Influence of an innovative and promising gas clarification process on the quality of stored extra virgin olive oils

# Influence of an innovative and promising gas clarification process on the quality of stored extra virgin olive oils

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**Abstract:**

Filtration of extra virgin olive oil is a process that may improve preservation of the quality during storage. In the current study, different aliquots of extra virgin olive oils were subjected to filtration with a traditional filter press or an innovative patented alternative process of clarification by insufflating inert gas such as nitrogen and argon; all treated samples and, as control unfiltered ones, were stored for one year to evaluate the effects of these technologies on the quality of oil during shelf-life. Basic quality indexes, diglycerides, phenolics and volatiles, as well as the sensory characteristics of samples, were determined at 4 month intervals during storage. According to the volatile compounds, phenolics and sensory analysis, the novel technique had a beneficial effect on the storage of extra virgin olive oils; accordingly, this process could be exploited by the olive oil industry.

**Keywords:** *extra virgin olive oil, filtration, inert gas clarification, volatiles, phenolics*, *shelf-life*

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## 1. Introduction

Extra virgin olive oil (EVOO) is the extract from high-quality olives that can be freshly consumed without any further treatment. Olive oil stability is related to conservation of so-called dynamic parameters during the useful life of the product. During the autoxidation process a series of compounds are formed, causing off-flavors, rancidity, loss of nutritional value and consumer rejection of the food product (Andreou et al., 2017). The main endogenous factors responsible for the high oxidative stability of virgin olive oil (VOO) is the characteristic content in fatty acids, and, as recognized in many studies, the presence of certain minor components, such as phenolic compounds (Bendini, Cerretani, Salvador, Fregapane, & Lercker 2009a; Boskou, 2006; Psomiadou, & Tsimidou, 2002). Moreover, it has also been reported in the literature that the stability of EVOO is influenced by the presence of suspended solids and vegetative water that remain in the product after the extraction process, which can lead to fermentation and off-flavors, such as fusty-muddy sediments or winey, that declassify the product (Bendini et al., 2013; Bubola, Koprivnjak, & Sladonja 2012). In addition, exogenous factors can strongly affect the shelf-life of EVOOs, such as the availability of oxygen, temperature and light during the storage. These latter factors influence the oxidative decomposition of triglycerides, thus forming peroxide compounds that evolve into secondary oxidation products leading to the rancid off-flavor (García, Brenes, García, Romero, & Garrido, 2003).

In order to minimize the negative effects linked to the presence of suspended or emulsified compounds, filtration is a process allowed by European Community (EEC Reg. 1638/98) as pre-treatment before bottling to enhance the quality and appearance of virgin olive oil during storage (Jabeur, Zribi, Abdelhedi, & Bouaziz, 2017; Lozano-Sánchez, Cerretani, Bendini, Gallina-Toschi, Segura-Carretero, & Fernandez-Gutierrez, 2012). The effects of filtration on the EVOO quality have been addressed by different authors. It has been reported that the filtration process reduces the phospholipid and water content that can render EVOO cloudy during storage; at the same time, the decrease of water content enhances olive oil stability because the oxidation process is lower during storage and reduces the hydrolysis rate of triglyceride to liberate free fatty acids (Spyros, Philippidis, & Dais, 2004; Brenes, García, García & Garrido, 2001). Depending on the EVOO composition and as a result of the water reduction after filtration, some authors have found that the hydrolysis rate of triglycerides and of phenolic compounds, such as secoiridoids, is lower in filtered than in the unfiltered oil. However, the content of simple phenolic compounds such as hydroxytyrosol (Hyty) during storage was higher in unfiltered olive oil than in the filtered one, while other phenolic compounds seems to increase after filtration. On the other hand, unfiltered EVOO develops

sensory defects earlier than filtered EVOO during storage (Gomez-Caravaca, Cerretani, Bendini, Segura-Carretero, Fernández-Gutiérrez, & Lercker, 2007; Fregapane, Lavelli, León, Kapuralin, & Salvador, 2006).

As an alternative to the filtration process, a clarification technique has been developed by the University of Bologna together with Sapio, a private Italian company that supplies gas for industrial and research sectors. This patented clarification system is based on inserting a flow of inert gas from the bottom of the filter tank containing the cloudy virgin olive oil directly to the center of the virgin olive oil mass. The gas flow generates circular bubble movements that enhance the separation of suspended solids and vegetative water (Bendini et al., 2013; Cerretani, Rocculi, Bendini, Romani, & Bacci, 2009). One of the advantages of this system over other kinds of filtration techniques is that the inert gas flow avoids direct contact with organic materials or filtration aids with the EVOO. Moreover, even after the clarification, the treated oil can remain in the storage tanks under inert gas. Therefore, the shelf-life of oil could be potentially extended compared to a non-filtered or traditionally-filtered product (Lozano-Sanchez, Cerretani, Bendini, Segura-Carretero, & Fernandez-Gutierrez, 2010). One of the main drawbacks is represented by the cost of the process (Bendini et al., 2013): to reduce it, an adequate recycling system of the inert gas needs to be designed in order to re-use the same gas for subsequent processes.

The effect of traditional filtration and the innovative clarification systems on the quality of EVOOs was previously studies by Lozano-Sanchez et al. (2012), reporting that the water content decreased in treated samples. It was also shown that the total phenolic compounds increased following all adopted treatment systems, especially after clarification with argon. In addition, the oxidative stability of both filtered and clarified samples was lower than that in unfiltered oil. Regarding sensory attributes, fruity attributes and pungency were slightly enhanced after clarification (Gila, Beltrán, Bejaoui, Aguilera, & Jiménez, 2017; Lozano-Sanchez et al., 2012).

Despite this, there is no study on the effects of the innovative clarification system on the chemical and sensory properties of EVOO during and after prolonged storage. Thus, the aim of this study was to analyze the influence of the innovative clarification system with nitrogen or argon flow on chemical quality parameters and sensory attributes of EVOO during one year of storage compared to samples obtained by a commercial filtration system. In order to achieve the purpose of this study, full characterization in terms of oxidative and hydrolytic status, sensory quality, water content, phenolic and volatile profiles have been carried out on unfiltered, filtered and clarified EVOOs: all analyses were performed at defined time intervals after subjecting a freshly produced EVOO to the different treatments (Ayyad, 2015a).

