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New Filtration Systems for Extra-Virgin Olive Oil: Effect on Antioxidant Compounds, Oxidative Stability, and Physicochemical and Sensory Properties

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ABSTRACT: The purpose of this work was to evaluate some new filtration systems in relation to the quality of extra-virgin olive oil (EVOO). Filtration processes were undertaken using a polypropylene filter bag and two different inert gas flows as filter aids (argon and nitrogen). Qualitative and quantitative variations of the glyceride composition, antioxidant and pro-oxidant compounds, and water content were correlated with the oxidative stability to establish the effect on EVOO shelf life. The influence on physicochemical and sensorial properties was also evaluated. After filtration, the oxidative stability was reduced. The behavior of the polyphenols and water content on the filtration process could explain the lowest oxidative stability of filtered EVOO. Moreover, the results of the sensorial analysis confirmed that filtration using inert gases did not decrease the intensity of the main positive sensory attributes. The results could help olive-oil producers to improve EVOO quality and establish optimal storage conditions.

KEYWORDS: *olive oil, filtration, filter bag, nitrogen/argon gas flow, oxidative stability, chlorophylls, tocopherols, polyphenols, sensory properties*

INTRODUCTION

Cloudy extra-virgin olive oil (EVOO) is the fresh olive juice obtained exclusively by mechanical and physical processes. The extraction process includes the following main steps: collecting, washing, and pressing of olives, malaxation of olive paste, centrifugation, decantation, storage, and packaging (bottling). However, European Community Regulation establishes the possibility of including EVOO filtration prior to the bottling of oil.[1] Filtration is especially important as a final step to remove suspended solids or moisture and make the olive oil more brilliant for consumer acceptance. Different filtration systems have been applied in the olive-oil industry: conventional filtration systems (filter tanks and filter presses) and cross-flow filtration (tangential flow filtration).[2,3]

Conventional filtration systems use organic or inorganic filter aids in conjunction with filtration equipment (tanks or presses) to enhance or enable suspended solids and water−oil separations. Diatomaceous earth and cellulose fibers are commonly used as filter aids,[3−5] which are deposited on the surface of the filtration equipment by filtering specially prepared mixes.[2] On occasion, nonpower filter aids such as cotton or cellulose paper may be used instead of diatomaceous earth and cellulose fibers in filter presses.[6]

Tangential filtration systems have been characterized by perpendicular flow through the membrane in several studies using olive oil and different membrane types at the laboratory scale.[7] However, this procedure is not widespread in the olive oil industry due to the characteristics, composition, and physicochemical properties of olive oil.

In recent years, two new filtration systems have been proposed as alternative processes to conventional and tangential filtering.[2] The first uses a filter-bag system as an innovative technique in the olive-oil sector. Olive oil is directly introduced into the filtration equipment constituted by the polypropylene filter bag. The fluid passes across the filter bag, and suspended solids are removed. The second system is a new filtration methodology based on the flow of an inert gas (nitrogen or argon), which is introduced directly in the center of the olive-oil mass. The gas insertion generates a circular movement of the oil mass that facilitates the separation of the suspended solids. It is important to underline that this new process prevents organic matters from coming into contact with the olive oil.

EVOO is a food credited with providing multiple health benefits for humans related mainly to minor components.[8−12] During these filtering operations, quantitative and qualitative changes take place, especially on these minor components, which are of great value in establishing the quality and health value of EVOO.[13,14] The effects of conventional and cross-flow filtration on the oxidative stability, sensorial and physicochemical characteristics, and antioxidant components have been reported in the past decade.[2,6,15,16] However, the impact of the

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new filtration systems on these characteristics has not been evaluated.

The aim of this work was to evaluate the new filtration systems, based on a filter bag and inert gas flow, in relation to the quality of EVOO with particular emphasis on water content, oxidative stability, pigments, antioxidant compounds, and physicochemical as well as sensory properties. This is the first available comparison between filtered and unfiltered EVOOs using new filtration systems.

MATERIALS AND METHODS

Samples. The EVOO used in this study was of the Peranzana olive variety. In November 2010, olives were collected in the south of Italy (Foggia, Apulia Region) and processed by continuous industrial plants equipped with a hammer crusher, a horizontal malaxator, and a two-phase decanter. Cloudy olive oil was filtered at room temperature using new filtration systems based on three different filter aids: (a) a polypropylene bag, (b) argon gas flow, and (c) nitrogen gas flow. Fifty liters of EVOO was filtered for each new filtration system in triplicate. Filtered samples were stored in bottles without headspace at room temperature and in darkness before analysis.

Filter-bag equipment (developed by Filterflo) consisted of two compartments, a cylindrical tube and filter bag, made up of a unique polypropylene bag (1−5 μm i.d.) and its support. The filter bag was introduced into the cylindrical tube, and the system was pressurized by hydraulic closure. Cloudy olive oil was directly conducted from storage tanks to filter tank, passing through the filter bag.

Filtration equipment based on the flow of an inert gas consisted of a filter tank and inert gas tank developed and patented by University of Bologna and Sapio.[17] Cloudy olive oil was placed in the 50 L filter tank and connected at the bottom to the tank of inert gas, using an insertion device. The flow rate of both gases injected directly into the center of the olive-oil mass was 15 L/min.

Chemicals and Apparatus. All chemicals were of analytical reagent grade. Potassium and sodium hydroxides, methanol, *n*-hexane, cyclohexane, isopropanol, and isooctane were purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from Fluka, Sigma-Aldrich (Steinheim, Germany). Hydranal-Tritan 2 and Hydranal-solvent oil were from Riedel-deHaën (Seelze, Germany). Standards of α-tocopherol, hydroxytyrosol, tyrosol, luteolin, apigenin, and quinic acid were purchased by Sigma-Aldrich (St. Louis, MO), and (+)-pinoresinol was acquired from Arbo Nova (Turku, Finland). Oleuropein was purchased from Extrasynthese (Lyon, France). Double-deionized water with conductivity less than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA). The vacuum pump used for this work was a Millipore pump model WP6222050 (Millipore).

Analytical Determination of the Quality Indices. Free acidity, peroxide value (PV), and UV spectrophotometric indices (K_{232} and K_{270}) were determined according to the analytical methods described in EEC Regulations 2568/91 and the following amendments.[1] All parameters were determined in triplicate for each sample. Free acidity was given as a percentage of oleic acid and PV expressed in milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg). Spectrophotometric determinations were made using an UV−vis 1800 instrument (Shimadzu Co., Kyoto, Japan). The K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively. Oil samples were previously diluted (1:10 v/v) in isooctane when the K_{232} extinction coefficient was analyzed.

