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A new extraction approach to correct the effect of apparent increase in the secoiridoid content after filtration of virgin olive oil

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ABSTRACT

In the current study, a new approach has been developed for correcting the effect that moisture reduction after virgin olive oil (VOO) filtration exerts on the apparent increase of the secoiridoid content by using an internal standard during extraction. Firstly, two main Spanish varieties (Picual and Hojiblanca) were submitted to industrial filtration of VOOs. Afterwards, the moisture content was determined in unfiltered and filtered VOOs, and liquid–liquid extraction of phenolic compounds was performed using different internal standards. The resulting extracts were analyzed by HPLC-ESI-TOF/MS, in order to gain maximum information concerning the phenolic profiles of the samples under study. The reduction effect of filtration on the moisture content, phenolic alcohols, and flavones was confirmed at the industrial scale. Oleuropein was chosen as internal standard and, for the first time, the apparent increase of secoiridoids in filtered VOO was corrected, using a correction coefficient (Cc) calculated from the variation of internal standard area in filtered and unfiltered VOO during extraction. This approach gave the real concentration of secoiridoids in filtered VOO, and clarified the effect of the filtration step on the phenolic fraction. This finding is of great importance for future studies that seek to quantify phenolic compounds in VOOs.

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1. Introduction

Virgin olive oil (VOO) is a natural product obtained exclusively through mechanical and physical operations. The process begins by collecting and washing olives, followed by crushing them to tear the flesh cells and thus let the oil escape. The resulting olive paste has to be mixed; in this stage the droplets of oil merge into larger drops until they form a continuous liquid phase, and then the oil can be separated from the other phases by centrifugation [1–3] and [4]. Immediately after centrifugation, the VOO produced is turbid from suspended solid plant-tissue particles and vegetable water emulsified in the oil, which can deteriorate its quality by facilitating hydrolysis or oxidation of lipid matrix. Recently, filtration was included in VOO-production process as a final step before bottling in order to make VOO more brilliant and maintain its quality [1,5] and [6].

Several filtration systems are used for VOO: conventional filtration systems, cross-flow filtration, and new patented approaches based on inert gas-flow filtration and filter bags [7] and [8]. At the industrial scale, the most widespread system is the conventional one, which employs filter aids in conjunction with filtration equipment (tanks or presses) to enhance or enable suspended solids and water–oil separation [5]. Filter aids for filter cake can be produced from a wide variety of raw materials. Traditionally, diatomite, known also as diatomaceous earth was used, the composition being largely silica (95–98%). Unfortunately, the sludge from this kind of filter cake represents a major source of pollution, and land disposal of this waste is forbidden. Consequently, in recent years, filter aids based on fibrous material are becoming more widely used. Normally, the fibrous products used to filter cloudy VOO are cellulose or mixtures of cellulose and lignin. Besides its ecological advantage, filtration by an organic filter aid is preferred due to its high performance in the filtration process [9–11].

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The effect of this step on VOO composition, particularly on polar phenol fraction, has been studied by several authors, due to the importance of these peculiar compounds on VOO oxidative stability and organoleptic quality [12–15]. Nevertheless, controversial results have been reported. On the one hand, no differences in the total polyphenol content have been found after VOO filtration, using gas-flow filtration as filter aids [6]. On the other, a laboratory study on the retentive power of inorganic and organic filter aids on phenolic compounds showed that a large number of polyphenols were retained in filter aids, lowering the total phenol content in filtered VOO [11]. The same trend for this fraction during filtration has been reported by other authors [16]. Furthermore, the effect of filtration on individual polyphenols in VOO has also been studied at laboratory scale [17]. The authors found that compounds belonging to the secoiridoid group, such as ligstroside aglycone and oleuropein aglycone, increased significantly after filtration with cotton in comparison to the unfiltered VOO. Five years afterwards, a pilot-plant-scale study using filter bags showed that secoiridoids in filtered VOO were responsible for the apparent increase in the total phenolic content [8]. Finally, the apparent increase in different compounds from the secoiridoid group after filtration was confirmed in a more recent study at the industrial scale [18]. The hypothesis proposed to explain secoiridoid behavior was that after filtration the reduction in VOO moisture content facilitated their extraction, triggering an apparent increase in the filtered VOO.

Thus, the objective of this work was to correct this apparent increase and then to evaluate what really happens to the phenolic compounds during VOO filtration. Taking into consideration the hypothesis proposed above, this work seeks to achieve the correction by using an internal standard in the extraction step, and then to quantify the real concentration of phenolic compounds in the filtered VOO. The analysis was made using HPLC-ESI-TOF/MS, which could provide information concerning the phenolic profile of the VOOs under study.

