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THE EFFECTS OF PACKAGE MATERIAL AND STORAGE TIME ON THE STABILITY OF VITAMIN C AND FLAVOR COMPOUNDS IN RECONSTITUTED ORANGE JUICE PRODUCED IN ALGERIA

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Abstract

Description of the subject: In the present work, the nutritional and sensory quality of reconstituted orange juice was studied, during storage in different packaging, under normal conditions of commercialization.
Objective : This study investigated the effects of three different packaging on aroma compounds and vitamin C stability under light and darkness exposure.
Methods : Samples of orange juice, packaged in the three most used packaging in the beverage industry; tetra-pack, glass and plastic (standard PET), are stored at the laboratory at room temperature in two conditions control; light exposure and total darkness. The monitoring of the quality during three months was realized using high-performance liquid chromatography (HPLC) and gas chromatography-time of flight Mass spectroscopy (GC-TOFMS) for vitamin C and flavor compounds determination, respectively.
Results : The results showed very important losses of aromas and vitamin C throught the plastic packaging materials against glass or tera-pack. In fact, significant losses ($p < 0.05$) of vitamine C in PET were about 96% at light exposure and 92% in darkness. Thus, losses of vitamin C in plastic materials (PET) have been correlated with their oxygen permeability and the sorption phenomena.
Conclusion : The best conservation of vitamin C was in packed juice tetra pack. The glass packaging was intermediary for losses of aromas and viamin C.
Keywords: orange juice; quality control; orange aroma; vitamin C stability.

EFFETS DE L'EMBALLAGE ET DU TEMPS DE STOCKAGE SUR LA STABILITÉ DES COMPOSÉS AROMATIQUES ET DE LA DE VITAMINE C DANS UN JUS D'ORANGE RECONSTITUÉ PRODUIT EN ALGÉRIE

Résumé

Description du sujet : Dans le présent travail, la qualité nutritionnelle et sensorielle d'un jus d'orange reconstitué en Algérie a été étudiée, pendant le stockage dans différents emballages, dans des conditions normales de commercialisation.
Objectifs : Cette étude a pour objectif principal d'étudier l'impact de trois emballages différents sur la stabilité des composés aromatiques et de la vitamine C dans des jus sous exposition à la lumière et à l'obscurité.
Méthodes : Des échantillons de jus d'orange, emballés dans les trois emballages les plus utilisés dans l'industrie des boissons; tétra-pack, verre et plastique (PET standard), sont stockés au laboratoire à température ambiante dans deux conditions de contrôle; l'exposition à la lumière et l'obscurité totale. La surveillance de la qualité pendant trois mois a été réalisée en utilisant la chromatographie en phase liquide à haute performance (HPLC) et la chromatographie en phase gazeuse (GC-TOFMS) pour la détermination de la vitamine C et des composés aromatiques volatils, respectivement.
Résultats : Les résultats ont montré des pertes très importantes d'arômes et de vitamine C à travers les matériaux d'emballage en plastique contre le verre ou le tera-pack. En fait, des pertes significatives ($p <0,05$) de vitamine C dans le PET étaient d'environ 96% à l'exposition à la lumière et 92% dans l'obscurité. Ainsi, les pertes de vitamine C dans les matériaux plastiques (PET) ont été corrélées avec leur perméabilité à l'oxygène et les phénomènes de sorption.
Conclusion : La meilleure conservation de la vitamine C était dans le tetra-pack. L'emballage en verre était intermédiaire pour les pertes d'arômes et de viamine C.
Mots clés: jus d'orange; contrôle de qualité; arôme d'orange; stabilité de la vitamine C.

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INTRODUCTION

The beverage sector is among the most dynamic sectors of the food industry in Algeria. Indeed, a wide range of products is offered to the consumer who is often lost between the different names and qualities displayed. Orange juice, one of the products of this sector, is the most consumed in the world and Algeria is no exception. This juice is known for its role of quenching, its taste both sweet and sour and especially for its vitamin C intake. However, like all juices, orange juice can undergo degradation reactions before reaching the consumer. These reactions, which are of a physical, chemical, enzymatic or microbiological nature, depend on several factors such as; the nature and condition of the product (fresh or processed), its packaging and storage [1]. In recent years, there has been an awareness of the importance of conditioning in the protection of the orange juice against the risks of external contamination, the maintenance of aromatic stability and the preservation of vitamin C contents. In fact, aromas or flavor and vitamin C are two important elements that composed orange juice. It is well known that citrus fruits are characterized by a complex mixture of volatile flavor compounds [2] which constitute their specific sensory quality and often considered a necessity for reconstituted juices [3]. Orange juices were analytically investigated in many recent studies where the focus was put on the identification of flavor compounds. Mastello *et al*., analysed pasteurised orange juices with a multi-assessment instrument approach based on gas chromatography and confirmed the presence of four aldehydes (hexanal, heptanal, octanal, citral), two esters (ethyl butanoate, methyl hexanoate), and four monoterpenes (α-pinene, D-limonene, linalool, α-terpineol) [4].While, Kim *et al.*, identified β-pinene, D-limonene, linalool, decanal and α- and β-terpineol, in commercial orange juices prepared by different processing methods [5]. But the quantification of all aromas compounds remains difficult in analytical chemistry given to their volatility and the complexity of the matrix which depends on the diversity of nature and the different geographic origin of the orange. Otherwise, vitamin C, or ascorbic acid, is mainly found in vegetables and fruits [6]. It is the most important water-soluble antioxidant that is required to maintain human health [6,7]. The recommended daily acceptance (RDA) for ascorbic acid is 100–120 mg/day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals [8]. The vitamin C content in orange juices range from 150 to 450 mg/l; and one glass of orange juice (200 mL) can deliver about 30–80% of recommended daily intake of vitamin C [9]. Thereby, the evaluation of flavor compounds and vitamin C is essential to define the organoleptic and nutritional quality of orange juice. So, in the present work, the quality of orange juice with respect to its packaging during storage under normal conditions of commercialization was studied. Thus, the effect of three different packaging; glass, plastic (standard PET) and tetra pack, on aroma compounds and vitamin C contents was investigated under light and darkness exposure.

