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Characterization of Natural Orange Juices Employing Physicochemical Properties and FTIR Spectroscopy: A Study with the Storage Time

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Research Article

ABSTRACT

In this work, the physicochemical properties and the infrared spectra of three different natural orange juices derived from the sour orange (*Citrus aurantium*), the common orange (*Citrus bigardia Riss*) and the sweet orange (*Citrus sinensis*) varieties, named A, C, and D respectively were studied at room temperature with the storage days during the 92 days. The physicochemical properties studied were the *pH*, refractive index, density, conductivity and the quantity of total soluble solids expressed as degree Brix. The natural A and C juices show a great quantity of bands attributed principally to the ascorbic and citric acid while the natural D juice evidence a higher content of bands associated with sucrose. The assignments of the bands observed in the infrared spectra of the natural A, C and D varieties in the 4000-400 cm^{-1} region was proposed. From the three natural studied juices, the D juice is the most instable with the time, as evidenced by the Infrared (IR) spectra and the physicochemical properties.

INTRODUCTION

The orange juices are of great importance in nutrition because they are sources of nutrients, such as vitamins C, B6, B9, proteins and minerals calcium, potassium and Mg, among other [1-3]. For this reason, the quality of these beverages should be permanently controlled and directed toward the safety and human health protection since the adulteration represents a serious problem actual [4,5]. From recent times, the Fourier Transform Infrared (FT-IR) spectroscopy is a rapid technique used to control falsifications in the final products [6-11] and, also as an alternative for the classification of orange varieties, as reported by Suphamitmongkol [12]. In this work, we have considered only the references of some recent papers taking into account the great diversity of related studies [1-12]. Industrially, the acid content in a natural juice is one of the important quality attributes in the orange fruit, being the ascorbic and citric acids the main organic acid components [2,13,14]. On the other hand, from the four varieties of oranges known, the bitter (sour) orange (*Citrus aurantium*), the sweet orange (*Citrus sinensis*), and the common orange (*Citrus bigardia Riss*), the most important for juice production is the sweet orange in its four varieties, namely the common orange, the acidless orange, the pigmented orange, and the navel orange [2]. Industrially, there is a significant difference between the commercial and natural orange juices because the pasteurization process in the commercial juices is very important for food safety and quality requirement previous to packaging and distribution while a fresh juice is highly susceptible to contamination and, for this reason, it should be consumed quickly [3]. The recent spectroscopic studies [15-20] reported for the ascorbic and citric acids and sucrose have permitted their vibrational assignments and their quick identifications by using Fourier Transform Infrared (FT-IR) and Raman spectroscopies. Consequently, the constituents of major proportion in an orange juice can be easily identified by using IR spectroscopy. Thus, recent studies on model orange juices combining the Fourier Transform Infrared (FT-IR) spectra with physicochemical properties at room temperature with the storage time were reported by Bichara [21]. Hence, these authors have performed the assignments of the bands observed in the spectra of aqueous solutions of sucrose containing ascorbic (AA) and citric acids (CA) in different concentrations together with the study of their physicochemical properties [21]. These results are very important

taking into account that the mixtures simulate diluted model orange juices. So far, for a fresh orange juice there are no experimental Infrared (IR) studies related to their main components in the 4000-400 cm^{-1} region combined with studies on their physicochemical properties during a storage time of 92 days. The changes performed in the Infrared (IR) spectra and in the physicochemical properties of the three natural juices with the storage days are of importance for known the stability of the different natural juices in relation to the diverse orange varieties and to the identification of its main components taking into account that they are the fruit juices of high consumption in many countries. Moreover, to know the profile of the infrared spectra of natural juices during the storage time is of importance to detect adulteration in these juices. For these reasons, the goal of this work is the characterization of three natural orange juices derived from the sour orange (*Citrus aurantium*), the common orange (*Citrus bigardia Riss*) and the sweet orange (*Citrus sinensis*) varieties by using Fourier Transform Infrared (FTIR) spectroscopy and some physicochemical properties simultaneously at room temperature in order to observe the modifications performed by the storage time during the 92 days. Then, the results for the three natural juices are compared with results obtained for model juices of different concentrations [21]. Here, the pH, refractive index, density, conductivity and the quantity of total soluble solids expressed as degree Brix are the physicochemical properties evaluated at room temperature.

MATERIAL AND METHODS

Natural Orange Juices

Three natural orange juices were prepared from fresh oranges obtained from different cultivars of San Miguel de Tucumán (Argentine) and of the sour orange (*Citrus aurantium*), the common orange (*Citrus bigardia Riss*) and the sweet orange (*C. sinensis*) varieties, named A, C, and D respectively. The compositions of these varieties of juices expressed as total solids, total sugars, acidity, and pectin contents of oranges were taken [3] and they can be seen in **Table S1** while in **Table 1** are presented the composition for a sweet orange juice according to Sandhu [3]. Here, these juices were used after of their manual extraction and, then their Fourier Transform Infrared (FTIR) spectra and physicochemical properties at room temperature were studied with the storage time during the 92 days. In all this study the juices were maintained free of the light and the heat.

Table S1. Total Solids, Total Sugars, Acidity, and Pectin Contents of Oranges[a,b].

Natural orange juices				
Variety	Total Solids, %	Total Sugars, %	Acidity[c]	Pectin, %[d]
C Orange (bitter)				
Edible portion	13.59	5.49	3.30	0.86
Peel and pith	27.27	5.86	0.46	0.89
Juice	10.72	5.74	3.77	
D Orange (sweet)				
Edible portion	12.98	7.88	0.79	0.59
Peel and pith	25.52	6.81	0.27	0.13
Juice	11.09	8.47	1.17	0.13

a: [3]
[b]Money and Christian.
[c]Acidity No. cc 0.1N Per 100 g
[d]Pectin, % as Calcium Pectate

Table 1. Composition of a sweet orange juice (*Citrus sinensis*) [3].

Valor nutricional por cada 100 g (*citrus sinensis*)			
Energía 50 Kcal. 200 kJ			
Carbohidratos	11.57 g	Vitamina B6	0.060 mg (5%)
Azúcares	9.35 g	Ácido fólico (Vit. B9)	30 µg (8%)
Fibra alimentaria	2.4 g	Vitamina B12	0 µg (0%)
Grasas	0.12 g	Vitamina C	53.2 mg (89%)
saturadas	0.015 g	Vitamina D	0 µg (0%)
monoinsaturadas	0.023 g	Vitamina E	0.18 mg (1%)
poliinsaturadas	0.025 g	Vitamina K	0 µg (0%)
Proteínas	0.94 g	Calcio	40 mg (4%)
Agua	86.75 g	Hierro	0.10 mg (1%)
Vitamina A	11 µg (1%)	Magnesio	10 mg (3%)
β-caroteno	71 µg (1%)	Manganeso	0.025 mg (1%)
Tiamina (Vit. B1)	0.087 mg (7%)	Fósforo	14 mg (2%)
Riboflavina (Vit. B2)	0.040 mg (3%)	Potasio	181 mg (4%)
Niacina (Vit. B3)	0.282 mg (2%)	Sodio	0 mg (0%)
Ácido pantoténico (B5)	0.250 mg (5%)	Zinc	0.67 mg (7%)

Physicochemical Properties

The equipments used in the determinations of pH, refractive index, density, conductivity and the quantity of total soluble solids expressed as degree Brix for the three natural orange juices at 25 ºC were reported in an above paper [21].

