J Am Oil Chem Soc (2008) 85:685–691
DOI 10.1007/s11746-008-1252-8

ORIGINAL PAPER

Modulation of Olive Oil Quality Using NaCl as Extraction Coadjuvant

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Received: 12 March 2008 / Revised: 8 May 2008 / Accepted: 15 May 2008 / Published online: 31 May 2008

Abstract The use of four concentrations of common salt (NaCl) used as coadjuvant for the extraction of virgin olive oil has been tested on a laboratory scale and the quality attributes of the oils obtained were compared to those obtained with talc as coadjuvant. The oils extracted from Picual fruits after NaCl addition were not significantly affected in terms of the physicochemical requirements established for extra virgin olive oil, the best level of quality of this produce. Addition of NaCl during the extraction process was positively correlated with the presence of *o*-diphenol compounds and the stability of the oils obtained. Moreover the use of NaCl resulted in a significant increase in contents of pigments (β-carotene, lutein and chlorophylls *a* and *b*) and volatile compounds in the oils.

Keywords *Olea europaea* · Olive oil · Quality · Phenolic compounds · Pigments · Aroma · Stability

Introduction

Increase in consumption of virgin olive oil (VOO) outside the Mediterranean region must be attributed not only to its potential health benefits [1], but also to its unique flavor. It is well established that volatile compounds and phenolic components have a direct influence on the flavor of VOO. Both, oil aroma and phenolic components are mainly determined by the chemical composition and biochemical status of the olive fruit. Our group has recently found that olive heat-treatments seemed to promote a partial deactivation of the lipoxygenase (LOX) system that leads to a net decrease in the contents of almost all volatile compounds [2] and also affect the colour of the oils obtained by increasing the content of both the carotenoids and the chlorophyllic compounds [3]. Experimental data obtained in our lab have also shown that olive seeds contain enzymatic activities metabolising 13-hydroperoxides other than hydroperoxide lyase, that give rise to a net decrease in the content of six-carbons unsaturated aldehydes observed during the olive oil extraction process. Olive seeds supplied this process with alcohol dehydrogenase activity and also contain an important quantity of alcohol acyltransferase that is responsible for the biosynthesis of 30–50% esters during the olive oil extraction process of intact fruits. These previous studies demonstrated that the technological procedure of stone removal may be used to modulate olive oil aroma giving rise to oils with a modified aroma composition, enriched in six-carbons aldehydes and alcohols and with a lower amount of volatile esters and five-carbons compounds [4]. The main classes of hydrophilic phenols found in VOO are phenolic alcohols, phenolic acids, lignans, flavonoids and secoiridoids. The occurrence of these hydrophilic phenols depends on the amount of glycosidic precursors present in the olive fruit and on the activities of several endogenous enzymes, such as β-glucosidase catalysing the hydrolysis of these phenolic glycosides and oxidoreductases catalysing the oxidative catabolism of phenolic compounds. The final phenolic content in the oil is strongly affected by processing parameters such as temperature, time, or exposure to air that could modulate the activity of those enzymes during the crushing and malaxation processes [5]. Thus, modulation of the phenolic content by olive fruit heat-treatments, probably mediated through a β-glucosidase inactivation,

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has been described [6], and in a similar way an improvement in VOO phenolic content has been achieved by means of stone removal that eliminates the important pool of oxidative enzymes located in the olive seed [7].

The aim of increasing the quality standards for VOO is continuously stimulating the development of new technological procedures to improve or modulate its sensory properties. In this sense, in the past few years different coadjuvants for the physical extraction of olive oil have been tested. A combination of pectolytic, cellulolytic and hemicellulolytic plant enzymes have been successfully used to break emulsions improving not only the oil yield but also the nutritional quality of the oil [8]. However, the use of coadjuvants with chemical or biochemical action in the olive pastes has been expressly excluded from the certified denomination of VOO under current EU regulation [9]. The unique additive authorised in olive oil extraction by the Spanish Government, and not affected by the restrictions of EU regulation, is micronised talc (hydrated magnesium silicate of particle size lower than 40 μm) due to its exclusive physical action. Very recently our group has reported the feasibility of using common salt (sodium chloride) as a coadjuvant for the physical extraction of olive oil with similar oil yields as micronised talc and with non-significant changes in the main physico-chemical parameters of the oil [10]. The presence of NaCl in the olive pastes increases the density and the ionic strength of the aqueous phase that could affect the solubility of certain compounds and may even modulate the activity of those enzymes actives during the malaxation process. The aim of this study is to further characterise the effect of common salt, used as coadjuvant during the olive oil extraction process, on the oil physicochemical indexes, stability against oxidation, pigment composition, aroma composition, phenolic profile and volatile components of VOO. Green-mature "Picual" fruits were used, because the oil with highest natural antioxidant content is extracted from these ripening level olives, but, at same time, these fruits offer lower yields than the riper ones. The use of common edible salt during oil extraction could allow one to obtain the highest possible amount of the best quality oil.

