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# Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange

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**Abstract**

This paper reports on the composition of aroma compounds and fatty acids and some physico-chemical parameters (juice percentage, acidity and total sugars) in five varieties of citrus: blood orange, sweet orange, lemon, bergamot and bitter orange. Volatile compounds and methyl esters have been analyzed by gas chromatography. Limonene is the most abundant compound of monoterpene hydrocarbons for all of the examined juices. Eighteen fatty acids have been identified in the studied citrus juices, their quantification points out that unsaturated acids predominate over the saturated ones. Mean concentration of fatty acids varies from 311.8 mg/l in blood orange juice to 678 mg/l in bitter orange juice.


*Keywords:* Blood orange; Sweet orange; Lemon; Bergamot; Bitter orange; Aroma; Fatty acids; Lipids

## 1. Introduction

Tunisia is well known for its important production of citrus fruits (240 000 tons/2000/2001). This agricultural field is of great interest since it satisfies the needs of the country in fresh fruits lasting on an appreciable period of the year. Furthermore, the exports of this fruit over the same period was 23 477 tons.

As dictated by the merchandising requirements, the product "fruit" needs to be of a high quality. Organoleptic quality is determined by the following parameters: aroma, content in juice and in sugars as well as the acidity that must be high for lemons and limes but moderate for other fruit productions.

The current study aims at highlighting some physico-chemical features of citrus juice extracted from common known Tunisian citrus fruit varieties of a particular industrial and nutritional value. The following varieties are important in this respect:

The blood orange, a variety of *Citrus sinensis,* is of exceptional quality when the variety is cultivated in the region of the Cap-Bon in Tunisia (Swingle, 1943). The sweet orange, also a variety of *Citrus sinensis* has a smooth peel, a soft pulp, a weakly winged leafstalk (Swingle, 1943). The lemon (*Citrus limon* L.) has acidic pulp, of fine texture and coloured pale yellow (Swingle, 1943). The bergamot (*Citrus bergamia*), appeared in the south of Italy before 1700 and is defined as a hybrid of *Citrus aurantium* and *Citrus limon* L. for some authors or *Citrus aurantium* and *Citrus aurantifolia* for others. Its greenish yellow skin is very acidic. The bergamot owes its commercial importance to the particular quality of its essential oils, since it constitutes the basis of the best perfumes (Chapot, 1962). The bitter orange (*Citrus aurantium*), although resembling the orange, differs by several characters. Its pulp is acidic and the albedo is more bitter. Essential Oil relative to the skin and leaves has a very characteristic pleasant odor (Chapot and Praloran, 1955).

According to some bibliographic data, intervarietal differentiation has been established by multivariate pattern recognition involving amino acids (Aristoy et al., 1989), flavanone glycosides (Mouly et al., 1994), and flavor constituents (Maccarone et al., 1998).

In this investigation, we aimed at characterizing juices of the previous mentioned varieties by reference to the percentage of juice, total sugars, acidity, aroma, total lipids and fatty acids.

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## 2. Results

Citrus juices have been the subject of many studies (Baldwin, 1993; Nisperos-Carriedo et al., 1992; Mouly et al., 1994). Indeed, Arnao et al. (1998) underlined the antioxydant capacity of citrus juices. In addition, Rice Evans et al. (1997) studied their flavonoid compositions.

### 2.1. Juice rate, acidity and total sugars

Blood orange constitutes the group characterized by the highest juice rate if compared to that of sweet orange and bergamot group and lemon and bitter orange group. When referring to acidity, the five citrus varieties analysed constitute 4 main groups: blood orange group (10.17 g/l), sweet orange (1.09 g/l) and bergamot (0.71 g/l) group, lemon group (40.29 g/l) and bitter orange group (49.92 g/l). However, when considering their total sugar contents, the citrus varieties are classified into two groups, the first including blood orange, sweet orange and bitter orange having the highest sugar quantities, compared to the second constituted by lemon and bergamot (Table 1).

