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Liquid Carbon Dioxide Use in the Extraction of Extra Virgin Olive Oil From Olive Paste

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Abstract

In this study the use of liquid carbon dioxide, CO_2, for extraction of oil from olive paste (*Peranzana cultivar*) were examined and extracted oil was compared with oils obtained by centrifugation, pressure and use of chemical solvent.

It is well known that the use of CO_2 has many advantages: miscibility with a wide range of molecules, food safety, non-flammability, absence of residues in the extract, possibility of total solvent recovery and no production of olive mill waste water that are highly polluting for the environment and require expansive disposal.

Samples were subjected to the following analyses: determination of Free Fatty Acids (FFA), Peroxides Value (PV), Spectrophotometric Indices, Fatty Acids Composition (FA), determination of biophenols content and determination of Volatile Organic Compounds (VOCs). All samples showed FFA, PV and ΔK values within the limits established by law for extra-virgin olive oil. The use of CO_2 did not catalyze hydrolysis, oxidation and condensation of double bonds. Centrifuged oils and oils extracted with carbon dioxide presented the lowest PV and FFA values. Extraction with liquid carbon dioxide contributed to an increasing of phenolic content with a value of 270.5 mg/kg, a value twice that of the oils extracted with centrifugation (135.3 mg/kg) or pressure methods (173.2 mg/kg). Oil extracted with liquid carbon dioxide showed the greatest amount of t-2-octenal and t-2-heptenal, giving herbaceous and pungent notes. Moreover the presence of aromatic compounds such as limonene, generally absent in olive oils, was only detected in the sample extracted with liquid carbon dioxide.

Keywords: liquid carbon dioxide, extra-virgin olive oil, biophenols, *Peranzana cultivar*

1. Introduction

Extra virgin olive oil (EVOO) is the product obtained by processing of the fruit of Olea Europeae. Reg. CE 1531/2001 and further modifications recognizes different types of olive oil, ranging from EVOO to unedible oil, depending on parameters fixed for each category.

Virgin oils preserve the quality of the fruit as they are obtained from fresh olive drupes by using only physical procedures, including centrifugation or pressure extraction methods.

To obtain oils of high quality, in recent years new extraction procedures have been experimented, like the application of compressed fluids, such as CO_2. The use of liquid carbon dioxide is taking an important role in food industry, in fact it is already used for many application (Roselius et al., 1974; Zizovic et al., 2007). The wide use of CO_2 is due to its advantages: miscibility with a wide range of molecules, food safety, the non-flammability, absence of residues in the extract and the possibility of total solvent recovery. The aim of this study was to examine the effects of CO_2 application in the extraction of oil from olive paste and to compare obtained oils with those extracted by use of common solvent (hexane) and by conventional systems (centrifugation and pressure) from the same olive paste.

2.Materials and Methods

2.1 Sampling

Olive paste from Peranzana cultivar, collected in November (CROP, 2011), was centrifuged and pressed to obtain the samples OFRC and OFRP, respectively. The same olive paste was extracted by liquid carbon dioxide (sample CDO) using a specific extractor at Centro Sperimentale per la Valorizzazione delle produzioni olearie e vitivinicole, Cercola (Napoli) Italy, and by hexane (sample SOLV).

2.2 CO_2 Extraction Procedure From Olive Paste

The extraction with CO_2 was performed as described by Romano et al. (2009). Two different solid-liquid ratios (1:5 and 1:10) were used to evaluate the yield of extraction and three different tests were carried out:

a) Extraction of oil from olive paste and calculation of the yield every 30 min with replacement of solvent (discontinuous process). Solid-liquid ratio 1:5.

b) Extraction of oil from olive paste and calculation of the yield every 30 min with replacement of solvent (discontinuous process). Solid-liquid ratio 1:10.

c) Extraction of oil from olive paste and calculation of the yield after 180 min without replacement of solvent (continuous process). Solid-liquid ratio 1:10.

