J Am Oil Chem Soc (2009) 86:1137–1140
DOI 10.1007/s11746-009-1445-9

LETTER TO THE EDITOR

Influence of Vertical Centrifugation on Extra Virgin Olive Oil Quality

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Received: 27 April 2009 / Revised: 10 July 2009 / Accepted: 15 July 2009 / Published online: 5 August 2009

Abstract The qualitative effects of vertical centrifugation (VC), i.e., the last step of the extra virgin olive oil (EVOO) extraction process, were investigated on an industrial scale by sampling EVOOs before and after VC. Several parameters were determined to evaluate EVOO quality. Vertical centrifugation results in a marked loss of volatile aromatic compounds, whereas only a slightly variation was recorded in the hydrophilic phenols concentration.

Keywords Vertical centrifugation · Extra virgin olive oil · Washing water · Dissolved oxygen · Olive oil aromatic compounds

Introduction

Extra virgin olive oil (EVOO) is the main vegetable fat source in the diet of the Mediterranean region [1]. Characteristic aroma, taste, color and nutritive properties of this product distinguish it from other edible vegetable oils [2]. Olive oil quality is strictly related to the extraction process [3–5], which consists of crushing the olives to a paste, paste malaxation, paste centrifugation to obtain an oily must (oil that contains small amounts of residual vegetative water and impurities). The oily must, especially in the two-phases extraction system, requires a further cleaning that is performed by washing the oil in a vertical centrifuge with lukewarm tap water added [3]. The first three process phases have been well studied in terms of effects on the oil quality, but information on VC is rather limited. Garcia et al. [6] and Di Giovacchino et al. [7] focused the study on the effect of VC on hydrophilic phenols whereas any statistic validation of the results was not attempted. In a previous work [8], we found that the processing steps contribute differently to the amount of dissolved oxygen in olive oil, and the VC step afforded the highest oxygenation effect. However, an evaluation of the influence of VC on the overall oil quality was not done, as important parameters such as fatty acids composition, pigment content, the HPLC phenolic profile and volatile components concentration, were not measured. Therefore, the aim of this work was to evaluate the effect of VC on olive oil quality with respect to these quality parameters.

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Materials and Methods

Experimental Procedure

Trials were performed on an industrial scale by means of a two-phase continuous centrifugation plant model Jumbo2 (Pieralisi, MAIP spa, Jesi - Ancona, Italy). To ascertain the effect of VC on olive oil quality, three trials were performed by sampling the oils before (BVC) and after the vertical centrifuging (AVC), i.e., three pairs of samples

were compared. The trials were performed by processing 1,500 kg of olives cv. *Frantoio* at medium ripeness and under good sanitary conditions, the olives were harvested near Florence. The olives were homogenized and then divided into three batches of about 500 kg, each batch corresponding to one out of the three trials. Processing conditions were 45 min malaxation time, 28 °C malaxation temperature, 3,500 centrifugation speed for horizontal decanter feed of the olive paste at 1,000 kg h^{-1} . The oily must was cleaned by a vertical centrifuge operating at 6,500 rpm and fed with 0.25 L tap water/kg oily must. The oils were sampled in quadruplicate (250 cm^3) before and after VC at different times during separation in each trial (i.e., every 4 min). Then, they were merged and homogenized to obtain a single sample per trial. Samples were stored in green screw-cap glass bottles in the dark until chemicals analyses, i.e., 1 week. Immediately after processing the dissolved oxygen concentration was measured on all samples and repeated 1 week later along with the other analyses.