### 2.2. Aroma

The survey of citrus varieties by their juice aroma content, permits to conclude on the existence of two groups. A group including the citrus varieties with the highest aroma quantity: sweet orange (344.46 μg/ml) and bergamot (355.94 μg/ml). A second group represented by lemon (140.75 μg/ml), blood orange 157.55 μg/ml) and bitter orange (99.52 μg/ml), characterized by the lowest aroma quantities.

The following monoterpene hydrocarbons were found in all the aromas analyzed: α-pinene, limonene, β-pinene and p-cymene.

Limonene is the most abundant component of this fraction for all the aromas examined; its contents vary from 63% in the blood orange aroma to about 90% in bitter orange aroma. The monoterpene percentage sum permits to split the studied varieties into two groups, a group constituted by blood orange and bergamot and a second including the sweet orange and the bitter orange. For the lemon, it can be among both groups.

Other aroma compounds have been identified in the juices analyzed such as methanol, isopropanol, butyl acetate, 3-heptanone, ocimene, nonanol, linalool, α-terpineol, valencene, α-terpinene, β-ionone, terpinen-4-ol, and p-cymene: as far as the butyl acetate compound is concerned, it exhibits its highest rate in bergamot juice. Nevertheless, the same compound was not recorded for both sweet and bitter orange. The 3-heptanone was detected in all of the juices under-investigation excepting the sweet orange. The ocimene was absent in sweet orange and bergamot. In the same way, nonanol was also missing in sweet orange and bergamot juices but also in bitter orange. Linalool, was identified in all the analyzed juices but its highest quantity was signaled in bergamot. The α-terpineol displays its highest rate in blood orange, however it was missing in bergamot juice. Valencene is a sesquiterpene present with a low rate in the different juice aromas; α-terpinene was identified in lemon, bergamot and bitter orange juices. This last citrus was individualized by the most important quantity of this previous monoterpene. β-Ionone was absent in the sweet orange and was present in trace in all the other juices. Terpinen-4-ol and p-cymene were present in almost the same percentage in all the aromas examined (Table 2).

### 2.3. Effect of variety on fatty acids and on total lipids

Our present study showed that lipid contents of the citrus juices are negligible at a percentage of about 0.20%. For the content of fatty acids, there is not a significant difference among varieties; it varies from 311,80 mg/l in the sweet orange to 678 mg/l in bitter orange juice. In addition, the quantification of fatty acids in citrus juices points out the abundance of 4 fatty acids (palmitic, oleic, linoleic and linolenic) constituting 74.90% of the total acids in blood orange juice (C16:0: 12.79%, C18:1: 20.11%, C18:2: 29.14%, C18:3: 12.86%). The aforementioned fatty acids represent 65.74 of the total acids in sweet orange (C16:0: 10.15%, C18:1: 15.46%, C18:2: 19.97%, C18:3: 20.15%). They are of 71.24 in lemon juice (C16:0: 14.20%, C18:1: 21.04%, C18:2: 26.22%, C18:3: 9.77%). Moreover, in bergamot juice, they constitute 79.50% (C16:0: 15.34%, C18:1: 33.98%, C18:2: 24.00%, C18:3: 6.16%) and of about 63.00% in bitter orange juice (C16:0: 17.40%, C18:1: 23.38%, C18:2: 16.35, C18:3: 5.55%).

In addition, we have noticed that the unsaturated acids are more abundant than the saturated ones. Thus, the major blood orange component is linoleic (29.14%), followed by oleic (20.11%) and linolenic (12.86%).

For sweet orange, the major acids are as follows: linolenic (20.15%), linoleic (19.97%) and oleic (15.46%). Among lemon, a noteworthy prevalence of

Table 1
Juice percentage, acidity and total of blood orange, sweet orange, lemon, bergamot and bitter orange

| Varieties | Blood orange | Sweet orange | Lemon | Bergamot | Bitter orange |
|---|---|---|---|---|---|
| % Juice | 50.167a | 45.600b | 27.733d | 35.560c | 26.533d |
| Acidity (g/l) | 10.17c | 1.09d | 40.29b | 0.710d | 49.920a |
| Total sugars (g/l) | 127.993a | 123.750a | 75.983b | 72.760b | 122.467a |

Means in the same row followed by a different letter are significantly different.

