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Influence of Extraction Techniques on Physical-chemical Characteristics and Volatile Compounds of Extra Virgin Olive Oil

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Abstract: The purpose of this study was to investigate three types of extraction methods of extra virgin olive oil (EVOO) from the same cultivar (Ortice olive cultivar): traditional or pressing (T) system, decanter centrifugation (DC) system and a patented horizontal axis decanter centrifugation (HADC) system. Oil samples were subjected to chemical analyses: free acidity, peroxide value, ultraviolet light absorption K_{232} and K_{270}, total polyphenols, antioxidant capacity, volatile compounds and olfactory characteristics by electronic nose. The two centrifugation systems showed better free acidity and peroxides value but total polyphenol content was particularly high in extra virgin olive oil produced by patented HADC system. Same volatile substances that positively characterize the oil aroma were found in higher amount in the two centrifugation systems, although some differences have been detected between DC and HADC system, other were found in higher amount in extra virgin olive oil produced by T system. The electronic nose analysis confirmed these results, principal component analysis (PCA) and correlation matrix showed the major differences between EVOO produced by T and HADC system. Taken together the results showed that DC and HADC systems produce EVOO with better characteristics than T system and patented HADC is the best extraction system.

Key words: extra virgin olive oil, extraction technologies, quality, volatile compounds, electronic nose

1 Introduction

Virgin olive (*Olea europaea*) oil is recognized as one of the best vegetable oils given its nutritional benefits in the human diet. Its oxidative stability, sensory quality and health properties stem from a prominent and well-balanced chemical composition. Relevant is the presence of antioxidants which ensure the delay of fatty acids oxidation and unpleasant flavours production[1]. Indeed, virgin olive oil is more stable than other edible oils because of its high content of phenolic compounds, α-tocopherol, carotenoids and monounsatured fatty acids[2]. Moreover, the presence of polyphenols provides a strong added value which resides in their protective action against cancer, cardiometabolic and neurodegenerative diseases[3–5]. As shown in a recent study, the polyphenols present in the olive oil may increase the levels of nerve growth factor and brain-derived neurotrophic factor in mouse brain. Such factors play a key role in learning and memory processes and in the proliferation and migration of endogenous progenitor cells present in the rodent brain[6].

The good chemical composition through the use of mechanical processes, preserve the integrity of the molecules. However it is influenced by several different factors, including the geographical origin[7], the ripening stage[8, 9], the soil features[10], the olive cultivar[11–14], the agronomic techniques[15] and the extraction process[16]. Moreover, processing, which includes milling, paste malaxation and separation of the oil phase, also has an important role in determining the chemical composition of EVOO[17, 18].

Volatile compounds represent another important category of substances which confer typicality to the olive oil. Volatile compounds are low molecular weight compounds

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(less than 300 Da) which vaporize readily at room temperature. Some volatile compounds reach the olfactory epithelium, dissolve into the mucus and may bond with olfactory receptors to give an odor sensation[19]. The major volatile compounds reported in virgin olive oils are the C6 and the C5 volatile compounds. The aroma of olive oil is attributed to aldehydes, alcohols, esters, hydrocarbons, ketones, furans and, probably, other as yet unidentified volatile compounds[20]. Cultivar, geographic region, fruit maturity, processing methods and parameters influence the volatile composition of olive oil. Extraction methods and technical conditions, in particular the malaxation time and temperature, produce olive oils with different flavours[20].

Mechanical EVOO extraction techniques have been developed in order to reduce manpower, and consequently, costs. Pressing and centrifugation have been widely recognized as the best extraction techniques. Positive effects on the concentration of polyphenols have been noted as a result of the extraction process carried out with a system equipped with vertical malaxators working at low oxygen pressure[21] and with a decanter that excludes the need for further oil centrifugation[22]. In addition, the characteristics and operating parameters of the systems have been shown to affect both extraction yield and the quality of the extracted oil[23–26]. In the last 30 years, centrifugation has taken over and has become the most widespread extraction technique, inasmuch entails a reduction in the manufacturing costs and, at the same time, allows high production and shortens the storage time of olives before processing, with beneficial effects on the final product[27]. Moreover a particular centrifugation typology, considered also in our study, a patented device that does not need any vertical centrifugal separator at the end of the process[28–31] is described as the one that is able to better preserve the chemical-nutritional characteristics of extra virgin olive oil.

