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Quality control of virgin olive oils with regard to different storage and shipment conditions

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Quality Control of Virgin Olive Oils with Regard to Different Storage and Shipment Conditions

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Ph.D. Thesis

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LIST OF PAPERS

1- Ayyad, Z., Valli, E., Bendini, A. and Gallina-Toschi, T. (2015). Influence of filtration and clarification systems on quality of stored extra virgin olive oil. *Research paper in progress*.

2- Ayyad, Z., Valli, E., Bendini, A. and Gallina-Toschi, T. (2015). Filtered and clarified virgin olive oils: evolution of their quality during the storage under different conditions. *Research paper in progress.*

3- Ayyad, Z., Valli, E., Bendini, A., Adrover-Obrador, S., Femenia, A. and Gallina-Toschi, T. (2014). Extra virgin olive oil stored in different conditions: Focus on diglycerides. *Italian Journal of Food Science, in press.*

4- Ayyad, Z., Valli, E., Bendini, A., Accorsi, R., Manzini, R. and Gallina-Toschi, T. (2015). Effect of temperature fluctuation in the virgin olive oil oxidation quality and shelf life. *Research article in progress.*

5- Ayyad, Z., Valli, E., Bendini, A., Accorsi, R., Manzini, R., Bortolini, M., Gamberi, M. and Gallina-Toschi, T. (2015). Quality changes of vegetable oils after simulations of shipment in different containers. *Research article in progress.*

6- Manzini, R., Accorsi, R., Ayyad, Z., Bendini, A., Bortolini, M., Gamberi, M., Valli, E. and Gallina-Toschi, T. (2014). Sustainability and quality in the food supply chain. A case study of shipment of edible oils. *British Food Journal*, (116), pp. 2069-2090.

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Afaneh, I.A., Abbadi, J., Ayyad, Z., Sultan, W. and Kanan, K. (2013). Evaluation of selected quality indicators of extra virgin olive oil bottled in different packaging materials upon storage under different lighting conditions. *Journal of Food Science and Engineering*, (3), 267-283.

LIST OF ABBREVIATIONS

EVOO: Extra virgin olive oil.

VOO: Virgin olive oil.

Hyty: Hydroxytyrosol.

Ty: Tyrosol.

DOA: Decarboxymethyl oleuropein aglycone.

FFA: Free fatty acids %.

PV: Peroxide value.

TG: Triglycerides.

DG: Diglyceride.

FLT: Fluctuated temperature.

ST: Static temperature.

IS: internal standard

TP: Total amount of phenolic compounds.

TBARs: Thiobarbituric acid reactants.

EVOO Q: EVOO samples to final destination (Quebec).

EVOO LA: EVOO samples to final destination (Los Angeles).

1. BACKGROUND

Virgin olive oil (VOO) is a product with high economic and nutritional value because of its superior organoleptic characteristics (taste and aroma) and minor compounds (phenols and tocopherols) contents, which distinguish it from other vegetable oils. Olive oil producers, exporters, and sellers suffer from rapid quality loss of their products when exposed to storage or transportation phases. Uncorrected conditions during storage and transportation can reduce the shelf life of VOO and lead to serious health consequences and significant economic losses. After production, preserving the initial characteristics of VOO during storage and distribution is a priority of scientific research in the olive oil industry. The original quality of VOO may change during shipment, but real-time study of such changes can be complex because of logistic reasons. Therefore, simulated shipment studies could be helpful to investigate the effects of shipment on the quality of transported VOO. These studies may also suggest practical solutions to protect the original quality of shipped oil. Several researchers studied the effects of filtration before bottling on the quality of VOO and highlighted many advantages in terms of quality and consumer acceptance. By contrast, some researchers determined the possible side effects of filtration or clarification on the oxidation stability of freshly produced VOO. Despite contradicting results, simulation studies of actual storage under different conditions can provide information about changes in the quality and storage stability of filtered or clarified VOO.

2. OBJECTIVES

The different research lines in this study aimed to:

1. Study the influence of filtration or clarification as pretreatment before bottling on the quality of VOO under long-term storage and different storage conditions (lighting and temperature).
2. Evaluate the influence of these conditions on the freshness of stored VOO.
3. Investigate changes in different chemical, physical, and sensory parameters of VOO subjected to different stress conditions (temperature and storage). These stress conditions include storage of VOO under different temperatures and lighting, simulated shipments of edible oils and VOO from olives grown in Italy to different destinations (Los Angeles, USA and Quebec, Canada), accelerated storage of olive oil under fluctuating temperatures, and storage of filtered and clarified olive oil under different conditions (dark and light).

4. Analyze the efficiency of using protection techniques (temperature-controlled containers) on the quality of shipped VOO.

3. METHODOLOGY

Different samples of VOO were obtained from different sources (producers and markets) and prepared according to the research case studies in the experimental section.

Case study 1: Fresh VOO samples were obtained from an Italian producer, filtered/ clarified, filled in glass bottles, and stored in the Department of Agricultural and Food Science (DISTAL) laboratories (Bologna University).

Case study 2: VOO samples were obtained from a Spanish producer as detailed under "Experimental Section 2."

Case study 3: VOO samples were extracted from olive fruits of 'Canino' cultivar produced in Italy and prepared in the Department (DISTAL) laboratory mill; storage simulation was conducted in cooperation with the Mechanical Engineering Department (Bologna University).

Case study 4: VOO samples were extracted from olive fruits of 'Canino' cultivar produced in Italy and bottled in the Department (DISTAL) laboratory mill; shipment simulation was conducted in the Mechanical Engineering Department (Bologna University).

All olive oil samples were analyzed according to the specific aim of each case study by applying different analytical methodologies. Free acidity and peroxide values (PV) were assessed with titrimetric assays. Pigments, total phenols, and *ortho*-diphenols were evaluated using spectrophotometric tests, with specific absorbance determined at 232 and 270 nm. Chromatographic approaches were used to determine the profiles of the following minor compounds: diglycerides (DGs) via GC-FID, volatile compounds via SPME/GC-MS, tocopherols via HPLC-UV, and phenols via HPLC-DAD/MS. Color coordinates were identified via CIELab color analyzer, and water amount was evaluated using oven-drying method. Sensory characteristics were analyzed by a trained panel (professional DISTAL panel recognized by the Italian Ministry). All determinations were performed in triplicates by using the protocols specified in each experimental section. All analysis was performed in the laboratories of the Department of Agricultural and Food Sciences (DISTAL) (University of Bologna), unless specified in the respective experimental sections.

4. SUMMARY OF RESULTS AND KEY FINDINGS

The following research lines were involved in the respective experimental sections of the thesis.

- ✓ In the **first section**, the influence of different filtration systems (innovative and traditional) and storage conditions on the quality of extra VOO (EVOO) was investigated. EVOO samples were categorized as follows: (1) filtered using a commercial filtration system, (2) clarified with inert gases (argon and nitrogen), and (3) the remaining part considered as unfiltered. All samples were analyzed at time zero, bottled in clear glass bottles, and stored in the dark or diffused daylight at room temperature for 1 year. Basic quality parameters, such as water content, diglyceride isomerization, profiles of volatiles, tocopherols, polar phenols, and pigments, and sensory properties were periodically analyzed at scheduled times during storage. After 12 months of storage, the total phenol and *ortho*-diphenol contents significantly decreased in all samples under dark and light conditions. Secoiridoid derivatives and tocopherols remained significantly high in samples clarified with inert gases, whereas hydroxytyrosol significantly increased in the unfiltered sample. Chlorophyll was drastically depleted in all samples stored under light conditions. Water amount was significantly low in sample clarified with inert gases, followed by filtered and then unfiltered samples. DGs isomerization was mainly dependent on storage time. C_6 and C_5 LOX volatiles (volatile products of lipoxygenase pathway) in sample clarified with inert gases showed no significant variation at the end of storage under dark condition, whereas volatile compounds significantly decreased in the unfiltered and filtered samples. Positive attributes (fruity, bitter, and pungent perceptions) also sharply reduced in the unfiltered sample at the end of storage. Furthermore, samples filtered and samples clarified with inert gases did not show significant loss in sensory quality. By contrast, all samples stored under light condition were characterized by sensory defects, particularly rancidity, at the end of storage.

- ✓ In the **second section**, the DGs isomerization was investigated in EVOO during storage under different conditions. Aliquots of EVOO were stored for 14 months under four different conditions: 20 °C in dark, 20 °C in light, 4 °C to 6 °C in light, and 20 °C in light with argon in the headspace. The samples were analyzed at scheduled times during storage.

After 14 months of storage, 1,2-DGs decreased and 1,3-DGs significantly increased in all samples during storage and the former was predominant compared with the latter. Overall, EVOO stored at 4 °C to 6 °C in light showed the highest preservation of 1,2-DGs isomers. This finding indicated that storage temperature was the most significant factor for diglyceride isomerization.

- ✓ The **third section** of the experimental work focused on the effect of fluctuating versus constant accelerated storage temperature on quality parameters of VOOs. EVOO samples were subjected to a static temperature of 45 °C or fluctuating temperature (from 5 °C to 45 °C) for 1 month. The results showed that K_{232} of EVOO was significantly higher in sample stored at fluctuating temperature than that in static temperature. The total phenol content was also significantly low in samples stored at fluctuating temperature. Percentage of free fatty acids (FFA), *ortho*-diphenol content, and 1,2/1,3-DG ratio did not significantly vary between both stress conditions. These results revealed that oscillation in temperature and static high temperature may adversely affect olive oil quality.

- ✓ In the **fourth section**, quality changes (FFA, PV, total phenols, thiobarbituric acid, and color) were evaluated in VOO subjected to simulated shipments. Different container solutions and equipments were used to protect the transported edible oil. Oil samples were placed in containers that could control stress conditions to simulate shipment stages toward two different destinations, namely, Los Angeles (USA) and Quebec (Canada). The samples affected by thermal changes throughout the simulated journeys, such as heating and cooling cycles, were compared with the samples subjected to similar conditions but stored in thermally insulated containers. After shipment simulation, VOO shipped inside thermally insulated containers showed lower hydrolytic and oxidation degradation than those without insulation cover.

5. INTRODUCTION

Virgin olive oil (VOO) is extracted from the fruit of the olive tree *Olea europaea* by using physical operations without any further treatment other than washing, centrifugation, decantation, and filtration (EEC Reg. 1513/2001). VOO is mechanically produced by accumulating olives, washing, crushing, and then paste mixing, followed by centrifuging and decantation. The resulting liquid is further processed by optional filtration and then bottled before marketing (Bakhouche et al. 2014). Olive oil exerts various biological activities, including antioxidant, anti-inflammatory, and antibacterial. Olive oil is also an excellent source of oleic acid, vitamin E, and several antioxidant compounds. Moreover, olive oil consumption improves several health problems, such as cardiovascular diseases, inflammatory bowel disease, and high blood pressure (Addsomelife, 2012).

5.1 Olive oil global production

Olive oil production accounts for approximately 3% of the total world production of vegetable oils (Gunstone, 2011). The main olive oil producing countries are members of the International Olive Council (IOC) in the Mediterranean basin. Mediterranean regions are characterized by dry summers, mild winters, and proper exposure to the sunshine; these regions provide the optimal conditions that support the cultivation of the most common species of *O. europaea* trees and lead to the high production yield of VOO (Lozano-Sanchez et al. 2010). Mediterranean countries account for approximately 98% (2 million tons) of olive oil production worldwide in 2013, whereas other countries such as the USA, Canada, China, and Australia account for the remaining 2% (Barjol, 2013). An in-depth review of recent data available from the IOC (2013) showed that European Union (EU) countries account for approximately 76% of olive oil production worldwide (approximately 2.31 million metric tons; Fig. 1), among which Italy contributes approximately 30% of the total amount. Therefore, Italy is second to Spain as the main VOO producer in the EU.

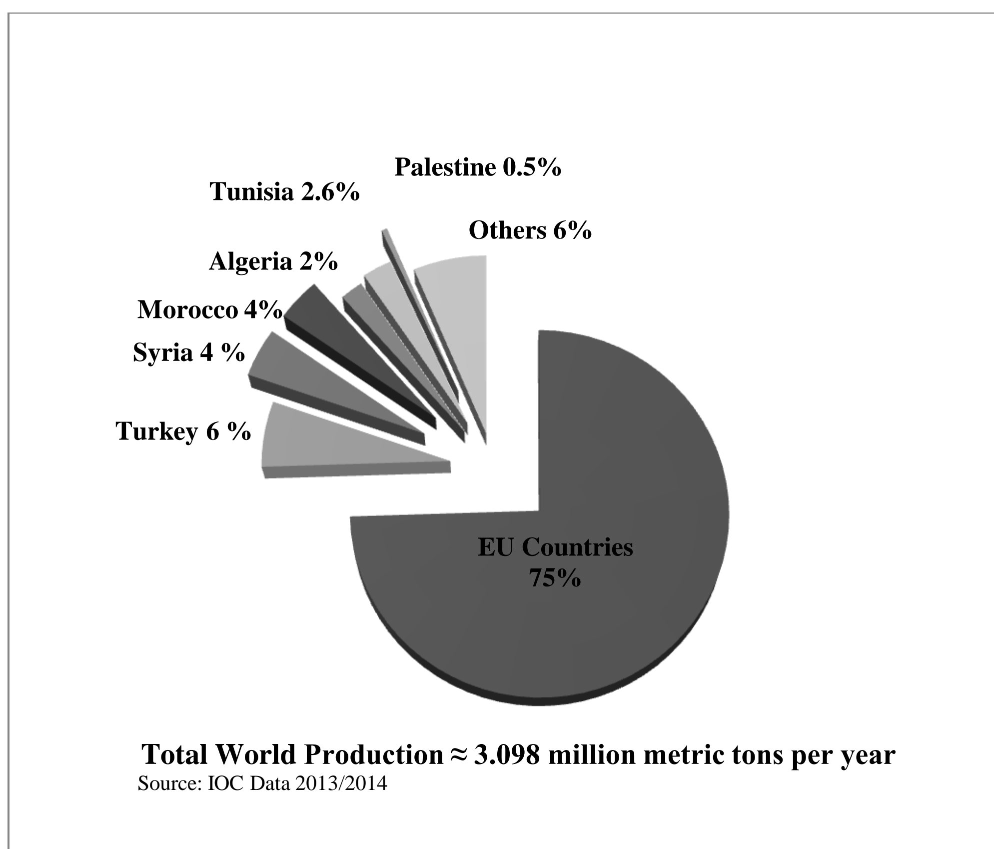

Fig. 1. World production of olive oil (percentage of total, 2013/2014; total: 3089000 metric ton). *Source: IOC Data, 2013/2014.*

5.2 Olive oil global consumption

The growth in the production rate of VOO has been accompanied by a similar trend in olive oil consumption. The EU is considered the largest consumer of olive oil worldwide (80% of the total world production). Within the EU, Italy and Spain are the predominant consumers, which consume approximately 40% of the global production of olive oil (NAOOA, 2012). As a non-European IOC member country, the USA is considered the third largest olive oil consumer worldwide, which consumes approximately 9% of the global production (IOC, 2013). The USA is the second largest importer of olive oil (approximately 200,000 tons) (USITC, 2013; IOC, 2013), followed by Brazil, Canada, and Japan (Aparicio and Harwood, 2013). On the basis of consumption per person, Greece, Spain, and Italy are the main VOO consumers, with annual ranges of 20, 12.6, and 10.9 L VOO per person, respectively (Butler, 2013).

5.3 Importance of VOO in Italy

Italian olive trees cover approximately 1,700,000 hectares, 80% of which are located in South Italy, (Fontanazza, 2004). Italian olive production is primarily used for oil extraction (95%) and secondarily for table olive production (5%) (Barmore, 2010). Moreover, current data extracted from the IOC (2013) show that Italy produced approximately 450,000 metric tons of olive oil in 2013. However, this amount accounts for approximately 16% of the global production of olive oil (USITC, 2013). Domestic consumption in Italy exceeds 600,000 metric tons, whereas Italy exports approximately 243,000 metric tons per year (IOC, 2013). Italian olive oil production participates in approximately 10% to 15% of Italian olive oil consumption (Gain Report, 2010). Therefore, Italy imports extra virgin olive oil (EVOO) from different countries, particularly Spain, to cover the domestic demand and exportation perspectives (Fontanazza, 2004). In fact, 60% of the produced Italian olive oil is extra virgin, which is olive oil of the highest quality (Barmore, 2010). Indeed, the average price of EVOO per liter costs approximately 3.12 Euros in 2013 (IOC, 2013).

5.4 Olive oil classification

One of the globally recognized classification of olive oil has been recently defined by the EU (EU Reg. 1348/2013) with its respective amendments (EEC Reg. 2568/1991). The proposed definitions of olive oil categories on the basis of their chemical and sensory properties are described as follows:

(1) VOO is the oil obtained from the olive fruit solely by mechanical means or other physical operations without any treatments other than washing or filtration. VOO has free acidity expressed as oleic acid not more than 2 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.

(2) EVOO is VOO with free acidity expressed as oleic acid not more than 0.8 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.

(3) Lampante VOO (LVOO) is VOO with free acidity expressed as oleic acid more than 2 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.

(4) Refined olive oil is olive oil obtained from VOOs using refining methods. This oil has free acidity expressed as oleic acid not more than 0.3 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.
(5) Olive oil composed of refined olive oils and VOOs is a mixture of refined olive oil and VOO. This oil has free acidity expressed as oleic acid less than or equal to 1 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.
(6) Olive pomace oil is the oil obtained by treating olive pomace with organic solvents. This oil has free acidity expressed as oleic acid not more than 1 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.
(7) Crude olive pomace oil is the oil obtained by treating olive pomace with organic solvents. The other characteristics of the oil are consistent with those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.
(8) Refined olive pomace oil is the oil obtained from crude olive-residue oil by refining methods. This oil has free acidity expressed as oleic acid not more than 0.3 g per 100 g; any other characteristics correspond to those fixed for this category in Annex I of the EU regulation EU Reg. 1348/2013.
Notably, only VOO, EVOO, pomace olive oil, and olive oil are fit for human dietary consumption.

5.5 Compositional properties of olive oil and their importance for olive oil quality

5.5.1 Triglycerides

VOO are mainly composed (≈99%) of lipid components of a glyceride nature (triglycerides). Triglycerides (TG) are composed of three fatty acids linked to a glycerol molecule via an ester linkage (Table 1) (Boskou, 2006). The excellent stability of VOOs against oxidation, if compared with other vegetable oils is related to two main properties: (1) the presence of minor compounds that act as natural antioxidants, such as tocopherols and polar phenolic compounds, and (2) the high ratio of monounsaturated fatty acids with respect to polyunsaturated fatty acids (Bendini et al. 2009a).

Table 1. Triglycerides found in significant proportions in olive oil

Triglyceride*	**Percentage in olive oil**
OOO	40%–59%
POO	12%–20%
OOL	12.5%–20%
POL	5.5%–7%
SOO	3%–7%
POP, POS, OLnL, LOL, OLnO, PLL, PLnO, and LLL	Very small amounts

*__O__, oleic acid; **P**, palmitic acid; **L**, linoleic acid; **Ln**, linolinic acid; **S**, stearic acid.
Source: Aparicio and Haward (2013).

5.5.2 Minor components

Minor compounds in olive oil are unsaponifiable lipid fractions that comprise approximately 1%–2% of the oil; these compounds contribute to the stability, unique flavor, and taste of VOO (Murkovic et al. 2004). The minor compounds in VOO include polyphenols, phospholipids, squalene, wax esters, terpene alcohols, glycosides, monoglycerides, DGs, FFA, volatile compounds, and water (Bianchi, 2002; Boskou, 2006). These minor components are influenced by different factors, such as olive fruit cultivar, climate, maturity degree upon harvest, and extraction techniques (Covas, 2008). These components are also affected by the storage conditions of the fruit before processing and after oil extraction (Bendini et al. 2009a). Moreover, the concentrations of the minor components and their relative percentages determine the characteristics and commercial categories of olive oil (Bianchi, 2002). Some of the minor components that contribute to olive oil stability are described in the following subsections.

5.5.2.1 Tocopherols

VOO contains four natural tocopherol isomers, namely, α, β, γ, and d, among which α-tocopherol is the predominant one (88.5% of the total amount), followed by β- and γ-tocopherols (Matthaus and Ozcan, 2011). The amount of tocopherols ranges from 100 to 300 mg per kg in the best-quality EVOO. Tocopherols are lipophilic phenols that effectively protect VOO from oxidative deterioration (Lozano-Sanchez et al. 2010; Lavelli et al. 2006). Tocopherols act as proton donors and free radical scavengers that interrupt the propagation of auto-oxidation (Aparicio and Harwood, 2013; Morello et al. 2004; Baldioli et al. 1996). Tocopherols can also act against

photo-oxidation as singlet oxygen quenchers, especially in the presence of chlorophyll pigments (Okogeri and Margari, 2002). Tocopherols are second to polar phenols (*ortho*-diphenols) in protecting olive oil against oxidation during storage (Psomiadou and Tsimidou, 2002a). Oxidation during storage significantly decreases the amount of tocopherols (Vacca et al. 2006; Morello et al. 2004; Gutierrez and Fernandez, 2002; Okogeri and Margari, 2002). The severity of tocopherol depletion in VOO is associated with the increase in the surrounding temperature; however, such cases could occur during the delivery stage of edible oil shipments (Manzini et al. 2014; Valli et al. 2013). Nissiotis and Tasioula-Margari (2002) investigated accelerated thermal oxidation at 60 °C and found that tocopherol content sharply reduces or even completely diminishes after a few hours. Similar observations were also reported by Lerma-Garcia et al. (2009). Moreover, the loss of tocopherols becomes prominent in VOOs as the oxygen level increases. Such a case could occur in frequently opened olive oil bottles (Krichene et al. 2010). Both studies showed that the decrease in tocopherol is less as compared with that in other polar phenols, particularly diphenols (Bendini et al. 2009a). Nevertheless, the loss of tocopherols during storage can occur even at low refrigerated temperatures (Samaniego-Sanchez et al. 2012). In addition, tocopherols have greater antioxidant activity than polar phenols in the presence of light, whereas tocopherols can act as singlet oxygen quenchers via a charge transfer mechanism (Psomiadou and Tsimidou, 2002b). Filtration affects the quantity and characteristics of different minor components of olive oil; however, there was no evidence that filtration could significantly affect the tocopherol content, or its antioxidant characteristics (Fregapane et al. 2006; Brenes et al. 2001). Bendini et al. (2013) and Lozano-Sanchez et al. (2012) reported similar observations after treating olive oil with gas clarification.

5.5.2.2 Pigments

VOO contains the green pigment chlorophyll and the yellow pigment carotenoid at concentrations of 1–40 mg per kg and 2 to 20 mg per kg, respectively (Gandul-Rojas and Mınguez-Mosquera, 2006). The pigment concentration of VOO is influenced by several factors, including fruit variety, ripening degree, processing, and storage conditions (Cerretani et al. 2008; Gallardo-Guerrero et al. 2005). For instance, filtration before bottling significantly decreases the amounts of chlorophyll and carotenoid in VOO (Lozano-Sanchez et al. 2012; Bottino et al. 2008). Furthermore, temperature elevation sharply decreases the amount of chlorophyll (Guillaume et al. 2014), thereby altering the natural color, clarity, and transmittance of VOO

(Sikorska et al. 2007). However, during storage of VOO in the dark, the amount of chlorophyll pigment simultaneously decreases in accordance with first-order kinetics, wherein degradation mostly occurs during the initial storage period (Gallardo-Guerrero et al. 2005). Chlorophyll does not disappear during storage or upon heating of VOO; rather, the green pigment is converted to its alternative brown pigment pyropheophytin (Guillaume et al. 2014; Rodney et al. 2012). The chlorophyll pigment may exert a slight antioxidant activity that protects olive oil during storage in the dark (Bendini et al. 2009a). However, chlorophyll acts as a pro-oxidant photosensitizer that accelerates photo-oxidation under light exposure (Psomiadou and Tsimidou, 2002b). Meanwhile, carotenoids, particularly β-carotene, have pronounced stability during storage; moreover, these pigments protect VOO by acting as free radical scavengers that slow down auto-oxidation and photo-oxidation (Velasco and Dobarganes, 2002). However, the significant degradation of carotenoid pigments is expected if the surrounding temperature exceeds 40 °C (Thakkar et al. 2009), as in the case of olive oil shipments (Valli et al. 2013).

5.5.2.3 Polar phenols

Polar phenolic compounds are composed of a benzene ring linked to one or more hydroxyl groups, including their functional derivatives (Aparicio and Harwood, 2013). These minor compounds contribute to the oxidation, stability, and organoleptic properties of VOO (Gutierrez et al. 2001; Tsimidou, 1998). The average content of phenolic compounds in VOO ranges from 50 mg per kg to 1000 mg per kg as gallic acid (Nissiotis and Tasioula-Margari, 2002). Several authors (Bendini et al. 2007; Servili et al. 2004; Tsimidou, 1998) have classified polar phenolic compounds in VOO into the following:

(A) Simple phenols

(1) Phenolic acids, such as syringic acid, vanillic acid, *p*-coumaric acid, *o*-coumaric acid, gallic acid, caffeic acid, protocatechuic acid, ferulic acid, *p*-hydroxybenzoic acid, cinnamic acid, and benzoic acid.

(2) Phenyl ethyl alcohols, such as 3,4-dihydroxyphenyl ethanol (3,4-DHPEA), hydroxytyrosol acetate, *p*-hydroxyphenyl ethanol (*p*-HPEA), tyrosol acetate, 3,4-dihydroxyphenyl ethanol glucoside, and vanillin.

(B) Complex oleuropein derivatives (secoiridoids), including a dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), a dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA), oleuropein aglycon (3,4-

DHPEA-EA), ligstroside aglycon, a *p*-HPEA derivative, a dialdehydic form of oleuropein aglycon, a dialdehydic form of ligstroside aglycon, and elenolic acid (free and glycoside-linked elenoic acid).

(C) Flavonoids (apigenin and luteolin).

(D) Lignans, including (+)-1-acetoxypinoresinol, (+)-pinoresinol.

Polar phenols have a major role in the sensory attributes and oxidation stability of VOO. For instance, tyrosol (Ty), Hyty, and other secoiridoid derivatives are mainly responsible for the bitter, pungent, and astringent taste of VOO (Carrasco-Pancorbo et al. 2005). Phenolic compounds, particularly those with Ty and Hyty in their structure, increase the stability of VOO during storage through their antioxidant activity. As antioxidants, phenolic compounds can interfere with the propagation step of lipid oxidation by donating a hydrogen atom to the formed free radicals (Tsimidou, 1998). The predominant phenolic compounds in olive oils are the secoridoid compounds, followed by flavonoids and phenolic alcohols (Gomez-Caravaca et al. 2007). However, the antioxidant activity of phenolic compounds differs depending on their structure, which is mostly affected by the presence of hydroxyl groups (Mancebo-Campos et al. 2014). Carrasco-Pancorbo et al. (2005) reported that phenolic compounds can be arranged in accordance with their antioxidant activity as follows: Hyty > decarboxymethyl oleuropein aglycone (DOA) > oleuropein aglycon > (+)-pinoresinol > ligstroside aglycon > Ty > elenolic acid > (+)-1-acetoxypinoresinol. The loss of phenolic compounds during storage, particularly Hyty, is associated with an increase in the surrounding temperature (Bendini et al. 2006). Such temperature elevation can occur during summer. The concentration of polar phenols, particularly *ortho*-diphenols, decreases during storage because of their hydrolytic activity and oxidation; in addition, the degradation of these compounds increases when olive oil is stored under light exposure (Cinquanta et al. 1997). Filtration and clarification affect the amount of phenolic compounds in VOO; however, the concentrations of phenolic compounds, particularly Hyty and Ty, decrease after the filtration of VOO (Lozano-Sanchez et al. 2010; Gomez-Caravaca, 2007). Nevertheless, the degradation of phenolic compounds is less in filtered than in unfiltered VOO during long-term storage (Fregapane et al. 2006).

5.5.2.4 Volatile compounds

Volatile fractions are low-molecular-weight compounds that are readily vaporized at room temperature; these compounds contribute to the characteristic aroma and flavor, especially the green and fruity sensory attributes as well as the undesired off-flavors, of VOO. Over a hundred volatile compounds have been identified in VOO, including hydrocarbons, alcohols, aldehydes, ketones, esters, acids, terpenes, and furan derivatives (Kalua et al. 2007). The composition and the concentration of the volatiles in VOO depend on several factors: (1) agronomic factors such as cultivar, maturity stage, soil, and climate conditions; (2) technological factors such as fruit storage, crushing, mixing, and extraction of olive oil; (3) factors that depend on VOO conservation (Bendini et al. 2009a). The volatile aroma of VOO is developed during olive oil extraction, particularly during crushing and malaxation, via the lipoxygenase (LOX) pathway (Angerosa et al. 2000). The LOX pathway involves a series of enzymatic oxidation reactions and the cleavage of monounsaturated and polyunsaturated C18 fatty acids, followed by alcohol production and esterification to produce ester volatiles (Kalua et al. 2007). Most volatile fractions formed by the LOX enzymatic pathway contain five or six carbon atoms (Kalua et al. 2007; Angerosa, 2002). C_6 aldehyde compounds are the major volatiles among the different groups of volatiles present in fresh VOO, whereas alcohol and ester volatiles are found in relatively small amounts. These volatile compounds are mainly responsible for the unique positive aroma of VOO (sweet, green, and fruity notes). Volatiles that belong to this group include (*E*)-3-hexenal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexan-1-ol, (*E*)-3-hexene-1-ol, (*Z*)-3-hexene-1-ol, (*E*)-2-hexene-1-ol, hexenyl acetate, and (*Z*)-3-hexenyl acetate (Dhifi, 2005; Angerosa, 2000, 2002). Long-term storage reduces the amount of (*E*)-2-hexenal, which is the predominant volatile of VOO (Cavalli et al. 2004). Moreover, improper storage conditions, such as increased temperature, light exposure, and increased oxygen level, lead to the formation of off-flavor volatile compounds because of hydroperoxide degradation in oxidized oil (rancid). Such oxidative volatiles include carboxylic acids, nonanal, octanal, 2,4-heptadienal, and 2-heptenal (Kaula et al. 2007). Consequently, the hexanal/nonanal ratio can be used as an excellent rancidity marker to evaluate the oxidation reactions and to discriminate oxidized olive oil from fresh oil (Angerosa et al. 2004; Kiritsakis, 1998). Furthermore, filtration and clarification generally slightly affect the concentration of volatile compounds that are initially formed by the LOX pathway (Bendini et al. 2013 Brkic-Bubola et al. 2012; Lozano-Sanchez, 2010).

5.5.2.5 Free fatty acids

FFA form in VOO because of the hydrolytic reaction of TG; the liberation of FFA in fresh olive oil is affected by several factors, including fruit diseases and improper storage of the fruit before oil extraction (Paradiso et al. 2010; El-Abassy et al. 2009). An increase in the amount of FFA in VOOs accelerates the oxidation rate and degradation of minor compounds, particularly secoridoid derivatives (Bendini et al; 2009a; Brenes et al. 2001). During storage, the hydrolysis rate of TG and the formation of FFA increase with temperature elevation, as well as with the presence of high amounts of vegetative water and lipolytic enzymes in veiled VOO (Di Giovacchino, 2013). However, filtration reportedly decreases the hydrolysis rate of TG (Fregapane et al. 2006). Thus, the susceptibility of VOO to develop off-flavors as a result of oxidation is reduced.

5.5.2.6 Diglycerides

DGs account for 1%–3% of fresh VOO; these compounds consist of 1,2-DGs and 1,3-DGs isomers. However, 1,2-DGs isomers are the predominant form in fresh VOO. A decrease in the amount of 1,2-DGs and an increase in the amount of 1,3-DGs during storage are associated with the length of the storage period (León-Camacho et al. 2013). Some authors have suggested that DG isomerization could be used as a marker to determine the freshness and genuineness of VOOs (Caponio et al. 2005; Pérez-Camino et al. 2001). Nevertheless, the degree of isomerization and formation of 1,3-DGs is notably affected by the initial quality of VOO, particularly its acidity (Spyros et al. 2004) and temperature elevation during storage (Cossignani et al. 2007).

5.6 Sensory analysis of VOO

The sensory sensation of VOO (flavor) arises from a combination of taste and smell; however, organoleptic properties have important roles in the consumer acceptance of VOO. Sensory sensation enhances the stimulation of taste receptors and the free endings of the trigeminal nerve that is responsible for the detection of bitter and pungent sensations. The analysis of minor components, such as volatile and phenolic compounds, is not included in olive oil classification despite the important contributions of these components to the quality and sensory characteristics of VOO. Given its importance as a classification tool (see Section 5.4), sensory analysis is used to evaluate VOO quality and consumer preferences (Angerosa, 2002; Aparicio and Luna, 2002).

"Panel test" is the official method used to evaluate the sensory properties of VOO; this test was developed by the International Olive Oil Council (COI/T.20 and its amendments). In brief, the proposed test involves 8 to 12 trained tasters who can identify, measure, and describe their sensation intensity, whether positive or negative, by smelling and tasting VOO samples. The tasters use a profile that shows the positive attributes (fruitiness, bitterness, and pungency) and the main sensory defects that could exist in VOO (fusty, musty, humid, rancid, frostbitten olives, winey, vinegary, and other defects). The evaluation sheets are provided with a scale from 0 to 10 to measure the sensation intensity of each attribute; after the evaluation, the median of each attribute is computed. Consequently, the category of the tested sample is distinguished in accordance of EU Reg. 1348/2013. The sensory characteristics of VOO are influenced by many factors, including cultivar, environment, cultivation techniques, ripening degree of the olive fruit, harvesting, transport and storage of olives fruits, processing techniques, storage, and packaging conditions (Kalua et al. 2007; Angerosa et al. 2004). Minor components, such as phenolic and volatile compounds, contribute to the odor and taste of VOO. In this regard, phenolic compounds, especially secoiridoid derivatives, contribute to the bitterness and pungency of olive oil (Bendini et al. 2007). Among the LOX volatile compounds, C_6 aldehydes, particularly (*Z*)-3-hexenal, and other compounds, such as (*Z*)-3-hexenyl acetate, highly correlate with sensory green notes (apple, artichoke, freshly cut grass, etc.); meanwhile, C_6 alcohols only correlate with ripe fruit and aromatic perception (Kalua et al. 2007). C_5 LOX compounds, such as 1-penten-3-one, are linked to fruity and sweet sensations, such as strawberry flavor (Bendini et al. 2009a).

However, VOO may contain some defects (off-flavors) because of the inadequate storage of olives before processing, as well as because of incorrect olive oil extraction. Such defects include the winey sensation that originates from sugar fermentation by the action of microbial enzymes, the fusty sensation caused by the conversion of some amino acids, and the musty sensation due to mold deterioration. Moreover, oxidation reactions are mainly associated with the rancid flavor (Bendini et al. 2012). During olive oil storage, a decrease in the sensory scores of the pleasant sensory attributes is associated with the decline in the amount of volatiles derived from the LOX pathway and the reduction in the phenol content because of oxidation reactions. Moreover, sensory defects may develop through fermentation by the activity of microbial enzymes during the long-term storage of unfiltered VOO (Gandul-Rojas and Minguez-Mosquera, 2006). Therefore, filtration positively influences the organoleptic properties of VOO. For instance, filtration blocks the development of sensory defects associated with the presence of suspended

impurities; filtration can also enhance and maintain the positive sensory attributes of VOO during long-term storage (Lozano-Sanchez et al. 2010, 2012).

5.7 Olive oil deterioration during shelf-life

Several factors influence olive oil stability by altering chemical compositions and organoleptic properties. Such factors include olive fruit infection, harvesting and extraction practices, oxygen concentration, temperature, and light (Velasco and Dobarganes, 2002). Hydrolytic rancidity, auto-oxidation, and photo-oxidation primarily cause lipid deterioration (Morales and Przybylski, 1999). Negative conditions, such as oxygen presence, temperature changes, and light exposure, may decrease the shelf-life of VOO. For example, temperature elevation during storage accelerates oxidation and thus the production of unpleasant volatile compounds (i.e., volatile acids) in VOO (Campos et al. 2007). In addition, light exposure during storage triggers photo-oxidation reactions that cause significant loss of VOO quality (Aparicio et al. 1999). Therefore, VOO should be stored at temperatures around 15 °C and away from light exposure (Piscopo and Poiana, 2012; Mendez and Flaque, 2007) to maintain its original quality for a long period.

5.7.1 Hydrolytic degradation

Hydrolytic rancidity can spontaneously occur in edible oils at room temperature; this reaction is triggered by the cleavage of fatty acids from TG molecules in the presence of water molecules. However, this reaction is accelerated by heat and may lead to the development of rancid off-flavors. This reaction can also occur at low temperatures (at low rates), depending on the moisture amount in the liquid oil phase that surrounds the fat molecules (Kristott et al. 2000). VOO from fruits has low FFA values. However, the prolonged preservation of fruit until processing may trigger the activity of hydrolytic enzymes (lipase) and slightly increase the free acidity of fresh VOO (Boskou, 2006).

5.7.2 Oxidative degradation

VOO can be consumed without any chemical treatments. VOO has high resistance to oxidation, unlike other vegetable oils, which are consumed after refining treatments. The superior resistance to oxidation of VOO is attributed to: (1) the high content of monounsaturated fatty acid relative to the polyunsaturated ones and (2) the presence of a wide range of natural minor components with antioxidant activities, particularly phenolic compounds, carotenoids, and tocopherols. These

minor compounds slow down oxidation reactions and prevent the decrease in the quality and nutritional value of VOO (Bendini et al. 2009a; Gutierrez and Fernandez, 2002; Velasco and Dobarganes, 2002).

5.7.2.1 Auto-oxidation

The formation of free radicals that subsequently react with triplet oxygen (auto-oxidation) leads to the formation of rancid volatile flavor compounds in edible oils. Auto-oxidation involves three main stages (Fig. 2):

(1) Initiation, in which free radicals are formed through the thermal or photo-decomposition of hydroperoxides or even by the presence of trace metals.

(2) Propagation, in which the formed free alkyl radicals react with oxygen to form peroxy radicals. This stage is accelerated by temperature elevation.

(3) Termination, in which new non-reactive products, such as the volatile compounds responsible for the rancid defects and other polymers, are formed.

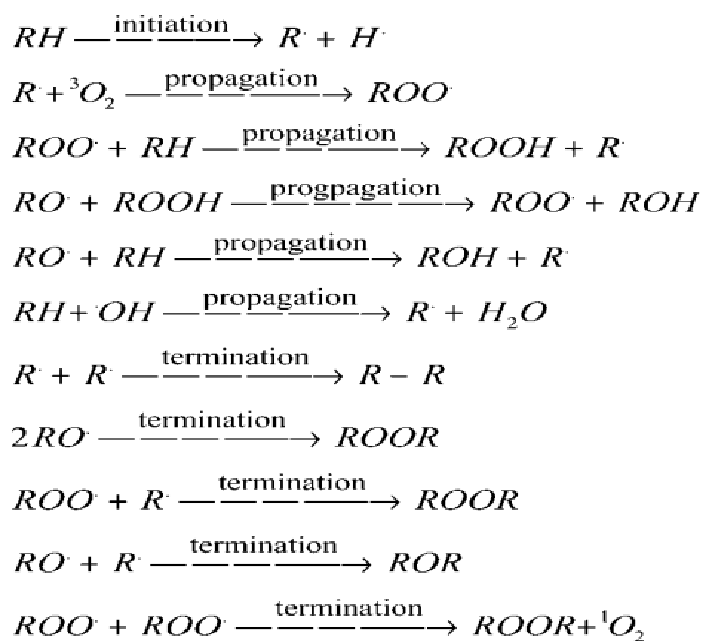

Fig. 2. Characteristic reactions during the initiation, propagation, and termination steps of oxidative degradation of fatty acids including triplet oxygen.
Source: Kanavouras et al. 2006.

In many papers (e.g. Bendini et al., 2009a; Kanavouras et al., 2006) are described the following conditions that determine the rate of oxidation reactions:

(1) Fatty acid composition. The reaction rate increases with increasing content of unsaturated fatty acids.

(2) FFA. FFA increase the velocity of the oxidation reaction by working as pro-oxidants.

(3) Oxygen concentration. The oxidation rate is approximately proportional to the partial pressure of oxygen. However, the concentration of oxygen in glass-bottled oil can be reduced by using inert gases in the headspace.

(4) Moisture. Very low or very high content of water can increase the rate of oxidation reactions. Intermediate moisture content has a protective action probably because of the decreased catalytic activity of oxidative enzymes.

(5) Temperature. The oxidation rate increases as the surrounding temperature increases.

5.7.2.2 Photo-oxidation

Photosensitized oxidation occurs in the presence of photo-sensitizers (chlorophylls) and visible light. Under light exposure, the oxygen is transferred to an excited singlet state (unstable strong reactive molecule). The formed singlet oxygen reacts with singlet-state unsaturated fatty acids that contain high electron densities and form a mixture of conjugated and non-conjugated hydro-peroxides, which readily degrade to produce undesirable oxidation by-products (Kanavouras et al. 2006). However, the singlet oxygen produced during photo-oxidation is approximately 1000–1500 times more reactive than the triplet oxygen formed during auto-oxidation; therefore, exposure to light sharply decreases the quality of VOO (Caponio et al. 2005).

5.8 Influence of storage length and conditions on EVOO quality

Storage time, storage conditions, and packaging materials influence the quality of the produced VOO (Afaneh et al. 2013; Piscopo and Poiana, 2012; Dabbou et al. 2011; Velasco and Dobarganes, 2002). Extensive research has been conducted to address various aspects of olive oil bottling and storage. To simplify the subject to some extent with this in mind, this section primarily considered studies on glass-bottled VOO during storage under different conditions. Moreover, this section aimed to provide insights into the theoretical and practical aspects of olive oil storage and their effects on different VOO quality parameters.

5.8.1 Influence of storage length and conditions (dark/light) on EVOO quality

Fernandez-Gutierrez et al. (2002) studied the determinant parameters and components of VOO storage to predict the storage time beyond which the oil becomes "no longer extra virgin." In their study, EVOO samples were stored for 6 months in glass bottles at 30 °C under an illumination of 800 lux for 12 h per day, whereas other samples were stored at 2 °C in the dark. The quality of the EVOO samples stored under light exposure significantly decreased. However, the tocopherol level decreased by approximately 97% of its initial content. In addition, chlorophyll content was completely depleted by the end of the storage period. Consequently, the samples exposed to light were declassed from the EVOO category in terms of the K_{270} index and because of the development of winey, muddy, and rancid sensory defects.

Similar findings were reported by Okogeri and Margari (2002), who studied the changes in the phenolic compounds of EVOO during storage in glass bottles under diffused light and in the dark. Their group showed that the samples exposed to light were declassed to the virgin category after 6 months of storage in terms of the peroxide and K_{232} values, whereas the samples stored in the dark exceeded the accepted limit for PV after 12 months of storage. Simultaneously, approximately 79% of the phenolic compounds and tocopherol were consumed after 6 months in the samples stored under diffused light. The phenolic fractions of the samples stored in the dark decreased by approximately 54%–62% after 8 months of storage.

Focusing on changes that occurred on the EVOO pigments and the phenolic components during storage in the dark at room temperature, Morello et al. (2004) highlighted that the positive sensory attributes decrease with time during storage. Furthermore, chlorophylls and carotenoid contents decreased by approximately 30% and 40%, respectively, after 12 months of storage. The total content of phenols, particularly secoiridoid derivatives, significantly decreased, whereas tocopherol completely disappeared at the end of the storage period.

This finding agreed with that of Gallardo-Guerrero et al. (2005), who studied the effect of storage conditions (15 °C in dark) on the original chlorophyll profile of Spanish VOO. They observed a sharp decrease in the total amount of chlorophyll pigments at the end of the storage period, which implied their conversion to pheophytin a.

Storage under maximum protective conditions was investigated by Mendes and Falque (2007), who evaluated the effect of storage time (6 months) in transparent and opaque glass bottles on VOO at 20–22 °C; their samples were placed under illumination and daylight (without a

headspace). Their group found that all samples stored in transparent glass bottles were no longer extra virgin after 6 months of storage because of the increased oxidation stability indices.

Caponio et al. (2005) also used DG isomerization as a beneficial parameter for tracing the quality and progressive aging of EVOO and studied the influence of light exposure on the quality of EVOO during storage for 12 months as compared with storage in the dark. The total phenolic content, which was expressed as gallic acid, decreased during storage in samples stored under light and in the dark. No significant differences were observed between the two storage conditions. However, the amount of chlorophyll pigment drastically decreased after 2 months of storage under light exposure and eventually disappeared after 4 months of storage. In addition, a progressive increase of 1,3-DGs isomers was observed during the given storage time. However, all of the samples stored under light exceeded the accepted limits of EVOO in terms of the K_{270} values at the end of the storage period.

To focus on the effects of different storage conditions on the sensory evaluation of positive attributes in EVOO, Sinesio et al. (2005) conducted a dynamic sensory evaluation of bitterness and pungency in VOO stored at 10–28 °C for 12–18 months. In their study, the bitterness and pungency attributes decrease during storage as a function of temperature. The intensity of the bitter taste notably decreased more than the pungency, which became the predominant sensation in the aged EVOO.

Vacca et al. (2006) evaluated the oxidation index of VOO to determine the effects of storage period and exposure conditions on the quality of EVOO samples stored in sunlight for 18 months. They observed a significant decrease in the minor components and pigments during storage. In particular, the samples exposed to light lost approximately 50% of their chlorophyll and polyphenol contents. In addition, the tocopherol and carotenoid content decreased by 30% and 20%, respectively, relative to their initial values. None of the stored samples exceeded the EU limits for commercial EVOO, which could be related to the high quality of the examined EVOO samples. Similarly, Del Caro et al. (2006) evaluated the influence of technology, storage, and exposure conditions on EVOO samples stored under normal light and in the dark for 16 months. They found that the total phenolic content decreased by approximately 40% after 16 months of storage. The samples exposed to light had lower final values than those stored in the dark. However, minor components such as chlorophylls, carotenoids, and tocopherols significantly decreased by approximately 50%, 20%, and 30%, respectively, with lower values in the samples exposed to light than in those stored in the dark.

Kalua et al. (2006) evaluated the effect of different storage conditions on the freshness and volatile compounds of VOO to determine the quality indices that can be used to discriminate storage conditions. They identified K_{270} as the quality index that can discriminate the storage conditions under light exposure because its value was significantly increased after storage for 12 months. However, their group proposed that (*E*)-2-hexenal, K_{232}, and K_{270} can indicate VOO freshness during storage. The following markers were also highlighted with respect to the storage conditions. (1) In the presence of oxygen, hexanal was a marker of storage under light exposure, FFA were markers of storage in the dark, whereas acetic acid and pentanal were markers of low-temperature storage. (2) In the absence of oxygen, octane was a marker of storage under light exposure, whereas Ty and hexanal were markers of storage in the dark. No indicative marker of low-temperature storage was recommended in the absence of oxygen.

Cosio et al. (2007) studied the evolution of different storage conditions (light and dark at room temperature) on EVOO quality. PV of samples stored under light conditions for a year exceeded 20 meq O_2 per kg oil, thereby declassing them from the VOO category to the lampante one. Conversely, samples stored in the dark exceeded the accepted limit for the VOO category in terms of PV after 2 years of storage.

Similar findings were reported by Sinelli et al. (2007), who studied the application of mid-infrared spectroscopy to evaluate EVOO freshness during a year of storage under light exposure and in the dark. The UV adsorption values of EVOO stored under light conditions exceeded the established limit of the respective legislations at the end of the storage period.

Romani et al. (2007) intensively investigated minor polar compounds and their antioxidant activities during the storage of EVOO in the dark at 18 °C for 18 months. Their group showed that 50% to 60% of the phenolic compounds (secoiridoid derivatives) were lost during the first six months of storage.

Vekiari et al. (2007) studied the effect of processing and commercial storage conditions (28 °C in the dark and under diffused daylight) on the quality indices of EVOO stored for 10 months. Their group showed that all of the samples stored under light lost their EVOO status after 3 months of storage.

Gomez-Alonso et al. (2007) evaluated the oxidation indices for VOO stored in open glass bottles in the dark at room temperature for 21 months. Their group found that all of the samples were declassed from the VOO category because of the increased K_{232} index. In addition, an average reduction of more than 50% of the total phenols was detected at the end of the storage period,

which was accompanied by a continuous increase in the amount of simple phenols (Hyty). However, an apparent decrease in tocopherol content (approximately 23% of its initial content) was detected at the end of the storage period.

Sacchi et al. (2008) studied the shelf-life of different vegetable oils stored under controlled room temperature (25 ± 4 °C) and exposed to diffused day and artificial light for 6 months. Their group showed that the total phenol content of VOO was not significantly decreased during the 6 months of storage as compared with the significant decrease in tocopherol content. Their results also showed that chlorophyll pigments were completely lost after 3 months of storage. However, 40% of the total carotenoids remained at the end of the storage period. Moreover, none of the stored samples exceeded the official EU limits for EVOO (EU Reg. 1348/2013).

Pristouri et al. (2010) studied the effects of different packaging materials and storage conditions on the quality of EVOO stored in the dark at 22 °C. Their group found that all of the samples were declassed in terms of their oxidation stability indices after 6 months of storage.

Baiano et al. (2014) observed changes in EVOO stored in the dark at 15–20 °C for 8 years. Their study showed that the sensory defects appeared after 6 years of storage and were accompanied by a decrease in fruity attributes. These attributes were reduced to zero after 7 years. By contrast, the other oxidation parameters (PV, K_{232}, and K_{270}) remained within the official limits of EVOO even after 7 years. After 8 years of storage, their group found that the total phenol content decreased by approximately 67% in relation to its initial value. In specific, Hyty, Ty, and DOA decreased by 50%, 40%, and 70%, respectively, after 8 years of storage.