MATERIALS AND METHODS

1. Chemicals and reagents

Ascorbic acid ($C_6H_8O_6$), potassium dihydrogen phosphate (KH_2PO_4), meta phosphoric acid (H_3PO_4) extra pure and methanol (CH_4O) (99,9%, of HPLC grade), were purchased from Sigma–Aldrich (Steinheim, Germany). n-Hexane (C6H14), VWR Prolabo chemicals 98,2%, Deionized water and Solvents of LC grade were filtered through 0.45µm filter membrane and degassed for 20 minutes by ultra-sonic cleaner. All other chemicals were reagent grade.

2. Orange juice

Samples of pure 100% orange juice were used in this work. It was purchased from an Algerian industry, as a final product beverage. The pure juice is commercialized in packed form (in tetra-pack), where the nutritional informations (in 100 ml) were indicated (187 KJ (44Kcal) energy, 0.8g protein, 10.0g carbohydrates, lipids < 0.1g, dietary fiber < 0.1g and sodium < 0.002g). The physical and chemical properties were shown in the table 1. The vitamin C concentration was about 240 mg/L. The juice is composed of water and orange juice concentrate without added sugar. Our choice was in relation to the richness of the nutrient product, especially in vitamin C, because the formula is the closest to natural orange juice.

Other samples of the beverage were directly packed in standard PET and glass. The total number of samples was 54, distributed in 3 lots of 18 samples for each package, where 9 were exposed to high light intensity and 9 were kept in darkness, at room temperature. The experiment time was 3 months, and after each month, 3 samples were taken to analyse in each packaging for each exposure condition.

Table 1: Some physico-chemical and microbiological properties of commercialized reconstituted pure 100% orange juice.

Physico-chemical parameters		**Microbiological parameters**	
Acidity	8.1 $g.L^{-1}$ ± 5 % ACM	Total mesophilic aerobic germs	0 in 1 ml
Brix	11.2	Anaerobic sulfito-reducers	0 in 10 ml
Density	1.040±0.003	Yeast	< 20
pH (25°C)	3.5 - 3.9	Molds	<10/100 ml
		Stability test	negative (-)

3. Vitamin C standard preparation

To measure the concentration of vitamin C in the juice, it was necessary to prepare a standard solution that allows us to identify vitamin C in relation to the retention time. The standard solution was prepared by dissolving 100 mg of ascorbic acid in 100 ml of the Potassium dihydrogen orthophosphate buffer solution (20 mM), therefore the concentration of the standard solution was 1 mg / ml [10].This standard stock was further diluted with methanol in order to obtain a calibration set consisted of five concentration levels. The calibration curve was prepared in the range of 30 -500 µg/ml.

4. Vitamin C extraction from orange juice

A liquid-liquid extraction was conducted to extract vitamin C from orange juice, following the method described in literature, with slight modifications [10, 11, 12]. Briefly, a volume of 5 ml of juice was mixed with 5 ml of mobile phase (methanol+ KH_2PO_4) using a vortex (5 min), centrifuged at 5000 rpm for 10 min and filtered through millipore filters (0.45 µm). 20 µl were injected onto the HPLC to evaluate the vitamin C content.

5. HPLC determination of vitamin C

The HPLC analysis of vitamin C in orange juice were performed on Agilent 1100 high-performance liquid chromatograph (USA) equipped with C18 column (150x4.6 mm, 5µm, Restek Pinnacle II). The mobile phase was composed of 20% methanol (solvent A) and 80% KH_2PO_4 buffer at pH 3 (solvent B) with a flow rate of 1 mL/min. The eluate was detected using an UV detector at 245 nm. Vitamin C was identified by comparing its UV spectrum and retention time with that of standard. Quantification of vitamin C was evaluated using the external standard method [12]. The percentage of vitamin C degradation (D) was determined using Eq. (1), where C_0 is the mean concentration at the beginning of exposure and Ct the mean concentration after t days of exposure :

$$D = (C_0 - Ct) \times 100 / C_0$$

Eq. (1)

6. Volatile flavor compounds extraction

Solvent extraction involves mixing a quantity of immiscible solvent in the water with an aliquot of orange juice. A volume (2:1) of orange juice and hexane solvent, respectively, was introduced into a separating funnel and subjected to agitation. The funnel is then put back on its support, and we wait until the two immiscible phases separate by decantation, once separated we remove the cap of the separating funnel, we open the tap and the organic phase is recovered in a clean container. The solvent, which becomes enriched in volatile compounds, is evaporated by rotavapor in order to concentrate the volatile compounds extracted.