The aim of this study is to determine how different types of extraction techniques influenced physical-chemical characteristics and volatile compounds of EVOO from Ortice cultivar and how these correlates with oil quality. Three types of EVOO extraction systems, described in material and methods section, are considered: traditional or pressing system; decanter centrifugation; horizontal axis decanter centrifugation.

2 Materials and methods

2.1 Reagents

For the determination of free fatty acids, peroxide value and for the determination of absorption coefficients, ethanol, potassium iodine, sodium thiosulfate, sodium hydroxide chloroform, acetic acid, starch solution 1% in water and isooctane were purchased from Carlo Erba, while diethyl ether was purchased from Sigma-Aldrich. For extraction and determination of total polyphenol content, methanol and Folin-Ciocalteu reagent were purchased from Carlo Erba.

2.2 Olive cultivar

Samples of EVOOs were obtained from olive fruits of Ortice an autochthonous cultivar, grown in the Campania region, in the province of Benevento. Olive fruits of the Ortice cultivar were collected during the same period of maturation and were processed. Then, samples were stored at T＜20℃.

For the olives sampling the fruits were picked in November 2012, harvested at the right ripeness, and processed within 24 h. The quantity of olives used for oil extraction was about 900 kg, 300 kg were used for each extraction system.

2.3 Extraction systems

2.3.1 T extraction system

First, the olives are ground into an olive paste using large millstones for 45-50 min. After grinding, the olive paste is spread onto fibre disks, which are stacked on top of each other and then placed into the press (Pieralisi, Ancona, Italy). Traditionally, the disks were made of hemp fibre, but nowadays they are made of synthetic fibres, which are easier to clean and maintain. These disks are then put on a hydraulic piston, forming a pile. Pressure is applied on the disks, thus compacting the solid phase of the olive paste and percolating the liquid phases (oil and vegetation water). The large part of wastewater is separated from the oil within 2-3 hours by decanting. The residual part of water is separated from the oil within 20 days by decanting.

2.3.2 DC extraction system

The method used for olive oil extraction involved a centrifugation system (Pieralisi, Ancona, Italy) to separate the three phases: solids, oil and water. In this method, the olives are crushed to a fine paste. The aromas are created during these steps through the action of fruit enzymes. In the three phase system the paste is pumped into an industrial decanter where the phases will be separated. Water is added to olive paste to facilitate the extraction process. For our extraction 90 L of water were added to the paste during the extraction procedure, with a flow rate of 360 L/h. The decanter is a large capacity horizontal centrifuge rotating approximately 3000 revolutions per minute (rpm). The high centrifugal force created allows the phases to be readily separated according to their different densities (solids＞vegetation water＞oil). Inside the decanter's rotating conical drum is a coil rotating a few rpm slower, pushing the solid materials out of the system. The separated oil and vegetation water are then rerun through a vertical centrifuge, working around 6000 rpm and separating the small quantity of vegetation water still contained in oil

and vice versa[27].

2.3.3 Patented HADC extraction system

The system consists in a horizontal axis centrifugation device EURO X15 D.E. (Officine Meccaniche Toscane, Florence, Italy) patented original device[28–31]. As reported by manufacturer this device does not need any vertical centrifugal separator at the end of the process, due to the renewed and exclusive design of both the scroll and the oil collecting system. The oil flowing out from the decanter is impurity free and the vegetation water does not contain oil at all. The decanter is fitted with a new oil collecting system from the chamber. Through a particular design of the scroll (a part of the spiral of this scroll is inverse), after the first extraction, the solid phase (olive husk) approaching the discharge is sent back to the central part of the drum (second separation chamber) where it has a further expansion with a second extraction (3600 rpm). Like this a further 1-1.8% of oil from the olive paste is recovered.

