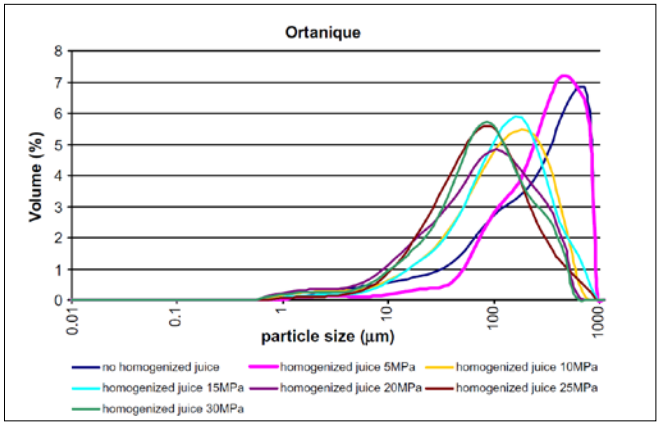

Figure 1. Effect of homogenization pressure on particle size of citrus juices.

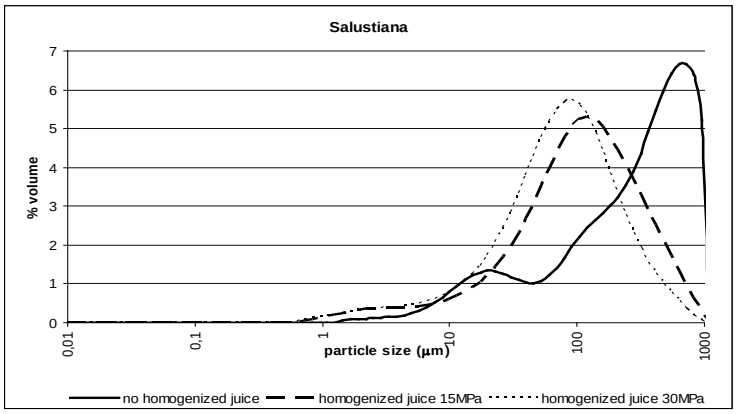

Figure 2. Effect of homogenization pressure on particle size of citrus juices.

The particle size parameters for Ortanique juice and Salustiana juice are shown in Tables 3 and 4, respectively. The mean D[4,3] and D_{peak} values for Ortanique juice and Salustiana juice decreased as the homogenization pressure increased. An analysis of variance with confidence level of 95% ($p < 0.05$) was conducted to determine the significance of the differences in these two variables as a function of homogenization pressure. For Ortanique juice, the analysis showed a significant effect of homogenization pressure on both D[4,3] and D_{peak}.

Table 3. Effect of homogenization pressure on the particle size of Ortanique juice (mean ± standard desviation).

Homogenization pressure (MPa)	*D*[4,3]	*D*peak

0	346.3 ± 12.1[a]	710.3 ± 18.5[a]
5	372.1 ± 1.9 [a]	503.0 ± 7.8[a]
10	171.01 ± 7.4[b]	206.5 ± 2.5[b]
15	180.6 ± 3.1[b]	166.9 ± 10.8[b]
20	130.3 ± 3.6 [c]	114.5 ± 1.7[c]
25	123.2 ± 8.6[c]	90.7 ± 1.3[c]
30	122.9 ± 2.2[c]	90.7 ± 1.2[c]

* Values with different superscript letters in a column are significantly different ($P \leq 0.05$).

Table 4. Effect of homogenization pressure on the particle size of Salustiana juice (mean ± standard desviation).

Homogenization pressure (MPa)	$D[4,3]$	D_{peak}
0	340.3 ± 8.5[a]	552.4 ± 41.8[a]
15	150.1 ± 4.8[b]	95.9 ± 1.4[b]
30	107.7 ± 4.1[c]	70.8 ± 1.0[c]

* Values with different superscript letters in a column are significantly different ($P \leq 0.05$).

The analysis of variance for D[4,3] in Ortanique juice clearly showed three sample groups: one group was composed by homogenized samples at 0 and 5 MPa, a second group was composed by homogenized samples at 10 and 15 MPa, and a third group was composed by homogenized samples at 20, 25, and 30 MPa (Fig. 3). The analysis of variance for Dpeak showed significant differences between juice samples homogenized at 0 and 5 MPa; the remainder of the samples were in the same groups as shown for D[4,3] (Fig. 3). In this case, the analysis showed four sample groups: one group was composed by unhomogenized samples, a second group was composed by homogenized samples at 5 MPa, a third group was composed by homogenized samples at 10 and 15 MPa, and a fourth group was composed by homogenized samples at 20, 25, and 30 MPa.

For both D[4,3] and D_{peak} in Salustiana juice, the biggest differences were observed between the unhomogenized samples, the samples homogenized at 5 MPa, and the remainder of the samples (Fig. 4). In case of Salustiana juice samples showed a significant effect of homogenization pressure in both D[4,3] and D_{peak}.