This type of extraction makes it possible to extract the total amount of volatile compounds provided that there is a high affinity of the volatile compounds for the solvent used [13].

7. GC/MS determination of volatile flavor compounds

The volatile flavor compounds were identified by gas chromatography-time of flight Mass spectroscopy (GC-TOFMS). The GC was equipped with split/splitless injector operated in the split mode (1/50). The analytical column was a DN-5 capillary column, 0.25 mm I.D., 30 m, 0.25 µm film thickness and the flow rate was 0.5 ml/min. The detector was a time of flight Mass spectrometer equipped with an electron ionization source. The source temperature was 200°C, and transfer line temperature was 250°C. A volume of 10µl was injected in the GC injection port held at 250°C. The GC oven was maintained at an initial temperature of 40°C for 5 min, then was ramped at 5°C/min to 160°C and held for 5 min. Finally it ramped at 8°C/min to 240°C for 2 min. As they eluted from the column, analytes were detected by the mass spectrometer.

8. Statistical analysis

The software Statistica (V10) was used for statistical analysis. Results of the packaging effect on the vitamin C contents were expressed as mean of three replicates ± standard deviation (n=3).

Differences between light and darkness exposure were evaluated by one-way ANOVA followed by Tukey's test ($p < 0.05$).

RESULTS

1. Detrmination of vitamin C content

In order to quantify vitamin C in orange juice, high-performance liquid chromatography was performed. The figure 1, representing the chromatogram of standard solution of 1mg AA /mL showed a specific peak at retention time (RT) of 1.82 min. The linear calibration curve was calculated over the concentration range of 30 -500 µg/mL (Fig.2). The mean (±SD) regression equation from three replicate calibration curves, was: y= 0.0019x. The coefficient of determination (r^2) was equal to 0.99, indicating suitability for quantification. Thus, the stability of vitamin C in orange juice was studied in terms of ascorbic acid (AA) concentration.

The Figure 3 illustrated vitamin C content versus storage time in the exposure conditions cited above for respectively; tetra-pack, glass and PET. The results revealed higher lossess (p<0.05) of vitamin C in PET compared to glass and tetra-pack. Indeed, the calculation of degradation percentage (table 2) showed the lowest values for PET which were 96.229% at light exposure and 92.234% in darkness, after three months storage.

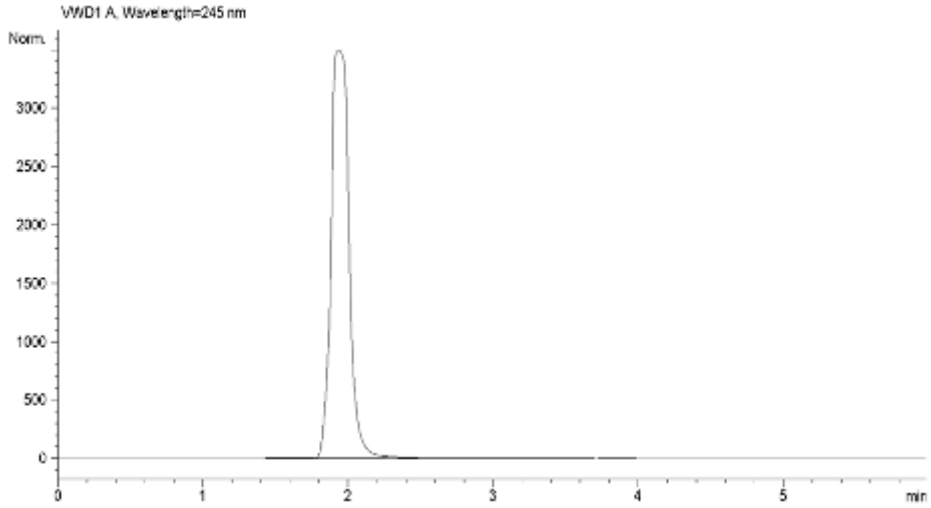

Figure 1 : HPLC Chromatogram of Standard Vitamin-C (AA), 1 mg/mL.