2. Materials and methods

2.1. Samples

The VOO samples used in this study were from Aceites Maeva Company (Aceites Maeva S.L., Granada, Spain). The extraction was made in November 2012 by a continuous industrial hammer crusher, a horizontal malaxator, and a two-phase decanter. For this work, 45,000 kg of VOO mixture was filtered using a conventional filtration process at the industrial scale. The mixture was from two of the main Spanish varieties, Picual 40% and Hojiblanca 60%. Cloudy VOOs were filtered at room temperature using the following organic filter aids: Vitacel® L-90 (30 kg, composed of 100 % cellulose) and Filtracel® EFC-950 (60 kg, composed of 70% cellulose and 30% lignin). The cake layer formed in conjunction with filter tank. For the filtration, a preliminary phase is required, during which a prepared combinations of filter aids and unfiltered VOO are mixed in a slurry tank. Afterwards, the slurry was circulated through filter tank and back to the slurry tank. The filter aids were kept in the filtration equipment and circulation continued until the cake layer formed and the effluent ran clear. Afterwards, filtration was conducted under a constant flow and increasing differential pressure in different steps designated as A, B, C, and D, with 12,000 kg each. During the last step (D) the filtrate was just 9000 kg, which depended on the availability of VOO in the company. A total of 24 unfiltered and filtered samples were collected for analysis (Fig. 1). To have representative results and eliminate confounding factors which could affect olive-oil composition, the moisture content and isolation of phenolic fraction from samples were determined without storage.

2.2. Chemicals and reagents

All chemicals were of analytical reagent grade. Methanol, *n*-hexane, sodium hydroxide and isopropanol were purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from Fluka, Sigma-Aldrich (Steinheim, Germany). Double-deionized water with conductivity of less than 18.2 MΩ cm was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Standards of hydroxytyrosol, tyrosol, luteolin, apigenin, taxifolin and quinic acid were purchased by Sigma-Aldrich St. Louis, MO, USA), and (+)-pinoresinol was acquired from Arbo Nova (Turku, Finland). Oleuropein, luteolin 7-glucoside and dihydrocaffeic acid were purchased from Extrasynthese (Lyon, France).

2.3. Moisture content

The moisture content was determined following the norms of the Spanish Association for Standardization and Certification (AENOR). Briefly, in a capsule, previously dried at 105 °C and cooled, 10 g of completely homogenized sample was weighed. The samples were placed in an oven (Memmert GmbH + CO.KG, Schwabach, Germany) at 105 °C for 21 h, after which the samples were removed and weighed. Next, they were returned in the oven and the operation was repeated until the weight was constant. The moisture content was calculated as the difference in weights [19].

2.4. Phenolic compound extraction

The phenolic compounds from the VOOs were extracted using a liquid–liquid extraction system following the method reported previously [18], with some modifications. As mentioned above, in an effort to correct the effect of moisture content on extraction of those analytes from the samples, different internal standards belonging to different phenolic families were tested: luteolin 7-glucoside, dihydrocaffeic acid, taxifolin, and oleuropein. Concentrations ranging from 5 mg L^{-1} to 15 mg L^{-1} of the internal standards were also tested. The extraction procedure was as described in the following protocol. VOO, with 50 μL of internal standard in methanol added, was dissolved in *n*-hexane (2.5 g in 5 mL). Afterwards, 5 mL of methanol/water (60/40, v/v) was added, and the mixture was vortexed and then centrifuged at 445.1 g during 10 min. The polar extract was evaporated to dryness in a rotary evaporator under reduced pressure at a temperature of 35 °C. The residue was dissolved in 0.25 mL of methanol/water (50/50 v/v) and finally filtered through a 0.2 μm filter before the HPLC analysis.

2.5. HPLC-ESI-TOF/MS phenolic analysis

The analysis to characterize the phenolic profile in filtered and unfiltered VOOs was performed in an Agilent 1200-HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, autosampler, a binary pump, and a diode array detector (DAD). The chromatographic separation of these compounds was performed on a 150 mm × 4.6 mm i.d., 1.8 μm, Zorbax Eclipse Plus RP-C18 column (Agilent Technologies, Palo Alto, CA, USA). The mobile phases used were water with 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^{-1} 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^{-1} to ensure reproducible results and stable spray. HPLC was coupled