Experimental Procedures

Plant Material

Olive fruits (*Olea europaea* L. cv. Picual) were harvested during the 2005–2006 season in Cabra (Córdoba) at the green-mature stage of ripening (RI = 1). Thirty kilograms of healthy fruits were transported to the Instituto de la Grasa, where the ripening index (RI) was carefully evaluated, using a subjective evaluation of the colour of the olive skin and flesh [10].

Oil Extraction

Olive fruits were randomly distributed in five treatment groups. Each treatment group was divided into four 1 kg batches (4 replicates per treatment) that were milled separately. A sample of 800 g paste was taken from each replicate and weighed in a metallic pitcher. A measure of 10 g of micronised talc were added to each 800 g paste samples and additionally 0.0 (control), 2.5, 5.0, or 10.0% w/w of common edible salt was added to each treatment group. The paste of each pitcher was homogenised for 1 min with a spatula, and the oil was extracted, using an Abencor analyser (Commercial Abengoa S.A., Seville, Spain). This unit, consisting of three basic elements: a mill, a thermobeater, and a pulp centrifuge, simulates on a laboratory scale the industrial process of VOO production [11]. After centrifugation the oil was decanted in a graduated tube to measure the volume obtained to calculate the oil yield, which was expressed as a percentage of the fresh weight.

Paste Analysis

From each replicate of the control treatment, samples of 50 g of the surplus paste was separately weighed in a previously weighed capsule and dried at 105 °C to constant weight. The oil of the dried paste was chemically extracted with hexane, using the Soxhlet method, to evaluate the total oil content of the paste. The results of total oil content are presented as a percentage of the paste's fresh weight. The extractability of the different treatments tested in each variety was calculated as the percentage of oil physically extracted from the total oil content of the fruit.

Oil Analysis

The titratable acidity, the peroxide index, the coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were determined from the extracted oils according to the European Union standard methods [12]. Induction time was measured by the Rancimat method, which evaluates the time (hours) of resistance to oxidation of 3 g oil samples exposed to a stream of dry air at a temperature of 100 °C [13].

Analysis of Phenolic Compounds

Phenolics of VOO were isolated by solid phase extraction on a diol-bonded phase cartridge (Supelco, Bellefonte, PA)

following a previously described procedure [6]. *o*-Coumaric and *p*-hydroxyphenyl-acetic acids were used as internal standards in this extraction procedure. Phenolic extracts were further analysed by HPLC in an AKTA Basic 10/100 liquid chromatographic system equipped with a UV-900 detector and a Mediterranea Sea 18 column (4.0 mm i.d. × 250 mm, particle size 5 μm) (Teknokroma, Barcelona, Spain). Elution was performed at a flow rate 1.0 ml/min, using as mobile phases water/phosphoric acid (99.5:0.5) (solvent A) and methanol/acetonitrile (50:50) (solvent B). Two phenolic extracts were isolated from each oil sample replicate and, subsequently, were analysed by HPLC. Quantification of phenols (except ferulic acid), cinnamic acid and lignans was carried out at 280 nm, while flavones and ferulic acid quantification was done at 335 nm. Response factors were calculated for each phenolic compound. Tentative identification of compounds was confirmed by HPLC-MS using a 126 pump with a 168 diode array detector (Beckman, Inc., USA) on-line with a MAT95's magnetic sector mass spectrometer (Finnigan Mat, Bremen, Germany) equipped with an ESI-II electrospray ionisation (ESI) interface with the same column and gradient conditions previously described [7].