Moreover, Guillaume et al. (2014) investigated the evolution of 1,2-DGs over time in EVOO samples under different storage conditions (dark and light) at 20°C and 30 °C for 24 months. They showed that the samples stored in clear glass bottles at 20 °C lost their extra virgin status in terms of K_{270} after 20 months of storage; in addition, the 1,2-DGs of these samples decreased from approximately 85% at the beginning of the study to approximately 46%, without any significant variation between the storage conditions. The authors highlighted the negative effects of storage at high temperatures on the quality and diglycerol isomerization of EVOO. However, all of the samples stored at 30 °C were declassed from the EVOO category after 16 months of storage, and the 1,2-DGs isomer was reduced from 85% to 30% at the end of the storage period.

5.8.2 Influence of storage length and conditions (high temperature) on EVOO quality

Di Giovacchino et al. (2002) studied the use of nitrogen gas instead of air in the headspace of glass-bottled EVOO stored in the dark at room temperature (12–20 °C) and at 40 °C to improve the stability of olive oil during storage. Their group demonstrated that the FFA of the examined oils did not significantly vary in the samples stored at room temperature with a 2% headspace (of air). Conversely, the FFA of the samples stored at 40 °C continuously increased by more than 1% after 24 months of storage with air or nitrogen gas in the headspace. Furthermore, the samples stored at 40 °C with nitrogen in the headspace were not declassed from the extra virgin category after more than 15 months.

Grigoriadou and Tsimidou (2006) examined the probability of using UV absorption alone to determine oxidative quality without needing PV for VOO stored in open glass bottles at 45 °C for 6 months. Among 40 EVOO samples, 35 were still classified as EVOO/VOO after 6 months of storage and 5 were classified as LVOO in terms of PV. On the basis of K_{232} and K_{270} extinction coefficients, 13 samples were classified as EVOO and 15 samples as VOO, whereas the rest of the samples were LVOO. They concluded a positive correlation between PV and K_{232} as well as between K_{270} and PV.

These findings agreed with those of Mancebo-Campos et al. (2007), who compared the behavior of VOO stored in open glass bottles under Rancimat accelerated conditions (using 3.5 g of each oil sample heated at 100 °C with an air flow of 10 L/h) and long-term room temperature storage. Their group found that the PV increased in a directly proportional manner to 20 meq O_2 per kg oil after 96–167 h of storage under Rancimat conditions. This phenomenon was accompanied by the depletion of *ortho*-diphenols. Meanwhile, samples stored at room temperature (25 °C) showed no significant variation at the end of the three-month storage period.

5.8.3 Influence of storage length and conditions (low temperature) on EVOO quality

Samaniego-Sanchez et al. (2012) explored storage conditions that might alter EVOO exposed to light at room and refrigerated temperatures for 9 months. Sensory evaluation revealed that the stored samples were no longer EVOO. For instance, the samples developed sensory defects (became winey and rancid) after 3 months of storage at both storage temperatures. At the end of the storage period, the total phenol and tocopherol concentrations generally decreased by 90% and 80%, with respect to their initial contents under both conditions.

Similarly, Ben-Hassine et al. (2013) studied the changes in the physicochemical and sensory characteristics of VOO during storage in fully filled clear and dark glass bottles at 8 °C and at the ambient temperature for 9 months. They observed decreases in phenolic compound content, tocopherol content, and fruity attributes. In addition, rancid flavor mainly developed in VOO stored in clear glass bottles. However, they also presented a marked reduction in the C_6 and C_5 LOX volatile compounds, particularly in samples stored at ambient temperature.

Brkic-Bubola et al. (2014) have recently studied content changes in the total phenols, *ortho*-diphenols, and volatile compounds of filtered EVOO stored in the dark in glass bottles with nitrogen in the headspace at room, refrigerated, and freezing temperatures. Among the quality indicators, only the hexanal/(*E*)-2-hexenal ratios of the samples stored at room temperature for 12 months showed significant changes. Tocopherol content slightly varied during the study period.

These previous reports clearly suggest that light is the most harmful factor that influences the quality of stored VOO (Guillaume et al. 2014; Sacchi et al. 2008; Vekiari et al. 2007; Sinelli et al. 2007; Mendes and Falque, 2007; Kalua et al. 2006; Del Caro et al. 2006; Sinesio et al. 2005; Caponio et al. 2005; Okogeri and Margari, 2002; Gutierrez and Fernandez, 2002), followed by temperature elevation (Mancebo-Campos et al. 2007; Grigoriadou and Tsimidou, 2006; Di Giovacchino et al. 2002). The storage of EVOO under light accelerates its retrogression to other lower quality grades, depending on the extent and severity of light exposure. Moreover, light exposure and temperature elevation result in the extreme degradation of minor components, particularly phenolic compounds, and lead to the development of off-flavor volatile compounds. Nevertheless, the replacement of air in the headspace with an inert gas could improve the resistance of EVOO to oxidation degradation (Giovacchino et al. 2002).

5.9 Influence of filtration on the quality and oxidative stability of VOO

Filtration removes suspended solids and humidity from VOO before bottling (Lozano-Sanchez, 2010). The possible use of filtration prior to bottling was established by the EU Regulation (EEC Reg. 1513/2001) to reduce VOO turbidity and improve its commercial value (Lozano-Sanchez et al. 2012).

5.9.1 Applications of filtration in the olive oil industry

Several filtration processes have been used in the olive oil industry. These methods can be classified into two main types in accordance with the material needed to be eliminated from

VOO. The first type aims to remove suspended solids. Such filtration processes are achieved by utilizing filtration aids, such as diatomaceous earth, which is usually mixed with oil in different steps to generate a filter membrane. During filtration, the accumulation of suspended solids with the filter aid generates a so-called filter cake membrane. Filtration is considered finished when the maximum partial pressure is reached. Organic powder filter aids can be replaced by a mechanically stable and elastic fibrous membrane based on cellulose or food grade plastic fibers. The use of this fiber membrane helps reduce process cost by approximately 70%. The other type of filtration aims to remove the humidity in the olive oil. However, a high amount of water in VOO can trigger hydrolytic reactions and promote the liberation of FFA. Nevertheless, all the filtration processes can remove part of the humidity found in VOO. Therefore, the use of sodium sulfate as a filter aid can almost completely remove the amount of water in VOO (Lozano-Sanchez et al. 2010). An Italian researcher (Filterflo of Binasco; Milano, Italy) has recently developed a filtration technique that involves the use of polypropylene filter bags. In this system, olive oil is pumped from the storage tanks to the filtration equipment, wherein the fluid passes across the filter bag and the suspended solids are removed. This system has several benefits over other older systems, including its wide range, versatility, and easy maintenance, which permit and guarantee an optimal level of oil clarity (Lozano-Sanchez, 2010). However, all of the previously mentioned systems for filtration utilize filter aids that come in contact with the VOO; thus, a novel filtration system based on the flow of inert gases, either nitrogen or argon, was developed by researchers at the University of Bologna (Cerretani et al. 2009). In their system, the inert gas (whether argon or nitrogen) insufflates from the bottom of the bulk oil tank, and its circular movement ensures the clarification of the filtered VOO. In addition, this system could improve the stability of the filtered oil when it is applied in large-scale companies because the oil could remain under inert gas in the headspace of large tanks after clarification until it is bottled (Lozano-Sanchez et al. 2010, 2012).

5.9.2 Influence of filtration on the quality of VOO

Numerous studies evaluated the effects of filtration on the quality of filtered oil as compared with unfiltered VOO. For instance, Lercker et al. (1994) suggested that the filtration of EVOO can reduce its oxidation stability. Accordingly, Gomez-Caravaca et al. (2007) have recently studied the effect of filtration using cotton, filter paper, and sodium sulfate on the phenolic compounds and oxidation stability of EVOO. Their group observed that the concentration of

Hyty and the measured oxidation stability index were significantly decreased despite the detected slight increase in the total amount of polar phenols. Moreover, filtration significantly decreased the water content of EVOO. In general, several studies suggested that filtration can decrease the oxidative stability of olive oil by altering the antioxidant properties of phenolic compounds (Bendini et al. 2009a). To compare the effect of different filtration systems on the quality of filtered oil, Lozano-Sanchez et al. (2012) investigated qualitative differences after the filtration of EVOO through a filter bag and an inert gas filtration system. Their results showed that the water content decreased in the filtered oil for all the adopted filtration systems as compared with the unfiltered samples. Among all samples, argon gas-clarified samples had the lowest amount of water. Their group also showed that the total amount of phenolic compounds increased after filtration, and this increase was significant after clarification with argon gas. Consequently, the observed oxidative stability of the filtered and clarified samples was lower than that recorded for unfiltered oil. In terms of the sensory attributes, the authors showed that the fruity and pungent attributes were enhanced after filtration, especially when nitrogen and argon gases were used as filter aids. Bendini et al. (2013) have recently studied changes in the different quality parameters and sensory properties after the clarification of EVOO via inert gas. The following results were highlighted: (1) no pronounced differences were observed between clarified and non-clarified samples in terms of the basic quality parameters and the total amount of polar phenols; (2) the clarified samples had lower water content than the non-clarified ones; (3) the samples clarified with inert gases were richer in LOX volatiles compared with the other samples; (4) the samples clarified with inert gases were rich in fruity attributes and contained fewer defects than the cloudy EVOO.

5.9.3 Influence of filtration processes on the quality of stored VOO

The previously mentioned studies were mainly focused on the determination of the direct effects of filtration on the quality of filtered VOO as compared with those produced without filtration. However, some studies in the last decade reported on the influence of the storage period on filtered versus unfiltered VOO by considering their oxidative stability as well as different chemical and sensory properties. For instance, Brenes et al. (2001) studied the effect of storage conditions on the acid hydrolysis of secoiridoid aglycons during the storage of filtered and unfiltered VOO in the dark for a year in a thermostatically controlled chamber at 30 ± 1 °C. Their group reported that the amount of phenolic compounds, particularly Hyty and Ty, rapidly

increased during the first 200 d of storage as a result of the degradation of secoiridoids during storage. They also proposed that the level of hydrolysis of complex phenols was lower in the filtered VOO samples than in the unfiltered samples. However, they showed that the degradation was more pronounced in tocopherols than in free phenols as a result of their oxidation during storage.

Other factors were examined by Tsimidou et al. (2005), who studied the possible loss of oxidative stability in cloudy VOO during storage in the dark for 9 months at 20 °C. Their research demonstrated that the total amount of phenolic compounds in the unfiltered oil was much higher than that in the filtered samples. However, the same study showed that the loss of polyphenols, particularly Hyty and Ty, was more marked in the filtered oil than in the unfiltered oil. In addition, the consumption of tocopherol and pigments in the unfiltered oil was less than that in the filtered VOO. This finding revealed the possible side effect of filtration on the stability of olive oil during storage.

The effects of filtration on VOO oxidative stability was studied by Fregapane et al. (2006) under different conditions. In their study, olive oil samples were stored in the dark with a 10% headspace at room temperature and at an accelerated storage temperature (40 °C). Their results showed that the unfiltered EVOO exceeded the accepted FFA limits by the respective regulations after 8 months of storage. However, the FFA of filtered VOO remained within the EVOO category regulations throughout the period of storage. Their work also showed that the polar phenol content increased during storage because of the hydrolysis rate of secoiridoid derivatives in the unfiltered samples. In addition, the decreased fruitiness attributes during storage were accompanied by the presence of rancid defects in the unfiltered olive oil sample as compared with the filtered one. This study showed that the unfiltered EVOO was initially declassed to VOO grade as compared with the filtered EVOO, which revealed the positive effect of filtration on the stability of EVOO during storage.

Stefanoudak et al. (2010) focused on the changes in the individual phenols and LOX volatiles of EVOO stored in the dark at room temperature for 15 months, with and without nitrogen in the headspace. The filtered oil had a higher PV and K_{232} at the end of storage, whereas unfiltered EVOO had a higher K_{270} value. However, except for the filtered EVOO with nitrogen in the headspace, all of the samples lost their EVOO status at the end of the storage time. With respect to the phenolic compounds, the degradation of secoiridoid derivatives was extremely high in the unfiltered samples with oxygen in the headspace. However, the changes in the LOX volatile

compounds, particularly (*E*)-2-hexenal, were negligible in the filtered samples at the end of the storage period.

In conclusion, the previously mentioned studies showed that filtration improved the appearance, sensory properties, and volatile characteristics of filtered VOO samples, whereas contradictory observations were obtained regarding the oxidation stability of particular compounds during storage. Thus, further research needs to adopt different storage conditions and filtration systems to elucidate the positive or negative effects of filtration or clarification on the quality of EVOO.
Additional literatures for each case study is included in the respective papers for each experimental section.

6. EXPERIMENTAL SECTIONS

Section 6.1: Influence of filtration/clarification and different storage conditions on the quality of VOO

6.1.1 Background

Filtration is a pretreatment process before bottling permitted by the European Community Regulation (EEC Reg. 1638/98) to enhance the quality and appearance of VOO during storage. Filtration removes materials, such as phospholipids and humidity, which cause the cloudy appearance of EVOO during storage (Bendini et al. 2009a). Filtration may enhance olive oil stability by decreasing water amount and reducing the hydrolysis rate of TG to liberate FFA (Fregapane et al. 2006). The shelf life of VOO is mainly affected by oxygen availability, storage temperature, and light exposure during storage (Garca et al. 2003). These factors can create conditions under which off-flavors and unpleasant olfactory notes are produced, which are due to olive oil deterioration caused by degradation of the initially formed mono-hydro peroxides into secondary oxidation products (unsaturated aldehydes). Nevertheless, some studies revealed that filtration may negatively affect EVOO quality; such effects include reduced chlorophyll pigment compared with the unfiltered EVOO (Bottino et al. 2008).

6.1.2 Objective of the study

This study was performed to investigate the quality and oxidative stability of different filtered and clarified EVOOs stored under different conditions (dark and light) for 12 months. This study also aimed to correlate filtration/clarification techniques with different quality parameters of VOO immediately after the process and during storage.

6.1.3 Materials and methods

6.1.3.1 Samples

EVOO was extracted from olives of ‘Canino’ cultivar from Lazio region (Italy) in October 2012 and then divided into four parts. An aliquot was subjected to a commercial system filter press (1.8 bar) to produce filtered EVOO sample. Aliquot of cloudy EVOO was clarified by directly injecting inert gases, namely, nitrogen or argon, into the center of EVOO mass by using a pilot clarification system (Fig. 3); this system was developed and patented by the University of

Bologna and Sapio (Cerretani et al. 2009). In this system, nitrogen gas was directly injected into the veiled EVOO bulk mass (pressure = 2 bar) to produce nitrogen-clarified EVOO. Another part of the veiled EVOO was injected with argon gas (flow = 12 l/min) to produce argon-clarified EVOO. The remaining EVOO was remained not filtered. Clarification and filtration were conducted at room temperature.

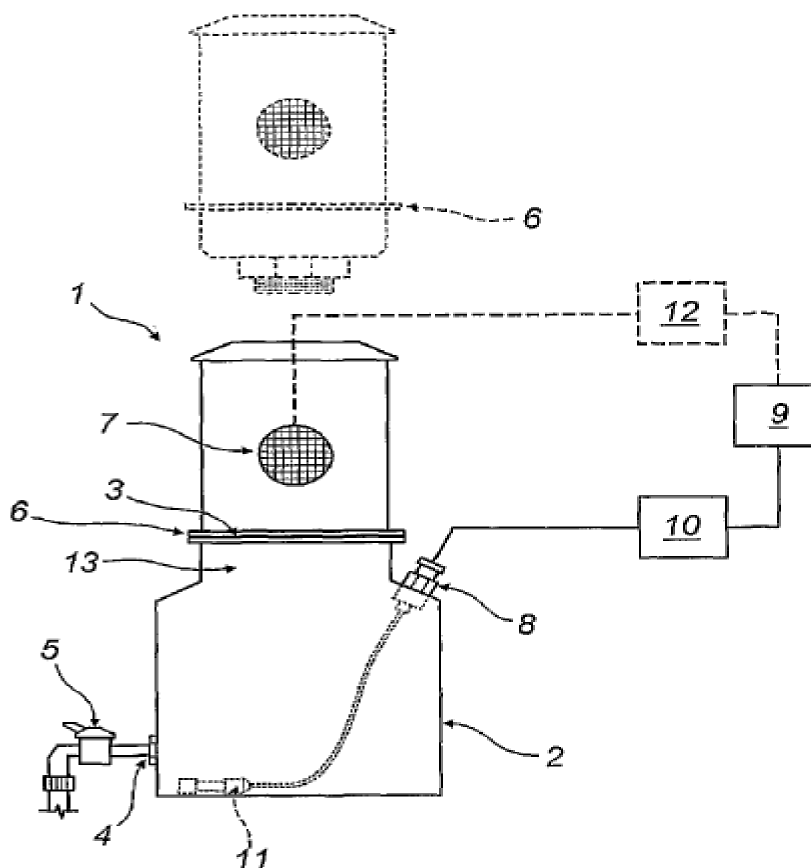

Fig. 3. Schematic diagram of laboratory inert gas clarification system.
Source: Cerretani et al. (2009)

1- Clarification apparatus
2- Edible oil tank
3-Inlet side from which the oil can supplied to the system
4- Drain side from which the oil can extracted after clarification
5- Outlet taps with valve
6- Lid
7- Check valve to prevent air from entering to the tank 2
8- Connector
9- A block scheme to describe the gas delivery system
10- Gas supplying valve
11- Outlet device from which the gas blow inside the bulk oil
12- Scheme block show the gas recovery (not described)
13-Oxygen and carbon dioxide measuring device

6.1.3.2 Storage conditions

All EVOO samples (filtered, clarified, and unfiltered) were filled in 250 ml glass bottles (with approximately 4% v/v of head space) immediately after production and filtration/clarification treatments. The hermetically sealed bottles were stored inside a storage room in the dark (the bottles were covered with aluminum foil) or under artificial and diffused day light. Storage started from January 01, 2013 to December 31, 2013. The monitored temperature ranges were 17 °C to 22 °C from January to the end of May, 30 °C to 36 °C from June to the end of August, and approximately 20 °C to 25 °C until the end of storage period. These ranges were established to simulate actual storage conditions on the market shelf during different seasons.

The samples were analyzed at time zero and after 4, 6, 8, and 12 months of storage under dark and light conditions for chemical and sensory evaluation (panel test). Analysis included basic quality parameters, HPLC profiles of phenolic compunds, *ortho*-diphenols, tocopherols, and chlorophylls, water amount, volatile compound content, and DGs isomerization. This experimental section was divided into two parts. In the first part (**Paper 1**), changes in basic quality parameters, phenolic profile, DGs isomerization, water amount, and volatile profile (normally generated in the LOX enzymatic pathway) during storage in the dark are discussed. This part also aimed to investigate the advantages and disadvantages of filtration/clarification during storage of EVOO. The second part (**Paper 2**) discusses the effect of light on the stored samples, particularly parameters and microcomponents that are highly affected by light exposure and associated with oxidative stability of EVOO. These parameters included basic quality indices, phenolic compounds (*ortho*-diphenols), volatile compounds related to oxidation reaction, chlorophyll, and tocopherols.

6.1.3.3 Analysis plan

Three bottles were removed from the storage room and analyzed in triplicates at each respective time. Each replicate was obtained from a separate bottle, and samples were collected from the geometrical center of each bottle. The samples were evaluated for their sensory properties (panel test) at time zero and at the end of storage.

6.1.3.3.1 Free fatty acids

FFA was determined and calculated according to the official methods described in the European Union Council Regulation (EEC Reg. 2568/91). Approximately 5 g of the oil sample was dissolved in 90 ml of diethyl ether–ethanol solution (2:1 v/v) neutralized with 0.1 NaOH solution. Few drops of phenolphthalein solution (1% in ethanol) were added. The oil and organic solvent solutions were titrated with 0.1 N standardized NaOH solution. The exact weight of the samples and titrate volume were determined to calculate the percentage of FFA on the tested replicate. The results were expressed as gram of oleic acid per 100 g of oil.

6.1.3.3.2 Peroxide value

PV was determined and calculated according to the official method described in the European Union Council Regulation (EEC Reg. 2568/91). About 2–5 g of the oil sample was dissolved in acetic acid–chloroform solution (2:1 v/v) and added with saturated potassium iodide (0.5 ml). The solution was immediately agitated and stored in the dark for 5 min. The solution was then added with distilled water (75 ml) and then 2 ml of 1% starch solution (1 g of starch dissolved in 100 ml of water). The solution was titrated with standardized 0.01 N sodium thiosulfate ($Na_2S_2O_3$). The exact weight of the sample and titrate volume were recorded to calculate the PV of the tested replicate. The results were expressed as milliequivalent O_2 per kilogram of oil.

6.1.3.3.3 Extinction coefficients

UV absorption coefficients (K_{232} and K_{270}) were analyzed according to the official methods described in the EEC Reg. 2568/91 and its successive amendments. The protocol was slightly modified to conserve solvent volume. Oil (0.1 g) was weighed into a 10 ml volumetric flask, and the volume was completed with isooctane. The absorbance of the oil sample was determined at 232 and 270 nm with a UV-vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). UV absorbance was used to calculate K_{232} and K_{270} values.

6.1.3.3.4 Total phenolic compounds extraction

Phenolic compounds were extracted according to the method of Pirisi et al. (2000). Sample (2 g) was dissolved in 1 ml of *n*-hexane and extracted three times with 2 ml of methanol–water solution (60:40 v/v). In each extraction, the mixture was shaken with a vortex mixer for 1 min and then centrifuged for 5 min at 3,000 rpm. The aqueous phase was collected and transferred into another test tube after each centrifugation cycle. *n*-Hexane (2 ml) was added to the collected

phenolic extract, mixed on the vortex, and then centrifuged for 5 min at 3,000 rpm. After the *n*-hexane phase was removed, the extract was evaporated using a rotary evaporator at 35 °C. The residue was dissolved with 5 ml of methanol–water solution (50:50 v/v). The extract was stored at -18 °C until used.

6.1.3.3.5 Total ortho-diphenol compounds

ortho-Diphenols were determined using the method of Rotondi et al. (2004). The phenolic extract (0.5 ml) (Section 6.1.3.3.4) was placed into a 5 ml volumetric flask, and the volume was filled with methanol–water solution (50:50 v/v). A portion (4 ml) of the solution was transferred into a test tube and added with 1 ml of sodium–molybdate solution (5% in ethanol–water solution, 50:50 v/v). The formed solution was thoroughly mixed on the vortex and maintained in the dark for 10 min. The mixture was then centrifuged for 10 min at 3,000 rpm, and the absorbance of the sample and blank was determined at 370 nm (Singleton and Rossi, 1965) with a UV−vis 6705 spectrophotometer (Jenway, United Kingdom). A standard calibration curve was also prepared using different concentrations of gallic acid and read as the samples. The results were calculated and expressed as milligram of gallic acid per kilogram of oil.

6.1.3.3.6 Extraction of phenolic compounds for HPLC determination

Polar phenolic compounds were extracted from EVOO samples following the procedure described by (Pirisi et al. 2000) and further modified by (Rotondi et al. 2004) using liquid- liquid extraction method. Sample (4 g) was dissolved in 2 ml of *n*-hexane and extracted three times with 2 ml of methanol–water solution (60:40 v/v). In each extraction, the mixture was shaken with a vortex mixer for 1 min and then centrifuged for 5 min at 3,000 rpm. The aqueous phase was collected and transferred into another test tube after each centrifugation cycle. *n*-Hexane (2 ml) was added to the collected phenolic extract, mixed on the vortex, and then centrifuged for 5 min at 3,000 rpm. After the *n*-hexane phase was removed, the extract was evaporated using a rotary evaporator at 35 °C. After evaporation, the dried residue was dissolved in 3 ml of methanol–water solution (50:50 v/v) and filtered through a 0.2-μm syringe filter (Whatman Inc.). The phenolic extracts were stored at −18 °C until use.

6.1.3.3.7 Determination of phenolic compounds via HPLC

Chromatography was performed with a 1100 series liquid chromatography instrument equipped with a quaternary pump and a UV–vis diode array and MS detectors (Agilent Technologies, Waldbronn, Germany). Phenolic compounds were separated on the reverse phase of the C18 100A Kinetex column (2.6 µm, 100 mm × 3 mm I.D., Phenomenex, Torrance, CA, USA). Gradient elution was conducted using a solvent system of water–formic acid (100:0.5 v/v) as mobile phase A and acetonitrile as mobile phase B. The total run time was 13 min, and the gradient elution was as follows: from 0 to 3 min, solvent B increased from 5% to 20%. Solvent B reached 40%, 60%, and 100% at 4, 9, and 10 min, respectively. At 13 min, 5% of solvent B was restored. The column was thermostated at 30 °C and equilibrated for 5 min prior to each analysis. An injection volume of 2.5 µl and a flow rate of 0.7 ml/min were used. The chromatograms were monitored at 240, 280, 320, and 345 nm. The following wavelengths were suitable for each group of compounds: 240 nm for elenolic acid; 280 nm for hydroxybenzoic acids, phenyl ethyl alcohols, secoiridoids, and lignans; 320 nm for hydroxycinnamic acids; and 345 nm for flavones.

The main phenolic compounds were identified by comparison with the relative retention times of reference standards. Other compounds (where the reference compound was not available) were identified with an ion trap mass spectrometer (Agilent) in the electrospray ionization mode. The working conditions for mass spectrometry were as follows: nebulizer gas pressure, 0.24 MPa; drying gas flow, 7 l/min at 300 °C; and capillary voltage, 2.5 kV. Nitrogen was used as a nebulizer and drying gas. Mass scans ion was performed within the *m/z* 100–900 in the negative and positive ion modes.

6.1.3.3.8 Tocopherol determination

Total tocopherols content was determined by using 0.5 g of the sample through HPLC-DAD according to the method described by Bendini et al. (2013). Tocopherols (α and γ forms) were calculated using the calibration curve of known concentrations of α-tocopherol (R^2 = 0.999). The results were reported in milligram of total tocopherol per kilogram of oil.

Sample preparation

Olive oil sample (0.5 g) was weighed into a 10 ml volumetric flask, and the volume was completed with isopropanol. The sample was agitated and then filtered with 0.45 µm filters before filling the vials for HPLC-DAD analysis.

Determination with HPLC-DAD

The sample (20 µl) was injected on HPLC-DAD (Agilent Technologies, Waldbronn, Germany). Tocopherol was separated with a Cosmosil NAP column (CPS Analitica, Milan, Italy) with dimensions of 150 mm × 4.6 mm i.d. at 120 Å. Analysis was performed with methanol–water solution (90:10 v/v, water (acidified with 0.2% phosphoric acid), and acetonitrile were applied as mobile phase A and B respectively, with a flow rate of 1 ml/min. Separation was performed at room temperature, with 35 min as the total run time. The separated peaks were determined with a DAD detector at 292 nm. A calibration curve was calculated with α-tocopherol standard at different concentrations (R^2 = 0.999). The results were expressed as milligram of α-tocopherol per kilogram of oil.

6.1.3.3.9 Water amount

Water amount was determined at 103 °C through air drying technique (ISO 662:1988). Oil sample (10 g) was weighed in an empty aluminum moisture dish (approximately 50 mm in diameter and 30 mm height, with a flat bottom). The samples were heated for 1 h in a drying oven at 103 ± 2 °C, and the dish was cooled in the desiccator and weighed. The sample was reheated for another 0.5 h, cooled, and then weighed again. The half-hour reheating, cooling, and weighing cycle may be repeated until the difference between the final successive weights was lower than 2 mg. The water amount was calculated with the following equation:

“weight of sample − weight of dried sample / weight of sample”

The result was expressed as milligram of water per kilogram of oil.

6.1.3.3.10 Determination of chlorophylls

Pigment composition was determined with a UV−vis 6705 Jenway spectrophotometer (United Kingdom). Determinations were performed at 670 nm according to the protocol described by Baccouri et al. (2008). Sample (1 g) was weighed into a 10 ml volumetric flask, and the volume was filled with isooctane. Chlorophyll was determined with a calibration curve of known concentrations of chlorophyll soluble in isooctane (R^2 = 0.999). Data were reported as milligram of chlorophyll per kilogram of oil.

6.1.3.3.11 Determination of volatile compounds via SPME-GC/MSD

Volatile compounds were determined via SPME-GC/MSD (Agilent 6890N, Santa Clara, CA, USA) coupled with quadrupole mass-selective spectrometry (Agilent 5973 N, Agilent Technologies) according the procedure described by Cerretani et al. (2008). Volatile compounds were identified with mass spectrometry by comparing their mass spectral data with the information obtained from the NIST Library (2005 version) and MS literature data. Volatile compounds were expressed as milligram of internal standard (IS) (4-methyl-2-pentanone) per kilogram of oil.

A VOO sample (1.5 g) was placed in a 10 ml vial containing a micro stirring bar and spiked with 0.15 g of 4-methyl-2-pentanone (IS). The vials were sealed with a silicon septum. The headspace in the vial was equilibrated for 2 min at 40 °C in a water bath with gentle agitation prior to insert the SPEME fiber for volatile extraction. The SPME fiber (divinylbenzen/carboxen/polydimethylsiloxane, 50/30 µm, 2 cm long from Supelco Ltd., Bellefonte, PA, USA) was then exposed to the sample headspace, where volatile extraction was performed at 40 °C for 30 min. After volatile adsorption, the fiber was injected into the GC sampling port in splitless mode. Thermal desorption of volatiles was attained after 3 min at 250 °C.

GC-MS conditions

Volatile compounds were separated with a ZB-WAX column (30 m, 0.25 mm i.d., 1 µm film thickness; Phenomenex, Torrence, CA, USA). The initial oven temperature was 40 °C for 10 min and then increased to 200 °C at a rate of 3 °C/min (maintained for 2 min). The temperature was further increased to 250 °C at a rate of 10 °C/min and maintained for 2 min before cooling to 40 °C. The temperatures of the ion source and transfer line were 230 °C and 250 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy within 30–250 amu mass

range at 2 scans/s. Volatile compounds were expressed as milligram of IS (4-methyl-2-pentanone) per kilogram of oil.

6.1.3.3.12 Determination of diglycerides

Sample preparation

The samples were prepared for gas chromatography (GC) determination according to method described by Sweeley et al. (1963). EVOO sample (0.1 g) was weighed into a 10 ml centrifuge tube and added with 0.5 ml of IS (prepared by diluting 2 g of dilaurin reagent in 1 l of chloroform solution). The solution was thoroughly mixed on the vortex, and 100 µl of homogenized solution was placed into another centrifuge tube. This solution was evaporated under a gentle nitrogen flow until complete dehydration. After evaporation, 0.2 ml of silylation reagent (3:1:9 v/v/ hexamethyldisiloxane: trimethylchlorosilane: pyridin) was added to the residue and allowed to react for 5 min in the closed tube in the dark. The liquid phase was then evaporated under a gentle nitrogen gas flow, and the residue was dissolved in 0.2 ml of *n*-hexane, centrifuged for 1 min at 2,000 rpm, and then transferred to GC vials. The samples were injected in the GC-FID to determine their DGs profile within the same day of sample preparation.

GC determination of DGs

Diacylglycerol profile was determined according to the modified version of the method suggested by Serani et al. (2001). A GC Carlo Erba MFC500 with Rtx-65TG (Restek, Bellefonte, PA) and a fused-silica capillary column (30 m length × 0.25 mm i.d. × 0.10 µm f.t.) coated with 35% dimethyl/65% diphenyl polysiloxane were used. The oven temperature was programmed from 250 °C to 320 °C at a rate of 2 °C/min and increased to 365 °C at a rate of 5 °C/min. The final temperature was maintained for 21 min. The injector and FID temperatures were set at 360 °C. Helium was used as the carrier gas at 130 kPa, and the split ratio was 1:70.

DGs identification

DGs were identified by comparing the peak retention times and GC traces with those of the DG standards and chromatograms reported in the literature (Bendini et al. 2009b; Serani et al. 2001). The results were expressed as milligram of each DG per 100 mg of oil and quantified using IS concentration.

6.1.3.3.13 Sensory analysis

Sensory analysis (panel test) was performed according to the EC Reg. 640/2008 by a trained group of eight expert tasters of the Department (DISTAL) of the University of Bologna. The brief of the analysis protocol was described in the Introduction section (paragraph 5.6).

6.1.3.3.14 Statistical analysis

The software XLSTAT 7.4.2 version (Addinsoft, USA) was used for analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) to elaborate data.

6.1.4 Summary of results and main findings

In **Paper 1** of this section, the effects of filtration/clarification and storage time of 12 months on EVOO quality are presented. The evaluated quality parameters included phenolic profiles, DGs, volatile compounds, and organoleptic properties.

In **Paper 2** of this section, the oxidative stability and possible changes in the filtered and clarified samples caused by storage under light exposure are discussed.

The changes in basic quality parameters did not exceed the limits for the EVOO category according to the EU Reg. 1348/2013 (Table 2). Nevertheless, the FFA value in the unfiltered sample was higher than that in the filtered and clarified EVOO samples at the end of storage.

Table 2. FFA, Free Fatty Acids (g oleic acid per 100 g oil), peroxide value (PV) (meq O_2 per kg oil), UV absorbance indexes (K_{232} & K_{270}) and oxidative volatile compound markers (hexanal/nonanal ratio) for all samples at time zero and after 12 months of storage in dark and light.

Letter (a-c) indicates the statistical differences between time zero and the respective sample after 12 months at each condition (light/dark) Letters (w - z) indicate the statistical differences among the samples at the same time and condition (light/dark). (*) indicate the significant higher value between dark and light of the same sample after 12 months.

Samples	FFA	PV	K_{232}	K_{270}	Hexanal/nonanal Ratio
Time zero					
Unfiltered	0.21 ± 0.00 b,x	10 ± 1 a,w	1.37 ± 0.09 b,y	0.10 ± 0.01 b,x	----------------
Filtered	0.21 ± 0.00 b,x	10 ± 0 a,w	1.69 ± 0.12 b,w	0.09 ± 0.00 b,x	----------------
Nitrogen clarified	0.21 ± 0.00 b,x	8 ± 1 a,x	1.58 ± 0.10 b,x	0.10 ± 0.00 b,x	----------------
Argon clarified	0.21 ± 0.01 a,b,x	9 ± 1 b,xw	1.43 ± 0.02 b,x	0.11 ± 0.00 b,w	----------------
After 12 months in dark					
Unfiltered	0.34 ± 0.00 a,w*	9 ± 1 a,x	2.13 ± 0.09 a,w	0.12 ± 0.00 a,x	1.20* ± 0.09 b,y
Filtered	0.26 ± 0.00 a,x*	10 ± 0 a,xw	2.31 ± 0.22 a,w*	0.14 ± 0.01 a,x	1.98 ± 0.34 b,y
Nitrogen clarified	0.23 ± 0.01 a,y	10 ± 1 a,x	2.37 ± 0.14 a,w*	0.17 ± 0.01 a,w	8.95* ± 1.24 a,w
Argon clarified	0.21 ± 0.00 a,y	12 ± 1 a,x*	2.11 ± 0.10 a,w	0.12 ± 0.01 a,x	6.44* ± 0.94 b,x
After 12 months under light					
Unfiltered	0.30 ± 0.00 a,w	13 ± 1 a,w*	1.85 ± 0.09 a,w	0.19 ± 0.00 a,w*	2.16* ± 0.21 b,wx
Filtered	0.24 ± 0.01 a,y	12 ± 0 a,w*	1.94 ± 0.17 a,w	0.19 ± 0.00 a,w*	2.4*± 0.19 b,wx
Nitrogen clarified	0.27 ± 0.00 a,x*	11 ± 1 a,w	1.96 ± 0.10 a,w	0.19 ± 0.01 a,w*	1.28 ± 0.20 b,y
Argon clarified	0.25 ± 0.01 a,y*	7 ± 1 b,x	1.96 ± 0.07 a,w	0.19 ± 0.00 a,w*	1.99 ± 0.05 b,x

Influence of filtration on minor components and sensory properties of EVOO

The amount of water in EVOO was significantly affected by filtration and clarification. These processes significantly decreased the amount of water in the following order: unfiltered > filtered > argon-clarified > nitrogen-clarified samples. In addition, the water amount in EVOO clarified with inert gases was significantly lower than that in commercially filtered EVOO sample (Table 3). These findings were in accordance with the results obtained by Lozano–Sanchez et al. (2012) and Bendini et al. (2013).

Table 3. Water amount (mg per kg), phenolic compounds (mg per kg), LOX generated volatile compounds (C_6 and C_5) expressed as mg 4-methyl-2- pentanone per kg of oil and diacylglycerols ratio for the samples at time zero and after 12 months of storage in the dark.

Letter (a-c) indicates the statistical differences between time zero and the respective sample after 12 months at each condition (light/dark) Letters (w - z) indicate the statistical differences among the samples at the same time and condition (light/dark).

Sample	Water Amount	Hyty	DOA	C6 LOX volatile	C5 LOX volatiles	1.2/1,3-DG ratio
Time zero						
Unfiltered	1485 ± 40 a,w	6.8 ± 0.4 b,x	277.4 ± 6.7 a,w	16.74 ± 2.35 a ,w	2.81 ± 0.27 a,w	27 ± 1 a,w
Filtered	763 ± 36 a,x	6.0 ± 0.8 b,x	289.4 ± 39.3 a,w	13.47 ± 0.16 a,x	1.84 ± 0.02 b,y	27 ± 0 a,w
Nitrogen clarified	190 ± 6 a,z	6.5 ± 0.5 a,x	310.9 ± 36.6 a,w	13.35 ± 0.15 a,x	2.07 ± 0.03 a,xy	23 ± 2 a,x
Argon clarified	260 ± 32 a,y	14.0 ± 1.9 a,w	287.2 ± 16.5 a,w	12.63 ± 0.18 b,,x	2.28 ± 0.05 b,x	25 ± 2 a,x
After 12 months in dark						
Unfiltered	771 ± 6 b,w	31.7 ± 0.1 a,w	76.3 ± 7.8 b,y	11.65 ± 0.44 b,x	1.28 ± 0.06 b,z	2 ± 0 b,w
Filtered	568 ± 44 b,x	7.7 ± 0.3 a,x	106.4 ± 11.8 b,x	11.28 ± 0.17 b,x	2.20 ± 0.08 a,x	2 ± 0 b,w
Nitrogen clarified	26 ± 6 b,z	6.9 ± 0.3 b,y	154.9 ± 9.1 b,w	12.58 ± 0.56 a,w	1.98 ± 0.10 a,y	2 ± 0 b,w
Argon clarified	85 ± 5 b,y	6.0 ± 0.0 b,y	171.4 ± 26.8 b,w	13.2 ± 0.13 a,w	2.76 ± 0.07 a,w	2 ± 0 b,w

Paper 1 of this section discusses the changes in minor compounds and sensory properties during 12 months of storage in the dark.

At the end of storage, the water amount significantly decreased in all samples, which could be due to normal settling of suspended materials, including water (Table 3). However, the samples used for chemical analysis were collected from the center of the bottle and water amount cannot be detected in samples clarified with inert gases. Simple phenols, particularly Hyty, significantly increased in the unfiltered sample and were approximately five times higher than their initial value after storage. Hyty also slightly varied in the filtered and argon-clarified samples, in comparison with the results obtained in the unfiltered sample. The significantly high formation rate of Hyty in the unfiltered sample can be related to the high amount of water that partially maintain the hydrolytic enzymes activity (Bendini et al. 2009a).

Main secoiridoid derivatives, such as DOA, sharply decreased at the end of storage. The amount loss was approximately 50%, 46%, 37%, and 23% for unfiltered, filtered, nitrogen-clarified, and argon-clarified samples, respectively (Table 4). DOA was significantly higher in samples clarified with inert gases than that in commercially filtered sample. The amounts of C6 and C5 volatiles also significantly decreased (Table 3) in the unfiltered and filtered samples. These LOX

volatiles were significantly higher in samples clarified with inert gas than those in commercially filtered samples or slightly differed from the results at time zero (**Paper 1**).

Table 4. Total phenols and *ortho*-diphenols content (mg gallic acid per kg oil), chlorophylls, and tocopherols content (mg per kg oil) for all samples at time zero and after 12 months of storage in dark and light.

Letter (a-c) indicate the statistical differences between time zero and the respective sample after 12 months at each condition (light/dark) Letters (w - z) indicate the statistical differences among the samples at the same time and condition (light/dark). (*) indicate the significant higher value between dark and light of the same sample after 12 months.

Samples	Total phenols	*ortho*-diphenols	Total tocopherols	Chlorophylls
Time zero				
C	308 ± 6 a,w	143 ± 2 a,w	309 ± 1 a,w	29 ± 1 a,y
F	307 ± 22 a,w	126 ± 9 a,w	301 ± 7 a,w	32 ± 0 a,x
Nc	279 ± 1 a,x	146 ± 9 a,w	283 ± 6 a,w	28 ± 2 a,y
Ac	297 ± 4 a,wx	158 ± 13 a,w	282 ± 1 a,w	37 ± 1 a,w
After 12 month in the dark				
C	172 ± 6 b,w	92 ± 13 b,w	211 ± 20 b,x*	27 ± 1 a,w*
F	194 ± 24 b,w	89 ± 10 b,w	233 ± 9 a,w*	24 ± 1 b,w*
Nc	170 ± 16 a,w	90 ± 14 b,w	239 ± 4 a,w*	19 ± 1 b,x*
Ac	165 ± 6 a,w	93 ± 5 b,w	223 ± 20 a,w*	20 ± 2 b,x*
After 12 months under light				
C	171 ± 1 b,x	90 ± 2 b,w	140 ± 10 b,x	4 ± 1 b,w
F	189 ± 10 b,wx	85 ± 4 b,w	187 ± 0 a,w	4 ± 0 b,w
Nc	207 ± 12 a,w*	99 ± 5 b,w	192 ± 5 a,w	1 ± 0 b,x
Ac	171 ± 18 b,x	87 ± 3 b,w	186 ± 4 a,w	1 ± 0 b,x

At the end of storage, the initial sensory scores decreased in all stored samples but the changes in the filtered and clarified samples were lower than that in the unfiltered sample. The scores for fruity, bitter, and pungent attributes remained higher in the filtered and clarified samples than those in the unfiltered sample (**Paper 1**). With regard to sensory evaluation, the results revealed that the filtered and clarified EVOO samples stored in the dark were not significantly different.

The results in Table 2 further showed that the 1,2/1,3-DG ratio significantly decreased in all samples at the end of storage. None of the stored samples in dark showed any sensorial defect and remained within the accepted limits established for the EVOO category (EU Reg. 1348/2013) (Table 5).

Table 5. Changes in organoleptic assessment for olive oils during storage for 12 months as evaluated by Panel test according to the EC. Reg 640/2008, by a fully trained group of 8 expert tasters from University of Bologna.

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Samples	Fruity	Bitter	Pungent	Defect (rancidity)
Time zero				
Unfiltered	4.20	4.20	4.40	--------
Filtered	4.65	6.50	6.55	--------
Nitrogen clarified	4.45	4.75	6.75	--------
Argon clarified	4.90	6.25	6.40	--------
After 12 months in dark				
Unfiltered	2.20	2.60	2.10	--------
Filtered	3.40	4.10	3.90	--------
Nitrogen clarified	2.40	3.90	3.90	--------
Argon clarified	2.40	3.30	3.50	--------
After 12 months under light				
Unfiltered	2.1	2	2	1.7
Filtered	2.3	3.3	2.6	1.5
Nitrogen clarified	2.3	3.8	3.2	0.9
Argon clarified	2.4	1.6	1.6	0.9

Paper 2 of this section discusses the oxidative stability and the possible changes in the filtered and clarified samples caused by storage under light exposure.

Phenolic compounds (*ortho*-diphenols) are powerful antioxidants present in EVOO (Bendini et al. 2007). These compounds significantly decreased during storage (Table 4) but were not significantly different among stored samples or between storage conditions (dark/light). The total tocopherol content (Table 4) significantly decreased in all samples stored under dark and light conditions. This class of compound (tocopherol) remained significantly higher in the filtered, nitrogen-, and argon-clarified samples than that in the unfiltered samples. Hence, filtration and clarification with inert gas may protect lipophilic phenols; this finding was also reported by Bendini et al. (2013).

With regard to EVOO pigments, chlorophyll content (Table 4) remained constant in all samples stored under dark condition but significantly decreased in the filtered and clarified samples at the end of storage. Chlorophyll pigment was significantly depleted in all samples stored under light condition. After storage, the amount of chlorophyll remained higher in the unfiltered samples stored under both conditions than those in samples filtered and clarified with inert gas.

Olive oil contains volatile compounds caused by oxidation reactions, such as octane, nonanal, hexanal, and 2,4-heptadienal (Kiritsakis, 1998; Vichi et al. 2003a). Some researchers proposed hexanal/nonanal as an indicative marker of progressive oxidation (Kiritsakis, 1998; Morales et al. 1997). At the end of storage, rancid volatiles were detected in all samples stored under both conditions (Table 2) and were higher in the unfiltered and filtered samples than those in samples clarified with inert gases. The carboxy cyclic volatiles were also significantly higher in the unfiltered samples under both conditions than those in the filtered, nitrogen- and argon-clarified samples (**Paper 2**, Table 2). These results could be attributed to the presence of high amounts of suspended solids and oxygen in the unfiltered EVOO sample (Kalua et al. 2007; Kanavouras et al. 2006).

The hexanal/nonanal ratio (Table 2) was lower than 2 in the unfiltered EVOO sample after storage under dark condition. This ratio in nitrogen- and argon-clarified samples was threefold to fourfold higher than that in the filtered sample stored under dark condition. Moreover, the amounts of oxidative markers for volatiles were significantly higher in all samples stored under diffused daylight condition than those stored under dark condition. These results were confirmed with sensory evaluation, in which all samples stored under light condition contained sensory defects, particularly rancidity. Thus, these samples were declassed from EVOO status.

6.1.5 Conclusions

The hydrolytic reactions were more pronounced in the unfiltered EVOO than those in the filtered/clarified EVOO at the end of storage. Nevertheless, basic physicochemical parameters of the stored samples did not exceed the limits established by the European Union relations for the extra virgin category. Clarification significantly reduced the water amount in EVOO than commercial filtration. This process did not affect phenolic compounds, tocopherols, C6-LOX, and organoleptic properties of EVOO, despite the presence of circulating gas bubbles in the bulk oil. The presence of oxidative volatile compounds was significantly lower in the clarified EVOO than that in commercially filtered samples, whereas the unfiltered EVOO considered oxidized oil in term of nonanal/ hexanal ratio. After storage of 12 months, tocopherol content was lower in the unfiltered EVOO than that in the filtered and clarified samples.

Clarification with inert gases can effectively protect phenolic compounds, particularly DOA, compared with commercial filtration. The unfiltered sample lost most of its initial phenolic contents.

The amount of tocopherols and the scores for sensory properties were higher in the filtered/ clarified samples than those in samples stored without clarification or filtration.

C_6-LOX volatiles were maintained constant after storage compared with those in the commercially filtered or unfiltered EVOO. In general, qualitative decay in samples stored under light condition was more pronounced than those stored under dark condition at the end of 12-month storage. All samples stored under light condition were downgraded to virgin oils because of developed sensory defect (rancidity).

6.2 Experimental Section 2: Diacylglycerol isomerization in EVOO in relation to different storage conditions

6.2.1 Background

DGs, which are minor components in VOOs, originate from incomplete biosynthesis and partial hydrolysis of TG. Fresh VOO contains predominantly 1,2-DGs isomers, but this isomer decreases during storage because it isomerizes to form 1,3-DGs isomers (Fronimaki et al. 2002). Despite that the presence of DGs in VOO is an indicator of its freshness and quality during storage (Serani et al., 2001), this parameter is excluded in the European regulation (EU Reg. 1348/2013).

6.2.2 Objectives of the study

This study aimed to investigate the effects of storage conditions on diacylglycerols isomerization. Changes in 1,2/1,3-DG ratio and the concentration of, 1,2-DGs and 1,3-DGs isomers in different EVOO samples were evaluated during 14 months of storage under different conditions. This research further investigated the influence of different variables (temperature, lighting, and headspace conditioning atmosphere) on these compounds to determine the degree of EVOO freshness and thus establish useful markers.

6.2.3 Materials and methods

6.2.3.1 Olive oil samples

EVOO samples were extracted from olive fruits of a Spanish cultivar (‘Arbequina’) by using a three-phase industrial decanter. The samples were filled into 250 ml hermetically sealed clear glass bottles with 2 ml headspace.

6.2.3.2 Storage conditions

The sample bottles were divided into four groups and stored under the following conditions, to simulate the conditions of a supermarket shelf.

1. The first group was stored in a thermostatic chamber at 20 °C in the dark (Cond. 1).
2. The second group was stored in a thermostatic chamber at 20 °C under diffused light (600 Lux for 12 h/day, 11 W, 595 lm, 6,400 K) (Cond. 2).
3. The third group was stored in a refrigerated chamber at 4 °C to 6 °C and exposed to diffused light (600 Lux for 12 h/day, 11 W, 595 lm, 6,400 K) (Cond. 3).
4. After replacing the air in the headspace with argon, the fourth group was stored in a thermostatic chamber at 20 °C and exposed to diffused light (600 Lux for 12 h/day, 11 W, 595 lm, 6,400 K) (Cond. 4).

6.2.3.3 Analysis plan

The samples were analyzed in triplicates after 2, 4, 6, 8, 10, 12, and 14 months of storage under the four conditions on the basis of their different diacylglycerol classes. A newly closed bottle was used for sampling at each respective time of analysis and discarded after use.

6.2.3.3.1 Sample preparation

Samples for GC determination were prepared according to the method described by Sweeley et al. (1963). EVOO (0.1 g) was weighed into a 10 ml centrifuge tube and added with 0.5 ml of IS (prepared by diluting 2 g of dilaurin reagent in 1 l of chloroform solution). The solution was thoroughly mixed on the vortex. About 100 µl of homogenized solution was placed into another centrifuge tube and evaporated under gentle nitrogen flow until complete dehydration. After evaporation, 0.2 ml of silylation reagent (hexamethyldisiloxane/trimethylchlorosilane/pyridin, 3:1:9 v/v) was added to the residue and allowed to react for 5 min in the closed tube under dark condition. The liquid phase was then evaporated under gentle nitrogen gas flow. The residue was subsequently dissolved in 0.2 ml of *n*-hexane, centrifuged for 1 min at 2,000 rpm, and transferred into GC vials. The samples were injected in GC-FID to determine their DGs profile within the same day of sample preparation.