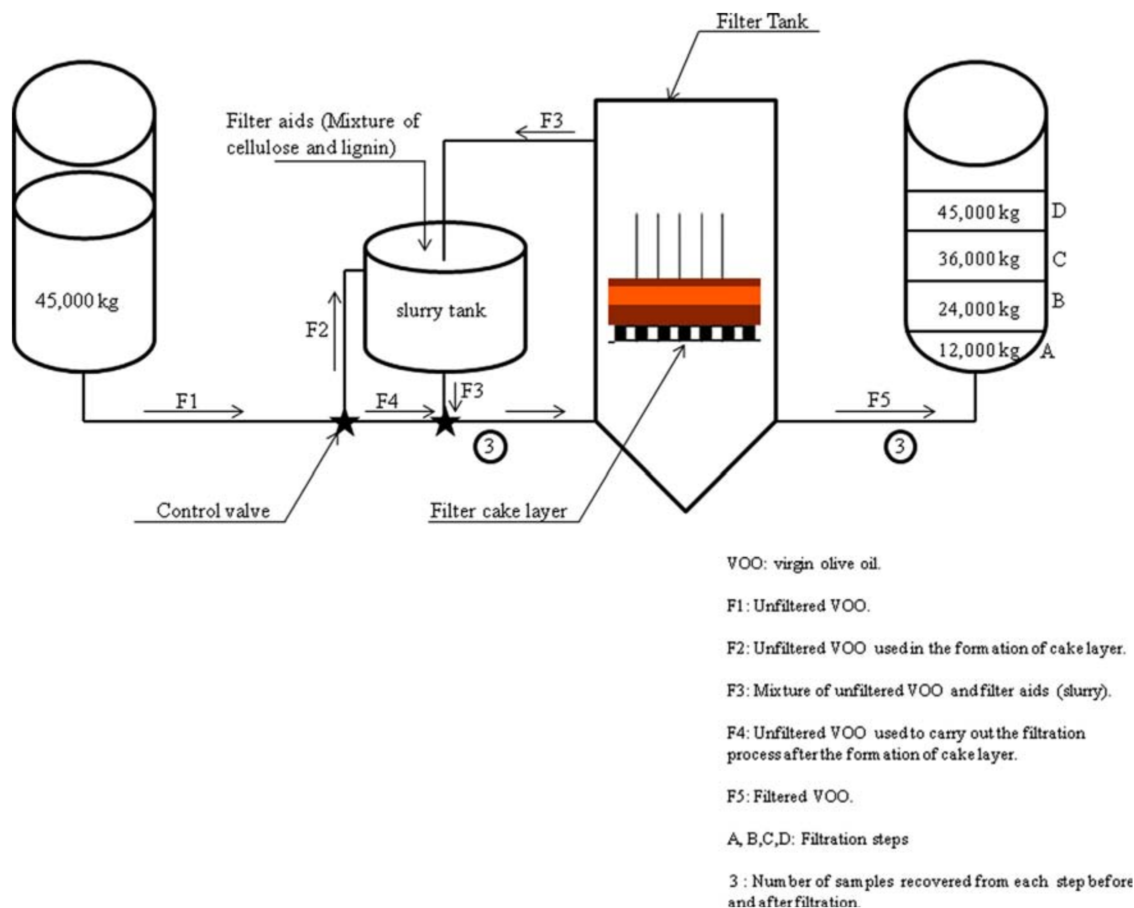

Fig. 1. Industrial filtration diagram.

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.

External mass-spectrometer calibration was performed with sodium acetate clusters (5 mM sodium hydroxide in water/isopropanol 1/1 (v/v), with 0.2% of acetic acid) in quadratic high-precision calibration (HPC) regression mode. The optimum values of the source and transfer parameters were established according to the method published previously [20]. The widely accepted accuracy threshold for confirmation of elemental compositions was set 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 TOF analyzer, and the information reported in the literature [21,22] and [23]. Quantification was made by HPLC-ESI-TOF/MS. Seven standard calibration curves of the main compounds found in the samples were prepared using seven commercial standards. Stock solutions at a concentration of 1000 mg L^{-1} for each phenolic compound were first prepared by dissolving the appropriate amount of the compound in methanol and then serially diluted to working concentrations. All calibration curves showed good linearity over the study range ($r^2=0.993$). The individual concentrations were determined using the area of each individual compound (three replicates) and by interpolation of the corresponding calibration curve. Regarding the secoiridoid group, their real concentration in filtered VOO was determined with a correction coefficient (Cc) calculated using the following equation:

$$Cc = \frac{A\ IS_{FL}}{A\ IS_{UF}}$$

where $A\ IS_{FL}$ is the area of the internal standard obtained in filtered VOO, and $A\ IS_{UF}$ is the area of the internal standard determined in the unfiltered VOO.

Afterwards, the area of all secoiridoids in filtered VOO was divided by the correction coefficient (Cc), and the quantification was performed as previously described using the new areas. Results were given in mg of analyte per kg of VOO.

2.6. Statistical analysis

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

3. Results and discussion

3.1. The time course of moisture content

As shown in Table 1, the moisture content was sharply reduced using organic filter aids. Meanwhile, the highest moisture values were registered in unfiltered VOO, which varied from 0.132 to

Table 1
Time course of virgin olive oil moisture content during filtration process.

Filtration steps	Moisture (%)		Reduction percentage
	UF[a]	FL[b]	((UF − FL)/UF × 100
A	$0.132^c \pm 0.001$	$0.074^e \pm 0.002$	44%
B	$0.125^c \pm 0.002$	$0.077^e \pm 0.002$	39%
C	$0.120^c \pm 0.003$	$0.083^e \pm 0.002$	31%
D	$0.123^c \pm 0.004$	$0.086^e \pm 0.001$	30%

Values with different letters in a line are significantly different at a 95% confidence level ($p \leq 0.05$).
[a] Unfiltered.
[b] Filtered.

0.120%, the lowest ones being registered in filtered VOO, varying from 0.086 to 0.074%.

