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Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO

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Abstract

The volatile profiles of seven Tunisian and four French virgin olive oils were established by solid phase micro-extraction (SPME) and gas chromatography (GC), using flame ionisation and mass spectrometer detectors. Eighty-six compounds were identified and characterized, representing 97.4–99.9% of the total GC area. (*E*)-2-hexenal, the main compound extracted by SPME, characterized the olive oil headspace for approximately 90% of the oils examined. Significant differences in the proportions of volatile constituents from oils of different varieties were detected. The results demonstrated that the accumulation of the different metabolites in the oils obtained from the various cultivars was strictly connected with the varietal parameters on the basis of the enzyme differences genetically determined. This feature made possible the differentiation of the examined cultivars on the basis of the percent of each metabolite.

Keywords: Virgin olive oil; Headspace-solid-phase microextraction (HS-SPME); Volatile compounds; (*E*)-2-hexenal; Lipoxygenase pathway

1. Introduction

Olive oil is a very versatile product. Long known to many generations in the Mediterranean world as essential to their health and diet, it is now widely appreciated around the world for its nutritional, health and sensory properties. Olive cultivation is widespread throughout the Mediterranean region and is important for the rural economy, local heritage and the environment. The olive oil sector plays an important role in the Tunisian economy, providing both employment and export revenue. Indeed, with an annual production of 170.000 tonnes, Tunisia is the world's fourth largest producer of olive oil.

Sensory characteristics are used to define virgin olive oil quality. In fact, virgin olive oil is characterized by a unique flavour, which represents one of the most important qualitative aspects of this vegetable oil, and plays a major role in consumer approval. Although a full description of the organoleptic characteristics of the oil is only obtainable through sensory analysis, the quali-quantitative determination of the volatile compounds can provide very useful information on product quality. The study of volatile compounds has been successfully used for the quality control of olive oils, particularly for the detection of adulterants (Lorenzo, Pavón, Laespada, Pinto, & Cordero, 2002a) or rancidity (oxidation) (Jiménez, Beltrán, & Aguilera, 2004; Morales, Rios, & Aparicio, 1997) or to determine their origin (Lorenzo et al., 2002b).

The volatile fraction of virgin olive oil consists of a complex mixture of more than one hundred compounds (Morales et al., 1994; Vichi, Castellote, Pizzale, Conte, Buxaderas

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& López-Tamames, 2003), among which are saturated and unsaturated aldehydes, alcohols, esters, ketones, hydrocarbons and terpenic hydrocarbons.

Usually, these compounds are determined by GC, employing different techniques, such as direct injection, static headspace and dynamic headspace, the last one being the most used, and it has contributed mainly to the knowledge of the aroma composition of virgin olive oil (Morales et al., 1994).

Solid-phase microextraction (SPME) has been introduced as an alternative to dynamic headspace as a preconcentration method prior to GC analysis. This is a faster method, solvents are not required. Developed by Arthur and Pawliszyn (1990) for water analyses and then applied to food analysis, it has been recently used in food flavour analysis.

The aim of this study was to compare different Tunisian and French olive oil samples by the characterization of their volatile compounds. Seven monovarietal olive oils from the North of Tunisia (Nebeur–El Kef) and four French (Nice) were used in this study. The results obtained by SPME were then compared. This is the first study of aroma chemical composition of virgin olive oils from Jarboui, Ain Jarboua, Reguregui, Rekhami, Neb Jmel and Bidh Hmam and the first comparison with French PDO.

2. Materials and methods

2.1. Sampling

Samples, obtained from homogeneous olive fruits (*Olea europaea* L.) of seven Tunisian olive cultivars (Regregui, Rekhami, Jarboui, Ain Jarboua, Neb Jmel, Bidh Hmam and Chétoui) were picked by hand at a known ripening degree during the crop season 2004/2005. Chétoui is a main variety cultivated in the north of Tunisia, while the others are minor varieties. These cultivars were grown in the locality of Nebeur from the region of El Kef in the North of Tunisia in the same region, under the same growth conditions. Two other samples of Chétoui and Jarboui cultivars were collected from the locality of Teboursok. Only healthy fruits, without any kind of infection or physical damage, were processed. The olives were washed and deleafed, crushed with a hammer crusher, and the paste mixed at 25 °C for 30 min, centrifuged without addition of warm water and then transferred into dark glass bottles.

Four PDO virgin olive oils ''Olive de Nice'' were exclusively produced from the ''Cailletier'' variety in a specific geographical area, with fixed processes (harvesting dates, growing practices, storage duration and extraction methods), physical (peroxide and acid contents) and sensorial analysis. Samples were supplied by the ''Syndicat Interprofessionnel de l'olive de Nice'' (SION), from Alpes Maritines, France.

All samples were stored at 4 °C in darkness using amber glass bottles without headspace prior to analysis.

2.2. Oil samples analyses

Free acidity, peroxide value and standard absorbance values at 232 and 270 nm were determined according to the European Communities official methods (EEC, 1991).

2.3. Volatile compounds analyses

2.3.1. SPME analysis

Fibres were obtained from the Supelco Company (Bellefonte, PA). The fiber used for the extraction of the volatile components was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm. Before use, the fibre was conditioned, as recommended by the manufacturer. The olive oil (5 g) was placed in a 20 ml vial closed by PTFE/silicone septum (Interchim). Before extraction, the stabilization of the headspace in the vial was accomplished by equilibration for 60 min at 25 °C. The extraction was carried out at 25 °C (room temperature).

To determine the optimal adsorption time of the fibre with the sample headspace, the fibre DVB/CAR/PDMS was exposed for time periods of 10, 30, 60, 90 and 120 min. A sampling time of 90 min was chosen to perform the analysis (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2003).

After extraction, injections were performed using a SPME autosampler (CTC Analytics, Swiss). The fibre was thermally desorbed into a GC and left in the injection port (equipped with a 0.75 mm i.d. inlet liner) for 4 min. The injector was set at 250 °C and operated in the splitless mode for 2 min unless otherwise stated. Before sampling, the fibre was reconditioned for 5 min in a washing port at 250 °C and blank runs were done periodically during the study.

