Chapter

Terpene Compounds of New Tunisian Extra-Virgin Olive Oil: Effect of Ripening Stage

Bechir Baccouri and Imene Rajhi

Abstract

The volatile profiles of Tunisian virgin olive oils were established by solid phase micro-extraction (SPME) and gas chromatography (GC), using flame ionisation and mass spectrometer detectors. Terpenes compounds were identified and characterized. Limonene, the main terpene compound extracted by SPME, characterized the studied olive oil. Significant differences in the proportions of terpenes constituents from oils of different maturity index were detected. The results demonstrated that the accumulation of the terpenes compounds in the studied oils obtained from different ripeness stage was strictly connected with the ripeness stage.

Keywords: virgin olive oil, headspace-solid-phase microextraction (HS-SPME), volatile compounds, terpenes

1. Introduction

Virgin olive oil is characterized through its distinctive perfume, which is synthesized after olive fruits are crushed during industrial oil production. Extra virgin olive oil (EVOO) is unique for its high monounsaturated fatty acids levels and the existence of a wide range of minor components responsible for their organoleptic characteristics and health properties [1]. EVOO attract the interest of the scientific community for its health properties; is an indispensable element of the Mediterranean diet [2]. Its consumption is correlated with a lower incidence of a number of diseases correlated to inflammatory processes such as cardiovascular diseases, diabetes, arthritis, Alzheimer and certain types of cancer [3].

A potential interacting impact of phenolic compounds on EVOO aroma release and perception has been recently described. Volatile minor components are consequently responsible for the aroma of EVOO whereas phenolics are closely related to the bitter and pungent sensory notes [4].

Aldehydes and alcohols of six straight-chain carbons (C6), as well as their corresponding esters, are the most important compounds in EVOO volatile compounds, also quantitatively or qualitatively. Linolenic (LnA) and Linoleic (LA) acids are the main substrates for this synthesis. Lastly, terpenes found in the volatile fraction of EVOO seem not to be important contributors to EVOO aroma due to their low concentration and high odor threshold [1, 5].

Baccouri et al. [6] revealed that Solid-phase microextraction (SPME) of the head space (HS) in combination with mass spectrometry (GC–MS) and gas

chromatography is a very powerful technique that is used quite regularly for the analysis of aroma compounds in foods. HS-SPME–GG–MS has been applied to study the volatile of products derived from the olive fruit, such as oil or table olives [7]. Actually, SPME-GC–MS is the technique of reference to validate the discriminating power of new e-sensing technologies such as the electronic nose for olive oil [8–10].

The main triterpenes present in EVOO are two hydroxyl pentacyclic triterpene acids (oleanolic and maslinic acid) and two dialcohols (uvaol and erythrodiol) [2]. These compounds are mostly found in the epicarp of drupes, therefore, pomace olive oil extracted from olive pomace after the first press with the use of solvents or other chemical processes generally contains 10-fold higher concentrations than EVOO [11].

In in vitro studies, EVOO triterpenes have been described as potent inhibitors of LDL oxidation [12] and to possess antiatherogenic properties via preventing LDL-supporting thrombin generation [3]. A role of these compounds in atherosclerosis protection has been further suggested in a feed work with apolipoprotein (apo) E knockout (KO) mice developing a spontaneous atherosclerosis that mimics most of the features of human atherogenesis [11].

EVOO triterpenes together with hydrocarbons and lignans inhibited cell proliferation and DNA synthesis in Caco-2 colon cancer cell cultures induced through oleic acid, as oleic acid in deficiency of growth factors was capable to induce Caco-2 propagation [13]. In addition, pentacyclic triterpenes from olives established an antiproliferative, and proapoptotic action on on HT-29 colon cancer cells and MCF-7 human breast cancer cells [14].

2. Material and methods

2.1 Sampling

This work was carried out on the study of Effect of ripening period on Terpene compounds of new Tunisian extra-virgin olive oil obtained through controlled crossings on Meski variety. Preliminary work evaluating the oil fatty acid composition of the oil of 50 hybrids showed the performance of these cultivars (9d) among the studied descendants. This new cultivars have an improved oil composition compared to that of Chemlali, the most abundant variety in Tunisia. Samples, obtained from homogeneous olive have picked by hand at a known ripening degree during the crop season 2018/2019. Healthy fruits, without any infection or physical damage, were processed. The olives were washed, deleafed and 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.

2.2 Analysis of volatile compounds: HS–SPME analysis

Before use, the fibre was conditioned; the fibre used for the extraction of the volatile components was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 mm.

5 g olive oil was placed in a 20 ml vial closed by PTFE/silicone septum. 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 room temperature, with magnetic stirring (900 trs/min). 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 [5].

The injections were performed using a SPME autosampler. The fibre was thermally desorbed into a GC and left in the injection port for 4 min. The injector was set at 250 °C and operated in the splitless mode for 2 min unless otherwise stated. The fibre was reconditioned for 5 min in a washing port at 250 °C and blank runs were done periodically during the study [5].

