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Volatile and Non-Volatile Compounds of Single Cultivar Virgin Olive Oils Produced in Italy and Tunisia with Regard to Different Extraction Systems and Storage Conditions

Cinzia Benincasa, Kaouther Ben Hassine,
Naziha Grati Kammoun and Enzo Perri
[1]*CRA-OLI Olive Growing and Olive Oil Industry Research Center Rende,*
[2]*Institut de l'Olivier, Sfax*
[1]*Italy*
[2]*Tunisia*

1. Introduction

Virgin olive oil has a fundamental role in the markets of alimentary oils because of its unique aroma, its stability and its healthy benefits. In this chapter the attention will be focused on Tunisian and Italian single cultivar olive oils.

The oils under investigation were produced by different extraction systems and characterised for their volatile and non-volatile compounds (Benincasa et al., 2003; Cerretani et al., 2005; Garcia et al., 1996). It is well known that volatile and non-volatile components of products of plant origin are dependent on genetic, agronomic and environmental factors. There are few reports (Angerosa et al., 1996, 1998a, 1998b, 1999; Morales et al., 1995; Solinas et al., 1998) on the evaluation of the relationships between the aroma components of virgin olive oil with the metabolic pathways and varietal factors. Olive ripening process and, to some extent, the fruit growing environment, affect also the composition of the volatile compounds of the oil (Aparicio & Morales, 1998; De Nino et al., 2000; Guth & Grosh, 1993; Montedoro & Garofalo, 1984; Morales et al., 1996). Volatile and non-volatile compounds are retained by virgin olive oils during their mechanical extraction process from olive fruits (*Olea europaea* L.). Non-volatile compounds such as phenolic compounds stimulate the tasting receptors such as the bitterness perception, the pungency, astringency and metallic attributes. Instead volatile compounds, stimulating the olfactive receptors, are responsible for the whole aroma of the virgin olive oil. The chromatograms of volatile compounds of olive oils were obtained by solid phase micro extraction-gas chromatography/mass spectrometry (SPME-GC/MS) (Hatanaka, 1993; Kataoka et al., 2000; Steffen & Pawliszyn, 1996). The method is based on the assay of the terminal species of the "lipoxygenase pathway" which are present in the volatile fraction of the sampled compounds (Hatanaka, 1993).

2. Materials and methods

2.1 Extraction of olive oil and storage

The olive oils investigated (60 Italian and 60 Tunisian) were single cultivar virgin olive oils (SCVOOs) produced in different regions of Tunisia (Chamlali Cv.) and Italy (Coratina Cv.). Olives were handpicked at the optimal olive ripening degree. Immediately after harvest, olive fruits were transported and cleaned, each fruit sample was divided into three portions of 20 Kg. One portion was extracted using pressure system (see paragraph 2.1.1), the second and the third were extracted by centrifugation systems, three and two phases, respectively (see paragraph 2.1.2 and 2.1.3). The oils obtained were stored in three types of packaging (opaque glass, transparent glass and polyethylene terephtalate PET) and monitored for six months.

2.1.1 Pressure system (PS)

Olives are ground into an olive paste using large millstones. In general, the olive paste stays under the stones for 45–50 minutes. After grinding, the olive paste is spread onto fibre disks, that are easier to clean and maintain, stacked on top of each other and then placed into the press. Afterwards, this pile of disks are put on a hydraulic piston where a pressure of about 400 atm is applied. By the action of this pressure, a olive paste and a liquid phase is produced.

Finally, the liquid phase containing oil and vegetation water is separated by a standard process of decantation.

2.1.2 Two-phase centrifugation (2P)

This system does not need water addition and produces a liquid phase (oil) and a solid waste-water-dampened phase (pomace). The olive paste is kneaded for 60 minutes at 27°C and the oil is extracted with a horizontal centrifugation decanter and separated by means of an automated discharge vertical centrifuge.

2.1.3 Three-phase centrifugation (3P)

This system allows the crushing of olives into a fine paste. This paste is then malaxed for 60 minutes in order to achieve the coalescence of small oil droplets. The aromas are created during these two steps through the action of enzymes. Then, the paste is pumped into an industrial decanter where the phases are separated. Water (500 liters per ton) is added to facilitate the extraction process with the paste. The high centrifugal force created into the decanter separates the phases readily according to their different densities (solid phase pomace, vegetation water, oil). The solid materials is pushed out of the system by the action of a conical drum that rotates with a lower speed. The separated oil and vegetation water are then rerun through a vertical centrifuge, which separates the small quantity of vegetation water still contained in the oil.

2.2 Analytical methods

The physic-chemical and organoleptic analysis of VOO were carried out according to the methods described by the European Union Regulations (UE 61/2011).

In particular, analysis of fatty acid methyl esters, total phenols, free acidity, peroxide number, conjugated dienes and trienes, sensory analysis and volatile compounds were conducted as described in the following paragraphs.