Colour was measured in the unhomogenized and homogenized samples of Ortanique juice and Salustiana juice. The effect of homogenization pressure on the CIE-L*a*b* parameters, psychrometric coordinates chrome (C^*_{ab}), and hue (h^*_{ab}) are shown in Tables 5 and 6, respectively. Brightness (L*) and colorfulness (C^*_{ab}) were greater in Salustiana juice than in Ortanique juice. Moreover, component a* was greater and component b* smaller in Ortanique juice than in Salustiana juice. This means a yellow colour for Salustiana juice and as red colour for Ortanique juice.

Table 5. Effect of homogenization pressure on CIE-L*a*b* parameters in Ortanique juice (mean ± standard desviation).

Homogenization pressure (MPa)	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}
0	52.01±0.03[a]	14.27±0.5[a]	74.06±0.13[a]	75.42±0.12[a]	79.09±0.06[a]
5	51.47±0.03[a]	14.455±0.014[a]	75.22±0.12[a]	76.59±0.12[a]	79.122±0.008[a]
10	53.72±0.04[b]	15.53±0.02[b]	80.45±0.05[b]	81.94±0.05[b]	79.08±0.02[a]
15	54.02±0.06[b]	15.47±0.06[b]	81.753±0.112[b]	83.20±0.110[b]	79.28±0.04[a]
20	53.83±0.03[b]	13.000±0.014[c]	80.502±0.015[b]	81.546±0.013[b]	80.8262±0.0112[b]
25	55.8±0.3[c]	17.815±0.019[d]	86.3±0.2[c]	88.±0.3[c]	78.33±0.09[c]
30	53.95±0.17[b]	15.19±0,06[b]	82.2±0.2[b]	83.6±0.2[b]	79.537±0.015[a]

* Values with different superscript letters in a column are significantly different ($P \leq 0.05$).

Table 6. Effect of homogenization pressure on CIE-L*a*b* parameters of Salustiana juice (mean ± standard desviation).

Homogenization pressure (MPa)	L^*	a^*	b^*	C^*_{ab}	h^*_{ab}
0	70.36±1.48[a]	4.88±0.18[a]	99.46±2.47[a]	99.58±2.46[a]	87.19±0.09[a]
15	72.13±1.13[a]	3.72±0.17[b]	95.17±1.63[b]	95.24±1.62[b]	87.76±0.06[b]
30	72.77±0.59[a]	8.33±0.23[c]	101.62±0.62[a]	101.96±0.62[a]	85.31±0.22[c]

* Values with different superscript letters in a column are significantly different ($P \leq 0.05$).

To determine the significance of the differences in colour coordinates as a function of homogenization pressure in Ortanique juice samples an analysis of variance was conducted. The analysis showed a significant effect of homogenization pressure on all of the variables examined.

CIE-L*a*b* coordinates increased with increases in homogenization pressure, up to 25 MPa. For to explain the drop observed in parameters for the homogenization pressure of 30 MPa we suggest that the aggregation of the smallest particles when the homogenization pressure of 30 MPa and the sedimentation of them after centrifugation step can affect L* a* and b* parameters. More analysis at different homogenization pressures would be necessary to confirm this effect. As observed for particle size parameters, sample groups were observed. One group was composed by homogenized samples at 0 and 5 MPa; a second group was composed by homogenized samples at 10, 15, 20, and 30 MPa; and a third group was composed by homogenized samples at 25 MPa.

On the basis of the psychrometric coordinates chrome (C^*_{ab}), the values tendency and samples groups are the same as for the CIEL*a*b* coordinates. On the basis of hue (h^*_{ab}), however, the differences were compensated.

The brightness of the Salustiana juice samples increased as the homogenization pressure increased. The values for a* and b* were greater in samples homogenized at 30 MPa than in nonhomogenized samples and were smaller in samples homogenized at 15 MPa than in nonhomogenized samples. In the case of Salustiana juice, like for Ortanique juice the L* a* and b* parameters increase with homogenization pressure. However, for the drop observed when 30 MPa homogenization pressure we suggest that the aggregation of the smallest particles when the homogenization pressure is 30 MPa and the sedimentation of them after centrifugation step can affect L* a* and b* parameters and make the colour more similar to unhomogenized juice. More analysis at different pressures would be necessary to confirm this effect.

We were unable to find any published studies of the relation between visual perception of colour changes in mandarin or orange juice and global colour differences (ΔE). We found a few studies that were conducted using similar juice products, which indicated that an DE value less than 1.0 unit was not possible to detect in Star Ruby grapefruit juices (Soon-Mi and Gun-Hee, 2002), whereas an ΔE value of 2.0 was detected in carrot juice (Hongmei et al., 2007, quoting Francis and Clydesdale, 1975). Thus, it is possible that colour is first detected at a

homogenization pressure of 5 MPa and becomes obvious at a pressure of 10 MPa. The greatest colour differences in Ortanique mandarin juice were found at a homogenization pressure of 25 MPa.