Fatty Acid (FA) Composition. The FA composition of filtered and unfiltered oil samples was determined as fatty acid methyl esters (FAMEs) after alkaline treatment, obtained by mixing 0.05 g of oil dissolved in 2 mL of *n*-hexane with 1 mL of 2 N potassium hydroxide in methanol; before the gas chromatographic analysis, the upper phase containing FAME was diluted 1:10 with *n*-hexane. A gas chromatograph (GC) Clarus 500 Perkin-Elmer (Shelton, CT) equipped with an autosampler, split/splitless injector, and flame ionization detector (FID) was used for this determination. The chromatographic analysis was performed according to Maggio et al.[18] Analytes were separated on a RTX-2330 capillary column (30 m × 0.25 mm i.d., 0.2 μm film thickness) from Restek (Bellefonte, PA) and helium as the carrier gas (flow rate, 0.8 mL min^{-1}; split ratio of 1:30, v/v). The injection volume into a split GC port was 1 μL. The column temperature was held at 140 °C for 5 min and then increased by increments of 2.5 °C min to 240 °C. The FID and the injector temperatures were both set at 250 °C. Gas chromatographic data were processed by the software Total-Chrom Navigator (version 6.2.1) from Perkin-Elmer, and the FA composition was expressed as weight percentage of total FAMEs present (g/100 g of FAME). Three replicates were prepared and analyzed per sample.

Water Content in Filtered and Unfiltered Olive-Oil Samples. The water content was analyzed with a TitroMatic 1S instrument (Crison Instruments, S.A.; Alella, Barcelona, Spain) following the method described by Gomez-Caravaca et al.[6] Filtered and unfiltered EVOOs were dissolved in a solution of chloroform/Hydranal-solvent 2:1 (v/v), and the titrating reagent (Hydranal-Titran 2) was added until the equivalence point was reached. Each sample was introduced three times, and the quantity of the sample was measured with the back-weighting technique. The quantity of water was expressed as mg of water/kg of oil (n = 3). The RSD of the water method was 1.5.

Oxidative Stability Index (OSI) Time. The oxidative stability under forced conditions was determined using an eight-channel oxidative stability instrument (OSI) (Omnion, Decatur, IL). The OSI time was determined according to Maggio et al.[18] Briefly, samples (5 g) were loaded onto each channel and heated at 110 °C under atmospheric pressure. At this stage, the air flow was injected into the center of the sample mass to bubble through the oil at 150 mL min^{-1}, generating a conductivity increase due to short-chain volatile acid formation. This increase, measured in distilled water channels directly connected to the sample ones, determined an induction period (OSI time), expressed in hours and hundreds of hours. OSI time was tested three times for each sample.

Pigments. The total chlorophyll and carotenoid contents were calculated by spectrophotometric method at 670 and 470 nm, respectively. The absorption at 670 nm is usually considered to be related to the chlorophyll fraction (pheophytin "a" as its major component) and 470 nm to the absorption of the carotenoid fraction (lutein). EVOOs (7.5 g) were accurately weighted and dissolved in 25 mL of cyclohexane, and their concentrations were calculated using the absorbance value and specific extinction coefficients (613 for pheophytin "a" and 2000 for lutein) according to Minguez-Mosquera et al.[19] Three replicates were prepared and analyzed per sample.

Antioxidant Compounds. Analytical methods to characterize the phenolic profile in filtered and unfiltered EVOOs were performed in an Agilent 1200-RRLC system (Agilent Technologies, Waldbronn, Germany) of the Series Rapid Resolution equipped with a vacuum degasser, autosampler, a binary pump, and a diode array detector (DAD) detector.

Regarding lipophilic fraction of phenols, 0.3 g of samples was dissolved in 10 mL of isopropanol and filtered through a 0.25 μm filter before the RRLC analysis to characterize the individual content of tocopherols. The chromatographic separation of these compounds was performed on a 150 mm × 4.6 mm i.d., 120 Å, Cosmosil π NAP column (CPS Analitica, Milan, Italy). The mobile phases used were water with 0.2% phosphoric acid as eluent A and methanol as eluent B in isocratic gradient (10:90, v/v). The flow rate was 1 mL/min, and analyses were made at room temperature. The injection volume in the RRLC was 20 μL. The total run time was 30 min. Tocopherols separated were quantified with DAD detector at 295 nm. A calibration curve was calculated by using six points of α-tocopherol at different concentrations, estimated from the amounts of the analytes in samples, and was linear over the range studied (r^2 = 0.999) . Results were given in mg of α-tocopherol per kg of oil.

Polar phenols were isolated from the EVOO using solid-phase extraction (SPE) with Diol-cartridges (bed weight 1000 mg, 6 mL of tube size), following the method described by Lozano-Sanchez et al.[20] EVOO (60 g) was dissolved in *n*-hexane and loaded into the column. The cartridge was washed with 15 mL of *n*-hexane. Finally, the sample

Table 1. Quality Indices and FA Composition of Filtered and Unfiltered EVOOs[a]

		filtered EVOO			
quality indices	unfiltered EVOO	filter bag	argon gas	nitrogen gas	legal Limits[b]
PV	6.82 a ± 0.12	2.46 b ± 0.04	1.84 b ± 0.01	1.84 b ± 0.06	≤20
K_{232}	1.56 a ± 0.09	1.61 a ± 0.04	1.64 a ± 0.05	1.60 a ± 0.08	≤2.5
K_{270}	0.11 b ± 0.00	0.15 a ± 0.00	0.16 a ± 0.00	0.10 b ± 0.00	≤0.22
FA	0.28 a ± 0.01	0.28 a ± 0.00	0.21 b ± 0.00	0.22 b ± 0.00	≤0.8
FA composition	unfiltered EVOO	filter bag	argon gas	nitrogen gas	legal limits/usual range[b]
C16:0	12.53 a ± 0.17	12.65 a ± 0.18	12.63 a ± 0.04	12.58 a ± 0.14	7.5−20
C16:1 n-9	0.09 a ± 0.00	0.09 a ± 0.00	0.09 a ± 0.01	0.09 a ± 0.02	
C16:1 n-7	0.75 a ± 0.05	0.75 a ± 0.01	0.74 a ± 0.01	0.74 a ± 0.01	0.3−3.5
C17:0	0.05 a ± 0.00	0.05 a ± 0.00	0.04 a ± 0.00	0.04 a ± 0.00	≤0.3
C17:1	0.08 a ± 0.00	0.07 a ± 0.00	0.08 a ± 0.00	0.07 a ± 0.00	≤0.3
C18:0	1.98 a ± 0.04	2.00 a ± 0.02	2.00 a ± 0.05	2.00 a ± 0.05	0.5−5
C18:1 n-9	71.10 a ± 0.23	71.34 a ± 0.03	71.30 a ± 0.00	71.42 a ± 0.07	55−83
C18:1 n-7	2.59 a ± 0.15	2.46 a ± 0.01	2.46 a ± 0.00	2.44 a ± 0.01	
C18:2 n-6	9.17 a ± 0.13	9.22 a ± 0.05	9.22 a ± 0.02	9.23 a ± 0.02	3.5−21
C20:0	0.41 a ± 0.03	0.35 a ± 0.02	0.36 a ± 0.00	0.36 a ± 0.02	≤0.6
C18:3 n-3	0.70 a ± 0.02	0.70 a ± 0.01	0.70 a ± 0.00	0.70 a ± 0.02	≤1
C20:1	0.30 a ± 0.01	0.31 a ± 0.02	0.30 a ± 0.01	0.31 a ± 0.02	≤0.4
MUFAs/PUFAs	7.53 a ± 0.08	7.56 a ± 0.05	7.52 a ± 0.02	7.56 a ± 0.04	
C18:1/C18:2	7.75 a ± 0.08	7.74 a ± 0.05	7.73 a ± 0.02	7.74 a ± 0.02	