6.2.3.3.2 GC Determination of diglycerides

Diacylglycerol profiles were determined according to the modified version of the method proposed by Serani et al. (2001) by using a GC Carlo Erba MFC500 equipped with Rtx-65 TG (Restek, Bellefonte, PA) fused silica capillary column (30 m length × 0.25 mm i.d. × 0.10 µm f.t.) coated with 35% dimethyl/65% diphenylpolysiloxane. The oven temperature was

programmed from 250 °C to 320 °C at a rate of 2 °C/min and then increased to 365 °C at a rate of 5 °C/min. The final temperature was maintained for 21 min. The temperatures of the injector and FID were set at 360 °C. Helium was used as the carrier gas at 130 kPa, and the split ratio was 1:70.

6.2.3.3.3 Diglycerides identification

DGs were identified by comparing peak retention times and GC traces with those of the DG standards and chromatograms reported in the literature (Bendini et al. 2009b; Serani et al. 2001). The results were expressed as milligram of each DG per 100 mg of oil and quantified with respect to IS concentration.

6.2.3.3.4 Statistical analysis

The software XLSTAT 7.4.2 version (Addinsoft, USA) was used for analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) to elaborate data.

6.2.4 Summary of results and main findings

Several studies in the literature focused on the effects of different storage conditions, such as time, temperature, and lighting, on DGs isomerization and initial quality of VOOs (Cossignani et al. 2007; Spyros et al. 2004; Pérez–Camino et al. 2001; Catalano et al. 1994). In the present study (**Paper 3**), the evolution of 1,2/1,3-DG ratio and the changes in the concentrations of 1,2-DGs and 1,3-DGs in EVOO samples were studied under the previously mentioned condition during 14 months of storage.

At the end of storage, 1,2/1,3-DG ratio significantly decreased in Cond. 1, which slightly differed from that in Cond. 2. The ratio in Cond. 3 (3.7) (Table 6) remained approximately two times higher than that in Cond. 1 and 2 (1.47 and 1.69, respectively). In Cond. 4, 1,2/1,3-DG ratio significantly decreased similar to that observed under other storage conditions. A similar 1,2/1,3-DG decreasing trend was also detected for 1,2-DGs, C34, and C36 isomers in Cond. 4. The amount of C36 DG isomers was higher than that of C34 DGs isomers because diolein is the predominant DG in VOO (Boskou, 2006).

The decrease in 1,2-DGs isomers was accompanied with an increase in 1,3-DGs, C36, and C34 isomers during the entire storage period (**Paper 3**). In the first period of storage (2–4 months),

samples stored at low temperatures presented high 1,2/1,3-DG ratio, followed by those stored under light condition at 20 °C with argon in the headspace (**Paper 3**). The 1,2/1,3-DG ratio, 1,2-DGs, C36, and C34 isomers remained twice higher in Cond. 3 with lower amount of 1,3-DGs isomers than those of the other samples stored at high temperatures (Conditions 1, 2, and 4).

Samples stored at 20 °C were further compared in terms of the headspace conditioning gas at the end of storage. The results indicated that 1,2/1,3-DG ratio was not significantly different among the samples. The advantage of using inert gas in the headspace was evident in the first period of storage (**Paper 3**). In-depth investigation on 1,2-DGs isomers also provided evidence regarding the benefits of using inert gas. 1,2-DGs, C36, and C34 isomers remained higher in samples with inert gas in the headspace than those with air.

Samples stored at 20 °C were also compared under light and dark conditions. 1,2/1,3-DG ratio was not significantly different among these samples, whereas 1,2-DGs, C36, and C34 isomers were high in samples stored under dark condition (Table 6).

Table 6. Evolution of 1,2 and 1,3-DGs isomers of C34 and C36 diglyceride and 1,2/1,3-DG ratio after 14 months of EVOO storage under different conditions (Cond 1-4*). The concentration of DGs was calculated as mg dilaurin per 100 mg of oil.

*Different letters (x-z) represent significant differences among mean values among the four storage conditions after 14 months of storage. *Cond. 1, stored at 20 °C in dark, Cond. 2, stored at 20 °C in light, Cond. 3, stored at 6-8 °C in light, Cond. 4 stored at 20 °C in light with argon in the headspace.*

Storage conditions	1,3 C34-DGs	1,3 C36-DGs	1,2 C34-DGs	1,2 C36-DGs	1,2/1,3-DG ratio
Cond 1	0.19 ± 0.02 x	0.49 ± 0.02 x	0.27 ± 0.02 yz	0.73 ± 0.05 y	1.47 ± 0.06 y
Cond 2	0.14 ± 0.01 y	0.32 ± 0.06 y	0.21 ± 0.01 z	0.57 ± 0.04 z	1.69 ± 0.14 y
Cond 3	0.13 ± 0.01 y	0.28 ± 0.03 y	0.39 ± 0.04 x	1.07 ± 0.03 x	3.70 ± 0.29 x
Cond 4	0.21 ± 0.04 x	0.53 ± 0.10x	0.31 ± 0.06 y	0.84 ± 0.15 y	1.56 ± 0.01 y

6.2.5 Conclusions

This study confirmed that the isomerization of DGs in EVOO was dependent not only on storage duration, but also on temperature. The results of this investigation (**Paper 3**) can be summarized into the following: (1) storage time and temperature are the main factors that influence isomerization and accumulation of 1,3-DGs isomers in VOO. (2) Replacing the headspace gas can decrease the isomerization rate in the initial months of storage. (3) The effect of storage conditions (dark or light) on isomerization was negligible compared with the effect of temperature elevation.

6.3 Experimental Section 3: Effects of temperature fluctuation in the oxidation quality and shelf life of VOO

6.3.1 Background

Studies on the effects of temperature fluctuation mainly focused on foods, such as meat and tomato, rather than VOO. Temperature is a main factor that affects VOO quality, and its increase and variation can consequently accelerate lipid hydrolysis and oxidation reactions. During storage, temperature variation induces quality loss and development of off flavors because of the formation of volatile and nonvolatile by-products (Bendini et al. 2009a). In the present study, selected VOO quality indicators were evaluated using samples from a similar batch during and after storage simulation under fluctuating and static temperatures for 720 h (30 d). The experimental plan was designed to investigate the effect of temperature fluctuation on VOO quality during a short period. Extreme temperature fluctuation may widely occur in different Mediterranean desert areas during day and night sequential change particularly in summer. In markets, olive oil can also be affected by climate change during winter season when heating systems are switched off at night or on weekends to save electrical energy.

6.3.2 Objectives of the study

This study aimed to (i) investigate the effects of temperature fluctuation on VOO quality, (ii) compare the influence of temperature fluctuation and accelerated constant storage temperature on VOO of different initial quality grades, and (iii) evaluate the effect of the initial quality of VOO on its oxidative stability during storage under different temperature stresses.

6.3.3 Materials and methods

6.3.3.1 Samples

EVOO extracted from ‘Canino’ cultivar in November 2012 and LVOO extracted from olive fruits of the same cultivar that was stored for 15 d before oil extraction.

6.3.3.2 Accelerated storage conditions

Storage simulation was started in February 2013. The samples from both VOO categories were stored at constant temperature (45 °C) for 30 d. Another set of samples from the same batch was stored under fluctuating temperature, which increased from 5 °C to 45 °C at a rate of 0.3333 °C/h for 5 d and decreased to 5 °C at the same rate for another 5 d. The total time for accelerated storage simulation was 720 h (Fig. 4). The lower temperature was chosen to avoid olive oil solidification at temperatures lower than 5 °C (Piscopo and Poiana, 2012).

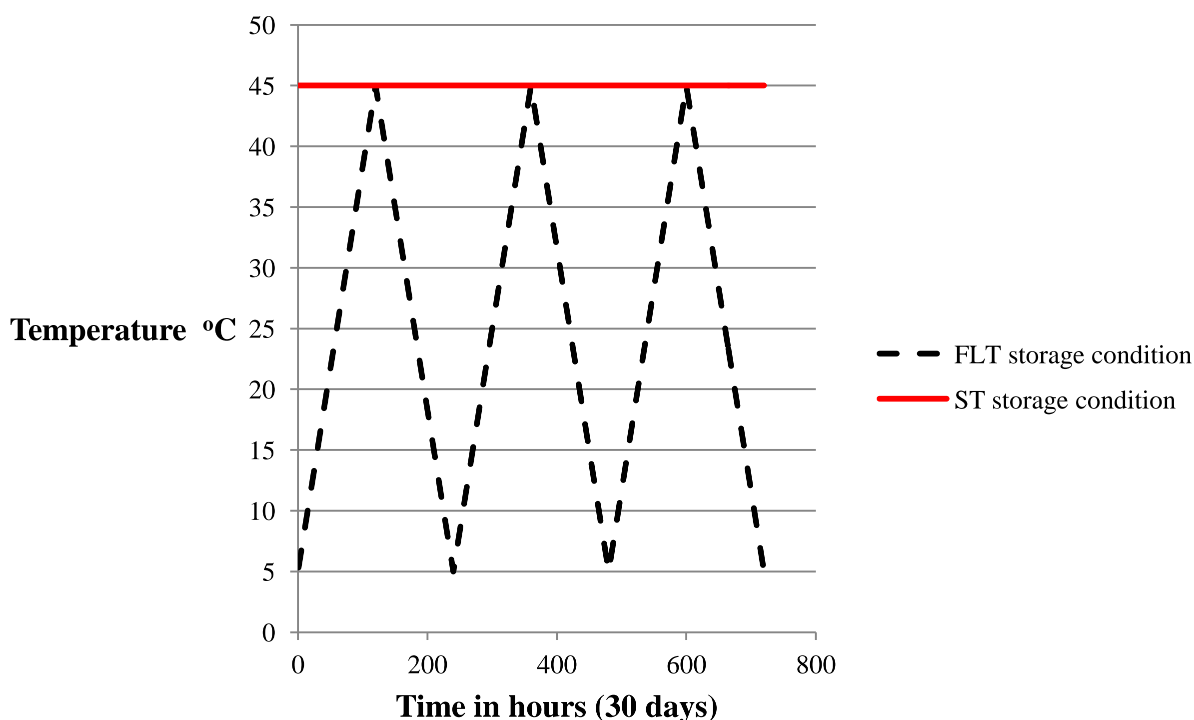

Fig. 4. Temperature profile for the ST and FLT storage conditions. Duration: 720 hours (30 days), highest temperature: 45 °C, lowest temperature: 5 °C.

6.3.3.3 Analysis plan

Each sample was analyzed before simulation. Two bottles of each sample were analyzed under accelerated storage conditions (constant temperature coded as ST or fluctuating temperature coded as FLT). Each of the two bottles contained samples from the same batch was used for chemical and sensory evaluation every 10 d. Chemical analyses were performed in triplicates for each type of sample at each respective time of analysis.

6.3.3.3.1 Free fatty acids

FFA was determined and calculated according to the official method described in EEC Reg. 2568/91. The protocol for determination was outlined in Section **6.1.3.3.1**.

6.3.3.3.2 Peroxide value

PV was determined and calculated according to the official method described in EEC Reg. 2568/91. The protocol for determination of PV was presented in Section **6.1.3.3.2**.

6.3.3.3.3 Extinction coefficient (K_{270})

UV absorption (K_{270}) was analyzed according to the official methods described in EEC Reg. 2568/91. The analysis protocol was summarized in Section **6.1.3.3.3**.

6.3.3.3.4 ortho-Diphenol compounds

ortho-Diphenol compounds were determined using the method reported by Pirisi et al. (2000), as presented in Section **6.1.3.3.5.**

6.3.3.3.5 Determination of diglycerides

The protocol for DGs analysis was illustrated in Section **6.1.3.3.12**.

6.3.3.3.6 Sensory analysis

Sensory analysis (panel test) was performed according to the EC Reg. 640/2008 by a trained group of eight expert tasters of the Department (DISTAL) of the University of Bologna. The brief of the analysis protocol was described in the Introduction section (paragraph 5.6).

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6.3.3.3.7 Statistical analysis

The software XLSTAT 7.4.2 version (Addinsoft, USA) was used for analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) to elaborate data.

6.3.4 Summary of results and main findings

Focusing on the EVOO sample under the experimental conditions. The results after 30 d (720 h) of accelerated storage simulation under both temperature conditions (static and fluctuation) showed that free acidity value significantly increased in the stored EVOO samples compared with their time-zero values (Table 7). Nevertheless, FFA did not significantly vary between similar samples stored under both conditions. Hydrolytic degradation was also evident in the results of DGs isomerization. 1,2/1,3-DG ratio showed sharp and significant decrease at the end of storage but did not vary between samples stored under fluctuating and static temperatures; the resultant ratio (Table 7) was also lower than 2. Furthermore, oxidation stability indices (Table 7) did not differ in EVOO samples stored under both temperatures conditions.

A sharp and significant decrease in *ortho*-diphenols, which are strongest antioxidants in EVOO (Gomez- Caravaca. 2007), was detected after 30 d of storage under both conditions (ST and FLT) (Table 7). Nevertheless, *ortho*-diphenols did not significantly differ in samples stored

under static and fluctuating temperatures. Sensory results (**Paper 3**, Fig. 4) further showed that static temperature resulted in a clear sensory defect of rancidity in the stored EVOO samples. However, samples stored under fluctuating temperature only exhibited decreased sensory score in fruity attributes.

Table 7. Free fatty acids (FFA) (g oleic acid per 100 g oil), 1,2/1,3-DG ratio, peroxide value (PV) meq O_2 per kg oil, *ortho*-diphenols content (mg gallic acid per kg oil) and Extinction coefficient K_{270} (± standard deviation) of the EVOO samples subjected to a static temperature 45 ^oC (ST), and fluctuated temperature (0-45 ^oC, each 10 days) (FLT).

**Different letters (A-C) indicate statistical significant differences between 0 and 30 days of accelerated storage for the same condition; letters (X-Y) indicate significant differences among the tow accelerated storage conditions (ST and FLT) related to the same storage time.*

		EVOO	
Quality parameters	**Storage time**	**ST**	**FLT**
FFA	0	0.37 ± 0.03 C	0.37 ± 0.03 C
	10	0.51 ± 0.02 B,X	0.53 ± 0.01 B,X
	20	0.61 ± 0.01 A,X	0.61 ± 0.00 A,X
	30	0.61 ± 0.00 A,X	0.61 ± 0.01 A,X
1,2/1,3-DG ratio	0	4.92 ± 0.09 A	4.92 ± 0.09 A
	10	1.08 ± 0.10 B,Y	1.71 ± 0.02 B,X
	20	0.72 ± 0.03 C,Y	1.56 ± 0.01 BC,X
	30	0.59 ± 0.01 C,Y	1.40 ± 0.20 C,X
PV	0	9. 6± 0.5 AB	9.6 ± 0.5 AB
	10	9.2 ± 0.8 B,X	9.1 ± 1.0 B,X
	20	10.9 ± 1.1 A,X	10.8 ± 0.3 A,X
	30	8.1 ± 0.1 B,X	9.3 ± 0.9 AB,X
K_{270}	0	0.17 ± 0.00 B	0.17 ± 0.00 B
	10	0.19 ± 0.00 B,X	0.18 ± 0.00 B,X
	20	0.20 ± 0.01 A,X	0.18 ± 0.00 B,Y
	30	0.20 ± 0.01 A,X	0.20 ± 0.00 A,X
ortho-diphenols	0	200 ± 7 A	200 ± 7 A
	10	102 ± 6 B,X	149 ± 8 B,X
	20	88 ± 3 B,X	81 ± 4 C,X
	30	60 ± 1 C,X	78± 2 C,X

6.3.5 Conclusions

A high temperature (45 °C) for a short period (approximately 18 h) affected the stored samples under fluctuating temperature during the 30-d simulation. The following conclusions were established. (1) The effects of temperature fluctuation on the quality of the stored VOO may be similar to the effects of elevated static temperature, particularly with regard to FFA, PV, K_{270}, and *ortho*-diphenols. (2) The EVOO sample was declassed to the virgin category on the basis of the sensory evaluation after simulation under static temperature. (3) DGs exhibited low isomerization under fluctuating temperature. Nevertheless, 1,2/1,3-DG ratio was also lower than 2, indicating that deteriorating EVOO freshness was approximately similar under both conditions. Temperature fluctuation produced minimal stress at high temperatures (for approximately 18 h during 30 d of simulation) respect with static experiment (720 h). These findings revealed that fluctuation in temperature have drastic effects on the quality of stored VOO as evident for static high storage temperature.

6.4 Experimental Section 4: Evaluation of the quality of VOO subjected to simulated shipments from Italy to different destinations

6.4.1 Background

To determine whether the quality of EVOO at final destination after shipping is similar to that in the country of origin has gained increasing attention. Protecting EVOO quality during cargo, shipment, or transportation and at final destination is an important consideration in the VOO industry, involving producers up to retailers. The long distance between producing and importing countries may cause quality degradation of the product. However, conducting quality assessment for EVOO during real shipment may be limited by different logistic reasons. These limitations could be resolved by performing simulated shipments to generate environmental stresses, which affect actual journeys, for assessing the quality of products at final destination. In the present work, two specific shipments were performed using two separate containers, namely, with and without thermal insulation. Bottled EVOOs were placed in the containers and shipped (simulated) to Los Angeles (USA) and Quebec (Canada).

6.4.2 Objectives of the study

This study **(Papers 5 and 6)** aimed to (1) determine quality changes and environmental effects caused by worldwide distribution of shipped EVOO in different containers and (2) assess the efficiency of using insulated containers during EVOO shipment affected by temperature variation. The purpose of the study was achieved by performing a simulated shipment from Italy to two different destinations, namely, Los Angeles (USA) and Quebec (Canada).

6.4.3 Materials and methods

6.4.3.1 Olive oil samples

Two simulated shipments were conducted using two glass bottles (1 l) of commercial EVOO. The samples were virtually shipped to two different destinations (Los Angeles and Quebec).

Destination 1: International shipment from Bologna (Italy) to Quebec (Canada). Simulation started on January 30, 2012 from the port of origin (Livorno) and terminated on March 1, 2012 at the port of final destination (Quebec) **(Papers 5 and 6)**.

Destination 2: International shipment from Bologna (Italy) to Los Angeles (USA). Simulation started on June 26, 2012 from the port of origin (Livorno) and terminated on August 2, 2012 at the port of final destination **(Paper 6)**.

6.4.3.2 Simulation

This study simulated the temperature conditions monitored during different logistic phases of shipment (handling, shipping, and final delivery) of commercial EVOO from Italy to Quebec and Los Angeles. Actual shipments were reproduced with closed-loop climate-controlled chambers. A set of samples (two EVOO bottles) was placed in the standard and thermally insulated containers. These containers were sequentially placed in climate-controlled chambers to reproduce and monitor temperature profiles during actual shipments. The temperature sensors inside the chambers can detect and record temperatures ranging from −20 °C to 65 °C. The integrated cooling system consists of an evaporator with 21 g of R600a ISO-butane as a refrigerant. A closed-loop algorithm was developed with LabView national instrument software to control actuators to reach specified chamber temperatures. The temperature profile of the international simulated shipment (Q) from Italy to Quebec (Canada) ranged from −12 °C to 20 °C during different logistic phases of simulated shipment (Fig. 5). In addition, the profile of the simulated shipment (LA) from Italy to Los Angeles ranged from 10 °C to 60 °C (Fig. 6).

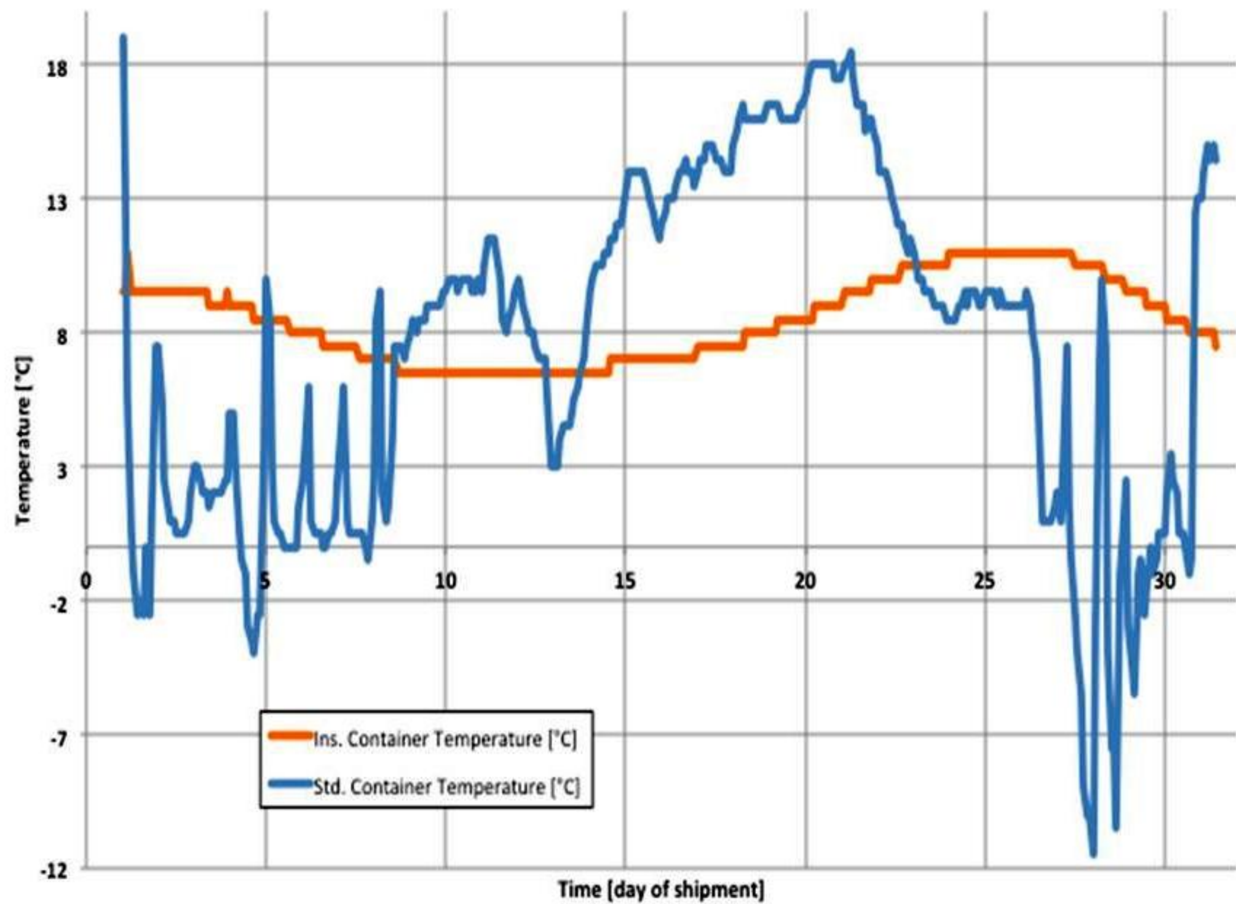

Fig. 5. Temperature profile monitored by using data loggers for Quebec simulation. Orange line: the temperature profile inside thermal insulated container. Treatments: handling, shipment, final delivery. Duration: one month, highest temperature: 11 ^{o}C, lowest temperature: 6.5 ^{o}C. Blue line: the temperature profile inside standard container. Treatments: handling, shipment, final delivery. Duration: one month, highest temperature: 19 ^{o}C, lowest temperature: -11.5 ^{o}C.

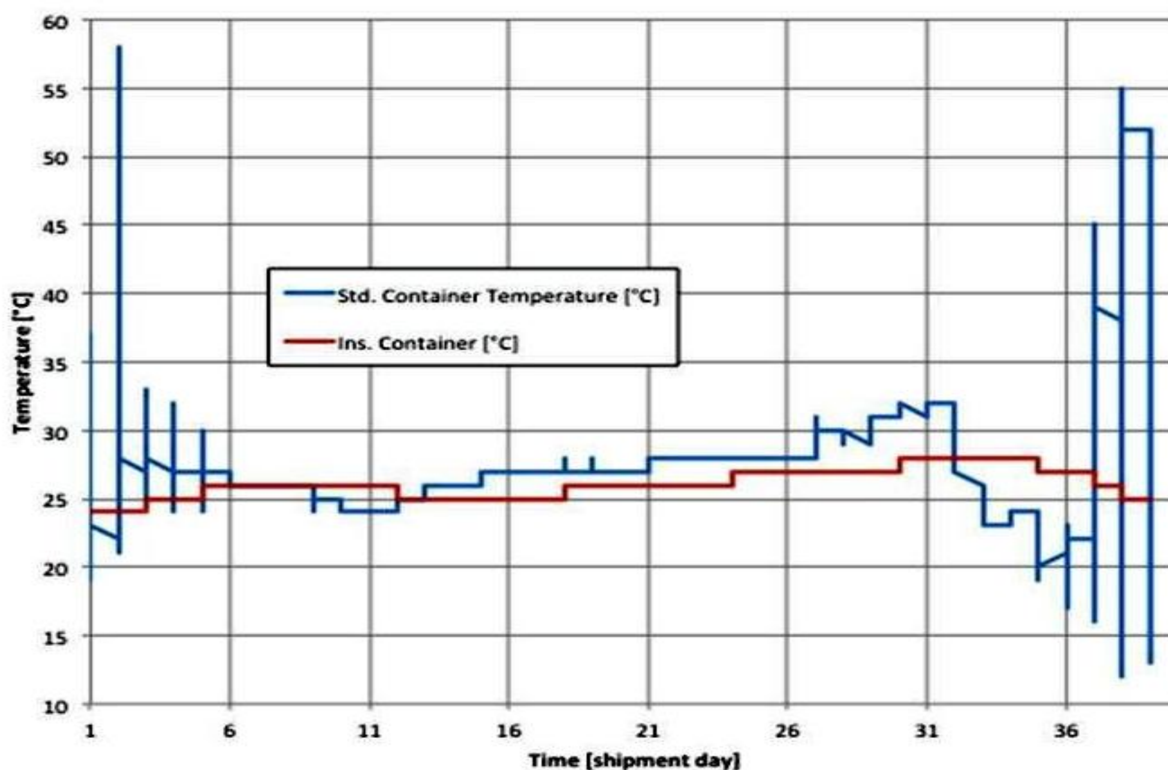

Fig. 6. Temperature profile monitored by using data loggers for Los Angeles simulation. Blue line : the temperature profile inside standard container. Treatments: handling, shipment, final delivery. Duration: 39 days, highest temperature: 58 ^{o}C, lowest temperature: 12 ^{o}C. Red line: the temperature profile inside thermal insulated container Treatments: handling, shipment, final delivery. Duration: 39 days, highest temperature: 27 ^{o}C, lowest temperature: 24 ^{o}C.

6.4.3.3 Analysis Plan

The corresponding samples were initially analyzed before shipment (designated as time zero samples) and after simulation inside standard (thermally unprotected) and thermally insulated containers (Accorsi et al. 2014). For each destination, two sample bottles from each container were used for chemical, physical, and sensory evaluation. Chemical and physical analyses of each sample were performed in triplicates at time zero and after simulation.

6.4.3.3.1 Free fatty acids

FFA was determined and calculated according to the official method described in EEC Reg. 2568/91: details in Section **6.1.3.3.1**

6.4.3.3.2 Peroxide Value

PV was determined and calculated according to the official method described in EEC Reg. 2568/91**:** details in Section **6.1.3.3.2.**

6.4.3.3.3 Total phenolic compounds determination

Phenolic compounds were extracted according to the method of Pirisi et al. (2000). Absorbance was determined at 750 nm by using a UV−vis 6705 spectrophotometer (Jenway, United Kingdom) through the method reported by Singleton and Rossi (1965). Sample (2 g) was dissolved in 1 ml of *n*-hexane and extracted three times with 2 ml of methanol–water solution (60:40 v/v). In each extraction, the mixture was shaken with a vortex mixer for 1 min and then centrifuged for 5 min at 3,000 rpm. The aqueous phase was collected and transferred into another test tube after each centrifugation cycle. *n*-Hexane (2 ml) was added to the collected phenolic extract, mixed on the vortex, and then centrifuged for 5 min at 3,000 rpm. After the *n*-hexane phase was removed, the extract was evaporated using a rotary evaporator at 35 °C. The residue was dissolved with 5 ml of methanol–water solution (50:50 v/v). Absorption was determined with a spectrophotometer, and a standard calibration curve was prepared using different concentrations of gallic acid. The results were calculated and expressed as milligram of gallic acid per kilogram of oil.

6.4.3.3.4 Thiobarbituric acid reactants (TBARs) content

TBARs content was determined in triplicates according to the AOCS Official Method Cd 19-90 (2006) and expressed as TBA value (milligram of malonaldehyde equivalent per kilogram of oil). Oil sample (50–200 mg) was weighed into 25 ml volumetric flask and dissolved with a small portion of 1-butanol. The solution volume was filled using 1-butanol. A portion (5 ml) of the sample dissolved in 1-butanol was transferred into a screw-capped test tube. The reagent solution (200 mg of 2-thiobarbituric acid dissolved in 100 ml of 1-butanol) was added, and the mixture was thoroughly mixed. The tubes were then placed in a water bath at 95 °C for 2 h. After cooling at room temperature, absorbance was determined at 530 nm by using 1 ml glass cuvettes with a UV-vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). The reagent blank was prepared simultaneous to sample preparation. TBA value was obtained using the following equation:

TBA = 50 × (absorbance of the sample−absorbance of the blank)/weight of the sample (mg)

6.4.3.3.5 Color coordinates (L, a*, and b*)*

EVOO sample was placed in a quartz dish and analyzed using a ColorFlex instrument with CIELab color analyzer (Hunterlab, Reston, VA, USA). The samples were analyzed in triplicates without dilution by using the method reported by Gómez–Caravaca et al. (2007). The results were expressed as *L**, *a**, and *b** chromatic coordinates. *L** ranges from 0 to 100 and represents brightness; *a** ranges from −120 to 120 and represents redness; and *b** ranges from −120 to 120 and represents yellowness.

6.4.3.3.6 Sensory analysis

Sensory analysis (panel test) was performed according to the EC Reg. 640/2008 by a trained group of eight expert tasters of the Department (DISTAL) of the University of Bologna. The brief of the analysis protocol was described in the Introduction section (paragraph 5.6).

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6.4.3.3.7 Statistical analysis

The software XLSTAT 7.4.2 version (Addinsoft, USA) was used for analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) to elaborate data.

6.4.4 Summary of results and main findings

In this study, shipment of EVOO to two different destinations was simulated. Each simulation was characterized by different environmental conditions. The first shipment involved a low temperature profile ranging from −12 °C to 18 °C (**Papers 5 and 6**), whereas the other shipment simulated a condition with high temperatures (10 °C to 60 °C) during different shipping stages. In both simulations, the basic quality parameters (FFA and PV) of EVOO significantly increased after shipment compared with those at time zero, particularly in samples shipped in standard containers (Table 8).

Table 8. FFA, Free Fatty Acids (g oleic acid per 100 g oil); PV, peroxide value (meq O_2 per kg oil); TBARs, thiobarbituric acid reactive substances value (mg of malonaldehyde equivalent per kg oil); TP, total phenols (mg gallic acid per kg oil), tested before simulation (time zero) and after simulation in insulated container and in standard container for EVOO samples to final destinations (EVOO Q, Quebec and EVOO LA, Los Angeles).

**Values (mean ± standard deviation) with different superscript capital letters in a column and for each sample are significantly different within different kinds of experimental conditions, at 0.05 level of significativity (Fisher test).*

Sample	Experimental status	FFA (G oleic acid 100 g^{-1})	PV (meq O_2 per kg)	TBARs (mg of malonaldehyde equivalent per kg)	TP (mg gallic acid per kg)
EVOO Q	**Time zero**	$0.52^{B} \pm 0.04$	$11.7^{C} \pm 0.7$	$0.013^{B} \pm 0.001$	$353^{B} \pm 35$
	Insulated container	$0.59^{A} \pm 0.01$	$13.1^{B} \pm 0.3$	$0.012^{B} \pm 0.001$	$372^{A} \pm 54$
	Standard container	$0.60^{A} \pm 0.01$	$17.0^{A} \pm 0.8$	$0.016^{A} \pm 0.001$	$478^{A} \pm 43$
EVOO LA	**Time zero**	$0.45^{B} \pm 0.01$	$8.8^{C} \pm 0.2$	$0.015^{C} \pm 0.001$	$259^{A} \pm 2$
	Insulated container	$0.45^{B} \pm 0.01$	$9.2^{B} \pm 0.1$	$0.028^{B} \pm 0.001$	$257^{A} \pm 8$
	Standard container	$0.48^{A} \pm 0.01$	$10.4^{A} \pm 0.1$	$0.040^{A} \pm 0.001$	$222^{B} \pm 3$

Nonetheless, both parameters remained within the accepted limits for EVOO according to the EU regulations (EU Reg. 1348/2013). Oxidative degradation was evident in the significant increase in TBARs values (Table 8) at the end of simulation toward both destinations. The total polar phenols also decreased after both simulations compared with those at time zero, particularly in samples shipped in standard containers (Table 8). The color of the EVOO samples changed after both simulations (Table 9).

Table 9. Color coordinates (L*, a*, b*) tested before simulation (time zero) and after simulation in an insulated container and in a standard container, for EVOO samples simulated to the two different destinations (EVOO Q, Quebec and EVOO LA, Los Angeles).

**Values (mean ± standard deviation) with different superscript capital letters in a column and for each sample are significantly different within different kinds of stresses, at 0.05 level of significativity (Fisher test).*

Samples	Experimental status	L*	a*	b*
EVOO Q	**Time Zero**	$54^{B} \pm 0.1$	$4.9^{A} \pm 0.0$	$80^{B} \pm 0.0$
	Insulated container	$55^{A} \pm 0.1$	$4.8^{B} \pm 0.0$	$84^{A} \pm 0.0$
	Standard container	$55^{A} \pm 0.1$	$4.6^{C} \pm 0.0$	$84^{A} \pm 0.0$
EVOO LA	**Time Zero**	$63^{A} \pm 0.0$	$4.3^{B} \pm 0.1$	$89^{A} \pm 0$
	Insulated container	$50^{B} \pm 1.4$	$6.8^{A} \pm 0.2$	$71^{C} \pm 1$
	Standard container	$52^{B} \pm 1.5$	$6.5^{A} \pm 0.2$	$79^{B} \pm 2$

The efficiencies of different containers throughout both simulations are demonstrated in Table 9.

Table 10. FFA, Free Fatty Acids (g oleic acid per 100 g oil), peroxide value (PV, meq of active oxygen per kg oil), thiobarbituric acid reactant substances content (TBARs, mg of malonaldehyde eq per kg oil) and total amount of phenolic compound (TP, mg gallic acid per kg oil), analyzed for the commercial extra virgin olive oils (EVOO Q and EVOO LA) samples. 1 means that the insulated container, significantly better performs than the standard container solution in terms of the selected quality parameter, 0 means no difference between both the containers. To establish such differences, analysis of variance (ANOVA) is performed at a 95% confidence level (Fisher LSD, $p < 0.05$).

**Three replications per sample were performed for each determination.*

	Quality parameters			
Samples	**FFA**	**PV**	**TBARs**	**TP**
EVOO Q	0	1	1	0
EVOO LA	1	1	1	1

During shipment from Italy to Los Angeles, samples transported inside insulated containers exhibited better quality in terms of FFA and oxidative stability indices at the target destination than samples shipped in standard containers. These results revealed the protective role of thermally insulated containers against temperature elevation, particularly at delivery stage. The

increase in temperature accelerated the oxidation reaction and the consumption of phenolic antioxidants (Bendini et al. 2007; Frankel., 1991). A similar trend for oxidation degradation indices was also obtained using thermally insulated container during shipment to Quebec. In this shipment, EVOOs were exposed to very low or freezing temperatures during the handling stage. The decrease in temperature could affect the properties of phenolic compounds, thereby reducing the oxidative stability of EVOO (Bendini et al. 2009a) (Fig. 5). The use of standard and thermally insulated containers for EVOO shipment to Quebec also showed similar performance in terms of FFA and TP quality parameters (Table 10).

6.4.5 Conclusions

The obtained results emphasized the efficiency of using thermally insulated containers during shipment of EVOO even at very low surrounding temperatures.

General conclusions

The effect of different stress conditions on VOO quality was investigated in this PhD study. This consecutive and complementary research was performed by subjecting VOOs of different categories and other edible oils (Experimental Section 5) to different chemical and sensory analyses. This study provides comprehensive information regarding the correlations between different quality components of olive oil and stress conditions. Identifying treatments crucial for determination of VOO quality and good practices in instrumental, sensory, and statistical analyses has been obtained. Many scientific manuscripts were written, submitted, and even published on the basis of the different case studies illustrated in this PhD thesis. Nevertheless, the case studies discussed in this work focused on different conditions that may potentially affect VOO quality after production and bottling. These cumulative studies provide useful information to predict protective action for sustaining high-quality VOO during shipment and storage.

In the first case study (Experimental Section 1), the effects of different filtration techniques and storage conditions on the quality of VOO were evaluated. Filtration and clarification may improve the quality of virgin olive oil during storage. The low water content of the filtered and clarified VOO may result in less occurrence of the hydrolytic reactions. These techniques can also preserve the sensory properties and the phenolic and volatile components in the filtered VOO compared with those in the unfiltered or unclarified stored VOO. Clarification may be further beneficial respect with commercial filtration, in which the clarified VOO contains high amounts of pleasant volatiles and low water content with slightly developed oxidized volatiles after storage. This study emphasizes the importance of maintaining the unfiltered, filtered, and clarified VOOs from light to preserve their quality.

The effect of storage time, treatments, and storage conditions on the isomerization of DGs was also evaluated (Experimental Section 2). In this investigation, isomerization and accumulation of 1,3-DGs isomer, an indicator of the freshness of VOO, are affected by the initial quality of VOO, storage time, and storage temperatures. This study shows that the effects of storage lightening conditions (Experimental Section 2) and filtration/ clarification treatments (Experimental Section 1) on isomerization are negligible.

The study in Section 3 focused on the effects of temperature fluctuation during storage on VOO quality. The findings reveal that fluctuating temperatures may adversely affect VOO quality, particularly EVOOs.

Another investigation was conducted regarding the quality changes occurring to VOO and other edible oils during shipment (Experimental Section 4). In this section, quality loss in transported VOO was investigated. The results reveal that edible oils must be shipped inside thermally insulated containers to reduce quality loss.

The scientific knowledge on the interactions of packaging materials, particularly high-density polyethylene (still widely used in Palestine), must be elucidated with regard to olive oil quality. The correlation between different groups of microorganisms and the quality of VOOs, particularly sensory properties, must also be assessed. Furthermore, whether filtration or clarification can decrease microbial load or eliminate harmful microorganisms must be investigated in future research.

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Ziad I.M Ayyad

Papers

I declare that any published manuscript attached to this PhD thesis is permitted to be used by the respective journal auditor.

Ziad Ayyad

Paper 1

Influence of filtration or clarification systems on quality of stored extra virgin olive oil

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Influence of filtration or clarification systems on quality of stored extra virgin olive oil

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Abstract:

Filtration of extra virgin olive oil (EVOO) is a pre-treatment generally adopted before bottling in order to facilitate the removal of suspended particles and decrease the moisture permitting the keeping quality and organoleptic properties during storage. In the current study, aliquots of EVOO were subjected to a filtration by filter press and a clarification by inert gas (nitrogen and argon) processes and were store in glass bottles for one year. Basic quality indexes, diglyceride isomerization, phenolic and volatile profiles, as well as sensory characteristics of filtered and clarified samples respect to unfiltered EVOO were determined within intervals of four months. The main results showed that at the end of storage, significantly higher concentrations of C_5 and C_6 LOX volatiles and phenolic compounds in particular secoiridoid derivatives remained in filtered and clarified samples significantly lower amount of water especially for gas clarified sample respect to unfiltered sample. Higher sensory scores were maintained in the filtered and clarified samples respect to unfiltered sample. The filtration and clarification showed advantages in term of maintaining higher amount of phenols, flavorful olive oil, and less susceptible to hydrolytic and organoleptic degradation during EVOO storage.

Key words: *extra virgin olive oil, filtration, clarification, water amount, volatiles, phenolic profile, dark*

Introduction:

It is well known that about 98% of EVOO composition is triglycerides while the other 2% include free fatty acids, squalene, sterols, phospholipids, phenolic compounds, volatile compounds as minor components (Boskou, 2006). Some of these minor components and the high content of monounsaturated fatty acids play a major role in keeping EVOO more stable against oxidation during storage (Bendini et al. 2009a). The main factors that can affect the shelf life of olive oil during storage are the availability of oxygen, elevated temperature and the action of light (Garca et al. 2003). These factors can favourite the oxidative decomposition of l triglyceride fraction and initially forming peroxide compounds that evolve into secondary oxidation products. Just produces virgin olive oil is the turbid extract that can be consumed without any further treatment like refining process. The remained suspended solid and vegetative water after extraction can facilitate the deterioration of EVOO due to their impacts on hydrolysis and oxidation reactions (Bubola et al. 2012). Filtration is a process allowed by European community regulation (EEC. Reg 1638/98) as pre-treatment before bottling to enhance the quality and appearance of virgin olive oil during storage (Lozano-Sanchez et al. 2012) thanks to the reduction of the negative effects of these suspended/emulsified compounds. Filtration removes some materials such as phospholipids and humidity that could make extra virgin olive oil cloudy during storage (Spyros et al. 2004). It is assumed that filtration enhances olive oil stability by decreasing the water content, thus reduces the hydrolysis rate of triglyceride to liberate free fatty acids (Fregapane et al. 2006). The effects of filtration on the quality of EVOO have been addressed by different authors. Brenes et al (2001) studied the effect of storage on the hydrolysis rate of complex phenols in filtered and unfiltered olive oils, finding that, in addition to the reduction of water content after filtration, the oxidation rate was higher in unfiltered olive oil than in filtered one during storage. Moreover, the hydrolysis rate of secoiridoid phenolic compound during storage was more pronounced in unfiltered oil. Likewise, Fregapane et al. (2006) studied the effect of filtration using filter paper on the stability of EVOO during storage. They concluded that, as a result of water reduction after filtration, the hydrolysis rate of triglyceride was lower in filtered oil than in the unfiltered one. In addition, the formation rate of simple phenolic compound (hydroxytyrosol; Hyty) during storage was higher in unfiltered olive oil than in the filtered one. Additionally, they showed that unfiltered EVOO developed sensory defects earlier than filtered EVOO during storage. On the other hand, they also showed that

filtered olive oil was more sensitive to oxidation than unfiltered EVOO. More sophisticated study about the effect of filtration on the phenolic compounds was performed by Gomez-Caravaca et al (2007); found that, water content and oxidative stability decreased significantly after filtration. Indeed, they showed that Hyty significantly decrease after filtration, while the other phenolic compounds seems to be increased after filtration. Recently, a new and innovative clarification technique has been developed by the University of Bologna and Sapio (Cerretani et al. 2009). This clarification system is based on inserting a flow of inert gases (nitrogen or argon) 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 solid and vegetative water (Bendini et al. 2013). Beside clarification, the advantages of this system over other kinds of filtration techniques, is that, the inert gas flow avoids the direct contact of organic materials or filtration aids with the virgin olive oil. Moreover, after clarification, the oil in the storage tanks remains under inert gas with little amount of oxygen, therefore the shelf life of oil could be extended (Lozano-Sanchez et al. 2010). The effect of different filtration/clarification systems such as filter bag and clarification systems using inert gases as a filtered aid (clarification with argon or nitrogen) on the quality of EVOO had been investigated by Lozano-Sanchez et al. (2012), who found that, water content was decreased in all treated samples among which, argon gas had the lowest value. They also showed that total phenolic compounds were increased by all adopted treatment systems and the significant increase was found after clarification with argon. In addition, the oxidative stability of filtered and clarified samples was lower than that in unfiltered oil. Regarding sensory attributes, they evidenced that, the fruity attributes and pungency were enhanced after filtration, especially when nitrogen and argon gas was used as filter aids. On 2013, Bendini and co authors showed that there were no pronounced differences between clarified and non clarified samples in term of basic quality parameters and total polar phenols. They also showed that, the clarified samples had lower water content than the unfiltered one. In addition, the clarified samples by inert gases were richer in lipoxygenase pathway (LOX) volatiles, fruity perception and contained fewer defects than cloudy EVOO. To the best of our knowledge, there is no intensive study on the effect of commercial filtration and clarification systems on the chemical and sensorial properties of virgin olive oil during prolong storage. The aim of this research work was to investigate the influence of commercial filtration system by using food grade plastic fibers and the new

clarification systems, (using a flow of nitrogen or argon gases) on the chemical quality parameters and sensory perception of EVOO during one year storage in glass bottles in dark. In order to achieve the purpose of this study, unfiltered, filtered and clarified EVOO were characterized for their oxidative and hydrolytic status, sensorial quality, water content, phenolic and volatile profiles. This analytical plan was performed at defined time intervals (after 4, 8 and 12 months) after subjecting a just produced EVOO to the previously mentioned different treatments and stress conditions.

Methodology

Samples:

Sabina DOP EVOO of Canino cultivar from Lazio region was extracted in October 2012. The oil was divided into 4 parts: an aliquot was filtered through a commercial system filter press (1.8 bars) to produce filtered EVOO sample (Cf). Aliquots of cloudy EVOO were clarified by injecting inert gases, nitrogen or argon directly into the center of the EVOO mass by using a pilot clarification system developed and patented by the University of Bologna and Sapio (Cerretani et al. 2009). In this system, the nitrogen gas was directly injected into the veiled EVOO bulk mass (P =2 bars) to produce nitrogen clarified EVOO (Nc). Another part of veiled EVOO was clarified using argon gas (12 L min^{-1}) to produce argon clarified EVOO (Ac). The rest was remained as unfiltered (Uf). Filtration and clarification treatments were performed at room temperature.

Storage simulation

All EVOO samples were bottled in 250 ml hermetically sealed glass bottles (with 4% head space) directly after production and filtration/ clarification treatments, then stored inside a storage room in the dark (the bottles were covered with aluminum foil). Storage duration was started from 1st of January 2013 and ended in 31 of December 2013. The temperature range during the one year storage period was 17- 22 °C from January to the end of May, 30- 36 °C from June to the end of August and around 20 to 25 °C from the first of September to the end of the storage period.

Samples were evaluated at time zero and after 4, 8 and 12 months of storage in the dark, for their chemical and the sensory properties. Three bottles of each kind of sample were removed from the storage room at each respective time of analysis and analyzed in triplicate (each replicate was

obtained from a separate bottle and the samples were collected from the geometrical center of each bottle).

Chemical analysis

Stored samples were analyzed for their: free acidity (FA) expressed as g oleic acid per 100 g of oil, peroxide value (PV) expressed as milli-equivalent O_2 kg^{-1} oil and UV absorption (K_{232}, K_{270}) 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 air oven technique (ISO 662:1988) and expressed as mg Kg oil^{-1}. Diglycerides (DGs) were determined by using GC-FID (Carlo Erba MFC500 with an Rtx-65TG,≈ Restek, Bellefonte, PA) according to a modified version of the method suggested by Serani et al. (2001). Identification of DGs was carried out by comparing the peaks retention time and the GC traces with those of the DGs standards and chromatograms reported in the literature (Serani et al. 2001; Bendini et al. 2009b). DGs chromatogram were quantified with respect to dilaurin that added as internal standard (0,5 ml of a solution 2 mg mL^{-1} of dilaurin dissolved in chloroform, added to 100 mg of oil). The displayed results were only the 1,2/1,3-DG ratio.

Volatile compounds were evaluated by SPME-GC/MSD (Agilent 6890N, Santa Clara, CA, USA)) coupled with quadrupolar mass selective spectrometry (Agilent 5973 N, Agilent Technologies), according to Cerretani et al. (2008). Volatile compounds identification was carried out using mass spectrometry by a comparison of their mass spectral data with the information from the NIST library (2005 version) and MS literature data. Volatile compounds were expressed as mg of internal standard (4-methyl-2- pentanone Fluka, Buchs, Switzerland) per kg of oil.

Extraction of phenolic compounds

Polar phenolic compounds were extracted from EVOO samples following the procedure described by (Pirisi et al. 2000) and further modified by (Rotondi et al. 2004) using liquid- liquid extraction method. EVOO sample (4 g) was dissolved by 4 ml methanol/water solution (60:40, v/v) and 2 ml of *n*-hexane in 20 ml centrifuge tube. The mixture was homogenized for 1 minute using vortex, and then centrifuged at 3000 rpm for 5 min. The methanol water phase was removed then, the *n*-hexane phase was extracted two times more with methanol/ water 60:40, v/v) solution. The companied polar fractions were washed with 4 ml *n*-hexane to remove the oil phase. The solvent was then evaporated using a rotary evaporator at 35 °C.

After evaporation, the dried residue was dissolved in 3 ml of methanol/water (50:50, v/v), filtered through 0.2 µm syringe filter (Whatman Inc) then, the phenolic extracts were stored at -18 °C until used.

Determination the phenolic compounds by HPLC

The chromatography 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 C18 100A Kinetex column (2.6 µm, 100 x 3.00 mm I.D, Phenomenex, Torrance, CA, USA). Gradient elution was carried out with a solvent system of water/formic acid (100:0.5 v/v) as mobile phase A and acetonitrile as mobile phase B; the total run-time was 13 minutes and 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 column was thermostated at 30 °C and equilibrated for 5 min prior to each analysis. An injection volume of 2.5 µL and a flow rate of 0.7 ml min^{-1} were used. The chromatograms were monitored at wavelengths: 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 main phenolic compounds were identified by comparison with the relative retention times of reference standards, whereas for the other compounds (where the reference compound was not available), identification was performed by an ion trap mass spectrometer (Agilent) in electro spray ionization mode. 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 within the *m/z* 100–900 range in the negative and positive ion mode.

Sensory analysis

The sensory analysis (COI-Panel Test) of all the EVOO samples was performed according to the EU Reg. 1348/2013), by a fully trained group of 8 expert tasters (DiSTAL, University of Bologna).

Statistical analysis

The software XLSTAT 7.5.2 version (Addinsoft, USA) was used to elaborate the data by analysis of variance (ANOVA, Fisher LSD, $p < 0.05$).