2.3.2. GC analysis

GC analyses were carried out using an Agilent 6890N Gas Chromatograph equipped with a FID and with fused-silica capillary columns HP-1 (polydimethylsiloxane, 50 m × 0.2 mm i.d., film thickness: 0.33 μm). The carrier gas was helium; constant flow 1 ml/min; oven temperature programmed from 60 °C to 250 °C at 2 °C/min and then held isothermal (20 min). The FID temperature was set at 250 °C.

Retention indices were determined with C5 to C26 alkane standards as references (retention times determined for SPME experiment: 20 s at 50 °C). Relative amounts of individual compounds are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these values and the standard deviation were determined for each component identified.

2.3.3. GC–MS analyses

Each oil was analyzed by GC–MS using an Agilent 6890N/5973N system, with fused-silica capillary columns HP-1 (50 m × 0.20 mm; film thickness: 0.5 μm). Oven conditions were the same as above for GC under the following

operating conditions: carrier gas helium; injector temperature, 250 °C, the ion source and the transfer line temperatures were 170 °C and 280 °C, respectively; splitless mode.

GC–MS analyses on a polar column were performed on a Hewlett-Packard 5890/5970A system with HP20M capillary column (50 m × 0.20 × 0.1 μm). Injector and transfer line temperature: 220 °C; other conditions were the same as above for the apolar column.

Retention indices were determined with C_5–C_{26} alkane standards as references. The mass spectra were performed at 70 eV over a mass range of 35–350 amu.

The identification of the constituents was based on comparison of the retention times with those of authentic samples, on computer matching against commercial (NIST 1998, Wiley 6N, MassFinder 2.1 Library 2001) libraries and our laboratory-made spectral library, built up from pure substances and MS literature data (Jennigs & Shibamoto, 1980; Joulain & König, 1998; Joulain, König, & Hochmuth, 2001) and then, confirmed by comparison of retention indices with published index data (BACIS, ESO 2000, 1999; Davies, 1990).

2.3.4. Statistical analysis

Data were computed with Pirouette™ 3.11 (Infometrix, Inc.), which is easy-to-use multivariate analysis software designed to facilitate the integration of chemometrics in chemical data treatment.

The data matrix involved in this study is of the following form: $C_{ij} = (OO_i, RI_J)$ with OO_i the ith element of the C_{ij} matrix, that is the ith olive oil sample, and RI_j the Jth element of C_{ij}, that is the area measured under the Jth peak with the Jth retention index.

Each oil was analyzed in triplicate and each analysis was used for the treatment. So the matrix was composed of 39 lines (13 olive oils: 9 Tunisian and 4 French) and 98 columns. All peaks of the chromatograms (identified and non-identified compounds) were used. Blank analyses were carried out during the study to remove the pollution peaks (siloxanes) of the matrix.

Before applying chemometric treatments, the data matrix was normalized to 100.

3. Results and discussion

3.1. Free acidity, peroxide value, UV spectrophotometric indices

The free fatty acid content of all analysed samples was below 1 and fell within the accepted value for extra virgin olive oils (EEC, 1991) (Table 1). The quantity of free acids is an important quality factor and has been extensively used as a traditional criterion for classifying olive oil in various commercial grades (Salvador, Aranda, Gomez-Alonso, & Fregapane, 2000). Oil with acidity >2% cannot be consumed directly and must be refined.

Peroxides are formed during oxidation. So, the peroxide value offers a measure of lipid oxidation. In oils studied, peroxide values ranged from 2.63 to 8.25 meq O_2/kg of oil, less than the upper limit of 20 established for the "extra virgin" olive oil (Table 1).

Measurements of absorbance at specific wavelengths in the UV region are used to provide information on the quality of olive oil. Extra virgin olive oil is required to have extinction coefficients at 232 and 270 nm, respectively, of less than 2.50 and 0.25.

The seven Tunisian oil samples studied had K232 and K270 absorbance values below the limit allowed by the EEC Regulations for classification as "extra virgin" olive oil (Table 1).

In all samples analysed, the values of the analytical parameters fell within the ranges established for the highest quality category "extra virgin" olive oil. As shown in Table 1, cultivars had no significant influence on these analytical parameters, which are basically affected (Kiritsakis, Nanos, Polymenoupoulos, Thomai, & Sfakiotakis, 1998; Ranalli & Angerosa, 1996) by factors causing damage to the fruits, e.g., olive fly attacks or improper systems of harvesting, carriage and storage of olives.

Table 1
Description of virgin olive oil samples

Cultivars	Origin	Acidity[a] % C18:1	Peroxide value[a] meq O_2/kg	K232[a,b]	K270[a,b]
Chétoui 1	Nebeur (Tunisia)	0.30 ± 0.05	2.63 ± 1.47	1.83 ± 0.04	0.20 ± 0.01
Neb Jmel	Nebeur (Tunisia)	0.10 ± 0.05	7.90 ± 3.53	1.63 ± 0.04	0.11 ± 0.01
Regregui	Nebeur (Tunisia)	0.25 ± 0.05	3.65 ± 0.57	1.81 ± 0.18	0.13 ± 0.01
Rekhami	Nebeur (Tunisia)	0.20 ± 0.05	4.96 ± 1.71	1.40 ± 0.01	0.10 ± 0.01
Ain Jarboua	Nebeur (Tunisia)	0.25 ± 0.05	4.99 ± 1.01	1.74 ± 0.04	0.10 ± 0.01
Jarboui 1	Nebeur (Tunisia)	0.15 ± 0.05	6.89 ± 2.59	2.11 ± 0.06	0.11 ± 0.01
Bidh Hmam	Nebeur (Tunisia)	0.25 ± 0.05	8.25 ± 3.01	1.68 ± 0.08	0.11 ± 0.01
Chétoui 2	Teboursok (Tunisia)	0.30 ± 0.05	3.65 ± 1.58	1.70 ± 0.03	0.11 ± 0.01
Jarboui 2	Teboursok (Tunisia)	0.60 ± 0.05	9.15 ± 2.46	2.13 ± 0.15	0.20 ± 0.01
PDO1	Nice (French)	–	–	–	–
PDO2	Nice (French)	–	–	–	–
PDO3	Nice (French)	–	–	–	–
PDO4	Nice (French)	–	–	–	–

[a] Data are means of three independent samples.
[b] For extra virgin olive oil K232 ⩽ 2.5 and K270 ⩽ 0.25.