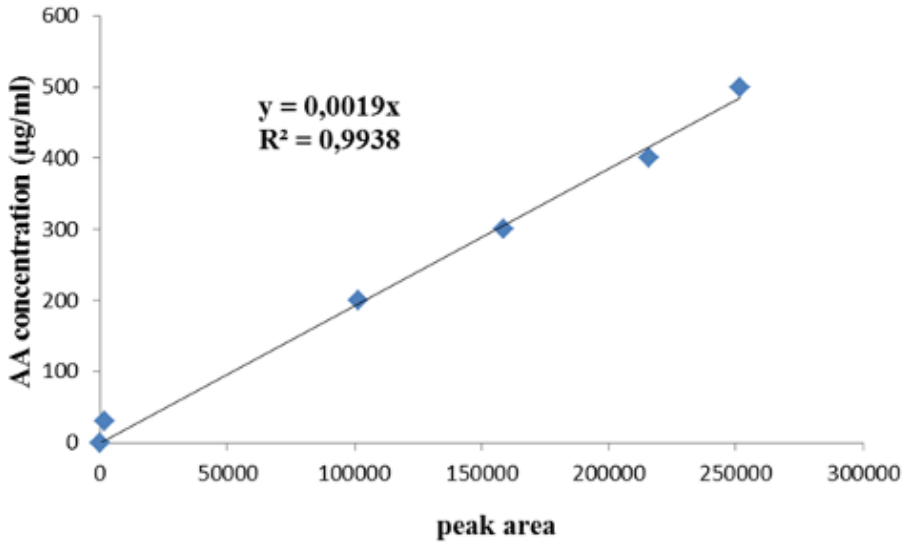

Figure 2 : Vitamin C standard calibration curve

Table 2 : Percentage of vitamin C degradation (D) in orange juice stored in different packaging at room temperature under hight light intensity and in darkness.

	Light exposure			Exposure in darkness		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Tetra-pack	9,129	33,371	62,108	1,379	5,075	5,863
Glass	10,471	68,263	95,683	8,179	19,529	37,996
PET	10,558	93,700	96,229	12,575	43,404	92,234

2. *Qualitative determination of volatile flavor compounds*

In our study, the analysis of the volatile compounds profile (fig. 4) of orange juice involves two steps, i) the extraction of the volatile fraction from its liquid matrix, and ii) the qualitative identification using GC-TOFMS. According to the chromatograms with integration and the compound list (Table 3), the volatile fraction of pure orange juice analyzed contains hydrocarbons such as D-limonene, monocyclic and aliphatic alcohols such as terpineol, some aldehydes and esters. Depending on the light intensity and regardless of the package permeability, changes in the aromatic profile of orange juice were observed in standard PET against glass and tetra-pack (fig. 5 and fig. 6).

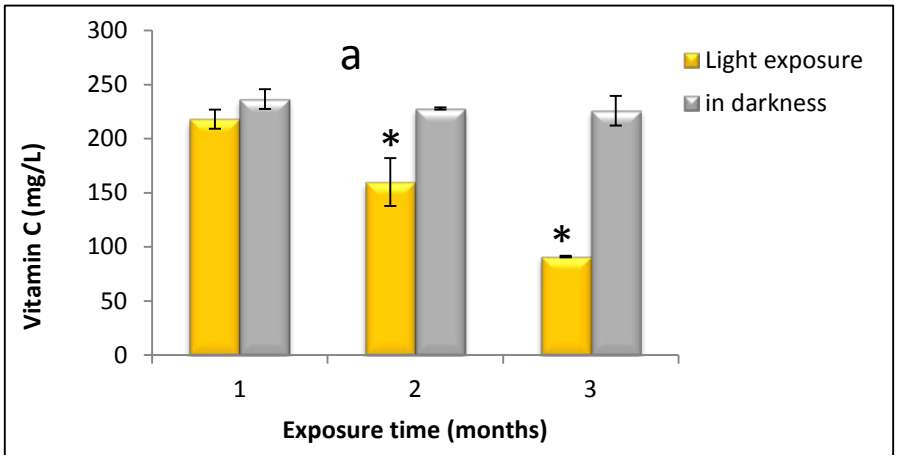

Figure 3 : Vitamin C contents in different packaging during three months time exposure.
a: Tetra-pack