These results were analyzed further by calculating the difference on moisture content between unfiltered and filtered VOOs for each step. The results were expressed as a percentage reduction (Table 1). The initial trend for the difference in moisture content was a sharp decrease. The maximum values were found after filtering 12,000 kg (44%) corresponding to the first filtration step (A), to reach 31% of the VOO belonging to the filtration step (C). Next, the moisture reduction showed minimal decreases and even remained constant in the last filtration step (D, 30%). The trend of this parameter during the filtration process could be explained by the saturation of filter cake. It should be taken into account that the cake layer used in this study was formed by 90 kg of organic filter aids; this amount did not change over the entire filtration cycle. Consequently, during filtration, the water absorption which raised the volume of filter-aid particles, and the trapped solid particles present in unfiltered VOO, lowered the volume and number of microscopic channels through which clarified oil could flow easily. This tendency during filtration affected the final moisture content in filtered VOO. Therefore, the monitoring of filter-cake saturation during filtration could be a key for determining the optimal time to add new filter aids in order to prolong the filtration cycle, increase the amount of VOO filtered per cycle, and maintain the moisture reduction stable.

3.2. Qualitative characterization of phenolic and other polar compounds in VOOs

Table 2 provides an overview of all the compounds characterized in a representative unfiltered VOO sample by HPLC-ESI-TOF/MS. These compounds are summarized together with their retention time, molecular formula, experimental and calculated mass (*m/z*), error, and msigma. Fig. 2 shows the base peak chromatogram (BPC) of the VOO phenolic extract. In the present work, a total of 23 phenolic compounds and another polar one were characterized following the procedure reported above in Materials and methods.

The phenolic compounds identified in the samples belong to different phenolic classes of phenolic alcohols, secoiridoids, lignans, flavones, and phenolic acids. The latter were represented only by *p*-coumaric acid eluted at a retention time of 13.51 min and yielded a deprotonated ion at *m/z* 163. Among the phenolic alcohols, oxidized form of hydroxytyrosol, hydroxytyrosol, and tyrosol were characterized, corresponding to the peaks (2), (3), and (4), respectively. The spectrum in the negative ionization mode also showed a deprotonated molecular ion at *m/z* 195 (peak7), corresponding to hydroxytyrosol derivative (hydroxytyrosol acetate).

The most representative complex phenols identified in VOO were oleuropein aglycone, ligstroside aglycone, and their derivatives, which belong to secoiridoid group. Hydroxylated, decarboxymethylated, dehydrated, and methylated forms of oleuropein aglycone (peaks 5, 10, 11, 17, 18, 23) as well as a decarboxymethylated and hydroxylated forms of ligstroside aglycone (peaks 14 and 16) were found in VOO samples. Deprotonated molecular ions at *m/z* 241 and 257 were identified as elenolic acid and its hydroxylated form, respectively.

With regard to lignans, three compounds were detected in samples under study, namely syringaresinol (peak 12), pinoresinol (peak 13), and acetoxypinoresinol (peak 15), which yielded deprotonated molecules *m/z* 417, 357, and 415, respectively. The last phenolic group detected was composed of flavones. The most noteworthy compounds identified in this group were luteolin and apigenin, which had retention times of 23.61 and 25.91 min, respectively. With respect to the presence of other polar compounds, quinic acid (peak 1) was found in the VOO samples.

3.3. The time course of VOO phenolic content during filtration

It should be taken into account that the phenolic compounds in filtered and unfiltered VOOs were firstly quantified without taking into consideration the internal standard used during extraction.

Regarding the total phenol content, differences among unfiltered and filtered VOOs were found for all filtration steps. However, these differences were statistically significant only in samples belonging to filtration steps B and C. The trend in the total phenolic content was linked to the behavior of the different polyphenol families detected in samples. As shown in Fig. 3, phenolic alcohols significantly decreased their concentration in filtered VOO. Indeed, the highest loss in this family was found in VOO belong to the first filtration step (A, 19%). However, less decrease in its concentration was registered during the last filtration step (D, 10%). The concentration in flavones after filtration also diminished significantly during all filtration steps. Indeed, the greatest decrease was reached in filtered VOO from the second filtration step (B, 35%) and the least reduction during the third filtration step (C, 10%). These results confirm those of our previous study conducted under the same conditions [18]. While phenolic alcohols and flavones significantly decreased their concentration in filtered VOO, the variation in the secoiridoid content was not significant in any of the VOOs from filtration steps A, B, C, and D. Nevertheless, during the first filtration step (A), the secoiridoid content in filtered VOO tended to increase, but tended to decrease during the remaining steps. This trend could be explained by the balance between the increase and the decrease of some compounds within the same family. Finally, no significant variations were found in lignan content after filtration in all the VOOs.

The analysis of the individual concentrations indicated that hydroxytyrosol and tyrosol were the main phenolic compounds responsible for the decrease in the concentration of this group during filtration. The greatest loss was found in filtered VOOs belonging to filtration steps (A) and (B) for hydroxytyrosol and tyrosol, respectively. Hydroxytyrosol acetate, a derivative of hydoxytyrosol, showed no significant variation in its content during filtration. Concerning flavones, luteolin decreased significantly, and these results repeated in all the filtration steps under study. However, the variation on apigenin content was significant only during filtration steps (B) and (D). In addition, the reduction effect of filtration on luteolin content was higher than for apigenin. Acetoxypinoresinol and pinoresinol belonging to the lignan family showed no significant content variation in the filtered VOOs. However, syringaresinol decreased its concentration during filtration steps B, C, and D.