3.2. Volatile compounds analyses

The flavour of extra virgin olive oil represents one of the most important qualitative aspects of this vegetable oil. Although a full description of the organoleptic characteristics of the oil is only obtainable through sensory analysis, the quali-quantitative determination of the volatile compounds can provide very useful information on product quality.

SPME has widespread application in analysis of volatiles (Kataoka, Lord, & Pawliszyn, 2000; Steenson, Lee, & Min, 2002) but has limited application to olive oils (Servili, Baldioli, Beglimini, Selvaggini, & Montedoro, 2000b, 2000a). In the analysis of volatiles of olive oil, dynamic headspace remains the preferred procedure (Ranalli, Contento, Schiavone, & Simone, 2001) but this work demonstrates the suitability of SPME in this role. Moreover, SPME offers the advantages of solventless recovery of volatiles, ease of operation and particularly reduces sample preparation time. Of the various SPME fibres, the performance of DVB/CAR/PDMS fibre in studying volatile compounds from olive oils has been demonstrated (Cavalli et al., 2003; Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2004) and was chosen for this study. But, the use of HS-SPME–GC, followed by chemometrics to study virgin olive oil quality, suffers some drawbacks. These difficulties mainly originated from the extraction method employed. Indeed, the state of fibre has to be frequently checked by carrying out blanks and visual fibre examination. But, in this study, the use of a SPME autosampler makes the automation of the procedure easier and reduces the risk of fibre damage during the sample preparation. In addition, the main drawback encountered in the SPME extraction derives from the heterogeneity of fibre lots. In this study, we used the same fibre to assure the good repeatability needed for chemometrics.

The analysis of volatile fractions from the seven Tunisian monovarietal olive oils by headspace solid-phase microextraction, separation and identification of their components by GC–RI and GC–MS, showed that the virgin olive oil aroma consisted of a complex mixture of more than seventy five compounds, representing 97.8–99.9% of the total GC area (Table 2). Furthermore, in the headspace of all oil samples, the isolated and identified compounds are mainly aldehydes with 31.6–72.9% of the total peak area percentage, alcohols (3.5–30.0%), esters (1.3–5.3%), as well as monoterpenes (0.2–1.6%) and sesquiterpenes (0.1–1.4%) and seven isomeric unsaturated hydrocarbons, known as pentene dimers (0.9–7.9%).

In this study, the products of the lipoxygenase pathway (LOX) were generally the major components of the volatile fraction and the sum of the areas of their peaks ranged between 72.7% and 88.3% of the total area (Table 2).

In all oil samples analysed, the major components of the volatile fraction of virgin olive oils, the main cause of the green odour note, were the C_6 compounds, which derive from the cascade of enzymatic reactions starting with the formation, by lipoxygenase action, of 13-hydroperoxides from linoleic and linolenic acid (Angerosa, D'alessandro, Basti, & Vito, 1998; Williams, Morales, Aparicio, & Harwood, 1998).

The different accumulations of metabolites from the lipoxygenase cascade have been reported by other authors (Angerosa, Basti, & Vito, 1999; Aparicio & Morales, 1998; Montedoro, Bertuccioli, & Anichini, 1978) to be highly dependent on the levels of enzymes involved, the extraction conditions, the storage time of olives, and the degree of ripening (Angerosa et al., 1998; Aparicio & Morales, 1998; Montedoro et al., 1978; Salas & Sanchez, 1999). In addition, climatic and environmental growth conditions may also influence the production of volatiles (Aparicio and Morales, 1998; Montedoro et al., 1978; Servili, Baldioli, Beglimini, 2000; Servili et al., 2000; Vichi et al., 2003). As the harvesting period, extraction conditions and geographical origin were similar for the samples studied, principal component analysis of bioformation volatiles was performed to evaluate the influence of genetic factors.

The chemical compositions of all Tunisian olive oil headspaces showed that C_6 aldehydes (hexanal, (*E*)-2-hexenal, (*Z*)-2-hexanal and (*Z*)-3-hexenal) were the most abundant compounds. The most notable feature for the distinction from European oils was (*E*)-hex-2-enal which was the dominant volatile in the profile of all published oils (Ranalli et al., 2001). For the Tunisian monovarietal olive oils involved in this study, (*E*)-2-hexenal was the major volatile in less than 70% of the tested oils (Table 2). It is the principal compound extracted by HS-SPME in Jarboui 1, Ain Jarboua, Rekhami, Regregui and Bidh Hmam olive oils (24.7–65.5%). The volatile fraction of Chétoui 1 oil was characterized by the dominance of (*Z*)-3-hexenal (22.2%). The major constituent of the volatile fraction obtained from Neb Jmel oil was identified as (*Z*)-2-hexenal (26.3%). Other C_6 compounds were identified in the headspace of the Tunisian olive oils analysed. Among them we identified hexanal (3.1–11.3%), (*Z*)-3-hexenol (2.4–6.6%), (*E*)-2-hexenol (0.8–13.1%), and hexanol (0.8–5.4%).

Hexyl and (*Z*)-3-hexenyl acetates are present in aroma of all Tunisian olive oil samples, but they are minor components when compared with aldehydes or alcohols. These esters are synthesized by alcohol acyltransferase within the LOX pathway. Moreover, a low level of esters in the Jarboui 1, Ain Jarboua, Rekhami, Regregui and Bidh Hmam varieties also indicates a lower content of alcohol acetyl transferase in the olive oils compared with Chétoui 1 and Neb Jmel varieties (Angerosa et al., 1999).