Infrared Spectra

The infrared (IR) spectra of the natural orange juices were recorded between Silver Chloride (AgCl) windows from 4000 to 400 cm^{-1}. FTIR GX1 Perkin Elmer spectrometer, equipped with Raman accessory and a Deuterated Triglycerine Sulfate (DTGS) detector cooled at liquid nitrogen temperature was used for all measurements. All spectra were recorded with a resolution of 1 cm^{-1} and 60 scans.

RESULTS AND DISCUSSION

Physicochemical Properties

Densities (d): Table 2 summarizes the studied physicochemical properties for the three natural orange juices at 25 ℃ with the storage time while **Figure 1** show the density variations with the time for the three juices. The comparisons with commercial orange juices and with model orange juices are shown in **Figure S1** (Supporting information). Note that the graphics for the A, C and D juices show clearly different behaviors on the densities in function of the time. Thus, it is important to observe for D a similar variation with the time than the commercial juices, it is a decrease quick in the density values from the first up to the 7 days. Later, an increase immediate in the density up to a maximum value in the 15 days continued with a decrease up to the 92 days is observed. On the contrary, the densities of the A and C juices increase slightly up to the 7 days, being higher the variation of C than A while the values decrease fast for C up to the 15 days but in lower proportion than A. When these variations are compared with those graphics studied for the commercial and model juices by Bichara [21], as observed in **Figure S1a**, we observed that: (i) the density of the D juice have a similar behaviour with the time than the commercial C2 juice up to the 30 days changing drastically the values up to the 92 days in similar form than B1, (ii) the density values for the A juice present a behavior similar to the model juices being more quick the variation from the 30 days than the other ones and, (iii) the sweet oranges C and D evidence different behaviour of the density with the storage time. The commercial C2 juice is constituted by higher quantity of orange juices, has a higher proportion of ascorbic acid and, besides no present conserving agent while, the natural D juice has an acidity lower but a higher content of total sugars. Probably, the difference between the natural D and the commercial C2 juice from the 30 days is justified in part by the presence of other components, such as proteins and vitamins. On the other hand, in the components of the model juices, these are ascorbic acid, citric acid and sucrose, sucrose have **(Figure S1b)** a higher proportion (2.5% of ascorbic acid, 2.5% of citric acid, 10% of sucrose and 85% of water) where the solution 1 is the more concentrated. A very important observation is that the behaviour of the density in A (sour orange) is similar to the model juice which has the highest proportion of sucrose while the behaviour of D is similar to a commercial juice. Thus, perhaps the similitude for these juices justifies the variations observed in the densities with the storage time because the density is a property depends on the volume.

Table 2. Physicochemical properties of commercial orange juices with the storage time.

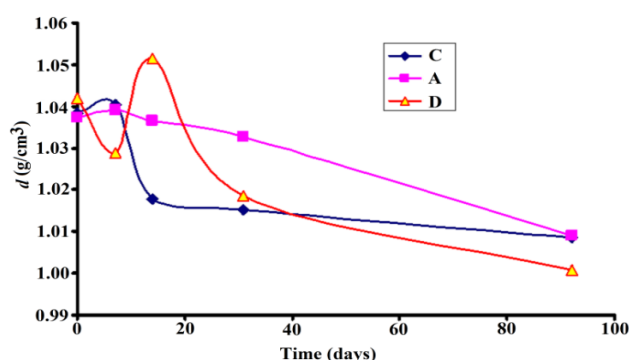| A			
T (days)	*d* (g/cm³)	pH	k (µS/cm)
0	1.0364	3.97	2.41
7	1.0367	4.15	2.41
14	1.0469	4.11	2.65
31	1.0302	4.06	3.23
92	0.9987	4.12	3.15
B1			
0	1.0427	3.24	1.832
7	1.04455	3.45	1.91
14	1.0533	3.49	2
31	1.0236	3.55	2.14
92	1.0005	3.67	1.95
B2			
0	1.0205	3.21	1.976
7	1.02249	3.14	1.99
14	1.0345	3.45	2.2

Figure 1. Variation of density against time, for the natural A, C and D juices at 25 ℃.
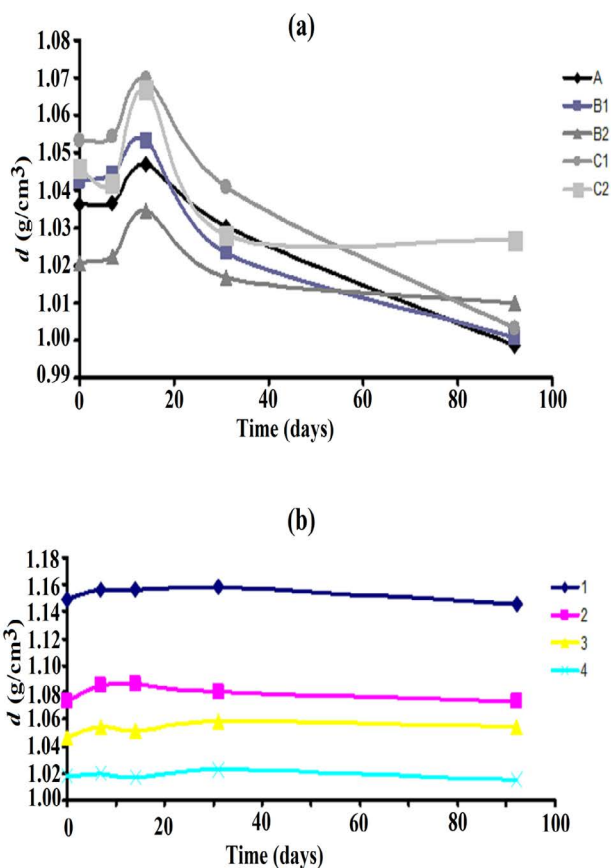
Figure S1. Variation of density against time, for (a) the commercial A, B1, B2, C1 and C2 juices and, (b) model 1, 2, 3 and 4 juices at 25 ºC [21,22].