## 2. Materials and methods

### 2.1. Samples

The olives selected for the extraction of the EVOOs were of the Canino cultivar and collected in the Lazio region (Italy). For the production of the oil it was used a two-phase system equipped with a decanter (Alfa Laval, Lund, Sweden).

The oil was divided into 4 aliquots: one remained as unfiltered (Uf), and one was filtered through a commercial filter press system (P= 1.8 bars, with the use of food grade plastic fibers) to produce filtered EVOO samples (Cf). The last two aliquots were clarified by insufflating inert gases, namely nitrogen or argon, directly into the center of the oil mass thanks to the adoption of a pilot clarification system developed and patented by the University of Bologna and Sapio (Cerretani et al., 2009). In the case of nitrogen gas, this was directly injected into the veiled EVOO bulk mass (P = 2 bars) to produce clarified EVOO (Nc), while the argon flow for the clarification of another aliquot (Ac) was set at 12 L $min^{-1}$. Both the filtration and clarification treatments were performed at room temperature for two hours (Ayyad, 2015a).

### 2.2. Storage simulation

All EVOO samples were filtered or clarified within three days after production, and immediately bottled in hermetically sealed clear glass bottles of 250 mL. The samples were stored inside a storage room covered with aluminum foil to avoid the negative effects of light exposure. The temperature range during the year of storage was 17-22 °C in November-May, 30-36 °C from June to the end of August and around 20-25 °C from September to the end of the storage period.

The chemical and sensory properties of samples were evaluated at time zero and after 4, 8 and 12 months of storage; for this purpose, three bottles of each sample were removed from the storage room at each scheduled time and then analyzed in triplicate. Aliquots of samples were withdrawn from the geometrical center of each bottle.

### 2.3. Chemicals

All solvents used were of high purity grade and furnished by Sigma–Aldrich (St. Louis, MO, USA), and Fluka (Buchs, Switzerland). HPLC-gradient grade solvents were also purchased from Sigma–Aldrich. Commercial standards, all of proper purity grades, were acquired from Sigma–Aldrich and Fluka.

## 2.4. Quality chemical parameters and water content

Free acidity (FA) expressed as g of oleic acid per 100 g of oil, peroxide value (PV) expressed as milli-equivalent $O_2$ $kg^{-1}$ oil and UV absorption coefficients ($K_{232}$, $K_{270}$) were determined according to the official methods of analysis described in the EEC Reg. 2568/91 and successive amendments. Water content was determined at 103 °C using an air oven (ISO 662:1988) and expressed as mg kg $oil^{-1}$. Diglycerides (DGs) were determined by a GC-FID Carlo Erba MFC500 (Milan, Italy) with an Rtx-65TG column (Restek, Bellefonte, PA) according to a modified version of the method reported by Serani, Piacenti, and Staiano (2001). Identification of DGs was carried out by comparing the retention time of peaks on the basis of chromatograms reported in the literature, while their quantification was realized by use of an internal standard, (0.5 mL of a 2 mg $mL^{-1}$ solution of dilaurin dissolved in chloroform, added to 100 mg of oil) (Serani et al. 2001). The results reported herein are the ratio between the sum of 1,2-DGs and the sum of 1,3-DGs.

## 2.5. Extraction of phenolic compounds

Polar phenolic compounds were extracted from EVOO samples following the liquid-liquid extraction procedure described by Rotondi, Bendini, Cerretani, and Mari (2004). After evaporation, the dried residue was dissolved in 3 mL of methanol/water (50:50, v/v). The phenolic extracts were filtered through a 0.2 µm syringe filter (Whatman Inc) and stored at -18 °C until analysis by HPLC.

## 2.6. Phenolic compounds determination

The chromatographic analysis was performed by an 1100 series liquid chromatography instrument equipped with a quaternary pump and UV-Vis diode array and MS detectors (Agilent Technologies, Waldbronn, Germany). The separation of phenolic compounds was carried out on a reverse phase 2.6 µm, 100 mm x 3.00 mm C18 100A Kinetex column (I.D; Phenomenex, Torrance, CA) thermostated at 30 °C and equilibrated for 5 min prior to each analysis. The mobile phases used were water/formic acid (99.5:0.5 % v/v) as eluent A and acetonitrile as eluent B; the gradient elution was as follows: from 0 to 3 min solvent B increased from 5% to 20%, at 4 min solvent B reached 40%, at 9 min solvent B reached 60%, and finally at 10 min solvent B was 100%; at 13 min 5% solvent B was restored. The total run-time was 13 minutes. The injection volume and flow rate were 2.5 µL and of 0.7 mL $min^{-1}$ respectively. The chromatograms were monitored at 240, 280, 320, and 345 nm. Each wavelength was suitable for each group of compounds: 240

nm was used for elenolic acid, 280 nm was used for hydroxybenzoic acids, phenyl ethyl alcohols, secoiridoids and lignans, 320 nm for hydroxycinnamic acids, and 345 nm for flavones.

The mass spectrometry working conditions were: nebulizer gas pressure, 0.24 MPa; drying gas flow, 7 L $min^{-1}$ at 300 °C; capillary voltage, 2.5 kV. Nitrogen was used as a nebulizer and drying gas. The mass scan/ion was performed in the negative and positive ion mode, within the *m/z* range from 100 to 900 (Ayyad, 2015a).

### 2.7. Volatile compounds determination

Volatile compounds were evaluated by SPME-GC (Agilent 6890N, Santa Clara, CA, USA) coupled to quadrupolar mass selective spectrometry (Agilent 5973 N, Agilent Technologies), according to Cerretani, Bendini, Salvador and Fregapane (2008). The identification was carried out on the basis of the NIST library (2005 version) and MS literature data. Volatile compounds were quantified by internal standard and the results were expressed as mg of 4-methyl-2-pentanone (Fluka, Buchs, Switzerland) per kg of oil.