With regard to secoiridoids, 11 compounds were quantified in filtered and unfiltered VOOs. These compounds showed different trends during filtration. Dehydro-oleuropein aglycone, oleuropein aglycone, ligstroside aglycone, and methyl oleuropein aglycone, increased significantly in concentration after filtration in all filtration steps, with the exception of step C and D, where the variation

Table 2
Main phenolic and other polar compounds identified in a representative extract of unfiltered virgin olive oil obtained by HPLC-ESI-TOF/MS.

Peak number	Compounds[a]	Retention time ($_{min}$)	Molecular formula	m/z calcd[b]	m/z Exptl[c]	Error (ppm)	msigma
1	Quinic acid	2.31	$C_7H_{12}O_6$	191.0561	191.0569	4.3	6.1
2	H-HYTY	3.94	$C_8H_8O_3$	151.0401	151.0398	1.6	7.2
3	HYTY	8.12	$C_8H_{10}O_3$	153.0557	153.0559	1.4	5.6
4	TY	9.90	$C_8H_{10}O_2$	137.0608	137.0605	2.2	9.4
5	H-D-Ol Agl or isomer	11.81	$C_{17}H_{20}O_7$	335.1136	335.1106	3.0	3.3
6	p-coumaric acid	13.51	$C_9H_8O_3$	163.0401	163.0384	1.7	6.7
7	HYTY-Ac	14.13	$C_{10}H_{12}O_4$	195.0663	195.0654	4.8	5.1
8	EA	15.14	$C_{11}H_{14}O_6$	241.0718	241.0709	3.7	2.4
9	H-EA	15.81	$C_{11}H_{14}O_7$	257.0667	257.0648	4.5	1.9
10	DOA	16.30	$C_{17}H_{20}O_6$	319.1187	319.1177	3.3	0.9
11	H-D-Ol Agl or isomer	16.64	$C_{17}H_{20}O_7$	335.1136	335.1114	2.3	2.4
12	Syringaresinol	18.22	$C_{22}H_{26}O_8$	417.1555	417.1533	2.2	4.4
13	Pin	18.91	$C_{20}H_{22}O_6$	357.1344	357.1349	1.6	9.2
14	D-Lig Agl	19.33	$C_{17}H_{20}O_5$	303.1238	303.1211	2.7	5.3
15	AcPin	19.41	$C_{22}H_{24}O_8$	415.1398	415.1373	2.5	4.3
16	H-D-Lig Agl	19.91	$C_{17}H_{20}O_6$	319.1187	319.1174	4.0	8.9
17	Dehydro Ol Agl	21.63	$C_{19}H_{20}O_8$	375.1085	375.1038	4.7	7.1
18	10-H-Ol Agl	23.02	$C_{19}H_{22}O_9$	393.1191	393.1170	2.1	2.4
19	Ol Agl	23.22	$C_{19}H_{22}O_8$	377.1242	377.1224	4.8	0.7
20	Lut	23.61	$C_{15}H_{10}O_6$	285.0405	285.0387	1.7	1.2
21	Lig Agl	25.60	$C_{19}H_{22}O_7$	361.1293	361.1259	9.3	7.0
22	Apig	25.91	$C_{15}H_{10}O_5$	269.0455	269.0435	2.0	1.7
23	Methyl Ol Agl	26.44	$C_{20}H_{24}O_8$	391.1398	391.1367	3.2	8.1

[a] H-HYTY, Oxidized hydroxytyrosol; HYTY, hydroxytyrosol; TY, tyrosol; H-D-Ol Agl, hydroxy decarboxymethyl oleuropein aglycone or isomer; HYTY-Ac, hydroxytyrosol acetate; EA, elenolic acid; H-EA, hydroxy elenolic acid; DOA, decarboxymethyl oleuropein aglycone; H-D-Ol Agl, hydroxy decarboxymethyl oleuropein aglycone or isomer; Pin, pinoresinol; D-Lig Agl, decarboxymethyl ligstroside aglycone; AcPin, acetoxypinoresinol; H-D-Lig Agl, hydroxy decarboxymethyl ligstroside aglycone; Dehydro Ol Agl, dehydro-oleuropein aglycone; 10-H-Ol Agl, 10-hydroxy oleuropein aglycone; Ol Agl, oleuropein aglycone; Lut, luteolin; Lig Agl, ligstroside aglycone; Apig, apigenin; Methyl Ol Agl, methyl oleuropein aglycone.
[b] m/z calcd: calculated mass.
[c] m/z exptl: experimental mass.