[a]PV, PV expressed as mequiv O_2/kg; K_{232} and K_{270}, specific absorptions at 232 and 270; FA, free acidity given as a percentage of oleic acid; and FA composition, weight percentage of total FAMEs present (g/100 g of FAME). Values with the same letter in a line are not significantly different at a 95% confidence level ($p \leq 0.05$). [b]Legal limits and/or usual ranges are in accord to the values reported in Reg. UE 61/2011.

was recovered by passing through 40 mL of methanol, and the solvent was evaporated under vacuum. The residue was dissolved with 2 mL of methanol and filtered through a 0.25 μm filter before the RRLC analysis. For the qualitative and quantitative characterization of phenolic extract, RRLC was coupled to a time-of-flight mass spectrometer detector microTOF (Bruker Daltonik, Bremen, Germany), which was equipped with a model G1607A ESI interface (Agilent Technologies) operating in negative ion mode. A 150 mm × 4.6 mm i.d., 1.8 μm, Zorbax Eclipse Plus RP-C18 column (Agilent Technologies, Palo Alto, CA) was used for analytical analysis. The mobile phases used were 0.25% acetic acid as eluent A and methanol as eluent B. The total run time was 27 min using a previously reported multistep linear gradient.[20] The flow rate was 0.80 mL/min, and consequently, the use of a splitter was required for the coupling with the MS detector, as the flow which arrived to the TOF detector had to be 0.2 mL/min to ensure reproducible results and stable spray.

External mass spectrometer calibration was performed with sodium acetate clusters [5 mM sodium hydroxide in water/2-propanol 1/1 (v/v), with 0.2% of acetic] in quadratic þ high precision calibration (HPC) regression mode. The optimum values of the source and transfer parameters were established according to Lozano-Sánchez et al.[20] The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 10 ppm for most of the compounds.

The phenolic compounds were identified by comparing both retention times and MS data from samples and standards. The remaining compounds for which no commercial standards were available were identified by the interpretation of the information generated by the DAD, TOF analyzer, and the information reported (most compounds were previously described in olive oil). Quantification was made by RRLC-ESI-TOF-MS. Seven standard calibration curves of the main compounds found in the samples were prepared using seven commercial standards. All calibration curves showed good linearity over the study range (r^2 = 0.995). The individual concentrations were determined using the area of each individual compound (three replicates) and by interpolation of the corresponding calibration curve. Results are given in mg of analyte per kg of oil.

Physicochemical and Sensorial Analysis. Color was expressed with the chromatic coordinates L^*,a^*,b^* adopted by the CIE in 1976, where L^* is the brightness varying from 0 to 100, while a^* (from green to red) and b^* (from blue to yellow) are two chromatic components ranging between −120 and 120.[21] The CIELab color space analyses were conducted using a ColorFlex instrument (HunterLab, Reston, VA). Three replicates were analyzed per sample.

Sensory analysis was performed by a fully trained analytical taste panel for virgin olive oil of Dipartimento di Scienze degli Alimenti of Università di Bologna (recognized by the Italian Ministry of Agriculture, Food, and Forestry Policy-Mipaaf). Quantitative descriptive analysis (QDA) was applied to identify different sensory profiles between tested EVOO. Each taster was asked to identify olfactory and gustatory characteristics in samples (specifying the difference in terms of major or minor presence of bitter and/or pungent attributes). Each oil sample was analyzed by 10 tasters during three different sessions using the sensory ballot reported by Cerretani et al.[22] Samples were randomly distributed among the assessors.

Statistical analysis. Data were analyzed using Origin (version Origin Pro 8 SR0, Northampton, MA) to perform one-way-analysis of variance (ANOVA) at a 95% confidence level ($p \leq 0.05$) to identify significant differences among all parameters analyzed in filtered and unfiltered EVOO.

RESULTS AND DISCUSSION

Compositional Analysis. Table 1 includes the legal quality indices and FA composition in samples. Specific absorptions at 232 and 270 nm, PV, and free acidity (FA) were widely within the legal limits for EVOO category. A slight increase in K_{270} values was evidenced for filtered EVOO using argon gas flow and filter-bag systems. In any case, filtration did not have effects on the K_{232} values. On the other hand, the PV was reduced to less than half of the genuine value in unfiltered EVOO. The FA values showed only a slight but significant decrease when the inert gas-flow filtration procedures were applied.

Regarding FA content, percentages of palmitic, oleic, linoleic, and linolenic acids covered the usual range in olive oils. No differences were found among samples concerning MUFAs/PUFAs and C18:1/C18:2 ratios. In any case, filtration had no effect on FA composition.

Table 2. Quantitative Results (Value = X ± SD)[a]

	unfiltered EVOO (mg analyte/kg)	filtered EVOO (mg analyte/kg)		
		filter bag	argon gas	nitrogen gas
water content	2618.33 a ± 21.52	882.13 c ± 3.02	856.66 c ± 5.50	1337.49 b ± 27.48
OSI time (h)	27.13 a ± 0.59	25.40 c ± 0.06	25.10 c ± 0.09	26.45 b ± 0.06
carotenoids	4.37 a ± 0.09	2.18 b ± 0.01	2.47 b ± 0.01	2.64 b ± 0.08
chlorophylls	9.59 a ± 0.09	2.88 b ± 0.01	3.29 b ± 0.04	3.74 b ± 0.10
total pigments	13.96 a ± 0.07	5.05 b ± 0.01	5.74 b ± 0.03	6.38 b ± 0.30
α-tocopherol	239.79 a ± 8.14	230.57 a ± 5.60	228.80 a ± 5.60	231.55 a ± 6.24
δ-tocopherols	55.52 a ± 0.82	56.80 a ± 3.17	57.21 a ± 0.72	56.10 a ± 0.07
total lipophilic phenols	295.31 a ± 8.84	287.37 a ± 7.89	286.02 a ± 6.18	294.26 a ± 7.58
total hydrophilic phenols	193.77 c ± 9.72	230.61 a ± 6.21	379.38 a ± 8.92	211.43 c ± 9.54

[a]Values with the same letter in a line are not significantly different at a 95% confidence level ($p \leq 0.05$).