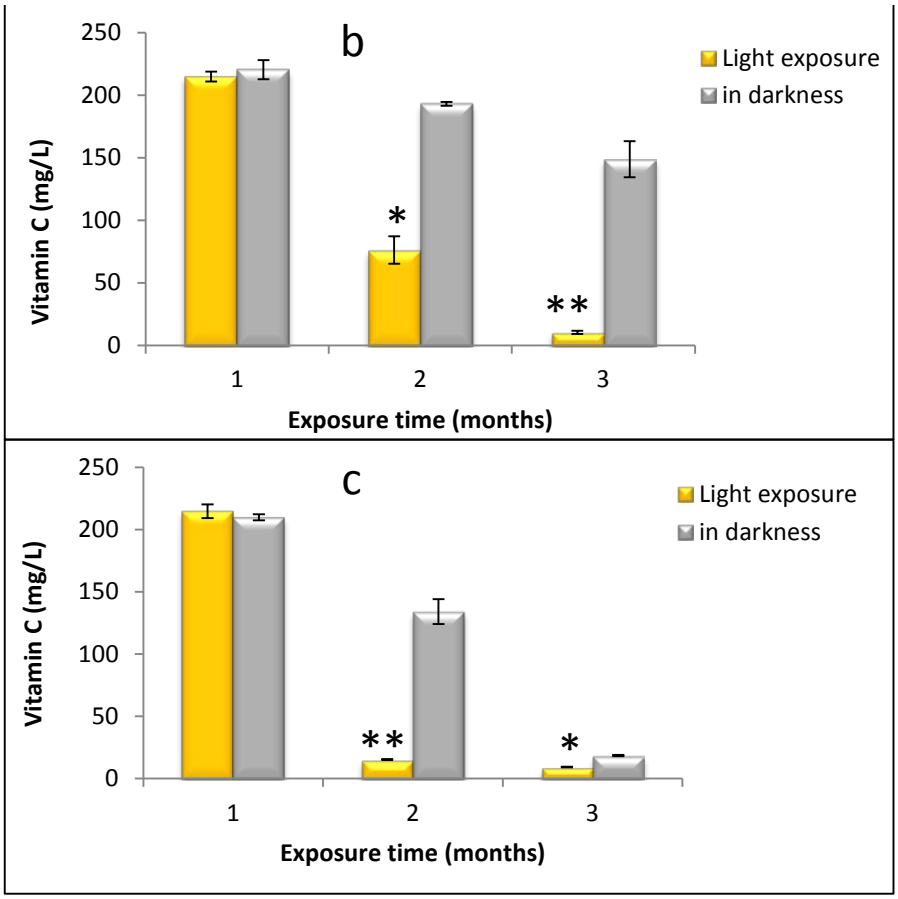

Figure 3 : Vitamin C contents in different packaging during three months time exposure. b: Glass, c: PET, (* $p < 0.05$; ** $p < 0.01$).