Table 2
Volatile compounds of varieties of blood orange, sweet orange, lemon, bergamot and bitter orange

| Total aroma quantity (μg/ml) volatile compounds | Blood orange 157.55b | | Sweet orange 344.46a | | Lemon 140.45b | | Bergamot 335.94a | | Bitter orange 99.52b | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mg/l | % | mg/l | % | mg/l | % | mg/l | % | mg/l | % |
| Methanol | 2.0563a | 1.3217a | 1.3627a | 0.3727a | 0.2843a | 0.2020a | 1.1633a | 0.3477a | 0.3703a | 0.2783a |
| Isopropanol | 2.828a | 1.8160a | 3.283a | 0.9053a | 0.169a | 0.1207a | 0.944a | 0.2767a | 0.602a | 0.8103a |
| α-Pinene | 4.42a | 2.858a | 22.81a | 6.372a | 0.39a | 0.271a | 4.75a | 1.392a | 2.65a | 1.498a |
| Butylacetate | 0.033b | 0.0220c | 0.000b | 0.0000c | 3.000b | 1.4723b | 16.577a | 4.9670a | 0.000b | 0.0000c |
| 3-Heptanone | 0.6200b | 0.3923bc | 0.0000b | 0.0000c | 0.4600b | 0.3427bc | 3.0933a | 0.9373ab | 1.2800b | 1.2467a |
| Limonene | 99.30b | 63.145b | 301.88a | 88.199a | 111.07b | 78.838ab | 243.93a | 72.880ab | 89.41b | 90.335a |
| Ocimene | 0.1333b | 0.0800b | 0.0000b | 0.0000b | 5.3100a | 3.8517a | 0.0000b | 0.0000b | 0.1767b | 0.4760b |
| Nonanol | 0.2200a | 0.1407a | 0.0000a | 0.0000a | 0.3900a | 0.2570a | 0.0000a | 0.0000a | 0.0000a | 0.0000a |
| Linalool | 0.250b | 0.158b | 0.063b | 0.018b | 0.017b | 0.015b | 34.430a | 10.231a | 1.387b | 1.461b |
| α-Terpineol | 7.473a | 4.711a | 2.453b | 0.705b | 1.807b | 1.304b | 0.000b | 0.000b | 0.583b | 1.068b |
| Valencene | 8.353a | 5.122a | 0.000a | 0.000a | 4.807a | 3.340a | 0.000a | 0.000a | 0.000a | 0.000a |
| α-Terpinene | 0.0000a | 0.0000b | 0.0000a | 0.0000b | 0.3400a | 0.4597b | 0.7500a | 0.2273ab | 0.9467a | 0.8020a |
| β-Ionone | 0.2467a | 0.1550a | 0.0000a | 0.0000a | 1.3200a | 0.3467a | 0.3933a | 0.1060a | 0.0300a | 0.0523a |
| β-Pinene | 0.8500a | 0.6237a | 0.3033a | 0.0800b | 0.2100a | 0.02000 | 0.0733a | 0.1407b | 0.1500a | 0.1600b |
| Terpinen-4-ol | 1.6833a | 1.0893a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.1633a | 0.2883a |
| p-Cymene | 18.193a | 11.622a | 8.930a | 2.368 | 1.993a | 1.747 | 19.397a | 5.623 | 0.937a | 0.759 |
| Monoterpene hydrocarbons | 78.329b | | 97.018a | | 85.308ab | | 80.143b | | 94.030a | |

Means in the same row followed by a different letter are significantly different.

linoleic acid (26.22%) over oleic (21.04%) and over palmitic (14.20%). Oleic acid is the most abundant compound in bergamot juice (33.98%) followed by linoleic (24.00%) and palmitic (15.34%). For bitter orange, oleic acid predominates (23.38%) over palmitic (17.40%) and over linoleic (16.35%).