2.3 Determination of Free Fatty Acids (FFA), Peroxides Value (PV) and Spectrophotometric Indices

The determinations of FFA, PV and Spectrophotometric indices were carried out according to Reg. UE 61/2011.

FFA were determined by titratation with NaOH and expressed as a percent of oleic acid.

PV were determined by iodometric assay and expressed as meq O_2/kg oil.

Spectrophotometric indices were measured as absorbance at 232 and 270 nm and ΔK was calculated as: K268-[(K262+K274)/2].

2.4 Fatty Acids Composition (FA)

Analysis of FA composition was performed by gas chromatography (GC) after derivatization to FA methyl esters (FAME) with 2N KOH in methanol, according to the IUPAC standard method (Marquez-Ruiz et al., 2008).

FAME were analyzed on a Perkin Elmer AutoSystem XL gas chromatograph (Perkin Elmer, Waltham, MA, USA) equipped with a PTV (programmed temperature vaporizer), a flame ionization detector, and a capillary column 100 m × 0.25 mm inner diameter, film thickness of 0.20 mm. Stationary phase 50% cyanopropyl methyl silicone (Supelco, USA). The carrier gas, helium, was introduced at a flow rate of 20 cm/s. The oven temperature program was as follows: 120 °C for 5 min, 5 °C/min ramp to 165 °C for 5 min; and then 10 °C/min ramp to 240 °C for 20 min. The split ratio was 1/60, and the flame ionization detector temperature was set at 260 °C.

The identification of the peaks was made using an external standard (Supelco TM 37 component FAME MIX) by comparing the retention times with those of the pure standard components.

2.5 Determination of Total Biophenols Content (TB)

TB were analyzed by a colorimetric method (Folin-Ciocalteau) after hydroalcolic extraction, as indicated by Blekas et al. (2002).

2.6 Biophenols by HPLC/DAD

Biophenols were detected by HPLC-DAD, according to the method suggested by International Olive Council (COI/T. 20/ Doc. n. 29, 2009).

2.7 Determination of Volatile Organic Compounds (VOCs)

2 ml of samples were analyzed by dynamic head space (DHS)/GC-MS. 100 microliter of a solution of 10mg/L of undecane, prepared in deodorized oil, were added as internal standard (I.S). The Gas Chromatograph used was an Agilent 6890N equipped with an Agilent 5973 N (Agilent technologies, Palo Alto, CA) mass spectrometer and a capillary column with 5%-phenyl-methylpolysiloxane (30 m × 0.25 mm id × 0.25 μm) HP-5 MS (Agilent technologies, Palo Alto, CA). The carrier gas used was helium at a flow rate of 1.2 mL/min. The oven temperature program was the following: 45 °C for 3 min, 10 °C/min ramp to 90 °C for 1 min; 20 °C/min ramp to 107 °C for 1 min and 20 °C/min ramp to 240 °C. VOC identification was obtained by comparing the mass spectra with those of the pure standards compounds available in the data system library (NIST 02 and WILEY 275). The quantification of the compounds was made by comparison of peak area with the area of the known amount of I.S.

2.8 Statystical Analysis

All determinations and experiments were performed in triplicate and the results are the average values of three determinations. The data obtained were analyzed by ANOVA using XLSTAT 2006, version 2006.6 (ADDINSOFT, Paris, France), to compare different oils. Differences at $P \leq 0.05$ were considered significant.

3. Results and Discussion

3.1 Yield Extraction

Discontinuous process using liquid carbon dioxide was carried out for 270 min. As can be seen in Figure 1, after 180 minutes of extraction, no important increase of yield was obtained. An increasing of solvent amount (1:10 ratio) corresponded to an increasing of yield after about 100 min of extraction.