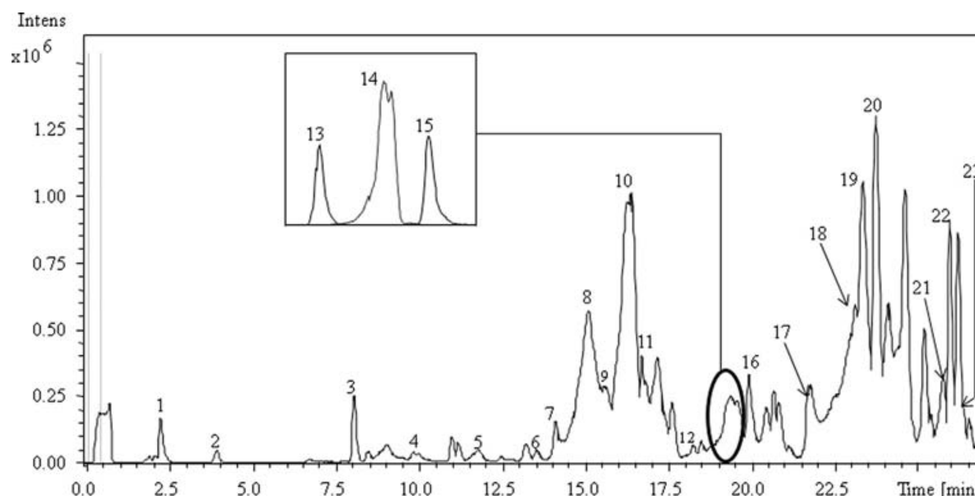

Fig. 2. Base-peak chromatogram (BPC) of representative unfiltered virgin olive oil phenolic extract obtained by HPLC-ESI-TOF/MS.

was not significant for dehydro-oleuropein aglycone and methyl oleuropein aglycone, respectively. The apparent increase in these compounds found in a previous work [8] was attributed to the lack of extraction method used. In a water-in-oil emulsion, polyphenols are stabilized around water droplets, and the affinity of the phenolic compounds for solvent extraction is lower than in a nonpolar matrix. However, the partial elimination of water during the filtration process permits a greater availability of polyphenols for extraction with a polar solvent mixture (methanol/water, 60/40) which results in the apparent increase in their concentration in filtered VOO. In the present experiment, the remaining compound belonging to this family as elenolic acid, decarboxymethyl oleuropein aglycone, and their hydroxylated forms, 10-hydroxy oleuropein aglycone and hydroxy decarboxymethyl ligstroside aglycone, maintained their concentration stable, with little variation in their content during some filtration steps. Nevertheless, their concentration decreased significantly during other filtration steps (Fig. 3). The results on the time course of this group of compounds during filtration proved unclear, due especially to the apparent increase in the concentration of some secoiridoids in the filtered VOO. Therefore, it is difficult to draw conclusions concerning the effect of this step on the VOO phenolic fraction, which is very important for VOO producers.

3.4. Correcting the effect of moisture reduction over the secoiridoids extraction from VOO

As a result of testing different internal standards in an effort to correct the effect of moisture content on extraction of phenolic compounds from the samples, luteolin 7-glucoside, taxifolin, and