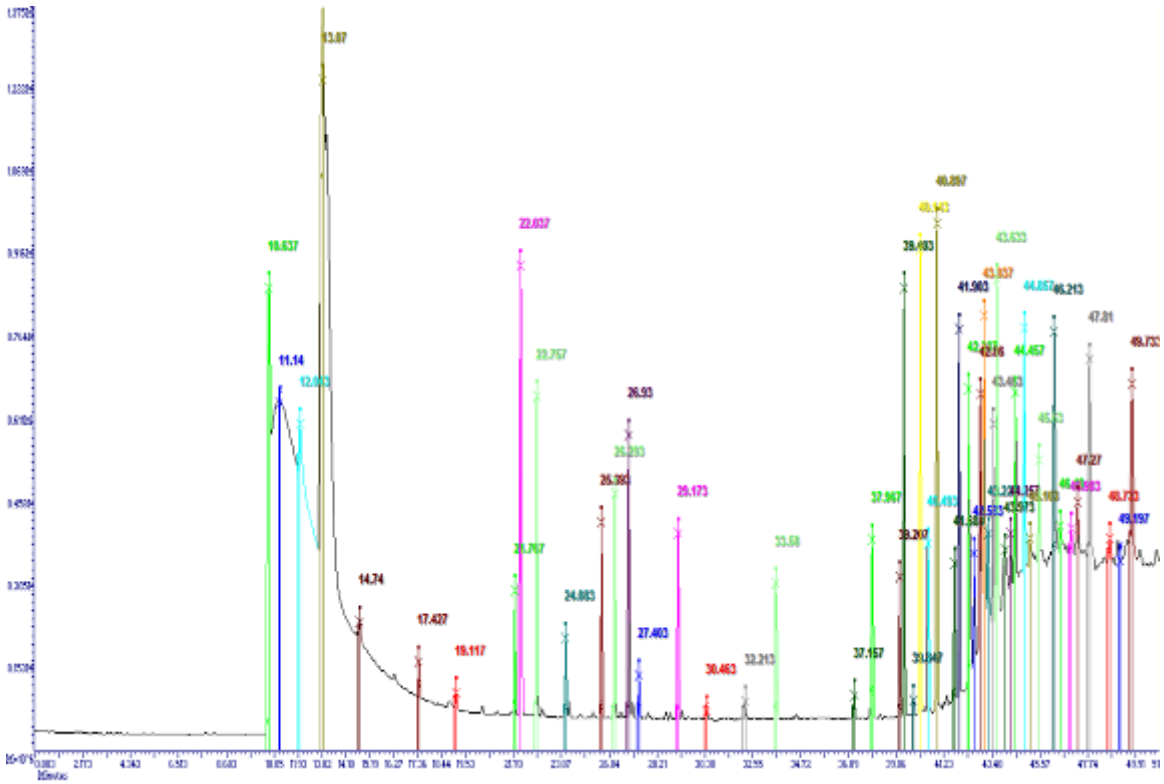

Figure 4: GC-TOFMS chromatogram of volatile flavor compounds in orange juice

Table 3: The flavor compounds identified in orange juice, using GC-TOFMS

Retention Time	Compound Name	Area Percent	Formula	Area
10.637	n-Hexane	11.413	C6H14	7069011.000
12.043	5-Hepten-1-yne, 6-methyl	7.572	C8H12	4689883.000
13.070	**D-Limonene**	4.267	C10H16	2643129.000
14.740	Cyclobutane, 1,2-diphenyl-	0.479	C16H16	296669.600
17.427	Cyclohexasiloxane, dodecamethyl-	0.238	C12H36O6Si6	147622.400
19.117	Pentane, 2,2,3,4-tetramethyl-	0.166	C9H20	103064.800
22.037	**Benzaldehyde**	5.811	C7H6O	3599113.000
24.083	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyl	0.620	C10H18O	383988.400
25.693	Cyclooctasiloxane, hexadecamethyl-	1.326	C16H48O8Si8	821061.600
26.293	**a-Terpineol**	1.905	C10H18O	1180190.000
26.930	Selina-3,7(11)-diene	2.588	C15H24	1603044.000
27.403	Undecane, 6-ethyl-	0.317	C13H28	196301.600
29.173	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimetyl-	1.861	C13H40O5Si6	1152971.000
30.463	Propanoic acid, 2-methyl-, anhydride	0.064	C8H14O3	39930.000
32.213	Dodecane, 2,6,10-trimethyl-	0.145	C15H32	90051.200
33.580	Heptasiloxane, hexadecamethyl-	2.294	C16H48O6Si7	1420894.000
37.157	Sulfurous acid, 2-ethylhexyl hexyl ester	0.178	C14H30O3S	110198.400
37.967	Cyclooctasiloxane, hexadecamethyl-	2.390	C16H48O8Si8	1480066.000
39.207	Dodecane, 1-iodo-	1.010	C12H25I	625448.400
39.403	**Hexadecanoic acid, methyl ester**	3.847	C17H34O2	2382980.000
39.847	2-Piperidinone, N-[4-bromo-n-butyl]-	0.121	C9H16BrNO	74787.600
40.143	Dodecane, 1-iodo-	3.931	C12H25I	2434799.000
40.493	Cyclononasiloxane, octadecamethyl-	1.688	C18H54O9Si9	1045592.000
40.897	Hexadecane, 1-iodo-	4.437	C16H33I	2748376.000
41.683	Dodecane, 1-iodo-	1.039	C12H25I	643324.000
41.903	2-methyloctacosane	3.217	C29H60	1992772.000
42.337	Dodecane, 1-iodo-	2.410	C12H25I	1492836.000
42.583	Hexadecanoic acid, 15-methyl-, methyl e	0.919	C18H36O2	569122.400
42.860	**9-Octadecenoic acid, methyl ester**	2.796	C19H36O2	1731688.000
43.037	2-methyloctacosane	2.881	C29H60	1784510.000
43.227	Dodecane, 1-iodo-	1.594	C12H25I	987288.800
43.453	9,15-Octadecadienoic acid, methyl ester,	1.678	C19H34O2	1039614.000
43.633	Hexadecane, 1-iodo-	3.701	C16H33I	2292611.000
43.973	Tetracosamethyl-cyclododecasiloxane	0.122	C24H72O12Si1	75812.800
44.257	Dodecane, 1-iodo-	0.525	C12H25I	325151.200
44.457	2-methyloctacosane	1.599	C29H60	990218.000
44.857	Hexadecane, 1-iodo-	2.454	C16H33I	1519982.000
45.103	Heptacosane	0.512	C27H56	317083.200

Following table 3: The flavor compounds identified in orange juice, using GC-TOFMS

Retention Time	Compound Name	Area Percent	Formula	Area
45.530	Tridecanol, 2-ethyl-2-methyl-	1.193	C16H34O	738688.800
46.213	2-methyloctacosane	2.862	C29H60	1772457.000
46.480	Heptacosane	0.434	C27H56	268520.400
46.983	Heptacosane	0.499	C27H56	309141.200
47.270	Heptadecane, 2,6,10,15-tetramethyl-	0.453	C21H44	280822.000
47.810	Hexadecane, 1-iodo-	3.615	C16H33I	2238931.000
48.733	Dodecane, 1-iodo-	0.367	C12H25I	227023.200
49.197	L-Alanine, N-[N-[N-[(4-nitrophenyl)meth	0.006	C23H34N4O6	3718.000
49.733	Tridecanol, 2-ethyl-2-methyl-	2.794	C16H34O	1730303.000