Conductivities (k): Figure 2 shows the conductivity values for the three studied natural juices at 25ºC against at the storage time during the 92 days. There are differences notable in the values during the studied time, thus, in the first 15 days the conductivity values increase for D while for A and C decrease in the first 7 days and later increase up to the 15 days. For A and D, after the 15 days and up to the 92 days the conductivities of both juices decrease with the time while only for C an increase in the values is observed between 15 and 92 days, as showed in **Figure 2**. When these natural juices are compared with the conductivity values corresponding to the commercial and model juices, studied [21] and presented in **Figures S2a and S2b**, the behaviors show similitude and differences among them. Hence, in the first 15 days the variations for A and C are similar to the commercial B2 juice; it is the values decrease to the 7 days and then increase up to the 15 days. For D, the behaviour of the conductivity is similar to the remains commercial juices up to the 30 days and, then, the values decrease for A and D up to the 92 days. The increase in the conductivity values could be related in part to the presence of H^+, OH^-, ascorbate and citrate ions in the medium and to the A^{2-} species derived from the ascorbic acid decomposition, in similar form to the more diluted model juice, because in aqueous medium the decomposition process is accelerated. Whereas, the decrease in the conductivity could be justified by the presence of the A and A_2 species derived from the decomposition of the ascorbic acid and, also to the di-hydrate and penta-hydrate sucrose formation, as reported by Max and Chapados [22]. Here, the similitude between A, C and the commercial B2 juice are attributed to a higher quantity of ascorbic acid while the D juice has a higher quantity of soluble solid, as indicated in **Tables 1 and 2**. This way, the formation of H bonds and sugar in aqueous solution justifies the presence of higher neutral species that diminishing the conductivity.
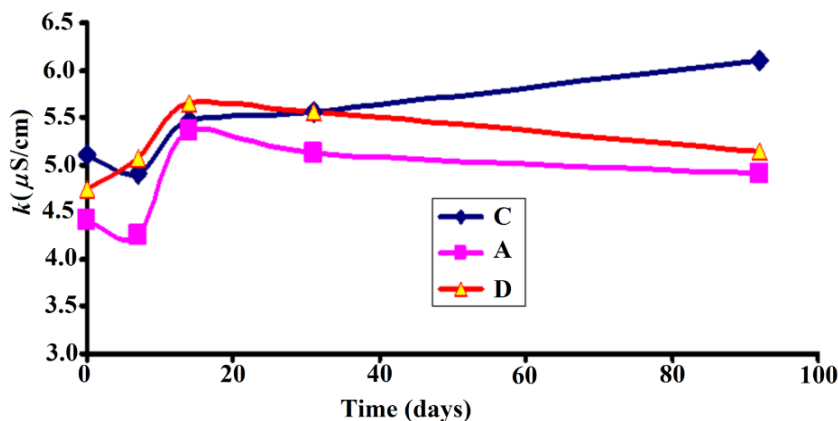

Figure 2. Variation of conductivity against time, for the natural A, C and D juices at 25 ºC.
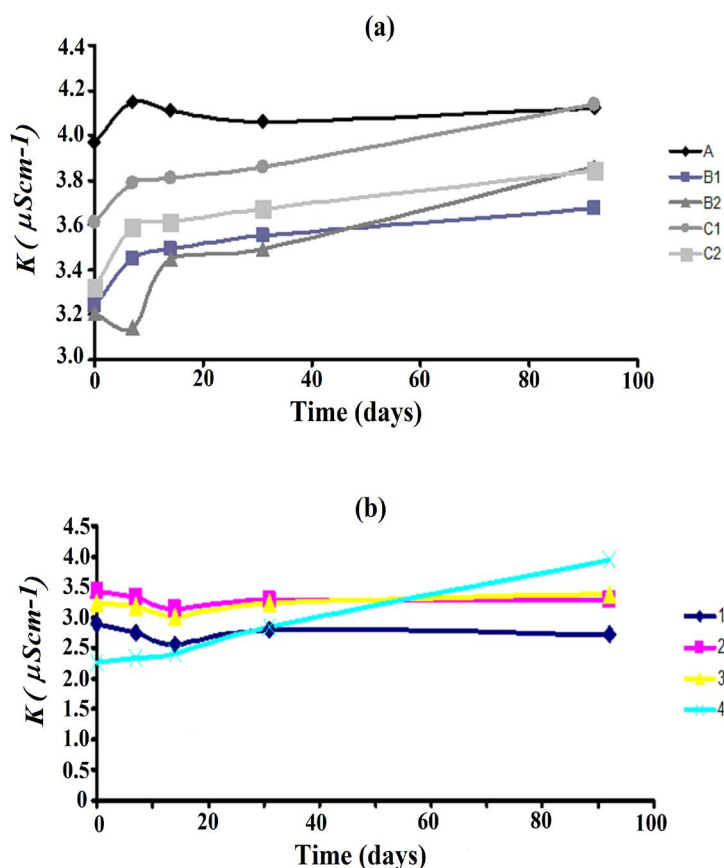
Figure S2. Variation of conductivity against concentration, for (a) the commercial A, B1, B2, C1 and C2 juices and, (b) model 1, 2, 3 and 4 juices at 25 ºC [21,22].

Values pH: Figure 3 shows the pH variations for the natural juices at 25ºC versus the storage time during 92 days while the values obtained for the three juices can be observed in **Table 2**. Initially, the natural A and C juices present similar behaviors with the time up to the 30 days and, later, the values for A increase with the time but remain practically constant for C. As it is expected and as observed in **Table 1**, the C juice product of an orange *bitter* has lower pH values and, for this, it has the higher acidity than A and D in spite the value of the acidity expressed as 0.1N per 100 g is 3.77 different from D whose values is 1.17, as observed in **Table S1**. On the contrary, the variation of the *pH* with the storage time for D is different from A and C up to the 15 days and, then the values increase from the 15 to 92 days showing a medium acidity between A and C. When these natural juices are compared with the commercial juices we observed in the A and C juices behaviours similar to the commercial A, B1, C1 and C2 juices up to the 30 days and, later, the values increase strongly for A but remain practically constant for C **(Figure S3a)**. While the behaviour of the pH values of D in the first 15 days is similar to the commercial B2 juice. On the other hand, when the natural juices are compared with the model juices the variation of the *pH* with the storage time for D are similar to the more concentrated solutions 1, 2 and 3 **(Figure S3b)**. Possibly, this decrease in the *pH* values during the first 15 days are associated with a higher acid concentration, as in the model juices cases. The presence of other components in the natural juices could be justifying the low acidity of these juices than the model ones. **Figure S3a** show clearly the decrease in the pH values with the storage time for B2. Probably, the presence of the ascorbic acid, citric acid and sucrose support the variations in the pH values for B2 and the juices model. These results justify the increase in the conductivity in the aqueous medium and the decreasing in the pH values due to the acid species. These results agree with those obtained by means of conductivity values.
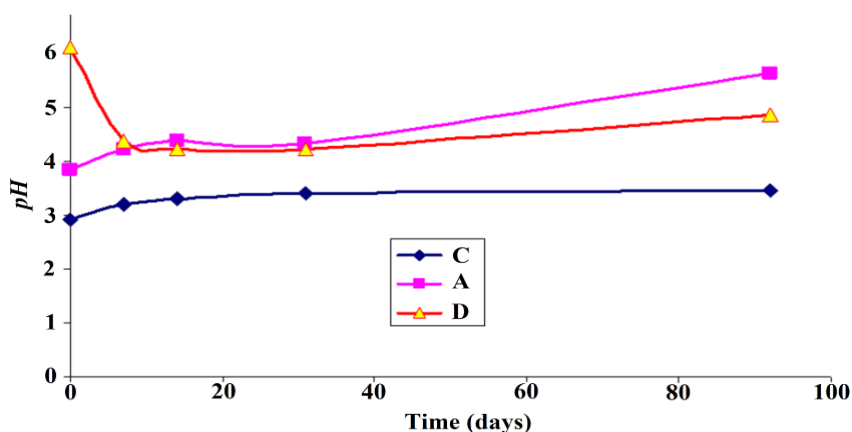