The saturated fatty acids such as: C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C20:0, and C22:0 (except for bitter orange) are present at a percentage ranging from 0 to about 4%.

The mean rate of each acid appears to be statistically different in some varieties. In fact, sweet orange is distinguished from other varieties by the lowest rate of oleic and palmitoleic, and the highest rate of linolenic and arachidic acids. Bergamot is characterized by the highest content of palmitic acid. Blood orange has the most important percentage of palmitoleic acid. According to their highest stearic acid rates, lemon and bitter orange are differentiated from the other varieties (Table 3).

## 3. Discussion

A comparison of these previous results with those of the literature points out several analogies together with some differences. Blood orange, which is considered the most important origin of juice, is characterized by the highest rate of juice (50.16%). Indeed for French orange, its juice percentage is of 42% (Dupaigne, 1971). For lemons, the content of juice determines their harvested period, it is of 30% in USA (Hodgson, 1967; Reuther and Castano, 1969; Cassin et al., 1969).

The freshly harvested lemon juice (pH = 2.3) contains 53.3 mg /ml of citric acid and 3.5 mg/ml of malic acid. In the case of oranges, the total sugar concentration increases whereas the acidity decreases during fruit growth. The acidity of Washington Navel orange juice is of 11–13 mg/ml; for Valencia orange, it is of about 11–13 mg/ml (Praloran, 1971). The Brix percentage of mature Valencia orange juice is of 13.8% (Johnson et al., 1996) and it is of 12% for Italian blood orange juice (Arena et al., 1998). Such results are in agreement with previous data (Table 1).

Notwithstanding the negligible role of hydrocarbon in determining the olfactory character of the essential oils, it has an important characterizing function and some of the components of this fraction can provide useful information on the genuineness and quality of the oils (Mondello et al., 1995). In this context, Dugo (1993) noted that monoterpene hydrocarbons percentage is about 97% in bitter orange aroma and that the oxygenated compounds such as alcohols, aldehydes and esters have the lowest percentages, ranging from 1.80 to 2.20%. For the aroma citrus fruits, the limonene importance has been confirmed by several studies. Its percentage varies from 50% in lemon to 93% in bitter orange to about 97% in grapefruit aroma (Dugo, 1993). Thus, limonene contents in Valencia orange aroma is about 106 ppm. It is much more important than the valencene (3.6 ppm), α-pinene (0.1 ppm) and linalool (0.68 ppm) (Hernandez et al., 1992). In fact, Tonder et al. (1998), signaled that limonene is the most abundant compound in aroma orange juice (88.9%) which is in conformity with our results (Table 2). For β-pinene, it

Table 3
Values of total lipids (TL), total fatty acids (TFA) and methyl esters of varieties of blood orange, sweet orange, lemon, bergamot and bitter orange