In addition to C_6 compounds, the aroma of Tunisian monovarietal virgin olive oils contains reasonable amounts of various classes of C_5volatile compounds (Table 2). The detection of these compounds (pentene dimers, pentenols and C_5 carbonyl compounds) indicates the existence of an additional branch of the LOX pathway which leads to the production of C_5 compounds through the alkoxy radical. This additional branch is active during olive oil aroma

Table 2
Compounds identified by HS-SPME and GC–MS

Components[a]	RI[b]		Varieties									Nice PDO			
	HP-1	HP20M	Chétoui 1	Jarboui 1	Regregui	Rekhami	Ain Jarboua	Neb Jmel	Bidh Hmam	Chétoui 2	Jarboui 2	1	2	3	4
Ethanol	577	871	0.9[c] ± 0.1[d]	0.8	0.5 ± 0.1	0.6 ± 0.1	2.1 ± 1.4	1 ± 0.1	2.0 ± 0.1	0.4	33.6 ± 1.9	2.1 ± 0.3	2.2 ± 0.2	2.8 ± 0.1	1.9 ± 0.1
Propan-2-one	580	–	–	–	–	–	–	–	–	0. 3 ± 0.1	–	–	–	–	–
Pentene	583	550	1.5 ± 0.1	0.3	–	0.4	0.5	0.4 ± 0.4	0.9	0.1	–	0.9	0.1	tr[e]	0.2 ± 0.2
Acetic acid	591	1400	3.4 ± 1.1	–	0.4 ± 0.1	–	–	–	0.6 ± 0.2	0.4 ± 0.1	3.6 ± 0.2	0.7 ± 0.1	0.4 ± 0.2	1.2 ± 0.1	0.5 ± 0.4
2-Methylpentane	593	–	0.3 ± 0.4	–	1.5 ± 0.1	–	–	–	–	0.1	2.3 ± 0.2	–	–	–	–
3-Methylpentane	596	–	1.1	–	0.6	–	–	–	–	–	2.5 ± 0.2	–	–	–	–
n-Hexane	594	595	–	–	–	0.2	0.4	–	1 ± 0.1	–	3.3 ± 2.8	–	–	–	–
Butan-2-ol	597	–	–	tr	–	–	–	–	0.3	–	–	–	–	–	–
3-Methyl butanal	628	–	–	0.4	–	–	0.3	0.2	–	0.2	–	tr	0.1	–	–
2-Methyl butanal	638	863	–	0.6	0.3	–	0.6	0.4	–	0.2	–	tr	0.1	0.2	–
Pentanal	645	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
Pentan-2-one	656	–	2.6 ± 0.1	–	–	–	–	–	–	–	2.4 ± 0.3	–	–	–	–
Pent-1-en-3-one	656	–	3.8 ± 0.2	–	1.6 ± 0.3	–	–	–	1 ± 0.2	–	–	2.4 ± 0.4	1.4 ± 0.5	1	0.2 ± 0.3
Pent-1-en-3-ol	658	1114	–	1.4	–	0.9	2.1 ± 0.1	2.7 ± 0.2	–	2.5 ± 2.2	–	–	–	–	–
Unknown	669	–	–	–	–	–	–	–	–	–	–	0.6 ± 0.1	0.9 ± 0.1	1.7 ± 0.1	–
Pentan-3-one	670	–	–	1.1 ± 0.1	0.4	0.5	3 ± 0.1	2.6 ± 0.2	0.3	1.2 ± 0.1	tr	–	–	–	0.7 ± 0.1
Heptane	700	–	–	–	tr	–	–	0.1	tr	–	0.1	–	–	0.1	tr
Pent-2-enal[f]	715	–	0.3	0.1 ± 0.1	tr	tr	0.4	0.4	tr	0.4	–	0.3 ± 0.1	–	–	–
3-Methyl butanol	714	720	–	–	–	–	–	–	–	–	0.2 ± 0.1	–	0.5 ± 0.1	0.5	0.1
2-Methyl butanol	717	–	–	tr	–	–	0.3	–	–	–	0.1	–	0.4	0.3	–
(*E*)-Pent-2-enal	723	–	0.5	0.4	0.1	0.1	0.3 ± 0.1	0.8	0.2	0.6	–	0.2	0.2	–	0.1
Pentan-1-ol	743	–	–	–	–	–	0.5	–	–	–	0.2	0.8 ± 0.1	–	–	–
(*Z*)-Pent-2-enol	745	1279	1.9 ± 0.1	1.6 ± 0.1	0.5 ± 0.1	0.7	2.2	2.8 ± 0.1	0.4	3.2 ± 0.1	1.0	0.4	0.5	0.7	0.8
Toluene	750	976	4.2± 0.1	1.8 ± 0.1	4.2 ± 0.2	2.1 ± 0.1	6 ± 0.1	3.3 ± 0.2	1.9 ± 0.1	1.8	8.8 ± 0.3	0.5	0.9 ± 0.1	1.5 ± 0.1	0.3 ± 0.1
(*Z*)-Hex-3-enal	769	1105	22.2 ± 2.1	–	2.4 ± 0.2	0.6 ± 0.1	2.3 ± 0.2	11± 1.7	0.7 ± 0.1	25.4 ± 2.5	0.6	3.6 ± 0.5	–	–	–
Hexanal	772	1035	7.4 ± 0.4	11.4 ± 4.0	8± 0.2	3.2 ± 0.1	3.6 ± 0.2	6.3 ± 0.6	5.8 ± 0.2	4.5 ± 0.3	4.1 ± 0.2	–	6.4± 0.6	5.5 ± 0.4	3.8 ± 0.5
Oct-1-ene	785	–	–	–	–	–	–	–	–	–	–	–	0.1	0.2	–
Octane	800	769	1.6	0.5	0.6	0.1	0.9 ± 3.1	3.1 ± 0.1	0.9	0.5	1.7 ± 0.1	0.2	0.8 ± 0.1	1.1 ± 0.1	0.3
Oct-2-ene	810	–	–	tr	–	–	–	0.1	tr	–	–	–	–	0.1	–
(*Z*)-Hex-2-enal	817	–	2.1± 0.2	0.9	0.8 ± 0.1	0.4	0.4	26.3 ± 0.4	0.5	2.1 ± 0.1	–	–	–	–	–
(*E*)-Hex-2-enal	824	1180	14.3 ± 0.5	56.8 ± 2.3	60.5± 0.3	64.9 ± 1.1	24.7 ± 0.3	0.8	65.5 ± 1.8	20.0 ± 1.2	7. 9 ± 0.1	78.3 ± 0.6	70.5 ± 0.5	8.3± 0.2	61.6 ± 4.2
(*Z*)-Hex-3-enol	834	1343	2.5 ± 0.2	2.6 ± 0.1	3.9 ± 0.2	2.4 ± 0.1	6.6 ± 2.5	5.3 ± 0.2	–	1.4 ± 0.5	0.9 ± 0.1	–	–	0.7 ± 0.1	–
(*E*)-Hex-2-enol	844	1365	–	2.5 ± 0.1	0.8 ± 0.2	5.1 ± 0.2	13.1	1	2.3 ± 0.1	tr	5.6 ± 0.1	1.7 ± 0.3	3.4 ± 0.3	52.0 ± 0.8	15.8 ± 4.1
Hexanol	847	1316	0.9	3.1 ± 0.2	1.4 ± 0.2	2.1 ± 0.1	5.5 ± 0.3	2± 0.1	0.9 ± 0.3	0.1 ± 0.1	5.6 ± 0.3	1.1 ± 0.3	2.9 ± 0.3	11.4 ± 0.1	7.2 ± 1.5
p-Xylene	852	1104	0.2	–	0.1	–	–	tr	tr	0.2	–	0.4 ± 0.1	1.2 ± 0.1	2.4	0.7 ± 0.1
o-Xylene	875	880	–	–	–	–	–	–	–	–	–	0.7 ± 0.3	0.7 ± 0.1	0.6	0.4
Heptane-2-one	863	–	tr	tr	0.1	tr	0.2 ± 1.1	0.1	tr	0.1	0.2	–	–	–	–
Heptanal	875	–	–	tr	0.2	–	tr	–	0.1	–	0.1 ± 0.1	–	–	–	–
(*E*,*E*)-Hexadiene-2,4-al[f]	878	1353	0.9 ± 0.2	0.1	0.1	0.1 ± 0.1	–	0.9 ± 0.2	0.2 ± 0.1	1.3 ± 0.2	–	–	–	–	–
3,4-Diethylhexa-1,5-diene[f]	892	–	1.0 ± 0.1	0.5	0.3	0.4 ± 0.1	0.9 ± 0.1	1	0.3	1.1	0.2	0.1	tr	–	0.1
3,4-Diethylhexa-1,5-diene[f]	896	–	0.7	0.3	0.2	0.3 ± 0.1	0.6	0.7	0.2	0.9	0.2 ± 0.1	–	tr	–	–
Nonane	898	–	–	tr	–	–	0.1	–	–	–	tr	tr	tr	0.2	tr
Methyl hexanoate	905	–	–	tr	tr	tr	0.1	tr	tr	–	tr	–	–	–	–
Unknown	908	–	0.7 ± 0.1	–	0.1	–	–	–	tr	1.1 ± 0.1	–	–	–	–	–
(*E*)-Hept-2-enal	926	–	0.5	0.4	0.2	0.1	0.2	tr	0.9 ± 0.1	6.1 ± 0.5	0.1	0.2	0.5 ± 0.1	0.6	0.2
α-Pinene	928	978	–	–	–	–	–	0.5 ± 0.1	–	6.0 ± 0.6	0.8	0.1	0.2	0.2	0.1
3-Ethylocta,1-5-diene[f]	931	1047	4.0± 0.1	2.0 ± 0.2	1.4	2.5 ± 0.3	4.1 ± 0.2	4.2 ± 0.2	1.4± 0.1	tr	0.7	0.7 ± 0.1	0.4	0.3	0.6 ± 0.1
3-Ethylocta,1-5-diene[f]	938	1054	3.9 ± 0.1	2.3 ± 0.2	1.4± 0.1	2.1 ± 0.2	4.0± 0.3	4.7 ± 0.3	1.7 ± 0.1	0.1	tr	0.5 ± 0.1	0.3	0.3	0.5
Heptanol	952	–	–	–	–	–	–	–	–	–	tr	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