### 2.8. Sensory analysis

The sensory analysis (COI Panel Test) of all samples was performed according to the EU Reg. 1348/2013 by a fully trained group of 8 expert tasters of the Professional Committee of olive oil tasters of the Department of Agricultural and Food Sciences of the University of Bologna.

### 2.9. Statistical analysis

All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons. The software XLSTAT 7.5.2 version (Addinsoft, USA) was used to elaborate the data by analysis of variance (ANOVA, Fisher LSD, $p < 0.05$). Significant differences (at p-level <0.05) among medians at different storage time (within the same sample) and at the same storage time (0 and 12 months) were explored by means of the nonparametric Kruskal-Wallis test followed by the multiple comparison (Statistica-StatSoft, version 7). The same letters (a-d) denote no significant differences during storage, within the same sample (P<0.05). The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) (P<0.05).

## 3. Results and discussion

### 3.1. Changes in quality parameters and water content

Basic quality parameters were established to estimate the changes in hydrolytic and oxidative state of EVOO samples after filtration or clarification for a storage time of 12 months. As shown in Table 1, a slight but significant increase in free acidity was observed in unfiltered, filtered and clarified with $N_2$ samples. Over time, this fact probably affects the susceptibility to oxidation and degradation of the complex phenolic compounds (Lozano-Sanchez et al., 2010). At the end of storage, the unfiltered EVOO sample showed a significantly higher FA value than filtered and clarified samples. This could be attributed to its higher water content and to the presence of lipase and other hydrolytic enzymes in the suspended materials present in the unfiltered sample which favor degradation of triglycerides (Fregapane et al., 2006, Brenes et al., 2001; Shimizu, Kudo, Nakajima, & Matsuo, 2008).

Regarding oxidation stability parameters, PV showed relative stability during storage of EVOO samples. On the other hand, $K_{232}$ and $K_{270}$ coefficients showed only a small increase, in particular after 8 months of storage (Table 1). The differences in PV and $K_{232}$ parameters at time zero and after 12 months among the different samples were not relevant. All stored samples remain, even at the end of storage period, within the established EU (Reg. EU 1348/2013) limits for EVOOs.

Water content in EVOO may range between 0.03 to 0.2%, depending on several factors (Ragni, Berardinelli, Cevoli, & Valli, 2012). It was assumed that the presence of water in VOO is responsible for the persistence of dispersed and suspended materials which reduce the consumer attractiveness of virgin olive oil (Lercker, Frega, Bocci, & Servidio, 1994). Moreover, water may induce degradation of minor compounds during storage and contribute to the perception of flavor defects, in particular vinegary perception (Dais, 2013).

As shown in Table 1, an important reduction of water content occurred in clarified or filtered EVOO samples compared to the unfiltered one. The water content in the same sample decreased gradually during storage time, probably as a result of the settling of suspended materials that are reach in water (actually the analyzed aliquots were collected from the geometrical center of each bottle). These results are in agreement with those presented by Bubola, Lukic, Mofardin, Butumovic and Koprivnjak (2017).

Comparing filtration and clarification, it was found that both treatments were very efficient in reducing the water content, with the latter being more efficient than the first.

The content in DGs, especially the 1,2-1,3-DG ratio, can be generally considered as an indicative freshness parameter related to EVOO storage; in agreement with previous results (Serani et al., 2001; Ayyad, Valli, Bendini, Adrover-Obrador, Femenia, & Gallina-Toschi, 2015). According to the data shown in Table 1, the 1,2/1,3-DG ratio underwent a significant decrease after 4 months of storage in all samples, after which the

change was slight and not significant for up to 12 months. Furthermore, there was no evidence that filtration or clarification could affect DG isomerization.

### 3.2. Changes in phenolic compounds

The results are shown in Table 2. Hyty and Ty increased significantly with storage time in Uf samples, from 6.8 to 31.7 mg $kg^{-1}$ and from 5.0 to 41.3 mg $kg^{-1}$ for Hyty and Ty, respectively. Considering the EVOO submitted to the different treatments, Hyty and Ty concentrations both reached their highest concentrations at month 8 in $N_2$ clarified samples, while in those clarified with Ar a slight decrease during storage was observed. The highest Hyty and Ty amounts found in the unfiltered sample compared to the other samples after 12 months of storage could be associated with the preservation of hydrolytic enzyme activity linked to the high water content in unfiltered samples (Bendini et al., 2009a) that was augmented by the temperature increase recorded in the summer season (34 $^{o}$C) (Fregapane et al., 2006). On the other hand, the amount of CA was similar for all samples and constant during storage.

Among the secoiridoid derivatives, decarboxymethyl oleuropein aglycon (DOA) and oleuropein aglycon (OA) are well known as the most active phenolic compounds as antioxidants against oxidation reactions (Lozano-Sanchez et al., 2012). As shown in Table 2, the major secoiridoid derivatives present in these samples were DOA and OA. During storage, the amount of DOA decreased in all samples, with the highest percentage of depletion found in the Uf and Cf samples (73 % and 63 % of the amount at time zero, respectively). For Nc and Ac samples, the DOA concentrations decreased by 50 and 40 % respectively.

The higher concentrations of DOA in both clarified samples found at the end of storage vs. the filtered and unfiltered samples could indicate that clarification has a positive impact in slowing down the degradation of these complex phenolic compounds that are among the main contributors to the oxidative stability of olive oils (Bendini, Cerretani, Vecchi, Carrasco-Pancorbo, & Lercker, 2006).

In agreement with previous studies, OA was the most stable secoiridoid during storage (Brenes et al., 2001; Fregapane et al., 2006). The concentration of this compound, in all samples at the beginning of the experiment ranged from 79 to 92 mg $kg^{-1}$. During storage, clarification with $N_2$ led to lesser loss of OA.

Regarding the other secoiridoid derivatives, the variation in LA content during storage was similar in all samples, while DLA tended to disappear during extended storage.

Considering the other phenolic compounds, EA decreased significantly during storage in all the samples, possibly as a result of oxidation reactions, with the lowest loss in its concentration during storage in Cf samples.