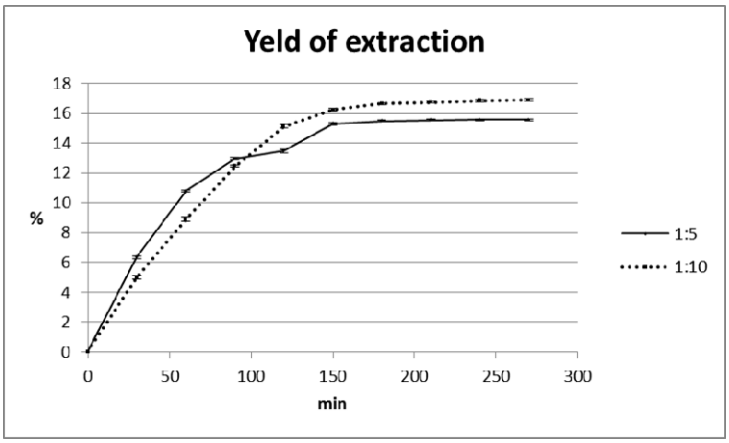

Figure 1. Yield of extraction (%) obtained using liquid carbon dioxide during discontinuous extraction with 1:5 and 1:10 solid-liquid ratio

Discontinuous procedure showed higher yield values respect to continuous extraction. In fact the yield was 15.49% and 16.64%, for 1:5 and 1:10 ratio, respectively, against the 6.9% and 8.15% for 1:5 and 1:10 ratio, respectively, of the continuous extraction after 180 minutes of extraction.

Oils extracted by physical methods showed comparable yields with those obtained with use of liquid carbon dioxide. In fact the first ranged from 17 to 19% (g oil/ 100 g olive paste), while oils extracted by liquid CO_2 ranged from 16 to 17% after 270 min of extraction.

3.2 FFA, PV and Spectrophotometric Indices

Free fatty acids, peroxide values and Spectrophotometric Indices are reported in Table 1.

Table 1. Free fatty acids (FFA), Peroxides Value (PV) and Spectrofotometric indices of oils extracted by pressure (OFRP), centrifugation (OFRC), hexane (SOLV) and oil extracted by liquid carbon dioxide (CDO)

	OFRP	OFRC	SOLV	CDO
FFA (%)	$0.45^{a} \pm 0.00$	$0.25^{c} \pm 0.01$	$0.36^{b} \pm 0.00$	$0.30^{c} \pm 0.00$
PV (meq O_2/kg)	$6.34^{a} \pm 0.06$	$4.65^{c} \pm 0.18$	$5.36^{b} \pm 0.04$	$4.74^{c} \pm 0.03$
ΔK	$-0.005^{b} \pm 0.000$	$-0.005^{b} \pm 0.000$	$0.005^{b} \pm 0.000$	$-0.006^{a} \pm 0.000$
K_{232}	1.47±0.02	1.47±0.01	1.48±0.01	1.47±0.00
K_{270}	0.12±0.00	0.11±0.00	0.18±0.01	0.13±0.00

a-c: Different letters for the same parameter correspond to statistically significant differences ($P \leq 0.05$).

All samples analyzed showed acidity values less than legal limit provided for EVOO (≤ 0.8 g oleic acid/ 100 g oil). Oil extracted by pressure method (OFRP) showed the highest value (0.45%). CDO and OFRC showed the

lowest acidity values, 0.30 and 0.25%, respectively, and no statistically differences ($P < 0.05$) were observed.

In a fresh oil hydroperoxides presence can be an index of oxidation level due to collection, storage and processing of olives (Kiritsakis et al., 1998). PV of all samples was in the legal limit required by law for EVOO ($\leq$ 20 meq O_2/ kg oil).

The sample OFRP showed the highest PV (6.34 meq O_2/ kg oil), while centrifuged oils and oils extracted with carbon dioxide presented the lowest values, 4.65 and 4.74, respectively. In all analyzed samples, spectrophotometric indices, indicating oxidation level, were below the legal limits. Only SOLV had a positive ΔK (0.005) but this value was lower than 0.01.