Components[a]	RI[b]		Varieties									Nice PDO			
	HP-1	HP20M	Chétoui 1	Jarboui 1	Regregui	Rekhami	Ain Jarboua	Neb Jmel	Bidh Hmam	Chétoui 2	Jarboui 2	1	2	3	4
Hexanoic acid	957	1798	–	0.1	–	–	tr	–	0.1	0.1	tr	–	0.1	0.2 ± 0.1	tr
Octan-3-one	961	–	–	tr	–	–	–	–	tr	–	0.2	tr	0.1	0.3	0.1
(*E*,*E*)-Hepta-2,4-dienal[f]	963	–	–	–	–	–	tr	–	0.2	–	–	–	tr	–	–
Octane-2-one	964	1249	–	tr	0.1	0.2	0.2	0.2	0.1	0.2	0.1	–	–	tr	–
β-Myrcene[f]	978	–	–	–	–	–	–	–	–	–	–	tr	–	–	–
Octanal	976	–	–	tr	0.3	–	–	–	tr	–	0.2 ± 0.1	–	–	–	–
Unknown	980	–	–	–	–	–	0.9 ± 0.1	–	–	–	–	–	–	–	–
(*E*)-Hex-3-enyl acetate	983	1285	4.0 ± 0.2	0.7 ± 0.1	1.0. ± 0.1	0.9± 0.1	2.0 ± 0.2	3.8 ± 0.3	0.5	0.9 ± 0.1	0.2	0.2	0.3	0.1	tr
Deca-3,7-diene[f]	986	–	1.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.6 ± 0.1	3.7 ± 0.3	2.0± 0.1	0.8 ± 0.1	3.2 ± 0.5	–	0.4 ± 0.1	0.3	0.4 ± 0.01	1.0 ± 0.2
Deca-3,7-diene[f]	988	–	3.3± 0.1	1.9 ± 0.2	1.3 ± 0.1	2.6 ± 0.2	2.3 ± 0.2	3.8 ± 0.3	1.6	6.0 ± 0.9	0.5	0.6 ± 0.1	0.3 ± 0.1	1 ± 0.01	0.5 ± 0.1
Deca-3,7-diene[f]	988	–	2.2 ± 0.1	1.3 ± 0.4	0.9 ± 0.1	1.4 ± 0.1	0.3 ± 0.2	2.8 ± 0.2	1.4 ± 0.1	3.8 ± 0.5	0.7	–	0.2	–	–
Hexyl acetate	991	1241	1.3 ± 0.1	1.4 ± 0.3	0.4	0.8 ± 0.1	tr	1.2 ± 0.1	0.8 ± 0.1	–	0.4	–	0.4 ± 0.1	–	0.2 ± 0.2
Unknown	991	–	–	–	–	–	–	–	–	–	–	0.7 ± 0.1	–	–	–
Decane	997	–	tr	tr	0.1	tr	0.5 ± 0.1	0.1	tr	0.1	0.2	tr	tr	0.1	tr
Unknown	1013	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–
Limonene	1016	1165	0.9 ± 0.2	tr	tr	–	0.5 ± 0.5	0.2	0.5 ± 0.4	1.1 ± 0.6	2.9 ± 2.5	0.8 ± 0.9	0.5 ± 0.8	tr	0.8 ± 0.9
Unknown	1020	–	–	–	–	–	–	–	–	–	–	–	–	0.7 ± 0.9	–
(*E*)-Oct-2-enal	1025	1247	–	–	–	–	–	–	0.1	–	–	–	–	0.1	–
(*Z*)-β-Ocimene	1032	–	–	tr	–	–	0.1	0.3	tr	0.3 ± 0.1	–	–	–	–	tr
(*E*)-β-Ocimene	1034	1223	0.6	0.1	0.2	0.4	tr	tr	0.6	–	1.3 ± 0.1	0.2	0.2	0.3	0.1
γ-Terpinene	1042	1214	–	–	–	–	–	–	–	–	–	tr	0.1 ± 0.1	0.1 ± 0.1	tr
Octan-1-ol	1046	–	–	tr	–	–	–	–	tr	–	–	–	–	–	–
Heptanoic acid	1049	–	–	tr	–	–	tr	0.2 ± 0.2	tr	tr	0.3 ± 0.3	–	–	–	–
Nonanal	1074	1358	0.4	0.5	0.6 ± 0.1	0.2	0.3	0.5	0.5 ± 0.1	0.5	0.9 ± 0.2	0.3	0.8 ± 0.1	1.5 ± 0.1	0.5 ± 0.1
Undecane	1094	–	tr	tr	–	–	tr	tr	tr	0.1	0.1 ± 0.1	tr	tr	tr	–
4,8-dimethyl nonatriene	1100	–	tr	0.3	–	–	0.2	0.2	0.7 ± 0.1	–	2.5 ± 0.1	–	0.1	0.1	–
Allocimene[f]	1111	–	tr	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-Allocimene	1122	–	tr	–	–	–	–	tr	tr	tr	tr	–	–	–	–
Octanoic acid	1148	–	0.2 ± 0.1	tr	–	–	tr	tr	tr	0.1 ± 0.1	0.2 ± 0.3	–	–	–	–
Methyl salicylate	1159	1726	tr	–	–	–	tr	tr	–	–	–	–	–	–	–
Benzoic acid	1168	–	0.5	0.2 ± 0.1	0.1 ± 0.1	–	–	0.5 ± 0.1	0.2	0.4	0.4	0.4 ± 0.1	0.5 ± 0.1	0.5	0.4 ± 0.1
Dodecane	1196	–	tr	tr	–	tr	tr	tr	0.1	0.1	0.1	tr	–	tr	–
Octyl acetate	1200	–	–	–	–	–	–	tr	–	–	–	–	–	–	–
Unknown	1200	–	0.2	0.1	0.2	0.2	1.7 ± 0.2	–	0.1	–	0.5	–	0.3	–	–
(*E*)-Dec-2-enal	1235	1600	–	–	–	–	–	0.2	0.2	–	–	–	–	–	–
Nonanoic acid	1253	–	0.2 ± 0.1	tr	–	tr	tr	0.1 ± 0.1	tr	0.3 ± 0.5	0.2 ± 0.4	–	–	–	–
Tridecane	1297	–	tr	tr	–	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr
Sesquiterpene	1361	–	–	tr	–	–	0.2	tr	–	0.1	–	–	–	–	–
β-Patchoulene	1362	1868	–	–	–	0.2	–	–	–			–	–	–	–
α-Copaene[f]	1370	1462	tr	tr	tr	0.9	0.4	0.1	–	0.2	0.2	0.2	0.2	0.2	tr
Tetradecane	1394	–	tr	tr	tr	0.1	tr	tr	tr	0.1	0.3	tr	tr	tr	–
Dodecanol	1428	–	–	–	–	–	–	–	–	tr	–	–	–	–	–
α-Muurolene[f]	1492	1691	–	–	0.2 ± 0.3	0.3	0.3	–	–	–	–	–	–	–	tr
α-Farnesene	1495	1726	0.4	–	0.4 ± 0.3	tr	tr	0.3	0.6 ± 0.5	–	0.5	tr	0.1	–	–
E-α-Bergamotene	1495	–	–	–	–	–	–	–	–	–	–	tr	–	0.2	tr
n-Pentadecane	1499	–	–	–	–	–	–	tr	–	–	–	–	tr	tr	–
γ-Bisabolene[f]	1582	1898	–	–	tr	–	–	tr	tr	–	–	–	–	–	–
Identified compounds			48(98.9%)	56(99.9%)	47(99.7%)	43(99.9%)	56(97.4%)	59(99.6%)	60(99.9%)	52(98.6%)	56(99.5%)	42(98.7%)	47(98.8%)	46(97.4%)	40(100%)
Non identified compounds			2(0.9%)	1(0.1%)	2(0.3%)	1(0.1%)	2(2.6%)	0	1(0.1 %)	2(1.4%)	1(0.5%)	2(1.3%)	2(1.2%)	2(2.4%)	0