2.2.1 Fatty acid methyl ester analysis (FAMEs)

FAMEs analysis were carried out after performing alkaline treatment obtained by dissolving the oil (0.05 g) in n-hexane (1 mL) and adding a solution of potassium hydroxide (1 mL; 2 N) in methanol (Christie, 1998). FAMEs were analyzed by gas chromatography by mean of a Shimadzu 17A chromatograph equipped with detector flame ionization and a capillary column. Peaks were identified by comparing their retention times with those of authentic reference compounds.

The fatty acid composition was expressed as relative percentages of each fatty acid calculated considering the internal normalization of the chromatographic peak area.

2.2.2 Total phenols analysis

Total phenols content was determined according to the method developed by Gutfinger (1981). Briefly, an amount of olive oil (2.5 g) was dissolved with hexane (5 mL) and extracted with a solution of methanol and water (5 mL; 60/40). The mixture was then vigorously agitated for 2 minutes. Folin-Ciocalteu reagent (0.5 mL) and bi-distilled water (4.8 mL) were added to the phenolic fraction. The absorbance of the mixture was measured at 725 nm and results were given as mg of caffeic acid per Kg of oil.

2.2.3 Free fatty acids, peroxides, ultra-violet light absorption

Acidity value, peroxide value (PV) and ultra-violet light absorption, conjugated diene (K232) and conjugated trienes (K270), were determined according to the Regulation EEC/ 2568/91 of the European Union Commission (EEC, 1991).

2.2.4 Sensory analysis

Olive oils were evaluated by a panel according to the official method for the Organoleptic assessment of virgin olive oil referenced COI/T.20/Doc. No 15/Rev. 2.

2.2.5 SPME-GC/MS analysis

Aroma components of products of plant origin are dependent on genetic, agronomic and environmental factors (Benincasa et al., 2003). The complexity of the mass-chromatograms in terms of number of components might represent a drawback when different samples are to be matched. Therefore, in order to consider the minimum set of components that mostly reflect the biogenesis of an oil (Aparicio & Morales, 1998), hexanal (1), 1-hexanol (2), (E)-2-hexenal (3), (E)-2-hexen-1-ol (4) and (Z)-3-hexenyl acetate (5) were chosen as markers of linoleic and linolenic acids specific lipoxygenase oxidation [(path A and B), Fig. 1].

2.2.5.1 Preparation of samples and standard solutions

A solution (200 mg/Kg) was prepared by dissolving 0.04 g of each analytes (see paragraph 2.2.5) in 200 g of commercial seeds oil. In the same manner a solution containing the internal standard (ethyl isobutyrate) was prepared.