Figure 3. Variation of pH against time, for the natural A, C and D juices at 25 ºC.
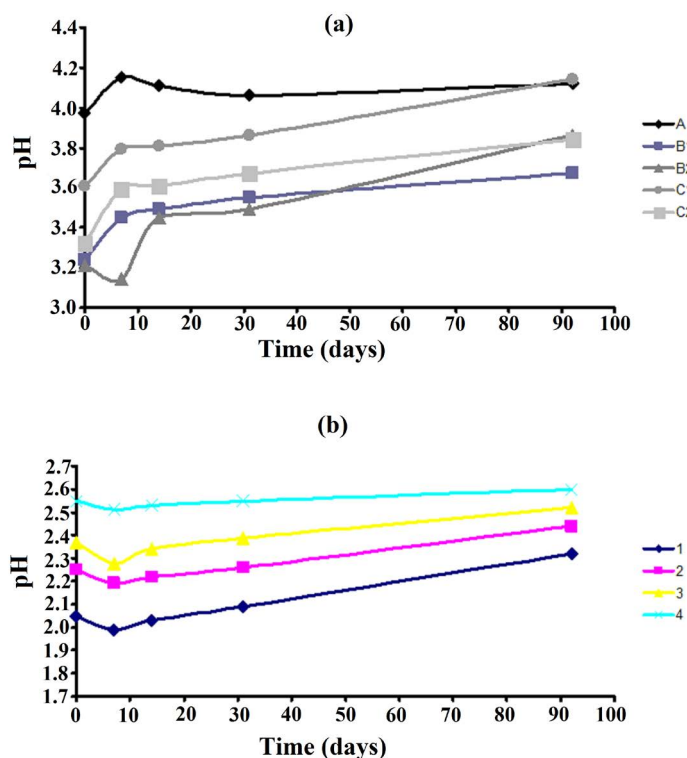
Figure S3. Variation of *pH* against concentration, for (a) the commercial A, B1, B2, C1 and C2 juices and, (b) model 1, 2, 3 and 4 juices at 25 °C [21,22].

Refractive index (n): The graphics of the refractive index for the natural A, C and D juices against to the storage time during 92 days at 25ºC are presented in **Figure 4** while the comparisons with commercial and model juices can be observed in **Figure S4**. First, the behaviour of *n* versus the time are different in the three juices, presenting the more important modifications the C juice. Thus, for this juice *n* decreases significantly with the time from the first up to the 15 days and then the values increase until the 30 days and, from here diminish newly the values up to the 92 days. The A and D juices, both products of sweet oranges, present minima to the 7 days while both present maxima in different times, A has a maximum approximately at the 20 days while D to the 15 days. The results show clear differences in the compositions of both juices. This way, when the three juices are compared with the commercial and model juices, presented in **Figure S4**, A and D show behaviours similar to the commercial juices while for C, the variation of *n* with the time is similar to the commercial B2 and to the most diluted solution of the model juices. Probably the low quantity of soluble solids in the commercial B2 justifies such variations because the solution 4, which is the most diluted solution of the model juices, shows drastically lower proportion of ascorbic acid, citric acid and sucrose during the storage time than solution 1, 2 and 3. Thus, C is the natural juice with lower values of *n* while A show strong decreasing of *n* with the time.
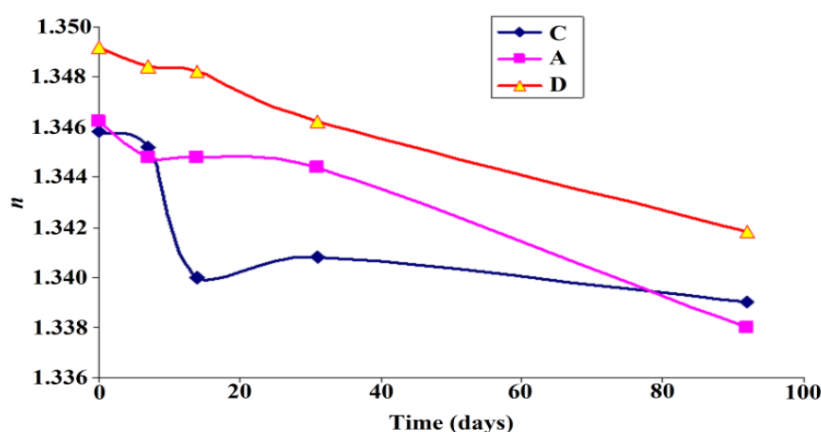

Figure 4. Variation of refractive indexes against time, for the natural A, C and D juices at 25 °C.

Quantity of soluble solid (ºBrix): In this work, the quantity of total soluble solids for the three natural juices was expressed as degree *Brix* and studied at 25ºC against at storage time during 92 days, as can be seen in **Figure 5**. On the other hand, the graphics for the natural juices were compared with those corresponding to different commercial and model oranges juices **(Figure S5)**. Note that the behaviours of the soluble solids for the three juices are similar to the variations of *n* by the time, given in Figure 4, a result different from those obtained for the commercial juices **(Figure S5a)**. A very important result for the D juice is observed in the ºBrix values due to that it has a higher quantity of soluble solid than A and C, as decrypted in **Table S1**. Probably, the low quantity of soluble solids in C can be justified in part by the ascorbic acid decomposition because it juice has low *pH* values and, for this, lower acidity than A and D. Moreover, these latter juices have behaviours similar than the commercial juices showing A and D decrease quick of the soluble solid with the time, being it more notable in A. Thus, the three juices after about 30 days show different slopes, as observed in **Figure 5.**

Figure S4. Variation of refractive index against concentration, for (a) the commercial A, B1, B2, C1 and C2 juices and, (b) model 1, 2, 3 and 4 juices at 25 ºC [21,22].

Figure 5. Variation of the soluble solids (ºBrix) against time, for the natural A, C and D juices at 25 ºC.
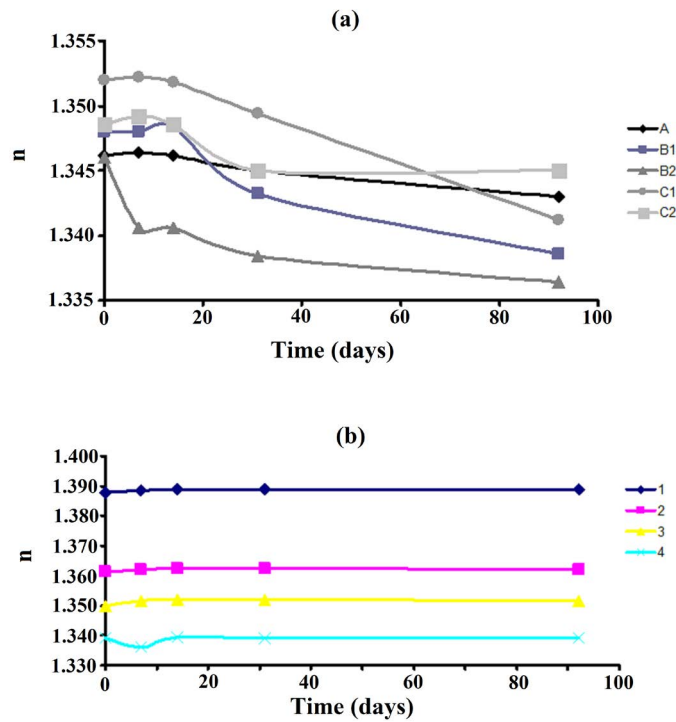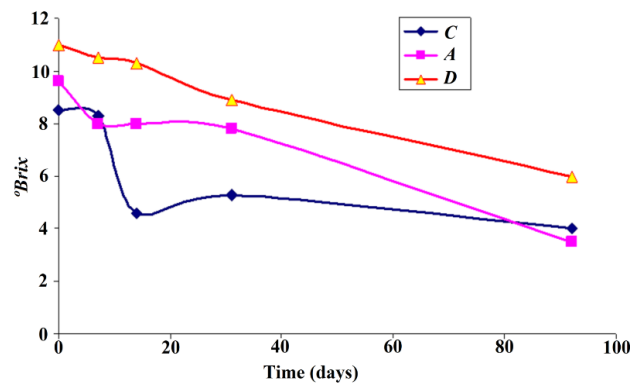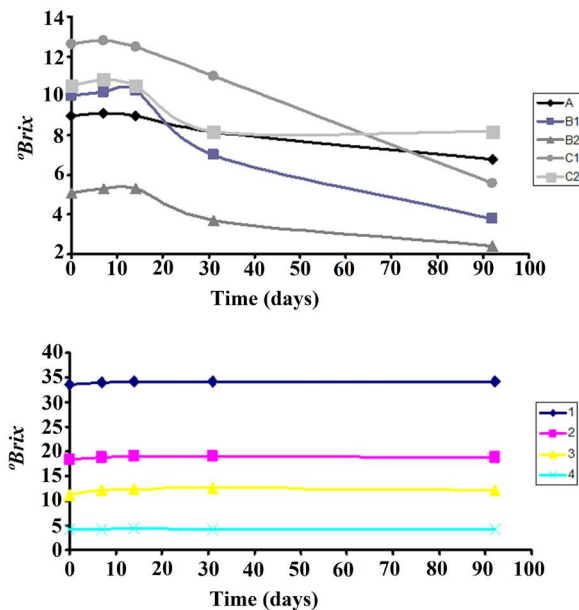