Chemicals Analyses, Determinations and Data Elaboration

Dissolved oxygen concentration (mg L^{-1}) was measured in duplicate at the same olive oil temperature (20 °C) by a portable oxygen analyzer model InTap4000 (Mettler-Toledo S.p.A, Italy) [8]. Humidity concentration (wt.%) was determined according to the AOCS Ca 2c–25 (1997) gravimetric method [9]. Free acidity (FA), peroxides value (PV), UV specific extinction coefficients and fatty acid composition were determined according to the analytical method of the European Official Method of Analysis (European Regulation EEC 2568/91, 1991) [10]. Total chlorophyll (Chlo) concentration (mg kg^{-1}) was determined spectrophotometrically according to Pokorny et al. [11] and expressed as pheophytin α. Hydrophilic phenols profile was determined according to the method of the SSOG Technical Commission [12]. The method allows extraction and HPLC quantification of natural and oxidized derivatives of oleuropein and ligstroside, lignans, flavonoids and phenolic acids. For the sake of simplicity, the identified compounds were grouped in, simple phenols (Sph, i.e., hydroxytyrosol, tyrosol), secoiridoids (Seco, i.e., decarboxymethyl oleuropein aglycone dialdehyde and oxidized forms, decarboxymethyl ligstroside aglycon dialdehyde and oxidized forms, oleuropein aglycon aldehyde and hydroxylic forms and oxidized form, oleuropein, oleuropein aglycon dialdehyde form, ligstroside aglycon dialdehyde form, ligstroside aglycon aldehyde and hydroxylic form and oxidized form), lignans (Lig, i.e., pinoresinol, 1-acetoxy-pinoresinol) and the summation of flavonoids and phenolic acids (F&Phac, i.e., vanillin, luteolin, apigenin, methyl-luteolin, vanillic ac., para-coumaric ac., ferulic ac., cinnamic ac.). The HPLC system was as already described [13], analytical conditions were as reported in the method. Gas chromatography-mass spectrometry analysis of volatile compounds was performed by an Agilent 7890 GC equipped with an Agilent 5975C inert XL MSD quadrupole mass spectrometer, after automated SPME sampling. A supelco stableflex PDMS/DVB/CARBOXEN fiber (30 μm) was used for SPME extraction. Preliminary experiments (data not reported) indicated that this fiber was superior over other fibers commercially available as it provided the best compromise between reproducibility, minimal carryover effect, and least selectivity toward polar/nonpolar or quick/late eluting compounds. The aim was, in fact the characterization of the broadest possible range of volatile compounds in the EVOOs under study. Olive oil samples (3 g) were weighed in 10-mL magnetic screw cap vials, supplemented with 100 mg of internal standard solution (ethyl nonanoate) and individually heated at 80 °C upon analyses. Then, volatile compounds from the headspace were adsorbed onto the fiber for 45 min. Analytical conditions were: injector and transfer line at 240 °C; temperature programmed at 35 °C for 1 min, 5 °C increase per minute up to 150 °C, 10 °C increase per minute up to 240 °C, isotherm 240 °C for 6 min. The compounds were quantitated with calibration lines obtained with pure standard compounds at different concentration after the addition of the internal standard. According to Sanchez-Ortiz et al. [14], volatile compounds were clustered into different classes as related to the polyunsaturated fatty acid and the LOX pathway branch origin. Quantitative data for every volatile class are the sum of the contents of the following compounds: C6/LnA [(*E*)-hex-2-enal; (*Z*)-hex-3-enol; (*E*)-hex-2-enol]; C6/LA [Hexanal; hexan-1-ol]; C5/LnA [pent-1-en-3-one; pent-1-en-3-ol; (*E*)-pent-2-enal]; Esters [3-Hexen-1-ol acetate; ethyl acetate; 2-hydroxybenzoate]; Others [pentanal]. Determinations for all the parameters were repeated in duplicate and the two values averaged. Statistical significance of the investigated treatment was evaluated through paired *t* test, i.e., three pairs to compare between BVC and AVC.