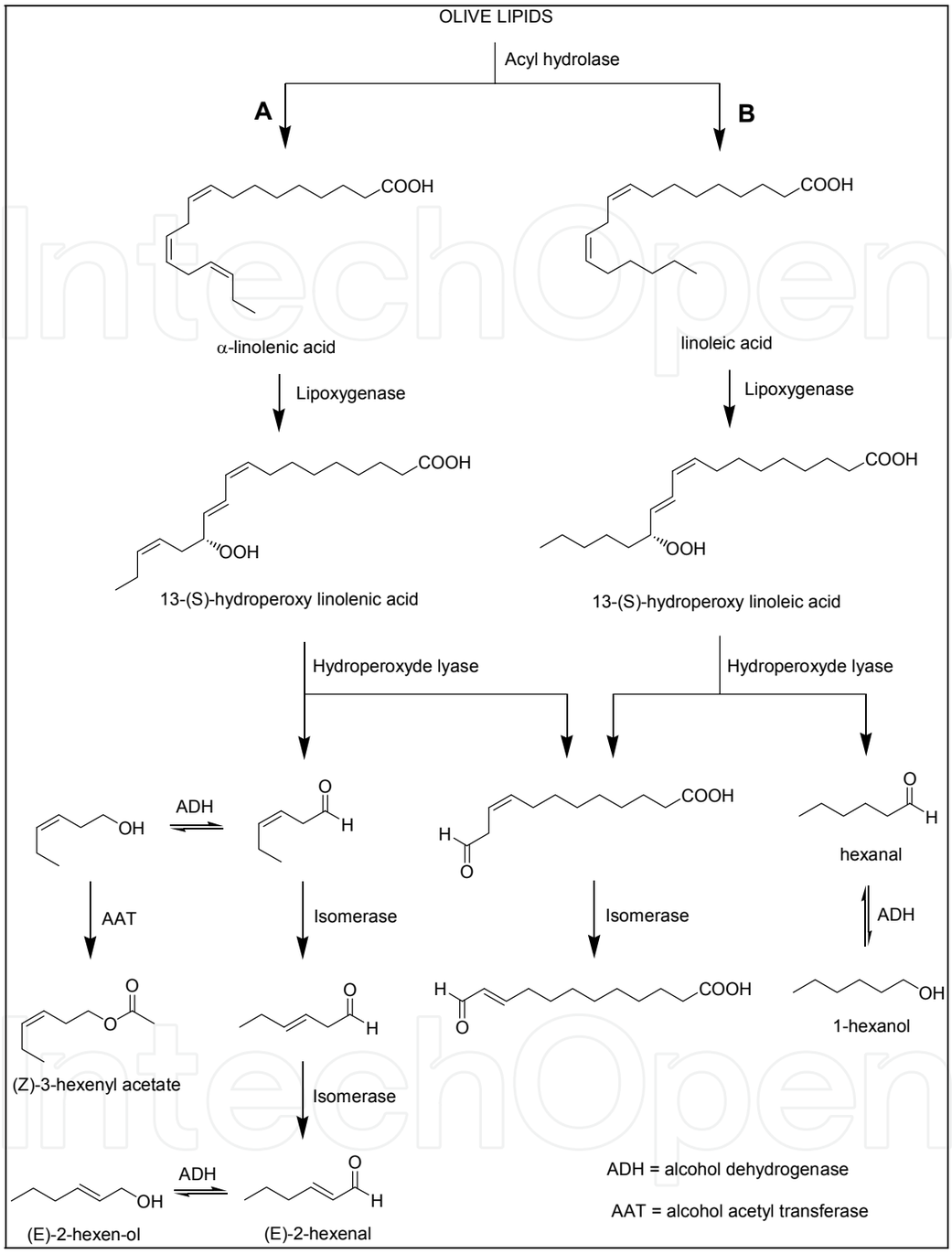

Fig. 1. Linoleic and linolenic acids specific lipoxygenase oxidation.

2.2.5.2 Experimental procedure and instrumentation

The assay of secoiridoid glycosides, such as oleuropein, in virgin olive oil has been proposed as a marker of quality (De Nino et al., 1999, 2005; Perri et al., 1999). With reference to the works previously mentioned, the chromatogram of volatile compounds was considered a useful target. Only the peaks with a certain threshold value (S/N equal to five)

were taken into account and integrated. Identification of analytes was made by comparison of their mass spectra and retention times with those of authentic reference compounds.

The experimental work was carried out using a Varian 4000 Ion Trap GC/MS system (Varian, Inc. Corporate Headquarters, U.S.A.) equipped with a CP 3800 GC. Volatile components were adsorbed by means of a divilbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber and separation was obtained by means of a capillary column FactorFOUR (Varian VF-5ms). The ion trap temperature was set at 210 °C with an ionization time of 2 ms, reaction time at 50 ms and scan rate at 1000 ms. The transfer line temperature was set at 230 °C. The column was a 30 m Chrompack CP-Sil 8 CB low bleed/MS (0.25 mm i.d., 0.25 μm film thickness). The GC oven temperature was initially held at 40 °C for 3 min, then ramped at 1 °C/min to 70 °C and finally ramped at 20 °C/min to 250 °C and held for 8 min. The carrier gas was helium at 1 mL/min. Analyses were performed in splitless mode. Mass spectra were collected in EI in positive mode.

2.2.5.3 Quantitative analysis

The calibration curves were obtained by covering two concentration range: 0.4-4 mg/Kg with six steps at 0.4, 0.8, 1.5, 3, 4 mg/Kg for each analyte, with 1.5 mg/Kg of internal standard and 5-150 mg/Kg with six steps at 5, 10, 25, 50, 100, 150 mg/Kg for each analyte, with 40 mg/kg of internal standard. Each experimental value corresponds to the average of three replicates.

The quantitative assay was performed by selecting the area of the ionic species as follows: m/z 41, 56, 67, 72, 82 for hexanal; m/z 55, 56, 69 for 1-hexanol; m/z 55, 69, 83, 97 for (E)-2-hexenal; m/z 57, 67, 82 for (E)-2-hexen-1-ol; m/z 67, 82 for (Z)-3-hexenyl acetate, respectively and m/z 71, 88, 116 for the internal standard.

2.2.5.4 Statistical analysis

The data obtained for each compound were subjected to statistical analysis. Statistical treatment was performed by STATGRAPHICS Plus Version 5.1 (Statistical Graphics Corporation , Professional Edition - Copyrigth 1994-2001). The approach chosen to analyse the set of data obtained was Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Also, in order to check possible differences between the oils, two-way analysis of variance (ANOVA) was performed considering, as main factors, the nationality of the sample and the type of storage container. Moreover, to evaluate significant differences between averages, Tukey test was performed on the oil quality parameters. Differences were considered statistically significant for $P \geq 0.01$ and $P \geq 0.05$. The values obtained for free acidity and FAMEs were analyzed after arcsine transformation in order to meet assumptions for ANOVA.

3. Results and discussions

3.1 FAMEs analysis

VOOs under investigation showed the typical profile of fatty acids of the areas of production. In general, the oils were dominated by palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18: 1) and linoleic acid (C18: 2). The observed values do not show a particular pattern that can indicate the mode of extraction and the type of packing. It is well known, in fact, that fatty acids are dependent on genetic factors, soil and climate (Christie, 1998; Dabbou, et al., 2010; Gharsallaoui, et al., 2011; Manai, et al., 2007).

3.2 Quality parameters

The extraction system has a significant effect on the physical and chemical parameters of the oil: the pressure system can preserve well the colour and the antioxidants of the olive oil, but may affect negatively the sensory profile. From the results obtained, olive oils were characterised by significant differences in free acidity and phenol content (Figures 2 and 3).