Alcohols (%)	6.2	12.2	7.1	11.8	32.4	14.7	5.9	7.7	47.3	83.1	78.7	16.2	66.2
Aldehydes (%)	48.7	71.5	73.6	69.7	33.1	47.7	75.2	61.3	13.9	5.9	9.8	68.4	25.7
Alkane (%)	3.1	0.7	2.9	0.6	2.1	3.5	2.2	1.0	10.8	0.6	1.1	1.9	0.5
Alkenes (%)	18.5	9.6	6.4	11.3	16.7	19.9	9.0	15.2	4.8	2.5	2.0	2.4	2.9
Terpenes (%)	2.1	0.3	0.8	1.8	1.6	1.4	1.8	1.7	5.7	1.4	1.3	1.0	1.2
Esters (%)	5.4	2.2	1.9	1.7	2.2	5.2	1.3	0.9	0.6	0.2	0.8	0.1	1.0
Ketones (%)	6.5	1.2	2.2	0.7	3.3	3.0	1.5	1.7	2.9	2.4	1.5	1.0	1.0
Acids (%)	4.3	0.4	0.5	0.1	0.1	0.8	1.0	1.3	4.8	1.1	1.0	2.0	0.9

[a] Order of elution and percentages of components are given on apolar column (HP-1).
[b] Retention indices as determined on HP-1 column and on HP20M column using the homologous series of *n*-alkanes.
[c] Peak area % (percent normalized areas) determined by HS-SPME–GC/FID analysis.
[d] Standard deviation.
[e] Trace (<0.1%).
[f] Correct isomer not characterized.

biogeneration (Angerosa, Mostallino, Basti, & Vito, 2000). Pentan-1-ol was also characteristic of the Ain Jarboua sample (0.5%).