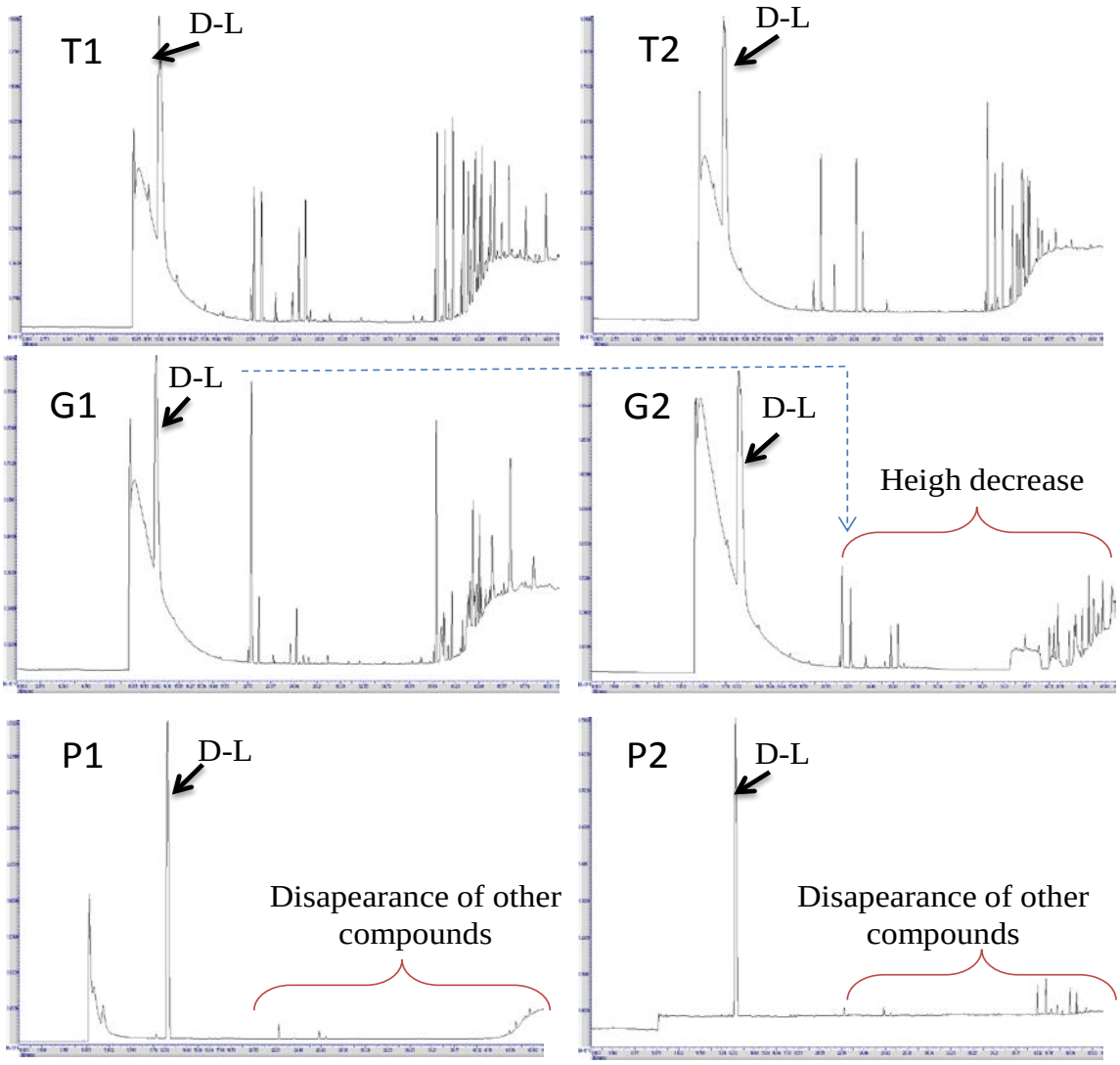

Figure 5: GC-TOFMS chromatograms of flavor compounds in orange juice stored under light in: Tetra-pack (T1:1 month, T2:3 months), Glass (G1:1 month, G2:3 months) and in PET (P1:1 month, P2:3 months). D-L: D-Limonene