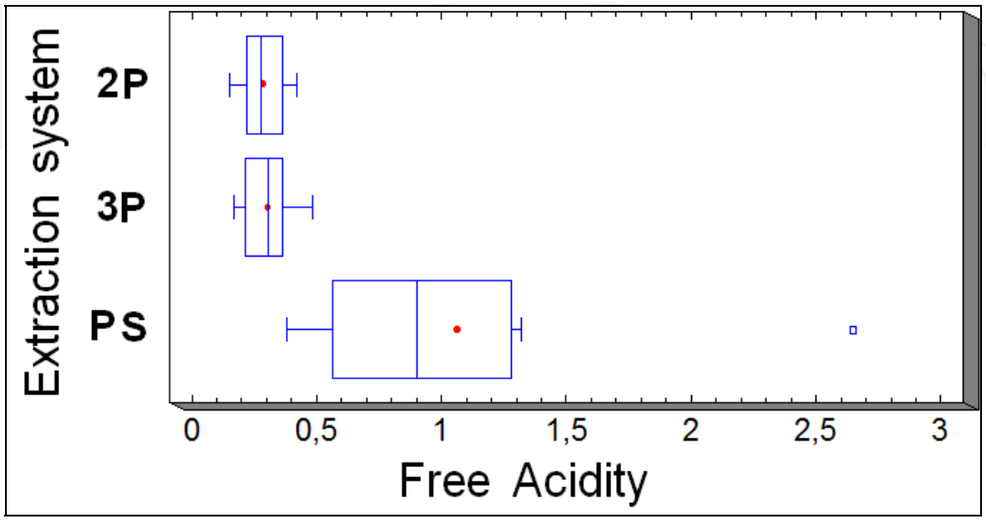

Fig. 2. Box plot of Tunisian VOOs. Free acidity is plotted vs the extraction system.

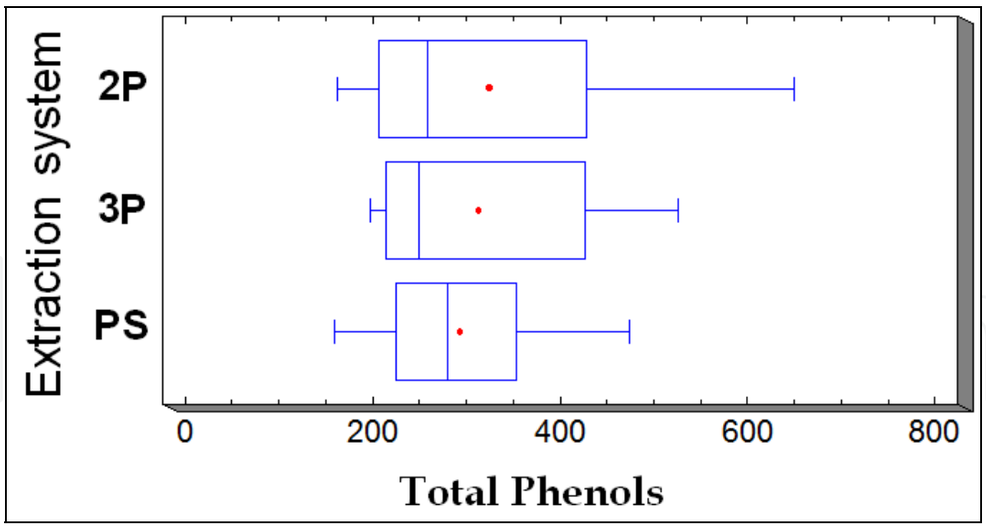

Fig. 3. Box plot of Tunisian VOOs. Total phenols are plotted vs the extraction system.

In general, oils produced with the pressure system have higher free acidity levels than the same oils produced by centrifugation (2P and 3P) and sometime cannot be classified as Extra Virgin Olive Oil (Fig. 2). Moreover, they are often characterised by a lower content of phenols (Fig. 3). In a similar way, the peroxide and K232 and K270 extintion coefficient values were higher than the same oils produced by centrifugation methods.

3.3 SPME-GC/MS and sensory analysis

Cultivar and extraction systems have a considerable effect on sensory attributes of virgin olive oil. A typical mass chromatogram of the volatile component of one of the analyzed sample is reported in Fig. 4, while the bar chart of Fig. 5 shows the distribution of volatile compounds at five and six carbon atoms that mostly contribute to the olive oil aroma.