3.3 FA Composition

The most representative fatty acid in olive oil is oleic acid with a percentage of 72%. However, its concentration is very variable, from 60 to 80%, due to different conditions (Aguilera et al., 2005). Palmitic acid (C16:0) is generally present in average values of 14-15%, while the linolenic acid in olive oils is contained in a percentage not exceeding 10%. The analysis of fatty acid composition showed significant differences between the oils extracted by physical methods, compared to oil obtained through the use of liquid CO_2 especially in the content of palmitic acid, present in an average concentration of 18 % in CDO, while the samples OFRC and OFRP contained minor amounts ranging from 13.73 to 13.96%.

CDO presented the lowest percentage of long chain fatty acids (LCFA) with a value of 0.73% respect to all the other samples that ranged from 1.06 to 1.76 %. On the other hand, CDO showed the greatest percentage of medium-chain fatty acids (MCFA). This could be due to the different solubility of the fatty acids in the solvent. Also, CDO contained the highest amount of saturated fatty acids (SFA) with a value of 20.46% respect to the other samples ranged from 16.77 to 17.42%.

3.4 Biophenols

The agronomic techniques and processing of olives can modify the concentration of polyphenols in the oil (Montedoro et al., 1989; Servilli et al., 1999; Servilli et al., 2000). Italian oils range between 40 and 900 mg/kg (Servilli & Montedoro, 2002) while Greek oils between 20 and 339 mg/kg, expressed as caffeic acid (Blekas et al., 2002).

In olive oil, the presence of phenolic compounds is an index of quality. These molecules give the oil the classic bitter taste (Gutieärrez-Rosales, 2003). As shown in Figure 2, results reported an higher amount of TP in OFRP (173.2 mg/kg caffeic acid) than in OFRC (135.3 mg/kg caffeic acid). This can be due to the addition of water during centrifugation of olive paste that changes the distribution of phenols, dissolving mainly in the aqueous solution (Servilli et al., 1999). Oils extracted with pressure method generally contains higher amount of phenolic compounds than oils obtained with centrifugation method (Servilli & Montedoro, 2002). Extraction with liquid carbon dioxide contributed to an increasing of phenolic content, in fact CDO sample showed the highest TP content (270.5 mg/kg), a value twice that of the oils extracted with centrifugation (135.3 mg/kg) or pressure methods (173.2 mg/kg). This can be due to the fact that little amount of liquid carbon dioxide is soluble in water, consequently the carbon dioxide has a higher affinity towards the mentioned molecules. According to Wiebe and Gaddy (1940), CO_2 solubility changes as a function of temperature and pressure. Considering the working conditions of the extractor (50 bar and 25 ± 2 °C) CO_2 solubility in water was about 32-28 ml/g. SOLV sample showed the lowest amount of TP (74.45 mg caffeic acid/kg).

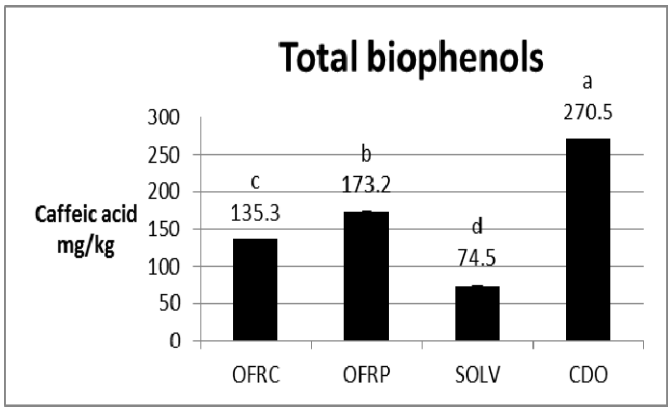

Figure 2. Total biophenols concentration in oil extracted by centrifugation (OFRC), pressure (OFRP), hexane (SOLV) and oil extracted by liquid carbon dioxide (CDO)

a-d: Different letters correspond to statistically significant differences ($P \leq 0.05$).