Figure S5. Variation of soluble solids (ºBrix) against concentration, for (a) the commercial A, B1, B2, C1 and C2 juices and, (b) model 1, 2, 3 and 4 juices at 25 ºC [21,22].

Infrared Spectra: Figure 6 shows the variations of the IR spectra of the natural A, C and D juices recorded on the first day compared with the corresponding to the mixture of ascorbic and citric acids and sucrose (model juice) in solid phase while from **Figures 7-9** show the variations of each spectrum with the storage time. The observed wavenumbers and the assignments for the natural orange juices studied compared with the bands observed in the more concentrated juice model can be seen in **Table 3**. **Figure S6** shows clearly the differences among the spectra for the A, C and D natural juices, in the form, positions and intensities of the bands observed while in the **Figures S7-S9** it is possible to observe the comparisons with the Infrared (IR) spectra for ascorbic acid, citric acid and sucrose. Note that **Figure S7** shows for the A juice a higher proportion of the ascorbic and citric acids than C and D while a high content of sucrose is expected in the D juice, as can be seen in **Figure S9**, and as expected because it juice is of sweet orange.
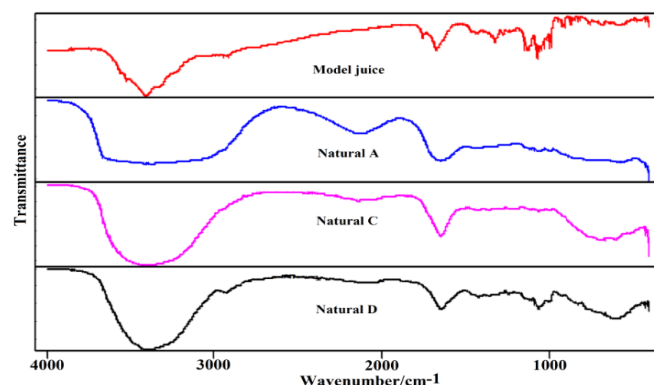
Figure 6. Comparisons of the Infrared spectra of the model juice in solid phase with those corresponding to the natural A, C and D juices at 25 °C.
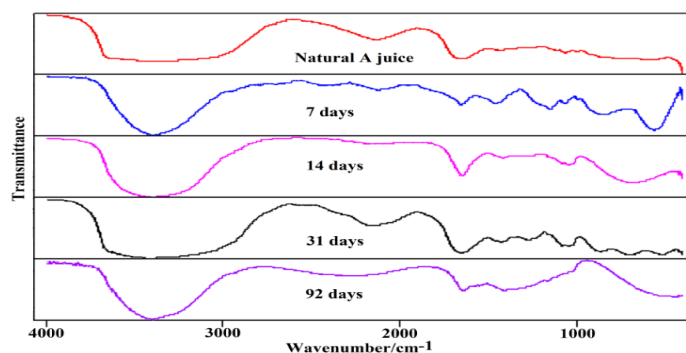

Figure 7. Comparisons of the Infrared spectra of the natural A with the storage time at 25 °C.
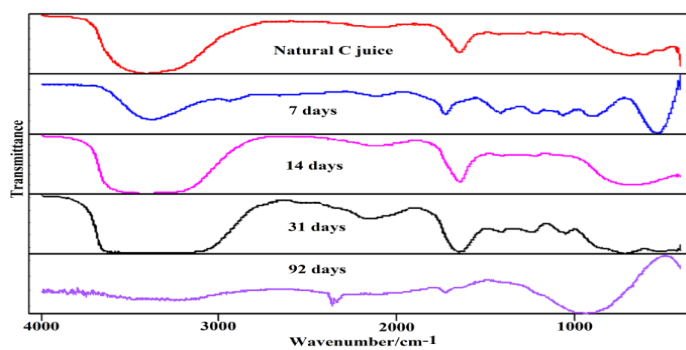

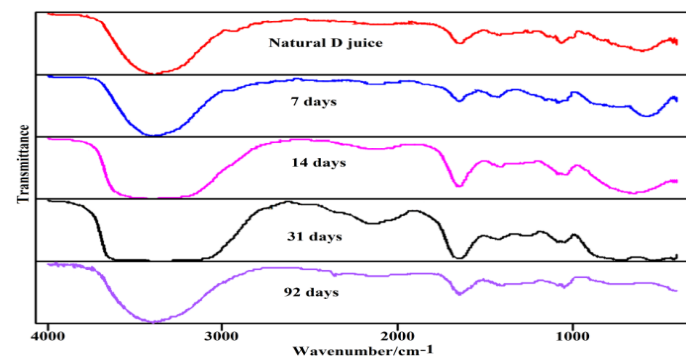**Figure 8.** Comparisons of the Infrared spectra of the natural C with the storage time at 25 °C.

Figure 9. Comparisons of the Infrared spectra of the natural D with the storage time at 25 °C.

Table 3. Physicochemical properties of natural orange juices with the storage time.