According to the different classes of phenolic compounds identified (see Figure 1), namely secoiridoids and phenyl ethyl alcohols, it is possible to observe that the content in secoiridoids for the clarified samples after 12 months of storage was higher than in unfiltered ones. This supports that clarification has a beneficial effect in the preservation of the secoiridoids during storage. At the same time, a consistent increase in the amount of the phenyl ethyl alcohols was found in the unfiltered sample, confirming possible degradation of secoiridoids.

### 3.3. Changes in LOX volatile compounds

Volatile compounds in EVOO are influenced by various factors, including cultivars, fruit maturity, geographical region, processing and storage conditions (Angerosa et al., 2004). Volatile compounds responsible for the positive aroma perception in VOO are mainly produced by the oxidation of unsaturated fatty acid via the lipoxygenase pathway (LOX) (Kalua, Allen, Bedgood, Bishop, Prenzler, & Robards, 2007). Positive perceptions from volatiles are attributed to aldehydes, esters, hydrocarbons, ketones, and alcohols. Among the different categories, 6 carbon volatile compounds like hexanal, (*E*)-2-hexenal and hexan-1-ol, as well as groups of 5 carbon volatiles derived by the secondary LOX pathway, are the main volatile compounds found in VOO (Kiritsakis, 1998; Angerosa, 2002). In addition, after filtration and clarification, a reduction in $C_6$ and $C_5$ was seen (Lozano-Sanchez et al., 2010).

The volatile compounds found in EVOO samples are shown in Table 3A and 3B (Ayyad, 2015a). The main aldehydes identified were hexanal and (*E*)-2-hexenal. High concentrations were found for (*E*)-2-hexenal. It could be observed that clarification with inert gases, similar to traditional filtration, allowed a greater stability of this compound during storage compared to the unfiltered sample. On the other hand, the increase in amounts found for Nc and Ac samples during storage could be due to oxidation reactions.

The total $C_6$ and $C_5$ alcohols showed a significant decrease in Uf and Cf samples during storage, while for the samples clarified by inert gases they remained practically constant. These results were comparable to those presented by other authors (Di Giovacchino, Mucciarella, Constantini, & Ferrante, 2002; Cavalli, Fernandez, Lizzani-cuvelier, & Loiseau, 2004; Stefanoudaki, Williams, & Harwood, 20100). In addition, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol concentrations remained stable from the beginning to the end of the experiment in both Nc and Ac samples. (*Z*)-2-pentene-1-ol and pentene dimers for Cf increased significantly at the end of storage and remained without significant variation in Nc samples. The presence of 1-penten-3-ol could be associated with the fruity perception of olive oil (Aparicio & Luna, 2002). On the other hand, the reduction in (*E*)-2-hexenal during storage is due mainly to the loss of freshness (Youssef,

Ben Youssef, Mokhtar, & Guido, 2011). It is well known that microorganisms, mainly yeasts, found especially in unfiltered samples migrate into the oil together with the solid particles of the fruit and micro-drops of vegetation water (Ciafardini, & Zullo, 2002). In this regard, it may be presumed that in the unfiltered sample (particularly considering the water content) the microorganisms that survive during oil storage are responsible for the reduction of (*E*)-2-hexenal in (*E*)-2-hexenol through the action of alcohol dehydrogenase. This could explain the anomalous increase of (*E*)-2-hexenol and the simultaneous decrease in (*E*)-2-hexenal seen only in the Uf sample during storage.

### 3.4. Changes in sensory attributes

After filtration and clarification with inert gases, an intensification of sensory attributes was observed, see table 4 (Ayyad, 2015a). The fruity intensity was more pronounced after clarification, even if not in a significant way, in particular for the Ac sample; this trend was in agreement with Lozano-Sanchez et al. (2010). During storage, there was a decrease in the sensory scores evaluated over time for all samples: this alteration was slower in filtered and clarified samples than in unfiltered ones (Jabeur, Zribi, & Bouazid, 2017). This behavior indicates that filtration and clarification might help to maintain the positive sensory attributes. Comparing all stored samples at the end of storage (Table 4), it was found that fruity, bitter and pungent attributes remained higher, even if not always significantly, in filtered and clarified samples than in unfiltered EVOO. The general higher fruitiness perception in Cf and clarified samples compared to the unfiltered one could be linked to the higher concentrations of (*E*)-2-hexenal and 1-pentene-3-one as these compounds are closely associated with fruity and green notes of EVOO (Angerosa et al., 2004; Bubola et al., 2012). The most evident effect is related to a trend in the intensities of bitter and pungent attributes that remained higher in filtered and clarified samples than in the unfiltered oil, in agreement with the less dramatic degradation of secoiridoids observed during storage. At the end of the storage period, none of the samples showed any sensory defects and remained within the accepted EU limits for the EVOO category (Reg. EU. 2095/2016).

## 4. Conclusions

This investigation highlights that clarification can have a beneficial effect in storage of EVOO compared to unfiltered oils. Hydrolytic degradation, evaluated in terms of increase in free acidity, was more pronounced in unfiltered EVOO than in clarified and filtered samples. A significant decrease in water content associated with filtration and clarification was found, especially for the inert gas clarified samples. The decrease in the

water content up to a certain value, as in the case of argon clarified sample, could be beneficial in maintaining the oxidative stability of EVOO. Lower degradation rates of secoiridoid phenolic compounds over time were found in clarified samples than in filtered ones as well as higher concentrations of (*E*)-2-hexenal and 1-pentene-3-one in filtered and clarified samples compared to the unfiltered one were observed. These trends contributed in maintaining the positive sensory attributes of oil. In general, filtration and clarification help in preserving the initial quality of the analyzed EVOO during storage, such as sensory attributes, compared to the unfiltered sample. Moreover, clarification has advantages over commercial filtration systems, since the volatiles linked to positive attributes were not altered during storage of inert gas clarified samples; these latter showed lower water content and higher secoiridoid levels compared to unfiltered sample. It is very important to plan future investigations to confirm these promising results for clarified samples, especially by increasing the storage time to get closer to the real condition of the commercial products. Definitively it will be important to focus on the economic aspects related to this process in order to favor its application in an industrial framework.