Flavonoids narirutin, hesperidin and didymin were analyzed in Ortanique mandarin juices no homogenized and homogenized at 5, 10, 15, 20, 25 and 30 MPa frozen and stored during five months and also in Ortanique mandarin juices no homogenized and homogenized at 5, 10, 15, 20, 25 and 30 MPa without freeze and store.

An auto-scaled chromatogram was obtained for 75 min, being time retention of narirutin 46 min and 48 min and 59 min approximately time retention for hesperidin and didymin. The retention time for cumaric acid was 49 min.

The effect of homogenization pressure on the flavonoid content of Ortanique juice is shown in Tables 7 and 8.

Table 7. Effect of homogenization pressure on the flavonoid content in Ortanique juices that has been frozen and stored during five months (mean ± standard desviation).

Homogenization pressure (MPa)	Narirutin (mg/L)	Hesperidin (mg/L)	Didymin (mg/L)
0	47.1 ± 4.9	45.0 ± 7.4	10.1 ±1.3
5	43.3 ± 1.9	33.5 ± 7.5	8.5 ± 0.4
10	45.2 ± 0.4	34.2 ± 4.9	9.2 ± 0.2
15	46 ± 1.2	33.7 ± 2.1	9 ± 0.3
20	47 ± 0.8	33 ± 0.6	10 ± 0.2
25	46.4 ± 1.4	29.7 ± 0.4	9.5 ± 0.1
30	47.3 ± 0.2	28.9 ± 0.3	9.5 ± 0.2

Table 8. Effect of homogenization pressure on the flavonoid content in fresh Ortanique juices that has not been frozen and stored (mean ± standard desviation).

Homogenization pressure (MPa)	Narirutin (mg/L)	Hesperidin (mg/L)	Didymin (mg/L)
0	41,745 ± 0,017[a]	116,6 ± 3,2[a]	14,0 ± 0,3[a]
5	41,70 ± 2,03[a]	121,4 ± 5,9[a]	15,7 ± 1,4[a]
10	41,94 ± 1,12[a]	115,7 ± 1,9[a]	18,8 ± 2,1[a]
15	43,60 ± 0,16[a]	134,1 ± 0,7[a]	16,5 ± 0,2[a]
20	47,1 ± 0,2[a]	112,7 ± 1,8[a]	16,5 ± 0,2[a]
25	46,7 ± 1,4[a]	79,9 ± 1,9[a]	14,2 ± 0,5[a]
30	50,57 ± 0,13[a]	106,3 ± 0,3[a]	16,437 ± 0,108[a]

Narirutin was the most abundant flavonoid in the juice samples frozen and stored during five months. However, like the references consulted (Sentandreu, 2006) noted that hesperidin is the most abundant flavonoid in fresh mandarin juice. The juice samples were obtained by heating the fruit at 63 ºC for 15 sec, and then samples were then frozen at -18 ºC until analyzed. According to Dhuique-Mayer et al. (2007) hesperidin has a very high thermal stability, and no significant losses of this flavonoid were observed with heat treatment at 90 ºC for 240 min. It is possible that the lower content of hesperidin observed after five months of freeze and store is the result from the freezing and storage processes.

The contents of the flavonoids narirutin and didymin were not affected by homogenization, whereas the content of hesperidin was affected in the juice five months frozen and stored. An analysis of variance with confidence level of 95% ($p < 0.05$) was conducted to determine the significance of the differences in flavonoid contents as a function of homogenization pressure. The analysis showed a different effect of homogenization pressure in all three of these flavonoids.

In the analyzed fresh juice, without freeze and store, the contents of the flavonoids were not affected by homogenization pressure but after five months frozen and stored juice the content of hesperidin was. Significant differences in the hesperidin content were observed between the nonhomogenized juice and the homogenized juices, but no significant differences were observed between the homogenized juices. No significant differences in the narirutin and didymin contents were observed between the juice samples. It is possible that the structural differences between hesperidin and the rest of flavonoids determine the stability of this molecule in solution during storing period.

4. Conclusion.

On the basis of the observed effects of homogenization pressure on particle size, colour, and flavonoid content of the juice samples, the following conclusions were drawn: the homogenization pressure affected the particle size distribution and colour of the citrus juices, which made it possible to define different sample groups on the basis of the applied pressure. In fresh juice the contents of the flavonoids were not affected by homogenization pressure but after five months stored juice the content of the flavonoid hesperidin was. To develop functional fresh fruit, the application of a homogenization pressure of at least 20 MPa to mandarin juice 1) is suitable for use in a posterior vacuum impregnation operation, because of the decrease in particle size; 2) does not negatively affect the colour of juice; and 3) has practically no influence on the total flavonoid content.

Acknowledgments.

This research was supported by the Spanish Government (Ministerio de Educación y Ciencia, project AGL2006-05809ALI) and FEDER founds. The authors also acknowledge the Conselleria de Agricultura, Pesca y Alimentación (Generalitat Valenciana) for its contribution throughout the AGROALIMED program and to the Politechnical University of Valencia for its contribution throughout the UPV22 project.

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