Work by Angerosa et al. (2004) has also demonstrated the relevance of C_5 compounds in the aroma of olive oil, and showed that these compounds, especially pent-1-en-3-one, strongly affect most of the taste and odour attributes. Moreover, a low amount of C_5 ketones, and pentene dimers also affects the aroma. So, in the samples studied, the main amount of this compound is present in the headspace of Chétoui 1 sample (3.8%), and it is absent in the headspace of Jarboui 1, Ain Jarboua, Rekhami, and Neb Jmel. The results show that the percentage of C_5 compounds differed according to the cultivar.

The hydrocarbons of olive oils have been studied by different authors as possible markers to distinguish virgin olive oil from different olive varieties or different geographical origins (Aparicio & Luna, 2002; Ben Temime, Campeol, Luigi Cioni, Daoud, & Zarrouk, in press; Bortolomeazzi, Berno, Pizzale, & Conte, 2001; Guinda, Lanzon, & Albi, 1996). In the oils analysed, great differences were found, but mainly in the contents of terpenic hydrocarbons (mono- and sesquiterpenes) and the sum of their areas accounted for 0.3–2.1% of the total peak area percentage. This component could play a very important role in the fragrance of this precious food (Vichi et al., 2003; Zunin et al., 2004). Also, the levels of the alkanes from C_9 to C_{15} found in the samples did not allow their differentiation, in spite of their absence in some samples.

Quite low amounts of volatile aromatic hydrocarbons, such as toluene and xylene isomers, were found in the headspaces of olive oils examined in the present study (Table 2). The origin of these compounds in virgin olive oil is largely unknown. Some studies on the presence of these aromatic hydrocarbons in virgin olive oil have been carried out by other authors, revealing that they might arise from both exogenous contamination and endogenous pathways (Biedermann, Grob, & Morchio, 1995; Morchio, Spadone, & Braco, 1994).

Other minor volatile compounds were observed in some of the virgin olive oils studied. Among them, the hydrocarbons octane and octene and the aldehydes heptanal, octanal, nonanal, (*E*)-2-heptenal and 2,4-heptadienal isomers are due to autoxidation reactions (Morales et al., 1997) that inevitably start after the virgin olive oil has been extracted. However, in the extra virgin olive oils analysed, the amounts of compounds formed from oxidation reactions were quite low (Table 2). Some products deriving from sugar fermentation and amino acid transformation were also found. They were ethanol and acetic acid (Angerosa, 2002) and branched aldehydes, alcohols and acids. The latter are thought to be produced by moulds during olive fruit storage (Angerosa, 2002).

As the harvesting period, environmental and extraction conditions were similar for the seven studied samples, the results indicate, the strict dependence of olive oil aroma on genetic factors.

The volatile fraction of Chétoui oil obtained from Teboursok was characterized by the dominance of two compounds, (*Z*)-3-hexenal (25.4%) and (*E*)-2-hexenal (20.0%), and a higher level of α-pinene (6.0%) than the other samples analysed in this study. The major constituent of the volatile fraction obtained from Jarboui 2 oil was identified as ethanol (33.6%). This high level of ethanol can be explained by the quality of the olive fruits, and is mainly due to fermentation before olive oil extraction.

The chemical compositions of the three French olive oils (Samples PDO1, PDO2, and PDO4) were characterised by the dominance of (*E*)-2-hexenal (61.6–78.4%) (Table 2). Other C_6 aldehydes, such as (*Z*)-3-hexenal, which was present only in the headspace of the PDO1 sample (3.6%), and hexanal was absent. The amounts of hexanal in the PDO2 and PDO4 samples were, respectively, 6.4% and 3.8%. In addition, the main C_6 alcohols detected were hexanol with 1.1–7.2% of the total area percentage, and (*E*)-2-hexenol (1.7–15.8%).

Finally, the analysis of sample PDO3 showed the dominance of (*E*)-2-hexenol (52.0%). Other compounds present in a relatively high concentration were (*E*)-2-hexenal (8.3%), hexanal (5.5%) and hexanol (11.4%). The contents of esters in the four French olive oils examined in the present study were very low compared to the Tunisian olive oils (Table 2).

These results indicated that strict dependence of olive oil aroma on the enzymatic store, which is genetically determined (Angerosa, 2002). Furthermore, it is important to note that Tunisian olive oils had volatile compositions which were similar to French ones. This feature indicates that the LOX pathway had the same importance and is the predominant pathway of volatiles biogeneration in Tunisian and French virgin olive oils.

It should be remembered that the volatile compounds present at higher concentrations are not always the main contributors to oil aroma (Angerosa et al., 2004). Each one of the aroma compounds of virgin olive oil is related to one or more sensory attributes.

3.3. Chemometrics

Principal component analysis is used in exploratory analysis. It gives graphical representations of inter-sample and inter-variable relationships and provides a way to reduce the complexity of the data.

The application of the PCA algorithm to data showed three distinctive groups (Fig. 1). The first group is composed of Bidh Hman, Rekhami, Jarboui 1, Regregui varieties and French PDO olive oils. The second group is characterised by the Tunisian varieties, Ain Jarboua, Chétoui 1 and 2 and Neb Jmel. Moreover, we observed the presence of a third group composed of a single sample (Jarboui 2) located on the top centre of the scores-plot. The first group is located on the left-bottom side of the score-plot whereas the second group is located in the symmetrical position. This result implies a great difference in terms of volatile compounds characterizing the headspaces of these two groups.