2.3 GC–MS analyses

Each oil was analysed by GC–MS using an Agilent 6890N/5973N system, with fused-silica capillary columns HP-1 (50 m X 0.20 mm; film thickness: 0.5 mm). The identification of the constituents was based on comparison of the retention times with those of authentic samples. Several structures were also confirmed by standard compounds injection. All chemicals were purchased from Fluka or Sigma–Aldrich (Saint Quentin Fallavier, France).

3. Result and discussion

Sesquiterpene hydrocarbons were a major class of compounds identified in the EVOOs samples. Monoterpenes and sesquiterpenes are the lower molecular weight representatives of the terpenoid compounds; they are produced by two and three isoprene units, respectively.

Studied EVOOs made during ripeness process were exposed. Ten sesquiterpenes (**Table 1**), acyclic, monocyclic, bicyclic and tricyclic, were studieded. The variables which were more decisive to discriminate among ripeness stages were sesquiterpenes and aldehydes, such as limonene, α-agarofuran, α-muurolene, *trans*-α-bergamotene, α-farnesene, α-copaene, β-selinene, β-elemene, β-dihydroagarofuran, β-caryophyllene, (Z)-β-farnesene, δ-cadinene, (*E,E*)- (*E*)-β-ocimene, (*Z*)-3-hexenal and nonanal. This finding is in very good agreement with previous studies on other varieties [2, 8].

Several terpene hydrocarbons (mono- and sesquiterpenes) were often detected, and they totally accounted for 2.9–13.5% of the whole volatiles (**Table 1**). (E,E)-α-farnesene (0.1–0.7%), a mono-unsaturated sesquiterpene, was the main one. Besides (E,E)- α-farnesene, other important sesquiterpenes were cyclosativene and α-muurolene, a tetra-unsaturated sesquiterpene that has already been detected in Spanish oils, mainly in those obtained from olives of the Hojiblanca variety [15].

Sesquiterpene hydrocarbons, tended to increase during the maturation process. The highest value (1.3%) was registered in EVOOs obtained from fruits at MI = 6, and the lowest (0.1%) in EVOOs from fruits at MI = 2.

During maturation process, α-copaene remained almost constant during the two maturation stages followed the general trend described above, varying from 0.2% (MI = 2) to 0.4% (MI = 6). further sesquiterpene, such as β-slinene, γ-muurolene, cyclosativene, α-ylangene and α-cubebene, appeared in small amounts only at the highest MI value (**Table 1**).

Monoterpene hydrocarbons, represented by limonene, p-cymene and (E)-β-ocimene, showed a constant increment of its levels during the three maturation stages. Limonene showed a constant increment of its levels passing from 2.2 to 9.4%.

This strong dependence on maturity process, makes terpenes good candidates suitable for the discrimination of oils with different ripeness index. Vichi et al. [15] demonstrated that the amounts of α-muurolene, α-copaeneand and α-farnesene may be used to construct a decisional tree that successfully classifies Western-Liguria extra-virgin olive oils from further Mediterranean oils. Vichi et al. [15] confirmed also for the first time that the enhance of sesquiterpene is Maturation-dependent in olive, and consequently that ripening must be taken carefully into

Constituents	RI	9d Im2	9d Im4	9d Im6
p-cymene	028	0,2	1,7	2,6
limonene	032	2,2	3,9	9,4
(E)-β-ocimene	052	0,2	0,2	0,2
α-cubebene	352			0,1
cyclosativene	370			0.2
α-ylangene	371			0,1
α-copaene	377	0,2	0,2	0,4
β-selinene	487			0,2
α-muurolene	499			0,2
(E,E)-α-farnesene	507	0,1	0,2	0,5
Monoterpene hydrocarbons		2,6	4,3	12,2
Sesquiterpene hydrocarbons		0,3	0,4	1,5
Total terpene hydrocarbons		2,9	4,7	13,7
erythrodiol		0,5	0,8	2,2
uvaol		0,1	0,4	0,9
erythrodiol +uvaol		0,57	1,2	3,14

Table 1.
Terpene composition of the studied olive oil at different stage of maturity.

account when analyzing terpenes [16]. Our results are in agreement with the study of Vichi et al. [15]. These hydrocarbons may be used as markers to distinguish EVOO of different geographical origins [10].

The volatile fraction of the oil from Sfax (South of Tunisia) was characterized by the pre-eminence of α-copaene (24.5%) that may be used as markers to differentiate EVOO of different sites. The other main compounds detected were (E,E)- α -farnesene (6.8%), α -muurolene (4.8%), cyclosativene (3.0%), aromadendrene (1.8%) and longicyclene (1.7%).

A comparison with literature data on the chemical composition of olive oils is complicated because of the big variability of the volatile profiles. In fact, it has been reported that the concentrations of compounds depend on the enzymatic activity though external parameters (soil, climate, harvesting and extraction conditions) may alter the inherent olive oil sensory profile. The variation in levels of C6 aldehydes and alcohols for oil samples from different soils implies that pedologic conditions may influence the activity of alcohol dehydrogenase (ADH).