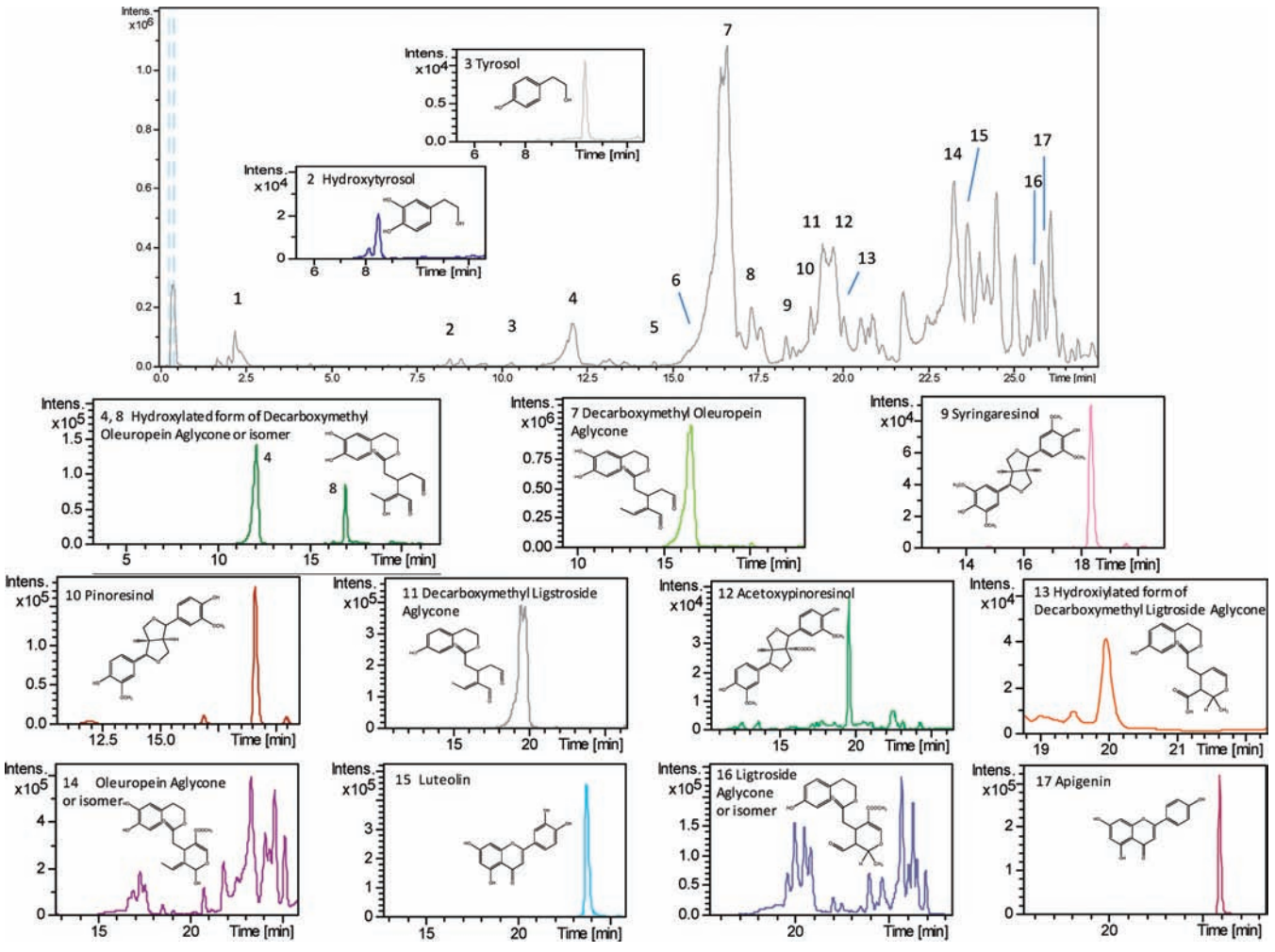

Figure 1. Top: base peak chromatogram (BPC) of the representative Peranzana EVOO phenolic extract obtained by RRLC-ESI-TOF-MS. Bottom: EIC of the main phenolic compounds identified in samples and their structures. Each compound has been numbered using the elution order obtained by RRLC-ESI-TOF-MS.

Water Content and OSI Time. Table 2 includes water content, OSI, pigments, and antioxidant compounds in samples. As expected, the highest water content was registered in unfiltered olive oil. The quantity of water significantly decreased in all filtered samples. Among these, olive oils filtered using argon gas flow and a filter bag had the lowest polar-phase contents. These systems could be considered the most effective ones for reducing the water content.

There was a slight tendency of OSI time to decrease after filtration. This effect was more pronounced when the argon gas and filter bag were used as filter aids than when nitrogen gas was used. These differences could be related to the relation between water content and antioxidant capacity of polyphenols. Indeed, different authors have demostrated that polar-phenolic compounds oriented in the water-in-oil emulsion interface are more protective against oxidation.[23] As a consequence, the

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Table 3. Main Phenolic and Other Polar Compounds Identified in Peranzana Monovarietal EVOO by RRLC-ESI-TOF

peak	compds	retention time (min)	m/z exptl	m/z calcd	molecular formula	error	σ
1	quinic acid	2.2	191.0564	191.0561	$C_7H_{12}O_6$	−1.6	0.014
2	hydroxytyrosol	8.5	153.0546	153.0557	$C_8H_{10}O_3$	7.6	0.006
3	tyrosol	10.3	137.0608	137.0608	$C_8H_{10}O_2$	0.3	0.035
4	hydroxylated form of decarboxymethyl-oleuropein aglycon or isomer	12.1	335.1140	335.1136	$C_{17}H_{20}O_7$	−1.0	0.004
5	hydroxytyrosol acetate	14.5	195.0654	195.0663	$C_{10}H_{12}O_4$	4.6	0.013
6	elenolic acid	15.3	241.0713	241.0718	$C_{11}H_{14}O_6$	2.9	0.039
7	decarboxymethyl oleuropein aglycon	16.3	319.1204	319.1187	$C_{17}H_{20}O_6$	−5.3	0.027
8	hydroxylated form of decarboxymethyl-oleuropein aglycon or isomer	16.6	335.1132	335.1136	$C_{17}H_{20}O_7$	1.3	0.010
9	syringaresinol	18.3	417.1540	417.1555	$C_{22}H_{26}O_8$	3.5	0.030
10	pinoresinol	19.0	357.1335	357.1344	$C_{20}H_{22}O_6$	2.4	0.007
11	decarboxymethyl ligstroside aglycon	19.4	303.1247	303.1229	$C_{17}H_{20}O_5$	−2.9	0.015
12	acetoxypinoresinol	19.5	415.1366	415.1398	$C_{22}H_{24}O_8$	7.8	0.023
13	hydroxylated form of decarboxymethyl- ligstroside aglycon	19.9	319.1179	319.1187	$C_{17}H_{20}O_6$	2.7	0.026
14	oleuropein aglycon	23.1	377.1266	377.1242	$C_{19}H_{22}O_8$	−6.4	0.046
15	luteolin	23.6	285.0416	285.0405	$C_{15}H_{10}O_6$	−4.1	0.024
16	ligstroside aglycon	25.5	361.1283	361.1293	$C_{19}H_{22}O_7$	2.9	0.019
17	apigenin	25.8	269.0460	269.0451	$C_{15}H_{10}O_5$	−1.8	0.034