A (sour orange)			
T (days)	*d* (g/cm^3)	pH	n 25ºC
0	1.0374	3.84	1.3462
7	1.0391	4.24	1.3448
14	1.0365	4.38	1.3448
31	1.0326	4.34	1.3444
92	1.009	5.64	1.338
C (common orange)			
0	1.0383	2.92	1.3458
7	1.0405	3.2	1.3452
14	1.0179	3.32	1.34
31	1.0153	3.4	1.3408
92	1.0085	3.46	1.339
D (sweet orange)			
0	1.0421	6.13	1.3492
7	1.0289	4.38	1.3484
14	1.0516	4.23	1.3482
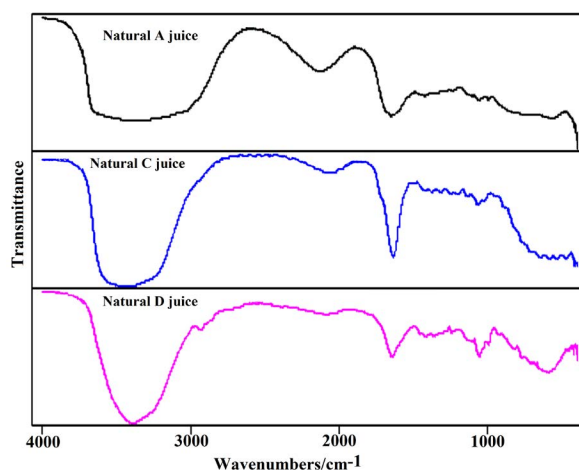
Figure S6. Infrared spectra of the natural A juice 1 at the first day (upper), for C (medium), and for D (bottom).
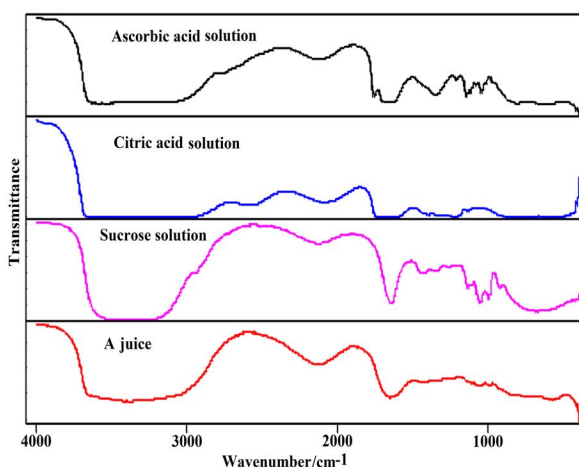
Figure S7. Infrared spectrum for the natural A compared with the corresponding to ascorbic acid, citric acid and sucrose.

Natural Orange A Juices: The Infrared (IR) spectra for this juice recorded each week from the first day can be seen in **Figure 7**. It is important to note the different variations that experiment this juice with the storage days especially during the 7 and 31 days, as observed in the physicochemical studies. The numbers of bands increase notably in this two week as a consequence of the decomposition products of ascorbic and citric acids and for this reason, increase the density and the pH in both times while decrease the conductivity. The broad Infrared (IR) bands at 3402 and 1654 cm^{-1} are strongly related with the presence of those two acids, as shown in **Figure S7**. The first band is assigned to the expected OH stretching modes of both acids while the band at 1654 cm^{-1} is assigned simultaneously to the C=O stretching mode of the ascorbic acid and to the OH deformation modes corresponding to the water molecules [17,18,20].

Natural Orange C Juice: Table 4 shows that the positions of the bands associated with the OH stretching and deformation modes for this juice are located at 3416 and 1650 cm^{-1}, respectively. In **Figure S8** can be seen the higher presence of acids in C and, for this juice, as for A, the more important variations are also observed at the 7 and 31 days, as shown in the different spectra of **Figure 8**. The probable formed species

are those derived from of ascorbic acid which are the un-oxidized H_2A, the HA^- and A^{2-} anions, the oxidized A and dimeric A_2 forms, in accordance with reported data [17,18,20]. The increasing of the density and in the *pH* values and the decreasing in the conductivity, *n* and *ºBrix* values the 7 days justified the modifications in the bands observed in the IR spectrum fro this juice.
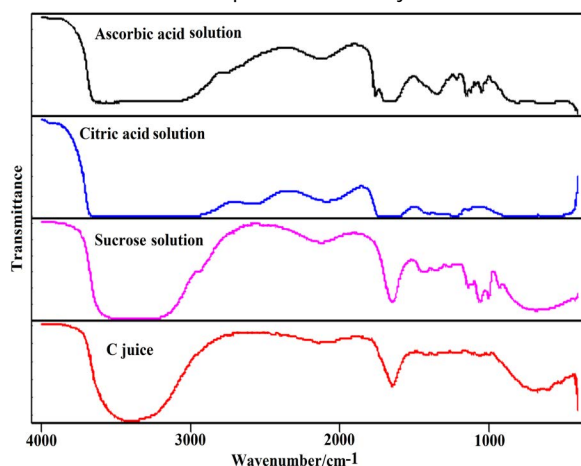
Figure S8. Infrared spectrum for the natural C compared with the corresponding to ascorbic acid, citric acid and sucrose.
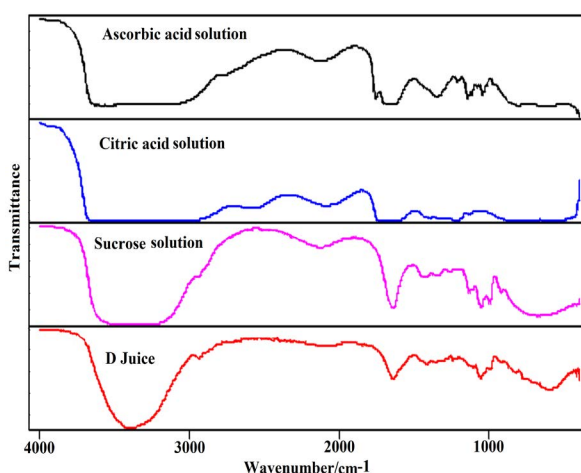
Figure S9. Infrared spectrum for the natural D compared with the corresponding to ascorbic acid, citric acid and sucrose.

Table 4. Observed wavenumbers (cm^{-1}) and assignments for all the natural orange juices [17,18,20,22].

Ascorbic acid (H_2A)[a]		Citric acid[b]		Sucrose[c]		Mixture[d]		Natural orange juices[e]		
IR,Raman	Assignment	IR,Raman	Assignment	IR,Raman	Assignment[c]	IR Solid	IR Solut	A	C	D
3523s	vO-H (H_2A, HA^-, A^{2-}, A)	3535	v O-H	3564 m	$v(O-H)_w$	3565	3538 vs,br	3642		
		3498	v O-H	3469 sh	v O-H	3525				
3409 vs	vO-H (H_2A, HA^-, A)			3394 s	v O-H	3412		3402	3416	3392
3316 s	vO-H (H_2A, HA^-)	3350	v O-H	3337 s	v O-H	3389	3302 vs.br			
		3247	v O-H	3257 sh	v O-H	3322				
3217 m	vO-H (H_2A)			3132 m	v_aCH_2	3226	3087 vs,br		3249	3266
3030 s	vO-H (H_2A)	3035	vaCH$_2$ op	3052 sh	vC-H	3034		3044		
3002 m	vaCH$_2$ (H_2A), vC-H (A)	2994	vaCH$_2$ op	2993 w	v_aCH_2	2997				
2978 m	va CH$_2$ (H_2A)	2975	vaCH$_2$ ip	2970 w	v_aCH_2	2971				
2960 w	va CH$_2$ (A^{2-}, A)	2961 w	vsCH$_2$ op	2958 sh	vC-H			2958		
2944 m	vC-H (H_2A), va CH$_2$ (HA^-)	2933 w	vO-H	2943 m	v_sCH_2	2944	2945 sh			2940
2916 m	vC-H (H_2A, HA^-), vs CH$_2$ (H_2A)			2916 m	vC-H	2915				
2903 vs	vC-H (H_2A, HA^-), vs CH$_2$ (H_2A, A^{2-}, A)			2902 m	v_sCH_2		2984 sh			2894
2854 w	vO-H(H_2A),vsCH$_2$(HA^-)vC-H(H_2A,HA^-,A^{2-},A)			2849 sh						2859
2737 w	vC-H (A^{2-})						2763 sh			
2641 sh	vO-H (A^{2-})						2650 sh			