**Figure 1:** Changes in total phenyl ethyl alcohols (Ty + Hyty) and secoiridoids (DOA + DLA + OA + LA) during storage of different EVOO samples in dark from 0 to 12 months.

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) ($P<0.05$).

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**Conflict of interest**

Authors have no competing interests to declare.

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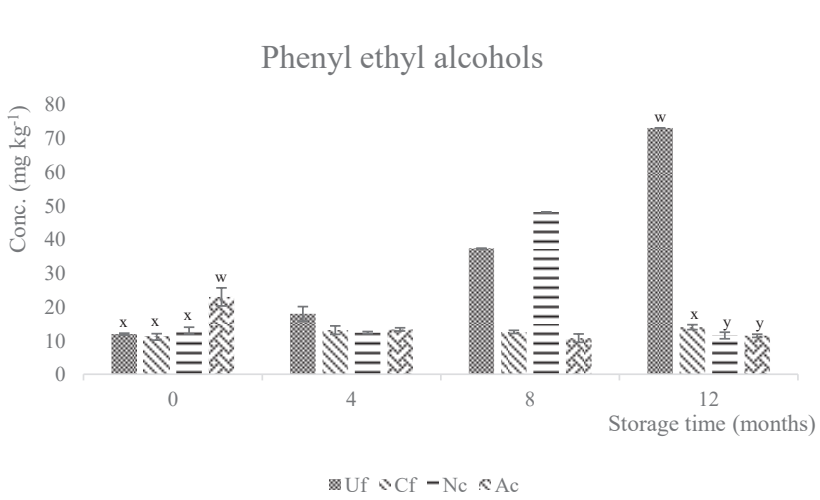
**Figure 1**


Phenyl ethyl alcohols
Conc. (mg kg-1)
80
70
60
50
40
30
20
10
0
x
x
x
w
w
x
y
y
0
4
8
12
Storage time (months)
Uf
Cf
Nc
Ac

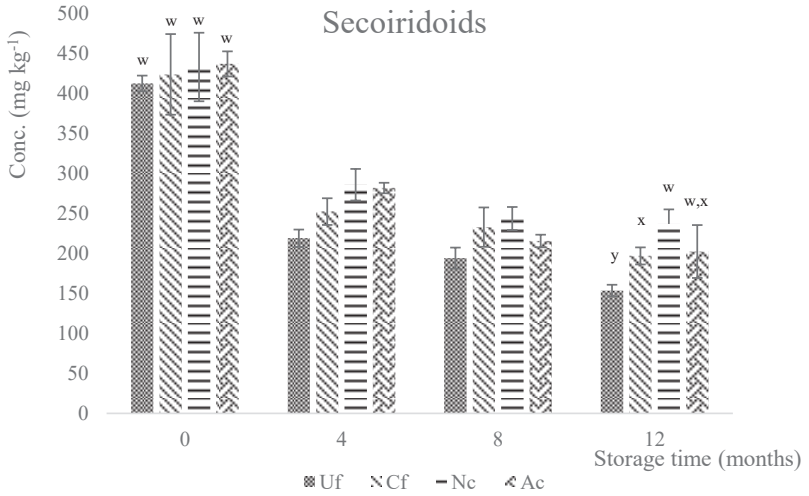

Secoiridoids
Conc. (mg kg-1)
500
450
400
350
300
250
200
150
100
50
0
w
w
w
w
y
x
w
w,x
0
4
8
12
Storage time (months)
Uf
Cf
Nc
Ac

**Table 1**

**Table 1:** Values of FA (g oleic acid 100 $g^{-1}$ oil), PV (meq $O_2$ $kg^{-1}$ oil), $K_{232}$, $K_{270}$, 1,2/1,3-DG ratio and water content (mg $kg^{-1}$ oil) registered during storage of different EVOO samples.

| Samples | Storage time (months) | FA | PV | $K_{232}$ | $K_{270}$ | 1,2/1,3-DG ratio | Water content |
|---|---|---|---|---|---|---|---|
| Uf | 0 | 0.21 ± 0.00 c,w | 10 ± 1 ab,w | 1.37 ± 0.09 b,y | 0.1 ± 0.01 bc,x | 26.8 ± 1.1 a,x | 1485 ± 40 a,w |
| | 4 | 0.27 ± 0.01 b | 7 ± 0 c | 1.9 ± 0.25 a | 0.09 ± 0.00 c | 6.9 ± 0.1 b | 885 ± 7 b |
| | 8 | 0.28 ± 0.02 b | 11 ± 1 a | 2.06 ± 0.34 a | 0.11 ± 0.01 ab | 2.5 ± 0.3 c | 878 ± 17 b |
| | 12 | 0.34 ± 0.00 a,w | 9 ± 1 b,x | 2.13 ± 0.09 a,w | 0.12 ± 0.00 a,y | 2.2 ± 0.2 c,w | 771 ± 6 c,w |
| Cf | 0 | 0.21 ± 0.00 c,w | 10 ± 0 a,w | 1.69 ± 0.12 b,w | 0.09 ± 0.00 c,x | 34.0 ± 6.5 a,w | 763 ± 36 a,x |
| | 4 | 0.24 ± 0.01 b | 8 ± 1 b | 1.48 ± 0.15 b | 0.1 ± 0.00 b | 6.7 ± 0.1 b | 705 ± 71 a |
| | 8 | 0.25 ± 0.00 b | 11 ± 0 a | 2.3 ± 0.17 a | 0.13 ± 0.00 a | 3.3 ± 0.0 b | 668 ± 62 ab |
| | 12 | 0.26 ± 0.00 a,y | 10 ± 0 a,wx | 2.31 ± 0.22 a,w | 0.14 ± 0.01 a,x | 2.2 ± 0.1 b,w | 568 ± 44 b,x |
| Nc | 0 | 0.21 ± 0.00 c,w | 8 ± 1 ab,x | 1.58 ± 0.10 c,wx | 0.1 ± 0.00 c,x | 23.3 ± 1.6 a,x | 190 ± 6 a,z |
| | 4 | 0.24 ± 0.01 b | 8 ± 1 b | 1.51 ± 0.12 c | 0.1 ± 0.00 c | 4.3 ± 0.2 b | 29 ± 9 b |
| | 8 | 0.24 ± 0.00 b | 9 ± 1 ab | 2.14 ± 0.11 b | 0.13 ± 0.01 b | 2.5 ± 0.1 c | 26 ± 6 b |
| | 12 | 0.29 ± 0.00 a,x | 10 ± 1 a,x | 2.37 ± 0.14 a,w | 0.17 ± 0.01 a,w | 1.7 ± 0.0 c,w | nd |
| Ac | 0 | 0.21 ± 0.01 b,w | 9 ± 1 bc,wx | 1.43 ± 0.02 c,xy | 0.11 ± 0.00 b,w | 24.7 ± 1.7 a,x | 260 ± 32 a,y |
| | 4 | 0.25 ± 0.01 a | 7 ± 1 c | 1.74 ± 0.04 b | 0.1 ± 0.00 b | 5.4 ± 0.7 b | 229 ± 16 a |
| | 8 | 0.25 ± 0.01 a | 10 ± 1 ab | 1.91 ± 0.23 ab | 0.13 ± 0.00 a | 2.7 ± 0.2 c | 85 ± 5 b |
| | 12 | 0.22 ± 0.01 b,z | 11 ± 1 a,w | 2.11 ± 0.10 a,w | 0.12 ± 0.01 a,xy | 1.9 ± 0.0 c,w | nd |