2.4 Analytical determination

2.4.1 Miscellaneus

Free acidity, peroxide value and ultra-violet light absorption K_{232} and K_{270} were determined by the methods reported in the European Union Commission Regulation[32]. To determine free acidity, 5 g of oil were added to 50 mL of ethyl alcohol-diethyl ether (1:1 v/v) mixture and neutralized with 0.1 N KOH. Data obtained were expressed as g of oleic acid per 100 g of oil.

For peroxide value determination, 1 g of oil, weighed precisely, was added to 25 mL of an acetic acid-chloroform (3:2 v/v) mixture. Next, 1 mL of a saturated solution of KI was added to this mixture and the sample was put in the dark for 5 min. Afterwards, 75 mL of deionized water and 0.5 mL of starch solution, 10 g/L aqueous dispersion as indicator, were added to the mixture and the sample titrated with 0.01 N sodium thiosulfate to complete bleaching. Data obtained were expressed as mEq of O_2 per kg of oil.

To determine spectrophotometric indices, K_{232}, K_{270} and ΔK, 0.25 g of olive oil, weighed precisely, were put into a 25 mL volumetric flask. The flask was made up to volume with isooctane for spectrophotometry. Samples were analyzed in a quartz cuvettes (optic length of 1 cm), using the DU 730 spectrophotometer, Beckman Coulter (Brea, California).

2.4.2 Total phenols

Total phenols were extracted following the method proposed by Montedoro *et al.*[33]. Ten grams of olive oil were put into a test tube and 10 mL of a methanol:water mixture (80:20 v/v) were added. The sample was then homogenized with a TecnoKartell TK 3S vortex for 1 min, and centrifuged at 5000 rpm for 10 min at room temperature. The methanol extract was collected with a pipette and transferred to a 50 mL flask. The same procedure was repeated twice. The sample was filtered through Whatman filter paper (Watman Inc. Clifton, NY) with 60-65 μm porosity. An aliquot of 0.2 mL of sample was taken for polyphenol analysis, using the Folin-Ciocalteu reagent. The phenol content was determined spectrophotometrically (DU 730 spectrophotometer) at 760 nm according to the method of Del Caro *et al.*[24] and the concentration was expressed as mg of gallic acid per kg of oil by comparison with a standard response curve.

2.4.3 Sample preparation for headspace solid-phase microextraction-gas cromatography (HS-SPME-GC) analysis

The SPME fibre (PDMS-100 μm, polydimethylsiloxane) was conditioned according to the manufacturer's recommendations prior to its first use. To a 20 mL headspace vial was added 5 mL of samples, together to 3 g of NaCl and octan-3-ol, in hydro-alcoholic solution (1/1, v/v) at 100 μg/L, as internal standard. The solution was homogenized with a vortex shaker and then loaded onto a Gerstelautosampling device. The program consisted of swirling the vial at 250 rpm for 5 min at 40℃, then inserting the fibre into the headspace for 30 min at 40℃ as the solution was swirled again, then transferring the fibre to the injector for desorption at 240℃ for 30 min.

2.4.4 Gas chromatography-mass spectrometry (GC/MS) analysis

Gas chromatographic analyses were carried out using a 7890 Agilent GC system coupled to an Agilent 5975 inert quadrupole mass spectrometer equipped with a Gerstel MPS2 autosampler. The capillary column used was a HP-Innowax (Agilent technologies) (30 m length × 0.25 mm id. 0.50 μm film thickness) and the carrier gas was Helium. A splitless injection system was used. The initial oven temperature was set to 40℃ for 1 min.