Table 4. Quantitative Results of the Individual Phenolic Compounds (Value = X ± SD)[a] in the Unfiltered and Filtered Peranzana Monovarietal EVOOs

compds	unfiltered EVOO (mg analyte/kg)	filtered EVOO (mg analyte/kg)		
		filter bag	argon gas	nitrogen gas
total phenyl alcohol contents	2.36 b ± 0.11	2.28 c ± 0.11	3.64 a ± 0.15	2.77 b ± 0.10
hydroxytyrosol	0.94 b ± 0.03	0.61 c ± 0.04	1.10 a ± 0.04	0.94 b ± 0.05
tyrosol	1.02 c ± 0.09	1.20 c ± 0.11	2.02 a ± 0.09	1.44 b ± 0.06
hydroxytyrosol acetate	0.40 b ± 0.02	0.48 a ± 0.03	0.52 a ± 0.05	0.39 b ± 0.01
total secoiridoid contents	181.40 b ± 9.30	219.52 c ± 17.12	364.96 a ± 10.67	199.35 b ± 5.34
hydroxy D-oleuropein aglycon	1.19 c ± 0.04	0.73 d ± 0.05	2.85 a ± 0.09	1.50 b ± 0.13
elenolic acid	0.62 b ± 0.05	0.33 d ± 0.02	0.87 a ± 0.05	0.50 c ± 0.04
oleuropein aglycon	47.19 b ± 2.84	48.20 b ± 3.99	93.48 a ± 5.53	44.49 b ± 2.84
decarboxymethyl oleuropein aglycon	108.59 c ± 5.55	135.58 b ± 9.08	198.12 a ± 6.91	115.88 c ± 3.50
hydroxy D-oleuropein aglycon	0.27 b ± 0.01	0.34 b ± 0.02	1.05 a ± 0.00	0.31 b ± 0.01
ligstroside aglycon	3.08 c ± 0.19	3.62 b ± 0.18	4.92 a ± 0.39	2.91 c ± 0.10
decarboxymethyl ligstroside aglycon	20.29 c ± 1.58	30.53 b ± 2.68	63.34 a ± 2.76	33.50 b ± 1.66
hydroxy D-ligstroside aglycon	0.24 b,c ± 0.01	0.21 c ± 0.014	0.37 a ± 0.02	0.27 b ± 0.01
total lignan contents	4.16 b ± 0.12	3.70 c ± 0.18	4.45 a ± 0.14	4.05 b ± 0.08
pinoresinol	2.45 a,b ± 0.09	2.11 c ± 0.09	2.58 a ± 0.10	2.42 b ± 0.05
acetoxypinoresinol	0.70 a ± 0.01	0.54 c ± 0.02	0.63 b ± 0.03	0.63 b ± 0.01
syringaresinol	1.01 b ± 0.04	1.04 b ± 0.09	1.24 a ± 0.04	1.00 b ± 0.04
total flavone contents	5.84 b ± 0.17	5.11 c ± 0.15	6.33 a ± 0.12	5.26 b ± 0.17
luteolin	3.53 b ± 0.15	3.32 b ± 0.17	4.13 a ± 0.09	3.45 b ± 0.11
apigenin	2.31 a ± 0.08	1.79 b ± 0.09	2.20 a ± 0.05	1.81 b ± 0.08
other polar compounds: quinic acid	1.12 c ± 0.07		4.14 a ± 0.14	2.34 b ± 0.18

[a]Values with the same letter in a line are not significantly different at a 95% confidence level ($p \leq 0.05$).

oxidative stability of virgin olive oil is lower when the water content is decreased after filtration.

Pigments and Antioxidant Compounds. Filtration systems affected the amount and nature of pigments in olive oil. Regarding carotenoids, significant differences were found between filtered and unfiltered olive oils. All filtration systems reduced to half or less the content tested in crude olive oils. The chlorophyll concentration in all filtered olive oils was 3-fold lower than unfiltered ones, perhaps because these compounds are removed together with the by-product generated after filtration. Future research is needed to evaluate the composition of these wastes.

Regarding phenolic fraction, the most important lipophilic phenols identified by RRLC-DAD in olive oil were α- and δ-tocopherols. Experimental data showed similar values of both α- and δ-tocopherols in filtered and unfiltered olive oil by different systems. In any case, new filtration systems had no effect on lipophilic phenol content. These results were consistent with those reported by other authors who evaluated the effect of conventional filtration system on the tocopherol content.[15,24]

The total polar phenolic content, tentatively calculated as the sum of the individual phenolic-compound concentrations, was increased by all the filtration systems. However, significant

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differences were found only in samples filtered with the filter bag and argon gas flow. The apparent increase could be attributed to the reduction in the water content. In water-in-oil emulsion, hydrophilic polyphenols are stabilized around water droplets, and the affinity of the phenolic compounds to solvent extraction is lower than nonpolar matrix. However, the partial elimination of water during the filtration process permits a greater availability of hydrophilic polyphenols for extraction with a polar solvent mixture.[2] This apparent rise in levels of these compounds by filtration has also been mentioned by different authors on applying the conventional filtration systems at the laboratory scale.[6,15,25] It should be considered that total polar phenols apparently increased after filtration due to the extraction in unfiltered EVOO and did not allow the complete recovery of these analytes due to the higher water content.