Analysis of Volatile Compounds

Olive oil samples from control and 5.0% NaCl treatments were conditioned to room temperature and then placed in a vial heater at 40 °C. After 10 min equilibrium time, volatile compounds from the headspace were adsorbed onto a SPME fibre DVB/Carboxen/PDMS 50/30 μm (Supelco Co., Bellefonte, PA, USA). Sampling time was 50 min at 40 °C. Desorption of volatile compounds trapped in the SPME fibre was done directly into the GC injector. Volatiles were analysed using a HP-6890 gas chromatograph equipped with a fused silica capillary column DB-Wax (60 m × 0.25 mm J&W, Scientific, Folsom, CA, USA). Operating conditions were as follows: N_2 as carrier gas, injector and detector at 250 °C, and the column was held for 6 min at 40 °C and then programmed at 2 °C/min to 120 °C. Quantification was performed using individual calibration curves for each identified compound by adding known amounts of different compounds to re-deodorised high oleic sunflower oil. High oleic sunflower oil was selected due to its natural low volatile content. To further minimise the presence of volatile compounds sunflower oil was heated at 40 °C for 1 h under vacuum. The total absence of volatiles was assessed by a GC analysis. Compound identification was carried out on a HRGC-MS Fisons series 8000 (Fisons Instruments, Manchester, UK) equipped with a similar stationary phase column and two different lengths, 30 and 60 m, matching against the Wiley/NBS Library and by GC retention time against standards.

Pigment Analysis

Contents of chlorophyllic compounds and major carotenoids of VOO samples were determined in each replicate by dissolving 0.1 g of olive oil in 370 μL of ethyl acetate and resolving the sample by HPLC on a Beckman System Gold Programmable Solvent Module 126 coupled to a diode array detector Module 168, according to the method by Pérez et al. [14]. The column was a Beckman Ultrasphere ODS (C18) (250 × 2 mm), 5 μm, operated at 30 °C, fitted with a 20-μL injection loop in a Rheodyne valve. A two-step gradient elution utilised solvents A: acetonitrile/H_2O (90:10) and B: ethyl acetate, programmed at 0.5 mL/min with detection at 430 nm.

Statistical Analysis

Data were statistically evaluated using Statgraphics Plus 5.1 software (Manugistic Inc., Rockville, MD). One way analysis of variance was carried out on all data of each oil

Table 1 Oil yield, oil extractability (100 × oil yield/total oil content) and physico-chemical quality parameters of the oils physically extracted from "Picual" olives at the mature-green stage of ripening, using talc (1%) plus different NaCl concentrations as technological coadjuvant (total oil content 16.5%)

NaCl[a,b] (%)	Oil yield (%)	Extractability (%)	Acidity (% oleic)	Peroxides (mg O_2/kg)	K_{232}[c]	K_{270}[c]	Stability (h)
0.0	8.80 ± 0.22 a	53.33 ± 2.54 a	0.18 ± 0.06	9.35 ± 1.68 a	1.37 ± 0.04 a	0.105 ± 0.014 a	75.2 ± 4.5 a
1.0	8.45 ± 0.27 a	51.22 ± 2.91 a	0.13 ± 0.08	8.35 ± 1.84 a	1.42 ± 0.03 ab	0.109 ± 0.012 a	103.6 ± 5.5 b
2.5	10.67 ± 0.32 c	64,67 ± 2.44 c	0.17 ± 0.07	10.10 ± 1.48 a	1.46 ± 0.04 bc	0.135 ± 0.011 b	105.9 ± 5.3 b
5.0	9.72 ± 0.30 b	58.91 ± 2.32 b	0.21 ± 0.06	8.75 ± 1.59 a	1.50 ± 0.04 c	0.141 ± 0.013 b	103.4 ± 6.5 b
10.0	10.00 ± 0.38 b	60.61 ± 2.24 b	0.22 ± 0.08	13.99 ± 1.32 b	1.60 ± 0.05 d	0.165 ± 0.012 c	105.0 ± 6.5 b

[a] Each value represents the mean value of four replicates

[b] For each parameter, two values followed by different small letters are significantly different according to the Duncan's multiple-range test, absence of a small letter means no significant effect ($P \leq 0.05$) of the treatments detected by ANOVA

[c] K_{232} and K_{270} values represent the extinction coefficients of olive oil at these wave lengths

Table 2 Content of phenol compounds in the oils physically extracted from "Picual" olives at the mature-green stage of ripening, using different NaCl concentrations as a technological coadjuvant