The temperature was increased in four steps: 40-60℃ at 2℃/min; 60–150℃ at 3℃/min, 150-200℃ at 10℃/min and 200-240℃ at 25℃/min; the final temperature was maintened for 7 min. The injector, the quadrupole, the source and the transfer line temperature were maintained at 240℃, 150℃, 230℃ and 200℃, respectively. Electron ionization mass spectra in full-scan mode were recorded at 70eV electron energy in the range 40-300 amu. Peaks were identified using both the NIST 98 and Wiley libraries. Quantification was performed by using the relative concentration in μg/kg of the internal standard, calculated as the ratio between each compound area and the internal standard area. The samples were analyzed in triplicate and blank runs were made by using an unfilled vial every two analysis.

2.4.5 Electronic nose analysis

A commercial portable electronic nose 3 (PEN 3), including the Win Muster software for data mining, Air sense Analytics Inc. (Schwerin, Germany), was also used to analyze the olfactory characteristics of the oils. The instrument was equipped with an array of 10 metal oxide semi-

conductors (MOS) type chemical sensor, the sensors differed in thickness and chemical composition to provide selectivity towards volatile compound classes as previously reported[34].

The sensor response is expressed as resistivity (Ω). The MOS sensors rely on changes in conductivity induced by the adsorption of molecules in the gas phase and on subsequent surface reactions. They consist of a ceramic substrate coated by a metal oxide semiconducting film, and heated by a wire resistor. Due to the high operating temperatures (200-500℃) the organic volatiles transferred to the surface of the sensors are totally combusted to carbon dioxide and water, leading to a change in the resistance. The high temperature allowed no interference from water and fast response and recovery times[35]. The detection limit of hot sensors was in the range of 1 mg/kg as indicated by the instrument supplier.

An aliquot of 3 mL of each sample was placed in air tight 35 mL glass vial, sealed with a PTFE/silicone septum and a screw cap, stored at 25℃ for 1 h to equilibrate, and analyzed at the same temperature. The measurement device sucked the gaseous compound from the headspace of the sample trough the sensory array at 400 mL/min for 200 s. The period of measurement allowed a steady state in the sensors response, the analyses were performed by using recording data in the steady state. A second pump transported the filtered air to the sensor array at 600 mL/min for 400 s to rinse the system between two consecutive samples. The results have been displayed in a two dimensional view[36] as shown (Fig. 2).

2.4.6 Statistical analysis

Data were reported as mean of three independent measurements ($n = 3$) ± standard deviation. Significant differences among different extraction systems were determined by an analysis of variance which applied a Duncan's multiple range test with a 95% significance level ($p < 0.05$), using the SPSS programme, release 11.0 for Windows (SPSS, Chicago, IL, USA). For the electronic nose analysis eight independent measures were performed for each sample. Principal component analysis (PCA) and correlation matrix of the data were performed by using the Win Muster software. PCA defines the structure of variance-covariance of a data set through a system of coordinates whose number of dimensions is less than the number of original variables. Correlation matrix (Table 3) shows the discrimination power measuring the severability of classes. In this process the overlapping pattern data is examined, values of discrimination indexes are in the range of 0 and 1 as previously reported[34]. Values lower than 0.5 show a rather bad severability of the classes by the measurement, while high values indicate a good severability of classes, the most the value approaching to 1, values of discrimination indexes ≥ 0.95 are considered significant.

3 Results and discussion

3.1 Chemical parameters

The olive oils were classed as "extra virgin" due to the free acidity values (Table 1). The peroxide value, K_{232}, K_{270} and ΔK of all oils were within the limits established by the European Commission Regulation[50] and International Olive Oil Council (IOC) Resolution[37].

The free acidity of the oil obtained with the T system was higher than the free acidity of the oil obtained with the DC and HADC systems (Table 1). The oil obtained with the DC system showed the lowest acidity value (0.22% free fatty acids). In the T system, both oil and vegetable water are extracted and remain together until they are separated by decanting[38, 39], a procedure which may favour the hydrolysis of triglycerides, resulting in an increase of free fatty acids level.