Behavior of Individual Polar Phenols in Filtration Process. Figure 1 shows the chromatogram of olive-oil polyphenolic extract and extracted ion chromatogram (EIC) of the main hydrophilic polyphenols identified in the samples. Table 3 includes the polar compounds identified in Peranzana olive-oil samples and the information generated by TOF analyzer. A total of 17 compounds were characterized in EVOO phenolic extract. Among these, 15 compounds were from different polyphenolic families (simple phenols, flavonoids, lignans, and secoiridoids). Peaks 2 and 3 were identified as the phenolic alcohols hydroxytyrosol and tyrosol, respectively. Peak 5 had a deprotonated molecule at m/z 195, corresponding to a derivative of simple phenols (hydroxytyrosol acetate), which has previously been described in the literature.[26]

The main secoiridoids identified in samples were decarboxymethyl oleuropein aglycone (peak 7), oleuropein aglycone (peak 14), ligstroside aglycone (peak 16), and their hydroxylated forms (peaks 4, 8, and 13). Elenolic acid (peak 6), considered a derivative of secoiridoid group, was also identified in all samples. Concerning lignans, the spectra generated for peaks 9, 10, and 12, yielded deprotonated molecules at m/z 417, 357, and 415, respectively. According to literature reports, these peaks were assigned to syringaresinol, pinoresinol, and acetoxypinoresinol.[20,27]

With regard to flavons, peak 15 had a deprotonated molecule at m/z 285 and 23.6 min and peak 17 at m/z 269 and 25.8 min, which were identified as luteolin and apigenin, respectively. Concerning the other polar compounds, quinic acid was also identified in the extracts deriving from all EVOO.

Table 4 includes quantitative results of the individual hydrophilic polyphenols. The phenolic compounds hydroxytyrosol, tyrosol, luteolin, apigenin, and (+)-pinoresinol, as well as quinic acid, were quantitated by the calibration curves drawn from their respective commercial standards. The other phenolic compounds, which had no commercial standards, were tentatively quantitated using other compounds with similar structures. The secoiridoid and lignan groups were quantitated using oleuropein and (+)-pinoresinol standards, respectively. The elenolic acid concentrations were expressed as oleuropein. It should be taken into account that the response of standards may differ from that of the analytes with similar structure present in samples, and consequently, the quantitation of these compounds is an estimation of their actual concentration.

The concentration of most polar-phenolic compounds seemed to increase after filtration. Among these, mainly hydrophilic phenols belonging to secoiridoid group were responsible for the apparent increase in the total polar phenolic content. The amounts of oleuropein aglycone, decarboxymethyl oleuropein aglycone, and decarboxymethyl ligstroside aglycone increased the most after filtration. These increases have also been mentioned by other authors when conventional filtration systems were applied.[6] However, this effect is related to the fact that filtration reduces the water content, and the polar phenols are more prone to solvent extraction, as noted above. Indeed, the higher concentrations of secoiridoids and derivatives were detected in filtered samples with argon gas flow, showing the lower content in water.

Regarding filter-bag system, while the total content on secoiridoids was also increased in filtered EVOOs, this filtration procedure did not have the same effect on the other phenolic compounds. Indeed, phenyl alcohols, lignans, and flavones were decreased after filtration using the polypropylene filter bag. Given that some of these compounds were not decreased by the inert gas-flow filtration system, it could be surmised that filter-bag filtration had the highest retention power of these minor compounds. The origin of this difference could be attributed to the different nature of filter aids. Future research is warranted to evaluate the phenolic composition in wastes generated during filtration by different systems.

With respect to other polar compounds, quinic acid was also increased in filtered EVOO by inert gas flow (argon and nitrogen), while an opposite trend was found for the filter-bag system. This compound was removed from the olive oil matrix when polypropylene filter bag was used as the filter aid.

Relationship between EVOO Oxidative Stability and Antioxidant Compounds. EVOO oxidative stability is influenced by different parameters, mainly temperature, exposure to light, the presence or absence of oxygen, FA composition (unsaturation rate), and composition on minor components, which may be antioxidant and pro-oxidant constituents.[28]

In terms of the FA composition, the same trend was found in EVOO with all of the filtration systems: filtered and unfiltered EVOO did not significantly differ. In this way, filtration processes did not affect the glyceride composition, and all olive oils (filtered and unfiltered) were constituted by the same glyceride matrix. Therefore, the oxidative stability depended on the concentrations of pro-oxidant and antioxidant constituents: chlorophylls, tocopherols, and polar phenols.

Among these, chlorophylls may act as protectors, capturing free radicals in the dark, improving oil stability. Indeed, the chlorophylls may interrupt the chain reaction of autoxidation by acting as electron donors to the free and peroxy radicals generated in the early phases. However, when exposed to light, these compounds stimulate the autoxidation by activated oxygen, producing singlet oxygen and generating allyl hydroperoxides, thereby decreasing the shelf life of the oil.[29] Thus, the behavior of chlorophylls depends on the storage conditions.

Because the amount of these compounds in filtered oils appears to be lower, it suggests that their oxidative stability in darkness would be lower than for unfiltered EVOOs. Nevertheless, when oil is exposed to light, filtration reduces susceptibility to oxidative reactions. Consequently, special attention should be given to storage conditions.

On the other hand, it is well-known and widely accepted that in the EVOO matrix the antioxidant activity of chlorophylls is lower than other minor constituents such as tocopherols and polar phenols.[28,30] Indeed, it has been reported that OSI values depend on the qualitative and quantitative compositions of FAs, tocopherols, and polar-phenolic compounds.[31] Given that

Table 5. Values of $L^*a^*b^*$ Coordinates of the EVOOs[a]

	unfiltered	SD	filter bag	SD	argon gas	SD	nitrogen gas	SD
L^*	41.14 d	0.82	46.37 c	0.23	53.28 a	0.39	51.33 b	0.95
a^*	3.01 a	0.27	2.34 b	0.05	1.98 b	0.03	2.35 b	0.19
b^*	64.89 c	0.98	74.39 b	0.42	82.16 a	0.51	75.65 b	1.52

[a]Values with the same letter in a line are not significantly different at a 95% confidence level ($p \leq 0.05$). SD, standard deviation.

the qualitative and quantitative compositions in FAs and tocopherols were very similar for all of the samples, the differences in oxidative stability could be linked to the polar-phenolic compounds and their antioxidant capacity.

Hydrophilic phenols and, in particular, *ortho*-diphenols are reported to be the highest contributors to oxidative stability in virgin olive oils.[28,30,32−34] The *ortho*-diphenolic compounds characterized in these samples were hydroxytyrosol, hydroxytyrosol acetate, oleuropein aglycone, decarboxymethyl oleuropein aglycone, and their hydroxylated derivatives and luteolin. The major total polar phenol content and *ortho*-diphenols composition were obtained in filtered samples, which showed the lowest OSI time values. No correlation was found between a major content in hydrophilic phenols and OSI time values.