Compound[a,b] (mmol/kg oil)	NaCl concentrations (%)				
	0.0 (control)	1.0	2.5	5.0	10.0
p-Coumaric acid	0.0073 a	0.0076 a	0.0102 b	0.0101 b	0.0078 a
Vanillic acid	0.0029 b	0.0026 b	0.0018 a	0.0022 a	0.0023 a
Cinnamic acid	0.0053 a	0.0068 b	0.0081 b	0.0102 c	0.0102 c
Ferulic acid	0.0053	0.0047	0.0062	0.0048	0.0065
Hydroxytyrosol acetate	0.0146 e	0.0113 c	0.0071 a	0.0124 d	0.0078 b
Hydroxytyrosol	0.0216 b	0.0100 a	0.0079 a	0.0073 a	0.0079 a
Tyrosol	0.0115 b	0.0076 a	0.0066 a	0.0078 a	0.0069 a
DGO[c]	0.3040 b	0.2956 b	0.1730 a	0.1840 a	0.1575 a
DGL[d]	0.1325 c	0.1171 bc	0.0619 a	0.0928 b	0.0621 a
AGO[e]	0.5230 a	0.7200 b	1.1470 c	1.4410 d	1.7960 e
AGL[f]	0.0701	0.0772	0.0730	0.0693	0.0791
Luteolin	0.1568 a	0.2485 b	0.3389 c	0.2556 b	0.2206 ab
Apigenin	0.0923 a	0.1199 ab	0.1536 b	0.1229 ab	0.1176 a
o-Diphenol derivatives	1.038 a	1.285 b	1.674 c	1.901 d	2.190 e
Secoiridoid derivatives	1.029 a	1.210 b	1.455 c	1.787 d	2.095 e
Total phenolic compounds	1.375 a	1.638 b	2.005 c	2.230 d	2.492 e

[a] Each value represents the mean value of four replicates

[b] For each compound, two values followed by different small letters are significantly different according to the Duncan's multiple-range test, absence of a small letter means no significant effect ($P \leq 0.05$) of the treatments detected by ANOVA

[c] Dialdehydic form of decarboxymethyl oleuropein aglycone

[d] Dialdehydic form of decarboxymethyl ligstroside aglycone

[e] Aldehydic form of oleuropein aglycone

[f] Aldehydic form of ligstroside aglycone

quality variable studied. If a significant ($P \leq 0.05$) effect was obtained by ANOVA, separation of the means was carried out using Duncan's multiple range test ($P \leq 0.05$).

Results and Discussion

Oil Yield

The physical extraction of oil from olive fruits at the mature green stage is difficult in all olive varieties giving rise to the so-called "difficult olive pastes". At the green ripening stage the high cellulose concentration of the cell wall and the high water content of the olive mesocarp cells induce the emulsion of the oil, reducing notably the efficacy of the centrifugation [15]. In a previous work [10] the efficacy of talc (1.2%) as a coadjuvant in the extraction of several olive cultivars was compared to that of NaCl (0.6 and 1.2%). Although the use of these low NaCl concentrations significantly improved the oil extraction, in most cultivars the highest oil yield was obtained with talc (Picual, verdial, Manzanilla and Hojiblanca) with the exceptions of Arbequina and Lechin in which NaCl proved to be a better coadjuvant than talc. Table 1 shows the oil yield and extractability obtained with green-mature fruits (RI = 1) when a combination of talc and NaCl is used as technological coadjuvant. The use of common salt at ≥2.5% combined with the use of micronised talc (1.2%) significantly improved the oil extraction from mature-green olives. Data obtained suggest that the increase of the electrostatic charge and density induced in the hydrophilic phase of the paste by the presence of NaCl synergistically helped the action of the talc in reducing the oil emulsion. However, the level of extractability achieved (64.67%) was lower than those normally obtained (≥75%) with ripe (RI = 5) "Picual" fruits [10]. It is well known that the seasons with high production of fruit always are followed by seasons with very poor production (olive alternation) and an early harvesting would reduce this effect and allows the extraction of oils with higher concentrations of natural antioxidants, pigments and green volatile compounds but it also affects the oil yield [16].