The two centrifugation systems revealed a lower peroxide value with respect to T system, according to a previous study[22]. The lower oxidation stress exerted by centrifugation systems explains the lower peroxide content in both oils.

Regarding to the UV spectrophotometric indices, the conjugated dienes (K_{232}) and trienes (K_{270}) of all oils showed values within the limits for EVOO (Table 1) even if low dif-

Table 1 Physicochemical quality parameters evaluated in EVOO samples obtained with different extraction systems.

	T	DC	HADC	Regulations EU[50] Resolution IOC[37]
Free fatty acids [g/100g]	0.60 ± 0.03^{a}	0.22 ± 0.03^{c}	0.40 ± 0.02^{b}	≤ 0.8
Peroxide value [meq/kg]	11.23 ± 0.35^{a}	9.80 ± 0.28^{b}	9.31 ± 0.08^{b}	≤ 20
K_{232}	1.95 ± 0.03^{b}	2.27 ± 0.02^{a}	2.13 ± 0.04^{b}	≤ 2.5
K_{270}	0.19 ± 0.01^{b}	$0.20 \pm 0.01^{a, b}$	0.21 ± 0.01^{a}	≤ 0.22
ΔK	−0.004	−0.006	−0.002	≤ 0.01

Values are the means ± SD. Different letters indicate significant differences ($p < 0.05$) between the extraction systems

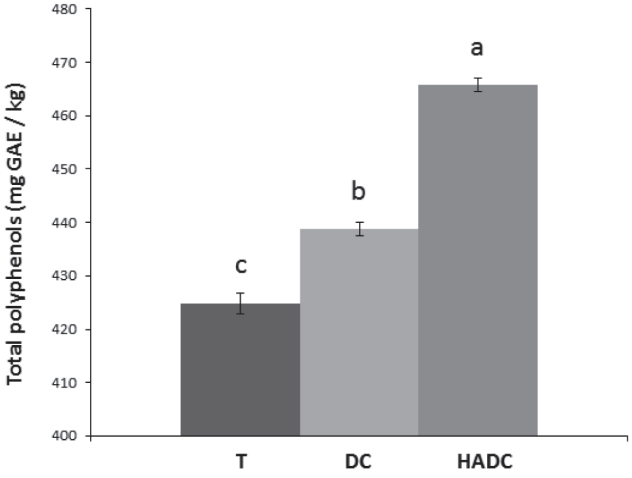

Fig. 1 Total polyphenol contents of the three EVOO from the three extraction methods. The columns indicate the mean ± SD. Different letters indicate significant differences ($p < 0.01$).

ferences in the values of K_{232} and K_{270} were found among the extraction systems. K_{232} value was higher in the oil obtained with the DC system and K_{270} was higher in the oil obtained with the HADC system. These results confirm in part what was already reported in previous papers[22, 27]. Also ΔK values are all within the limit fixed by the actual European Regulation and IOC resolution.

Polyphenol content varied from 424.87 mg GAE/kg for oil obtained with the T system, to 465.81 mg GAE/kg in the oil obtained with the HADC system (Fig. 1). The range of polyphenol content for Ortice cultivar is higher than that obtained with any extraction system[22, 27, 40], but in agreement with the Database of Italian monovarietal oils[41].

Phenolic substances do not only affect virgin olive oil stability, they also contribute to oil flavour and aroma, and give the typical bitter taste of olive oil, which is a positive attribute in the sensory evaluation of virgin olive oils[26] and a typical organoleptic characteristic of the Ortice cultivar[41].

The oil yields (% of the fruit weight) were: 17.2%, T system; 18.6%, DC system and 19.8%, HADC system.

3.2 Volatile compounds

3.2.1 GC/MS analysis

Table 2 shows the amount of volatile compounds of the three oils. C6 volatile compounds (aldehydes and alcohols), originated in the lipoxygenase pathway, are connected to positive sensory characteristics of olive oil and contribute to the green olive oil aroma[42]. C5 volatile compounds which originate in the additional branch of the lipoxygenase pathway[43] contribute to the pleasant aroma and positively correlate with bitterness and pungency of virgin olive oils[20].