Results and discussions:

Changes in basic quality parameters

Basic quality parameters were set in order to estimate the changes in hydrolytic and oxidation quality of EVOO samples after filtration or clarification and during the storage period of 12 months. Such parameters are; free acidity (FA) which was measured to investigate the hydrolysis process of triglycerides. The increase in acidity probably increases the susceptibility to oxidation and degradation of the complex phenolic compounds (Lozano-Sanchez et al. 2010). Peroxide value (PV) and the extinction coefficients (K_{232}, K_{270}) which were used to evaluate the oxidation status of the stored EVOO samples. FA results (Table 1) demonstrated that, after 12 months of storage, filtered and clarified EVOO samples showed a slight increase in FA contents where the argon clarified sample contained the lowest amount. However, the unfiltered EVOO sample showed slight, but significantly higher value in terms of free acidity than the filtered and clarified samples. This behavior could be attributed to the higher water content of the unfiltered sample (Fregapane et al. 2006), in addition to the presence of lipase and other hydrolytic enzymes in the suspended materials (Brenes et al. 2001; Shimizu et al. 2008) which favoured the degradation process in triglycerides. These results were in agreement with previous literature (Fregapane et al. 2006; Stefanoudaki et al. 2010; Bendini et al. 2013). Peroxide value (Table 1) showed relative stability during the storage of EVOO samples, on the other hand, K_{232} and K_{270} (Table 1) showed a significant increase, in particular, after 8 months of storage, for all the stored samples. Comparing all stored samples in term of oxidation stability parameters, it was found that differences in PV and K_{232} after 12 months storage in darkness was not distinguishable or not significant. On the contrary, K_{270} was significantly higher in filtered and nitrogen clarified sample respect to argon clarified sample and unfiltered one. These results were in agreement with previous work (Fregapane et al. 2006). In addition, these results could be explained by the decrease in the efficiency of phenolic compounds as antioxidants after filtration and clarification process (Bendini et al. 2007). Moreover, decreasing water content to a certain value as in the case of argon clarified sample could be beneficial in maintaining the oxidative stability of

EVOO. However, all stored samples remain within the limits of EU regulations (EU Reg 1348/2013) at the end of storage period.

Changes in water content

Water content in EVOO range in between 0.03 to 0.2%, depending on the production and filtration processes (Ragni et al. 2012). It was assumed that the presence of water in the virgin olive oil is responsible for the persistence of dispersed and suspended materials which reduce the consumer attractiveness of virgin olive oil (Lercker et al. 1994). Moreover, water may induce degradation of minor compounds during the storage and contribute to the perception of flavour defects, in particular vinegary perception (Dais, 2013). As shown in Table 1, water content of EVOO samples was significantly reduced after subjecting veiled EVOO to filtration and clarification processes, as expected, in the following order: (Uf > Cf > Ac > Nc sample). During the storage period of 12 months, water content continued decreasing gradually, probably as a result of normal settling of suspended materials, including water where the samples were collected from the geometrical center of each bottle at each respective time of analysis. In comparison between filtration and clarification treatments it was shown that clarification with inert gases was more efficient in decreasing the water content than commercial filtration system. At the end of storage time, water was below the limit of quantification in the clarified samples. These results were inconsistent with previous work (Caravaca et al. 2007; Fregapane et al. 2006; Lozano-Sanchez et al. 2010; Bendini et al. 2013).

Changes in the phenolic compounds.

Phenol compounds in particular the secoiridoid derivative and ortho diphenols such as Hyty are the main contributors in oxidative stability of the olive oil (Bendini et al. 2006). Among the secoiridoid derivatives, decarboxymethyl oleuropein aglycon (DOA) and oleuropein aglycon (OA) are the most active phenolic compounds as antioxidant against oxidation reactions. A special emphasis on the effect of filtration or clarification on Hyty, DOA, and OA behavior during storage, the obtained results (Table 2) showed that the initial amounts of simple phenols account about 3.5, 3.6, 4, 6.5 % of the total phenols determined by HPLC in Uf, Cf, Nc and Ac samples respectively. During storage, these compounds showed slight differences on commercial filter and argon clarified samples at the end of storage period respect to their initial values, while nitrogen clarified sample showed a slight significant increase in amount after 8 months of storage. On the other hand, Hyty showed a marked and significant increase in the unfiltered

sample, where the amount was about 5 times its initial concentration after 12 months of storage. This behavior at which Hyty increased in amount for unfiltered sample after 8 months, might be linked to the effect of the increase in the average temperature that was recorded in the summer season (34 ^{o}C) (Fregapane et al. 2006). The apparent and significant higher rate of Hyty formation in unfiltered sample respect to other stored samples could be linked to the water content in the unfiltered sample which partially maintained the hydrolysis enzymes activities (Bendini et al. 2009a). As depicted in (Table 2), the concentration of the main secoiridoid derivatives (DOA) decreased sharply after 4 months of storage, where the values showed about 23% and 37% loss for the Ac and Nc stored samples respectively, while about 46 % loss was observed for Cf sample. On the other hand, DOA content in Uf sample decreased by a half at the end of storage period of 12 months. OA compound was observed to be the most stable secoiridoid compound among the complex phenols during storage. These results were consistent with Brenes et al. (2001); Fregapane et al. (2006). The other secoiridoid derivative and EA decreased significantly during storage as a result of oxidation reactions in all stored samples. In comparison between filtered and clarified samples respect to the main secoiridoid compound (DOA), however, the amount of this compound remained significantly higher in inert gases clarified samples than Cf samples at the end of storage period while the Uf sample showed the lowest amount. The higher concentrations of DOA in filtered and clarified samples indicate that filtration and clarification could have a positive impact in term of slowing down the degradation of complex phenolic compounds.

Changes in 1,2/1,3-DG ratio

The content in DGs can be considered a good indicative freshness parameter during EVOO storage (Serani et al. 2001). The results in (Table 1) showed that 1,2/1,3-DG ratio underwent a sharp and significant drop after 4 months of storage in all samples, after which, the change was slight and not significant. Furthermore, there was no evidence that filtration or clarification could affect DGs isomerization, whereas the isomerization was affected mainly by the time of storage as previously shown by Ayyad et al. (2015).

Changes in LOX volatile compounds

Volatile compounds in EVOO are influenced mainly by various factors, including cultivars, fruit maturity, geographical region, processing and storage conditions (Angerosa et al. 2004). Volatile compounds that have responsibility for the positive aroma perception in the virgin olive oil are

mainly produced by the oxidation of unsaturated fatty acid throughout the lipoxygenase pathway (LOX) (Kalua et al. 2007). Positive perceptions coming from volatiles are attributed to aldehydes, esters, hydrocarbons, ketones and alcohols. Among the different categories, 6 carbons volatile compounds like hexanal, (*E*)-2-hexenal, hexan-1-ol as well as groups of 5 carbons volatiles are the main volatiles found in virgin olive oil (Kiritsakis, 1998; Angerosa, 2002). After filtration and clarification treatments, generally, there was a reduction in C_6 and C_5 (Lozano-Sanchez, 2010). Nevertheless, it is important to highlight that, there is no negative effect of clarification treatment of EVOO with inert gas on the volatile compounds as a result gas bubbles in the bulk of the oil. During storage, filtered and inert gases clarified samples, total aldehydes volatiles concentration showed a decreasing and increasing trends during the storage period where the maximum value was recorded at the month 8 of storage. The total C_6 alcohols showed significant decrease in Cf sample during storage, while these volatiles increased significantly in the inert gases clarified samples. C_5 alcohols, (*Z*)-2-pentene-1-ol and pentene dimers for Cf rose up significantly at the end of storage and remained without significant variation in the inert gases clarified samples. Significant alteration of LOX aldehyde volatile compounds was shown in Uf sample associated with a gradual increase in the total C6 alcohols for the same sample in particular the (*E*)-2-Hexen-1-ol. The results were comparable to those presented by many authors (Di Giovacchino et al. 2002; Cavalli et al. 2004; Stefanoudaki et al. 2010). A similar trend was also noted for C_5 volatiles (Table 3) in the Uf sample especially, 1-penten-3-ol, which associated with fruity perception of olive oil (Aparicio & Luna, 2002). However, the reduction in (*E*)-2-hexenal and the C_5 alcohols correlated mainly to the alteration of EVOO freshness during storage (Youssef et al. 2011). On the contrary, the unfiltered sample C_5 alcohols decreased significantly at the end of storage time. It is important to underline that, after the end of the storage period, the different categories of LOX C_6 and C_5 volatiles in addition to ketones did not show a significant decrease for inert gas clarified samples as evidenced for the commercial clarified samples or unfiltered. The apparent increase in (*E*)-2- hexenal at the months 8 of storage in particular, for Cf and inert gases clarified samples might be attributed to the increase in the temperature recorded in the storage room (34 ^{o}C) during summer season. Such an increase in temperature favoured the decomposition of 13-hydroperoxides of linoleic, linolenic acid from which hexanal and (*E*)-2-hexenal are originated by the activity of LOX enzymes (Di Giovacchino et al. 2002). Moreover,

the activity of such enzymes was higher in filtered olive oil samples since filtration removes impurities and inhibitory substances as already observed by (Georgalaki et al. 1998).

Changes in sensory attributes

After filtration and clarification with inert gases, there were intensification of sensory attributes (fruity, bitter and pungent) (Table 4). This intensity was more pronounced after clarification, in particular for Ac sample. This trend was in agreement with Lozano-Sanchez et al. (2010). During storage, there was a decrease in the sensory scores evaluated during the time for all stored samples, whereas the alteration was slower in filtered and clarified samples respect to unfiltered one. This behaviour indicates that filtration and clarification might participate in maintaining the positive sensory attributes. Comparing between all stored samples at the end of storage (Table 4), it was found that fruity, bitter and pungent attributes were remained higher in filtered and clarified samples than unfiltered EVOO. The higher fruitiness preception in Cf and clarified samples respect of the unfiltered one could be linked to the higher concentrations of C_5 and C_6 alcohols, (*E*)-2-hexenal, 1-pentene-3-one in filtered and clarified samples respect to the unfiltered one at the end of storage time. However, these compounds are highly associated with fruit and green notes of EVOO (Angerosa et al 2004; Bubola et al. 2012). At the end of the storage period, none of the stored samples showed any sensorial defects and remained within the accepted limits for extra virgin olive oil category (EEC. Reg 61/2011).

Conclusions

By the intensive investigation on the impact of different filtration and clarification systems on the quality of EVOO stored for 12 months in closed bottles in the dark, it could be concluded generally, that C_6 and C_5 volatiles were remained slightly unchanged after storage in inert gases clarified samples and contributed in keeping the pleasant sensory attribute of stored oil. A significant decrease of water content associated with the filtration and clarification, where the water content in the inert gas clarified samples was the lowest among all the stored samples. Lower degradation rates of secoiridoid phenolic compounds during the time was found in filtered and clarified samples respect to the unfiltered one. Hydrolytic degradation in term of increase in free acidity in unfiltered EVOO was more pronounced in clarified, filtered samples. Filtration/ clarification help in preserving the freshness of EVOO in term of sensory attribute during storage than unfiltered sample. Moreover, clarification has advantages over commercial filtration

system, where the positive attributes volatiles did not altered during storage of inert gases clarified samples, in addition to their lower water content respect to Cf stored samples.

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Table 1: FA, free acidity (g oleic acid 100 g^{-1} oil); PV, peroxide value (meq O_2 kg-^{1} oil); K_{232}, K_{270} specific extinction coefficients. 1,2/1,3-DG ratio and water content (mg kg^{-1} oil) during storage of different EVOO samples in dark during storage for12 months.

Source of variation	ST	FA ± sd*	PV ± sd	K_{232} ± sd	K_{270} ± sd	1,2/1,3-DG ratio ± sd	Water content ± sd
	0	0.21 ± 0 c,w	10 ± 1 ab,w	1.37 ± 0.09 b,y	0.1 ± 0.01 bc,x	27 ± 1 a,x	1485 ± 40 a,w
Uf	4	0.27 ± 0.01 b	7 ± 0 c	1.9 ± 0.25 a	0.09 ± 0 c	7 ± 0 b	885 ± 7 b
	8	0.28 ± 0.02 b	11 ± 1 a	2.06 ± 0.34 a	0.11 ± 0.01 ab	2 ± 0 c	878 ± 17 b
	12	0.34 ± 0 a,w	9 ± 1 b,x	2.13 ± 0.09 a,w	0.12 ± 0 a,y	2 ± 0 c,w	771 ± 6 c,w
	0	0.21 ± 0 c,w	10 ± 0 a,w	1.69 ± 0.12 b,w	0.09 ± 0 c,x	34 ± 6 a,w	763 ± 36 a,x
Cf	4	0.24 ± 0.01 b	8 ± 1 b	1.48 ± 0.15 b	0.1 ± 0 b	7 ± 1 b	705 ± 71 a
	8	0.25 ± 0 b	11 ± 0 a	2.3 ± 0.17 a	0.13 ± 0 a	3 ± 0 b	668 ± 62 ab
	12	0.26 ± 0 a,y	10 ± 0 a,wx	2.31 ± 0.22 a,w	0.14 ± 0.01 a,x	2 ± 0 b,w	568 ± 44 b,x
	0	0.21 ± 0 c,w	8 ± 1 ab,x	1.58 ± 0.1 c,wx	0.1 ± 0 c,x	23 ± 2 a,x	190 ± 6 a,z
Nc	4	0.24 ± 0.01 b	8 ± 1 b	1.51 ± 0.12 c	0.1 ± 0 c	4 ± 0 b	29 ± 9 b
	8	0.24 ± 0 b	9 ± 1 ab	2.14 ± 0.11 b	0.13 ± 0.01 b	2 ± 0 c	26 ± 6 b
	12	0.29 ± 0 a,x	10 ± 1 a,x	2.37 ± 0.14 a,w	0.17 ± 0.01 a,w	2 ± 0 c,w	nd
	0	0.21 ± 0.01 b,w	9 ± 1 bc,wx	1.43 ± 0.02 c,xy	0.11 ± 0 b,w	25 ± 2 a,x	260 ± 32 a,y
Ac	4	0.25 ± 0.01 a	7 ± 1 c	1.74 ± 0.04 b	0.1 ± 0 b	5 ± 1 b	229 ± 16 a
	8	0.25 ± 0.01 a	10 ± 1 ab	1.91 ± 0.23 ab	0.13 ± 0 a	3 ± 0 c	85 ± 5 b
	12	0.22 ± 0.01 b,z	11 ± 1 a,w	2.11 ± 0.1 a,w	0.12 ± 0.01 a,xy	2 ± 0 c,w	nd

* Uf: unfiltered EVOO sample; Cf: commercial filtered EVOO sample; Nc: nitrogen clarified EVOO sample; Ac: argon clarified EVOO sample.
*Values (mean ± standard deviation) with different 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). ST: Storage time in months. nd: not detected.

Table 2: Changes in phenolic compounds (mg kg-1) during storage of different EVOO samples in dark during storage for12 months.

Source of variation	ST	Hyty	Ty	CA	DOA	TyDer	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	277 ± 7 a,w	97 ± 7 a,w	24 ± 1 a,y	10 ± 1 a,y	85 ± 3 a,wx	40 ± 6 a,w	58 ± 1 a,wx
	4	9.1 ± 1.0 c	8.8 ± 1.2 c	1.02 ± 0.04 b	132 ± 2 b	47 ± 2 b	19 ± 1 b	5 ± 0 b	64 ± 9 b	18 ± 2 b	60 ± 1 a
	8	16.6 ± 0.0 b	20.7 ± 0.0 b	1.06 ± 0 a,	110 ± 5 c	41 ± 7 b	13 ± 1 c	2 ± 0 c	62 ± 9 b	20 ± 1 b	25 ± 1 b
	12	31.7 ± 0.1 a,w	41.3 ± 0.1 a,w	1.11 ± 0.01 a,w	75 ± 8 d,y	41 ± 1 b,w	8 ± 0 d,y	nd	59 ± 3 b,w	19 ± 0 b,wx	19 ± 4 c,x
Cf	0	6 ± 0.8 b,x	5.1 ± 0.2 c,y	1.16 ± 0.02 a,x	289 ± 39 a,w	84 ± 9 a,x	27 ± 1 a,x	12 ± 0 a,x	92 ± 9 a,w	30 ± 3 a,x	41 ± 4 a,y
	4	7.3 ± 1.1 a	5.7 ± 0.1 b	1.05 ± 0.11 ab	155 ± 16 b	50 ± 2 b	20 ± 2 b	5 ± 0 b	73 ± 1 b	19 ± 2 b	39 ± 2 a
	8	6.9 ± 0.2 ab	5.7 ± 0.3 ab	1.04 ± 0.02b	134 ± 26 bc	49 ± 4 b	13 ± 1 c	nd	77 ± 1 b	22 ± 2 b	24 ± 1 b
	12	7.7 ± 0.3 a,x	6.2 ± 0.4 a,x	0.97 ± 0.06 b,x	106 ± 12 c,x	43 ± 2 b,w	10 ± 0 d,xy	nd	70 ± 8 b,w	21 ± 2 b,w	24 ± 0 b,w
Nc	0	6.5 ± 0.5 bc,x	6.4 ± 0.5 b,x	1.17 ± 0.07 a,x	311 ± 37 a,w	87 ± 7 a,wx	32 ± 1 a,w	8 ± 0 a,z	79 ± 3 a,x	34 ± 5 a,wx	51 ± 8 a,xy
	4	6.9 ± 0.1 b	5.4 ± 0.2 c	0.99 ± 0.04 c	194 ± 8 b	70 ± 7 b	20 ± 2 b	5 ± 1 b	68 ± 13 a	19 ± 4 b	29 ± 4 b
	8	20.8 ± 0 a	27.2 ± 0 a	1.09 ± 0.00 ab	158 ± 15 b	45 ± 3 c	10 ± 2 c	nd	67 ± 1 a	18 ± 0 b	23 ± 0 bc
	12	5.9 ± 0.3 c,y	5.5 ± 0.6 c,x	1.03 ± 0.03 bc,wx	155 ± 9 b,w	46 ± 2 c,w	13 ± 1 c,wx	nd	65 ± 12 a,w	17 ± 2 b,x	18 ± 4 c,x
Ac	0	14 ± 1.9 a,w	8.9 ± 1.2 a,w	1.34 ± 0.10 a,w	287 ± 16 a,w	75 ± 2 a,x	25 ± 2 a,xy	20 ± 0 a,w	91 ± 1 a,w	30 ± 1 a,x	61 ± 7 a,w
	4	7.7 ± 0.4 b	5.6 ± 0.2 b	1.01 ± 0.01 b	219 ± 3 b	51 ± 6 b	19 ± 2 b	5 ± 0 b	38 ± 5 b	20 ± 2 b	48 ± 9 b
	8	5.8 ± 0.6 bc	4.9 ± 0.6 b	1.00 ± 0.02 b	163 ± 4 c	45 ± 6 b	13 ± 1 c	nd	35 ± 0 b	17 ± 3 b	14 ± 2 c
	12	6.0 ± 0.0 c,y	6.0 ± 0.0 b,x	1.08 ± 0.05 b,w	171 ± 26 c,w	41 ± 10 b,w	13 ± 3 c,w	nd	29 ± 0 c,x	20 ± 1 b,wx	16 ± 1 c,x

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

*Values (mean ± standard deviation) with different 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). ST: Storage time in months. nd: not detected.

Table 3: Changes in volatile compounds (expressed as mg 4 methyl-2-pentanone kg−1 oil) during storage of different EVOO samples in dark for 12 months.

Source of variation	ST*	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	0.2 ± 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 ± 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	0.1 ± 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	0.2 ± 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	0.2 ± 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

Table 3 continued

Source of variation	ST*	Main C5 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 a	0.19 ± 0.01 b	0.54 ± 0.1 b	0.6 ± 0.07 c	2.01 ± 0.07 b
	8	0.18 ± 0.01 b	0.18 ± 0 b	0.33 ± 0.01 c	1 ± 0.04 b	2.1 ± 0.08 b
	12	0.08 ± 0.01 c,z	0.19 ± 0.01 b,z	0.15 ± 0.01 d,z	0.7 ± 0.08 c,z	1.28 ± 0.06 c,z
Cf	0	0.15 ± 0 d,y	0.19 ± 0.01 c,x	0.7 ± 0.02 a,w	0.79 ± 0.03 c,y	1.84 ± 0.02 c,y
	4	0.2 ± 0 a	0.19 ± 0.01 c	0.53 ± 0 b	0.67 ± 0.04 d	1.64 ± 0.04 d
	8	0.17 ± 0 c	0.3 ± 0.02 a	0.68 ± 0.02 a	1.24 ± 0.07 a	2.39 ± 0.07 a
	12	0.18 ± 0 b,w	0.22 ± 0.01 b,y	0.69 ± 0.1 a,w	0.96 ± 0.02 b,y	2.2 ± 0.08 b,x
Nc	0	0.12 ± 0.01 c,z	0.19 ± 0 c,x	0.38 ± 0.01 a,y	1.41 ± 0.04 b,w	2.07 ± 0.03 b,xy
	4	0.18 ± 0 a	0.2 ± 0.01 c	0.3 ± 0.02 b	0.76 ± 0.09 c	1.27 ± 0.13 c
	8	0.15 ± 0.01 b	0.36 ± 0 a	0.37 ± 0.02 a	1.7 ± 0.03 a	2.43 ± 0.03 a
	12	0.09 ± 0 d,y	0.32 ± 0.01 b,x	0.28 ± 0 b,y	1.39 ± 0.08 b,x	1.98 ± 0.1 b,y
Ac	0	0.57 ± 0.01 a,w	0.19 ± 0 b,x	0.57 ± 0.01 b,x	0.85 ± 0.04 c,y	2.28 ± 0.05 b,x
	4	0.13 ± 0 c	0.21 ± 0.01 a	0.47 ± 0.02 c	0.74 ± 0.01 d	1.6 ± 0.02 c
	8	0.17 ± 0 b	0.42 ± 0.01 c	0.61 ± 0 a	1.5 ± 0.04 b	2.69 ± 0.05 a
	12	0.1 ± 0 d,x	0.34 ± 0 c,w	0.43 ± 0 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.

*Values (mean ± standard deviation) with different 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). ST: Storage time in months.

Table 4: Changes in organoleptic assessment for olive oils during storage for 12 months as evaluated by Panel testing according to the EU. 1348/2013), by a fully trained group of 8 expert tasters from Bologna University.

Source of variation	**ST**	Fruity	Bitter	Pungent
	0	4.20	4.20	4.40
Uf sample	4	4.30	4.25	4.40
	8	2.65	3.10	3.05
	12	2.20	2.60	2.10
	0	4.65	5.50	6.55
Cf sample	4	4.05	4.75	4.15
	8	4.15	4.30	5.50
	12	3.40	4.10	3.90
	0	4.45	4.75	5.75
Nc sample	4	3.80	4.65	3.95
	8	3.20	3.60	3.45
	12	2.40	3.90	3.90
	0	4.90	5.25	6.40
Ac sample	4	3.80	3.80	4.60
	8	2.40	1.60	1.60
	12	2.40	3.30	3.50

ST: Storage time in months.
+Extra virgin olive oils are expected to have a median of positive attributes more than 1 with 0 sensory defects.

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

Paper 2

Filtered and clarified virgin olive oils: Evolution of their quality during the storage under different conditions

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Filtered and clarified virgin olive oils: Evolution of their quality during the storage under different conditions

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Abstract

This research study was carried out to investigate the effects of storage time and the exposure conditions on the quality of filtered and inert gases clarified extra virgin oil during a 12 month of storage in dark and under diffused day light respect to the changes occurred in the quality of unfiltered extra virgin olive oil. Different quality parameters were evaluated during storage. The results showed that at the end of storage time, the sample stored under light contained significantly lower amounts of tocopherol, chlorophylls and developed oxidation products. The results also showed that isomerization of diglycerides were not affected by the filtration/ clarification processes and the storage conditions as influenced by the time of storage. Moreover unfiltered samples stored under light contained significantly lower amounts of tocopherols, lower positive sensory attribute scores and developed more oxidative volatiles than the filtered and inert gases clarified samples stored in dark. Overall results showed that, there was a protective effect of filtration and clarification on the stored extra virgin olive oil. At the end of storage, all the samples exposed to light underwent a drastic loss of quality and declassed from olive oil category in term of sensory evaluation.

Key words: *extra virgin olive oils, storage conditions, dark, light, oxidative volatiles, orthodiphenols, filteration, clarification, chlorophyll, tocopherols*.

1. Introduction

Virgin olive oil is the juicy extract from the Olea Europaea originated in the Mediterranean region that merely produced by mechanical means without any treatment other than washing (Boskou, 2006). The freshly produced virgin olive oil is distinguished from the other vegetable oils by its characteristic aroma, taste and color. Due to its organoleptic and nutritional properties, as well as the great tendency of the consumers to include extra virgin olive oil (EVOO) in their diet, thus, preserving olive oil with a minimal loss of these properties considered one of the greatest concerns of the olive oil industry sector (Salvador et al. 1999). Just produced virgin olive oil contains suspended solids, humidity, phospholipids, proteins that make olive oil cloudy and less attractive to the consumers. For this reason, filtration is the suggested process before bottling to remove or decrease the quantity of these substances therefore making clear the virgin olive oil (Lozano- schenze et al. 2010). Another process that was recently developed to remove the humidity and suspended solids from virgin olive oils is the clarification with inert gases (nitrogen or argon) (Bendini et al. 2013). This clarification process according to Cerretani et al. (2009) has some advantages, where, it avoids the contact between oil and the filtration aid in addition to its effect on decreasing the presence of oxygen in the bulk oil. Moreover, clarification with inert gases guaranteed that the bulk oil will remain under inert gas when applied in a large scale production companies. Regarding the susceptibility to oxidation, the presence of minor components, in particular phenolic compounds, side by side with its high content of monounsaturated respect to the polyunsaturated fatty acids enhances the resistance of virgin olive oil against oxidation reactions compared to other vegetable oils (Bendini et al. 2009). During storage, the oxidation stability of virgin olive oil mainly depends on the presence of pro-oxidant substances, the presence of oxygen, temperature and light exposure (Di Giovacchino et al. 2002). However, these factors can affect the shelf life of EVOO, therefore, leading to quality deterioration as a result of oxidative and hydrolytic degradations. (Psomiadou & Tsimidou., 2002). Above all factors that cause deterioration of stored EVOO, light exposure considered the most drastic factor that determine the commercial life of the stored olive oil. However, the singlet oxygen formed in the photo-oxidation reactions is 1000-1500 times more reactive than the triplet oxygen produced by auto-oxidation that occurred in the dark (Afaneh et al. 2013).

The effects of storage conditions (dark and light) on the quality of stored EVOO have been investigated recently by many authors (Caponio et al; 2005; Mendez and Falque., 2007; Dabbou et al. 2011; Afaneh et al. 2013; Ayyad et al. 2015). However, all of these researches revealed

that the quality and oxidation stability of the stored EVOO was highly affected when the samples stored under light in comparison with those stored in the dark**.** Some researches were carried out to determine the effect of storage time on the quality of filtered EVOO versus the unfiltered one (Brenes et al, 2001; Fregapane et al. 2006) who all showed that filtration process could prolong the shelf life of virgin olive oils and protect more the minor components, in particular phenolic compounds in the stored oil respect to the unfiltered virgin olive oil. In addition, the both mentioned studies revealed that, the free fatty acid content in the filtered EVOO after storage was significantly lower than that recorded for unfiltered oil. Most of the studies concerning the quality of filtered and inert gases clarified EVOO vs uinfiltered virgin olive oils were carried out on fresh olive oil samples directly after filtration (Lozano-Sanchez et al. 2012; Bubola at al., 2012; Bendini et al. 2013) or during the storage in dark condition (Brenes et al. 2001; Fregapane et al. 2006). The aim of the current study was to investigate the effects of storage period and exposure conditions (dark and light) on the quality of prolonged stored, filtered and clarified extra virgin olive oil in comparison with unfiltered one. The investigation was performed by tracing some quality markers including phenolic compounds, pigments, oxidative stability, diglycerides isomerization, sensorial properties during one year of storage at room temperature.

2. Material and methods

2.1 Preparation of extra virgin olive oil samples

A homogeneous sample of extra virgin olive oil of the cultivar “Canino” from Lazio region was extracted in October 2012. Part of which, was filtered using commercial filter press (F.lli Marchisio and C.S.p.A- Italy) to produce filtered extra virgin olive oil sample (filtered). Other parts were clarified using innovative inert gas filtration system patented by the University of Bologna and Sapio (Cerretani et al. 2009). In the inert gas clarification process, the inert gas (argon or nitrogen) was insufflated from the bottom of five liters capacity extra virgin olive oil reservoir (12 L min^{-1}) to produce (argon clarified) and nitrogen clarified; P= 2 bars). The rest of the sample was remained without filtration (control). Directly after filtration/ clarification, the EVOO samples were filled in a transparent glass bottle, leaving 4% head space, then, the bottles were hermetically sealed and stored either in the dark or exposed to the diffused day light at room temperature for 12 months. Chemical analyses were performed at time zero and after 6 and 12 months of storage. Organoleptic properties were evaluated directly after extraction and filtration/ clarification treatments and at the end of the storage period. At each respective time of

analysis, three replicates were obtained from three separate sealed bottles and the samples were collected from the geometrical center of each bottle.

2.2 Evaluation of the basic quality indexes

Basic quality indexes of EVOO samples, free fatty acids (FFA) expressed as g oleic acid 100 g^{-1} of oil, peroxide value (PV) expressed mili-equivalent O_2 kg^{-1} and spectrophotometric indices (K_{232} and K_{270}) were determined according to the analytical methods described in the European Commission Regulation (EEC. Reg 2568/91).

2.3 Determination of total phenol and ortho-diphenols

Phenolic compounds were extracted following the procedure modified by (Rotondi et al. 2004). The total and *ortho*-diphenols (*ortho*-Dp) were determined spectrophotometrically using UV−vis 6705 jenway spectrophotometer (United Kingdom) at 370 nm, according to Singleton and Rossi., (1965). The concentration of *ortho*-Dp was expressed as milligram of gallic acid per kilogram of oil (mg gallic acid/kg oil) using gallic acid calibration curves (R^2 = 0.995).

2.4 Determination of tocopherols

The contents of total tocopherols were determined using 0.5 g of sample and carried out by HPLC equipped with diode array (DAD) detector according to the method described by Bendini et al. (2013), α and γ tocopherols were calculated using a calibration curve of known concentrations of α -tocopherol (R^2 = 0.999). Results were expressed as mg of total-tocopherols per kg of oil.

2.5 Determination of chlorophylls

The determination of chlorophylls compositions was carried out spectrophotometrically using UV−vis 6705 jenway spectrophotometer (United Kingdom) at 670 nm, according to the protocol described by Baccouri et al. (2008). Chlorophylls were determined using a calibration curve of known concentrations of chlorophyll (Carlo Erba- Italy) soluble in isooctane (R^2 = 0.999). Data were reported as milligrams of chlorophylls kg oil^{-1} (mg kg^{-1}).

2.6 determinations of oxidation markers volatile compounds

Volatile compounds were evaluated by SPME-GC/MSD (Agilent 6890N, Santa Clara, CA, USA) coupled with quadrupolar mass selective spectrometry (Agilent 5973 N, Agilent Technologies), according to the method described by Cerretani et al. (2008), volatile compounds identification was carried out by mass spectrometry depending on a comparison of their mass spectral data with the information from NIST library (2005 version) and MS literature data.

Volatile compounds were expressed as mg of internal standard 4-methyl-2-pentanone (Fluka) per kg of oil.

2.7 sensory evaluations

EVOO samples were analyzed quantitatively and described for their grade category at each respective time of evaluation by the official panel group of Bologna University according to the method described in the European Commission Regulation (EU Reg. 1348/2013).

2.8 statistical analyses

Statistical analysis of the three replicates of each sample was elaborated by analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) using XLSTAT software version 7.5.2 (Addinsoft, USA).

3. Results and discussion

3.1 changes in the basic quality parameters

The evaluated basic quality indicators (Free fatty acid, Peroxide value, K_{232}, K_{270}) of the Control, filtered, nitrogen and argon clarified samples, at time zero and after one year of storage in the dark and under diffused daylight were located within the fixed limits for extra virgin olive oil category (FA% ≤ 0.8, PV ≤ 20 mac Kg-1, $K_{232} \leq 2.5$, $K_{270} \leq 0.22$) according to (EU 1348/2013) regulations (data not shown).

3.2 changes in *ortho*-Dp content

Hydrophilic phenolic compounds are very active radical scavenging antioxidant found in the virgin olive oil, however, phenolic compounds that belong to the ortho-diphenols group in particular hydroxytyrosol is considered the strongest antioxidant among the polar phenols (Bendini et al. 2007). For instance, phenolic antioxidants inhibit auto-oxidation of lipids by quenching the formed peroxy radicals (Psomiadou and Tsimidou., 1998). The results demonstrated in Table 1 showed a significant decrease in the ortho- diphenolic compounds during the storage time for all the stored samples under both conditions, without being significant, the statistical differences between samples under their respective conditions. This behavior could be related to the consumptions of these compounds as a result of their antioxidant activities (Bendini et al. 2007).

3.3 changes in total tocopherol contents

Lipophilic compound (tocopherols) mainly α- tocopherol has an important role as an antioxidant and contributes in prevention of the oxidation degradation, but with less antioxidant power than *ortho*-Dp such as hydroxytyrosol (Bendini et al. 2009). In fact tocopherols retard the oxidation

reaction by acting as both, electron donor and even as electron acceptor scavenging the singlet oxygen, in particular against photo oxidation reactions (Morello et al. 2004). The results (Table 1) showed that, the total tocopherols decreased significantly during the storage period for all the samples stored in the dark and under diffused day light. Indeed, it was significant, the variation between the different filtered, nitrogen clarified, argon clarified and control samples in accordance with the previously reported results of previous authors (Fregapane et al. 2006; Lozano-Sanchez et al. 2012).

On the other hand, all the samples stored under light showed significant loss of tocopherols in comparison with the same samples stored in the dark. These results were in agreement with Brenes et al. (2001). It’s important to highlight that, after the end of storage, the total tocopherols remained significantly higher in the filtered, nitrogen clarified and argon clarified samples than that stored without filtration. This implies that filtration or clarification with inert gas tend to protect lipophilic phenols as already observed by Bendini et al. (2013). At the end of storage, there were no significant variations between filtered and inert gases clarified samples in term of tocopherol contents.

3.4 changes in the pigments (chlorophyll)

Chlorophylls and carotenoids are the olive oil pigments that act as antioxidant when the oil stored in dark against outo-oxiadation (Kiritsakis and Dugan, 1984). While, in the presence of light chlorophylls may work as a photo-sensitizer and accelerates the oxidation reactions (Psomiadou and Tsimidou 2002). However, during storage, chlorophyll pigment did not disappear, otherwise the conversion of chlorophyll to pheophytins and pyropheophytins cause in fact, the fading of the green color of the virgin olive oil (Cuppett et al. 1997). The results presented in Table 1 showed that the chlorophyll content remained practically stable for all samples stored in the dark during the first 6 months of storage, after which, filtered, nitrogen and argon clarified samples exhibited a slight significant decrease at the end of storage time. On the contrary to the samples stored in the dark, all the samples stored under diffused daylight showed a strong significant depletion of the chlorophyll pigment during the storage period. Moreover, in the presence of light, chlorophylls act as photo-sensitizer leads to the formation of highly reactive singlet oxygen that in turn accelerates the production of hydroperoxides. Nevertheless, the decomposition of hydro-peroxides triggers the formation of high amounts of secondary oxidation products (Kiritsakis and Dugan., 1984). After 12 months of storage the amounts of chlorophyll remained in the control samples stored under both the conditions were higher than

that for the filtered and inert gas clarified samples. These results were in accordance with previously reported results by (Gutiarrez and Fernandez., 2002; Morello et al. 2004; Vacca et al. 2006; Caponio et al. 2005).

3.5 changes in the oxidative markers volatile compounds

In spite of the fact that, Peroxide values, K_{232} and K_{270} determines the oxidative changes of the virgin olive oil, on the other hand, the changes in these parameters could be slow to detect oxidative status of virgin olive oil. In general, the sensory evaluation can detect changes and even declass olive oil from its initial category before the verified change in the oxidation indices exceeds the accepted limit according to the EU regulations (EU Reg 1348/2013). For this reason, volatile compounds in particular those arise as a result of the oxidative deterioration could help in detecting the early stages of olive oil oxidation (Kalua et al. 2007). The volatile compounds that were identified in the oxidized olive oil include octane, nonanal, hexanal and 2,4-heptadinal (Kiritsakis, 1998; Vichi et al. 2003). Other volatiles like carboxylic acid volatiles such as (hexanoic acid, nonanoic acid and ocatanoic acid) also detected after 12 months of storage (Vichi et al. 2003). Excluding hexanal, the volatile compound which is also generated as a result of both oxidation reaction and enzymatic (LOX) pathway, these compounds could cause rancid off flavor in the virgin olive oil and indicate the oxidative rancidity (Kalua et al. 2007). In addition, some authors proposed also hexanal/nonanal as an indicative marker for progressive oxidation of the virgin olive oil (Morales et al. 1997; Kiritsakis, 1998). The results depicted in Table 2 showed that, the rancid volatiles were not present in the control, filtered, nitrogen and argon clarified samples directly after extraction and bottling except the presence of small amounts of octane in the control sample. However, during storage there were significant increases in octane and nonanal volatiles for all samples stored under both the conditions. On the other hand, (*E*)-2 heptenal and carboxylic acid volatiles present after the end of storage time in most of the stored samples. At the of storage it was observed that, the control sample and commercially filtered sample stored in the dark, contained higher amounts of rancid volatile than the nitrogen and argon clarified samples. Moreover, the carboxycyclic volatiles were significantly higher in the control samples in the dark and under light than that recorded for filtered, inert gas clarified samples. These results could be attributed to the presence of higher amounts of suspended solids and oxygen in the unfiltered olive oil sample (Kalua et al. 2007; Kanavouras et al, 2006). Concerning the hexanal/nonanal ratio, it was found that, this value was remained about 3 to 4 fold in nitrogen and argon clarified samples than the value registered for the filtered one stored in

the dark. On the other hand, control sample stored in the dark showed hexanal/nonanal value lower than two at the end of storage period which considered oxidized, according to Morales and Aparicio., (1997). As can be seen from the displayed results (Table 2), at the end of storage all the samples stored under diffused daylight contained as expected significantly higher amounts of oxidation markers volatiles than the respective samples stored in the dark. In addition, the development of the rancid volatiles during storage was associated with a depletion of C_6 LOX volatiles in the control and filtered samples both in the dark and under light. On the contrary, the inert gases clarified samples stored in the dark showed an intensification of such pleasant volatiles at the end of storage, which can be explained by the effects of some LOX enzymes (Di Giovacchino et al. 2002).

3.6 changes in the diglycerides isomerization.

The dominant form of diglycerides found in the just produced olive was the 1,2-DGs form in particular 1,2- diolein, in general, its isomerization to the 1,3-DGs form affected mainly by the time and the temperature of storage (Ayyad et al. 2015). Table 3 shows the amounts of 1,2 and 1,3-DGs isomers of C34 and C36 diglycerides forms in control, filtered, nitrogen and argon clarified samples stored in the dark and under diffused day light.

The 1,2-DGs in particular, the C36 diglyceride form, account the majority of the diglyceride contents after extraction. During storage, there was a significant increase in the formation of both, the C34 and C36 isomers of 1,3-DGs form in all of the stored samples under both the conditions as a result of the progressive isomerization from 1,2-DGs to 1,3-DGs form. However, 1,3-DGs considered more stable if compared with 1,2-DGs isomer (Caponio at al., 2005). At the end of the storage, the amounts of 1,2-DGs C34 and C36 isomers remained about 2 fold the amount of the respective 1,3-DGs isomers. In addition, there were no obvious differences between the storage conditions (dark and light). It’s important to note that, the isomerization process seems to depend mainly on the storage time in agreement with the results recorded by many authors (Pérez-Camino et al. 2001; Caponio et al. 2005).

3.7 changes in the organoleptic properties

Typical results of sensory analysis at time zero after bottling and at the end of storage time are shown in Table 4. The fruitiness, pungency and bitter attributes decrease during the storage time for all the samples stored under both the conditions. It is important to highlight that the positive attributes remained higher in the filtered, nitrogen and argon clarified samples after the end of storage period of 12 months, which implies that, commercial filtration and inert gas clarification

could protect the pleasant sensory properties of the treated virgin olive oil (Bendini et al. 2013). Regarding light storage condition, it was observed that all the samples produced sensory defects, in particular, rancidity, thus declassed from the extra virgin olive oil category. (EC Reg. 61/2011).

4. Conclusions

This research project was dedicated to emphases the effects of storage time and storage conditions on the quality of the filtered and inert gases clarified extra virgin olive oil in particular, the olive oil stability against oxidation. Accumulation of different oxidative volatile markers was more pronounced in the unfiltered samples at the end of storage time at which, the inert gas clarified samples contained the lowest amount. At the end of storage, the hexanal/ nonanal ratio the inert gases clarified samples remained higher than those recorded for the filtered sample stored in the dark, while the lowest ratio was shown in the unfiltered samples. There was a decrease in the amounts of the minor component and pigments during the storage under both the conditions, where the filtered and clarified samples preserved more the tocopherols than the unfiltered sample. At the end of storage, there was no evidence that the diglyceride isomerization during the storage was affected by filtration. Sensory scores of filtered and clarified samples remained higher than those evaluated for the unfiltered one.

Regarding the filtration and clarification processes it could also suggest that there were beneficial effects of these processes in terms of protecting more, the micro component and the pleasant sensory attributes in the stored oil. In addition to its role in decreasing the rate of developments of the oxidative volatiles especially in the inert gases clarified samples. The result also showed that, the extreme negative effects on the quality of stored EVOO were recorded when the samples stored under light at which, the stored oil after 12 months was no longer considered extra virgin olive oil.

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Table 1: Total *ortho*-diphenols content (*ortho*-Dp) (mg gallic acid kg^{-1} oil and total tocopherols (mg kg^{-1} oil) for all samples during storage time of 12 months in the dark and under diffused daylight.

+ Letters (a - d) indicate the statistical differences (at 0.05 level, Fisher test) during storage time for the respective sample (light/dark). + Letters (w-z) indicate the statistical differences among the samples at the same time and condition (light/dark after 12 months of storage). + (*) indicate the significant higher value between dark and light of the same sample after 12 months. *D: Dark. L: Light.*

Quality parameter	**Sample**	**Storage condition**	**Storage time (months)** 0	6	12
ortho-Dp	*Control*	*D*	143 ± 2 a	121 ± 2 b	92 ± 13 c,w
		L	143 ± 2 a	120 ± 2 b	90 ± 2 c,w
	Filtered	*D*	126 ± 9 a	127 ± 2 a	89 ± 10 b,w
		L	126 ± 9 a	105 ± 10 b	85 ± 4 c,w
	Nitrogen clarified	*D*	146 ± 9 a	136 ± 7 a	90 ± 14 b,w
		L	146 ± 9 a	117 ± 3 b	99 ± 5 c,w
	Argon clarified	*D*	158 ± 13 a	113 ± 18 b	92 ± 7 b,w
		L	158 ± 13 a	124 ± 7 b	87 ± 3 c,w
Total tocopherols	*Control*	*D*	309 ± 1 a	273 ± 10 b,x	211* ± 20 c,x
		L	309 ± 1 a	215 ± 15 b,y	140 ± 10 c,x
	Filtered	*D*	301 ± 7 a	283 ± 4 b,x	233* ± 9 c,w
		L	301 ± 7 a	192 ± 4 b,y	187 ± 0 b,w
	Nitrogen clarified	*D*	283 ± 6 a	280 ± 0 a,x	239* ± 4 b,w
		L	283 ± 6 a	219 ± 6 b,y	192 ± 5 c,w
	Argon clarified	*D*	282 ± 1 a	244 ± 36 a,x	233* ± 14 b,w
		L	282 ± 1 a	219 ± 2 b,y	186 ± 4 c,w
Chlorophylls	*Control*	*D*	29 ± 1 a	31 ± 3 a	27* ± 1 a,w
		L	29 ± 1 a	10 ± 2 b	4 ± 1 c,w
	Filtered	*D*	32 ± 0 a	31 ± 4 a	24* ± 1 b,w
		L	32 ± 0 a	6 ± 1 b	4 ± 0 b,w
	Nitrogen clarified	*D*	28 ± 2 a	24 ± 3 a	19* ± 1 b,x
		L	28 ± 2 a	8 ± 0 b	1 ± 0 c,x
	Argon clarified	*D*	35 ± 2 a	28 ± 8 a	20* ± 4 b,x
		L	35 ± 2 a	8 ± 0 b	1 ± 0 c,x

Table 2: Evolution of oxidation markers volatile compounds (expressed as mg 4 methyl-2-pentanone kg−1 oil) for all samples during storage time of 12 months in the dark and under diffused daylight.

+ Letters (a - d) indicate the statistical differences (at 0.05 level, Fisher test) during storage time for the respective sample (light/dark). + Letters (w-z) indicate the statistical differences among the samples at the same time and condition (light/dark after 12 months of storage). + (*) indicate the significant higher value between dark and light of the same sample after 12 months. SC: Storage condition. D: Dark. L: Light. ST : Storage time in months. nd: not detected.

Samples	SC	ST	Total C_6 volatile	Octan	Nonanal	Hexanal	(*E,E*) – 2,4 heptadienal	Total rancid volatiles	carboxylic acid violatiles	Hexanal/ Nonanal
Control	***D***	0	15.72 ± 2.31 b	0.09 ± 0.01 c,	Nd	0.67 ± 0.11 a,	nd	0.48 ± 0.09 b,	nd	-----
		6	10.63 ± 0.19 a	0.37 ± 0.02 b,	0.16 ± 0.02 b,y	0.56 ± 0.05 a,	nd	0.83 ± 0.06 a,y	nd	3.41 ± 0.52 a,
		12	11.65 ± 0.44 a,w	0.59 ± 0.06 a,w	0.22 ± 0.01 a,w	0.26 ± 0.01 b,y	0.06 ± 0 a,w	0.9 ± 0.06 a,x	0.75 ± 0.03 a,w	1.2* ± 0.09 b,y
	L	0	15.72 ± 2.31 a	0.09 ± 0.01 c,	Nd	0.67 ± 0.11 a,	nd	0.48 ± 0.09 c,	nd	-----
		6	11.81 ± 0.24 ab	0.79 ± 0.08 b,	0.22 ± 0.03 b,x	0.57 ± 0.03 a,	0 ± 0 b,	1.17 ± 0.11 b,x	0 ± 0 b,	2.67 ± 0.26 a,
		12	12.85 ± 0.96 b,w	1.8* ± 0.06 a,w	0.26* ± 0.02 a,x	0.57* ± 0.03 a,w	0.07 ± 0.01 a,x	2.59* ± 0.03 a,w	1.36* ± 0.04 a,w	2.16* ± 0.21 b,wx
Filtered	***D***	0	13.47 ± 0.16 a	nd	Nd	0.79 ± 0.02 a,	nd	0.27 ± 0.03 c,	nd	----
		6	11.42 ± 1.48 b	0.37 ± 0.02 a	0.15 ± 0.03 b,	0.78 ± 0.06 a,	nd	0.83 ± 0.04 b,x	0 ± 0 b,y	6.4 ± 0.28 a,
		12	11.28 ± 0.17 b,w	0.42 ± 0.05 a,x	0.24 ± 0.01 a,w	0.67 ± 0.11 a,wx	Nd,x	0.93 ± 0.03 a,wx	0.48 ± 0.01 a,x	1.98 ± 0.34 b,y
	L	0	13.47 ± 0.16 a	nd	Nd	0.79 ± 0.02 b,	nd ,	0.27 ± 0.03 c,	nd	----
		6	11.89 ± 0.22 b	0.74 ± 0.05 b	0.2 ± 0.01 b	0.91 ± 0.03 a,	nd	1.19 ± 0.05 b,x	0 ± 0 b,x	4.58 ± 0.39 a,
		12	11.58 ± 0.56 b,w	0.98* ± 0.08 a,z	0.34* ± 0.02 a,w	0.58 ± 0.03 c,wx	0.22* ± 0.02 a,w	1.64* ± 0.1 a,y	0.76* ± 0.04 a,x	2.4*± 0.19 b,wx
Nitrogen clarified	***D***	0	13.63 ± 0.15 b	nd	Nd	0.59 ± 0.07 b,	nd	0.18 ± 0.01 c,	nd	
		6	11.89 ± 0.22 c	0.12 ± 0 b,y	0.1 ± 0.01 a,y	0.98 ± 0.05 a,	nd	0.44 ± 0.02 b,y	nd	9.46 ± 0.64 a,
		12	15.16 ± 0.2 a,w	0.67 ± 0.05 a,w	0.09 ± 0.01 a,x	0.83* ± 0.14 a,w	0.06 ± 0 a,w	0.82 ± 0.04 a,y	0.2 ± 0.01 a,z	8.95* ± 1.24 a,w
	L	0	13.63 ± 0.15 a	nd	Nd	0.59 ± 0.07 b,	nd	0.18 ± 0.01 c,	nd	----
		6	11.42 ± 0.28 b	0.75 ± 0.12 b,x	0.17 ± 0.02 b,x	1.14 ± 0.06 a,	nd	1.09 ± 0.13 b,x	nd	6.83 ± 0.86 a,
		12	10.6 ± 0.76 b,x	1.15* ± 0.01 a,y	0.22* ± 0.03 a,x	0.27 ± 0 c,y	0.11* ± 0.01 a,x	1.73* ± 0.07 a,y	0.3 ± 0.04 a,z	1.28 ± 0.2 b,y
Argon clarified	***D***	0	12.63 ± 0.18 b	nd ,	nd ,	0.56 ± 0.01 b,	nd	0.17 ± 0.02 b,	nd	----
		6	12.63 ± 0.31 b	0.17 ± 0.01 a,y	0.09 ± 0.01 a,y	1.14 ± 0.12 a,	nd	0.53 ± 0.01 a,y	nd	12.24 ± 1.66 a,
		12	13.2 ± 0.13 a,w	0.16 ± 0 a,y	0.1 ± 0.01 a,x	0.66* ± 0.03 b,x	0.06 ± 0 a,w	0.49 ± 0.03 a,z	0.32 ± 0 a,y	6.44* ± 0.94 b,x
	L	0	12.63 ± 0.18 a	0 ± 0 c	0 ± 0 b	0.56 ± 0.01 b	nd	0.17 ± 0.02 c	nd	----
		6	12.07 ± 0.71 a	0.91 ± 0.06 b	0.21 ± 0.03 a	1.26 ± 0.09 a	nd	1.33 ± 0.02 b	nd	6.05 ± 1.15 a
		12	11.9 ± 0.44 a,x	1.53* ± 0.04 a,x	0.25* ± 0.01 a,x	0.49 ± 0.04 b,y	0.06 ± 0 a,y	2.06* ± 0.02 a,x	0.41* ± 0.04 a,y	1.99 ± 0.05 b,x

Table 3: Evolution of 1,3 and 1,2 C34, C36 DGs isomers (mg 4-methyl-2-pentanone/kg oil) for all sample during storage time of 12 months in the dark and under diffused daylight.