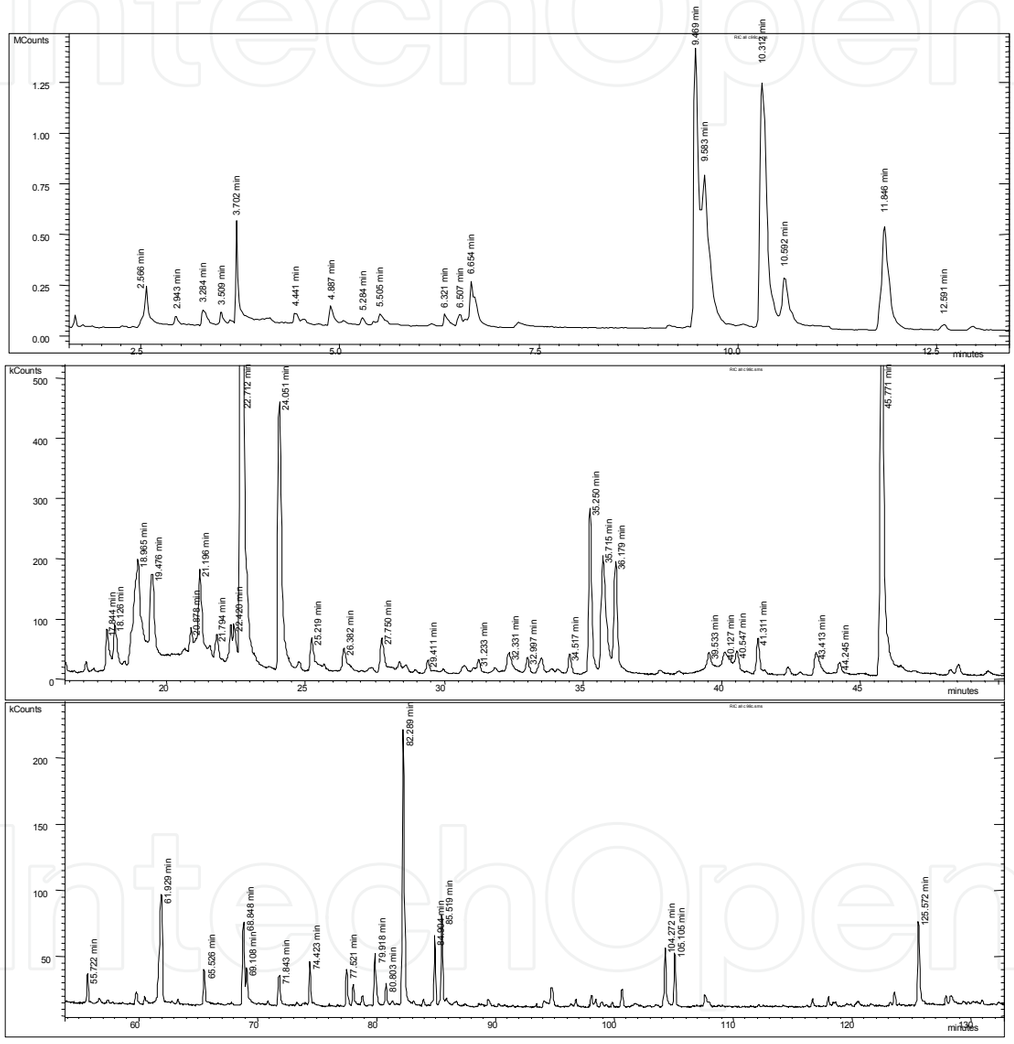

Fig. 4. A typical chromatogram of volatile compounds of one of the analysed samples.

According to the five markers selected as active components of the SPME-GS/MS chromatograms (see paragraph 2.2.5), the distinction of the VOOs under investigation was allowed. Even if both Italian and Tunisian oils were fruity with bitter and pungent characteristics, VOOs of Coratina Cv showed an higher values of fruitiness and bitterness intensity with a clear pungency mainly when they were extracted in centrifugation systems. In fact, these systems can produce olive oils with better organoleptic profiles.

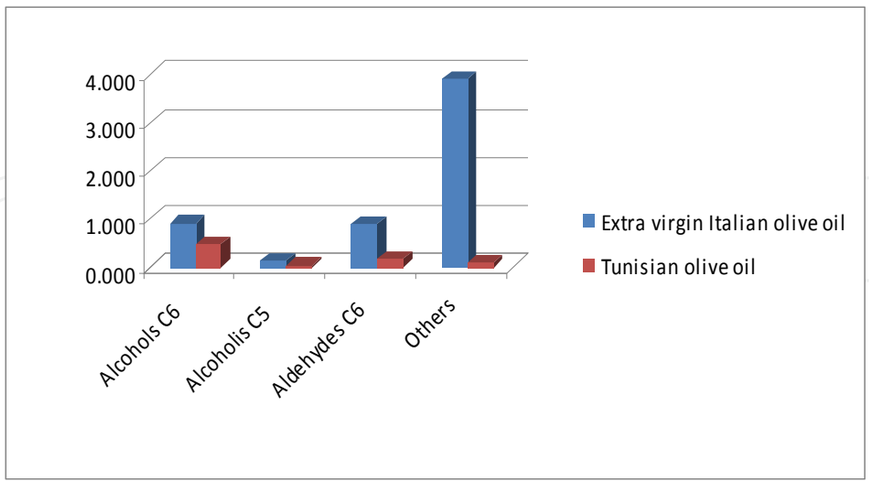

Fig. 5. Bar chart of volatile compounds analysed by SPME-GC/MS. The Cvs under investigation are Coratina and Chamlali from Italy and Tunisia respectively.