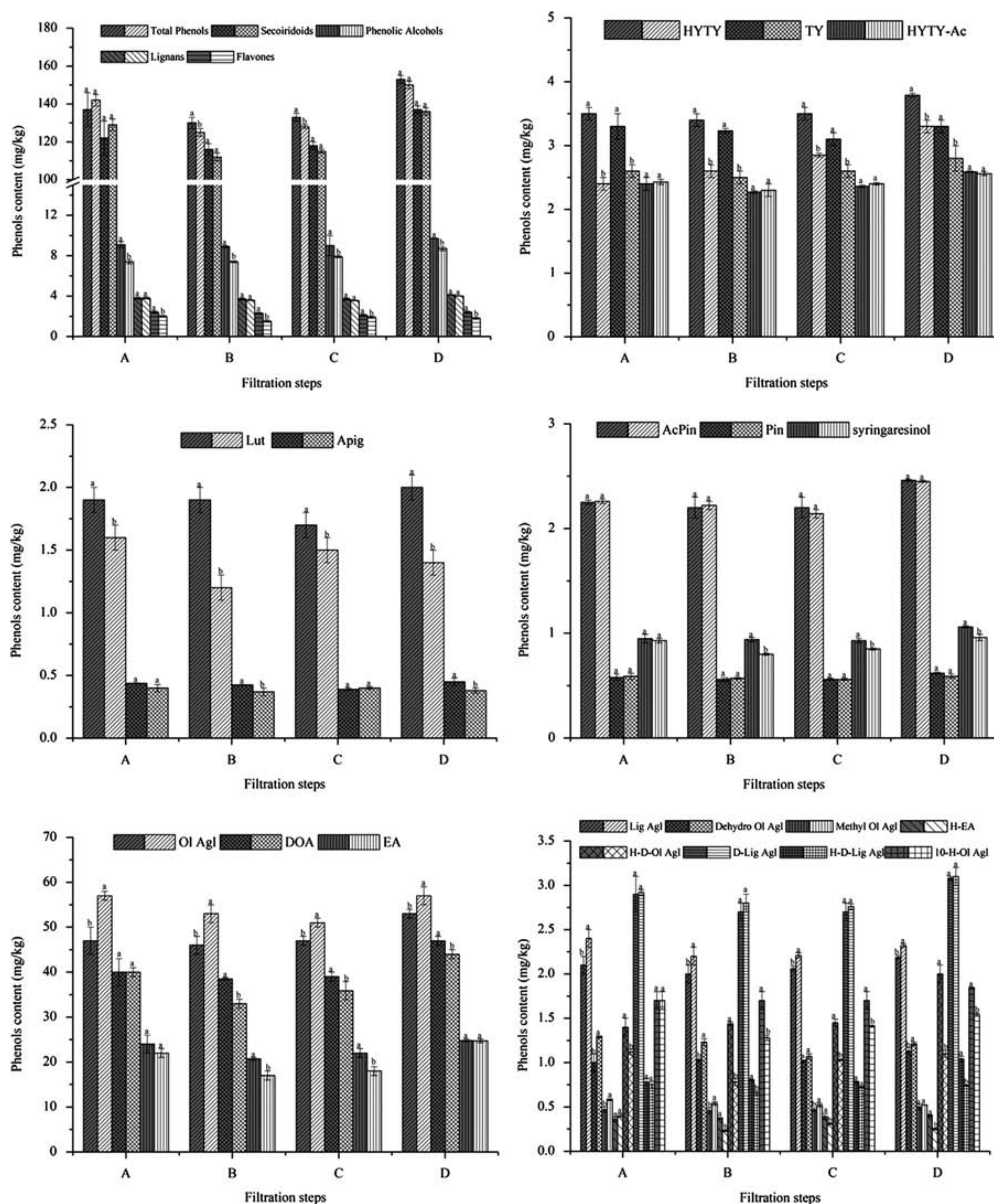

Fig. 3. Time course of virgin olive oil phenolic compounds during filtration process. HYTY, hydroxytyrosol; TY, tyrosol; HYTY-Ac, hydroxytyrosol acetate; Lut, luteolin; Apig, apigenin; AcPin, acetoxypinoresinol; Pin, pinoresinol; Ol Agl, oleuropein aglycone; DOA, decarboxymethyl oleuropein aglycone; EA, elenolic acid; H-EA, hydroxy elenolic acid; H-D-Ol Agl, hydroxy decarboxymethyl oleuropein aglycone or isomer; D-Lig Agl, decarboxymethyl ligstroside aglycone; H-D-Lig Agl, hydroxy decarboxymethyl ligstroside aglycone; Dehydro Ol Agl, dehydro-oleuropein aglycone; 10-H-Ol Agl, 10-hydroxy oleuropein aglycone; Lig Agl, ligstroside aglycone; Methyl Ol Agl, methyl oleuropein aglycone; gray, unfiltered VOO; white, filtered VOO; values with the same letter are not significantly different at a 95% confidence level ($p \leq 0.05$).

dihydrocaffeic acid were eliminated because their behavior was not similar to that of the compounds apparently increasing in filtered VOO, which made the correction impossible using these three standards. However, an apparent increase in oleuropein content was found in filtered VOO in comparison to the unfiltered one. Besides, the best results using oleuropein were found with a concentration of 10 mg L^{-1}. In consideration of these results, oleuropein was chosen as internal standard for the correction of the secoiridoid concentration in filtered VOO. Fig. 4 showed the time course of individual secoiridoid concentrations, the secoiridoid family, and total phenols during the industrial filtration process. The concentrations presented in this figure were those found after correction using oleuropein as internal standard during phenolic compound extraction. The use of the correction coefficient (*Cc*) allowed to observe that all the compounds belonging to this family tended to decrease after filtration. Indeed, oleuropein aglycone and ligstroside aglycone known as the main secoiridoids detected in VOO, showed no significant decrease on their content in filtered VOO from filtration steps A, B, C, and D. The same results were found for methyl oleuropein aglycone and