| | Blood orange | | Sweet orange | | Lemon | | Bergamot | | Bitter orange | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mg/l | % | mg/l | % | mg/l | % | mg/l | % | mg/l | % |
| C8:0 | 1.432a | 0.3097a | 1.710a | 0.8190a | 4.101a | 0.8523a | 1.034a | 0.2297a | 1.814a | 0.2310a |
| C10:0 | 12.872a | 3.580a | 9.686a | 3.559a | 1.759a | 0.406a | 0.579a | 0.1160a | 10.594a | 1.650a |
| C12:0 | 9.676a | 2.3783a | 3.254a | 1.0493ab | 5.915a | 1.3283ab | 4.354a | 0.6370ab | 16.987a | 2.2853a |
| C13:0 | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 2.265a | 0.361a |
| C14:0 | 3.05a | 0.816a | 9.3a | 2.299a | 16.67a | 3.976a | 12.98a | 1.826a | 28.21a | 3.976a |
| C14:1 | 0.274a | 0.074a | 0.320a | 0.230a | 0.928a | 3.111a | 20.375a | 0.416a | 2.003a | 1.94a |
| C15:0 | 0.185b | 0.0550b | 0.0000a | 0.0000b | 9.762a | 2.1873b | 3.167a | 0.5140ab | 9.565a | 1.2463ab |
| C16:0 | 49.57a | 12.793ab | 31.07a | 10.151b | 69.68a | 14.203ab | 94.68a | 15.347a | 121.99a | 17.399a |
| C16:1 | 29.62a | 7.3550a | 12.51a | 3.9193c | 22.61a | 4.4300bc | 34.18a | 5.5777abc | 42.90a | 6.4700ab |
| C18:0 | 2.183a | 0.5973b | 2.489a | 0.9793b | 14.061a | 2.9770a | 5.118a | 0.9660b | 18.356a | 2.8663a |
| C18:1 | 79.37a | 20.113ab | 46.70a | 15.460b | 111.23a | 21.042ab | 221.48a | 33.987a | 164.20a | 23.385ab |
| C18:1t | 0.0000a | 0.0000a | 0.0000a | 0.0000a | 2.138a | 0.4930a | 5.101a | 0.6087a | 0.0000a | 0.0000a |
| C18:2 | 116.3a | 29.143a | 60.71a | 19.972bc | 130a | 26.224ab | 146.42a | 23.999ab | 112.72a | 16.357c |
| C18:3δ | 22.194a | 5.581a | 22.474a | 6.577a | 29.484a | 5.972a | 29.595a | 4.834a | 21.920a | 3.534a |
| C18:3 | 49.48a | 12.860b | 63.40a | 20.156a | 47.11a | 9.768cb | 37.26a | 6.167c | 35.81a | 5.553c |
| C20:0 | 17.22ab | 4.250b | 47.43a | 14.690a | 19.70ab | 3.855b | 7.23b | 0.903b | 14.06ab | 3.046b |
| C20:1 | 0.18a | 0.055a | 0.000a | 0.000a | 0.000a | 0.000a | 23.25a | 2.858a | 11.99a | 1.746a |
| C22:0 | 0.000b | 0.000b | 0.000b | 0.000b | 0.000b | 0.000b | 8.28b | 1.012b | 51.87a | 7.941a |
| TFA | 393.8a | | 311.8a | | 497.3a | | 637.1a | | 678a | |
| TL | | 0.193a | | 0.196a | | 0.196a | | 0.193a | | 0.166a |

Means in the same row followed by a different letter are significantly different.

has been identified by Mondello et al. (1995) in bergamot juice (11.93%), in lemon aroma juice (12.40%) and in sweet orange juice (0.04%). The quantity of α-pinene in bitter orange juice is of 0.5%, in bergamot and lemon aroma juices is about 2% (Mondello et al., 1995). p-Cymene is detected in bergamot juice aroma (0.68%) and in lemon juice aroma (0.27%).

The purpose of this study was not to develop a set of aroma active components for the considered citrus juices, but was to characterize and differentiate between these juices in order to ovoid commercial falsifications. Although, not all the compounds have aroma activity, For example, there were over 180 FID peaks in orange essence oil, but fewer than 60 compounds exhibited aroma activity. Limonene, myrcene and valencene total about 96.5% of the total FID peak area, but account for less than 10% of the total aroma activity (Coleman et al., 1969; Haypek et al., 2000). In addition, mandarin juice contains over 51 volatiles (Nijssen, 1996), but the key aroma impact compounds are: thymol, b-pinene, g-terpinene and methylanthranilate (Wilson and Shaw, 1981).