Volatile compounds are distributed in a very different concentration in Italian and Tunisian olive oil samples. The flavour of Coratina VOOs was stronger than Chamlali VOOs. In particular, statistical evaluation showed that hexenal (Fig. 6), 1-hexanol (Fig. 7), produced by the lipoxygenase pathway, could discriminate the two VOOs.

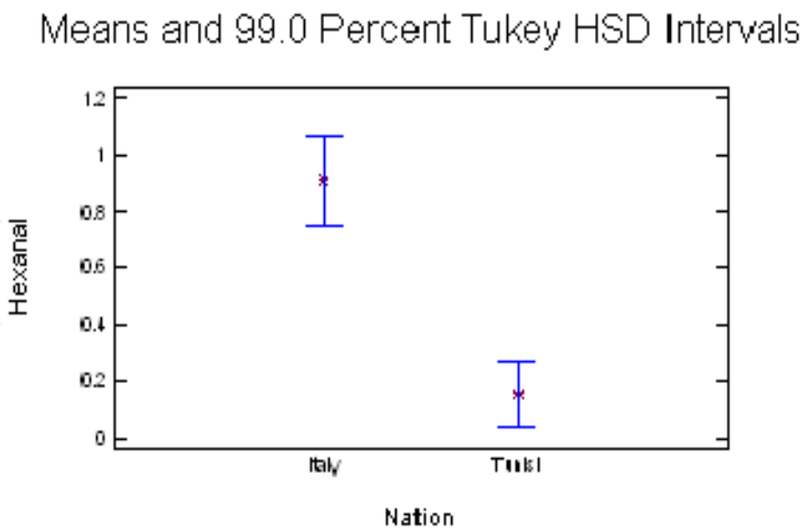

Fig. 6. Biplot of hexenal at 99% confidence level. The genotype was the main factor considered. The Cvs under investigation are Coratina and Chamlali from Italy and Tunisia respectively.

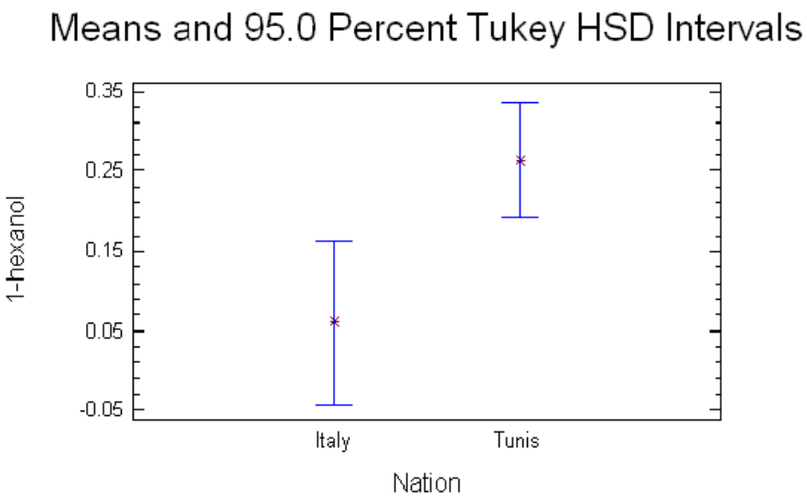

Fig. 7. Biplot of 1.hexanol at 95% confidence level. The genotype was the main factor considered. The Cvs under investigation are Coratina and Chamlali from Italy and Tunisia, respectively.

The VOOs tested by the panelists produced the aromagrams of Figures 8 and 9. According to the panel jury, Coratina olive oils extracted by centrifugation (2P and 3P) were very fruity with a good level of bitterness and astringency. These latter attributes seem to disappear when a pressure system is employed.

Chamlali olive oils extracted by a pressure system were found slightly defected while olive oils extracted by centrifugation systems were fruity with same level of bitterness and astringency. All these results matched those obtained by SPME-GC/MS (see paragraph 3.3).

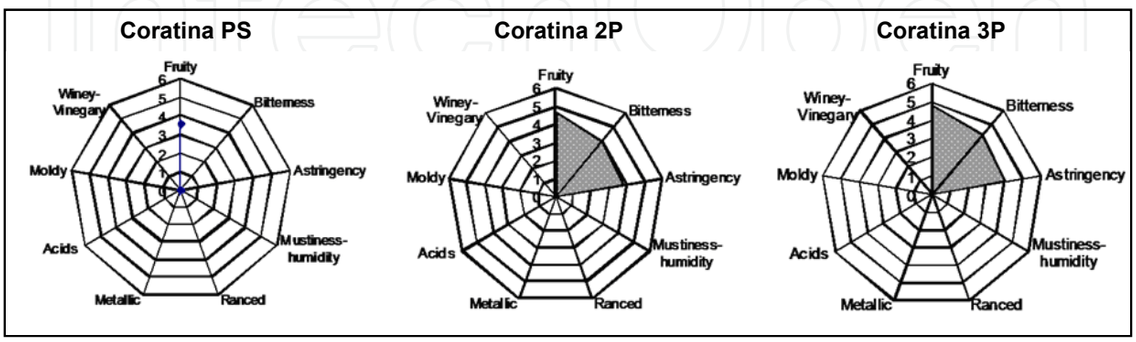

Fig. 8. Sensorial wheels of Italian olive oils of Coratina Cv. extracted by pressure system (PS) and centrifugation two phase and three phase systems (2P and 3P, respectively).