Oil Quality

In general, whereas the values of titratable acidity were not significantly affected by the use of NaCl during oil

extraction, the parameters which measure the level of oxidative oil deterioration (peroxide value, and ultraviolet absorbance) clearly increased with the addition of higher NaCl concentrations during the paste malaxation (Table 1). Nevertheless, the values obtained are considerably far from the limits established for extra VOO (20 mequiv O/kg for peroxide value; 2.50 and 0.22 for K_{232} and K_{270}, respectively) [12]. Furthermore, in spite of this, the stability values, measured as resistance to the oxidation by the Rancimat method, were significantly higher in the oils obtained with NaCl. The higher ionic charge of the hydrophilic phase induced by the presence of 1–10% salt in the olive pastes may increase the amount of antioxidant amphiphilic compounds in the oil, which would be mainly solved in the waste water under a conventional extraction without NaCl.

Phenolic Compounds

Table 2 shows the phenolic profiles of the oils studied. Total phenolic, *o*-diphenol and secoiridoid derivatives were proportionally increased in the oils obtained after NaCl addition. The clearest effect of the addition of NaCl during the malaxation of olive pastes was the increase of AGO, that doubled its content with 2.5% NaCl. Furthermore, the oil content of flavones (apigenine and luteoline) was also positively correlated with NaCl, presenting a maximum value, when the concentration of salt was 2.5%. The significant increase of *o*-diphenol compounds, whose antioxidant effect is well documented [17] might explain the NaCl-induced increase of oil stability. Recently, the effect of salts on the solubility in water of four different phenolic compounds (gallic acid, protocatechuic acid, vanillic acid and vanillin) was experimentally modelled [18]. Increasing salt concentrations greatly reduced the solubility of phenolic compounds in water and could have affected the partition coefficients of these compounds between the aqueous and oil phases coexisting during olive malaxation [19, 20]. Thus, the different effects of NaCl on each phenolic compound would depend on its specific partition coefficient between oil and water phases that ranges from 0.0006 for Oleuropein to 1.5 for AGO [20]. During the processes of olive milling and malaxation the phenolic glucosides oleuropein and ligstroside are hydrolised by endogenous β-glucosidases giving rise to secoiridoid derivatives that may be further oxidised by oxidoreductases [7]. In this sense, it should be noticed that an inhibitory effect of NaCl on a catechol oxidase from green olives has been reported [21]. Although modulation of biochemical factors could offer interesting possibilities for modifying the olive oil phenolic profile, limiting water solubility of phenolic compounds could also be a basis for designing alternative processes for enriching olive oil with these natural antioxidants [19].

Pigment Content

In general, common salt resulted in a significant increase in oil content of both carotenes (lutein and β-carotene) and chlorophylls (*a* and *b*) with the only exception being that the lutein content of the oil extracted using 1% NaCl was significantly lower than that of the control oil (Fig. 1). The increase of pigment accumulation in the oil induced by the addition of NaCl could be explained by two processes which are not necessarily exclusive. First of all a high saline concentration could possibly cause a better pigment release from the chloroplast and/or chromoplast by a more effective breaking of their membranes, and second an increase in NaCl concentration could induce the inhibition of enzymes, such as chlorophylase or lypoxygenase, which has been associated with pigment destruction during olive processing [3]. It is important to point out that the observed pigment increase affects not only olive oil colour but also its nutritional properties since oils obtained with NaCl as a coadjuvant have twice as much lutein and β-carotene, well known as health promoting compounds, than control oils.