3.2.2 Aroma markers and other main volatile substances

A previous study reports five aroma markers that characterize the oil produced from olives at three different stages of ripening[44]. In our study, the variable is represented by the oil extraction system and not the ripeness index, nevertheless the same markers strongly characterize the aroma, with the exception of Z-3-hexemyl acetate which was not found in the effected HS-SPME-GC/MS analysis. Hexanal, (*E*)-2-hexenal, 1-hexanol and (*E*)-2-hexen-1-ol contribute to the smell green leaves and grass smell of virgin olive oil[45]. These compounds arise during the oil process production and contribute to the typical oil aroma[20]. (*E*)-2-hexen-1-ol also contributes to the sweet, fresh mouth feel smell.

The oil obtained with the T system was particularly rich in alcohols. The dominant components of alcohol aroma marker were 1-hexanol and (*E*)-2-hexen-1-ol. Regarding the aldehyde components (*E*)-2-hexenal was the dominant of the EVOO aroma obtained with the DC and HADC systems, and hexanal concentration is similar in both the oil centrifugation systems while it is lower in the T system of oil extraction.

In Table 2 other volatile substances found in considerable amount are reported. Isoamyl alcohol is present in the T system at the concentration of about 200 μg/kg, while its concentration was very low in the EVOOs obtained with DC and HADC systems, moreover EVOO produced with HADC system showed very low values of ethanol respect to the EVOO obtained with T and DC systems. Isoamyl alcohol and isobutyl alcohol are off-flavours, it is possible to find them in EVOOs although in moderate amount[25], noteworthy they were both find in higher amount in T EVOO, with respect to DC oil and HADC oil.

3.2.3 Electronic nose

The electronic nose analysis is a useful method to characterize EVOOs. The electronic nose equipped with MOS sensors was used to characterize EVOOs obtained by different extraction methods, to detect adulterations and defects, to compare different kind of edible oil products[46–49]. In the present study we used such a technique to compare the "fingerprints" of the aromas arising from the EVOOs obtained by the three different extraction methods.

Figure 2 shows a PCA of the response the 10 electronic nose sensors array to the headspace of the samples. Each sample is represented by a cluster of eight different measures. The processed data show a shift of the groups by differently processed EVOOs along the first and the second principal component. The figure also shows the percentage of the variance explained for both components, with the value of the total variance 98.95%. All the clusters are in the same quadrant and the discrimination indexes (Table 3) never exceed 0.95 value, showing that olfactory characteristics are partially preserved in all cases[34]. In Fig. 2 is possible to observe that the clusters representing the

Table 2 Volatile compounds (μg/kg) of EVOO samples identified by HS-SPME-GC/MS analysis.