This trend in oxidative stability may be attributed to the influence of the water content in the polar-phenol extraction and its antioxidant activity. First, as indicated above, the lower water content in filtered EVOO to facilitate phenolic compound extraction gave higher polar-phenol concentrations than did unfiltered EVOO. Second, polar antioxidants are more effective in a water-in-oil emulsion system due to their orientation of polar-phenolic compounds at the water−oil interface, and the active surface of water droplets influences protection against the oxidation of oil. Thus, water content reduction after filtration provides a less antioxidant capacity of hydrophilic phenols and consequently lower OSI time values.

Physicochemical and Sensorial Properties. Table 5 shows the oil color measurement by tristimulus coordinates L^*, a^*, and b^* of the CIELAB chromatic space. Concerning the a^* and b^* values, corresponding to the green and yellow zones, respectively, the filtered EVOOs after the application of argon gas-flow filtration registered values significantly different from samples treated by nitrogen gas and the filter bag, while the unfiltered EVOOs were the richest in green color (higher value of a^*) and the poorest in yellow (lowest value of b^*). These trends among unfiltered EVOOs and the filtered samples can be considered consistent with a general loss in pigments (spectrophotometric data relative to chlorophylls, carotenoids, and total pigments) due to the application of the different treatment systems.

The luminosity value (L^*) was significantly lower in unfiltered samples than EVOOs treated with inert gases. However, the luminosity observed for the sample filtered by filter bag reached a middle value. The increase of the L^* values agreed with the general reduction in the pigment content in the oils, the pigments being able to capture part of the light, instead of transmitting it.

The sensory profile of the filtered and unfiltered EVOO is depicted in Figure 2 according to the sensory attributes. None of the EVOO samples included in this study presented any organoleptic defects. In terms of bitter and pleasant flavors, quantitative descriptive sensory analysis evidenced a trend toward enhanced gustatory attributes due to filtration. The fruity olive attribute, expressed as the olfactory intensity value, showed the same intensity value in unfiltered EVOOs and

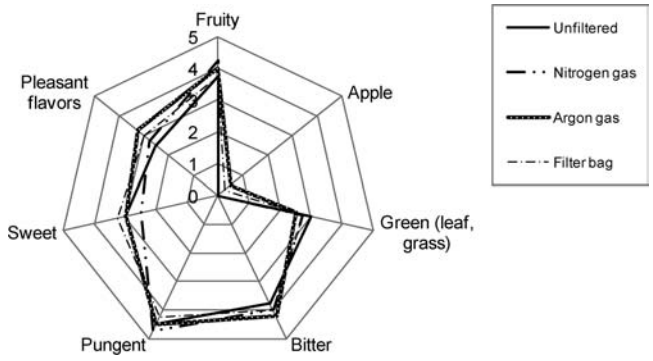

Figure 2. Sensory profiles of the filtered and unfiltered EVOOs.

those filtered by the bag system. This value, 3.75 for both kinds of samples, was lower than in samples filtered by nitrogen and argon gas flow (4.25 and 4, respectively).

Regarding apple and sweet attributes, the intensity was higher in samples filtered using argon gas flow and the polypropylene filter bag than in unfiltered EVOO samples. However, the intensity of the green perception diminished from unfiltered EVOOs (3) to filtered EVOOs (2.75, 2.5, and 2.75 for filter bag, argon, and nitrogen gas-flow system, respectively). Finally, the greater intensity of pungency was found in filtered samples, when nitrogen and argon gas were used as filter aids.

The positive and negative effects of the new filtration process on the shelf life and physicochemical and organoleptic properties of olive oil were tested. Although no effects on the glyceride matrix or tocopherol concentrations were found, other related compounds with the oxidative stability were affected. Indeed, the highest content in *ortho*-diphenols, which are reported to be the highest contributors to oxidative stability, was obtained in filtered EVOO. However, the lowest OSI time values were registered in filtered EVOO. It could be due to the fact that these compounds are a powerful antioxidants more protective against oxidation in a water-in-oil emulsion. Because the filtration reduces the water content, unfiltered olive oils may be more stable. This may be considered a negative effect of the filtration process. Nevertheless, it should be taken into account that EVOO is generally stored in the mill until commercialization. During this storage time, the higher polar phase content in unfiltered olive oils may increase the alteration of virgin olive oil, mainly affecting free acidity or generating off-flavors. On the other hand, new filtration processes reduce the chlorophyll content and consequently susceptibility to oxidative reactions when exposed to light. Special attention should be given to the storage conditions. Furthermore, it is important to emphasize that the results of the sensorial analysis confirmed that filtration using inert gas does not lower the intensity of the main organoleptic attributes that contribute to the consumer acceptance. Because this system avoids the use of organic materials that come into contact with oil, it could be a valuable alternative to conventional filtration processes.

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Notes

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REFERENCES

(1) European Community. Council Regulation No. 1513/2001 of 23 July 2001 amending Regulations No. 136/66/EEC and (EC) No. 1638/98 as regards the extension of the period of validity of the aid scheme and the quality strategy for olive oil. *Off. J. Eur. Comm.* **2001**, *L201*, 4−7.

(2) Lozano-Sanchez, J.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Fernandez-Gutierrez, A. Filtration process of extra virgin olive oil: Effect on minor components, oxidative stability and sensorial and physicochemical characteristics. *Trends Food Sci. Technol.* **2010**, *21*, 201−211.

(3) Masella, P.; Parenti, A.; Spugnoli, P.; Baldi, F.; Mattei, A. A predictive classification model for the management of virgin olive oil filtration at industrial scale. *Sep. Sci. Technol.* **2011**, *46*, 1709−1715.

(4) Sawalha, S.; Arraez-Roman, D.; Segura-Carretero, A.; Fernandez-Gutierrez, A. Characterization of phenolic compounds in diatomaceous earth used in the filtration process of olive oil by HPLC-ESI-TOF (MS). *Agro Food Ind. Hi-Tech* **2009**, *20*, 46−50.

(5) Lozano-Sanchez, J.; Segura-Carretero, A.; Fernandez-Gutierrez, A. Characterisation of the phenolic compounds retained in different organic and inorganic filter aids used for filtration of extra virgin olive oil. *Food Chem.* **2011**, *124*, 1146−1150.

(6) Gomez-Caravaca, A. M.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Fernandez- Gutierrez, A.; Lercker, G. Effect of filtration systems on the phenolic content in virgin olive oil by HPLC-DAD-MSD. *Am. J. Food Technol.* **2007**, *2*, 671−678.

(7) Bottino, A.; Capannelli, G.; Comite, A.; Ferrari, F.; Marotta, F.; Mattei, A.; Turchini, A. Application of membrane processes for the filtration of extra virgin olive oil. *J. Food Eng.* **2004**, *65*, 303−309.