+ Letters (a - d) indicate the statistical differences (at 0.05 level, Fisher test) during storage time for the respective sample (light/dark). + Letters (w-z) indicate the statistical differences among the samples at the same time and condition (light/dark after 12 months of storage). + (*) indicate the significant higher value between dark and light of the same sample after 12 months. *D: Dark. L: Light.*

Quality parameter	**Sample**	**Storage condition**	**Storage time months**		
			0	**6**	**12**
1,2 (C36) DGs	*Control*	*D*	90 ± 0.05 a	70 ± 0.01 b	5 ± 0.07 b,w
		L	90 ± 0.05 a	72 ± 0.02 b	6 ± 0.09 b,w
	Filtered	*D*	89 ± 0.00 a	70 ± 0.02 b	2 ± 0.04 b,w
		L	89 ± 0.02 a	62 ± 0.02 b	7 ± 0.03 b,wx
	Nitrogen clarified	*D*	89 ± 0.04 a	60 ± 0.03 b	2 ± 0.10 b,w
		L	89 ± 0.04 a	62 ± 0.05 b	3 ± 0.02 b,x
	Argon clarified	*D*	78 ± 0.16 a	66 ± 0.03 b	6 ± 0.01 b,w
		L	86 ± 0.04 a	58 ± 0.08 b	7 ± 0.01 b,wx
1,2 (C34) DGs	*Control*	*D*	19 ± 0.01 a	13 ± 0.01 b	4 ± 0.01 b,w
		L	19 ± 0.01 a	13 ± 0.00 b	5 ± 0.02 b,w
	Filtered	*D*	19 ± 0.00 a	13 ± 0.00 b	3 ± 0.00 b,w
		L	19 ± 0.00 a	12 ± 0.00 cy	3 ± 0.01 b,wx
	Nitrogen clarified	*D*	20 ± 0.00 a	11 ± 0.01 b	3 ± 0.02 b,w
		L	20 ± 0.00 a	11 ± 0.00 c	3 ± 0.00 b,x
	Argon clarified	*D*	16 ± 0.04 a	12 ± 0.01 cy	3 ± 0.00 b,w
		L	19 ± 0.01 a	11 ± 0.02 c	4 ± 0.00 b,w
1,3 (C36) DGs	*Control*	*D*	03 ± 0.00 c	10 ± 0.01 b	3 ± 0.00 a,x
		L	03 ± 0.00 c	09 ± 0.00 by	8 ± 0.04 a,w
	Filtered	*D*	03 ± 0.00 c	11 ± 0.00 b	2 ± 0.02 a,x
		L	03 ± 0.00 c	09 ± 0.00 by	2 ± 0.04 a,x
	Nitrogen clarified	*D*	03 ± 0.00 c	12 ± 0.01 b	1 ± 0.06 a,x
		L	03 ± 0.00 c	12 ± 0.00 b	5 ± 0.01 a,x
	Argon clarified	*D*	07 ± 0.06 c	20 ± 0.14 b	5 ± 0.00 a,xy
		L	03 ± 0.00 c	11 ± 0.02 b	4 ± 0.03 a,x
1,3 (C34) DGs	*Control*	*D*	01 ± 0.00 c	02 ± 0.00 b	7 ± 0.01 a,wx
		L	01 ± 0.00 b	02 ± 0.00 b	8 ± 0.01 a,w
	Filtered	*D*	01 ± 0.00 c	02 ± 0.00 b	7 ± 0.00 a,x
		L	01 ± 0.00 c	02 ± 0.00 by	7 ± 0.00 a,x
	Nitrogen clarified	*D*	01 ± 0.00 b	00 ± 0.00 b	0 ± 0.01 a,w
		L	01 ± 0.00 c	02 ± 0.00 b	8 ± 0.00 a,wx
	Argon clarified	*D*	02 ± 0.01 c	04 ± 0.03 b	7 ± 0.00 a,wx
		L	01 ± 0.00 c	02 ± 0.00 b	8 ± 0.00 a,w

Table 4: The median of organoleptic assessment for olive oils during storage for 12 months as evaluated by Panel testing according to the EU. 1348/2013), by a fully trained group of 8 expert tasters from Bologna University.
ST: Storage time in months. *D: Dark. L: Light.*
+Extra virgin olive oils are expected to have a median of positive attributes more than 1 with 0 sensory defects.

Source of variation	**ST**	Storage condition	Fruity	Bitter	Pungent	Sensory defects	
						Fusty/muddy sediment	Rancidity
	0		4.20	4.20	4.40	0	0
Control	12	D	2.20	2.60	2.10	0	0
		Light	2.1	2.0	2.0	1.8	1.7
	0		4.65	5.50	6.55	0	0
Filtered	12	D	3.40	4.10	3.90	0	0
		Light	2.3	3.6	2.6	1.6	1.5
	0		4.45	4.75	5.75	0	0
Nitrogen	12	D	2.40	3.90	3.90	0	0
clarified		Light	2.3	3.8	3.2	0	0.9
	0		4.90	5.25	6.40	0	0
Argon	12	D	2.40	3.30	3.50	0	0
clarified		Light	2.4	1.6	1.6	0	0.9

Paper 3

Extra Virgin Olive Oil Stored in Different Conditions: Focus on Diglycerides

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Extra Virgin Olive Oil Stored in Different Conditions: Focus on Diglycerides

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Key words: Extra Virgin Olive Oil, Diglycerides,1,2/1,3-DG ratio, GC, Storage conditions.

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4

5 **Abstract**

6 The effects of storage conditions of extra virgin olive oil (EVOO) on the isomerization of
7 diglycerides (DGs) have been investigated. Aliquots of EVOO were stored for 14 months under
8 four different conditions: at 20 °C in darkness and in light, at 4-6 °C in light and at 20 °C in light
9 with argon in the headspace. Samples were analyzed bimonthly: 12 DGs with C34 and C36 (1,2
10 and 1,3 isomers) were tentatively identified and quantified by GC-FID. After 14 months, a clear
11 tendency towards a decrease of 1,2-DGs and a significant increase of 1,3-DGs during storage was
12 observed for all samples. 1,2-DGs were always predominant compared to 1,3-DGs and, for both
13 types, C36 DGs were prevalent compared to C34 DGs. Overall, EVOO stored at 4-6 °C in light
14 showed the highest preservation of 1,2-DGs.

15

16 **Introduction**

17 Extra virgin olive oil (EVOO) is fresh olive (*Olea europaea L.*) juice obtained by mechanical and
18 physical processes (Lozano-Sanchez et al., 2012), and it is well known as one of the major
19 components of the diet of Mediterranean countries. EVOOs consist of triglycerides as the main
20 components (about 98%) and other minor components including diglycerides, free fatty acids,
21 squalenes, sterols, phospholipids, phenolics and different volatile compounds (Boskou, 1996).
22 Some of these minor components, in addition to a high content of mono-unsaturated fatty acids,
23 play a major role in keeping EVOO more stable against oxidation during storage compared to
24 other vegetable oils (Bendini et al., 2009a). Elimination of air in the head space, either by fully
25 filling the EVOO bottles or by its replacement with inert conditioning gas, has been found to add
26 marked improvement in terms of oxidation quality, stability, shelf life and slow down the
27 oxidation process of EVOO (Urda- Romacho 2009; Giovacchino et al., 2002).

Newly produced EVOO contains a low concentration of diglycerides (DGs) (1-3%), which are formed as intermediate products of the incomplete biosynthesis of triglycerides (Spyros et al., 2004) and partial hydrolysis of triglycerides. During storage many changes may occur in DG composition due to isomerization of 1,2-DGs, the predominant form in fresh EVOO, to 1,3-DGs (Sacchi et al., 1991). The effects of storage temperature and exposure to light during different periods of time on the quality of EVOO have been investigated by different authors (Velasco and Dobarganes, 2002; Mendez and Falque, 2007), while other studies have assessed the amount of DGs as an indicative parameter of the freshness of EVOO. Catalano et al. (1994) investigated DGs isomerization occurring in EVOO stored in darkness, at room temperature and at 4 °C. In particular, the results revealed that the 1,2-DGs remained less than 1.5 % after one year of storage for all samples analyzed, while about 10% and 45% of the samples stored at room temperature and at 4 °C, respectively, contained less than 0.4% 1,3-DGs. Furthermore, Pérez-Camino et al. (2001) studied the evolution of the two DG isomer classes in oils obtained from olives of different qualities stored at different temperatures, concluding that triacylglycerol hydrolysis and DG isomerization depended not only on the value of free acidity, but also on the storage temperature. In addition, the 1,3/1,2-DG ratio was a useful parameter for assessing the genuineness of EVOOs with low free acidity during early storage stages.

Another interesting study was carried out by Spyros et al., (2004), assessing olive oil through investigation of 1,2 and 1,3-DG isomerization during 18 months of storage at room temperature, at 5 °C with light and in darkness. The result of the isomerization process was mainly dependent on the initial quality parameters of the oil, and in particular the free acidity. Another study based on the evaluation of olive oil quality in relation to storage conditions through the analysis of DG isomerization was carried out by Cossignani et al. (2007) on samples produced from different olive cultivars stored at 15 °C and at 30 °C in darkness for 12 months. The results showed

52 important differences in the percentage of each individual DG and in the ratio among classes; in
53 particular, samples analyzed at time zero exhibited the highest percentage of 1,2-DGs and the
54 lowest of 1,3-DGs, whereas samples stored at 30 °C showed the highest content of 1,3-DGs
55 suggesting that temperature plays an important role in the isomerization process. More recently, a
56 study carried out by Caponio et al. (2013) investigated the effects of storage of EVOO in green
57 glass bottles in light and darkness for 24 months, providing evidence that the degree of
58 isomerization was affected by the initial hydrolysis level of the oil and by the storage time,
59 although other storage conditions did not show any effect. Overall, these results suggest that the
60 content of DGs and the ratio between isomers might be considered as possible markers to
61 establish the freshness state of an EVOO alongside with other quality parameters defined by
62 official regulations (EU Reg. 61/2011).
63 Therefore, the main aim of this study was to investigate the isomerization processes related to
64 diacylglycerols, and in particular the amounts of 1,2- and 1,3-DGs and relative C34 and C36 sub-
65 classes as well as the 1,2/1,3-DG ratio in EVOO during storage under different conditions for 14
66 months. The purpose was to investigate how these compounds were influenced by different
67 variables such as temperature, light and headspace gases.
68

69 **Materials and Methods**

70 *Samples*

71 EVOO samples used in this study were produced from olives of the Arbequina cultivar (Coop.
72 Sant Bartomeu, Soller, Spain) using an industrial plant working with a three-phase decanter.
73 Once in the laboratory, the EVOO was poured into 250 ml transparent glass bottles. The
74 headspace in each bottle was about 2 ml. The bottles were hermetically sealed and divided into
75 four batches. The first batch was stored in darkness inside a thermostatic chamber at 20 ºC

76 (Cond. 1*)*; the second batch was stored at 20 ºC under diffuse light (600 Lux for 12 h/day 11 W;
77 595 lm; 6400 ºK) simulating the conditions of a supermarket shelf (Cond. 2); the third batch was
78 stored in a refrigerated chamber at 4-6 ºC with diffuse light (Cond. 3); finally, the fourth batch
79 was stored with argon in the headspace of bottles at 20 ºC with diffuse light (Cond. 4). Samples
80 were analyzed in triplicate after 2, 4, 6, 8, 10, 12 and 14 months of storage after production.

81 *Basic chemical analysis*

82 Free acidity, peroxide value and UV absorption (K_{232}, K_{270}) were determined according to the
83 official methods described in EEC Reg. 2568/91 for all samples at the initial period of storage (2
84 months) and after the end of storage simulation (14 months).

85 *Gas chromatographic (GC) determination of diglycerides*

86 The silylated samples were prepared according to a previous work (Sweeley et al., 1963) and
87 DGs were determined according to a modified version of the method suggested by Serani et al.,
88 (2001) using a GC Carlo Erba MFC500 with a Rtx-65TG (Restek, Bellefonte, PA) fused silica
89 capillary column (30 m length x 0.25 mm i.d. x 0.10 µm f.t.) coated with 35 % dimethyl-65 %
90 diphenylpolysiloxane. The oven temperature was programmed from 250 to 320 °C at a rate of 2
91 °C min^{-1} and then increased to 365 °C at a rate of 5 °C min^{-1}. The final temperature was
92 maintained for 21 min. The injector and FID temperatures were both set at 360 °C. Helium was
93 used as carrier gas at a pressure of 130 kPa. The split ratio was 1:70. Identification of DGs was
94 carried out by comparing peak retention times and GC traces with those of DG standards and
95 chromatograms reported in the literature (Serani et al., 2001; Bendini et al., 2009b). The results,
96 expressed as mg of each DG per 100 mg of oil, were quantified with respect to dilaurin, added as
97 internal standard (0.5 mL of a solution 2 mg mL^{-1} of dilaurin dissolved in chloroform, added to
98 100 mg of oil).

99 *Statistical analysis*

100 The software XLSTAT 7.5.2 version (Addinsoft, USA) was used to elaborate the data by analysis
101 of variance (ANOVA, Fisher LSD, $p < 0.05$).

102 **Results and Discussion**

103 The free acidity, peroxide values and extinction coefficients (K_{232} and K_{270}), shown in Table 1,
104 indicated that at the end of the storage period all samples were within the accepted limits
105 established by EU regulations for the EVOO category (EU Reg. 61/2011).
106 Fig. 1 shows a comparison between the gas chromatography traces of DG fractions of EVOO
107 stored for 2 and 14 months in dark at 20 ^{o}C. Twelve different DGs were tentatively identified and
108 quantified as 1,2 and 1,3 isomers with 34 or 36 carbon atoms (C34, C36). Only a co-elution was
109 present (peak 11) between 1,3 isomers of the oleic-linoleic and linoleic-linoleic couples. The
110 peaks numbered from 1 to 6 (Fig. 1) were relative to C34 DGs whereas from 7 to 11 belonged to
111 C36, and palmitic-oleic (PO) and oleic-oleic (OO) were the most abundant DGs for the two
112 classes, respectively. Observing the GC traces (Fig. 1), it is also possible to note that the 1,2
113 isomers eluted before the 1,3 ones for both groups with 34 and 36 carbon atoms.
114 Fig.2 illustrates the evolution of 1,2/1,3-DG ratios, and Tables 2-5 highlight the trends of 1,2-
115 DGs (C34, C36) and 1,3-DGs (C34, C36) for EVOOs stored under the four different
116 experimental conditions. For the samples kept at 20 °C in darkness (Cond. 1), a rapid and
117 significant decrease was observed in the 1,2/1,3-DG ratio for the first 8 months; this ratio
118 continued to decrease slowly until the end of storage period (Fig.2). A similar trend was also seen
119 for the 1,2-DGs C34 and C36 under the same condition (Table 2), and the rapid decrease
120 continued for up to 8 months. At the end of storage period, total 1,2-DG remained about 60 %
121 (data not shown) of total DGs with higher amounts of C36 isomers, in particular diolein, which is
122 considered the predominant DG in olive oil (Boskou, 1996).

123 A comparable behaviour was observed for samples stored at 20 °C in light (Cond. 2).
124 Accordingly, the ratio of 1,2/1,3-DG decreased significantly from 5.43 to 1.69 after 10 months
125 (Fig.2). Moreover, the 1,2-DG C36 isomer (Table 3) decreased significantly from 0.79 to 0.57
126 mg per 100 mg oil at the end of storage period, although this decrease slowed after 10 months.
127 On the other hand, the 1,3-DG C36 isomer showed steady significant increase up to 12 months
128 (Table 3) and then remained with slight changes, until the end of storage. However, 1,3-DG C34
129 isomers showed a significant slight change toward increases, after 6 months of storage, reaching
130 about 0.14 mg per 100 mg sample after 14 months of storage (Table 2).
131 The results for samples stored at low temperature (4-6 °C) (Cond. 3) showed that, at the end of
132 the storage period, the 1,2/1,3-DG ratio remained about 2 times higher than the values for EVOO
133 samples stored at 20 ºC (Fig.2). Furthermore, the 1,2-DGs isomers C36 and C34 showed a
134 significant decrease from 2 to 14 months (Table 4). .
135 Regarding the samples stored with argon in the headspace (Cond. 4), the 1,2/1,3-DG ratio
136 decreased significantly during the first 8 months of storage, and minor changes were detected up
137 to the end of storage (Fig.2). Similarly, 1,2-DGs for both C36 and C34 classes decreased after 14
138 months of storage compared to the initial value, with a fluctuation trend trend (Table 5), while
139 1,3-DG C36 isomers showed a significant increase throughout the entire storage period.
140 By comparing the different conditions, after 2 months of storage the highest 1,2/1,3-DG ratio
141 corresponded to the sample stored at low temperature (4-6 °C), followed by the sample stored
142 under light at 20 °C with argon in the headspace (Fig.2). Moreover, during the first 4 months,
143 when EVOOs were stored at 20 °C under light without headspace modification (Cond. 2), the
144 sample exhibited a lower ratio than the respective sample stored in darkness (Cond. 1). The
145 results also highlighted the positive effect of using inert gas in the head space. The total 1,2-DGs
146 remained after 14 months (data not shown) of storage was about 1.5 times higher, in comparison

147 with their presence in EVOO stored under the same conditions, but with air in the head space.
148 The findings are in accordance with Spyros et al. (2004), suggesting that the length of storage
149 time plays an important role in isomerization changes of DGs, which is accelerated by
150 temperature.
151 The formation of oxidation products by photo-oxidation was confirmed by the high values of
152 K_{270} obtained for samples stored under diffuse light, especially for those stored at 20 °C after 14
153 months of storage (Table 1). It should be noted that, at the end of storage period, all the samples
154 remained within EVOO category parameters. As expected, free acidity (Table 1), which is
155 considered to be the main driving factor affecting DG isomerization (Pérez-Camino et al., 2001),
156 showed only a minor increase after 14 months of storage.
157 The results of this study showed that the isomerization of DGs in EVOOs depends not only on
158 the length of storage, but also on the temperature of storage. This finding is in agreement with the
159 studies of Pérez-Camino et al. (2001) and Cossignani et al. (2007). Moreover, the results showed
160 that after 14 months of storage at 20 °C (Cond. 1, 2 and 4) there were slight but not significant
161 differences in the 1,2/1,3 ratio among samples stored under diffuse light (Cond. 2 and 4) and for
162 those stored in darkness (Cond. 1), in spite of the fact that light exposure has an adverse effect on
163 the oxidation of EVOO (significantly higher K_{270} values were found for samples stored under
164 diffuse light). This result is in agreement with considerations noted by Afaneh et al. (2013).

165 **Conclusion**

166 The results of this study confirmed that the isomerization of DGs in EVOO depends not only on
167 the length of storage, but also on the temperature. By comparing the different conditions, it was
168 found that after 10-14 months of storage the 1,2/1,3-DG ratio remained higher for samples stored
169 at low temperature (4-6 °C). Moreover, the presence of argon gas in the headspace of the sample
170 was not sufficient to protect it from DG isomerization when the EVOO was exposed to light.

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220

221 **Fig. 1.** Example of full chromatogram of the EVOO sample at 20 °C in dark. A) GC tracing of
222 the diglyceride fraction of EVOO stored for 2 months at condition 1; B) GC trace of the
223 diglyceride fraction of EVOO stored for 14 months at condition 1. 1, 1,2-PO; 2, 1,2-PoO; 3, 1,2-
224 PL; 4, 1,3-PO; 5, 1,3-PoO; 6, 1,3-PL; 7, 1,2-OO; 8, 1,2-OL; 9, 1,3-OO; 10, 1,2-LL; 11, 13-OL +
225 1,3-LL. P = palmitic acid; Po = palmitoleic acid; O = oleic acid; L = linoleic acid.

226

227 **Fig. 2.** Trends of 1,2/1,3 DGs during the EVOO storage of 14 months at the four different
228 conditions (Cond 1-4)*. The concentration of DGs was calculated as mg dilaurin per 100 mg of
229 oil. Different letters (a-e) represent significant differences among mean values for a same
230 condition during the storage time (from 2 to 14 months). Different letters (x-z) indicate
231 significant differences among the four storage conditions after 14 months.
232 * Cond. 1, stored at 20 °C in dark, Cond. 2, stored at 20 °C in light, Cond. 3, stored at 6-8 °C in
233 light, Cond. 4 stored at 20 °C in light with argon in the headspace.

234

Table 1 Table 1. Results for free acidity (FA, g of oleic acid per 100 g of oil), peroxide values (PV, Meq O_2 Kg^{-1}) and extinction coefficient at 232 and 270 nm (K_{232}, K_{270}) at time zero and after 14 months of storage under the four different conditions (Cond. 1 - 4)*.

	2 months of storage				**14 months of storage**			
	FA	PV	K_{232}	K_{270}	FA	PV	K_{232}	K_{270}
Cond. 1	0.15 ± 0.01 b,x	11.63 ± 1.29 a,xy	2.11 ± 0.03 b,x	0.10 ± 0.00 b,z	0.20 ± 0.01 a,x	12.74 ± 0.55 a,y	2.34 ± 0.02 a,x	0.15 ± 0.01 a,y
Cond. 2	0.15 ± 0.01 b,x	14.00 ± 0.04 a,x	2.00 ± 0.09 b,xy	0.17 ± 0.01 b,x	0.20 ± 0.01 a,x	14.74 ± 1.02 a,xy	2.19 ± 0.07 a,y	0.18± 0.01 a,x
Cond. 3	0.10 ± 0.01 b,x	10.59 ± 0.01 b,y	1.94 ± 0.12 b, y	0.13 ± 0.00 b,y	0.17 ± 0.01 a,y	15.47 ± 0.80 a,x	2.19 ± 0.06 a,y	0.14 ± 0.00 a,y
Cond. 4	0.16 ± 0.01 b,x	14.00 ± 0.15 a,z	2.15 ± 0.04 b,x	0.17 ± 0.00 b,x	0.20 ± 0.01 a,x	14.70 ± 0.40 a,xy	2.24 ± 0.03 a,xy	0.18 ± 0.01 a,x

Table 2. Evolution of 1,2 and 1,3 isomers of C34 and C36 diglycerides during the EVOO storage of 14 months under condition 1 (at 20 ^{o}C in dark). The concentration of DGs was calculated as mg dilaurin per 100 mg of oil. Different letters (a-e) represent significant differences among mean values for a same isomer during the storage time (from 2 to 14 months).

Different letters (x-z) indicate significant differences among the four storage conditions after 14 months of storage.

Cond. 1				
Months of oil storage	1,3 C34-DGs	1,3 C36-DGs	1,2 C34-DGs	1,2 C36-DGs
2	0.09 ± 0.01 f	0.19 ± 0.03 e	0.48 ± 0.06 a	1.25 ± 0.14 a
4	0.11 ± 0.01 e	0.25 ± 0.02 de	0.47 ± 0.05 a	1.27 ± 0.16 a
6	0.13 ± 0.01 de	0.26 ± 0.01 d	0.38 ± 0.05 b	0.89 ± 0.07 b
8	0.13 ± 0.00 cd	0.33 ± 0.01 c	0.28 ± 0.01 c	0.77 ± 0.02 b
10	0.15 ± 0.00 bc	0.40 ± 0.07 b	0.28 ± 0.01 c	0.75 ± 0.05 b
12	0.16 ± 0.01 b	0.37 ± 0.03 bc	0.27 ± 0.01 c	0.74 ± 0.10 b
14	0.19 ± 0.02 a,x	0.49 ± 0.02 a,x	0.27 ± 0.02 c,yz	0.73 ± 0.05 b,y

Table 3. Evolution of 1,2 and 1,3 isomers of C34 and C36 diglycerides during the EVOO storage of 14 months under condition 2 (at 20 °C in light). The concentration of DGs was calculated as mg dilaurin per 100 mg of oil. Different letters (a-e) represent significant differences among mean values for a same isomer during the storage time (from 2 to 14 months). Different letters (x-z) indicate significant differences among the four storage conditions after 14 months of storage.

Cond. 2				
Months of oil storage	1,3 C34-DGs	1,3 C36-DGs	1,2 C34-DGs	1,2 C36-DGs
2	0.06 ± 0.00 e	0.15 ± 0.01 e	0.35 ± 0.01 ab	0.79 ± 0.15 cd
4	0.09 ± 0.02 d	0.21 ± 0.02 d	0.38 ± 0.05 a	1.06 ± 0.14 a
6	0.12 ± 0.02 cd	0.25 ± 0.02 d	0.37 ± 0.02 a	0.98 ± 0.05 ab
8	0.15 ± 0.01 ab	0.30 ± 0.01 c	0.32 ± 0.01 b	0.85 ± 0.07 bc
10	0.15 ± 0.01 a	0.36 ± 0.01ab	0.22 ± 0.01 c	0.69 ± 0.02 de
12	0.13 ± 0.00 bc	0.39 ± 0.00 a	0.23 ± 0.00 c	0.68 ± 0.00 de
14	0.14 ± 0.01 ab,y	0.32 ± 0.06 bc,y	0.21 ± 0.01 c,z	0.57 ± 0.04 e,z

Table 4. Evolution of 1,2 and 1,3 isomers of C34 and C36 diglycerides during the EVOO storage of 14 months under condition 3 (at 6-8 °C in light). The concentration of DGs was calculated as mg dilaurin per 100 mg of oil. Different letters (a-e) represent significant differences among mean values for a same isomer during the storage time (from 2 to 14 months). Different letters (x-z) indicate significant differences among the four storage conditions after 14 months of storage.

Cond. 3				
Months of oil storage	1,3 C34-DGs	1,3 C36-DGs	1,2 C34-DGs	1,2 C36-DGs
2	0.08 ± 0.01 c	0.14 ± 0.01 d	0.58 ± 0.08 a	1.09 ± 0.18 ab
4	0.08 ± 0.00 c	0.17 ± 0.01 c	0.46 ± 0.02 b	1.25 ± 0.06 a
6	0.12 ± 0.01 ab	0.18 ± 0.01 c	0.41 ± 0.02 bcd	1.01 ± 0.11 b
8	0.12 ± 0.01 ab	0.16 ± 0.01 cd	0.34 ± 0.04 d	0.9 ± 0.12 b
10	0.10 ± 0.03 bc	0.22 ± 0.00 b	0.38 ± 0.01 cd	1.08 ± 0.03 ab
12	0.14 ± 0.02 a	0.25 ± 0.02 b	0.45 ± 0.02 bc	1.03 ± 0.17 b
14	0.13 ± 0.01 a,y	0.28 ± 0.03 a,y	0.39 ± 0.04 cd,x	1.07 ± 0.03 ab,x

Table 5. Evolution of 1,2 and 1,3 isomers of C34 and C36 diglycerides during the EVOO storage of 14 months under condition 4 (at 20 °C in light with argon in the headspace). The concentration of DGs was calculated as mg dilaurin per 100 mg of oil. Different letters (a-e) represent significant differences among mean values for a same isomer during the storage time (from 2 to 14 months). Different letters (x-z) indicate significant differences among the four storage conditions after 14 months of storage.

Cond. 4				
Months of oil storage	1,3 C34-DGs	1,3 C36-DGs	1,2 C34-DGs	1,2 C36-DGs
2	0.07 ± 0.00 c	0.14 ± 0.02 d	0.41 ± 0.08 a	1.07 ± 0.18 ab
4	0.07 ± 0.01 c	0.18 ± 0.01 d	0.32 ± 0.02 bc	0.82 ± 0.13 cd
6	0.14 ± 0.00 b	0.31 ± 0.06 c	0.46 ± 0.05 a	1.09 ± 0.11 a
8	0.15 ± 0.01 b	0.37 ± 0.04 bc	0.29 ± 0.01 c	0.82 ± 0.02 d
10	0.21 ± 0.01 a	0.46 ± 0.04 ab	0.38 ± 0.01 ab	1.06 ± 0.21 abc
12	0.17 ± 0.01 b	0.48 ± 0.06 a	0.25 ± 0.01 c	0.70 ± 0.05 d
14	0.21 ± 0.04 a,x	0.53 ± 0.10a,x	0.31 ± 0.06 bc,y	0.84 ± 0.15 bcd,y

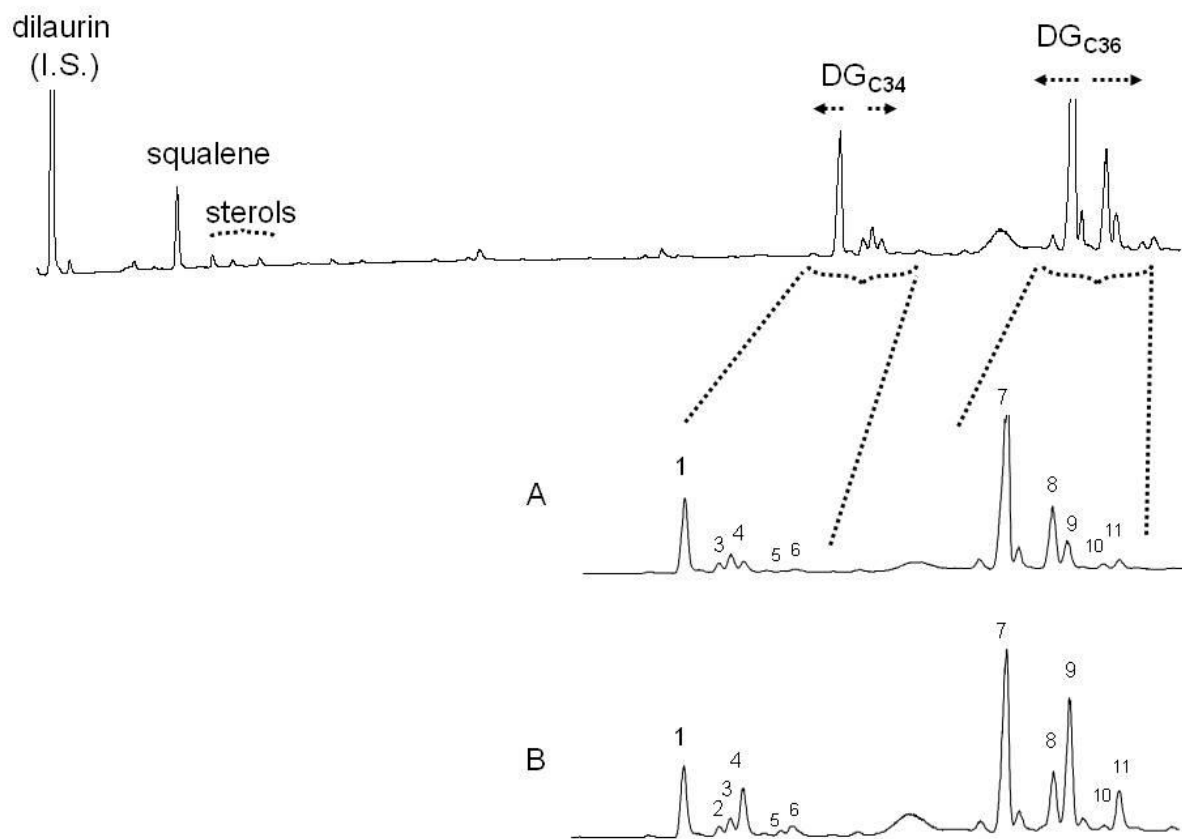

Figure 1

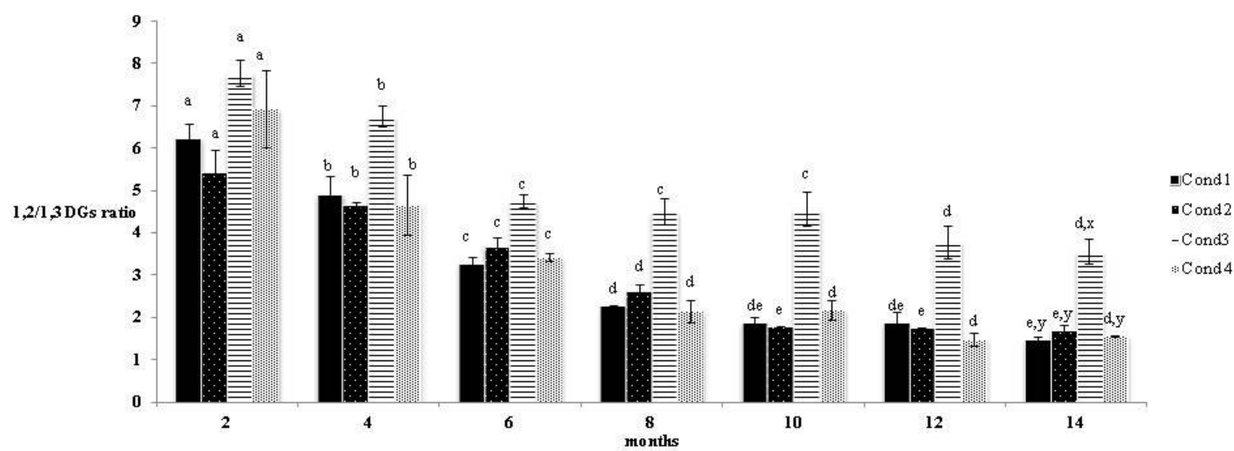

Figure 2

Paper 4

Effects of Temperature Fluctuation in VOO Oxidation Quality and Shelf life

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Effects of Temperature Fluctuation in VOO Oxidation Quality and Shelf-life

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Abstract:

High temperature and temperature fluctuations were the main factors that affect the virgin olive oil quality in terms of hydrolytic degradation, oxidation stability and olive oil freshness. In this study virgin olive oil samples of different quality grades (extra virgin and lampante virgin olive oils) were subjected to moderate accelerated storage conditions under static and fluctuated temperature for 30 days. Samples were analyzed for their chemical and sensory properties every 10 days of storage. The results revealed that, after 30 days of the simulation, there were no significant differences between extra virgin olive oil under both the conditions in term of acidity, peroxide value, K_{270} and *ortho*-diphenols content. The extra virgin olive oil sample was declassed to virgin category after the accelerated storage under static temperature. Lampante virgin olive oil showed a different behavior in term of k_{270} and peroxide value. Diglycerides results indicated that, the loss of freshness of extra virgin olive oil was nearly the same under both the conditions. This research highlighted that, the fluctuation in temperature has the similar effect as static high temperature in some chemical properties of extra virgin olive oil.

List of abbreviations

FFA: free fatty acid percentage
VOO: virgin olive oil
EVOO: extra virgin olive oil
LVOO: lampante virgin olive oil
ST: static temperature of 45 ^{o}C
FLT: fluctuated temperature (5-42 ^{o}C)
PV: peroxide value
ortho-dph: *ortho*-diphenols
DGs: Diglycerides

Keywords: *static storage temperature, fluctuating storage temperature, extra virgin olive oil, lampante virgin olive oil, acidity percent, peroxide value, extinction coefficient, diglyceride, ortho-diphenols, sensory evaluation*

Introduction:

Virgin olive oil (VOO) is the fresh extract of olive fruit produced solely by mechanical and physical processes and discriminated from other kinds of edible oils by its characteristic aroma and flavour as well as its organoleptic and nutritional properties (Cerretani et al. 2008). VOO consists predominantly from triglycerides, but contain also a range of minor component (phenolic compounds, free fatty acid, sterols, pigments and diglycerides). The quantity and evolution of these minor compounds, usually contribute to the determination of VOO quality and freshness during storage (Mancebo-Campos et al. 2007; Catalano et al. 1994). In addition to the VOO high content of mono-unsaturated fatty acids with respect to other vegetable oil, minor components are important for VOO stability against oxidation during storage (Bendini et al. 2009a). It's well known that during long storage time, several factors can affect the shelf life of VOO including, temperature, light, oxygen. These factors can initiate the oxidation deterioration, thus altering EVOO quality during storage (Mendez and Flaque. 2007). Temperature considered one of the main factors that affect the VOO quality. In fact, lipid hydrolytic and oxidation reactions are accelerated by the increase in temperature and temperature variations. Temperature variation during storage induce the quality loss and development of the off-flavour as a result of volatile and nonvolatile degradation by-products (Bendini et al. 2009). Therefore, VOO should be protected from temperature fluctuation in order to maintain its freshness and acceptability (Boskou, 2006). The temperature effects on the virgin olive oil quality during storage under normal and accelerated condition have been focused by many authors (Mancebo-Campos et al. 2007; Gomez-Alonso et al. 2004; Velasco and Dobarganes, 2002). Many researchers evidenced that EVOO can reserve its oxidation quality at "moderate" accelerated storage temperature (35-40 ^{o}C) without substantial modifications for not less than 6 weeks. Moreover, the resistance of VOO to the temperature variation depends on its initial quality. For instance, Mancebo-Campos et al. (2008) measured peroxide value (PV) and, K_{270} for different virgin olive oil samples and found that, the first EVOO sample under study was degraded from the extra virgin category after about 6 weeks.

The same observations were already found also by Pristouri et al. (2010) where the EVOO samples stored at 35 ^{o}C were lost its extra virgin quality within 3 months of storage. The same trend was also evidenced in a recent study carried out by Mancebo-Campos et al. (2014) where the upper limit of extra virgin was never reached before 16 weeks of storage at 40 ^{o}C. The

previous researches carried out on the effect of temperature fluctuation were mainly dealt with the frozen perishable food products that suffers from extreme fluctuation in temperature during storage (Alvarez and Canet, 2000; Gormley et al. 2001). To the best of our knowledge's, there were no published studies concerning the effect of temperature variation on olive oil. Such fluctuation in temperature may occur in the markets during the day and night sequential change. Such variation also might occur during the winter season at which, the temperature conditioning systems might be in function throughout the day and probably switches off at the night time, in addition, such case of temperature fluctuation could occur during virgin olive oil shipment and transportation.

The main objective of this study was to compare the influence of fluctuation in temperature on the quality of virgin olive oil of different categories with the effect of accelerated constant storage temperature. The study will investigate the effects of temperature changes on the quality of olive oil. This study compare selected virgin olive oil quality indicators during and after accelerated storage under fluctuated temperature with samples of the same batch held under static temperature throughout the 30 days..

Materials and methods

Samples

Hermetically sealed 250 ml bottles of extra virgin olive oil (EVOO) extracted from "Canino" cultivar in November 2012 and lampante virgin olive oil extracted from the olive fruits that were stored for 15 days after picking. Storage simulation was started in February 2013. The samples from both VOO categories were stored at constant temperature (45 °C) for 30 d. Another set of samples from the same batch was stored under fluctuating temperature, which increased from 5 °C to 45 °C at a rate of 0.3333 °C/h for 5 d and decreased to 5 °C at the same rate for another 5 d. The total time for accelerated storage simulation was 720 h (Fig. 4). The lower temperature was chosen to avoid olive oil solidification at temperatures lower than 5 °C (Piscopo and Poiana, 2012). All chemical analysis were performed in triplicates for each kind of sample at each respective time of analysis

Chemical analyses: The free fatty acids % (FFA %), peroxide value (PV) and UV absorption indexes (K_{270}), were analyzed according to the official methods described in EEC. Reg. 2568/91.

The total *ortho*-diphenols compounds (*ortho*-dph), were evaluated at 370 nm and expressed as mg of gallic acid kg^{-1} oil, respond to Pirisi et al. (2000) using UV−vis 6705 spectrophotometer (Jenway, UK). Diglyceride analyzed for silylated samples that prepared according to (Sweeley et al. 1963) and were determined according to a modified version of the method suggested by Serani et al. (2001) using a GC Carlo Erba MFC500 with a Rtx-65TG (Restek, Bellefonte, PA) fused silica capillary column (30 m length x 0.25 mm i.d. x 0.10 µm f.t.) coated with 35 % dimethyl-65 % diphenylpolysiloxane. The oven temperature was programmed from 250 to 320 °C at a rate of 2 °C min^{-1} and then increased to 365 °C at a rate of 5 °C min^{-1}. The final temperature was maintained for 21 min. The injector and FID temperatures were both set at 360 °C. Helium was used as the carrier gas at a pressure of 130 kPa. The split ratio was 1:70. Identification of diglycerids (DGs) was carried out by comparing peak retention times and GC traces with those of DG standards and chromatograms reported in the literature (Serani et al. 2001; Bendini et al. 2009b). The results, expressed as mg of each DG per 100 mg of oil, were quantified with respect to dilaurin, added as an internal standard (0.5 mL of a solution 2 mg mL^{-1} of dilaurin dissolved in chloroform, added to 100 mg of oil).

Sensory analysis: A sensory analysis of all the VOO samples was performed according to the ECC Reg. 640/2008 by a fully trained group of 8 expert tasters.

Statistical analysis. Analysis of variance (ANOVA) is performed with XLSTAT 7.5.2 (Addinsoft, NY, USA) at a 95% confidence level (Fisher LSD, $p < 0.05$).

Results and discussions

Effect of constant Vs fluctuation in temperature on Free acidity and DGs isomerization:-

A significant increase in FFA % (Table 1) was showed, up to 20 days for EVOO samples subjected to ST condition, the same trend was also observed for the sample stressed by FLT condition, followed by no significant changes during the last 10 days under both the conditions. These results, in fact, were associated with the high temperature during the 30 days of accelerated storage.

In comparison, between the both accelerated storage conditions regarding free fatty acid accumulations in EVOO samples, it was clearly found that there were no significant differences

between both conditions were recorded, at the end of the storage simulations. In fact that, the samples stored under fluctuated temperature were affected by 45 ^{o}C for about 18 hours during the overall storage period (Fig.1).

In respect to LVOO samples which were, contained a high amount of free fatty acids before starting the accelerated storage experiment. However, similar to EVOO sample, no significant variations between FLT and ST samples were detected after the storage period of 30 days. Diglyceride is the minor compounds that contribute in about 1- 3 % of the fresh virgin olive oil polar fraction. DGs accumulations as total amount and there isomerization behavior was considered as a beneficial indicator for assessing the olive oil freshness (Catalano et al. 1994). The results showed that 1,2/1,3-DG ratio (Table.1) for EVOO samples exhibited sharp significant decrease in the samples under both the conditions after 10 days of storage simulation. This behavior, highlight, the drastic effect of high temperature in the DGs isomerization process, despite, the fact that EVOO under FLT condition was subjected to high temperature (45 ^{o}C) for a shorter time (18 hours) than ST subjected EVOO (720 hours), the overall storage time. The result also showed that during storage time, the 1,2/1,3-DG ratio obtained in the EVOO samples under both the conditions, was less than “2” with significantly higher value in the FLT EVOO subjected sample. In the case of LVOO sample, the same trend as EVOO was recorded where of 1,2-DGs decreased sharply in the first 10 days of simulation, then little change where evidenced during the rest of the storage period. This result indeed, indicated that the effect of temperature on the DGs isomerization rate was marked at the initial time of samples exposure to high temperature, after which, the effect was diminished, toward inducing a sharp change in 1,2/1,3-DG ratio. At the end of storage time, there was no significant difference in 1,2/1,3-DG ratio in LVOO samples under both the conditions.

As already seen in the case of 1,2/1,3-DG ratio, total amount of 1,2-DGs C36 and C34 (Fig.2) showed about 40 and 30 % reduction in the EVOO samples under ST and FLT storage, respectively, during the first 10 days, then, minor changes were recorded, in particular, for the sample stored under FLT condition till the end of storage time. Similar behavior was achieved in term of increase in the total amount of 1,3-DGs C36 and C34 classes isomers, except that the increase of the same isomer was slight at FLT subjected EVOO sample. The results also demonstrated that there was equilibrium in the quantity of 1,2 and 1,3-DGs isomers after 10 days for the EVOO sample under ST condition which indicates the strong influence of temperature on

the isomerization process of DGs. On the other hand, such equilibrium has never been attained in the case FLT (EVOO) sample. Moreover Fig.2 showed that 1,2-DGs C34 decreased from 0.32 to 0.1 and from 0.32 to 0.16 mg 100^{-1} for EVOO samples under ST and FLT respectively. A similar trend was also observed for 1,2-DGs C36 that decreased from 1.27 to 0.47 and from 1.27 to 0.67 mg 100^{-1} for the same sample under ST and FLT storage condition respectively. On the other hand 1.3-DGs C34 increased from 0.07 to 0.19 and from 0.07 to 0.12 for the same sample under ST and FLT conditions respectively. A similar trend toward the increase in 1,3-DGs C36 isomers were recorded for the EVOO sample under both the conditions. Isomerization changes in DGs-C36 isomers were more pronounced, because of the fact that the C36 fatty acids like oleic acid are the predominant fatty acid in the olive oil (Boskou et al. 2006). These results indicated that, although the effect of static high temperature on DGs isomerization were higher than the effect of fluctuated temperature considering that the samples were affected by the temperature of 45 ^{o}C for 18 hours during the storage period. Nevertheless, the both conditions have almost similar effect on EVOO freshness as evidenced by DGs isomerization change.

Regarding LVOO sample that, before the storage, possessing a high amount of 1,3-DGs isomers (Fig. 2). This high amount of 1,3-DGs in LVOO sample, revealed the bad effect of the quality status of virgin olive oil in particular, high free fatty acid content on the DGs isomerization process (Pérez-Camino et al. 2001). In the case of LVOO sample as stated before, the effect of temperature was noted in the first 10 days, then a little change was observed in the last 10 days of accelerated storage under both the conditions. Furthermore, almost similar content of the 1.2-DGs C34, and 1,2 C36 was evidenced under the both conditions during the simulation of 30 days. Similar trends also showed for 1,3-DGs classes which were increased nearly, within the same level under both the storage conditions, this behavior could occure as a result of combined effects of temperature of storage and high free fatty acid content in LVOO samples.

Effect of constant Vs fluctuation in temperature on oxidation stability:-

Peroxide value showed frequent variation (Table.2) during the 30 days of storage under both ST and FLT conditions for EVOO sample, without being significant, the variation between ST and FLT (EVOO) samples. Indeed, the limit of 20 meq/kg oil was never reached after 30 days of storage. According to K_{270} results which indicate the secondary oxidation products. The significant increase was recorded after 20 and 30 days for EVOO samples under ST and FLT

conditions respectively (Table.2). No significant difference was recorded between both the thermal stress conditions at the end of the experiment. Concerning the LVOO, decrease in PVs were confirmed in term of increasing the K_{270} values after 30 days of storage. Although such higher value was not significant for the sample under FLT condition without being significant the difference between the samples under both condition. However, the increase of K_{270} values at the end of storage resulted from the accumulation of secondary oxidation products during the simulation period (Abbadi et al. 2014). After the end of storage time under both the conditions, EVOO K_{270} value was remained within the accepted limit by EEC Reg. 2568/1991 and the following amendments for EVOO category.

Effect of constant Vs fluctuation in temperature on, ortho-diphenols and sensory evaluation

ortho-dph is considered one of the most efficient phenolic compounds as an antioxidant (Boskou, 2006). These compounds have a high contribution to the protection of virgin olive oil against oxidation reactions (Bendini et al. 2007). The results (Fig. 3) showed generally a significant and dramatic degradation of *ortho*-dph compounds in the EVOO samples, under both conditions. These results indicated that an ultimate oxidation resistance was involved as a result of high temperature stress in this study. A loss of more than 60 % was recorded under FLT temperature, even though the samples were not exposed to the highest temperature (45 ^{o}C) all over the period of storage (about 18 hours). At the end of experiment, not significant, the variation in terms of *ortho*-dph content between the both stress conditions. Regarding the *ortho*-dph content of LVOO samples (Fig. 3), the results showed that, there were no significant differences observed between the samples subjected to both conditions at the end of storage. It is important to indicate that, the sharp increase in free acidity during simulation, especially for LVOO samples, in addition to the high temperature involved in the experiments, could accelerate the degradation of complex phenolic compounds and decrease the oxidative stability of EVOO (Brenes et al. 2001).

At the end of storage, FLT (EVOO) samples remained without any sensory defects, while the other sample subjected ST condition declassed to virgin olive oil (Fig. 4).

Conclusions:

According to the previously displayed results, after 30 days of accelerated storage condition under static and fluctuated temperature, EVOO samples results showed no significant differences

in FFA%, peroxide value and K_{270} between the ST and FLT conditions. The same behavior was observed for PV results of EVOO. The result showed that there was no significant variation in *ortho*-dph content and 1,2/1,3DG ratio in EVOO under the both conditions with a dramatic DGs isomerization change were evidenced under the both conditions. From this preliminary study results, it can be concluded that, the fluctuation in temperature could have harmful impact on virgin olive oil quality even though, the stress time at which the sample exposed to high temperature was less than that, under the static temperature storage condition.

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LEGENDS FOR FIGURES

Fig. 1: Temperature profile for the ST and FLT storage conditions. Duration: 720 hours (30 days), highest temperature: 45 ^{o}C, lowest temperature: 5 ^{o}C.

Fig .2 1,2 and 1,3 DGs glasses (34 and 36) behaviour (mg 100 oil^{-1}) for Extra virgin olive oil and Ordinary Virgin Olive Oil sample subjected to static temperature 45 ^{o}C and fluctuated temperature (5-45 ^{o}C) during 30 days.

Fig . 3 *ortho*-Diphenol behaviour (mg Kg oil^{-1}) for Extra virgin olive oil. (EVOO) (A) and lampante Virgin Olive Oil (LVOO) (B) subjected to static temperature 45 ^{o}C and fluctuated temperature (5-45 ^{o}C) during 30 days.