Results obtained with Folin-Ciocolteau method about TP content were confirmed by HPLC-DAD analysis. In Table 2 the concentration (mg/kg of tyrosol) of different phenolic compounds found in all samples was reported. Among extracted oils, CDO showed the highest amount of TP, with a value of about 103 mg/kg, while SOLV showed the lowest amount (15.81 mg/kg). As shown in the table, in all samples, except for SOLV, the largest amount of phenol compounds belonged to the family of secoiridoids. In oils extracted with classic physical methods the content of secoiridoids ranged from 30.68 in OFRP to 27.69 mg/kg in OFRC, while oil extracted with carbon dioxide showed a value about twice (62.71 mg/kg).

Table 2. Individual biophenol concentration (mg/kg tyrosol) in oils extracted by pressure (OFRP), centrifugation (OFRC), hexane (SOLV) and oil extracted by liquid carbon dioxide (CDO)

	OFRP	OFRC	SOLV	CDO
Phenolic alcohol	13.54^{a}±0.29	5.52^{c}±0.16	11.60^{b}±0.49	1.51^{d}±0.29
hydroxytyrosol	13.21^{a}±0.29	5.05^{b}±0.27	-	-
tyrosol	0.33^{c}±0.01	0.47^{c}±0.11	11.60^{a}±0.49	1.51^{b}±0.29
Phenolic acids	3.13^{b}±0.17	1.52^{c}±0.07	1.03^{c}±0.05	36.81^{a}±0.93
caffeic acid	2.64^{b}±0.18	1.14^{c}±0.08	0.81^{c}±0.05	35.67^{a}±0.86
vanillic acid	0.49^{a}±0.01	0.38^{b}±0.02	0.23^{c}±0.00	-
p-coumaric acid	-	-	-	1.13±0.07
Secoiridoides	30.68^{b}±0.60	27.69^{c}±1.72	3.17^{d}±0.06	62.71^{a}±0.27
hydroxyl tyrosyl acetate	2.66^{a}±0.04	$1.48^{a.b}$±0.00	0.21^{b} ±0.03	-
decarboximethyl oleuropein aglycon, aldehydic form	0.27^{b}±0.01	-	0.36^{b}±0.01	2.10^{a}±0.16
oleuropein	1.09^{b}±0.06	-	0.37^{c}±0.01	1.92^{a}±0.15
tyrosyl acetate	-	-	0.44±0.04	-
oleuropein aglycone, dialdehydic form	-	1.02±0.02	-	0.87±0.17
aglycone decarboxymethyl ligstroside, dialdehydic oxidized form	4.08^{a}±0.16	0.43^{d}±0.09	0.29^{c}±0.01	3.51^{b}±0.17
aglycone decarboxymethyl ligstroside, dialdehydic form	1.27^{a}±0.06	0.48^{c}±0.05	-	0.35^{b}±0.10
Ligstroside aglycone, dialdehydic form	-	0.84±0.07	-	-
oleuropein aglycone, aldehydic and hidroxilic oxidized form	-	-	0.36^{b}±0.01	1.67^{a}±0.05
oleuropein aglycone, aldehydic and hidroxilic form	20.37^{b}±0.50	22.16^{b}±1.91	0.45^{c}±0.03	51.46^{a}±0.26
LIGSTROSIDE aglycone, aldehydic and hidroxilic form	0.94^{b}±0.12	$1.29^{a.b}$ ±0.25	0.68^{b}±0.03	0.84^{b}±0.11
lignans	0.88±0.03	-	-	-
pinoresinol	0.88±0.03	-	-	-
flavones	1.31^{b}±0.13	10.99^{a}±0.19	-	1.34^{b}±0.54
apigenin	-	10.62^{a}±0.28	-	1.34^{b}±0.54
luteolin	1.31^{a}±0.12	0.37^{b}±0.09	-	-
Total	49.54^{b}±0.23	45.71^{b}±1.08	15.81^{c}±0.71	102.36^{a}±5.47

Among secoiridoids, Oleuropein, as described by Servilli et al. (2009), is one of the most abundant phenolic compounds in the olives while it is contained in little amount or absent in olive oils. In fact it was detected in concentration of 66.61 mg/ kg in the olive paste and in concentration of 1.09, 0.37 and 1.92 mg/kg in OFRP, SOLV and CO_2, respectively.