(8) Deiana, M.; Incani, A.; Rosa, A.; Corona, G.; Atzeri, A.; Loru, D.; Paola Melis, M.; Assunta Dessì, M. Protective effect of hydroxytyrosol and its metabolite homovanillic alcohol on H_2O_2 induced lipid peroxidation in renal tubular epithelial cells. *Food Chem. Toxicol.* **2008**, *46*, 2984−2990.

(9) Corona, G.; Deiana, M.; Incani, A.; Vauzour, D.; Dessià, M. A.; Spencer, J. P. E. Hydroxytyrosol inhibits the proliferation of human colon adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. *Mol. Nutr. Food Res.* **2009**, *53*, 897−903.

(10) Incani, A.; Deiana, M.; Corona, G.; Vafeiadou, K.; Vauzour, D.; Dessì, M. A.; Spencer, J. P. E. Involvement of ERK, Akt and JNK signalling in H2O2-induced cell injury and protection by hydroxytyrosol and its metabolite homovanillic alcohol. *Mol. Nutr. Food Res.* **2010**, *54*, 788−796.

(11) Deiana, M.; Corona, G.; Incani, A.; Loru, D.; Rosa, A.; Atzeri, A.; Paola Melis, M.; Assunta Dessì, M. Protective effect of simple phenols from extravirgin olive oil against lipid peroxidation in intestinal Caco-2 cells. *Food Chem. Toxicol.* **2010**, *48*, 3008−3016.

(12) Oliveras-Ferraros, C.; Fernández-Arroyo, S.; Vazquez-Martin, A.; Lozano-Sánchez, J.; Cufí, S.; Joven, J.; Micol, V.; Fernández-Gutiérrez, A.; Segura-Carretero, A.; Menendez, J. A. Crude phenolic extracts from extra virgin olive oil circumvent de novo breast cancer resistance to HER1/HER2-targeting drugs by inducing GADD45-sensed cellular stress, G2/M arrest and hyperacetylation of Histone H3. *Int. J. Oncol.* **2011**, *38*, 1533−1547.

(13) Koidis, A.; Boskou, D. The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 323−328.

(14) Tsimidou, M. Z.; Georgiou, A.; Koidis, A.; Boskou, D. Loss of stability of "veiled" (cloudy) virgin olive oils in storage. *Food Chem.* **2005**, *93*, 377−383.

(15) Fregapane, G.; Lavelli, V.; Leon, S.; Kapuralin, J.; Salvador, M. D. Effect of filtration on virgin olive oil stability during storage. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 134−142.

(16) Bottino, A.; Capannelli, G.; Mattei, A.; Rovellini, P.; Zunin, P. Effect of membrane filtration on the flavor of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 1109−1115.

(17) Cerretani, L.; Roculi, P.; Bendini, A.; Romani, S.; Bacci, A. Oil clarifying process and apparatus for implementing the process. World Patent Application WO 2009/107096, 2009.

(18) Maggio, R. M.; Valli, E.; Bendini, A.; Gomez-Caravaca, A. M.; Toschi-Gallina, T.; Cerretani, L. A spectroscopic and chemometric study of virgin olive oils subjected to thermal stress. *Food Chem.* **2011**, *127*, 216−221.

(19) Minguez-Mosquera, M. I.; Rejano, L.; Gandul-Rojas, B.; Sanchez, A. H.; Garrido-Fernandez, J. Color-pigment correlation in virgin olive oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 332−336.

(20) Lozano-Sanchez, J.; Segura-Carretero, A.; Menendez, J. A.; Oliveras-Ferraros, C.; Cerretani, L.; Fernandez-Gutierrez, A. Prediction of Extra Virgin Olive Oil Varieties through Their Phenolic Profile. Potential Cytotoxic Activity against Human Breast Cancer Cells. *J. Agric. Food Chem.* **2010**, *58*, 9942−9955.

(21) Kilic, K.; Onal-Ulusoy, B.; Boyaci, I. H. A novel method for color determination of edible b oils in L*a*b* format. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 157−164.

(22) Cerretani, L.; Biasini, G.; Bonoli-Carbognin, M.; Bendini, A. Harmony of virgin olive oil and food pairing: A methodological proposal. *J. Sens. Stud.* **2007**, *22*, 403−416.

(23) Bendini, A.; Cerretani, L.; Carrasco-Pancorbo, A.; Gomez-Caravaca, A. M.; Segura-Carretero, A.; Fernandez-Gutierrez, A.; Lercker, G. Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* **2007**, *12*, 1679−1719.

(24) Romero, C.; Brenes, M.; Garcia, P.; Garrido, A. Hydroxytyrosol 4-beta-D-glucoside, an important phenolic compound in olive fruits and derived products. *J. Agric. Food Chem.* **2002**, *50*, 3835−3839.

(25) Brenes, M.; Garcia, A.; Garcia, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609−5614.

(26) Ouni, Y.; Taamalli, A.; Gomez-Caravaca, A. M.; Segura-Carretero, A.; Fernandez-Gutierrez, A.; Zarrouk, M. Characterisation and quantification of phenolic compounds of extra-virgin olive oils according to their geographical origin by a rapid and resolutive LC-ESI-TOF MS method. *Food Chem.* **2011**, *127*, 1263−1267.

(27) Suarez, M.; Macia, A.; Romero, M.; Motilva, M. Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. *J. Chromatogr., A* **2008**, *1214*, 90−99.

(28) Mateos, R.; Trujillo, M.; Pérez-Camino, M. C.; Moreda, W.; Cert, A. Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. *J. Agric. Food Chem.* **2005**, *53*, 5766−5771.

(29) Giuliani, A.; Cerretani, L.; Cichelli, A. Chlorophylls in olive and in olive oil: Chemistry and occurrences. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 678−690.

(30) Mateos, R.; Dominguez, M.; Espartero, J.; Cert, A. Antioxidant effect of phenolic compounds, alpha-tocopherol, and other minor components in virgin olive oil. *J. Agric. Food Chem.* **2003**, *51*, 7170−7175.

(31) Bendini, A.; Valli, E.; Cerretani, L.; Chiavaro, E.; Lercker, G. Study on the effects of heating of virgin olive oil blended with mildly

deodorized olive oil: Focus on the hydrolytic and oxidative state. *J. Agric. Food Chem.* **2009**, *57*, 10055−10062.
(32) Velasco, J.; Dobarganes, C. Oxidative stability of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 661−676.
(33) Cerretani, L.; Bendini, A.; Gallina-Toschi, T.; Lercker, G.; Rotondi, A. Influence of two process variables on the oxidative stability of extra-virgin olive oils (cv. Peranzana) obtained from different extraction technologies. *Ind. Aliment. Italy* **2006**, *455*, 133−140.
(34) Bendini, A.; Cerretani, L.; Vecchi, S.; Carrasco-Pancorbo, A.; Lercker, G. Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions. *J. Agric. Food Chem.* **2006**, *54*, 4880−4887.