Results and Discussion

Vertical centrifugation cleaned the oil efficiently as it allowed the EVOO humidity concentration to be reduced significantly to a mean value of about 0.18% (Table 1), in agreement with the values already reported [15]. The dissolved oxygen concentration, standard quality parameters and fatty acid composition of BVC and AVC are shown in Table 1. Confirming the values observed in a previous

Table 1 Effect of vertical centrifugation on EVOO standard quality parameters, dissolved oxygen content, fatty acid composition and humidity concentration

Parameter	BVC	AVC	MD
Dissolved oxygen (mg L^{-1})	1.88 (0.66)	8.42 (0.97)	6.54 (0.99)**
FA (%)	0.34 (0.06)	0.24 (0.01)	0.10 (0.06)^{ns}
PV (mequiv O_2 kg^{-1})	4.93 (0.68)	6.87 (0.68)	1.93 (0.68)*
K232 (1%, 1 cm)	1.60 (0.04)	1.83 (0.04)	0.23 (0.07)*
K270 (1%, 1 cm)	0.14 (0.02)	0.16 (0.02)	0.02 (0.03)^{ns}
Humidity (wt.%)	0.42 (0.10)	0.18 (0.07)	0.24 (0.03)**
Fatty acids (%)			
Myristic	0.01 (0.00)	0.01 (0.00)	–
Palmitic	12.43 (0.21)	12.34 (0.35)	0.09 (0.14)^{ns}
Palmitoleic	0.97 (0.05)	0.97 (0.07)	0.00 (0.02)^{ns}
Heptadecanoic	0.04 (0.00)	0.04 (0.00)	–
9-Heptadecenoic	0.08 (0.00)	0.08 (0.00)	–
Stearic	2.07 (0.02)	2.09 (0.02)	0.02 (0.01)^{ns}
Oleic	75.39 (0.64)	75.40 (0.78)	0.01 (0.16)^{ns}
Linoleic	7.76 (0.40)	7.81 (0.41)	0.05 (0.01)
Arachidic	0.32 (0.01)	0.33 (0.01)	–
Linolenic	0.51 (0.00)	0.52 (0.01)	0.01 (0.01)^{ns}
11-Eicosenoic	0.24 (0.01)	0.25 (0.01)	0.01 (0.01)^{ns}
Behenic	0.10 (0.01)	0.10 (0.01)	–
Lignoceric	0.04 (0.00)	0.04 (0.01)	0.00 (0.01)^{ns}
trans-C18:1	0.02 (0.01)	0.01 (0.01)	0.01 (0.01)^{ns}
trans-C18:2 + trans-C18:3	0.03 (0.00)	0.03 (0.00)	–

Note: *BVC* oil before vertical centrifugation, *AVC* oil after vertical centrifugation, *MD* mean difference; data are means of three independent replicated experiments; standard deviations are reported in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01, *ns* not significant

Table 2 Effect of vertical centrifugation on minor components concentrations (mg kg^{-1}) in EVOO

Parameter	BVC	AVC	MD
Chlo	109.33 (14.57)	145 (12.17)	−35.67 (25.77)^{ns}
Sph	17.53 (3.72)	5.01 (0.46)	12.52 (3.27)*
F&PA	36.04 (3.17)	33.1 (2.81)	2.94 (1.64)^{ns}
Lign	69.57 (6.53)	68.87 (7.12)	0.70 (3.26)^{ns}
Seco	364.07 (71.89)	377.89 (63.87)	−13.82 (38.77)^{ns}
Total phenols	487.2 (80.97)	484.87 (73.61)	2.34 (40.0)^{ns}

Note: *BVC* oil before vertical centrifugation, *AVC* oil after vertical centrifugation, *MD* mean difference; data are means of three independent replicated experiments; standard deviation are reported in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01, *ns* not significant