1753 m	ν C=O (H_2A, HA^-, A^{2-}, A)	1756 vs	$vsC=O_1$	1733 w	δH_2O	1757				
		1708 vs	$vaC=O_3$	1714 w	δH_2O			1709		
1672 s	ν C=C (H_2A), δCOH (A^{2-}),			1662 vw	δH_2O	1674	1677 vs			
1667 vs	ν C=C (HA^-),ν C=O (A), δ CH_2 dim(H_2A)	1698 sh	$vsC=O_3$	1648 s	δH_2O			1654	1645	1650
				1538 vw	ν(C-C)				1546	1573
				1525 vw	δCH_2					
				1517 vw	δCH_2					
1495[b] m	νC-Cdim (H_2A),wagCH_2 (HA^-), δ CH_2(H_2A, A)	1493 vs	δsCOH	1496 vw	wagCH_2	1502			1503	
1459 m	wagCH_2 (H_2A), δCH_2 (H_2A, HA^-, A^{2-})	1469 w	$vaC-C_2$	1463 w	ρC-H	1465	1455 sh	1459	1460	1459
1438 sh	δCOH(HA^-),wagCH_2(A),δHOC(H_2A),vC-C(H_2A)	1430 m	vC-C, δCH_2 ip	1432 w	wagCH_2	1432		1431		
				1426 w	τ(O---H),τwH_2O(2)		1428 s		1422	1421
				1413 sh	τ(O---H),τwH_2O(2)			1419		
				1399 sh	ρC-H	1392				
1387 w	wag CH_2 (H_2A)	1389 m	δaCOH, wagCH_2op	1388 w	δ(O-H),ρ'(C-H)				1380	
1363 sh	δCOH (HA^-), wagCH_2 (A^{2-}), δOCH (A)	1365 w	wag CH_2 ip	1365 m	τ(O---H),τwH_2O(2)	1366		1365	1363	1368
1353 m	δCOH (H_2A, HA^-, A^{2-}, A), δ CCO (H_2A)	1358 w	ρCH_2 ip	1354 sh	ρC-H		1347 w			
1344 vw	δCCH(H_2A,HA^-,A^{2-}),δOCH (A) δCOH(H_2A,HA^-)	1340 sh	δs COH	1348 m	ρC-H	1343				
1321 m	δCOH (H_2A), δCCH (HA^-)	1325 vw	δs COH	1325 m	ρCH_2 δ(O- H)	1323	1323 sh			
1302 sh	ρCH_2 (H_2A, HA^-), δ OCH (A^{2-})ν C-O (H_2A), τw CC_2 (H_2A)	1308 w	wagCH_2 ip,δaCOH	1302 w	ρ'(C-H)	1306		1318	1320	1315
		1292 w	ρCH_2 ip, wagCH_2 ip	1293 w	ρC-H				1292	
1274 s	δOCH (HA^-,A^{2-},A),ρCH_2 (A), wagCH_2 (HA^-), δCOH(H_2A),δCCH(H_2A)			1271 w	δO-H	1276	1265 sh	1275		1280
1246 m	δCOH (H_2A), ρCH_2 dim (H_2A), δOCH (HA^-), ρCH_2 (A^{2-}, HA^-)	1242 m	vsC-O	1241 m	δO-H	1246		1253	1257	
							1233 m	1240	1242	1245
1221 w	τwCC_2 (H_2A), δ OCH (A^{2-}),δCOH(A)	1214 m	ρCH_2 ip	1212 w	δO-H	1227				
1197 w	δCOH (H_2A, HA^-), ν C-C (HA^-, A^{2-}), τw CC_2 (H_2A, A)			1191 vw	δO-H	1200			1192	1192
		1174 s	ν C-O	1172 m	δO-H	1177				
				1163 m	vC-O		1152 m	1162		
1139 m	τwCC_2 (HA^-, A^{2-}), ν C-O (H_2A, A), ν C-C (H_2A)	1140 s	vC-O	1141 m	vC-O	1140		1140	1138	1139
				1130 s	vC-O					
1120	νC-O (H_2A), δ COH (H_2A)			1126 m	vC-O	1120				
1112 s	νC-C (HA^-, A),ν C-C (A^{2-})			1115 m	vC-O			1112	1110	1110
				1105 s	vC-O		1107 w			
1074[b] m	vC-O (H_2A, HA^-, A^{2-}, A)	1081 w	δaCOH	1074 m	vC-O	1071	1079 sh	1080	1084	1078
1065[b] m	vC-O (H_2A), ν C-C (H_2A), τw CH_2 (A^{2-})			1069 vs	vC-O			1070		
		1053 m	vC-C	1055 s	vC-O	1054		1061	1065	1060
1046 sh	vC-O (H_2A, HA^-, A^{2-}, A)			1042 sh	τwH_2O(2),τ(O--H)					

		1036 sh	wagCH$_2$ op,twCH$_2$ op				1039 s	1033	1034	1040
1026 vs	vC-C (HA$^-$), v C-O (H$_2$A, A)			1022 w	vC-C	1028				
				1015 m	twH$_2$O(2),τ(O--H)	1015		1013	1015	
				1004 m	vC-O					
990 m	vC-O (HA$^-$, A^{2-}),v C-C (H$_2$A, HA$^-$, A) βC=O (A)			994 s	vC-O	991	998 sh	995	996	999
		966 vw	vs C-C$_2$	966 sh	vC-O	945		963		966
		945 w	vs C-C$_1$	946 w	τR$_1$(A6)	922	925 w	920	920	928
924 vw	twCH$_2$ (H$_2$A, A) τ (OH) (A^{2-}),v C-C (HA$^-$)	914 sh	twCH$_2$ op			912		908		
		904 w	twCH$_2$ op, vC-O	899 sh	τR$_1$(A5)	872			892	
870	vC-O (A)	881 w	twCH$_2$ ip	885 sh	v(C-O)	852				867
820^b m	vC-C (H$_2$A), twCH$_2$ (HA$^-$, A^{2-}, A), v C-O (HA$^-$)	842 vw	va C-C$_1$	836 s	δ(OCC), twCH$_2$, vC1-C5)	825			821	829
		795 sh	γCOO$_2$				799 vs	806		
783 vw	γC-C (H$_2$A, HA$^-$)			785 vw	wagH$_2$O(1)				774	776
756^b s	τ(OH)ip dim (H$_2$A), βC-O (A^{2-})			755 sh	βR$_1$(A6)	759				
722 m	γC=O dim (H$_2$A), γC-C (A)	729 sh	δCOO$_5$	722 w	βR$_2$(A6)	736				
711^b vw	γC=O (H$_2$A), βC-O (HA$^-$)			714 sh	βR$_3$(A6)		713 sh			714
697 s	tw CH$_2$ dim(H$_2$A), βC=O (H$_2$A), βR$_1$ (A^{2-}), v C-C (A), γC=O (HÁ$^-$)	700 w	δCOO$_4$	701 vw	ρH$_2$O					
686 w	βC=O (H$_2$A), v C-C (H$_2$A)	686 w	γCOO$_5$	686 w	δC-C-C	686			684	676
675^b m	v C-O (H$_2$A, HA$^-$), τ (OH) (HA$^-$)	666 vw	γCOO$_3$	666 sh	δO-C-O					
649	τ(OH)op dim(H$_2$A), γC=O (A^{2-})	640 w	τ(O-H)	644 w	ρH$_2$O	643		653		
628 m	βR$_1$ dim(H$_2$A), βC=O (A), βR$_1$ (HA$^-$)	627 vw	τ(O-H),δCOO$_3$	636 m	ρH$_2$O					632
591 w	γC-O (HA$^-$), v C-C (A^{2-})βR$_1$ (H$_2$A, A), βR$_2$(HA$^-$)	599 s	τ(O-H),δCOO$_5$, δCCC,ρCOO$_5$	594 m	βR$_1$(A5)	600			605	603
							581 vs	573		
565^b m	δOCH(H$_2$A), γC-O (H$_2$A, A^{2-}),βR$_2$ (H$_2$A, A^{2-}, A), wagCC$_2$ (A)	571 w	γCOO$_4$	570 w	δO-C-O	573				565
		541 w	δCOO$_1$	548 s	ρH$_2$O(1)	553				
		520 sh	γCOO$_6$	537 w	τO-H$_w$				520	
496 w	δCCO(H$_2$A), wagCC$_2$ (A^{2-})	504 w	γCOO$_1$	504 m	twH$_2$O			498	498	
				482 w	τO-H					
473 w	γC=O (A)	477 sh	γCOO$_6$	474 m	δO-C-C	474	477 w		473	
				467 w	wagH$_2$O					
449 w	ρCC$_2$ (H$_2$A), Wag CC$_2$(H$_2$A, HA$^-$) τ(OH) (A)	438 vvw	ρCOO$_4$	451 w	twH$_2$O			434		432
				412 s	τO-H	424	404 sh	420	418	420
396 w	τ(OH) (H$_2$A), βC-O (HA$^-$, A^{2-})	397 w	δCCC	393 s	τO-H				406	403
				378 s	τO-H					
363 m	τ(OH) (H$_2$A), γC-O (A^{2-})ρ CC$_2$ (A^{2-}), βC=O (A)	367 w	ρCOO$_3$, ρCOO$_1$	368 vs	δC-C-O					
				357 sh	δC-C-O					
340^b w	γC-O (H$_2$A, HA$^-$), γC=O (A) τ (OH) (H$_2$A), βC-O (A^{2-}), δ CCO (A)	348 w	ρCOO$_6$	342 sh	τO-H					