Different letters (A-C) indicate statistical significant differences between 0 and 30 days of accelerated storage for the same condition; letters (X-Y) indicate significant differences among the tow accelerated storage conditions (ST and FLT) related to the same storage time

Fig. 4: Sensory analysis radar diagram of EVOO before simulation (A), after 30 days (B) and LVOO (C) after 30 of storage under static and fluctuated temperature.

Table.1 free fatty acid % (FFA%)and DGs ratio (± standard deviation) for EVOO and LVOO samples subjected to static temperature 45 ^{o}C, and fluctuated temperature (0-45 ^{o}C, each 10 days).

*Different letters (A-C) indicate statistical significant differences between 0 and 30 days of accelerated storage for the same condition; letters (X-Y) indicate significant differences among the tow accelerated storage conditions (ST and FLT) related to the same storage time.

Sample	Stress Tim	Stress condition			
		ST	FLT	ST	FLT
		FFA%	FFA%	1,2/1,3-DG ratio	1,2/1,3-DG ratio
(EVOO)	0	0.37 ± 0.03 C	0.37 ± 0.03 C	4.92 ± 0.09 A	4.92 ± 0.09 A
	10	0.51 ± 0.02 B,X	0.53 ± 0.01 B,X	1.08 ± 0.1 B,Y	1.71 ± 0.02 B,X
	20	0.61 ± 0.01 A,X	0.61 ± 0.00 A,X	0.72 ± 0.03 C,Y	1.56 ± 0.01 BC,X
	30	0.61 ± 0.00 A,X	0.61 ± 0.01 A,X	0.59 ± 0.01 C,Y	1.40 ± 0.2 C,X
LAMPANTE Olive Oil	0	3.06 ± 0.18 B	3.06 ± 0.18 B	0.88 ± 0.00 A	0.88 ± 0.00 A
	10	3.30 ± 0.17 B,X	3.47 ± 0.00 A,X	0.42 ± 0.02 BC,X	0.45 ± 0.03 B,X
	20	3.74 ± 0.04 A,X	3.49 ± 0.06 A,Y	0.42 ± 0.00 B,X	0.44 ± 0.02 BC,X
	30	3.68 ± 0.04 A,X	3.62 ± 0.04 A,X	0.40 ± 0.01 C,X	0.40 ± 0.00 C,X

Table .2 Peroxide value and Uv absorption coefficient at wavelength 270 (± standard deviation) of EVOO and LVOO samples subjected to static temperature 45 °C, and fluctuated temperature (0-45 °C, each 10 days) during 30 days.

*Different letters (A-C) indicate statistical significant differences between 0 and 30 days of accelerated storage for the same condition; letters (X-Y) indicate significant differences among the tow accelerated storage conditions (ST and FLT) related to the same storage time.

Sample	Stress Tim	Stress type			
		ST	FLT	ST	FLT
		PV (meq Kg oil^{-1})	PV (meq Kg oil^{-1})	K_{270}	K_{270}
EVOO	0	9. 6± 0.5 AB	9.6 ± 0.5 AB	0.17 ± 0.00 B	0.17 ± 0.00 B
	10	9.2 ± 0.8 B,X	9.1 ± 1.0 B,X	0.19 ± 0.00 B,X	0.18 ± 0.00 B,X
	20	10.9 ± 1.1 A,X	10.8 ± 0.3 A,X	0.20 ± 0.01 A,X	0.18 ± 0.00 B,Y
	30	8.1 ± 0.1 B,X	9.3 ± 0.9 AB,X	0.20 ± 0.01 A,X	0.20 ± 0.00 A,X
LVOO	0	10.2 ± 0.3 A	10.2 ± 0.3 A	0.25 ± 0.01 C	0.25 ± 0.01 A
	10	7.6 ± 0.5 B,X	7.9 ± 0.1 C,X	0.26 ± 0.00 B,X	0.25 ± 0.02 A,X
	20	9.5 ± 0.9 A,X	9.8 ± 0.3 B,X	0.26 ± 0.00 B,X	0.25 ± 0.01 A,X
	30	8.3 ± 0.2 B,Y	9.7 ± 0.2 B,X	0.27 ± 0.00 A,X	0.26 ± 0.00 A,Y

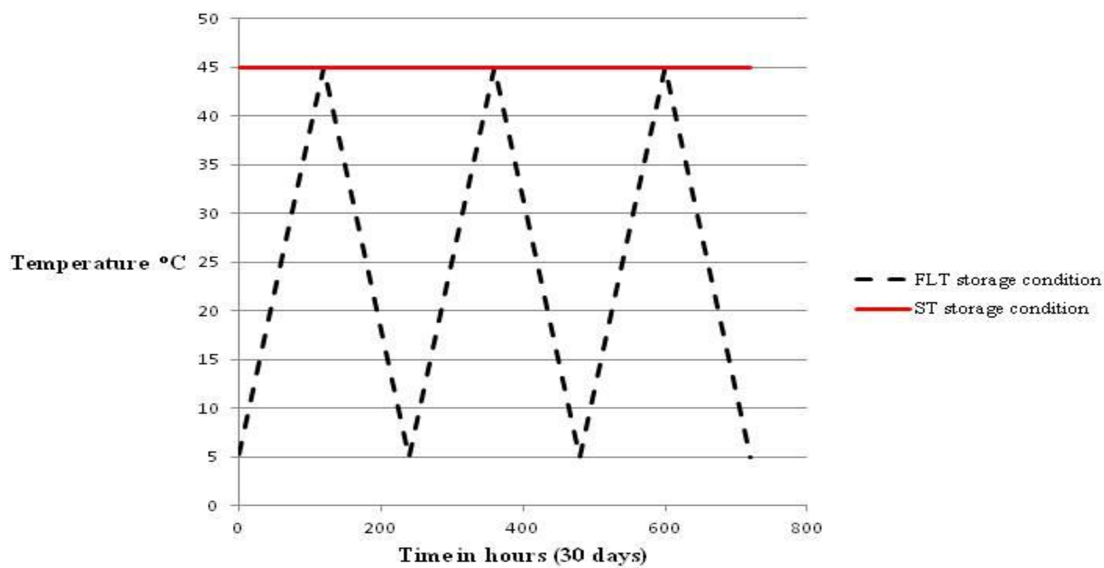

Figure 1

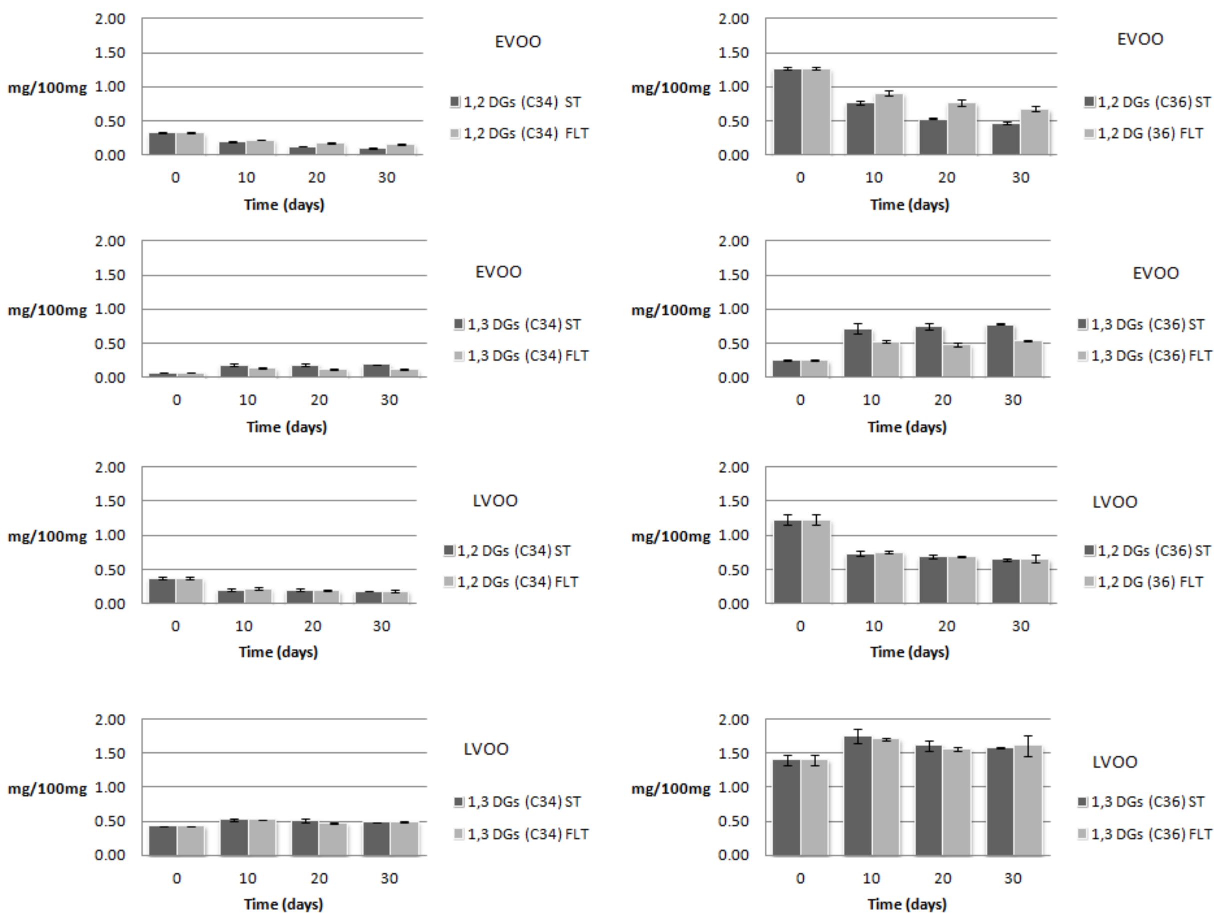

Figure 2

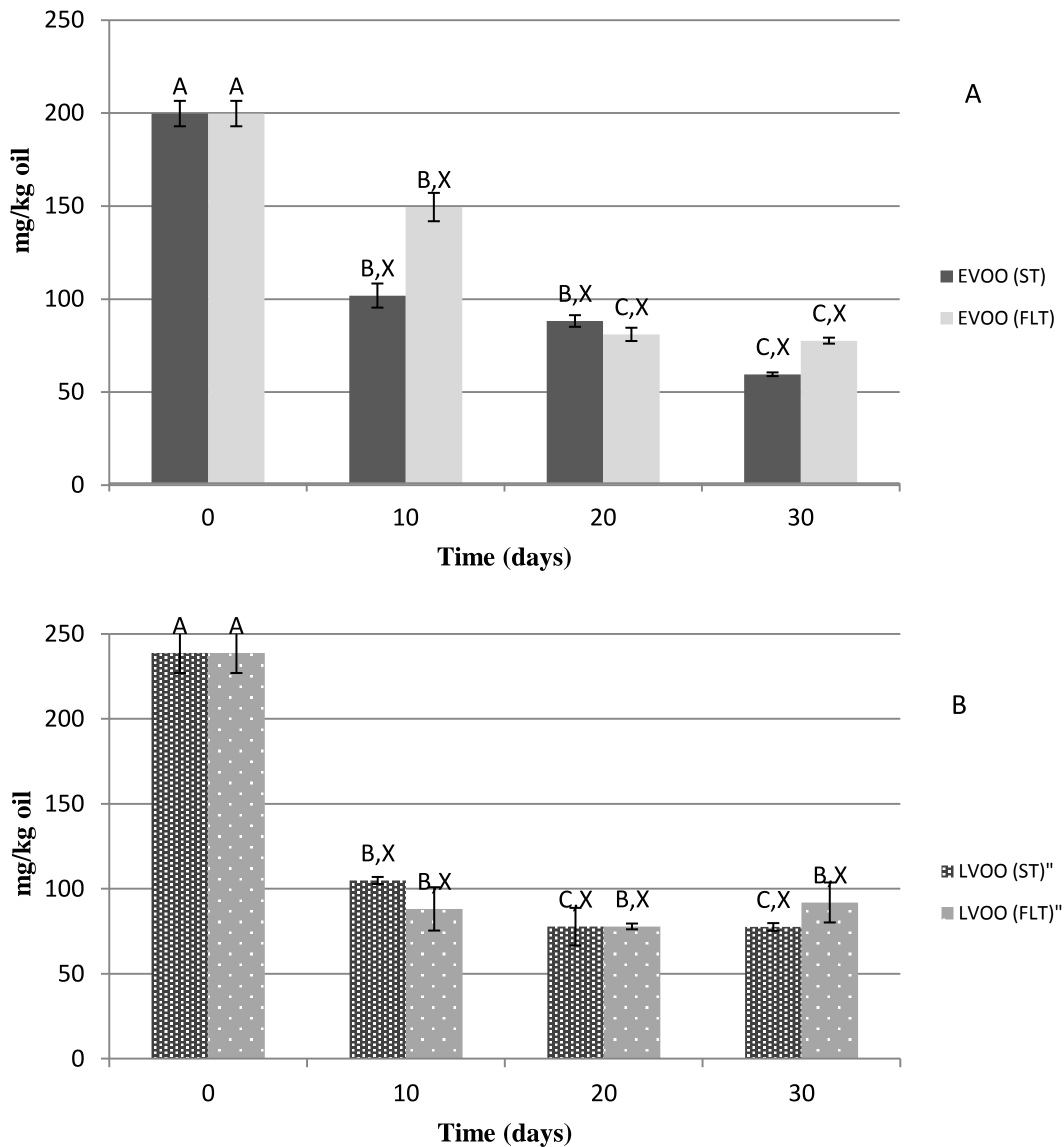

Figure 3

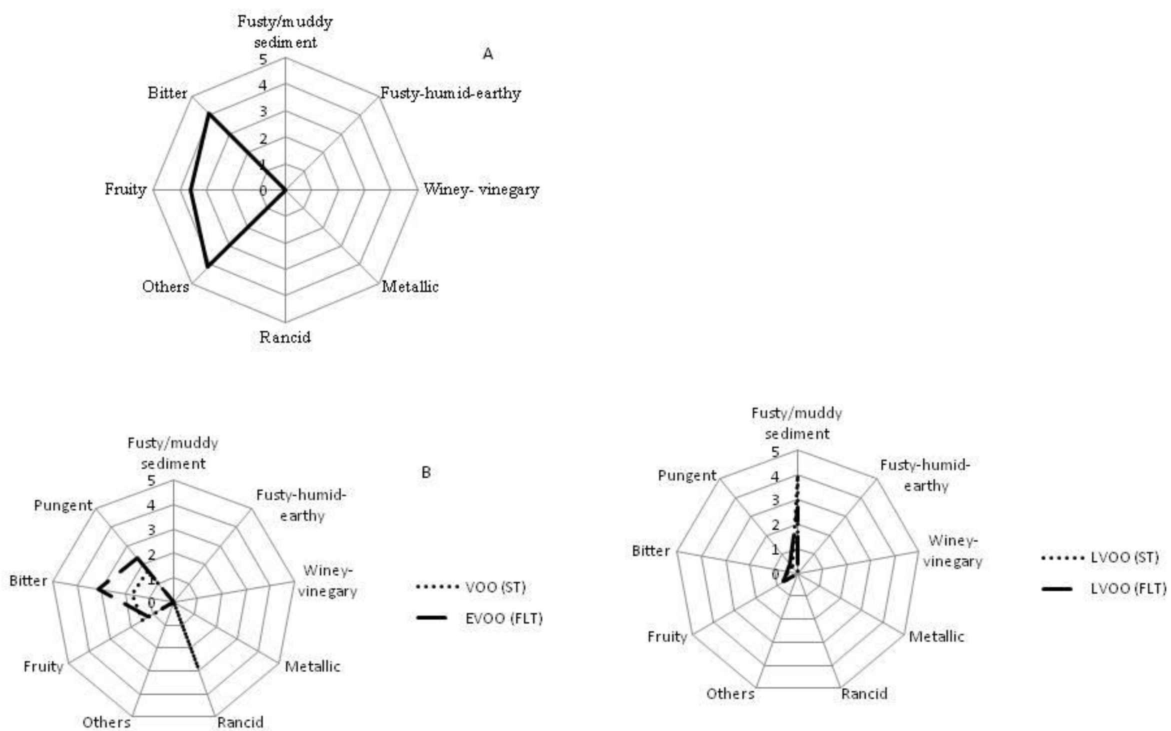

Figure 4

Paper 5

Sustainability and quality in the food supply chain.

A case study of shipment of edible oils.

British food Journal

Manzini R, Accorsi R, Ayyad Z, Bendini A, Bortolini M, Gamberi M, Valli E, Gallina Toschi T

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Sustainability and quality in the food supply chain. A case study of shipment of edible oils

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Abstract

Purpose – Modern supply chains collect and deliver products worldwide and link vendors and consumers over thousands of miles. In the food industry, the quality of products is affected by manufacturing/processing and logistics activities, such as transportation and packaging. Specifically, transportation is likely the most critical step throughout the "food journey" from farm to fork because of the potential stresses that affect the products during shipment and storage activities. The purpose of this paper is to present and apply an original assessment of quality, safety and environmental effects due to the international distribution of food products via different container solutions. A case study that examines the shipment of edible oils from Italy to Canada demonstrates that the quality of a product at the place of consumption can be significantly affected by the use of different containers.

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Design/methodology/approach – A simulation-based quality assessment, combined with a life cycle and environmental analysis, supports the logistic manager in the decision-making process in order to guarantee the highest level of product quality at the place of consumption.
Findings – The proposed approach and the illustrated case study demonstrate the importance of conducting safety and quality assessment combined with environmental analyses of sustainable food supply chains.
Originality/value – This paper highlights the interdependency of implications and decisions on food quality and environmental sustainability of supply chain processes and activities.
Keywords Supply chain, Food, Freight container, International shipment, Life cycle assessment (LCA), Edible oil
Paper type Research paper

1. Introduction

Global supply chains ship products worldwide by linking vendors and demand over thousands of miles. Consumers can detect the brand and the origin of the products according to the package label, which usually even reports certifications and standards. Despite the amount of data that tags the products, further details that describe the efficiency, quality and impact on the environment of transportation as well as other logistic activities have not yet been identified. This information might play a key role in marketing to affect the purchasing behavior of consumers.

Global food supply chains are expanding to match worldwide seasonal food production and demand, following a trend that is expected to accelerate in the future (Ahumada and Villalobos, 2009; World Bank, 2013). Food specialties will increase exportation because consumers seek quality, taste, flavor and specific healthy properties worldwide.

The quality and taste of food products depend both on harvesting and manufacturing/processing as well as on logistics, transportation and packaging processes (Manzini and Accorsi, 2013). In particular, transportation and packaging are critical issues in the "food journey" due to the stressors affecting the shipment and storage activities within the food supply chain (FSC). Quality (including customer service level and satisfaction), safety, sustainability and cost efficiency are the main targets of an effective FSC.

This paper aims to present an original assessment of the effects on quality and further environmental factors of adopting different container solutions to internationally distribute food products. This analysis is supported by the development and application of an original control system, which consists of an on-field monitoring activity combined with a laboratory simulation process and a chemical and sensorial plan of analysis of the food products that are virtually shipped to the consumers. The methodology and the technological devices that comprise this closed-loop control system are illustrated in Manzini and Accorsi (2013).

Manzini and Accorsi (2013) do not conduct chemical and sensorial analyses of food products in addition to the quality analysis of food products at the place of consumption and the environmental assessment due to logistic solutions. The novelty and principle improvement of this manuscript lies in the inclusion of these analyses.

To this end, a case study of a shipment of edible oils from Italy to Canada is illustrated. The results demonstrate that the quality is significantly affected by the use of a standard (or non-standard) container.

The remainder of the paper is organized as follows. Section 2 presents a review of the literature on topics and issues studied in this paper. Section 3 presents the original methodology developed, proposed and applied to conduct the proposed joint assessment. Sections 4-6 present the case study, the analyses conducted and the results

obtained in the selected international shipment of edible oil packages. Section 7 presents and discusses the main conclusions and further research issues.

2. Review of the literature

Global supply chains strengthened worldwide transportation trends and encourages companies to control more accurately the quality of products during the distribution activities. Despite the global financial crisis, the top ten exporters of agricultural products in 2011 recorded growth rates of 15 percent or more (World Trade Organization (WTO), 2012). This growth is partly due to the increase in the prices and the value of exported food products and the agri-food raw materials. In 2011, China, Japan and the USA recorded the highest percentage increases in food and drink imports at 27, 23 and 17 percent, respectively, which confirmed the trend over the last five years (WTO, 2012).

The increasing trend in the global food distribution highlights new challenges for shippers, importers and logistic providers. Logistic managers and practitioners determine the proper distribution system, the transportation means and the use of freight containers.

Various container systems are available depending on the requirements of the shipped products (i.e. natural, partially air-conditioned or temperature-controlled atmosphere) including ventilated container, refrigerated container and, further, sub-categories, including insulated containers equipped with thermal insulations.

The primary role of each container system is to preserve the cargoes from a wide range of environmental stresses, which can affect the storage, handling and transport as well as the associated operations. These stresses are classified as follows: static/dynamic mechanical stresses (e.g. vibration and pressure), climatic stresses (e.g. temperature, humidity, dust and ultraviolet light), biotic stresses and chemical stresses (Wild, 2012).

In FSC, the large set of decisions on logistics and operations, including packaging and containment issues, affect not only costs but also the quality of products and processes and the level of sustainability and safety of the supply system. These factors have direct and indirect impacts on the safety, health and well-being of consumers.

These effects motivated this manuscript, whose main goal is to assess the quality and safety of different edible oils shipped from Italy to Canada in response to the adoption of different container solutions. This analysis is supported by a life cycle assessment (LCA) of the available containment solutions. To further support these arguments, the authors present a brief discussion of quality and safety issues in FSC, sustainability issues in FSC, and edible oil properties and critical factors.

2.1 Quality, safety and sustainability

Food deterioration essentially depends on intrinsic and extrinsic factors, such as the storage temperature, the concentration of oxygen, the relative humidity, the solar radiation, the acidity, the microbial growth, the endogenous enzyme activities, and so on (Alasalvar *et al.*, 2001; Howard *et al.*, 1994; Zhang *et al.*, 2009). The chemical deterioration of food is caused by adverse reactions (e.g. oxidation) that affect sensitive components, such as polyphenols, fats, vitamins and flavorings (Xia and Sun, 2002). This process has negative consequences on the quality of food products, e.g. edible oils and cheeses (Fox *et al.*, 2004; Goff and Hill, 1993).

Consequently, food quality is determined by age and environmental conditions, which depend by the type of packaging, loading method and the availability of temperature-controlled packages, transportation modes, etc.

Virgin olive oils are rich in polyphenols (Bendini *et al.*, 2007), which play a favorable role in preventing cardiovascular disease and delaying cellular aging and death. This content can be sensitive to physical and environmental stresses.

The packaging and container system play a special role in the determination and control of quality and safety of food products, such as fruits and vegetables (Singh and Xu, 1993), wines (Robinson *et al.*, 2010) fish (Margeirsson *et al.*, 2012), etc.

Temperature is renowned as one of the most critical factors affecting the quality and the state of conservation of perishable foods. Rodriguez-Bermejo *et al.* (2007) analyzed maritime shipments by comparing the temperature records tracked within freight reefer or standard containers (SCs). This study tests and analyzes different experimental conditions, such as cooling modes, the onset of defrosting and two varying set points.

Several literature studies focussed on the influence of one-parameter stress (such as temperature, humidity, vibrations) on products (Xiang and Eschke, 2004; Chonhenchob *et al.*, 2012; Raghav and Gupta, 2003; Mahajerin and Burgess, 2010). However, contributions that integrate the environmental impacts of food transportation, on-field stress monitoring activity, laboratory simulation and chemical analysis of food products at the place of consumption are lacking. Therefore, this paper constitutes an original contribution whose main focus is on container solutions for edible oils.

Carter and Easton (2011) presented a systematic review of the literature on sustainable supply chain management. They analyzed the evolution of the SCM from a so-called "standalone" approach to a corporate social responsibility (CSR)-based approach.

The literature on operations and management as well as that on food science and technology increasingly focussed on original contributions to the environmental sustainability evaluation of new and existing food products, processes and systems. In particular, LCA assesses products and processes along the entire life cycle (LC) from a "cradle to grave" perspective and is based on the analysis of materials and energy flows at each phase of the LC (Institute for Environment and Sustainability (IES) *et al.*, 2010; International Standard Organisation (ISO), 1997, 1998). This approach consists of four analysis steps:

(1) goal and scope definition;

(2) life cycle inventory (LCI);

(3) life cycle impact assessment; and

(4) the interpretation of the results.

The analysis of the environmental impacts of transportation activities is widely debated in the literature (Corbett *et al.*, 2009; Chang *et al.*, 2013).

Recent studies conducted on environmental assessment and the LCA of food products, processes and systems are in Poritosh *et al.* (2009), Savino *et al.* (2013), Garnett (2013), Pawelzik *et al.* (2013), Herath *et al.* (2013) and Virtanen *et al.* (2011). Wognum *et al.* (2011) discussed new perspectives and challenges for sustainability in FSC.

2.2 Edible oils

Lipolysis and oxidation are the two unavoidable chemical processes during the supply chain that mainly influence and limit the shelf-life of edible oils. The lipolytic rate strictly depends on the quality of the raw materials (seeds and olives) because endogenous and exogenous lipases may act prior to the extraction of the oil. In the case of olive oils, this process occurs if the fruits are damaged, injured or not well preserved (Boskou, 1996).

Oxidation occurs mainly during extraction and storage (Morales and Przybylski, 2000). Lipid oxidation involves the interaction of fatty acids in triacylglyceride structure with molecular oxygen, giving rise, by a free radical mechanism, to the formation of hydroperoxides. These latter are unstable primary compounds that decompose to produce several secondary oxidation compounds, such as volatile and non-volatile products. In particular, adehydic molecules are responsible for negative effects on the sensorial properties of the oils due to the development of a rancid flavor (Frankel, 1991; Gallina Toschi *et al.*, 1997).

Temperature, which is the most varying environmental parameter throughout a global distribution chain, is one of the key factors that affects the rate of the oxidation process (Frankel, 1991). Temperature has harmful effects on the oxidation stability of edible oils. Indeed, the proper storage temperature for edible oils is between 10 and 18°C. In particular, the optimal condition for olive oil is 14-15°C (Piscopo and Poiana, 2012).

The increase in the temperature during storage for long time could promote autoxidation of oils and speeds the decomposition rate of hydroperoxides, since the rate of the reaction increases exponentially with the temperature (Bendini *et al.*, 2009). Such a result is obtained by Gómez-Alonso *et al.* (2004), by performing a kinetic study of the autoxidation in olive oils subjected to different temperatures (25, 40, 50, 60 and 75°C) during storage in darkness.

Nevertheless, very low temperatures (such as freezing) may negatively alter some micro-components (phenolic compounds) and the physical characteristics of olive oil (mainly due to the crystallization of triacylglicerols and waxes) (Bendini *et al.*, 2007). Moreover, a loss of oxidation stability and a decrease in sensorial quality may occur as a result of temperature variations, such as freezing and defrosting (Bendini *et al.*, 2007).

The literature overview summarized in Figure 1 highlights the multi-disciplinary approach adopted in this work, which integrates quality and safety aspects of food after supply chain operations combined with an evaluation of effects of packaging and container solutions on environmental factors. Specifically, this paper focusses on an international edible oil supply chain and applies a multi-disciplinary methodology to study the impacts of the logistic activities on the food LC over quality and environmental criteria.

3. Methodology

The adopted methodology evaluates the performance of an international shipment with a focus on the quality of the food product and the environmental sustainability of the packaging solution through a chemical and sensorial evaluation of the products. This analysis integrates the LCA methodology to evaluate the environmental effects of alternative shipping containers.

3.1 Container systems

The paper examines temperature-sensitive products that are not recognized as perishables by law. These products are usually shipped with reefer or controlled atmosphere containers. They are sometimes shipped with dry containers equipped with a wide variety of insulating materials (Singh *et al.*, 2012). The choice of the system container depends on the limitations of the classification of products set forth by law. The transportation requirements are largely determined by the water content of the product and its resulting interaction with the environment humidity and temperature (Isengard, 2009). The water content of a product is the percentage of water on the total mass. Goods are classified according to the water content classes (WCC) (Wild, 2012) reported in Table I.

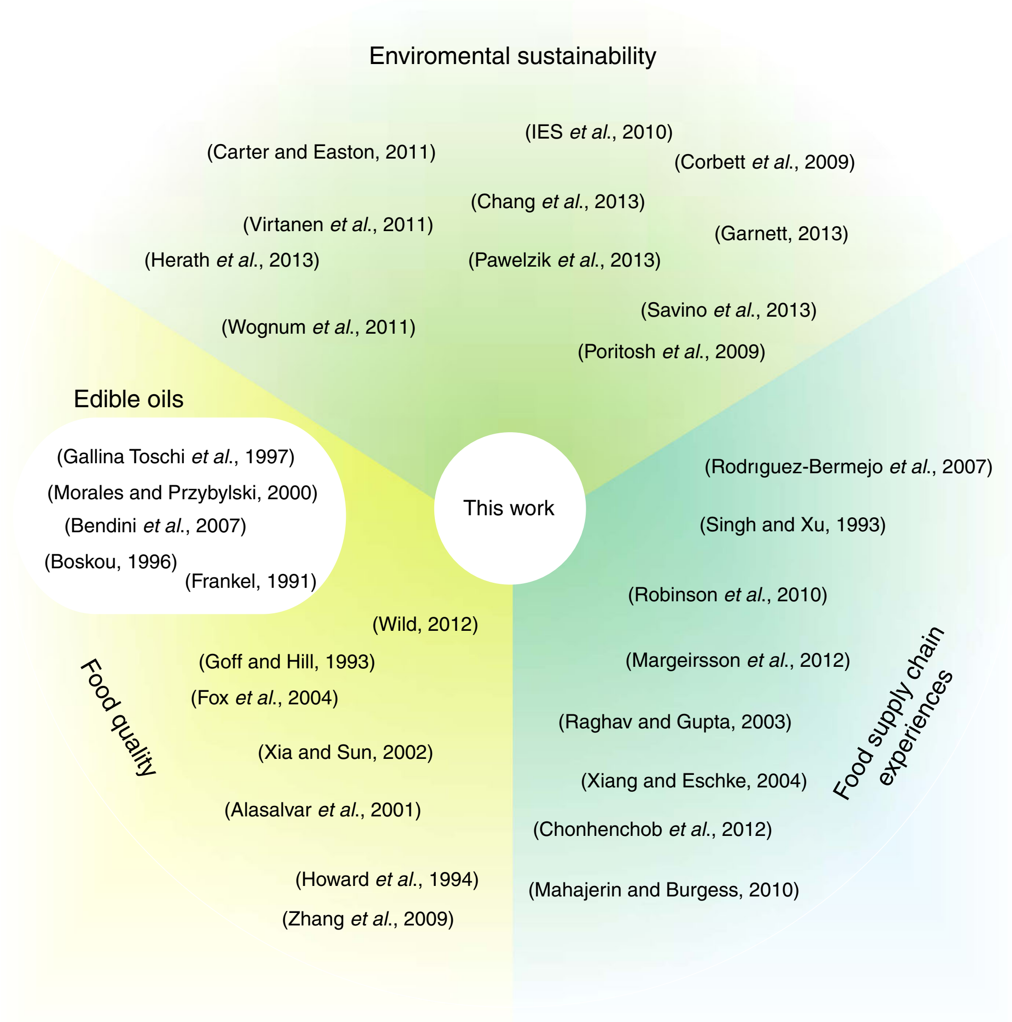


Figure 1.
Multi-disciplinary review of the literature

For high-water content products (i.e. WCC 3), the cooling process inhibits the growth of decay-producing microorganisms and restricts enzymatic and respiratory activities during the postharvest period to prevent water loss and to reduce ethylene production by decreasing the sensitivity of products to ethylene (Dincer, 2003). WCC 3 products require a cold chain (Agreement Transport Perishables, 1970) and are always shipped with reefer containers for food safety requirements. The most shipped products in reefer containers are bananas, meat, citrus fruit, fish and seasonal fruit, which account for 60 million tons/year (Wild, 2012).

Table I highlights that packed and sealed products, as bottled oil, wine, water and cosmetics belong to the WCC 0 and are usually shipped with dry containers (i.e. SC), even though they are well identified as temperature-sensitive products. A common belief retains that such products are completely protected by the combination of primary and secondary packaging (i.e. plastic or glass bottles and carton or cases), which isolate the products from environmental stresses. Despite the lack of legal constraints, some WCC 0 products (i.e. the temperature-sensitive products) might be considered candidates for other container systems in order to preserve their quality

WCC	WC	Description	Storage temperature	Container	Product category	Example		
WCC 0	0	Products containing no water	5-25°C	Standard container Bulk container	Raw material Beverage Healthcare General good	Metal Ceramic Plastic Glass		Bottled wine Bottled oil Bottled water Cosmetics Textile
WCC 1	0-1.5%	Products containing a little water	5-25°C	Standard container Bulk container Ventilated container	Agricultural raw material Spices	Sugar Cocoa		Salt Fertlizer
WCC 2	1.5-30%	Products with poor water content. Water is removed by natural or artifcial drying	5-15°C	Standard container Bulk container Ventilated container	Raw material Luxury product Animal raw material Paper	Coal Flavour	Wood	Fibers Chocolate
WCC 3	>30%	Products with high water content	0-4°C/-20°C	Reefer container	Perishables Frozen product	Fruit Fish	Vegetables	Meat Dairy

Table I.
Classification of product categories according to the WC and the system container

during transportation and supply chain operations. The literature widely discusses the need to change regulations and standards, which assign new categories of products to a specific container system (Panozzo and Cortella, 2008). For example, beverages require a particular temperature, humidity and ventilation conditions because of temperature-determined physical changes, such as ice expansion rupture or thermal dilatation. High humidity and temperature fluctuations can affect the product in terms of shelf-life reduction and/or packaging spoilage.

Table I illustrates the proper temperature range for the conservation of each product category. It reports that a range of 5-20°C is deemed optimal for beverages (Wild, 2012), and the choice of the container system can range over a wide set of alternatives.

Given a particular product category, many alternative container solutions are used to transport products, which results in widely different energy requirements and related greenhouse gas (GHG) emission profiles. This study suggests involving a multi-criteria perspective to determine the container system for long-range shipments that merges the concerns of the consumer (i.e. the quality of products) of supply chain actors (i.e. revenue) and of environment.

Three main container systems for food packages are:

- SC. Also known as general-purpose containers, dry cargo containers or box containers. SCs are typically sized at 20-foot equivalent units (FEU) (i.e. 6.10 meters long and 2.44 meters wide) and the 40-foot equivalent units (FEU) size (Singh *et al.*, 2012).
- Reefer container. This container is equipped with its own refrigeration unit, which normally relies on a three-phase electrical power supply that enables cold air to flow through and around the goods in the container.
- Insulated container. This category consists of generic SCs that are equipped with a thermal insulating liner. Section 4 illustrates the liner adopted by the case study discussed in this paper.

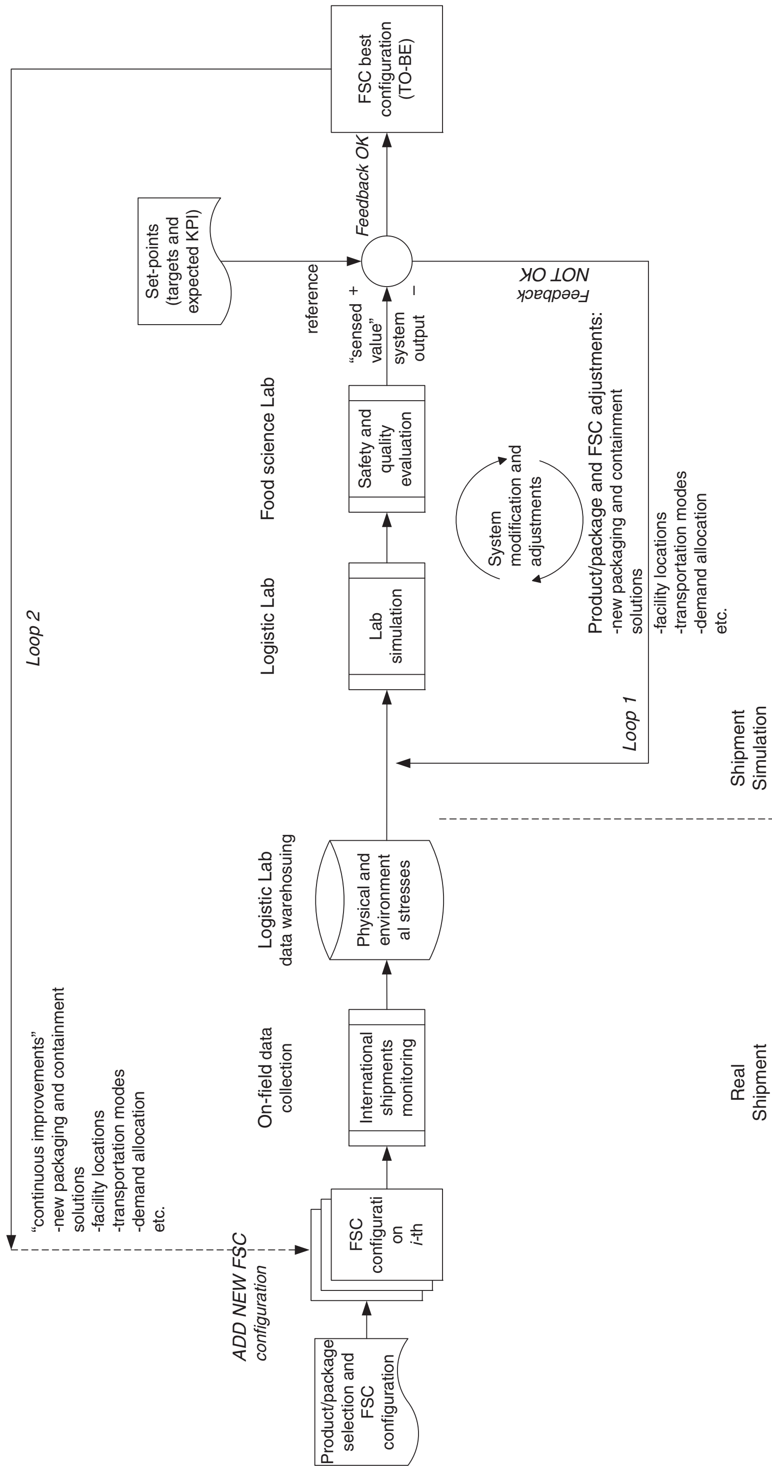
3.2 Monitoring and simulation

The quality, safety and environmental assessment in this study are supported by the development and application of a proactive ex-post and closed-loop control system. It takes inspiration from the control system presented by Manzini and Accorsi (2013) and introduces the so-called "safety and quality evaluation" phase.

This system consists of two main blocks (see Figure 2): the "monitoring block" and the "simulator block." The aim of the first block is to measure the physical and environmental conditions during the distribution activities.

The second block is the so-called simulation system (see the "Lab simulation" task in Figure 2). This climate room simulator virtually simulates the monitored physical and environmental stresses, e.g. time-dependent temperature variability, to measure the effects of logistic activities and intercontinental shipments. This simulation activity gives the analyst the opportunity to investigate the status of the quality and safety of a food product at the place of consumption even, if it is located far from the production site.

The closed loop system illustrated in Figure 1 is based on two different loops. The first loop addresses the laboratory chemical and sensorial analyses. The simulation activity gives the decision-maker the opportunity to measure the effects of different logistic decisions in a what-if laboratory environment. The so-called "sensed values," i.e. the output of the simulation run (task 2) followed by a chemical and



Source: Inspired to Manzini and Accorsi (2013)

Figure 2.
Closed-loop control system

sensorial analysis (task 3), are compared with the "expected (target) values." The simulation task simulates the environmental and physical stresses (i.e. temperature) on the selected food products/packages in agreement with the monitored values of the stresses collected on field in the "real shipment." The analyst can conduct task 2 and task 3 in a what-if environment supported by the development, introduction and evaluation of system modifications and FSC adjustments (e.g. new packaging and containment solutions).

The evaluation process, named task 3, consists of chemical and sensorial analyses that are designed to determine the level of quality and safety of the product/package at the point of consumption.

Both task 2 and task 3 are conducted on "time zero packages," whose production lot is the same as that of the packages shipped in the "real shipment" and subjected to the monitored levels of stresses. Consequently, the so-called "sensed value system output" (see Figure 2) is the output of chemical and sensorial analyses conducted at the point of consumption. As a result, the feedback is good ("Feedback OK" in Figure 2) when the expected values, or rather the performing KPIs, are generated. Otherwise ("Feedback NOT OK" in Figure 2), system modifications and adjustments are necessary.

In Figure 2, the second loop addresses the continuous improvement of the performance of the entire logistic system. The illustrated methodology finds application to analyze the impacts of the supply chain operations on the quality of food products, especially those with long-term expiration as bottled wine and oils, rather than those product that are shipped out-of-cold-chain.

4. Edible oil case study

The closed-loop system illustrated in Section 3 has been applied to the supply chain of a few Italian companies that distribute edible oils worldwide. The most suitable packaging and shipping solution for the international shipping of packaged edible oils is the intermodal freight container. The adopted tertiary package is the palletized unit load made of multiple layers of secondary packages, e.g. each made of six or 12 bottles of oils (the primary packages).

Packages of edible oils, e.g. extra virgin olive oil, are rarely shipped in reefer containers, and represent the proper case for the adoption of the illustrated methodology. The observed virgin olive oil is produced by mechanical lines without any chemical treatment to comply with the EU regulations (EU Commission Regulation 1348/2013, 2013).

This case study compares the performance of two different container solutions: the SC, and the previously defined insulated container, named IC. Insulated containers are basic dry containers equipped with a thermal liner that can partially or totally insulate cargo from climate stresses. This study focusses on a specific thermal liner based on multi-sheet heterogeneous films. The properties of the thermal liner are illustrated in Table II. The column named "Test Methods" indicates the standard adopted for measurement. The liner consists of two aluminum foils that are selected for their lightness, ductility, strength, resistance to environmental stresses (e.g. corrosion) and ability to protect from thermal shocks. The inner side of the liner consists of two polyethylene (PE) foils, which is a thermoplastic polymer usually adopted by the plastic industry. Because PE is inert at environmental temperatures, it does not encourage the growth of algae or bacteria. PE has a low thermal conductivity (i.e. 0.53 kcal/kg) and high flexibility, which enables it to absorb mechanical stress during handling and transportation activities. The woven fabric is the core of the thermal liner. The woven fabric prevents condensation on the liner, which enables air circulation between the inner and outer sides of the liner.

Table II. Thermal liner properties

Section		Properties		Test methods	Value
		Weight		In-House (Producer)	175 gsm ± 20 gsm
14-18 g/m²		Thickness		In-House (Producer)	200 µm ± 20 µm
25-30 g/m²		Water vapor transmission		ASTM F1249	<0.09 g/m²/day
		Emissivity (all surface)		ASTM E408	<0.03
46-51 g/m²	Allumium	Tensile strength		In-house (producer)	
			MD		650-850 (N/50 mm)
	PE		TD		550-750 (N/50 mm)
		Elongation		In-house (producer)	
	Woven fabric		MD		25-35%
			TD		15-25%
		Initial tear strength		ASTM D1004	
			MD		35-45
			TD		35-50
		Index puncture test		ASTM D4833	230-270 N

The previously illustrated monitoring activity (task 1 in Section 3) is conducted via the adoption of temperature sensors embedded within the food packages. Inside the container, which is loaded with 12 two-meters-high unit loads, two standard thermochrons (operating in a range from −40 to +85°C) are installed. The sensors are located as follows: one on the geometrical center of the compartment (at the midpoint and half the height), and the second on the door of the cargo (at half the height). The resulting temperature profiles are the average of the data from the two sensors. The chip embedded in these sensors integrates a 1-wire transmitter/receiver, a globally unique address, a thermometer, a clock/calendar, a thermal history log and 512 bytes of additional memory to store user data, such as a shipping certificate.

Figure 3 compares the trend of the monitored temperature during the selected international shipment from Bologna (Italy) to Quebec (Canada) for the SC solution and the IC. The port of origin is Livorno (Italy, mission started on January 30, 2012) and the port of destination is Quebec (mission stopped on March 1, 2012). The containers contained extra virgin olive oils and grape seed oils.

The graph in Figure 3 shows the critical impact of the handling operations at departure and arrival docks, where dangerous daily temperature fluctuations occur (−4°C at the port of Livorno and around −12°C at the port of destination).

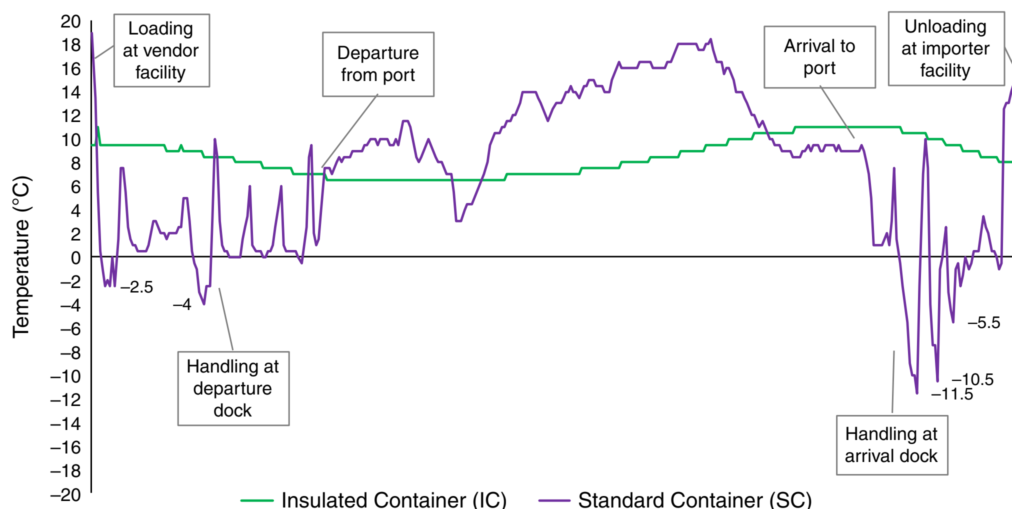


Figure 3. Temperature stress on standard container (SC) and thermal liner equipped container (IC)

The time spent at port docks is critic for the environmental conditions experienced by containers, and unfortunately this time is often not under direct control of neither logistic providers (i.e. carriers), nor of importers.

4.1 Samples and notations

Three samples of commercial extra virgin olive oils (respectively named S1, S2 and S3), one sample of refined grape seed oil (S4) and one sample of refined rice oil (S5) traveled from Italy to Canada. The samples are analyzed after the two different simulations runs: the samples virtually traveled from Italy to Canada within ICs or SCs. A non-simulated sample (named NS) is also analyzed for each type of oil. The non-simulated benchmarking samples are stored at 12°C and in absence of light (in a dark place) before they are analyzed.

4.2 Chemical and sensorial analyses

A brief discussion of the typology of analyses conducted on the selected simulated and NSs of edible oils is reported. The obtained results are reported in comparative and qualitative tables. The list of conducted analyses are grouped in the following subsections.

4.2.1 Chemical analyses. Free acidity (FA), which is an important parameter to determine the hydrolytic progress of triglyceride in edible oils, peroxide value (PV), which shows the development of primary oxidation compounds (hydro-peroxides) in edible oils and UV absorption indexes (K_{232}, K_{270}), that indicate the primary and secondary oxidation products in olive oils were evaluated in the analyzed samples. These parameters are computed according to the official methods described in European Community Commission Regulation 2568/91. For samples S4 and S5, the FA values obtained by the official method (and expressed as g oleic acid in 100 g of oil) are converted to mg KOH/g of oil in order to standardize and compare the results with the limits reported by the Codex Alimentarius for vegetable oils (Codex-Stan 210). The thiobarbituric acid reactant substances content is a measure of the secondary oxidation products of edible oils (thiobarbituric acid reactant (TBARs), mg of malonaldehyde, eq kg^{-1} oil) complied with the AOCS Official Method Cd 19-90 (2006). The oxidation stability test values (OSI), determined at 90°C, are expressed in hours (Maggio *et al.*, 2011). The total phenolic (TP) compounds, which represent the main antioxidant fraction found in olive oils expressed as mg of gallic acid kg^{-1} oil, respond to Pirisi *et al.* (2000) and are evaluated at 750 nm (Singleton and Rossi, 1965). The FA values strictly depend on the quality of the raw materials (olives, seeds), while PV, UV absorption indices (K_{232}, K_{270}), the TBARs substances content and OSI are important indices for evaluating the oxidative status of the oils. The TP compounds are related to the quality of extra virgin olive oil because of their antioxidant activity and healthy properties (Bendini *et al.*, 2007). A trial campaign of three replicates has been performed for each sample.

4.2.2 Sensory analysis. A sensory analysis of all the EVOOs (S1, S2 and S3) is performed according to the European Community, Commission Regulation (640/2008), (2008) by a fully trained group of 8 expert tasters. Sensory analysis is an essential tool for determining the commercial categories of oils obtained from olives, together with chemical parameters. The median and the robust standard deviation European Community, Commission Regulation (640/2008), (2008) are calculated for each attribute. If the value of the robust standard deviation exceeded 20 percent, the sensory analysis is repeated. Moreover, the analyses applied a triangle test that consisted of a standardized sensorial procedure (ISO, 2004) for determining perceptible differences or similarities

between two samples. In this case, the analysis shows the differences between the non-simulated EVOOs (NS) and the respective simulated samples (both SC and IC).

For the adequate interpretation of the results, an analysis of variance are performed with XLSTAT 7.5.2 (Addinsoft, NY, USA) at a 95 percent confidence level (Fisher LSD, $p < 0.05$) and Three replications were performed for each sample.

5. Quality and safety assessment

Table III reports the results obtained for the performance comparison of the SCs and the ICs in terms of chemical parameters that are linked to the quality and the oxidative status of samples S1, S2 and S3 (commercial extra virgin olive oils). Given a generic chemical metrics (e.g. acidity, phenolic content, etc.), a value of 0 indicates that the performance of the SC and the IC does not differ; $+1$ indicates that the IC performed significantly better than the SC solution in terms of the selected performance category, while -1 indicates that the SC performed better. Table IV refers to samples S4 and S5.