The triterpenic dialcohols (erythrodiol and uvaol), which are also part of the unsaponifiable fraction of the olive oil, are usually analysed together with the sterol fraction [1]. The erythrodiol and uvaol content of the studied olive oils varied according to maturity, ranging from 0.5 to 2.22% and from 0.1 to 0.92%, respectively (**Table 1**). The sum of erythrodiol and uvaol in all ripeness index was below the established limit of 4.5% for the "extra virgin" olive oil category. These results are consistent with the findings of other authors [15].

4. Conclusions

In conclusion, results demonstrated that the studied olive oils presents a elevated level of variability in terms of the volatile fraction. This aroma variability and the

high genetic diversity of the cultivar germplasm collection suggest that it is possible both to identify old olive cultivars that give rise to oils with a high organoleptic quality and to select optimal parents for olive breeding programs with the aim of finding new cultivars with improved oil aroma. The application of SPME to the analysis of virgin olive oil headspace allowed the detection of significant differences in the proportion of volatile constituents from oils of different maturity index. The results indicate that the ripeness time influence the quali-quantitative production of volatiles. These results permit to use the volatile composition as an indicator of each maturity stage.

Conflict of interest

The authors declare no conflicts of interest.

Author details

Bechir Baccouri[1]* and Imene Rajhi[2]

1 Laboratory of Olive Biotechnology, Centre of Biotechnology of Borj-Cédria, Tunisia

2 Laboratory of Legumes Centre of Biotechnology of Borj-Cédria, Tunisia

*Address all correspondence to: bechirbaccouri@yahoo.fr

References

[1] Baccouri B., Ben Temime S., Daoud D., M'sallem M. and Zarrouk M. (2007). Analytical characteristics of virgin olive oils from two new varieties obtained by controlled crossing on Meski variety. Journal of Food Lipids *14,* 19-34

[2] Ben Temime S, Manai H, Methenni K, Baccouri B, Abaza L, Daoud D, (2008), Sterolic composition of Chetoui virgin olive oil: Influence of geographical origin. Food Chem 110:368-374.

[3] R. K. Manoharan, J. Lee, and J. Lee, (2017). "Antibiofilm and antihyphal activities of cedar leaf essential oil, camphor, and fenchone derivatives against Candida albicans," Front. Microbiol. 8, 1476.

[4] Mills, J.J.; Chari, R.S.; Boyer, I.J.; Gould, M.N.; Jirtle, R.L. (1995). Induction of apoptosis in liver tumors by the monoterpene perillyl alcohol. Cancer Res., *53*, 955-979.

[5] Baccouri B, Zarrouk W, Khichene D, Nouari I, Ben Youssef N, Daoud D, (2007). Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (*Olea europea* L.). J Agron 6:388-396.

[6] Baccouri, B., Zarrouk, W., Krichene, D., Nouairi, I., Ben youssef, N., Daoud, D., Zarrouk, M., (2007b). Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (Olea Europea L.). J. Agron. 6 (3), 388-396.

[7] Baccouri, B., Guerfel, M., Zarrouk, W., Taamalli, W., Daoud, D., Zarrouk, M., (2011). Wild olive (olea europea L.) selection for quality oil production. J. Food Biochem. 35, 161-176.

[8] Baccouri, B., Ben Temime, S., Campeol, E., Cioni, P.L., Daoud, D., Zarrouk, M., (2007a). Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. Food Chem. 102, 850-856.

[9] Baccouri, B., Zarrouk, W., Guerfel, M., Baccouri, O., Nouairi, I., Krichene, D., Daoud, D., Zarrouk, M., (2008). Composition, quality and oxidative stability of virgin olive oils from some selected wild olives (Olea europaea L. subsp. Oleaster). Grasas Aceites 59 (4), 346-351.

[10] Ben Temime, S., Baccouri, B., Taamalli, W., Abaza, L., Daoud, D., Zarrouk, M., (2006). Location effects on Chetoui virgin olive oil stability. J. Food Biochem. 30, 659-670.

[11] Beltran G, Aguilera MP, Del Rio C(2005), Sanchez S and Martinez L, Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem 89:207-215.

[12] H. Sebai, S. Selmi, K. Rtibi, A. Souli, N. Gharbi, and M. Sakly, (2013). "Lavender (Lavandula stoechas L.) essential oils attenuate hyperglycemia and protect against oxidative stress in alloxan-induced diabetic rats," Lipids Health Dis. 12, 189.

[13] M. Božović and R. Ragno, (2017). "Calamintha nepeta (L.) Savi and its main essential oil constituent pulegone: Biological activities and chemistry," Molecules 22, 290.

[14] Urbina A., Martin M., Montero M., Carron R., Sevilla M., and L. Roman, (1990). "Antihistaminic activity of pulegone on the guinea-pig ileum," J. Pharm. Pharmacol. 42, 295-296.

[15] Vichi, S., Castellote, A.I., Pizzale, L., Conte, L.S., Buxaderas, S. & Lopez-Tamames, E. (2003). Analysis of

virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. Journal of Chromatography A, 983, 19-33.

[16] Heuberger, E.; Ilmberger, J.; Hartter, E.; Buchbauer, G. (2008) Physiological and behavioral effects of 1,8 cineol and (±)-linalool: A comparison of inhalation and massage aromatherapy. Nat. Prod. Commun., , *3*, 1103-1110.