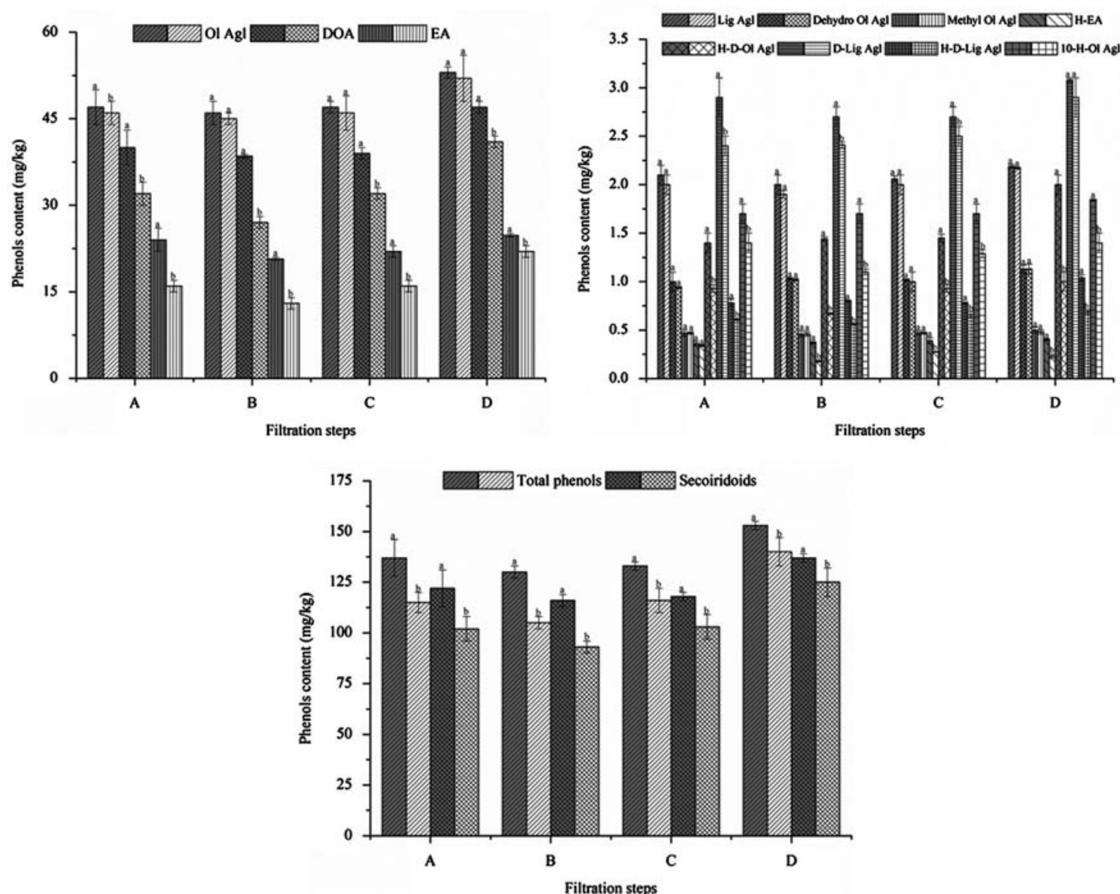

Fig. 4. Time course of secoiridoids and total phenols during filtration after correction using internal standard. Ol Agl, oleuropein aglycone; DOA, decarboxymethyl oleuropein aglycone; EA, elenolic acid; H-EA, hydroxy elenolic acid; H-D-Ol Agl, hydroxy decarboxymethyl oleuropein aglycone or isomer; D-Lig Agl, decarboxymethyl ligstroside aglycone; H-D-Lig Agl, hydroxy decarboxymethyl ligstroside aglycone; Dehydro Ol Agl, dehydro-oleuropein aglycone; 10-H-Ol Agl, 10-hydroxy oleuropein aglycone; Lig Agl, ligstroside aglycone; Methyl Ol Agl, methyl oleuropein aglycone; gray, unfiltered VOO; white, filtered VOO; values with the same letter are not significantly different at a 95% confidence level ($p \leq 0.05$).

dehydro oleuropein aglycone. The effect of filtration was stronger on the rest of oleuropein and ligstroside aglycone derivatives, showing a significant decline in their content after filtration during all filtration steps carried out, with the exception to hydroxy elenolic acid and decarboxymethyl ligstroside aglycone, which showed no significant decrease in their content in filtered VOOs from the filtration steps A and D, respectively. As a sum of individual concentrations of the compounds belonging to this group, the secoiridoid family showed a significant decline after filtration. Indeed, the greatest decrease was found in VOO from filtration step (B, 20%), while the lowest decrease occurred during the last filtration step (D, 8%). Only by use of the correction coefficient (*Cc*) was it possible to discern the real behavior of the secoiridoid family during the VOO industrial filtration, which was masked until now by an apparent increase in those compounds that, in fact, was a result of an analytical artifact in the extraction step promoted by the moisture reduction in the filtered VOO.

Finally, in an effort to establish the effect of filtration on total phenols, the individual concentration of phenolic alcohols, lignans, flavones, and the corrected values of the secoiridoid concentration were summarized and presented as total phenols in Fig. 4. The results showed a significant decrease in total phenol content in filtered VOOs belonging to all filtration steps A, B, C, and D. These results confirm those found previously, using the same filter aids as used in this study but at a laboratory scale, where the retentive power of filter cake on some phenolic compounds caused a decrease in total phenols after filtration [11].

4. Conclusions

In this study, the effect of industrial filtration on the decrease in moisture content, phenolic alcohols, and flavones reported in previous works was confirmed at the industrial scale.

However, the most important achievement of this work is the proposal, for the first time, of a correction coefficient (*Cc*) that allowed the correction of the effect of moisture reduction on the apparent increase of secoiridoids such as dehydro-oleuropein aglycone, oleuropein aglycone, ligstroside aglycone, and methyl oleuropein aglycone in filtered VOO, using oleuropein as the internal standard during phenolic extraction. This is of great importance for future studies seeking to quantify the phenolic compounds in VOOs.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.077.

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