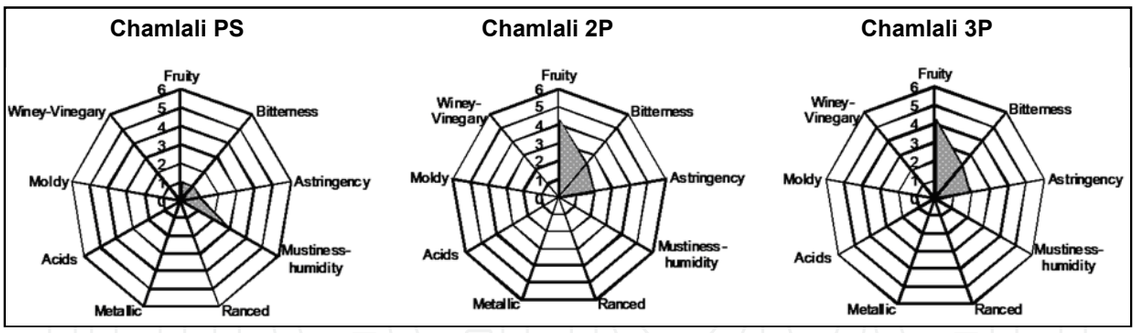

Fig. 9. Sensorial wheels of Tunisian olive oils of Chamlali Cv. extracted by pressure system (PS) and centrifugation two phase and three phase systems (2P and 3P, respectively).

Finally, the organoleptic analysis conducted on custemers demonstrated that consumers prefer olive oils extracted by centrifugation systems rather than olive oils obtained by pressure systems.

3.4 Olive oil storage

Soon after extraction, samples of the sixty Italian and sixty Tunisian VOOs were divided into three groups of twenty and stored in opaque glass, transparent glass and polyethylene terephtalate (PET) bottles. The storage of the oils in opaque glass bottles seemed to be better as it reduced oxidative changes and prolonged shelf life, while polyethylene terephtalate (PET) bottles were the package system that inhibits deterioration to a lesser extent. In fact, free acidity, over the period of six months, became higher when the oils were stored in PET bottles (Fig. 10 and Fig. 11).

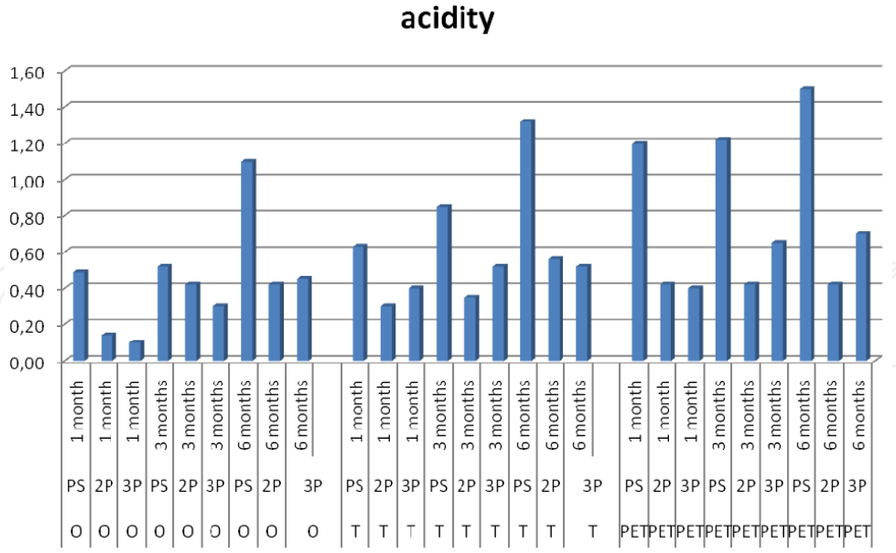

Fig. 10. Bar chart of free acidity of Coratina VOOs during a period of experimentation of six months and depending on the type of packaging utilized. The letters stand for: O opaque glass bottle, T transparent glass bottle, PET polyethylene terephtalate bottle and the extraction system employed: SP pressure system, 2P and 3P centrifugation system at two and three phases respectively.