Moreover, citrus juices are characterized by their negligible amounts of lipids (0.1%). The content of fatty acids in the studied citrus varieties fluctuates from 311.8 mg/l in blood orange juice to 678 mg/l in bitter orange juice. Although the content of fatty acids ranges from about 650–700 mg/l in blood juices to 115–191 mg/l in American blond juices (Stack et al., 1986); intermediate levels from 200 to 450 mg/l are also reported (Nicolosi Asmundo et al., 1987; Maccarone et al., 1996). Unsaturated fatty acids (C18:1, C18:2, C18:3) are the most abundant acids in the studied juices (Table 3). Their predominance (77%) over the saturated acids (4%), in blood orange, has been confirmed by Arena et al. (1998), although the major compound is linoleic (33%); its percentage is similar to that of our studied blood orange juice (29.14%). The mean levels of each acid appear to be statistically different in some varieties. In fact, bitter orange juice is characterized by the highest content of C22:0, whereas bergamot has the most abundant quantity of C20:0. For the other identified fatty acids there are no significant differences among the varieties under investigation.

The growing unripe fruit synthesizes high-molecular structures such as proteins, polysaccharides, lipids and flavonoids by carbohydrate metabolism initiated by photosynthesis in the leaves. During ripening, catabolic reactions predominate and the production of volatiles occurs during a short period and is influenced by internal and external factors (Tressl and Albrecht, 1986). In fact, many aroma compounds in fruits and plant materials derive from lipid metabolism. In addition, many species of plants have hydroperoxide lyase enzymes that cleave fatty acids hydroperoxides into two fragments (Vick and Zimmerman, 1976; Galliard and Phillips, 1976). If the substrate is a 13-hydroperoxy fatty acid, then the products are 12-oxo-*cis*-9-dodecenoic acid and either hexanal or *cis*-3-hexenal, depending on whether the hydroperoxide was derived from linoleic or linolenic acid respectively. For 9-hydroperoxy fatty acids the products of hydroperoxide lyase action are nonanoic acid and *cis*-3-nonenal (from linoleic) or *cis*, *cis*-3,6-nonadienal (from

linolenic). Most plants have active isomerase that quickly converts the *cis*-3-enal products into *trans*-2-enals. Both aldehyde isomers have distinctive aromas usually contributing to the odor of the plant tissue. In certain plant tissues alcohol dehydrogenase actively converts the aldehyde products to the corresponding alcohols, which also have characteristic odors. Mushrooms have an usual hydroperoxide lyase that uses the 10 (*S*)-hydroperoxide of linoleic acid as substrate to produce eight- and 10-carbon fragments, 1-octen-3-ol (the characteristic flavor of mushrooms) and 10-oxo-trans-8-decenoic acid (Wurzenberger and Grosch, 1984).

Notwithstanding the important role of lipids in the formation of aroma compounds, the oxidation of unsaturated fatty acids released from lipids during thermal treatment and storage causes significant alteration of flavor due to formation of unpleasant aroma compounds having low sensory perception thresholds, and carboxylic acids with a low number of carbon atoms (Galliard, 1975). For this reason, several studies concerning lipid compounds of citrus juice have been carried out.

## 4. Experimental

### 4.1. Sampling

Citrus fruits used in this study originate from an orchard in the Cap-Bon in the North East of Tunisia. The prospected material derived from trees that were abundantly irrigated regularly twice per month from March until October and supplied with ammonitrate (2 kg), potash (2 kg) and organic manures (5 kg). For every variety of citrus, a number of fruits ($n=30$) have been harvested from three selected trees. These citrus fruits were used for juice extraction which was achieved by means of a citrus fruit press. The total volume of extracted juice has been determined and used for the following analyses.

### 4.2. Acidity

In this experiment, the acidity was determined by the following volumetric method. The juice was neutralized by a NaOH solution (0.1 N) added by some drops of phenolphtaleine as indicator solution. Indeed, under neutral conditions, the NaOH solution turns the juice pink.