In general, the detailed results show a significant decrease in the chemical quality of all the edible oils because of the stress of temperature applied during the simulations. This loss is higher for the simulation in a SC than in an insulated container, as explained by the multiple reported analytical parameters. In particular, the IC solution has a significantly more protective effect than the SC solution in terms of the FA values (samples 2 and 5), PVs (samples 1 and 2), oxidative stability indices (samples 3 and 4),

Table III. Free acidity (FA, % oleic acid), peroxide value (PV, meq of active oxygen kg^{-1} oil), UV absorption indexes (K_{232} and K_{270}, specific extinctions at diene and triene UV zones), oxidation stability index (OSI, hours), total amount of phenolic compound (TP, mg gallic acid kg^{-1} oil), thiobarbituric acid reactant substances content (TBARs, mg of malonaldehyde eq kg^{-1} oil)

	FA	PV	K_{232}	K_{270}	OSI	TP	TBARs
S1	0	1	0	0	0	0	0
S2	1	1	0	0	0	1	1
S3	−1	0	0	0	1	−1	0

Table IV. Free acidity (FA, mg KOH g^{-1} oil), peroxide value (PV, meq of active oxygen kg^{-1} oil), thiobarbituric acid reactant substances content (TBARs, mg of malonaldehyde eq kg^{-1} oil), oxidation stability index (OSI, hours), analyzed for grape seed oil (S4) and rice bran oil (S5)

	FA	PV	TBARs	OSI
S4	0	0	0	1
S5	1	−1	0	0

total phenols (sample 2) and TBARs (sample 2) (see Tables III and IV). The general trends of the obtained results per each chemical parameter present rare exceptions (e.g. FA for sample 3) that deserve further compositional analysis on triacylglycerols, particularly involved in the crystallization process at low temperature.

If the chemical analyses would support the adoption of the IC solution for its protective effects, no significant variations on the resulting intensities of the positive sensorial attributes of fruitiness, bitterness and pungency are observed among the simulated and not simulated extra virgin olive oil samples; moreover, no sensorial defects are evidenced. Two are the reasonable arguments to motivate such results of the sensorial analysis. The former is that the samples show light intensity of the positive sensorial attributes before the simulation, so their natural decrease along the product shelf-life can be only slightly evidenced. The latter is the nature of the thermal stress experienced by the products in the simulated shipment: the low temperatures profiles experiences by the products (see Figure 2) did not occur the appearance of rancid defect, which are consequences of the exposure to high temperatures. Sensorial attributes are in fact more sensible to high temperature, than to low temperature, as far as heat accelerates the oxidation process. Conversely, storage at low and stable temperatures (6-11°C, see IC in Figure 2) may avoid significant changes in the chemical and sensory parameters of edible oils, especially verified for extra virgin olive oils (Li *et al.*, in press).

6. Environmental assessment

The LCA methodology quantifies the environmental and health impacts and the resource depletion issues that are associated with the entire LC of products and/or processes (IES *et al.*, 2010). This methodology is applied to the alternative container systems compared in this paper.

For the IC system, this analysis considers the whole LC of the thermal insulating liner, including the materials and the processes from manufacturing to the final disposal. A set of hypotheses are adopted for each phase of the LC in accordance with the general rules and standard guidelines. The benchmark of the analysis consists of the evaluation of the environmental impacts generated by a traditional shipment by SC in a typical vessel.

6.1 Goal definition and functional unit (FU)

The proposed study compares the environmental impact of the two alternative shipping containers adopted for a specific shipment from Italy to Canada. This shipment represents the so-called FU of the analysis. Any explored environmental impacts refer to the FU and are thus accordingly normalized and scaled.

6.2 Impact categories

The Environmental Product Declaration (2007) is adopted to compute the environmental impact. The EPD standard reports a set of impact categories, such as the equivalent Carbon dioxide equivalent (CO_2eq), as a metric of global warming potential to 100 years (GWP100), the gross calorific values, also referred to as the higher heating values, the GHGs, the ozone-depleting gases, the acidifying compounds, the gases creating ground-level ozone (photochemical ozone creation) and the eutrophicating compounds. These impact categories are evaluated for a defined population, system or activity by considering all relevant sources, sinks and storage solutions within the spatial and temporal boundaries of the population, system or activity of interest (Wright *et al.*, 2011).

6.3 Boundaries

Figure 4 highlights the boundaries of the LCA methodologies and remarks the environmental cost driver considered for the comparative analysis: the thermal liner LC (i.e. manufacturing, use and disposal) for the IC solution is the "additional" impact in comparison with the SC.

In particular, the light blue blocks represent the manufacturing and assembling processes for the SC. The IC results by the assembling of a SC with the thermal insulating liner. The light green blocks represent the IC LC from the manufacturing of the liner to its disposal. The environmental impacts of the IC LC are computed by a differential analysis with respect to the benchmark. The FU of the analysis is one shipment (i.e. $s = 1$ in Figure 4) that includes the whole LC of the liner from cradle-to-grave, while the impacts for the containers LC until its disposal (i.e. $1 < s \leqslant s^d$ in Figure 4) are not accounted.

A TEU container is considered for the analysis. This container measures 6.1 meters long and 2.4 meters wide, with an average load capacity between 15 and 22 tons. The size of the container determines the quantity of the insulating liner necessary to equip one shipment. This quantity is split into fractions for each of the liner components (i.e. aluminum foil, PE foil and woven fabric). A TEU container carries about 18 tons of products (i.e. oil bottles, cartons and pallets) for a total weight of 20 tons. The data obtained from the liner material, production and disposal activities creates the LCI for the SimaPro 7.1 software analysis by the EPD standard. Table V reports the comparison between the IC and SC solutions over the aforementioned environmental impacts. For the observed FU, the IC presents a higher load due to the thermal liner, and accounts the impacts for its raw material supply, assembling and disposal. Given a generic impact category, the column percent indicates the variance in percentage of the IC in comparison with the SC.

The insulating manufacturing processes are the raw materials (i.e. aluminum, PE, woven fabric) treatments and the thermoforming process composes the three materials into a unique foil. In the comparison, the vessel shipment does not represent a significant phase for the alternative containers, because the liner weight (i.e. approximately 8 kg) is negligible compared to the overall shipped load (i.e. 20 tons).

The insulating liner disposal step, which occurs after each shipment, consists of the transportation of waste to treatment sites and the end-of-life treatments. The former cost driver refers to the standard process provided by Ecoinvent databank (2010), while the latter considers the complete recycling of aluminum as well as the land filling (i.e. 50 percent) and incineration (i.e. 50 percent) of the remained fraction.

Table V highlights the influence of the adoption of the IC solution on the global warming potential and the exploitation of non-renewable fossil sources, which represent impact increases of 16 and 17 percent, respectively, compared to the SC solution.

Figure 5 concludes the analysis focussing on the LC activities that mostly affect the environmental performance of the IC. The remark from Figure 5 is that the raw materials composing the thermal liner are largely the main stressors for all the impact categories. Therefore, the adequate selection of the materials for non-reusable supplementary packaging or thermal liner is crucial to provide environmental care solutions that meet food preservation issues.

7. Conclusion

Logistics plays an increasingly important role in FSC, but this awareness must be shared between different actors in the chain. Food scientists widely debated on the effects on the quality of product resulting by the uncontrolled exposition to light,

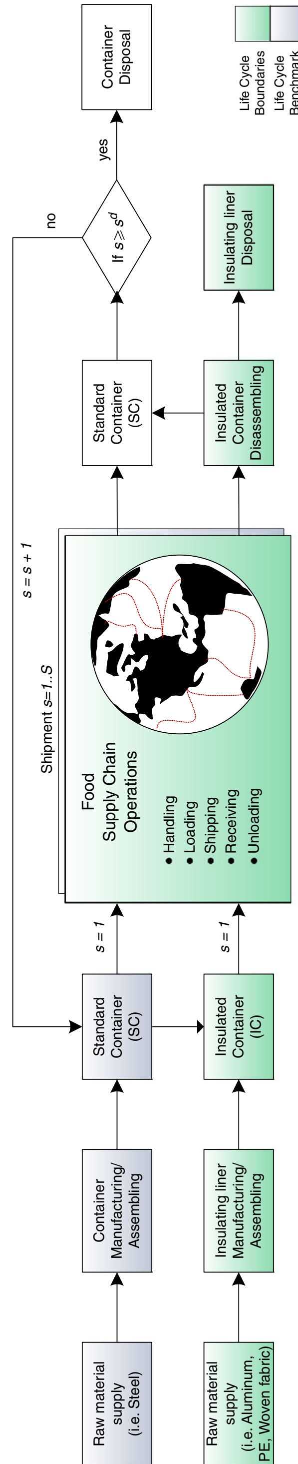


Figure 4.
Life cycle boundaries for CF comparative analysis

Table V. Impact categories differential analysis per container type

Shipping route (km)	Shipped weight (kg)	Container type	Environmental impact category	Unit of measure	Insulating liner manufacturing: Materials: Aluminium	Materials: PE	Materials: Woven cotton	Assembling: Thermoforming	Vessel shipment	Insulating liner disposal	Total	%
4,043	20,008	IC	Global warming (GWP100)	kg CO_2 eq	22.0343	8.3278	70.0362	5.5031	730.1409196	8.0539	844.0961	16
			Ozone layer depletion (ODP)	kg CFC-11 eq	1.32578E-06	9.69574E-08	1.3887E-06	3.08982E-07	9.16707E-05	6.66071E-08	0.0001	4
			Photochemical oxidation	kg C_2H_4	0.010984629	0.009194624	0.028242364	0. 002256196	0.923472391	0.000645944	0.9748	6
			Acidification	kg SO_2 eq	0.1	0.033424074	0.71984861	0.027055091	18.42388461	0.002819274	19.3112	5
			Eutrophication	kg PO_4 eq	0.009224604	0.002601517	0.097912353	0.002184557	1.575163614	0.014187785	1.7013	8
			Non-renewable, fossil	MJ eq	307.284	265.367	1,021.007	120.168	10,377.22623	6.832	12,097.8835	17
	20,000	SC	Global warming (GWP100)	kg CO_2 eq					729.84898		729.8490	
			Ozone layer depletion (ODP)	kg CFC-11 eq					9.16341E-05		0.0001	
			Photochemical oxidation	kg C_2H_4					0.92310315		0.9231	
			Acidification	kg SO_2 eq					18.416518		18.4165	
			Eutrophication	kg PO_4 eq					1.5745338		1.5745	
			Non-renewable, fossil	MJ eq					10,373.077		10,373.0770	

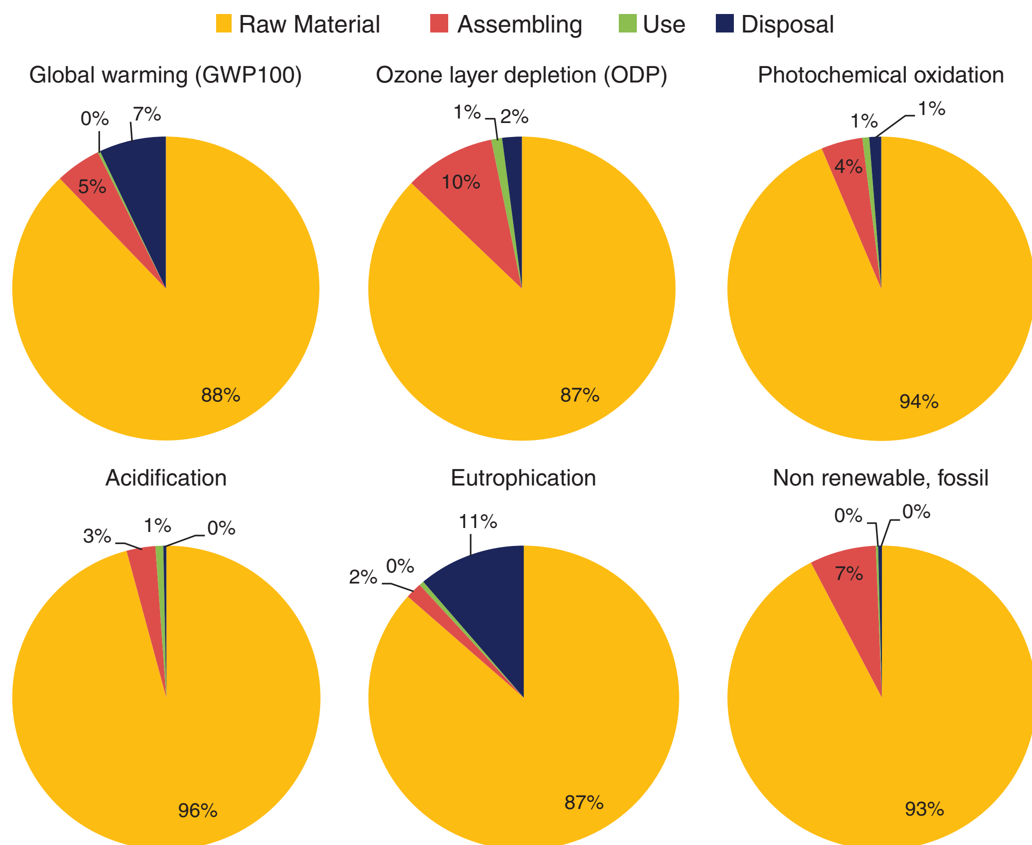


Figure 5.
Life cycle incidence on impact categories (IC)

thermal stress and mechanical shock. Over a multi-disciplinary approach, this paper aims to import these expertises by observing the role of supply chain operations and decisions (e.g. packaging, transport mode and container choice) on the quality of edible oils and on the environment through a LCA analysis.

The results of the chemical, sensorial and environmental assessment of edible oils shipped by two container systems are illustrated. The observed container systems are the SC and the SC equipped with an insulating thermal liner (IC).

The use of IC is necessary to control the temperature stress and to minimize its fluctuations, thus reasonably preventing the physical and chemical degradations of edible oils. The chemical analysis states that the IC solution protects the oils in terms of storage at more stable temperatures enabling the preservation of the hydrolytic and oxidative acceptable conditions. Preventing oxidation avoids the production of volatile and non-volatile adehydic molecules that are responsible of rancid flavors in the oils. IC solution also protects the level of total phenols, which play a favorable role in preventing cardiovascular disease and delaying cellular aging and death.

The results of the sensorial analysis, in terms of fruitiness, bitterness and pungency evidenced for extra virgin olive oils, do not highlight significant variations between the non-simulated and simulated samples. These results are expected given the low temperature stress experienced by products during transport activities, and given the observed samples characterized by a light intensity of positive attributes, determined by the grade of maturation of the oils. Further analyses are necessary to assess the variance of sensorial attributes on new samples.

According to the environmental assessment of the two container systems, the IC solution has a marginal added impact on the product distribution in comparison with the SC. For example, the impact on global warming of the IC is 16 percent higher than for the SC. For all the environmental impact categories, the proper definition of the thermal liner materials (or other supplementary packaging) is crucial to provide environmental care solutions to preserve product quality across transportation.

Further research are expected on the integration and food logistics issues (e.g. container loading, transportation modes, packaging solutions, storage conditions, delivery planning), with the assessment of food quality and safety perceptions by the consumer, as well as with environmental aspects. Consumers detect brand and origin of products from label and packages, reporting also the complied certifications and standards. Unfortunately, no reports are currently given about the efficiency, the quality, the environmental sustainability of manufacturing, consolidation and distribution processes. Such information should play a crucial role in marketing and brand promotion and should affect more and more the purchasing habits and prices.

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Further reading

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Paper 6

Quality changes in vegetable oils after simulating shipment in different containers

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Quality Changes in Vegetable Oils after Simulating Shipment in Different Containers

Running title: Simulated shipments of edible oils from Italy to USA and Canada

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ABSTRACT

BACKGROUND: Due to the long shipping routes between producing and importing countries and the possible degradation of the quality of vegetable oils, it is worthwhile to reproduce common and frequent shipments with the aim of predicting the final quality of products. The present study was conducted by simulating two specific shipments using two different containers, one without thermal insulation (standard container) and one with thermal insulation. In particular, bottled commercial vegetable oils were placed in containers and subjected to monitoring to simulate two real shipments to Los Angeles (USA) and Quebec (Canada) followed by analysis of chemical, physical, and sensory parameters. RESULTS: A higher degree of oxidation, in particular for samples shipped to Los Angeles in standard containers, was observed. A slight trend towards an increase in free fatty acidity was also found. No significant variations in water content, turbidity, or sensorial attributes were seen after the simulations, while significant changes were seen in chromatic coordinates. CONCLUSION: The thermal insulation container tested was effective in protecting samples from potential oxidative damage produced by variations in temperature during simulated shipping.

Keywords: simulated shipment, edible vegetable oils, thermal insulation, oxidation, food quality.

INTRODUCTION

Vegetable oils such as sunflower, palm kernel, and soybean oils are extensively used for cooking purposes. These types of fatty food products are more susceptible to oxidation than animal fat because of their content of unsaturated fatty acids.[1] In 2014, about 168 million tons of vegetable oil was produced worldwide [USDA (http://usda.gov)]. Among these, olive oils represented less than 3 % of the total amount of vegetable oils produced.[2] About 3.1 million tons of olive oil was produced globally, of which 2.3 million tons were produced in the EU and, 20 % of the total was produced in Italy [IOC (http://internationaloliveoil.org)]. Italy is considered as the dominant supplier of olive oils to Canada and USA, and about 72 % and 60 % of olive oil imported in 2014 to Canada and USA, respectively, was from Italy. Furthermore, in 2013, Italy exported around 243,000 metric tons of virgin olive oil [IOC (http://internationaloliveoil.org)]. During transportation by sea, the desired temperature for most edible oils is ambient temperature.[3] In particular, the recommended temperature for storage of extra virgin olive oil is around 15 °C, considering that solidification and crystallization of the product occurs at 3−4 °C.[4] Edible oils may suffer from deterioration in quality, which involve hydrolytic and oxidative modifications promoted by several factors, such as temperature and humidity in the stages of pumping and tank filling, in addition to the effect of light exposure for samples transported in clear bottles [BTM (www.cargohandbook.com)]. Raw edible oils, even after soft refining, as well as virgin olive oils contain a range of minor compounds such as chlorophylls, tocopherols, carotenoids, and phenolic compounds that function as natural antioxidants by enhancing the stability of the oil during storage.[5] Moreover, the monounsaturated/polyunsaturated fatty acid ratio, as well as the presence of phenolic compounds, make virgin olive oil more stable towards heat induced oxidation.[6,7] Moreover, the hydrolysis of acylglycerols, catalyzed mainly by an increase in temperature during storage, as well as the presence of moisture, oxygen, or light,[8] plays an important role in development of off-flavors, thus making edible oils unpalatable and shortening their shelf-life.[5] High temperatures increase the rate of oxidation, while very low freezing temperatures may also change the availability of some micro components, such as phenolic compounds, water distribution around crystals, and the physical characteristics of olive oil.[9] For instance, temperature variation may trigger loss of stability to oxidation and alter the sensory quality,[6] which implies deterioration and a reduced shelf-life. Several studies have been carried out on the simulated transportation of foodstuffs. For example, the effects of handling practices

during tomato transportation on the quality of fresh tomatoes at the final destination has been assessed.[10,11] Another study[12] investigated the effect of simulated shipment on wine in terms of flavor and volatile compounds. Regarding shipment of edible oils, an interesting report[13] studied the effect of bulk storage and transportation on the quality of palm oil, and found that during the 25 days of an actual journey at temperatures ranging between 37−55 °C, there was a slight increase in free acidity, while peroxide values were doubled at the final stage of the voyage. The effect of different thermal conditions registered in the food supply chain during transportation of edible oils was recently studied by our group.[14] In that study, we investigated the effect of simulated shipment on the quality of different types of edible oils from Italy to Taiwan, starting from the stage of truck loading and ending at the truck delivery phase. It was found that vegetable oils underwent a loss of quality and deterioration after the journey, especially in terms of primary and secondary oxidation products. The simulation runs were conducted using ad-hoc closed-loop controlled chambers[15] in order to measure and control the effects of transportation on the quality of edible oil. Moreover, we have also compared the performance of these containers.[16]

The present study evaluated the changes in quality of three kinds of vegetable oils (extra virgin olive oil, rice oil, and grape seed oil) after two different simulated shipments. Data on shipments was obtained using a thermal data logger to measure temperature during actual shipping and then reproduced in the laboratory. The first journey was characterized by high temperatures during 37 days of shipment from Italy to Los Angeles (USA), and the latter by lower temperatures during 30 days of shipment from Italy to Quebec (Canada). In particular, this study evaluated the ability of a thermal insulated container to protect the quality of the oils in both shipments. With this aim, quality parameters such as free acidity, oxidation indexes (peroxide value, thiobarbituric acid content, and oxidative stability index) as well as sensory analysis and other physicochemical parameters (water amount, turbidity, and CIElab color indexes) were evaluated before and after the simulated shipments.

EXPERIMENTAL

Samples

The two simulated shipments were carried out using three different kinds of commercial vegetable oils: extra virgin olive oil (EVOO), grape seed oil (GSO), and rice oil (RO). In

particular, two bottles (1 liter each) of oil were subjected to the simulated transports. The two bottles of each oil for each destination (Quebec, coded as “Q” or Los Angeles, coded as “LA”) contained edible oil coming from the same production line batch. Each was used for chemical, color, and sensorial evaluation (Fig. 1) before and after the simulations of shipping.

Simulation Process

This study had the scope of reproducing the temperature profile registered during the logistic phases (handling, shipping, and final delivery) of two shipments of commercial edible oils, from Italy to Quebec and from Italy to Los Angeles. The temperature profiles were reproduced using closed-loop climate-controlled chambers placed in standard or thermally insulated containers. The two container solutions have been previously described in a paper by the same research group.[17] The simulation chambers reproduced temperature cycles to fit the monitored temperatures registered during actual shipments. The temperature inside the chambers covers the possible range of -20 °C to 65 °C. The integrated cooling system consists of an evaporator utilizing 21 g of R600a iso-butane as a refrigerant. A closed-loop algorithm, developed with LabView National Instrument software, controls the actuators so that the chamber temperature reaches a defined set point. The first international simulated shipment (coded as “Q”) from Italy to Quebec started on January 30 from the port of origin (Livorno) and ended on March 1 at the port of final destination (Quebec); the temperature profile of this shipment is illustrated in Fig. 2. The second international shipment (coded as “LA”) from Italy to Los Angeles started on June 26 from the port of origin (Livorno) and ended on August 2 at the port of final destination; the temperature profile of this shipment is shown in Fig. 3.

Chemical, physical, and sensory analyses

Free acidity (FA) expressed as g oleic acid 100 g^{-1} oil and peroxide value (PV) expressed as milliequivalent O_2 kg^{-1} oil were determined for EVOO.[18] For the two other edible oils, free acidity values (AV) were obtained by the Codex Alimentarius official method[19] and expressed in mg KOH g^{-1} oil. Thiobarbituric acid reactant substance content (TBARs) was evaluated according to the AOAC method[20] and expressed as TBAR value (mg of malonaldehyde equivalent kg^{-1} oil). Total phenolic compounds (TP), expressed as mg of gallic acid kg^{-1} oil, were evaluated at 750 nm [21,22] by a calibration curve built with different concentration of gallic acid

(Sigma-Aldrich, St. Louis, Mo., USA) as standard, by using a UV−Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). CIELab color for EVOO samples was determined[23] using a Hunterlab (Reston, VA, USA) colorflex instrument and expressed as L*, a*, b* chromatic coordinates. Turbidity (TD) of samples was determined using a Ratio turbidimeter model 18900 (Hack, Colorado, USA) and expressed as nephelometric turbidity units (NTU). Water amount (WA) was determined at 103 °C using the air oven technique.[24] Sensory analysis of EVOO samples was performed[25] by a fully trained panel of 8 expert and trained tasters of the Department of Agricultural and Food Sciences of the University of Bologna.

Statistical analysis

All analyses were run in triplicate and expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using XLSTAT 7.5.2 software (Addinsoft, NY, USA) at a 95% confidence level (Fisher LSD, $p < 0.05$) to evaluate differences between means.

RESULTS AND DISCUSSION

Effect of simulated shipment on hydrolytic degradation

Free acidity is considered as an important parameter to determine the hydrolysis of triacylglycerol in olive oil. Moreover, acidity values are considered as a basic criterion to classify the different categories of olive oil. The results in Table 1 show that FA increased slightly during shipments to both destinations. In addition, there was a slight increasing trend in FA for EVOO LA shipped in a standard container compared with that before shipping, which was influenced by the increase in temperature during the simulated journey.[26] However, none of the shipped EVOO samples reached the limit of 0.8 % accepted for the extra virgin olive oil category.[18]

Acid value results (Table 2) of GSO stored in the standard container for both simulated shipments were significantly higher in comparison with the thermally insulated samples and that before shipping. Considering the RO samples shipped to Quebec which, before starting the simulation, had an AV higher than the accepted limit of 0.6 % for edible oils,[19] the AV registered for the sample stored in the standard container was significantly higher than both the respective values for samples with and without thermal insulation. The results for the RO sample to Quebec revealed a drastic effect of temperature variation, and in particular for low quality edible oils. In fact, as recorded during the simulation in a standard container to Quebec, the temperature

decreased to -10 °C (Fig. 2). Such low temperatures probably facilitate hydrolytic processes due to water droplets in the liquid phase that surrounds the lipid crystals.[5] In the case of RO in the simulated shipment to Los Angeles, on the other hand, the change in AV after simulation in both the standard and thermally insulated containers was not significant; in this case, the samples experienced a slight temperature fluctuation during 13 days of simulated shipment before reaching the final destination.

Influence of simulated shipment on oxidation stability

In order to estimate the effect of shipment on EVOO and other vegetable oils, oxidation quality was tracked by evaluating i) PVs, which indicate the increase in primary oxidation products, such as hydroperoxides, and ii) TBAR values, which detect the formation of malondialdehyde from fatty chains with three or more double bonds[8] and indicate the trend in secondary oxidation products in edible oil. As seen in Table 1, the PV was significantly higher in the EVOO sample for which the simulated shipment was conducted in a standard container compared to that shipped in a thermally insulated container for both destinations. TBARs values were also significantly higher when a standard container was used to transport EVOO samples compared with those subjected to simulation in a thermally insulated container for both destinations. These results suggest that thermally insulated containers have a beneficial effect, compared with a standard container, in terms of protecting EVOO samples against oxidative stress. Moreover, starting from similar values for both samples before shipping, higher TBARs values were reported for EVOO sent to Los Angeles compared with the sample sent to Quebec; this may be related to the higher temperature stress applied in the Los Angeles simulation (Figs. 2 and 3). Regarding the other vegetable oils, the PVs (Table 2) had higher values after simulation compared with those before shipping, for both destinations, except for RO shipped in a standard container to Quebec. Considering RO to Los Angeles, a higher increase was observed in PVs in a standard container compared with thermally insulated samples, which indicate more advanced formation of peroxides in the standard container. On the other hand, the lower PV values seen in RO to Quebec in a standard container compared with samples shipped in an insulated container reveals possible additional transformation of peroxides to secondary oxidation products, which was also confirmed by the increase in TBAR observed in the same sample (Table 2). The higher impact on oxidative status on all edible oils by the Los Angeles simulation is also demonstrated by considering the changes in total phenols in EVOO (Table 1): these minor components, in

addition to their nutritional role, act as antioxidants in EVOO.[9] Before simulation, EVOO samples contained about 353 and 259 mg gallic acid kg^{-1} oil, respectively, for samples sent to Quebec and Los Angeles (Table 1); after shipping, these values tended to decrease in standard container samples. This reduction was more pronounced for samples stored in the standard container after simulation to Los Angeles due to the effect of higher temperature stress than the non-thermally insulated journey (Fig. 3).

Influence of simulated shipment on physical and sensorial properties

Color changes in EVOO reflect the visual color appearance that is considered to be an important factor in consumer satisfaction.[27] The color of olive oils, in general, is principally affected by two classes of minor compounds, namely chlorophylls and carotenoids. The degradation of these compounds is due to different conditions of stress, such as temperature and light, which may alter color in addition to clarity and transmittance.[28] Color indexes were expressed as chromatic coordinates: L* corresponds to brightness and positive b* to yellowish color, while negative a* corresponds to light green color.[29] As seen in Table 3, there were significant changes in the brightness (L*) and b* indices for EVOO samples sent to Quebec after simulation in the standard and insulated containers (more bright and more yellowish). However, a reduction in L* values (meaning less bright oils) was seen in both shipping conditions for the simulated shipment to Los Angeles. A reduction was also observed for b* values (less yellow toward light blue) of samples shipped to Los Angeles, corresponding to the degradation of yellow chromophores (pigments), that function as natural antioxidants, such as carotenoids and pheophytins,[30] since oxidation is promoted by the increased temperature[31] during the simulation to Los Angeles (Fig. 3). As previously reported, degradation of natural pigments such as carotenoids occurs at around 40 °C.[32] Moreover, an increase in a* values (partial loss of green color toward redness) was recorded for samples sent to Los Angeles: such a partial loss of green color, in general, may correspond to partial degradation of chlorophylls, which are partially converted into other gray/brown compounds, and specifically to pyropheophytin a which is formed from pheophytin a due to degradation triggered by inadequate temperatures during the storage of oil.[33] Consequently, the increased degradation of chlorophyll and carotenoid pigments is likely related to the increased temperature (up to 58 °C) in the final stages of the Los Angeles simulation (see Fig. 3).

In addition, variations in water amount and turbidity were not significant (Table 3) in either

simulation. Sensory analysis, performed by a Panel test,[25] is an essential technique for the assessment of the quality of EVOO. The sensory evaluation (results not shown) indicated that no sensory defects developed after simulated shipment to Quebec or Los Angeles, and all samples remained within the “extra virgin” category in both thermally insulated and standard containers.

CONCLUSIONS

It is important to point out that this study is related to two specific simulations, and thus the results cannot be generalized to all shipments of vegetable oils to Los Angeles or Quebec. Shipping may affect the quality of edible vegetable oils if they are subjected to higher or lower temperatures, mostly depending on the container used. From parallel study of two simulated shipments to different destinations with different thermal conditions, it was found that thermal isolation is associated with significant benefits in terms of avoiding an increase in degradative reactions for edible oils, and especially on oxidative status. Considering the different parameters evaluated, the quality of the edible oils subjected to the simulation to Quebec was higher than those shipped to Los Angeles, which was due to the different thermal profiles of the two journeys. This suggests that a closed-loop simulation system is a useful tool to predict the quality of EVOO and other edible oils under different conditions and destinations. Such a simulation can also be profitably used to investigate the effects of transportation, packaging, containment solutions, and equipment on product quality. The aim of future studies is the adoption of a proposed ex-post simulation analysis on different edible oils having different ages, shipped in different periods of the year and to different destinations, in agreement with specific logistic decisions (storage, material handling, transportation modes, etc.) and packaging solutions including primary, secondary, tertiary packaging, and containment equipment.

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LEGENDS FOR FIGURES

Figure 1. Closed-loop protocol system for simulation of shipping.

Figure 2. Temperature profile monitored using data loggers for the Quebec simulation (in the world map, 1: Livorno port; 2: Quebec port) . A: Inside standard container; duration: 30 days; highest temperature: 19 °C; lowest temperature: -11.5 °C. B. Inside thermal insulated container; duration: 30 days; highest temperature: 11 °C; lowest temperature: 6.5 °C.

Figure 3. Temperature profile monitored using data loggers for the Los Angeles simulation (in the world map: 1, Genoa Port; 2, Panama Canal ; 3, Los Angeles Port). Duration: 37 days, highest temperature: 58 °C, lowest temperature: 11.5 °C.

Table 1. FA, free acidity (g oleic acid 100 g^{-1} oil); PV, peroxide values (meq O_2 kg^{-1} oil); TBARs, thiobarbituric acid reactive substances value (mg of malonaldehyde equivalent kg^{-1} oil); TP, total phenols (mg gallic acid kg^{-1} oil) tested before simulation and after simulation of shipping in insulated and standard containers for EVOO samples to the two final destinations (EVOO Q, Quebec and EVOO LA, Los Angeles).

Values (mean ± standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions ($P < 0.05$; Fisher's exact test).

Sample	Experimental condition	FA (g oleic acid 100 g^{-1})	PV (meq O_2 kg^{-1})	TBARs (mg of malonaldehyde equivalent kg^{-1})	TP (mg gallic acid kg^{-1})
EVOO Q	**Before shipping**	$0.52^{B} \pm 0.04$	$11.7^{C} \pm 0.7$	$0.013^{B} \pm 0.001$	$353^{B} \pm 35$
	Insulated container	$0.59^{A} \pm 0.01$	$13.1^{B} \pm 0.3$	$0.012^{B} \pm 0.001$	$372^{A} \pm 54$
	Standard container	$0.60^{A} \pm 0.01$	$17.0^{A} \pm 0.8$	$0.016^{A} \pm 0.001$	$478^{A} \pm 43$
EVOO LA	**Before shipping**	$0.45^{B} \pm 0.01$	$8.8^{C} \pm 0.2$	$0.015^{C} \pm 0.001$	$259^{A} \pm 2$
	Insulated container	$0.45^{B} \pm 0.01$	$9.2^{B} \pm 0.1$	$0.028^{B} \pm 0.001$	$257^{A} \pm 8$
	Standard container	$0.48^{A} \pm 0.01$	$10.4^{A} \pm 0.1$	$0.040^{A} \pm 0.001$	$222^{B} \pm 3$

Table 2. AV, acid values (mg KOH g^{-1}); PV, peroxide v

es (meq O_2 kg^{-1} oil); TBARs, thiobarbituric acid reactive substance values (mg of malonaldehyde equivalent kg^{-1} oil) of vegetable oil samples [grape seed oil (GSO) and rice oil (RO)] tested before and after simulation of shipping in insulated or standard containers to the two final destinations (coded as "Q" to Quebec and as "LA" to Los Angeles).

Values (mean ± standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions ($P < 0.05$; Fisher's exact test).

Sample	Experimental conditions	AV (mg KOH g^{-1})	PV (meq O_2 kg^{-1})	TBARs (mg of malonaldehyde equivalent kg^{-1})
GSO Q	Before shipping	$0.27^{C} \pm 0.00$	$4.2^{B} \pm 0.1$	$0.018^{A} \pm 0.001$
	Insulated container	$0.36^{B} \pm 0.03$	$6.3^{A} \pm 0.9$	$0.020^{A} \pm 0.003$
	Standard container	$0.43^{A} \pm 0.00$	$6.2^{A} \pm 0.1$	$0.017^{A} \pm 0.002$
RO Q	Before shipping	$0.74^{C} \pm 0.01$	$4.4^{B} \pm 0.2$	$0.017^{B} \pm 0.001$
	Insulated container	$0.86^{B} \pm 0.03$	$4.8^{A} \pm 0.1$	$0.016^{B} \pm 0.002$
	Standard container	$0.98^{A} \pm 0.08$	$4.1^{B} \pm 0.1$	$0.022^{A} \pm 0.003$
GSO LA	Before shipping	$0.24^{B} \pm 0.04$	$1.6^{B} \pm 0.0$	$0.018^{C} \pm 0.001$
	Insulated container	$0.24^{B} \pm 0.03$	$3.3^{A} \pm 0.5$	$0.020^{B} \pm 0.001$
	Standard container	$0.35^{A} \pm 0.02$	$3.0^{A} \pm 0.2$	$0.043^{A} \pm 0.001$
RO LA	Before shipping	$0.46^{A} \pm 0.01$	$3.3^{B} \pm 0.3$	$0.014^{B} \pm 0.001$
	Insulated container	$0.45^{A} \pm 0.03$	$3.5^{B} \pm 0.4$	$0.020^{A} \pm 0.001$
	Standard container	$0.51^{A} \pm 0.03$	$4.9^{A} \pm 0.4$	$0.020^{A} \pm 0.001$

Table 3. Color coordinates (L*, a*, b*); TD, turbidity (NTU); WA, water amount (mg kg^{-1} oil) before and after simulated shipping in an insulated and standard container for EVOO samples to the two final destinations (EVOO Q, Quebec and EVOO LA, Los Angeles). Values (mean ± standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions ($P < 0.05$; Fisher's exact test).

Samples	Experimental conditions	L*	a*	b*	TD (NTU)	WA (mg kg^{-1} oil)
EVOO Q	Before shipping	$54^{B} \pm 0.1$	$4.9^{A} \pm 0.0$	$80^{B} \pm 0$	$11.7^{A} \pm 0.2$	$719^{A} \pm 98$
	Insulated container	$55^{A} \pm 0.1$	$4.8^{B} \pm 0.0$	$84^{A} \pm 0$	$11.3^{A} \pm 0.1$	$621^{A} \pm 6$
	Standard container	$55^{A} \pm 0.1$	$4.6^{C} \pm 0.0$	$84^{A} \pm 0$	$11.5^{A} \pm 0.2$	$708^{A} \pm 92$
EVOO LA	Before shipping	$63^{A} \pm 0.0$	$4.3^{B} \pm 0.1$	$89^{A} \pm 0$	$11.6^{A} \pm 0.2$	$650^{A} \pm 30$
	Insulated container	$50^{B} \pm 1.4$	$5.8^{A} \pm 0.2$	$71^{C} \pm 1$	$11.5^{A} \pm 0.2$	$607^{A} \pm 64$
	Standard container	$52^{B} \pm 1.5$	$5.5^{A} \pm 0.2$	$79^{B} \pm 2$	$11.4^{A} \pm 0.1$	$562^{A} \pm 72$

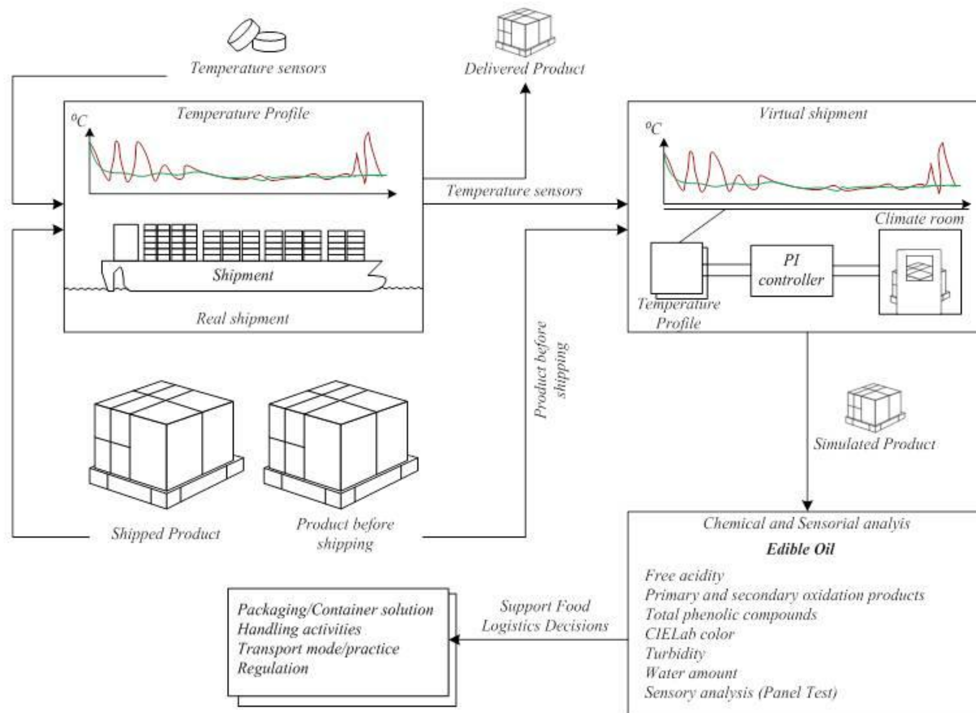

Figure 1

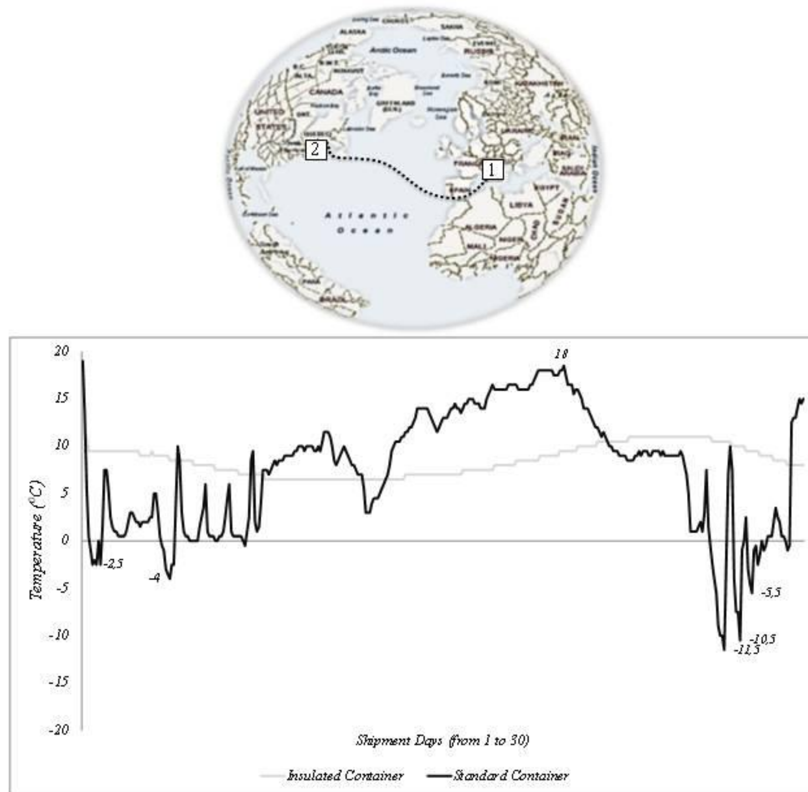

Figure 2

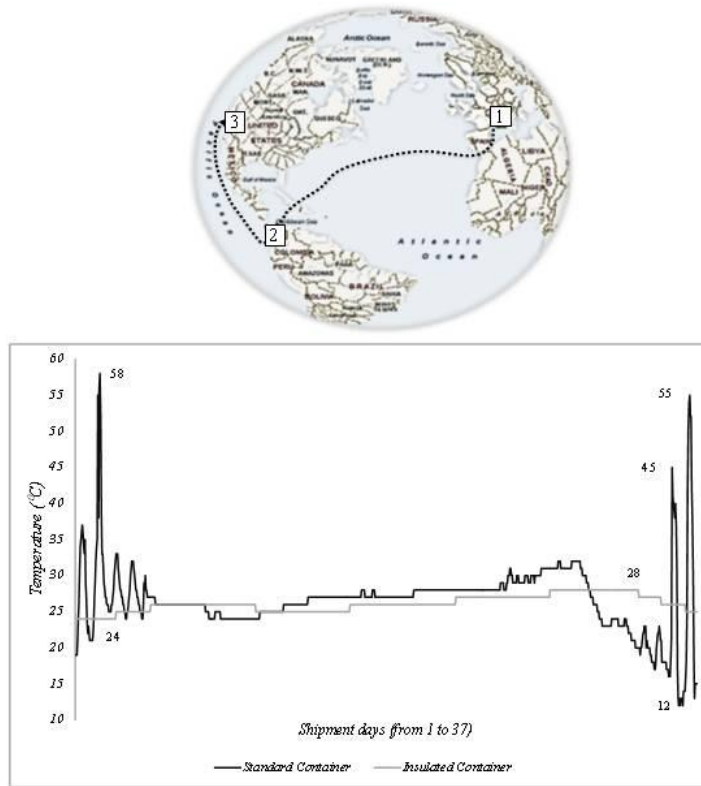

Figure 3

Annex I. Other publications related to the topic of the Ph.D. Thesis

I reported here the references of two papers related to the topic of this Ph.D. thesis,
extracted from previous studies but realized during my three-years-Ph.D. Course; they are foucused on the influences of different storage conditions and packaging materials on the quality of EVOO. My contribution to these works was to establish the reaearch plan, performing the chemical analysis, review the literatures, and writing the drafts of these articles with collaboration of the other co-authors

Abbadi, J., Afaneh, I., Ayyad, Z., Al-Rimawi, F., Sultan, W. and Kanaan, K. (2014). Evaluation of the Effect of Packaging Materials and Storage Temperatures on Quality Degradation of Extra Virgin Olive Oil from Olives Grown in Palestine. *American Journal of Food Science and Technology*, 5, pp.162-174.

Afaneh, I.A., Abbadi, J., Ayyad, Z., Sultan, W. and Kanan, K. (2013). Evaluation of selected quality indicators of extra virgin olive oil bottled in different packaging materials upon storage under different lighting conditions. *Journal of Food Science and Engineering*, 3, pp. 267-283.

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Evaluation of the Effect of Packaging Materials and Storage Temperatures on Quality Degradation of Extra Virgin Olive Oil from Olives Grown in Palestine

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Abstract The quality of extra virgin olive oil (EVOO) is intimately affected by packaging material and storage temperature. In this study, the influence of packaging materials and elevated temperature on EVOO quality was investigated during six months. At ambient temperatures, oil maintained EVOO when stored in glass, polyethylene terephthalate (PET), high density polyethylene (HDPE), cans and Pottery in terms of chemical tests (acidity, peroxide value, K_{232}, and K_{270}). Loss of phenols was the highest in pottery-stored oil and the lowest was found in glass-stored oil. Only PET-stored oil maintained the EVOO grade in terms of sensory evaluation when stored at room temperature. At elevated temperature, oil stored in all packaging materials lost extra virgin quality in terms of chemical tests. The loss of phenols was the largest in HDPE and smallest in cans-stored oil. Sensory evaluation, maintained glass-stored oil and PET-stored oil as EVOO. This study has reaffirmed that at both storage temperatures, the best container in maintaining the EVOO quality was glass and the worst was pottery. Grading of stored olive oil under investigation using sensory evaluation solely was not sufficient. Also it was clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil and could be used as a rapid indicator test.

Keywords: *Olea europaea L., olive oil, oil oxidation, stability indicators, storage conditions, packaging materials*

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1. Introduction

Olive trees (*Olea europaea* L.) is an important trees internationally, produce high nutritional and health quality edible oil. The global production of olive oil in 2012 was around 2,903,680 tons, from which around 22,950 tons are produced in Palestine. As olive oil production fluctuates from year to year, the mean annual production of olive oil globally during the recent ten years (2003-2012) was 2,946,288 tons and the average annual contribution in Palestine was 17,045 tons [1]. The European Union (EU) is the leading producer of olive oil and within the EU, the Mediterranean members are the biggest producers, accounting for 95% of world production and 85% of world consumption of olive oil [2].

Virgin and extra virgin olive oil is a genuine fruit juice obtained from olive drupes, using exclusively mechanical procedures, without further treatments or chemical additions. Several clinical data have shown that consumption of olive oil can provide heart health benefits, such as favorable effects on cholesterol regulation and LDL cholesterol oxidation, exerting anti-inflammatory, antithrombotic and antihypertensive effects [3]. Quality of olive oil is defined as the combination of its attributes that have significance in determining the degree of its acceptability by the consumer, and may be also defined from commercial, nutritional or organoleptic perspectives. The nutritional value of extra virgin olive oil (EVOO) originates from its high levels of oleic acid content and minor components, such as phenolic compounds that donate the oil its aroma [4]. Therefore, these quality parameters promote the consumption demands and price of olive oil in comparison with other edible oils ranking it superior among vegetable oils [5].

There is a need to develop reliable analytical methods to ensure compliance of olive oil quality with labeling, and to determine the genuineness of the product by the detection of eventual defects during adulterations, processing and storage conditions. Therefore, the International Olive Oil Council (IOOC) and European Communities Legislation (EC) define the identity characteristics of olive oil by specifying analytical methods and standard limit

values of the quality parameters such as peroxide value (PV), acidity, Ultra violet (UV) absorbance values (K_{232} and K_{270}) and organoleptic characteristics (odor, taste and color) for olive oils in order to improve product quality, expand international trade, and raise its consumption. The chemical tests and the organoleptic properties categorize olive oil into extra virgin, virgin, and lampant oil indicating its edible quality and marketable values. The extra virgin olive oil is the highest grade and must contains zero defects and greater than zero positive attributes as evaluated by a certified taste panel, and must have a free acidity of less than 0.8%, peroxide value doesn't exceed 20 milliequivalent O_2 kg^{-1} oil and should have clear flavor that reflect the fruit from which it is produced [6,7].

Quality of olive oil is potentially affected by different factors including genetic (tree variety), agronomic (ripening stage, fertilization, irrigation, and harvesting practices), health of the drupe [8], environmental (temperature, day length, and sunlight duration), geographical [9] factors, and finally the postharvest processing including packaging materials and storage conditions [6]. Furthermore, an important European regulation allows the Protected Denomination of Origin (PDO) labeling of some EU EVOOs and this designation guarantees that the geographical origin of the product is closely in conjunction with the quality of the product [11]. The complex interference of these factors make only 50% of the world's olive oil production is classified as extra virgin grade [12].

In order to fulfill the expectations of consumers, good quality control of olive oil should be assured in the course of production and storage line. The quality of olive oil decreases during storage, and is attributable to oxidation that lead to rancidity [5], and to hydrolytic degradations causing partial loss of healthy minor constituents [13]. Preserving the positive attributes of oil is a matter of great concern for the olive oil industry during the time elapsing from production to bottling, and up to purchasing and consumption [14,15], because the variation of storage conditions during olive oil storage and transportation affect its quality [8,16]. During shelf life of bottled extra virgin olive oil, the bottle must be adequately protective against autoxidation that cause rancidity [7]. Several types of plastic films or metal containers can be used, but glass bottles of different shape and color are the most common [14,17]. Although, extra-virgin olive oil is usually packaged in glass, or plastic bottles, these packages have some disadvantages because their bottled contents may be subjected to oxidation [16]. Accordingly, oil producers need to pay a great deal of attention to the type of containers they place the oils in, after production and to the storage conditions they are kept in, before sale [14]. The influence of glass and high density polyethylene on oil quality during storage was frequently studied [17], while little information is known about the effect of high density polyethylene (HDPE), cans, and pottery jars. The effect of different packaging materials on the quality of olive oil is previously reported [7,14,17]. In the other hand, the non-optimal storage conditions, such as those occurring on a store shelf, may alter the qualitative characteristics of the product to the extent that they may eventually illegally differ from those indicated on the label. Thus, an investigation of the type and magnitude of the alterations in oil undergoes during its shelf life at elevated temperature may provide useful information about optimum practical storage or transport conditions that sustain high quality of olive oil for maximum storage period [7].