The loadings plot (Fig. 2) gives some explanations for understanding this classification. Indeed, the first group, composed of four Tunisian monovarietal oils and all the French oils, is correlated with a molecule with a 824 retention index, identified as (*E*)-hex-2-enal by GC–MS and the second (three Tunisian varieties) is correlated with the

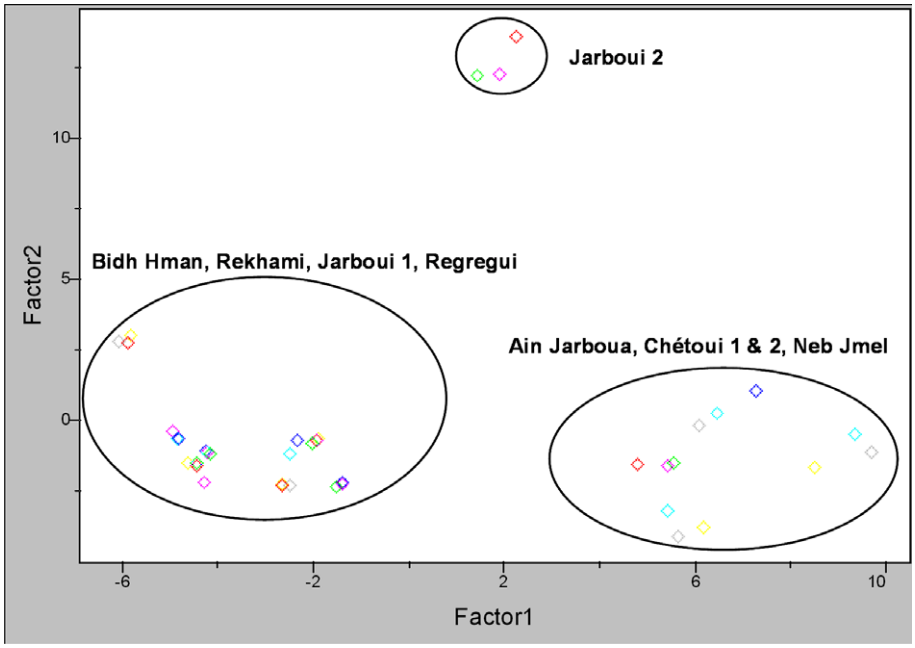

Fig. 1. Scores plots of PCA of Tunisian monovarietal and PDO Nice virgin oils.

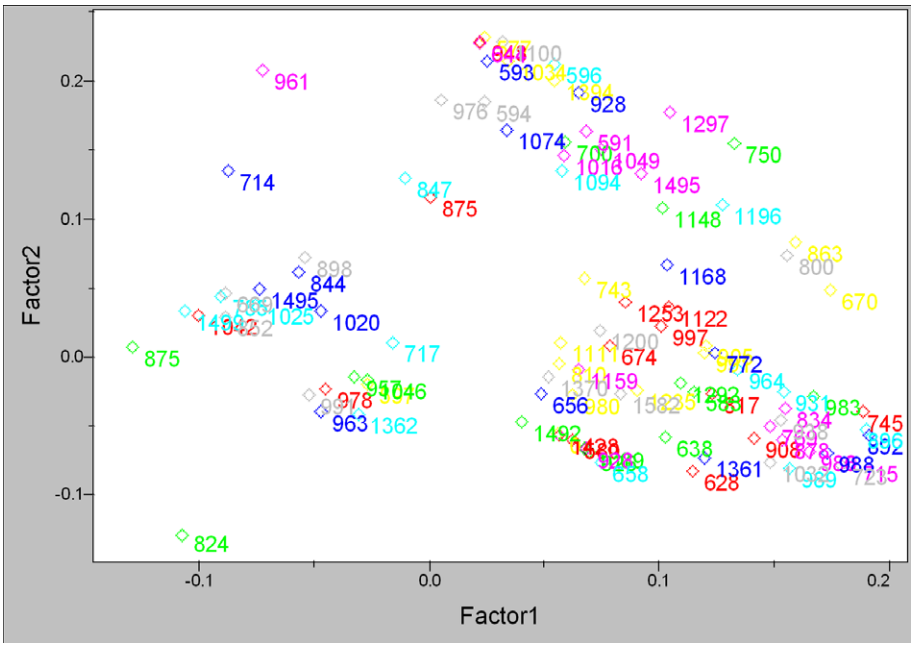

Fig. 2. Loadings plots of PCA of Tunisian monovarietal and PDO Nice virgin oils.

molecules with retention indices of 745, 892 and 896 identified as (*Z*)-pent-2-enol and the two isomers of 3,4-diethylhexa-1,5-diene, respectively. These different conclusions are in agreement with results presented in Table 2. The last group, composed of one sample (Jarboui 2), is correlated with five compounds (RI = 577, 844, 847, 593 and 1110). This classification was not surprising because this sample is characterized by a high content of ethanol (RI = 577, 33.6 ± 1.9%).

This principal component analysis of volatile compounds showed the aroma composition similarity of four Tunisian monovarietal virgin olive oils (Bidh Hman, Rekhami, Jarboui 1 and Regregui varieties) and French Nice PDO. Other Tunisian oils are very different from French PDO oils by a more important concentration of (*Z*)-pent-2-enol and isomers of 3,4-diethylhexa-1,5-diene.

Virgin olive oils from the same variety (Jarboui) can present a very different headspace composition, which illustrates the complexity of virgin olive oil aroma studies.

4. Conclusion

The application of SPME to the analysis of virgin olive oil headspace allowed the detection of significant differences in the proportions of volatile constituents from oils of different varieties. (*E*)-2-hexenal was the principal compound characterising the olive oil headspace for eight samples (Jarboui 1, Ain Jarboua, Rekhami, Regregui and Bidh Hmam, PDO1, PDO2, PDO4). The five other samples Chétoui 1, Chétoui 2, Neb Jmel, Jarboui 2 and PDO3 were characterized by (*Z*)-3-hexenal, (*Z*)-2-hexenal, ethanol, and (*E*)-2-hexenol, respectively. The results indicate that genetic factors and geographic region influence the volatile production. However, the study of a larger number of samples from various years of production would lend support to the results obtained by this first screening.

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