work [8] there was a remarkable oxygenation effect of the VC as indicated by the marked increase in dissolved oxygen concentration in the AVC vs. BVC immediately after processing. At the time of qualitative analyses, i.e., 1 week after the oil's production, the dissolved oxygen concentration was about 0 mg L^{-1} in all the samples. Contextually, significantly higher values of the oxidative indexes PV and K232 were recorded as results of VC, i.e., AVC showing values of about 40 and 14% higher than BVC, respectively. According to what those described by Parenti et al. [8] this condition could result in faster decay kinetics of EVOO during storage. No significant differences were found both in free acidity and fatty acid composition. Despite the strong oxygenation effect, VC slightly affects the minor components of EVOO as reported in Table 2. Significant differences were recorded only for simple phenols, whereas the main and most important phenolic class, i.e., the secoiridoids, showed similar concentrations between BVC and AVC. Similar results were in agreement with Garcia et al. [6], who reported only minor variation of non-orthodiphenols and orthodiphenols. By contrast, Di Giovacchino et al. [7] reported a decrease after VC both for total phenols and orthodiphenols concentration as a function of increasing amounts of washing water (from 0 to 80% of the oily must). This discrepancy could be related to the small water amounts added in the present experiment (about 25%). In fact, during processing hydrophilic phenols are subject to chemical-physical changes, i.e., combination of chemical and biochemical reactions (both enzymatic and not enzymatic hydrolysis and oxidation) related to operative conditions such as temperature and oxygen availability [4]. During malaxation and decanter centrifugation, they dissolve in the oil and water phases according to their relative affinities toward these phases (partition coefficient Kp) [16]. So, as described for three-phase decanting [3] the addition of water can result in a decrease in phenols of hydrophilic nature in relation to the compounds specific Kp. Rodis et al. [16] reports that EVOO phenols have very different Kp, i.e., oleuropein 0.0006, hydroxytyrosol 0.0100, tyrosol 0.0770, decarboxymethyl oleuropein aglycon dialdehyde 0.1890, oleuropein aglycon 1.4900. So, the very low Kp of simple phenols in comparison to other phenols such as secoiridoids, can contribute to explaining the observed decrease as a consequence of VC. The results of volatile compounds concentration are reported in Table 3. Significant differences were recorded both in the total volatile concentration and in the two volatile classes from the LOX pathway involving LnA conversion (both C5 and C6 compounds). These compounds are synthesized from nonesterified polyunsaturated fatty acids containing a (*Z*,*Z*)-1,4-pentadiene structure that in the first LOX step, by using molecular oxygen as cosubstrate, produces 13-hydroperoxide derivatives that are subsequently cleaved heterolytically by hydroperoxide lyase (HPL) to C6 compounds [5]. C5 compounds would be generated through an

Table 3 Effect of vertical centrifugation on volatile compounds concentrations (μg kg^{-1}) in EVOO

Volatile classes	BVC	AVC	MD
C6/LnA	97027 (4059)	81390 (1923)	15637 (2186)**
C6/LA	7990 (835)	9230 (2651)	−1240 (2819)^{ns}
C5/LnA	7207 (517)	5733 (305)	1473 (465)*
Esters	3227 (626)	3213 (297)	14 (664)^{ns}
Others	243 (21)	240 (50)	3 (59)
Total volatiles	115693 (5013)	99807 (3462)	15887 (4413)*

Note: *BVC* oil before vertical centrifugation, *AVC* oil after vertical centrifugation, *MD* mean difference; data are means of three independent replicated experiments; standard deviations are given in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01, *ns* not significant

additional branch of the LOX pathway that would involve the production of a 13-alkoxyl radical by a homolytic way [5]. Angerosa et al. [17] pointed out that, after a very fast biosynthesis of volatiles during the crushing step, the partition phenomena between the oil and water phases would be the main factor responsible for the variations of the volatile content in the oils during the malaxation step. In analogy, we can suppose that the observed decrease of C6/LnA and C5/LnA compounds was partly the result of volatiles partition between oil and water phases during VC. Moreover, as stated by the occurrence of strong oxygenation, we could presume that VC determines a stripping effect by air that partly removes the volatile compounds. This result was confirmed by the comparison of the overall odor of BVC and AVC oils. As a general trend the BVC samples showed more marked fruity notes. No defects were perceived by the tasters in any samples.

Conclusion

The experimental results showed that VC is an important step in EVOO processing that could determine important variations of some aspects of the product quality. This suggests the need for engineering suitable vertical centrifuges designed to limit the oxygenation effect, i.e., increase in dissolved oxygen concentration, and the loss of aromatic volatile compounds. This option could result in the production of EVOO of improved quality especially in terms of prolonged shelf-life and preservation of positive sensory notes.

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