The aldehydic and hydroxylic form of the aglycone oleuropein was the phenolic compound mainly present in all samples of olive oil, with the exception for SOLV.

ANOVA showed no significant differences in samples OFRC and OFRP, while there were significant differences with oils extracted with carbon dioxide and hexane.

The class of phenolic acids, containing caffeic acid, p-coumaric acid and vanillin, was the most abundant after that of secoiridoids only in CDO oils.

In all the other samples, the most abundant classes of phenols were phenolic alcohols and flavones.

Little amount of lignans were detected in oils extracted by pressure.

3.5 VOCs

The complex sensory profile of an oil depends on several variables.

The variety, the degree of ripening, the process of crushing, the kneading temperature, the amount of oxygen in contact with the paste, are all factors that influence the volatile component (Angerosa et al., 1998; Sanchez

-Ortiz et al., 2008).

The degree of maturation influences the activity and the amount of alcohol dehydrogenase, in fact as the degree of ripening increases, the enzymatic activity decreases (Chervin & Truett, 1999).

The lack of oxygen in contact with the olive paste and the temperature range significantly affect the activity of lipoxygenase (Kalua et al., 2005).

Several molecules belonging to the classes of aldehydes, ketones, alcohols, esters and other components have been found (Table 3). It is possible to observe that the aldehyde compounds, in particular hexanal in OFRP and CDO and trans-2-esenal in OFRC, were the most representative compounds in all samples analyzed.

Hexanal, which determines a hint of green apple and trans-2-hexenal reminiscent of the scent of green almond (Morales et al., 1995) are in fact substances that characterize a fresh olive oil.

CDO appeared to have the greatest amount of t-2-octenal which gives a herbaceous and spicy notes (Aprea et al., 2006), while in the other samples this compound was very low (1.09 μg/kg in OFRC) or absent (OFRP).

The trans-2-Heptenal, a pungent compound, was present in CDO in high amounts, about 6 μg/kg , while the other samples contained from 0.42 to 2.63 μg/kg .

Oils extracted by pressure showed the highest amount of alcohols (58.19 μg/kg). In the other samples their concentration was very low (0.32 and 3.35 μg/kg in OFRC and CDO, respectively).

Among esters, ethyl acetate and ethyl furan were the only two compounds found in CDO and absent in the other samples. Morales et al., 1995 reported that ethyl acetate is responsible of sweet smell of oils.

Among ketones compounds, 1-penten-3-one, molecule with a hint of sweet and strawberry (Aparicio et al., 1996), was detected only in oil OFRC and heptan-2-one, with smell of fruity, was detected only in CDO.

Among the “other compounds” the presence of limonene, generally absent in olive oils, was only detected in the sample extracted with liquid carbon dioxide, indicating the different solvent power of carbon dioxide respect the existing physical extraction methods.

Table 3. VOCs concentration (μg/kg) of oils extracted by pressure (OFRP), centrifugation (OFRC) and liquid carbon dioxide (CDO)