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
FA: Free Acidity; PV: Peroxide value; DG ratio: Diglycerides ratio
The results are expressed as means.
The same letters (a-d) denote no significant differences during storage, within the same sample ($P<0.05$).
The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) ($P<0.05$).
nd: not detected.

**Table 2:** Changes in phenolic compounds (mg $kg^{-1}$) of different EVOO samples during storage in the dark from 0 to12 months.

| Samples | Storage time (Months) | Hyty | Ty | CA | DOA | Pin | DLA | OA | LA | EA |
|---|---|---|---|---|---|---|---|---|---|---|
| Uf | 0 | 6.8 ± 0.4 d,x | 5.0 ± 0.2 d,y | 1.02 ± 0.01 b,y | 276.8 ± 7.0 a,w | 23.8 ± 0.8 a,y | 10.1 ± 0.6 a,y | 85.2 ± 3.3 a,wx | 39.7 ± 5.6 a,w | 57.7 ± 1 |
| | 4 | 9.1 ± 1.0 c | 8.8 ± 1.2 c | 1.02 ± 0.04 b | 132 ± 1.8 b | 19.0 ± 0.6 b | 4.8 ± 0.2 b | 63.6 ± 8.8 b | 18.0 ± 2.4 b | 59.8 ± 1 |
| | 8 | 16.6 ± 0.0 b | 20.7 ± 0.0 b | 1.06 ± 0.0 a | 109.8 ± 5.1 c | 12.9 ± 1.3 c | 2.1 ± 0.4 c | 62.0 ± 8.8 b | 19.8 ± 0.7 b | 25.4 ± 1 |
| | 12 | 31.7 ± 0.1 a,w | 41.3 ± 0.1 a,w | 1.11 ± 0.01 a,w | 75.3 ± 8.0 d,y | 8.2 ± 0.2 d,y | nd | 59.2 ± 3.3 b,w | 18.7 ± 0.4 b,wx | 18.7 ± 3 |
| Cf | 0 | 6 ± 0.8 b,x | 5.1 ± 0.2 c,y | 1.2 ± 0.0 a,x | 289.1 ± 39.2 a,w | 27.0 ± 1.1 a,x | 12.1 ± 0.4 a,x | 91.8 ± 9.0 a,w | 30.1 ± 2.9 a,x | 41.2 ± 3 |
| | 4 | 7.3 ± 1.1 a | 5.7 ± 0.1 b | 1.1 ± 0.1 ab | 155.1 ± 16.1 b | 19.7 ± 1.6 b | 4.9 ± 0.2 b | 72.5 ± 0.9 b | 19.2 ± 1.7 b | 39.0 ± 2 |
| | 8 | 6.9 ± 0.2 ab | 5.7 ± 0.3 ab | 1 ± 0.0 b | 134.1 ± 26.3 bc | 13.3 ± 0.7 c | nd | 76.5 ± 1.4 b | 21.6 ± 2.4 b | 24.0 ± 0 |
| | 12 | 7.7 ± 0.3 a,x | 6.2 ± 0.4 a,x | 1 ± 0.1 b,x | 105.6 ± 12.0 c,x | 10.3 ± 0.3 d,xy | nd | 69.9 ± 8.3 b,w | 20.8 ± 1.8 b,w | 23.9 ± 0 |
| Nc | 0 | 6.5 ± 0.5 bc,x | 6.4 ± 0.5 b,x | 1.2 ± 0.1 a,x | 310.6 ± 37.0 a,w | 32.0± 0.9 a,w | 8.1 ± 0.3 a,z | 79.1 ± 3.4 a,x | 34.4 ± 4.9 a,wx | 51.0 ± 8 |
| | 4 | 6.9 ± 0.1 b | 5.4 ± 0.2 c | 1.0 ± 0.0 c | 194.0 ± 8.1 b | 19.9 ± 1.8 b | 4.8 ± 0.6 b | 68.1 ± 13.3 a | 18.5 ± 3.5 b | 28.8 ± 3 |
| | 8 | 20.8 ± 0.0 a | 27.2 ± 0.0 a | 1.1 ± 0.0 ab | 158.4 ± 15.1 b | 10.3 ± 2.1 c | nd | 66.8 ± 0.6 a | 17.7 ± 0.5 b | 23.0 ± 0 |
| | 12 | 6.0 ± 0.3 c,y | 5.5 ± 0.6 c,x | 1.0 ± 0.0 bc,wx | 154.9 ± 9.0 b,w | 12.6 ± 0.8 c,wx | nd | 64.6 ± 12.5 a,w | 16.7 ± 1.9 b,x | 18.2 ± 3 |
| Ac | 0 | 14 ± 1.9 a,w | 8.9 ± 1.2 a,w | 1.3 ± 0.1 a,w | 287.4 ± 16.0 a,w | 25.3 ± 2.1 a,xy | 19.9 ± 0.0 a,w | 91.0 ± 0.9 a,w | 30.6 ± 1.4 a,x | 62.8 ± 7 |
| | 4 | 7.7 ± 0.4 b | 5.6 ± 0.2 b | 1.0 ± 0.0 b | 219.4 ± 3.0 b | 19.3 ± 2.1 b | 4.5 ± 0.1 b | 37.9 ± 4.5 b | 19.4 ± 1.6 b | 48.3 ± 8 |
| | 8 | 5.8 ± 0.6 bc | 4.9 ± 0.6 b | 1.0 ± 0.0 b | 163.4 ± 4.1 c | 13.3 ± 1.1 c | nd | 35.4 ± 0.4 b | 17.1 ± 3.4 b | 14.3 ± 2 |
| | 12 | 6.0 ± 0.0 c,y | 6.0 ± 0.0 b,x | 1.1 ± 0.1 b,w | 170.6 ± 26.3 c,w | 13.8 ± 2.5 c,w | nd | 29.5 ± 7.7 c,x | 19.4 ± 1.5 b,wx | 15.7 ± 1 |