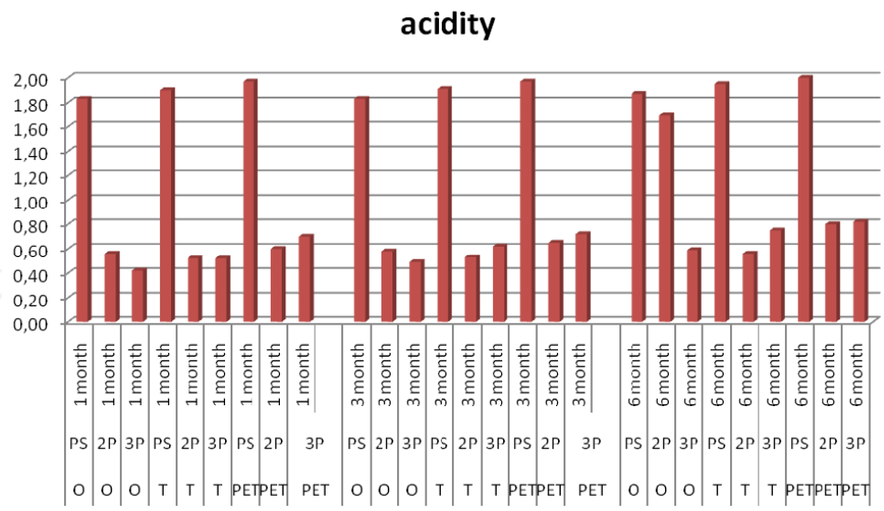

Fig. 11. Bar chart of free acidity of Chamlali VOOs during a period of experimentation of six months and depending on the type of packaging utilized. The letters stand for: O opaque glass bottle, T transparent glass bottle, PET polyethylene terephtalate bottle and the extraction system employed: SP pressure system, 2P and 3P centrifugation system at two and three phases respectively.

Chamlali VOOs were the samples that showed the higher indices of deterioration all over the period. The extraction system plays a key role on the value of the free acidity of an oil. In fact, oils extracted by pressure system have higher free acidity values which increase within the first month (Fig. 12).

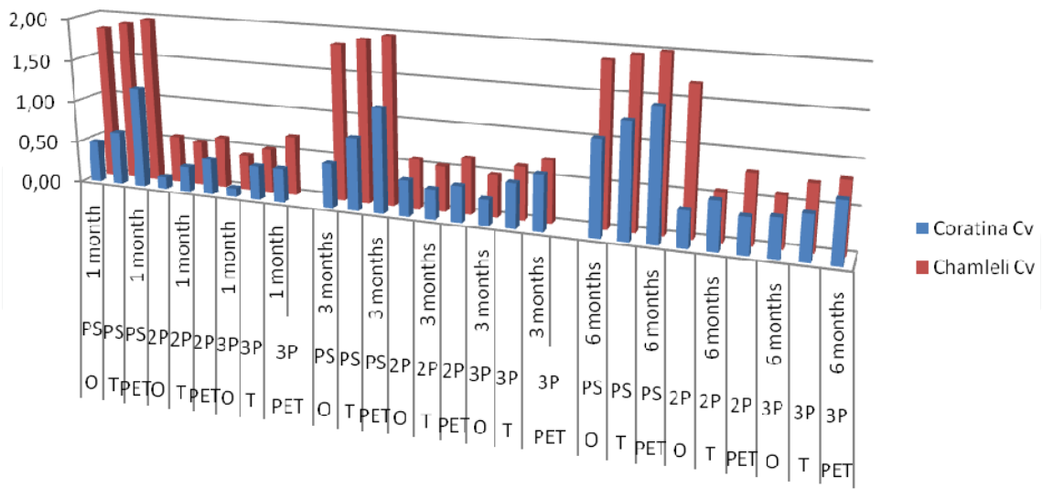

Fig. 12. 3D bar chart of free acidity of Coratina and Chamlali VOOs during a period of experimentation of six months and depending on the type of packaging utilized. The letters stand for: O opaque glass bottle, T transparent glass bottle, PET polyethylene terephtalate bottle and the extraction system employed: SP pressure system, 2P and 3P centrifugation system at two and three phases respectively.

4. Conclusions

The results obtained in this work and discussed in this chapter have shown how important is the method of extraction and the storage of an olive oil (Ben Hassine, et al., 2011; Romano, et al., 2008). A high-quality olive oil can be obtained preferring two phases extraction systems to the classical extraction ones where hydraulic pistons with a pressure of about 400 atm are applied and, storing it in dark glass bottles to better preserve its aroma and phenolic compounds.

5. Acknowledgment

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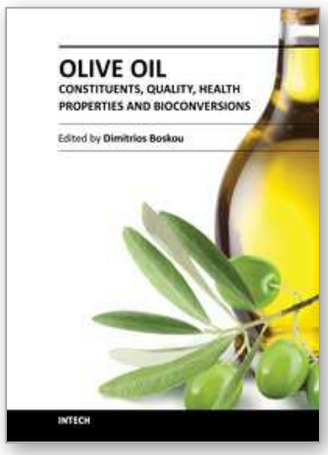

Olive Oil - Constituents, Quality, Health Properties and Bioconversions

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The health-promoting effects attributed to olive oil, and the development of the olive oil industry have intensified the quest for new information, stimulating wide areas of research. This book is a source of recently accumulated information. It covers a broad range of topics from chemistry, technology, and quality assessment, to bioavailability and function of important molecules, recovery of bioactive compounds, preparation of olive oil-based functional products, and identification of novel pharmacological targets for the prevention and treatment of certain diseases.

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Slavka Krautzeka 83/A
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Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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