		323 vw	δCCC	329 w	τO-H				
				320 sh	δO-C-C				
292ᵇ w	βC-O(H₂A), δCCO (A²⁻), βC=O (A), γC-O (HA⁻)	306 w	δCCC	309 vw	τw (O-C)				
		254 sh	δCCC	252 sh	δ(OCC), ρ'(C-C)				
238ᵇ m	τ(OH) dim(H₂A),δ CCC (A²⁻), τ (OH) (H₂A, HA⁻, A), β C-O(HA⁻)	247 vw	δCCC	241 sh	δO-C-C				
223 m	τ(OH) (H₂A, HA⁻), β C-O(H₂A), τCC (A²⁻), δ CCC (A)	237 vw	δCCC	235 m	δO-C-C				
		212 w	δCCC op	212 vs	δO-C-C				
208 m	δCCC (H₂A), τ (O-H) (A²⁻)			204 sh	νOH---Ow				
180 w	γC-C dim(H₂A)			180 vvw	τw₂O(2),τ(O---H)				
				172 vw	νOH---Ow				
				166 vvw	τR₁(A5)				
163 m	τCCCO dim(H₂A)			160 vw	τO-Hw				
148 m	γC-O(H₂A,) δ OCH (H₂A),	140ᵇ	vs (O-H--O)#	153 vvw	τO-Hw				
138 m	τR₁ (H₂A, HA⁻,A)			138 vvw	τR₃(A6)				
122 w	τR₁ (H₂A), τCCCC (HA⁻)			129 vvw	νOH---Ow				
113 w	τ CCCO (H₂A), τR₂ (A²⁻), δ CCC (A)	105ᵇ	νa(O-H--O)#,δCCC ip	114 vvw	OH---Ow				
91 m	τ CCCO (H₂A), τCCCC (HA⁻)	88ᵇ	δCCC ip	90	τwO-C				
81 s	τR₂ (H₂A) ,τCCCO (A²⁻), τCCCC (A)			82	τwO-C				
73 s	τR₂ (H₂A, τR₁ (A²⁻), τCCCO (A), τw Ring(HA⁻)	68ᵇ	τwCC ip	70	τR₂(A5)				
43 vw	τw Ring (H₂A), τCCCC (A²⁻), τR₂ (A), τR₁ (HA⁻)	44ᵇ	τwCC op	41	τ(O---H)				

Natural Orange D Juice: Figure S8 shows the comparisons of the Infrared (IR) spectra for this juice with those corresponding to the ascorbic and citric acids and sucrose while **Figure 8** shows the variations observed in the spectra for this juice with the storage time. In particular for this juice notable variations are observed in the positions of the bands, as shown in both Figures. First, in the higher wavenumbers region the position of the band at 3392 cm⁻¹ attributed to the OH stretching modes of the components are in agreement with the strong band observed in the spectrum of sucrose at 3394 cm⁻¹ and the band at 1650 cm⁻¹ associated with the hydrate-sucrose at 1648 cm⁻¹ support the higher presence of sucrose in this natural juice, as expected because this juice is prepared from the sweet orange. Also, in the 1500-400 cm⁻¹ region for this juice are observing a higher presence of bands associated with those corresponding to sucrose. During the studied time it is possible to observe changes significant in all the spectra presented in **Figure 9**. The permanent changes in the density values, the increase in the conductivity values and the decreasing in the *pH*, *n* and ºBrix values justify all the modifications observed in the Infrared (IR) spectra for this juice during the storage time.

CONCLUSIONS

In the present work, three natural orange juices derived from the sour orange (*Citrus aurantium*), the common orange (*Citrus bigardia Riss*) and the sweet orange (*Citrus sinensis*) varieties, named A, C and D, respectively, were characterized by using physicochemical properties and Fourier Transform Infrared (FTIR) spectra with the storage time during 92 days. The natural A and C juices show bands attributed to the ascorbic and citric acid and exhibits notable variations in the physicochemical properties especially at the 7 and 30 days supported by the new Infrared (IR) bands during these times while the infrared spectrum of the natural D juice evidence a higher content of sucrose and during all the studied time the infrared spectra show change in the form, positions and intensities of the observed bands. Thus, the changes in the density values, the increase in the conductivity values and the decreasing in the pH, *n* and ºBrix values reveal the high instability of this juice with the storage time. The significant decreasing in the *n* and *ºBrix* values for all the natural juices confirm that the fresh juices are highly susceptible to decomposition and, for this reason, it should be consumed quickly. In this study, we demonstrated that the Fourier Transform Infrared (FTIR) spectroscopy can be easily used for differentiation of natural juices if its technique is used in combination with the determination of their physicochemical properties. The assignments of the bands observed in the infrared spectra of the sour orange (*Citrus aurantium*), the common orange (*Citrus bigardia Riss*) and the sweet orange (*Citrus sinensis*) varieties in the 4000-400 cm⁻¹ region was proposed. In conclusion, from the three natural studied juices, the D juice is the most instable with the time, as evidenced by the Infrared (IR) spectra and the physicochemical properties.

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