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
Hyty; hydroxytyrosol; Ty: tyrosol; CA: caffeic acid; DOA: decarboxymethyl oleuropein aglycon; Pin: (+)-pinoresinol; DLA: decarboxymethyl ligstroside aglycone; OA: oleuropein aglycone; LA: ligstroside aglycone; EA: elenolic acid.
The same letters (a-d) denote no significant differences during storage, within the same sample ($P<0.05$).
The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) ($P<0.05$).
nd: not detected.

**Table 3**

**Table 3 A:** Changes in C6-LOX volatile compounds (expressed as mg 4 methyl-2-pentanone $kg^{-1}$ oil) during storage of different EVOO samples in the dark for 12 months.

| Samples | Storage time (Months) | Main Aldehydes | | Main C6 Alcohols | | | |
|---|---|---|---|---|---|---|---|
| | | Hexanal | (*E*)-2-Hexenal | Hexan-1-ol | (*E*)-2-Hexen-1-ol | (*Z*)-3-Hexen-1-ol | Sum C6-LOX volatiles |
| **Uf** | 0 | 0.67 ± 0.11 a,w | 14.1 ± 2.06 a,w | 0.23 ± 0.04 b,w | 0.40 ± 0.07 d,w | 0.20 ± 0.03 a,w | 15.72 ± 2.31 a,w |
| | 4 | 0.61 ± 0.05 a | 9.18 ± 0.70 bc | 0.51 ± 0.02 a | 1.47 ± 0.21 c | 0.21 ± 0.02 a | 11.98 ± 0.83 b |
| | 8 | 0.48 ± 0.05 b | 11.32 ± 1.02 b | 0.48 ± 0.00 a | 2.27 ± 0.07 b | 0.23 ± 0.05 a | 14.78 ± 1.04 a |
| | 12 | 0.26 ± 0.01 c,y | 8.00 ± 0.25 c,z | 0.47 ± 0.03 a,w | 2.78 ± 0.17 a,w | 0.14 ± 0.01 b,x | 11.65 ± 0.44 b,y |
| **Cf** | 0 | 0.79 ± 0.02 a,w | 11.88 ± 0.13 b,x | 0.19 ± 0.01 c,x | 0.32 ± 0.00 a,wx | 0.17 ± 0.01 a,w | 13.47 ± 0.16 b,x |
| | 4 | 0.72 ± 0.01 ab | 10.17 ± 0.36 c | 0.22 ± 0.01 b | 0.30 ± 0.03 a | 0.18 ± 0.01 a | 11.58 ± 0.35 c |
| | 8 | 0.73 ± 0.03 ab | 14.32 ± 0.23 a | 0.26 ± 0.00 a | 0.33 ± 0.03 a | 0.18 ± 0.00 a | 15.81 ± 0.19 a |
| | 12 | 0.67 ± 0.11 b,wx | 10.06 ± 0.15 c,y | 0.13 ± 0.02 d,y | 0.31 ± 0.01 a,x | 0.10 ± 0.01 b,y | 11.28 ± 0.17 c,y |
| **Nc** | 0 | 0.59 ± 0.07 b,x | 12.36 ± 0.10 b,wx | 0.21 ± 0.01 c,wx | 0.24 ± 0.06 b,x | 0.19 ± 0.01 bc,w | 13.63 ± 0.15 b,wx |
| | 4 | 0.84 ± 0.06 a | 10.45 ± 0.67 c | 0.24 ± 0.01 b | 0.29 ± 0.01 b | 0.20 ± 0.01 b | 12.02 ± 0.71 c |
| | 8 | 0.87 ± 0.01 a | 13.54 ± 0.07 a | 0.27 ± 0.00 a | 0.36 ± 0.03 a | 0.22 ± 0.00 a | 15.26 ± 0.07 a |
| | 12 | 0.83 ± 0.14 a,w | 13.61 ± 0.23 a,w | 0.21 ± 0.01 c,x | 0.28 ± 0.00 b,x | 0.18 ± 0.01 c,w | 15.16 ± 0.20 a,w |
| **Ac** | 0 | 0.56 ± 0.01 d,x | 11.39 ± 0.17 c,x | 0.20 ± 0.01 d,wx | 0.29 ± 0.00 c,x | 0.18 ± 0.01 c,w | 12.63 ± 0.18 c,x |
| | 4 | 1.06 ± 0.06 a | 10.79 ± 0.18 d | 0.23 ± 0.01 b | 0.36 ± 0.04 b | 0.20 ± 0.01 b | 12.63 ± 0.25 c |
| | 8 | 0.90 ± 0.03 b | 14.76 ± 0.15 a | 0.28 ± 0.00 a | 0.45 ± 0.04 a | 0.24 ± 0.00 a | 16.63 ± 0.21 a |
| | 12 | 0.66 ± 0.03 c,x | 11.88 ± 0.11 b,x | 0.21 ± 0.01 c,x | 0.27 ± 0.02 c,x | 0.18 ± 0.01 c,w | 13.2 ± 0.13 b,x |