The juice acidity is expressed in grams of citric acid by liter of juice. In other words, it is also expressed by the millilitres of NaOH (0.1 N) having neutralized 5 ml of juice. According to the following equation:

$$n_a v_a = n_b v_b$$

where: $n_a$: normality of the acidic solution (juice), $n_b$: normality of the solution of NaOH (0.1 N), $v_a$: volume of the acidic solution (juice), $v_b$: volume of the solution of NaOH (0.1 N), we will have then:

$$\text{Acidity (g/l)} = n_a = n_b v_b\,(\text{ml})\, M_r / v_a\,(\text{ml}) P.$$

with: $M_r$: molecular weight of the citric acid (192 g), $P$: number of $H^+$ protons carried by the citric acid (3).

### 4.3. Total sugars

The percentage of Brix is defined as being the content in sugar expressed in g for 100 g of juice. This parameter has been determined by direct reading on a Brixstix (BSR100) refractometer.

### 4.4. Aroma extraction

Aroma was extracted according to the protocol described by Tonder et al. (1998). In order to survey the juice aroma content of citrus fruits, 30 g of juice were extracted with 30 ml of a mixture of ether–pentane:$C_4H_{10}O$–$C_5H_{12}$ (1:1, v:v). A known quantity of 2-undecanon was added as an internal standard. The mixture was extracted under magnetic stirring for 30 min. After standing for 15 min, the sample was frozen at −20 °C. When the water phase was frozen, the organic phase was recovered in a quickfit pear-shaped flask provided by a Vigreux-column then placed in a water-bath regulated at 36 °C until concentration of the sample to a minimum volume of about 100 μl.

### 4.5. Aroma analysis

The extracts were analyzed by GC–FID on a capillary column (30 m×0.25 μm×250 μm) with a stationary phase made of PEG. Carrier gas was $N_2$, flow rate 1.6 ml $min^{-1}$. The analysis was performed using the following temperature program: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C $min^{-1}$, and isotherm at 225 °C during 10 min. Injector and detector temps were held, respectively, at 250 and 300 °C.

Surfaces of peaks and percentages of the different compouds are determined using a HP Chemstation (Rev. A. 0401) software. This software also permits to fix the analytic parameters (flow, temperature, beginning and end of an analysis...). Fifty-four reference aroma compounds have been analyzed in the same conditions mentioned above. The determination of their retention times permits the identification of the aroma compounds detected in our studied juices.

### 4.6. Lipid extraction

Orange juice (10 ml) was extracted with $CHCl_3$–MeOH (2:1; v:v) under magnetic stirring for 30 min.

The organic phase was poured off and completely evaporated under vacuum. The residue was stored in 0.5 ml of toluol–EtOH (4:1) for further analysis (Marzouk, 1991).

### 4.7. Lipid analysis

The fatty acids gas chromatography analysis requires their transformation into methyl esters. An aliquot (200 μl) of the preserved solution was evaporated, then 2 ml of hexan, a known quantity of methyl heptadecanoate as an internal standard and 0.5 ml of sodium methylate (1%) were added. After stirring during 1 min and standing for 2 min, the mixture was neutralized by 0.2 ml of $H_2SO_4$ (1 N), then the methyl esters were washed with 1.5 ml of distilled water. The superior phase was poured off and evaporated under vacuum (Carreau and Dubacq, 1978). Fatty acids methyl esters were analysed by GC–FID in the same capillary column. The analysis conditions were as follows: carrier gas $N_2$, flow rate 1.5 ml $min^{-1}$, oven temperatures isotherm at 150 °C for 1 min, from 150 to 200 °C at the rate of 15 °C $min^{-1}$, from 200 to 225 °C at the rate of 2 °C $min^{-1}$ and isotherm at 225 °C during 2 min. Injector and detector temperatures were held, respectively, at 250 and 275 °C. The identification of the different fatty acids in the juice is achieved by referring to the standard chromatograms.

### 4.8. Statistical analyses

All analyses were done in triplicate and the statistical comparison of data was performed by ANOVA to reveal significant differences for each parameter among varieties.

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