	T	DC	HADC
Esters			
ethyl acetate	11.15 ± 0.08[a]	1.05 ± 0.04[b]	2.07 ± 0.69[b]
ethyl benzene	4.25 ± 1.22[a]	1.75 ± 0.21[b]	1.35 ± 0.06[b]
n-hexyl acetate	1.81 ± 0.07[a]	0.92 ± 0.01[b]	0.81 ± 0.01[b]
3-hexen-1-ol acetate	2.02 ± 0.04[b]	2.86 ± 0.07[a]	1.38 ± 0.04[c]
Ketones			
acetone	6.58 ± 0.43[a]	2.73 ± 1.23[a]	8.29 ± 4.79[a]
3-pentanone	39.11 ± 1.91[a, b]	42.80 ± 2.16[a]	36.76 ± 0.63[b]
6-methyl-5-hepten-2-one	1.11 ± 0.02[a]	1.16 ± 0.30[a]	1.51 ± 0.05[a]
Aldeydes			
acetaldehyde	3.98 ± 0.02[a]	1.67 ± 0.84[b]	2.00 ± 0.25[b]
butanal-2-methyl	nd[b]	1.86 ± 0.99[a]	1.65 ± 0.01[a]
butanal-3-methyl	nd[b]	1.41 ± 0.76[a]	0.89 ± 0.01[a]
hexanal	16.62 ± 4.12[b]	47.18 ± 4.03[a]	46.57 ± 0.63[a]
(*E*)-2-pentenal	1.10 ± 0.02[c]	7.17 ± 0.13[a]	2.35 ± 0.13[b]
heptanal	0.66 ± 0.04[a]	0.72 ± 0.02[a]	0.54 ± 0.00[b]
(*E*)-2-hexenal	37.57 ± 2.59[c]	590.26 ± 16.70[a]	486.93 ± 5.82[b]
octanal	1.26 ± 0.07[a]	0.97 ± 0.01[b]	0.84 ± 0.04[b]
(*E*)-2-heptenal	nd[b]	1.63 ± 0.06[a]	1.82 ± 0.06[a]
nonanal	4.21 ± 0.53[b]	6.19 ± 0.08[a]	4.56 ± 0.16[b]
2,4-hexadienal	nd[b]	3.80 ± 0.28[a]	3.05 ± 0.81[a]
2,4-heptadienal	1.18 ± 0.12[b]	2.44 ± 0.19[a]	1.09 ± 0.09[b]
benzaldehyde	2.06 ± 0.06[a]	1.78 ± 0.10[b]	1.98 ± 0.02[a, b]
Alcohols			
ethanol	173.94 ± 13.01[a]	143.48 ± 11.47[a]	4.76 ± 0.01[b]
isobutyl alcohol	18.24 ± 1.79[a]	nd[c]	2.74 ± 0.06[b]
isoamyl alcohol	200.58 ± 6.58[a]	2.47 ± 0.18[b]	12.60 ± 0.06[b]
1-penten-3-ol	6.02 ± 0.45[c]	18.92 ± 0.33[a]	16.47 ± 0.15[b]
1-pentanol	4.51 ± 0.30[a]	2.32 ± 0.09[c]	3.86 ± 0.13[b]
(*E*)-2-penten-1-ol	1.69 ± 0.19[b]	3.78 ± 0.15[a]	1.94 ± 0.06[b]
(*Z*)-2-penten-1-ol	13.53 ± 6.02[a]	16.79 ± 1.09[a]	16.24 ± 0.73[a]
1-hexanol	173.00 ± 15.83[a]	32.44 ± 2.56[b]	61.29 ± 1.24[b]
(*E*)-*trans*-3-hexen-1-ol	1.82 ± 0.13[a]	0.56 ± 0.07[c]	1.10 ± 0.16[b]
(*Z*)-*cis*-3-hexen-1-ol	67.77 ± 3.92[a]	12.14 ± 1.57[b]	10.52 ± 0.06[b]
(*E*)-2-hexen-1-ol	114.40 ± 11.35[a]	92.33 ± 1.52[b]	88.15 ± 1.16[b]
(*Z*)-2-hexen-1-ol	0.91 ± 0.03[a]	nd[c]	0.74 ± 0.02[b]
2-ethyl-hexanol	1.99 ± 0.20[a]	0.76 ± 0.02[b]	1.65 ± 0.03[a]
benzene methanol	1.58 ± 0.22[a]	0.72 ± 0.01[b]	0.93 ± 0.11[b]
phenethyl alcohol	10.04 ± 0.62[a]	1.14 ± 0.04[b]	1.96 ± 0.09[b]
Alkenes			
3-ethyl-1,5-octadiene	7.59 ± 1.34[c]	52.67 ± 0.91[a]	39.40 ± 2.12[b]
limonene	0.95 ± 0.05[a]	nd[b]	nd[b]
(*E*)-4,8-dimethyl-1,3,7-nonatriene	3.50 ± 0.12[c]	11.26 ± 0.30[a]	8.46 ± 0.23[b]
cis-sabinene hydrate	1.11 ± 0.05[a]	nd[b]	nd[b]
valencene	1.17 ± 0.26[a]	0.23 ± 0.01[b]	0.99 ± 0.12[a]
α-muurolene	0.95 ± 0.13[a]	0.54 ± 0.02[b]	0.84 ± 0.01[a]
α-farnesene	0.63 ± 0.05[a]	0.38 ± 0.01[b]	0.36 ± 0.01[b]

nd, not detectable. Values are the means ± SD. Different letters indicate significant differences ($p < 0.05$) between extraction systems.