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
The same letters (a-d) denote no significant differences during storage, within the same sample ($P<0.05$).
The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) ($P<0.05$).
nd: not detected

**Table 3 B:** Changes in C5-LOX volatile compounds (expressed as mg 4 methyl-2-pentanone $kg^{-1}$ oil) during storage of different EVOO samples in dark for 12 months

| Samples | Storage time (Months) | Main C6 Alcohols | | | | |
|---|---|---|---|---|---|---|
| | | 1-penten-3-ol | (Z)-2-penten-1-ol | 1-penten-3-one | Pentene dimers | Sum of C5 volatiles |
| Uf | 0 | 0.17 ± 0.02 b,x | 0.26 ± 0.03 a,w | 0.82 ± 0.13 a,w | 1.24 ± 0.13 a,x | 2.81 ± 0.27 a,w |
| | 4 | 0.21 ± 0.00 a | 0.19 ± 0.01 b | 0.54 ± 0.10 b | 0.60 ± 0.07 c | 2.01 ± 0.07 b |
| | 8 | 0.18 ± 0.01 b | 0.18 ± 0.00 b | 0.33 ± 0.01 c | 1.00 ± 0.04 b | 2.10 ± 0.08 b |
| | 12 | 0.08 ± 0.01 c,z | 0.19 ± 0.01 b,z | 0.15 ± 0.01 d,z | 0.70 ± 0.08 c,z | 1.28 ± 0.06 c,z |
| Cf | 0 | 0.15 ± 0.00 d,y | 0.19 ± 0.01 c,x | 0.70 ± 0.02 a,w | 0.79 ± 0.03 c,y | 1.84 ± 0.02 c,y |
| | 4 | 0.20 ± 0.00 a | 0.19 ± 0.01 c | 0.53 ± 0.00 b | 0.67 ± 0.04 d | 1.64 ± 0.04 d |
| | 8 | 0.17 ± 0.00 c | 0.30 ± 0.02 a | 0.68 ± 0.02 a | 1.24 ± 0.07 a | 2.39 ± 0.07 a |
| | 12 | 0.18 ± 0.00 b,w | 0.22 ± 0.01 b,y | 0.69 ± 0.10 a,w | 0.96 ± 0.02 b,y | 2.20 ± 0.08 b,x |
| Nc | 0 | 0.12 ± 0.01 c,z | 0.19 ± 0.00 c,x | 0.38 ± 0.01 a,y | 1.41 ± 0.04 b,w | 2.07 ± 0.03 b,xy |
| | 4 | 0.18 ± 0.00 a | 0.20 ± 0.01 c | 0.30 ± 0.02 b | 0.76 ± 0.09 c | 1.27 ± 0.13 c |
| | 8 | 0.15 ± 0.01 b | 0.36 ± 0.00 a | 0.37 ± 0.02 a | 1.70 ± 0.03 a | 2.43 ± 0.03 a |
| | 12 | 0.09 ± 0.00 d,y | 0.32 ± 0.01 b,x | 0.28 ± 0.00 b,y | 1.39 ± 0.08 b,x | 1.98 ± 0.10 b,y |
| Ac | 0 | 0.57 ± 0.01 a,w | 0.19 ± 0.00 b,x | 0.57 ± 0.01 b,x | 0.85 ± 0.04 c,y | 2.28 ± 0.05 b,x |
| | 4 | 0.13 ± 0.00 c | 0.21 ± 0.01 a | 0.47 ± 0.02 c | 0.74 ± 0.01 d | 1.60 ± 0.02 c |
| | 8 | 0.17 ± 0.00 b | 0.42 ± 0.01 c | 0.61 ± 0.00 a | 1.50 ± 0.04 b | 2.69 ± 0.05 a |
| | 12 | 0.10 ± 0.00 d,x | 0.34 ± 0.00 c,w | 0.43 ± 0.00 d,x | 1.79 ± 0.06 a,w | 2.76 ± 0.07 a,w |

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
The different lower case letters (a - d) indicate the statistical differences for each sample during the storage time, letters (w-z) indicate the statistical differences among different samples all at time zero and all after 12 months, at 0.05 level (Fisher test). nd: not detected.

**Table 4:** Intensities of the main positive sensory attributes of EVOO during storage from 0 to 12 months.

| Samples | Storage time (Months) | Fruity | Bitter | Pungent |
|---|---|---|---|---|
| Uf | 0 | 4.2 a,w | 4.2 a,w | 4.4 a,w |
| | 4 | 4.3 a | 4.3 a | 4.4 a |
| | 8 | 2.7 b | 3.1 ab | 3.1ab |
| | 12 | 2.2 b,w | 2.6 b,w | 2.1 b,w |
| Cf | 0 | 4.7 a,w | 5.5 a,x | 6.6 a,x |
| | 4 | 4.1 a | 4.8 a | 4.2 ab |
| | 8 | 4.2 a | 4.3 a | 5.5 ab |
| | 12 | 3.4 a,w | 4.1 a,w | 3.9 b,x |
| Nc | 0 | 4.5 a,w | 4.8 a,wx | 5.8 a,wx |
| | 4 | 3.8 a | 4.7 a | 4.0 ab |
| | 8 | 3.2 a | 3.6 a | 3.5 b |
| | 12 | 2.4 a,w | 3.9 a,w | 3.9 ab,x |
| Ac | 0 | 4.9 a,w | 5.3 a,wx | 6.4 a,x |
| | 4 | 3.8 ab | 3.8 a | 4.6 ab |
| | 8 | 4.0 ab | 3.5 a | 4.2 ab |
| | 12 | 3.1 b,w | 4.0 a,w | 3.6 b,wx |

Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.

The same letters (a-d) denote no significant differences during storage, within the same sample ($P<0.05$).

The same letters (w-z) denote no significant differences between samples at the same storage time (0 and 12 months) ($P<0.05$).

*Graphical Abstract