	DESCRIPTORS	OFRP	OFRC	CDO
3-methyl butanal	Apple	0.70±0.06	-	-
2-methyl-2-butenal		-	-	0.58±0.08
pentanal	Woody, Bitter	-	0.96^{a}±0.06	1.04^{a}±0.01
hexanal	Green Apple, Grassy	53.92^{c}±1.20	80.95^{b}±2.38	168.17^{a}±7.28
trans-2-hexenal	Bitter Almonds	12.35^{c}±1.10	150.59^{a}±1.61	42.64^{b}±0.04
trans-2-octenal	Herbaceous, Spicy	-	1.09^{b}±0.07	5.88^{a}±0.06
octanal	Fatty	1.81^{a}±0.07	0.84^{b}±0.22	2.17^{a}±0.03
trans-2-heptenal	Pungent, Soapy	0.42^{a} ± 0.00	2.63^{b}± 0.04	6.08^{c} ± 0.67
heptanal	Oily	1.17^{ab}±0.31	0.41^{b}±0.11	1.42^{a}±0.53
∑aldehydes		69.95^{b}±0.28	237.47^{a}±4.5	221.9^{a}±8.63
1-propanol		-	0.32±0.01	-
3-methyl butanol	Woody, Sweet	12.72±2.50	-	-
1-hexanol		23.55^{a}±2.28	-	3.35^{b}±0.31
3-hexen-1-ol	Green leaf, Nuts	7.40±0.71	-	-
2-hexen-1-ol	Cut green grassy	2.95±0.20	-	-
2-methyl-1-butanol	Winey, Spicy	11.57±4.93	-	-
∑alcohols		58.19^{a}±3.80	0.32^{b}±0.01	3.35^{b}±0.31
ethyl acetate	Sticky, Sweet	-	-	8.18±0.01
ethyl furan		-	-	1.69±0.12
hexyl acetate	Sweet, Fruity	2.31^{a}±0.47	1.17^{b}±0.27	-
3-hexen-1-ol acetate	Green leaf, Nuts	4.51^{a}±0.33	4.78^{a}±0.05	-
∑esters		6.82^{b}±0.79	5.95^{b}±0.31	9.87^{a}±0.13
1-penten-3-one	Sweet, Strawbarry	-	2.39±0.24	-
6-methyl 2-heptanone	Fruity	0.95±0.08	-	-
heptan-2-one	Sweet, Fruity	-	-	1.93±0.13
3-methyl-cyclohexen-1-one		-	0.37±0.011	-
∑ketones		0.95^{b}±0.08	2.76^{a}±0.25	1.93^{a}±1.60
limonene		-	-	5.38±0.27
3-ethyl heptane		-	-	1.75±0.04
3-methyl hexane		-	-	1.94±0.42
3-methyl decane		-	-	0.46±0.04
heptane		-	38.39±3.27	-
buthyl-1-cycloexene		0.36±0.23	-	-
6-methyl-2-undecene		0.68±0.04	-	-
3-5 dimethyl-1-hexene		0.18±0.02	-	-
1-1 dimethyl-2-allyl, ciclopropane		0.69±0.17	-	-
4-8 dimethyl, 1-7-nonadiene		1.18^{a}±0.10	0.42^{c}±0.01	0.64^{b}±0.04
3-ethyl,1-5-octadiene		-	1.17^{a}±0.29	1.32^{a}±0.11
propylciclohexane		1.57^{b}±0.31	-	9.08^{a}±0.45
2,4-dimethyl-1-heptene		-	-	9.33^{a}±0.44
methyl cyclohexane		-	6.87±0.85	-
4-methyl heptane		0.88^{b}±0.55	-	5.35^{a}±1.17
∑ other compounds		5.54^{c}±1.43	46.85^{a}±4.41	32.25^{b}±2.10

a-c: Different letters for the same parameter correspond to statistically significant differences ($P \leqslant 0.05$)

- Under detection limit (< 0.01%).

4. Conclusions

Results showed that oils extracted with liquid CO_2 presented various advantages: limited hydrolysis and oxidative reactions, higher concentration of biophenols respect to the classic methods, presence of aromatic compounds such as limonene and, very important, no production of olive mill waste water that are highly polluting for the environment and require expansive disposal.

The use of CO_2 do not catalyzed hydrolysis, oxidation and condensation of double bonds so that the values of FFA, PV and ΔK were within the limits established by law for EVOO, as the other analyzed samples.

Our results confirmed that this extraction method influenced the pattern of phenolic compounds in EVOO. The addition of water during centrifugation method can eliminate important molecules, such as oleuropein, that are distributed in the vegetable water rather than oil (Servilli & Montedoro, 2002). In fact, when no water was added during process (pressure method and carbon dioxide extraction), oleuropein was detectable.

CO_2 can be considered an alternative to the use of chemical solvents because the extracted oil is qualitatively better in biophenolic content and also safer considering the possibility that traces of the most common solvent used may remain in the finished product, while CO_2 can be totally removed and recovered.

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