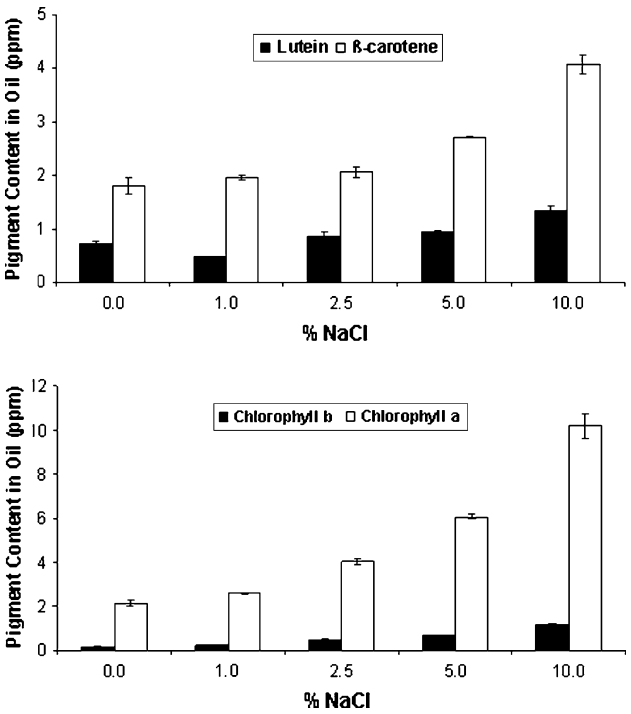

Fig. 1 Content on pigments of the oils physically extracted from "Picual" olives at a mature-green stage of ripening, using different NaCl concentrations as a technological coadjuvant

Table 3 Volatile compounds content of the oils physically extracted from "Picual" olives at the mature-green stage of ripening, without NaCl and using NaCl (5%) as a coadjuvant

Volatile compound[a,b] (ng/g olive oil)	Control	NaCl (5.0%)
(*E*)-Hex-3-enal	240.60 ± 21.19 a	391.99 ± 37.61 b
(*Z*)-Hex-3-enal	625.95 ± 117.38 a	1,200.18 ± 352.15 b
(*Z*)-Hex-2-enal	496.72 ± 49.21 a	1,062.41 ± 474.18 b
(*E*)-Hex-2-enal	16,522.72 ± 551.82 a	18,761.01 ± 272.60 b
∑ Aldehydes C6/LnA	17,885.99 ± 739.60 a	21,415.59 ± 1,136.54 b
(*E*)-Hex-3-enol	4.23 ± 3.72	2.23 ± 0.11
(*Z*)-Hex-3-enol	152.44 ± 4.39 a	183.38 ± 8.71 b
(*E*)-Hex-2-enol	21.00 ± 1.91 b	8.70 ± 0.78 a
∑ Alcohols C6/LnA	177.67 ± 10.01	194.30 ± 9.60
Hexanal	985.87 ± 37.31	2,022.47 ± 253.94
Hexanol	23.67 ± 0.43	24.52 ± 3.36
∑ C6/LA	1,009.54 ± 37.74 a	2,046.99 ± 257.30 b
Pentene dimers	5,131.87 ± 280.48 a	6,422.66 ± 513.27 b
Pent-1en-3-one	841.08 ± 81.37 a	1,431.05 ± 86.66 b
(Z)-Pent-2-enal	65.70 ± 8.61	93.30 ± 26.10
(E)-Pent-2-enal	149.84 ± 4.73 a	238.56 ± 13.94 b
Pent-1-en-3-ol	335.65 ± 19.20 a	686.69 ± 154.71 b
(Z)-Pent-2-en-1-ol	587.46 ± 19.83 a	1,239.37 ± 22.16 b
(E)-Pent-2-en-1-ol	127.70 ± 16.74	144.77 ± 2.47
∑ C5/LnA	7,239.29 ± 430.97 a	10,256.41 ± 819.30 b
∑ LOX esters	69.30 ± 5.84	71.59 ± 4.41
∑ Non-LOX esters	169.44 ± 51.85	253.31 ± 111.28

[a] Each value represents the mean value of four replicates ± SD

[b] For each compound, two values followed by different small letters are significantly different according to the Duncan's multiple-range test

Volatile Composition

The volatile profile of VOO obtained after NaCl addition compares favorably with that of control oil (Table 3). The increase of C6 and C5 aldehydes and alcohols that provide the green notes characterising VOO flavor, in oils obtained after NaCl addition may be assumed to be a positive effect of the treatment. Most VOO key volatiles are produced at the moment of tissue disruption of the olive pulp, at crushing, through the lipoxygenase pathway [22]. Although the malaxation process seems to be less important in terms of aroma biosynthesis [2], the incidence temperature and time of kneading has been reported by several authors as critical for the release of the volatiles previously formed and the transfer of these compounds to the oil [5]. In this sense, the increase of NaCl concentration in the water phase would favour the transfer of these volatile compounds to the oil, giving rise to VOO significantly richer in green odor notes.

Acknowledgments We would like to thank M.C. Martínez and M.M. Pascual for their technical assistance. This work was supported by Research Project AGL2005-03959 from Programa Nacional de Recursos y Tecnologías Alimentarias funded by the Spanish Government.

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