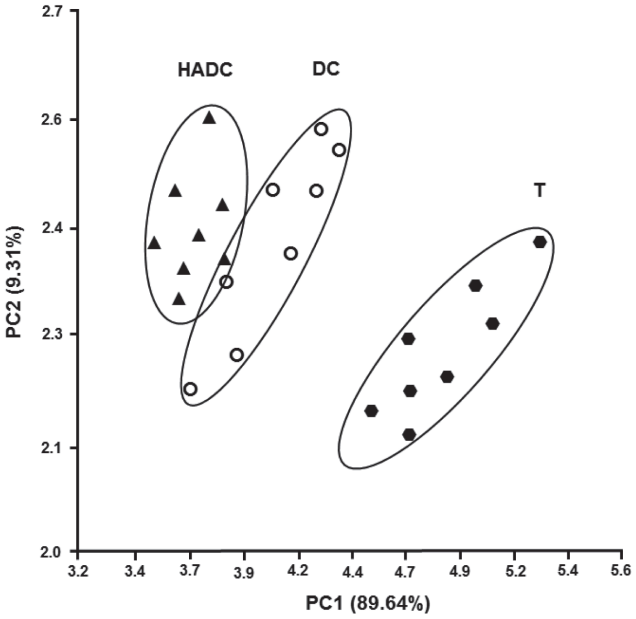

Fig. 2 PCA plot of the scores produced by the 10 sensor responses along the first two principal components. The number in parentheses indicate the proportion of the total variance explained by each principal component. T, DC and HADC as reported in material and methods section.

Table 3 Correlation Matrix among the groups.

	T	DC	HADC
T	0.000		
DC	0.632*	0.000	
HADC	0.863*	0.357	0.000

The numbers indicate the Discrimination Indexes. Different values ($p \geq 0.5$) are marked with an asterisk.

EVOOs produced by DC and HADC systems partially overlap. This observation corresponds to the discrimination index value of 0.357 (Table 3), considerably lower than 0.5, indicating that the olfactory characteristics of the two classes are quite similar. On the contrary, it is possible to observe a clear separation of the cluster representing the EVOO produced by T system with respect to DC and HADC systems. Along the x-axis is expressed the main variance of the PCA (89.64%), taking in account the shift along the axis, the higher distance is observed between HADC cluster and the cluster representing the EVOO produced by T system. This observation is confirmed by correlation matrix that shows the highest value of discrimination index between T and HADC classes.

4 Conclusions

In this study, the two centrifugation systems show better value of free acidity and peroxides. Total polyphenol content, follows the same trend ad is particularly high in EVOO produced by patented HADC, never characterized in previous studies.

Regarding the volatile substances, some of them that positively characterize the oil aroma were found in higher amount in DC and in HADC EVOO, other were found in higher amount in T EVOO, on the contrary isoamyl alcohol that negatively characterize oil aroma was found in higher amount only in T EVOO.

The results of the electronic nose confirm these data in fact DC and HADC oil show more similarity respect to T, in particular HADC oil is the most different respect to T EVOO. The ethanol was found almost exclusively in T and DC EVOOs in agreement with PCA and correlation matrix results that don't show the higher difference between T and DC clusters.

Our data show that DC and HADC systems produce EVOO with better characteristics the than T system especially regarding chemical parameters, in particular EVOO produced by patented HADC shows better characteristic out of the three systems.

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