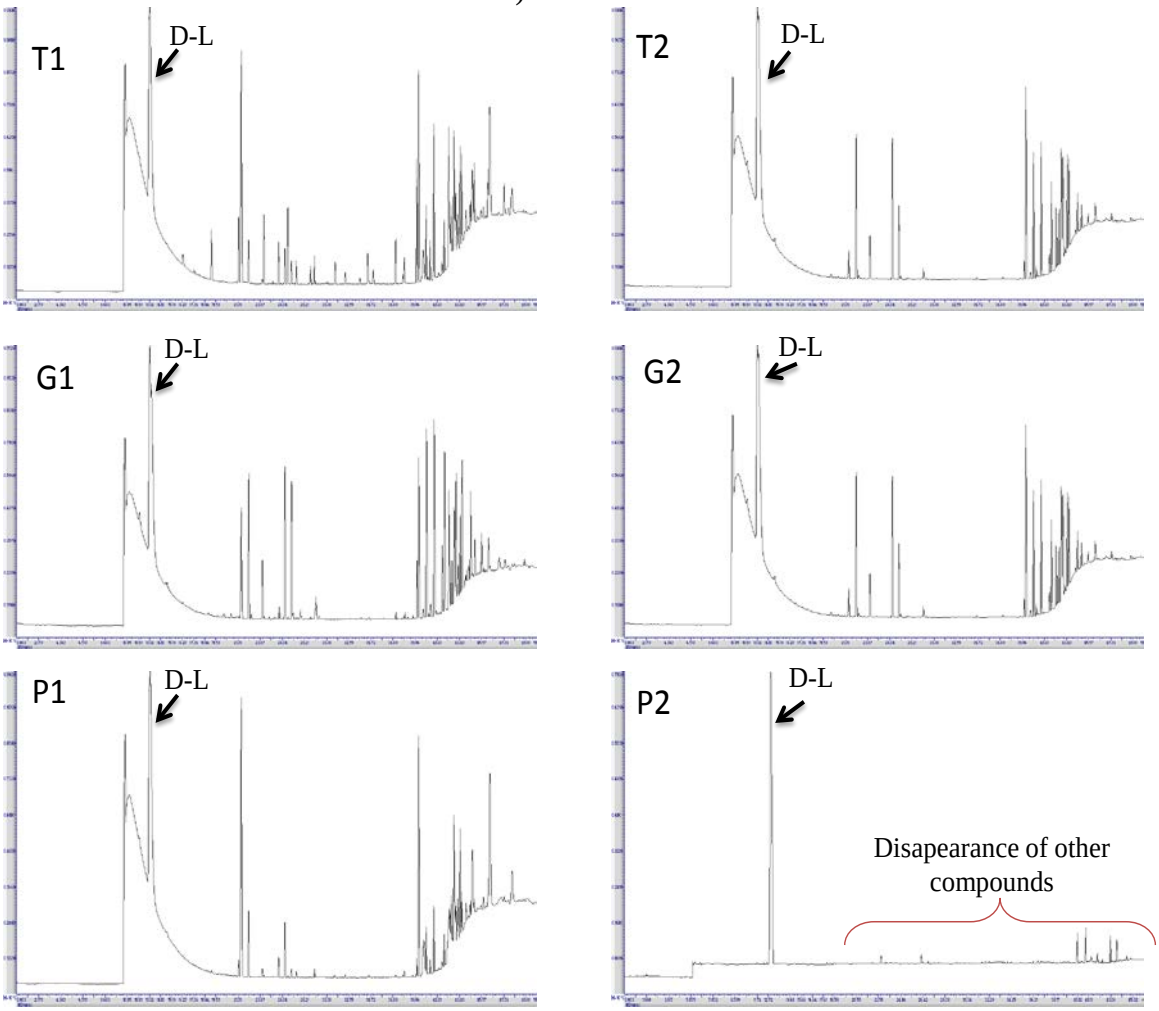

Figure 6: GC-TOFMS chromatograms of flavor compounds in orange juice stored in darkness, in: Tetra-pack (T1:1 month, T2:3 months), Glass (G1:1 month, G2:3 months) and in PET (P1:1 month, P2:3 months). D-L: D-Limonène.

DISCUSSION

During exposure of three months, under light and in darkness, at room temperature, ascorbic acid concentrations in all juices were gradually decreased with time at a rate depending on the package material and storage temperature. In fact, since the experiments were carried out during the period between april-may and june, the temperature at that time in Algeria, increased remarkably and reached 37 to 40°C in june, which affected directly orange juice quality and certainly other beverages and foods stored at room temperature. Moreover, when juice was exposed under light, a faster degradation of ascorbic acid was noticed compared to darkness, in all samples in the three packages.

Concerning tetra-pack, conservation in the light or in the dark is not a determining factor in the degradation of vitamin C. On the other hand, preservation time and temperature play a role in the stability of vitamin C. So, we obtained, after three months, a degradation of 62.108% under light against 5,863 %, in darkness. This degradation of ascorbic acid was related to the oxygen transfer, the conservation time and the hight room temperature which was more than 30°C in june [3]. It is also necessary to take into consideration the electric power failure which is frequent during this period, thus causing the shutdown of the cooling and air conditioning systems.

The main factors that can affect ascorbic acid loss in juices include temperature, salt and sugar concentrations, pH, oxygen, light, metal catalysts, initial concentration of ascorbic acid, microbial load and protection provided by container [14, 15]. Previous studies showed that Vitamin C can exist in two active forms L-ascorbic acid and its oxidation product, dehydroascorbic acid.

In a dilute acid medium, such as the beverage products, L-ascorbic acid is susceptible to oxidation, and two reactions which involve a loss of Vitamin C activity under these conditions [16,17]. The first step in the degradation of L-ascorbic acid would be the oxidation of L-ascorbic acid to dehydroascorbic acid [18]. No vitamin C activity is lost in this step. However, dehydroascorbic acid can undergo two additional types of reactions (oxydation and transformation) which result in a loss of Vitamin C activity [16].

For flavor determination, comparing with our results, Kim *et al*. [5], have also identified D-limonene, α-and β-terpineol, some aldehydes and esters, using Dynamic Headspace Sampling followed by Gaz Chromatography-Mass Spectrometry analysis, in four commercial orange juice products prepared by different processing methods [5]. Among these hydrocarbons it has been possible to identify D-limonene which is a terpene hydrocarbon responsible for the fresh smell characteristic of the orange, being the most abundant aroma compounds [2, 4].

Indeed, we notice losses of hydrocarbons and alcohols in PET, from the first month and arriving at the third month, there is only the D-limonene which persists with a very intense late peak in the 12 th minute of the analysis. While in the glass and the tetra-pack, the aromatic fraction is conserved with a slight variation in the areas of the peaks, certainly due to the opening of the bottles during the sampling test or during extraction. However, the presence of D-limonene is remarkable in all chromatograms. It is very stable over time. In fact, the presence of D-limonene in pasteurised orange juice was confirmed in accordance with olfactometry assessment in the processed juice, using two-dimensional gas chromatography-accurate mass time-of-flight MS (GCxGC-accTOFMS) [4].

CONCLUSION

In our study, we evaluated the stability of vitamin C and the flavors in an orange juice marketed in Algeria, depending on the time and type of packaging. During exposure, under hight light intensity and in darkness, at room temperature, vitamin C contents were gradually decreased with time at a rate depending on the package material and storage temperature.

In fact, we noticed higher lossess of vitamin C in PET compared to glass and tetra-pack. Our results are in agreement with those shown in several studies involving time and temperature as well as packaging material, as crucial factors for the conservation of beverages. The qualitative determination of flavors with GC-TOFMS revealed that the volatile fraction of pure orange juice analyzed contains hydrocarbons such as D-limonene, monocyclic and aliphatic alcohols such as terpineol, some aldehydes and esters. But, the results showed the persistance of D-limonene after three months storage, under light and in darkness. The elucidation of the aroma pattern contributes to a better understanding of orange juices specially the reconstituted ones which still presenting variability depending on the specific matrices linked to number of factors such as the diversity of nature and geographic origin. In perspective, we intend to continue this study by a quantitative evaluation of flavor compounds and establishing a comparative study concerning nutritional and sensorial quality, between local and imported orange juice.

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