Although the effect of storage conditions, time and their consequences were studied for olive oils produced in many countries [9,18], there is no published studies - to our knowledge- corresponding to the effect of packaging materials and storage conditions on the quality of Palestinian olive oils except a recent investigation done by the research group of this investigation under different situations [15]. Therefore, the aim of this study is to evaluate different packaging materials (Glass bottles, PET plastic bottles, HDPE plastic bottles, tin plates, and pottery jars) in terms of their protective ability for quality indices of Palestinian extra virgin olive oil (acidity, peroxide value, K_{232}, K_{270}, phenolic compounds, sensory score 6.5) stored under different storage temperatures (18°C and 37°C) in a six months stability study. Additionally it is aimed to find the potential correlations between chemical quality indices with sensory evaluation test to optimize olive oil evaluation.

2. Materials and Methods

2.1. Experimental Design

Olive fruits of the cultivar 'Nabali Baladi' were handpicked in late October 2008 from an olives orchard located in Salfeet district of a Mediterranean climatic region of Palestine. The fruits were selected with no defects and at an optimal stage of ripening (5.5 N detachment force, 3.8 pigmentation index, and 57.5% water content). Washed olives were processed using stone mill and hydraulic press. The initial whole oil sample was filled temporarily in two 20-liter HDPE containers and directly transported to the laboratories of Al-Quds University. Extra virgin quality of the extracted oil was proved (peroxide value < 20, acidity $< 0.8\%$, $K_{232} < 2.5$, and $K_{270} < 0.25$, iodine value 75-94, refractive index 1.4677-1.4700, Table 1). The 40 liters extra virgin olive oil was distributed into subsamples (300-ml each) that were bottled in different packaging materials (amber glass bottles, polyethylene terephthalate (PET), high density polyethylene (HDPE), tin plate cans hermetically sealed, and pottery jars with covers), maintaining 2% head space in each bottle. Bottled oil was stored under different storage temperatures (18 ± 1°C and 37 ± 1°C); in thermostatic and ventilated incubators (with 100 Lux normal white light inside for around 10 hours daily simulating the condition on shelves). The samples were rearranged weakly to insure uniform spacial distribution of the bottles. The bottles (in four replicates for each treatment) of different packaging materials were randomized in a complete randomized design (CRD) in each storage condition. The effect of each of these factors (packaging materials and temperature storage conditions) on the stability of the extra virgin olive oil was studied in a non orthogonal design by monitoring oil quality indicators that include: acidity (percent as oleic acid), peroxide value, ultraviolet extinction coefficients (K_{232} and K_{270}), total phenolic contents (expressed as mg of

gallic acid kg^{-1} oil), and sensory attributes (Panel test) during six months of the experimental period (0, 30, 60, 90, and 180 days of storage).

Table 1. Quality of olive oil sample initially used in the study

Quality parameter	Value	Unit
Acidity	0.38	g oleic acid per 100 g oil (%)
Peroxide value	10.49	equivalent O_2 per kg oil
Iodine value	82.63	ml I_2 per 100g oil
Saponification value	188	mg KOH per g oil
K_{232}	1.68	absorbance
K_{270}	0.158	absorbance
Density	0.919	g per ml oil
Refractive index	1.46675	-
Sensorial evaluation	0 defect, 4.7 fruity, 5 pungency, 4.5 bitterness	

2.2. Determination of Oil Quality Indicators

Acidity (g oleic acid 100 g^{-1} oil) and peroxide value (milliequivalent O_2 kg^{-1} oil) were determined according to the AOAC [19]. Ultraviolet light absorption indexes (K_{232} and K_{270} extinction coefficients) were determined using the methods described in IOOC [20]. Total Phenol compounds were extracted according to Georgios et al, 2006 [21] and analyzed according to AOAC [19], and their content (mg gallic acid kg^{-1} oil) was determined spectrophotometrically at 765 nm. Sensory evaluation was run by taster team for sensory analyses in the Palestinian Standard Institution laboratory, Ramallah, Palestine. The test was performed by the analytical panel done by 13 trained technicians, working according to the method defined by the Standard IOOC [20]. The results obtained based on the ranking according to the median of notes from the tasters. Each bottle in each treatment was analyzed monthly for each mentioned chemical quality indicator up to six months. The sensory evaluation was inspected in three periods (0, 3, and 6 months).

2.3. Statistical Analysis

Four bottles of each treatment were independently analyzed in each sampling time. The results are expressed as mean ± standard deviation. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of different storage conditions and different packaging materials were carried out using the GLM procedure considering a fully randomized design, treating main factors (packaging materials and storage conditions) separately using one-way analysis of variance. The Bonferroni procedure was employed with multiple t-tests in order to maintain an experiment wise of 5%.

Initially Pearson correlations were calculated to test the relation among quality indicators of stored olive oil at each storage condition separately and when data were pooled. The NOMISS option was used in order to obtain results consistent with subsequent multiple regression studies.

3. Results

3.1. Acidity

Our findings reveal that, acidity of EVOO increased dramatically with increasing storage time in all studied storage containers stored at elevated temperatures, except for that stored in pottery jars, where the highest acidity value was reached after 90 days, then was significantly reduced after 135 and 180 days of storage (Table 2). After 30 days of storage, glass containers retain the highest acidity values followed by pottery followed by cans followed by PET and the least was found in HDPE but the values were statistically not significant in both types of plastic containers. At the end of storage time, only glass and cans exceeded the limit for the extra virgin grade (0.8 % oleic acid), where they shared the highest acidity values in stored oil (0.81 and 0.82 for glass and cans respectively). At the end of storage period, the least acidity value was found in oil stored in pottery, while both types of plastic containers retained the same intermediate acidity values.

At room temperature, acidity of stored EVOO increased slightly but significantly with increasing time of storage. At the end of storage period, the least acidity value was reported in oil stored in pottery, while the other containers maintained similar values significantly. All storage containers protected stored EVOO in terms of acidity and maintained its extra virgin grade throughout storage period. Comparing the acidity in the same container type at the same storage time but different temperature treatments, acidity was higher under elevated temperature in all packaging materials.

Table 2. Acidity (% as oleic acid) at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	0.38 E, a (0.38 C), a	0.38 D, a (0.38 C), a	0.38 D, a (0.38 E), a	0.38 E, a (0.38 D), a	0.38 C, a (0.38 D), a
30	0.58* CD, a (0.42 CB), a	0.44 C, c (0.42 B), a	0.41* C, c (0.54 B), a	0.46* D, cb (0.40 C), a	0.50* B, b (0.39 C), a
45	0.57* D, a (0.42 CB), c	0.47* C, cb (0.42 B), c	0.50 B, b (0.50 C), a	0.59* C, a (0.47 CB), b	0.41 C, c (0.42 BC), c
90	0.63* C, b (0.43 B), c	0.53* B, c (0.49 A), ba	0.50 B, c (0.50 C), a	0.71* B, a (0.48 B), cb	0.53* A, c (0.45 CBA), ba
135	0.71* B, a (0.52 A), ba	0.54* B, b (0.50 A), b	0.57* A, b (0.43 D), a	0.73* B, a (0.53 BA), a	0.49 B, c (0.50 BA), b
180	0.81* A, a (0.53 A), a	0.58* A, b (0.51 A), a	0.57* A, b (0.58 A), a	0.82* A, a (0.56 A), a	0.49* B, c (0.51 A), b

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.2. Peroxide Value

Our results highlighted that, PV of stored EVOO at elevated temperature showed different responses in different packaging materials as a function of storage time (Table 3). It fluctuated in glass containers; where it decreased significantly after 30 days of storage in dramatic manner tell 90 days of storage, then it increased and out-yielded the initial value but without significant difference. PV decreased drastically with time of storage in oil stored in both types of plastic containers, while increased in oil stored in pottery continuously with time of storage and overcame the limit of EVOO grade (20 milliequivalent O_2 kg^{-1} oil) before 135 days of storage. All storage containers except pottery retained the EVOO quality in terms of peroxide value during the experiment when stored at elevated temperature.

At ambient temperature, PV decreased significantly with time in oil stored in glass, PET and cans, while it didn’t change significantly in oil stored in HDPE, and it was significantly elevated in oil stored in pottery. At the end of storage time, PV of oil stored at elevated temperature was found significantly higher than that stored at room temperature in glass and pottery, while the opposite was recorded for oil stored in cans. In the other hand, both types of plastic containers maintained peroxide values similar at both storage conditions.

Table 3. Peroxide value (as milliequivalent O_2 kg^{-1} oil) at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 A, a (10.50 A), a	10.50 C, a (10.50 C), a
30	10.50 A, (9.90 A), a	8.10 A, a (8.27 B), a	10.87 A, a (8.70 A), a	10.53* A, a (8.20 B), a	10.8 7* C, a (8.37 D), a
45	7.87* B, cb (7.37 B), b	9.03* BA, cb (8.50 B), a	9.50* A, ba (8.63 A), a	7.30 B, c (8.37 B), a	11.10* C, a (8.13 D), a
90	6.23* C, d (8.23 B), b	8.13* B, c (8.63 B), b	8.83 A, b (9.37 A), b	5.90* B, d (8.17 B), b	16.53* B, a (11.63 CB), a
135	6.73* B, b (8.37 B), c	8.3 B, b (8.67 B), c	9.77* A, b (9.23 A), b	6.20* B, b (8.87 B), cb	22.73* A, a (12.57 B), a
180	10.87* A, b (8.43 B), c	8.43 B, c (8.87 B), c	9.77 A, b (10.03 A), b	6.17* B, d (7.70 B), d	21.83* A, a (14.10 A), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.3. Ultraviolet Extinction Coefficients

3.3.1. Extinction Coefficient at 232 nm (K_{232})

The extinction coefficient K_{232} of olive oil stored in HDPE at elevated temperature under study, increased continuously and significantly with extending time of storage (Table 4). The same response was recorded for oil stored in pottery in the first 135 days but this extinction coefficient was slightly and significantly decreased after 180 days compared to the previous measurement. In glass bottles, K_{232} fluctuated during storage, where it decreased significantly after 45 days, then reached its peak after 135 days, where it was significantly higher than the initial measurement, and at the end of the experiment went back to a value similar to the initial one. In PET, K_{232} showed a trend of increment during the experiment with a higher significant value at the end of the experiment compared with the baseline measurement. This quality index of oil stored in cans fluctuated during storage period; where the initial and final measurements were statistically similar. The extra virgin grade in terms of K_{232} (<2.5) was maintained in oil stored in glass, PET and cans even though they were stored for six months at elevated temperature. But oil stored in HDPE quitted this grade in terms of this quality index at the end of the experiment and that stored in pottery, exceeded 2.5 after 135 days and was marginal to the critical limit at the end of the storage period.

At ambient storage temperature, the extinction coefficient K_{232}, decreased slightly but significantly within the respective testing dates in oil samples stored in all packaging materials under study except for pottery jars, where a significant increase was reported after 90 days in pottery and the rate of increase was maintained tell the end of the experiment. None of the samples stored in either packaging material at ambient temperature exceeded the higher limit of K_{232} determining extra virgin quality of olive oil. Values of K_{232} measured at each testing time for each packaging material was found significantly higher in oil stored at elevated temperature compared to oil stored at ambient temperature, and this was true for all packages under study.

Table 4. K_{232} at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	2.02 B, a (2.02 A), a	2.02 C, a (2.02 A), a	2.02 D, a (2.02 BA), a	2.02 BC, a (2.02 A), a	2.02 E, a (2.02 D), a
30	2.04* B, b (1.77 D), b	2.03 C, b (2.03 A), a	2.09* D, ba (2.04 BA), a	2.15* A, a (2.02 A), a	2.09* D, ba (2.03 D), a
45	1.90* C, c (1.74 D), c	2.31* A, ba (2.01 BA), ba	2.26* C, b (2.08 A), a	1.91 D, c (1.84 B), cb	2.40* C, a (2.05 D), a
90	1.92* C, e (1.85 C), c	2.16* B, c (2.02 A), b	2.33* C, b (1.75 C), d	1.94* DC, d (1.88 B), c	2.37* C, a (2.23 B), a
135	2.34* A, c (1.90 CB), c	2.17* B, d (1.98 CB), b	2.49* B, b (1.95 BA), b	1.92 D, e (1.96 A), b	2.62* A, a (2.10 C), a
180	2.04* B, e (1.96 BA), b	2.31* A, c (1.96 C), b	2.60* A, a (1.87 CB), c	2.09* BA, d (1.83 B), c	2.48* B, b (2.36 A), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.3.2. Extinction Coefficients at 270 nm (K_{270})

Extinction coefficient measured at 270 nm (K_{270}) of stored olive oil at elevated temperature increased progressively in significant values with increasing time of storage in all studied packaging materials under study (Table 5). At the end of the experiment, the highest K_{270} value was found in oil stored in HDPE, followed by pottery without significant difference, followed by PET, followed by cans, and the least value was recorded in oil stored in glass bottles. All storage containers deteriorate stored olive oil and quitted from extra virgin grade in terms of K_{270} (< 0.2) when oil was stored at elevated temperature but at different storage periods. PET bottles retained stored oil as extra virgin in terms of K_{270} for less than 135 days, and that stored in glass and cans for less than 90 days, and for that stored in HDPE and pottery for less than 45 days.

At ambient temperature, K_{270} was slightly increased in oil stored in glass, PET, and pottery, while it was not affected in oil stored in cans, but was significantly decreased in oil stored in HDPE. None of packaging materials under investigation elevated K_{270} of stored olive oil to the critical limit of extra virgin grade when oil stored at ambient temperature for six months. K_{270} values of oil stored at elevated temperature was higher than that stored at room temperature in all packaging materials under study in most storage periods.

Table 5. K_{270} at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	0.160 D, a (0.160 B), a	0.160 E, a (0.160 B), a	0.160 F, a (0.160 CB), a	0.160 D, a (0.160 A), a	0.160 D, a (0.160 C), a
30	0.187 CB, a (0.180 A), ba	0.193 DC, a (0.197 A), a	0.200* E, a (0.180 BA), ba	0.187* C, a (0.160 A), c	0.160* D, b (0.173 CB), cb
45	0.180* C, c (0.163 B), a	0.230* B, a (0.190 A), a	0.217* D, b (0.203 A), a	0.183* CB, c (0.163 A), a	0.213* C, b (0.197 A), a
90	0.203* BA, dc (0.160 B), bc	0.190* D, d (0.187 A), a	0.230* C, a (0.147 C), c	0.210* A, cb (0.167 A), ba	0.220* C, ba (0.187 BA), a
135	0.210* A, b (0.160 B), b	0.207* C, b (0.180 B), a	0.263* B, a (0.153 C), b	0.210* A, b (0.170 A), ba	0.247* B, a (0.167 C), ba
180	0.197 BCA, c (0.180 A), a	0.260* A, b (0.187 A), a	0.290* A, a (0.137 C), b	0.200* BA, c (0.170 A), a	0.280* A, a (0.190 BA), a

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

3.4. Total Phenolic Compounds

Storage at elevated temperature significantly reduced total phenolic compounds of EVOO stored in all packaging materials under study (Table 6). Total phenols were significantly and highly reduced at all consequent storage periods in oil stored in PET, HDPE, and pottery, while in glass and cans, the successive reduction of phenolic compounds were reported until 135 days of storage but were significantly elevated at the end of storage period. Comparing phenolic compounds contents of stored olive oil at the end of storage period related to their initial contents in the same packaging material, the most reduced contents of phenolic compounds was found in HDPE followed by pottery followed by PET followed by glass and the least was recorded in oil stored in cans.

At room temperature storage condition, phenolic compounds were dramatically and significantly reduced with consecutive increase of storage period. At the end of storage period, the largest loss of phenolic compounds was found in pottery followed by HDPE, followed by cans and PET, and the least reduction of phenolic contents was recorded in glass.

Table 6. Total phenols at elevated temperatures compared to room temperature (between brackets)

days	Glass	PET	HDPE	Cans	Pottery
0	213.3 A, a (213.3 A), a	213.3 A, a (213.3 A), a	213.3 A, a (213.3 A), a	213.3 A, a (213.3 A), a	213.3 A, a (213.3 A), a
30	194.3* B, b (203.0 B), b	213.7 A, a (206.3 BA), b	196.7* CB, b (207.7 B), ba	190.0* CB, bc (214.3 A), a	185.3 B, c (185.3 B), c
45	188.7* C, c (201.3 B), b	212.7* A, a (202.33 CB), b	197.7* B, b (201.7 C), b	184.7* DC, cd (213.7 A), a	182.7* B, d (178.6 CB), c
90	182.7* D, c (200.3 CB), a	202.7* B, a (199.7 CB), a	188.0* DC, b (183.3 E), bc	181.7 DC, c (195.3 B), ba	143.7* C, d (170.0 DC), c
135	180.3* D, b (196.3 DC), a	197.0* B, a (195.0 C), ba	183.0* D, b (191.7 D), b	179.3* D, b (194.7 B), ba	140.7* C, c (161.3 D), c
180	191.7* CB, ba (196.0 D), a	182.7* C, c (184.6 D), b	123.3* E, b (167.3 F), c	196.7* B, a (184.3 B), b	133.0* D, c (161.0 D), d
Reduction‡ (%)	10.1 (8.1)	14.3 (13.5)	42.2 (21.6)	7.8 (13.6)	37.6 (24.5)

Different capital letters within each column or small letters within each line indicate significant difference ($p < 0.05$, $n = 4$). * Indicates significance between different temperature treatments in the same cell of the table at a given P level ($p < 0.05$).

‡Total reduction of phenolic compounds at the end of storage period based on the initial contents of total phenols.

Total phenols contents in oil stored in all packaging materials under study was found less in oil stored at elevated temperature than that stored at ambient temperature when compared in the same packaging material and the same testing date after all consecutive storage periods except for oil stored in cans at the end of the storage period where the opposite was recorded.

3.5. Sensory Evaluations

Sensory evaluation was done for all samples subjected to storage conditions in three periods (before storage, after three months, and after six months of storage). Sensory evaluation (Table 7) reveals that, oil stored in glass

sustained the extra virgin grade under elevated temperature throughout the experiment, and also at room temperature till 90 days and then turned to virgin grade because of the appearance of sensory defects (Figure 1). The fruity of the glass-stored oil at both storage conditions decreased consequently with increasing time of storage. Caned oil responded the same at both storage conditions, and lost the extra virgin grade before 90 days of storage then remained in the virgin grade throughout the experiment, because fruity of oil was lost and sensory defects appeared during storage. Oil stored in PET sustained the extra virgin grade for six months without sensory defects but with marginal loss in fruity. In HDPE, oil at both storage conditions became virgin after 90 days. Oil fruity decreased largely before 90 days of storage but the sensory defects appeared at the end of the experiment. Because of the complete loss of oil fruity, and the appearance of high level of sensory defects (>2.5), oil stored in pottery quitted from the virgin grade at both storage conditions before 90 days of storage.

Table 7. Olive oil grading according to the sensory evaluation for oil samples stored at elevated temperatures compared to room temperature bottled in different packaging materials

Container	days	Sensory Defects		Sensory Fruity		Olive oil grade	
		Elevated T	Room T	Elevated T	Room T	Elevated T	Room T
	0	0.0	0.0	4.9	4.9	EVOO	EVOO
Glass	90	0.0	0.0	1.9	2.0	EVOO	EVOO
	180	0.0	0.8	1.0	1.0	EVOO	VOO
	0	0.0	0.0	4.9	4.9	EVOO	EVOO
Can	90	1.5	1.5	0.0	0.0	VOO	VOO
	180	2.3	2.3	0.5	0.5	VOO	VOO
	0	0.0	0.0	4.9	4.9	EVOO	EVOO
PET	90	0.0	0.0	3.0	3.0	EVOO	EVOO
	180	0.0	0.0	2.6	2.6	EVOO	EVOO
	0	0.0	0.0	4.9	4.9	EVOO	EVOO
HDPE	90	0.0	0.0	1.2	1.2	EVOO	EVOO
	180	1.9	1.9	1.3	1.3	VOO	VOO
	0	0.0	0.0	4.9	4.9	EVOO	EVOO
Pottery	90	3.0	3.0	0.0	0.0	OVOO	OVOO
	180	3.5	3.5	0.0	0.0	OVOO	OVOO

EVOO is extra virgin olive oil, VOO is virgin olive oil, OVOO is ordinary virgin olive oil.

Table 8. Olive oil grading according to the sensory evaluation and other stability indices for oil samples stored at different temperatures bottled in different packaging materials

Container	days	Acidity	PV	K_{232}	K_{270}	Sensory evaluation		Olive oil grade
						Defect	Fruity	
Elevated temperature								
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Glass	90	0.63	6.23	1.92	0.20	0.0	1.9	EVOO
	180	**0.81**	10.88	2.04	0.19	0.0	1.0	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Can	90	0.71	7.30	1.94	0.21	**1.5**	**0.0**	VOO
	180	**0.82**	6.21	2.09	0.20	**2.3**	0.5	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
PET	90	0.53	8.13	2.16	0.19	0.0	3.0	EVOO
	180	0.58	8.44	2.31	**0.26**	0.0	2.6	OVOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
HDPE	90	0.50	8.82	2.33	**0.23**	0.0	1.2	VOO
	180	0.57	9.77	**2.60**	**0.29**	**1.9**	1.3	OVOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Pottery	90	0.53	16.53	2.37	**0.22**	**3.0**	**0.0**	OVOO
	180	0.50	**21.81**	2.48	**0.28**	**3.5**	**0.0**	OVOO
Room temperature								
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Glass	90	0.51	8.42	1.82	0.22	0.0	2.0	EVOO
	180	0.66	8.23	2.07	**0.27**	**0.8**	1.0	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Can	90	0.47	8.17	1.88	0.17	**1.5**	**0.0**	VOO
	180	0.56	7.72	1.83	0.17	**2.3**	0.5	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
PET	90	0.43	7.99	2.03	**0.24**	0.0	3.0	VOO
	180	0.52	8.55	1.85	**0.23**	0.0	2.6	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
HDPE	90	0.49	9.42	1.73	0.18	0.0	1.2	EVOO
	180	0.56	10.84	1.80	0.21	**1.9**	1.3	VOO
	0	0.38	10.49	2.02	0.16	0.0	4.9	EVOO
Pottery	90	0.51	11.63	2.23	0.19	**3.0**	**0.0**	O VOO
	180	0.42	14.12	2.36	0.19	**3.5**	**0.0**	O VOO

EVOO is extra virgin olive oil, VOO is virgin olive oil, OVOO is ordinary virgin olive oil.

3.6. Pearson Correlation with Oil Quality Parameters as Affected with Temperature Treatments

Pearson correlations between quality parameters of olive oil stored at room temperature (Table 9) show that peroxide value was positively and significantly correlated with K_{232} extinction coefficient but the correlation with phenolic contents was significantly negative, and also insignificantly correlated with K_{270}. K_{232} was significantly and positively correlated with K_{270} but was significantly and negatively correlated with phenolic contents. There was no significant correlation found between K_{270} and phenolic contents. At elevated temperatures, peroxide value was significantly and positively correlated with both extinction coefficients, and was also significantly and negatively correlated with phenolic contents. K_{232} was highly correlated with both K_{270} and phenolic compounds content but the correlation with the formers was positive while the correlation with the later was negative. K_{270} and phenolic contents was highly negatively correlated with each other. Pearson correlation between quality parameters of olive oil stored at both room and elevated temperature when all data was pooled (Table 10) shows that peroxide value was highly positively and significantly correlated with K_{232}. K_{270} was significantly and positively correlated with peroxide value and K_{232}. Phenolic contents showed highly negative and significant correlation with all quality parameters under study (peroxide value and K_{232}, and K_{270}).

Table 9. Pearson coefficients between quality parameters of oil stored at room temperature (above the diagonal) and at elevated temperature (below the diagonal)

	Peroxide	K_{232}	K_{270}	Phenols
Peroxide	-	0.608***	-0.055	-0.372***
K_{232}	0.559***	-	0.458***	-0.251*
K_{270}	0.311**	0.789***	-	-0.103
Phenols	-0.530***	-0.643***	-0.734***	-

Table 10. Pearson coefficients between quality parameters of olive oil stored at both room temperature and elevated temperature (pooled data)

	K_{232}	K_{270}	Phenols
Peroxide	0.550***	0.285***	-0.499***
K_{232}		0.779***	-0.546***
K_{270}			-0.584***

4. Discussion

The value of EVOO that determines it's commercial and health quality originates from its high oleic acid contents and the presence phenolic compounds that donates it the special aroma and antioxidant activity [12,22]. Olive oil quality and stability are principally affected by lipid oxidation, generating off-flavor (rancidity) and reduction in oil nutritional value causing health risks and even toxicity for consumers. Lipid peroxidation produces toxic compounds which causes lung damage. In addition to this effect, reactions between peroxidized lipids and proteins have been shown to cause loss of enzyme activities, polymerization, accelerated formation of brown pigments and the destruction of essential amino acids such as histidine, lysine, tryptophan and methionine. Aldehydes, ketones, hydrocarbons and furans, are known as the cleavage products of hydroperoxides, cause reduction in protein solubility, and reduction in nutritional value of proteins. As well, lipid oxidation provokes a decrease in nutritional values of some vitamins such as A, D, E and K. From the health point of view, lipid radicals and oxidation products contribute in aging, DNA damage, Parkinsonism, carcinogenesis, and coronary heart diseases [23].

As lipids oxidize, they form hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products, such as aldehydes, ketones, acids, and alcohols. In many cases, these compounds adversely affect flavor, aroma, taste, nutritional value, and overall quality. The oxidation process of triglycerides is complex because it always takes place by chain reactions either in dark involving free radicals, called autoxidation, or light-dependent reactions known as photooxidation [22]. Many catalytic systems such as light, temperature, enzymes (lipase), metals, and microorganisms, can accelerate lipids oxidation [24]. Variation during olive oil storage and transportation that enhance lipid oxidation is common, and may be attributed to natural or climatic condition and to extreme storage conditions [8,18]. In addition to storage conditions, the retention of oil quality for an extended period of time that allows its worldwide distribution is also highly affected by the type of packaging material [25]. Knowledge about packaging materials, and their interactions with the bottled oil, along with a deeper understanding of the oxidation pathways under various storage conditions provide necessary information for improving the quality of packaged olive oil during shelf life and transportation [17].

Therefore, in order to fulfill the consumer's requirements, good quality control of olive oil should be assured in the course of production and storage processes. The quality of olive oils is interpreted in terms of measurements of analytical parameters for which certain limit values are set. The most important quality requirements of olive oil in commercial transactions are: acidity, peroxide value, K_{232}, K_{270}, and total phenolic content in addition to the sensory evaluation. These parameters have been evaluated for the Palestinian olive oil samples under investigation as stability-indicators in terms of storage time in response to different packaging materials and storage temperatures.

4.1. Acidity

Acidity is mainly determined by titration using potassium hydroxide that measure the amount of free fatty acids (FFA's) present in the oil as oleic acid which is the major component in the triglycerides present in the olive oil, and should be less than 0.8% if the oil is extra virgin [26]. Although, acidity values are used as a basic criterion for classifying different categories of olive oil, it was not considered as the best criterion for evaluating olive oil quality by some investigators [27]. Acidity reflects oil stability and susceptibility to rancidity. The hydrolytic rancidity of oil due to presence of water and the catalytic action of the lipase (often derived by microorganisms) in oil as mentioned above, partially degrade triglycerides giving glycerol and free fatty acids, which increase acidity.

In agreement with our findings, acidity of EVOO stored in glass increased with increasing storage time but didn't

exceed the limits during storage at room temperature [28,29], but exceeded the limit at elevated temperatures [28,30]. While in contrary to our results, other reporters [30,31] found that acidity of oil stored in glass didn't change significantly during storage at room temperature. Several studies conducted on olive oil shelf life attested the glass as the best material for the storage [32], in terms of its acidity, especially when oil was stored in the dark with respect to other packages [33]. As acidity values of oil stored at room temperature in glass, PET, HDPE, and cans didn't differ significantly in our experiment, other investigators clearly indicated the glass as the best (less value) in terms of acidity in the following ranking Glass > HDPE > PET [34]. Metal containers have the same water resistant properties as glass and may protect the product from oxygen, light, and microorganisms that could increase the acidity of oil through increasing the rate of hydrolysis of triglycerides. But when oil was stored at elevated temperature, our results reported both glass and cans as the worst packaging materials in terms of acidity of stored oil which exceeded the extra virgin grade limit, while plastic materials (PET and HDPE) where found better and pottery was reported as the best. This can be explained by the high thermal conductivity of glass and cans compared to plastic ones, and for the cooling effect of pottery on stored oil.

4.2. Peroxide Value

Peroxide value (PV), a measure of total peroxides in olive oil (meq. O_2 kg^{-1} oil) is a major guide of oil quality. The official determination method is based on the titration of iodine liberated from potassium iodide by peroxides present in the oil. In other words, the peroxide value is a measure of the active oxygen bound by the oil which reflects the hydroxyperoxide value, and measures the degree of lipid peroxidation. The higher the number means the greater degradation due to oxidation with an upper limit of 20 meq. O_2 kg^{-1} oil, but levels higher than 10 may mean less stable oil with a shorter shelf life [35]. In lipid oxidation reactions, many free radicals and oxygen species, such as singlet oxygen are involved. The main substrates for these reactions are unsaturated fatty acids and oxygen. The free radical mechanism of lipid oxidation is usually described in a three stages chain reaction including initiation, propagation, and termination steps. Initiation starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid molecule, by the catalytic effect of light, heat, or metal ions to form a free radical, where direct reaction of fatty acid molecule with oxygen does not take place frequently, because of the high activation energy. The resultant free radical reacts with atmospheric oxygen to form an unstable peroxy free radical may in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide. A new alkyl free radical initiates further oxidation and contributes to the chain reaction, and this chain reaction is called propagation stage of autoxidation. The chain reaction may be terminated by formation of nonradical products resulting from combination of two radical species. The propagation stage in autoxidation process includes an induction period when hydroperoxides formation is minimal. The rate of oxidation of fatty acids increases in relation to their degree of unsaturation, therefore, oils that contain high proportions of polyunsaturated fatty acids may experience instability problems. The breakdown products of hydroperoxides, such as alcohols, aldehydes, ketones, furans, esters, lactones and hydrocarbons, generally cause off-flavors, and may also interact with other food components and change their functional and nutritional properties [36].

In accordance with our results, other investigators [5,30,37] found that PV of oils bottled in glass and PET stored at room temperature fluctuated during storage time and did not exceed the official limit during six months of storage. In the other hand, a linear increase in PV with storage time at room temperature in oil stored in glass bottles [9,10] and in tin plates [38] was reported. In the same line with our results in oil stored in glass, PET, and cans at room temperature and elevated temperature, PV decreased significantly with increasing storage time [7]. But in contrast with our findings except for oil stored in pottery, other scientists [22] reported an increase in the PV of oil samples stored under elevated temperature. In accordance with our results, fluctuation in the PV of oil samples stored at elevated temperature [10], and at shelf [15] was reported. The decrease in the PV with increasing time in many testing dates observed in our results in different packaging materials and at both storage temperatures, can be explained by the degradation of primary oxidation products (peroxides) to form secondary oxidation products which can be detected by K_{232} values. The results of PV was correlated with that obtained by K_{232} (Table 9, Table 10) and agreed with other reporters [7,28,39]. Generally, during the beginning of storage, PV in different packaging materials increased as a consequence of the action of both diluted and headspace oxygen in the containers and additionally, the temperature which induce a rapid deterioration of oil in terms of PV. After a period of storage, the PV progressively decrease because of the degradation of primary products into secondary products, which is more obvious in the samples packed in cans and glass containers and less in those packed in plastic and pottery. The oil samples packed in pottery and stored at both room and elevated temperatures have higher peroxide values compared to those stored in other containers. These results may point to the probable intrusion of oxygen and water through pottery, although it is impermeable to light with low thermal conductivity that retain primary oxidation products for longer time and delay their destruction to produce secondary oxidation products.

4.3 Ultraviolet Extinction Coefficients

Determination of the absorption coefficients in the ultraviolet region (232 nm and 270 nm) reflects the stage of oxidation for olive oil during storage [40], in which the shelf-life of virgin olive oil is determined by the increase in the K_{232} absorption coefficient [41], or by means of the time required to reach the upper legal limit of K_{270} absorption coefficient [7, 42]. Primary oxidation products in olive oil (fatty acid hydroperoxides and oxidized triacylglycerols) are measured as peroxide value and K_{232} absorption coefficient (measure the conjugated dienes), while secondary oxidation products (aldehydes, alcohols, ketones and hydrocarbons) are detected by K_{270} absorption coefficient [40,42]. Hydroperoxides are the initial

products of oxidation -very sensitive and comparatively unstable- and used as indicator of the early stages of oxidative deterioration in the oxidation process [17,43], while the K_{270} index is used to study the behavior of the secondary oxidation products by the formation of dimers and polymers of triacylglycerides [42].

For instance, an increase in K_{232} and K_{270} values is very common between extraction of olive oil and its consumption as affected by storage time and conditions [22]. It is documented that heat affects olive oil quality by increasing the trienes formation, measured by K_{270} [9], more than the dienes measured as K_{232} [5]. In agreement with our findings, K_{270} values were affected by the heat exposure conditions more than that of K_{232}, with higher values reported in the samples stored at elevated temperature than in those kept at ambient temperature [44]. Such a response is due to the degradation of primary oxidation products (peroxides) to form secondary oxidation products, as K_{232} representing the amount of conjugated dienes of the primary oxidation products [7,28,39] and are transferred to trienes measured by K_{270} [45].

Our findings are in agreement with previously reported results [37] which found that K_{270} of oil stored in glass bottles and PET containers at elevated temperatures, exceeded the limit of extra virgin grade after two and three months of storage for glass and PET respectively. Also in the same line with our results, other investigators [30] reported an increase in K_{270} of oil samples stored in glass and PET at room temperature throughout the storage, but in contrary with our findings, they found that K_{270} values exceeded the limit (0.2) after two months of storage. The increase in K_{232} with increasing time of oil -in contrary with our findings- was reported [30,37] when oil bottled in glass and PET container stored at room temperature but the values did not exceed the official limit, and values in glass overcame that in PET. Because of the significant variation of K_{270} values during olive oil storage as a response to oil oxidation, this parameter may be of capital importance to control the quality of stored extra virgin olive oils in terms of determining the time at which they will lose their “extra” category [7].

4.4. Total Phenolic Compounds

Extra virgin olive oil, is one of the few oils being consumed without any chemical treatment. It has high resistance to oxidative deterioration mainly due to its fatty acid composition -high monounsaturated to polyunsaturated ratio- and to the presence of natural antioxidants, especially phenolic compounds, carotenoids, and tocopherols, therefore delay the oxidation of lipids and the production of the undesirable volatile compounds [8,22]. During oil storage, the hydrolysis, esterification and oxidation deplete the minor constituents, because of the action of phenolic compounds as antioxidants mainly at the initial stage of autoxidation [46] by scavenging free radicals and chelating metals. Accordingly, the determination of the minor constituents in olive oil is essential for the analytical assessment of its quality and self protection potential.

In agreement with previous reports [7,28,47], our findings showed that total polyphenolic contents of extra virgin olive oil under investigation decreased during storage in all means of packaging materials and storage conditions (Table 6); due to degradation of these compounds that was well fitted to first order kinetics. At the end of storage period, the phenolic compounds of samples stored at elevated temperature showed significantly higher reduction than those stored at ambient temperature [5,17,30,32] in all types of packaging materials except those were stored in cans. Some reporters [30] found that total phenols of oils bottled in glass and PET container didn’t show significant decrease during storage, while others reported an increase in phenolic compounds contents with increasing time of storage [10,28], a situation found in our findings when oil was stored in glass and cans after 180 days of storage compared to the previous sampling date (135 days), which could be due to hydrolysis of secoiridoid derivatives in oil. As phenolic compounds act as natural antioxidants in oil and inhibit autoxidation of lipids (RH) by trapping intermediate peroxyl radicals [48], their reduction during storage is a result of oil oxidation [38].

The stability of virgin olive oil also depends on the presence of pro-oxidant substances as well as on factors linked to the storage conditions, namely the presence of oxygen, temperature and above all light exposure, therefore, the level of degradation of an oil results from a balance of all these factors [14]. The phenolic compounds act by giving an electron so that they can interrupt the radical reaction occurring with oxidation. The carotenoids act as electron acceptors, quenching the singlet oxygen. Finally, tocopherols act both as electron donors, slowing down the oxidative reaction, and as electron acceptors, determining the singlet oxygen quenching or scavenging, with consequent inhibition of the oxidation of lipids [49]. At the beginning of storage time, olive oil under this study contained 214 ± 1.5 mg kg^{-1} oil of total phenolic compounds, and this value was in consistent with the data (121-410 mg kg^{-1}) reported previously [15]. Afterwards, the total content of phenols decreased as a function of time, with various degree of reduction among the storage containers, and the decrease was more pronounced under elevated temperature storage condition. Table 6 showed that the lowest difference between the initial and final antiradical activity (percentage loss of total phenols) at ambient temperature was in glass bottles (8.1%), followed by PET (13.5%) and cans (13.6%), followed by HDPE (21.6%), and the highest reduction was found in pottery (24.5%) stored at room temperature. But concerning the reduction of phenolic compounds in glass and cans was more pronounced after 135 day of storage in a reduction percentage similar to each other and to PET (15.5% and 15.9% for glass and cans respectively). It was previously reported that glass bottles kept more phenolic compounds than that stored in PET containers [15]. The reduction of antioxidants in plastic containers could be due to their permeability to oxygen and the migration of active compounds between oil and packaging material. The large reduction found in oil stored in pottery could be due to the penetration of both oxygen and moisture which both accelerate the hydrolysis of fatty acids, formation of radicals and the depletion of antioxidants.

At elevated temperature, the highest reduction in phenol compounds was found in HDPE (42.2%), followed by pottery (37.6%), followed by PET (14.3%), followed by glass bottles (10.1%), and the least reduction was found in

cans (7.8%). Although cans and glass bottles have the highest thermal conductivity, they showed the least reduction in phenolic compounds. This can be discussed by the effect of oxygen penetration on the oxidation of oil and the consequent reduction of antioxidant compounds including total phenols and the more oxygen penetration through PET, HDPE, and pottery stated clearly that phenolic compound loss intensity during storage is directly proportional to the attitude and degree of oxidation occurred in the presence of oxygen.

4.5. Correlation among PV, K_{232}, K_{270}, and Phenolic Compounds

As the oxidation process of olive oil triglycerides occur as a consecutive chain reaction, and each stage in this oxidation pathway could be monitored by quality indicator(s), olive oil quality indices are correlated to each others. PV is correlated with the K_{232} value not only at time zero but also during storage. The significant correlation between K_{232} and peroxide value is expected as both parameters reflect primary oxidation products of the oil and therefore positive correlation was observed and was previously reported [44]. Therefore, for safety issues, PV determination could be excluded from the routine control of olive oil and replaced by K_{232} determination and the use of unwanted chemicals used in PV analysis could be avoided. No significant correlation was found between K_{270} and peroxide value as K_{270} reflects the secondary oxidation products of the oil. Regarding the negative correlation between peroxide value and phenolic content, this correlation is expected because when phenolic content decreases (by oxidation), the peroxide value increases and this explain why a negative correlation was observed. K_{232} and K_{270} are positively correlated which implies that there is a direct relationship between primary oxidation products and secondary oxidation products i.e. as primary oxidation products increases, secondary oxidation products increases too [44].

A close look at Pearson coefficients of quality indicators of oil stored at elevated temperature as compared to ambient temperature (Table 9) reveals that the correlation was stronger at the former storage condition as compared to the later, indicating that, the deterioration rate at elevated temperature is higher. Moreover the correlation between PV and K_{232} -which both indicate the primary oxidation products- are similar at both temperature treatments while the correlation between both mentioned indicators and K_{270} which indicates secondary oxidation products was higher at elevated temperature (there was no correlation between PV and K_{270} at room temperature). This highlight that the rate of transfer from primary oxidation products to secondary oxidation products is higher at elevated temperature as compared to that at room temperature [9]. This was also clearly observed in the presence of high negative Pearson coefficients at elevated temperature between total phenols and K_{270} (secondary oxidation products) compared to insignificant correlation between both indicators at room temperature. Also the correlation between the phenols in one hand and both PV an K_{232} (primary oxidation products) in the other hand was more negative at elevated temperature as compared to that at room temperature. This indicate that the formation of both primary and secondary oxidation products contribute to the depletion of phenolic compounds at higher temperature while the main contributor in the depletion of phenolic compounds under room temperature was the presence of primary oxidation products proving the importance of phenolic compounds as antioxidants in early stages of autoxidation [46].

4.6. Sensory Evaluation

The consumer expresses his judgment on olive oil quality considering some sensory characteristics, such as the pungent taste, fruity and mild flavor. A wide range of preferences within this context can be found, because the sensory quality may match cultural aspects or simple dietary habits. Characteristic aroma and in particular green and fruity features of olive oil originates from many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase pathway which takes place during the oil extraction process [50,51]. Beside volatile compounds, non-volatile compounds such as phenolic compounds also stimulate the tasting perception of bitterness and pungency. The concentrations of volatile compounds depend on the enzymatic activity [52], and though, the external parameters (e.g. climate, soil, harvesting and extraction conditions) may alter the inherent olive oil sensory profile [53]. The aroma of olive oil is attributed to aldehydes (hexanal, trans-2-hexenal, acetaldehyde), alcohols (methanol, hexan-1-ol, 3-methylbutan-1-ol), esters (methyl acetate, ethyl acetate, hexyl acetate), hydrocarbons (2-methylbutane, hexane, nonane), ketones (2-butanone, 3-methyl-2-butanone, 3-pentanone), furans and other undefined volatile compounds. The major volatiles in virgin olive oils are C6 and C5 volatile compounds [50,54].

Evaluating the quality of stored olive oil in terms of its grade of virginity as influenced by different packaging materials using both chemical and sensory tests is shown in Table 8. At room temperature, the best type of container was shared by glass and HDPE (sustained EVOO grade for more that 90 days and was found VOO after six months of storage), followed by cans and PET (was found VOO after 90 days and 180 days), and the worst container was pottery which was found ordinary virgin olive oil (OVOO) after 90 days of storage. At elevated temperature, glass containers were superior and pottery was inferior while the other types of containers were intermediate.

Considering both chemical and sensory tests (Table 8), results reveal that, the quality of olive oil stored at room temperature deviated from the extra virgin grade because of the absorption coefficient K_{270} (which was the only determinant chemical test) along with the sensory evaluation parameters (presence of sensory defect and/or absence of sensory fruity, Figure 1). At elevated temperature (Table 8), the most relevant chemical test contributed in the loss of oil quality was K_{270} followed by sensory evaluation parameters, followed by acidity and both PV and K_{232} were the least contributors. Table 8 revealed that, grading of stored olive oil under investigation using sensory evaluation without chemical analysis is not sufficient. Also it is clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil.

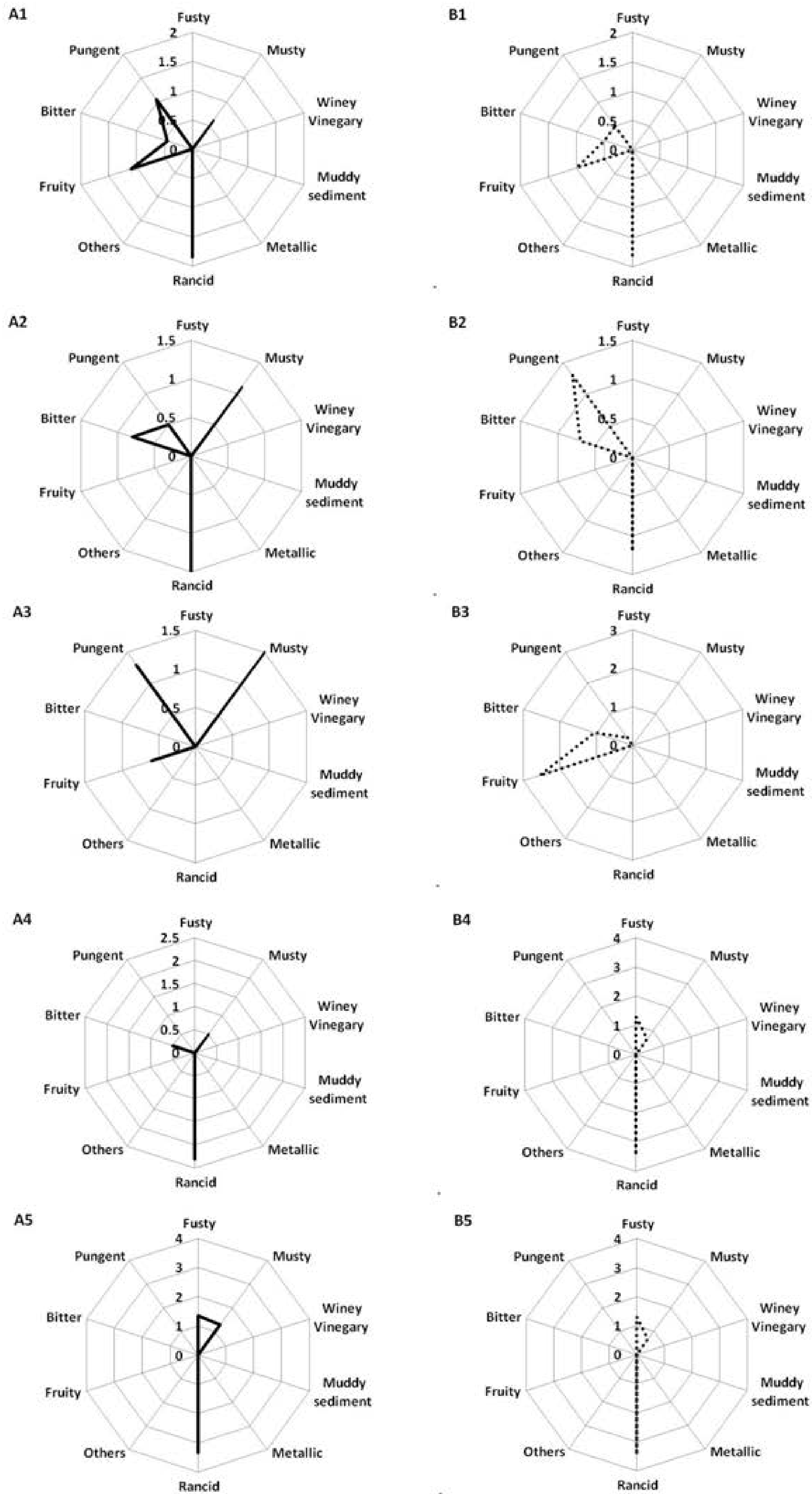

Figure 1. Evaluation of sensory attributes for EVOO stored at elevated temperature (A), and at room temperature (B) in glass bottles (1), PET (2), HDPE (3), cans (4), and pottery (5)

It was found that EVOO stored in glass bottles at low temperature maintained the extra virgin quality, whereas for that stored at elevated temperature (30°C) presented a sharp decrease in sensory score and lost its extra quality after less than two months of storage and become lambent due to loss of the positive attributes (fruity apple, green) and appearance of the negative ones (winy, muddy, rancid) [22]. A group of researchers [28] found a decrease in fruitiness during one year of oil storage and the rancid defect appear after 10-12 months at room temperature. Other investigators [55] found that the bitterness and pungency of virgin olive oil stored in glass bottles at increasing temperatures for 12-18 months decreased during storage time and the intensity of depletion was positively correlated with the increase in temperature of storage. Another research team [47] found that storage of olive oil in amber glass at low temperature results in lower amount of hexanal (off-flavor), but at ambient temperatures, positive attributes decrease throughout storage time.

5. Conclusions

As final statements and as a consequence of the results reported herein, olive oil storage and packaging are final steps of the production process and are as important as the other steps. The packaging material should ensure protection from storage conditions in order to maintain the olive oil quality. This study has reaffirmed that at ambient storage temperature, the best container in maintain the quality of stored oil is glass followed by HDPE, followed by both cans and PET, and the worst was pottery. At elevated temperature, glass was found the best primary packaging material, followed by PET, followed cans, followed by HDPE, and the worst container was pottery.

Deterioration agents can decrease the quality of olive oil during storage, so a correct control and monitoring of some quality indicators can be useful to predict the olive oil shelf life. The quality of olive oils is interpreted in terms of measurements of analytical parameters for which certain limit values are set. It was concluded that, grading of stored olive oil under investigation using sensory evaluation without chemical analysis is not sufficient. Also it is clear that the absorption coefficient K_{270} was the most sensitive determinant chemical test that determines the quality of stored olive oil.

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Abbreviations

EVOO: Extra virgin olive oil; **PET:** Polyethylene terephthalate; **HDPE:** High density polyethylene; **VOO:** Virgin olive oil; **EU:** European union; **LDL:** Low density lipoprotein; **IOOC:** International olive oil council; **PV:** Peroxide value; **EC:** European communities; **UV:** Ultra violet; **CRD:** Complete randomized design; **AOAC:** Association of official analytical communities; **FFA:** Free fatty acid.

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Evaluation of Selected Quality Degradation Indices for Palestinian Extra Virgin Olive Oil Bottled in Different Packaging Materials upon Storage under Different Lighting Conditions

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Abstract: The effect of packaging materials and lighting conditions on quality of extra virgin olive oil (EVOO) was investigated during six months. The results highlighted an influence of light and type of packaging material on EVOO-quality with storage time. At shelf, all packages maintained EVOO at the end of storage in terms of acidity, peroxide value, K_{232}, while K_{270} exceeded limit of EVOO in glass and PET-stored oil. Loss of phenols was the highest in glass-stored oil and the lowest in high-density polyethylene (HDPE)-stored oil. In terms of sensory evaluation, glass-stored oil lost EVOO grade after three months and its edible compliance after six months, while HDPE-stored oil maintained EVOO grade 90 days and was virgin after six months. In extended lighting, acidity, peroxide value and K_{232} did not exceed EVOO grade, while K_{270} exceeded EVOO grade after 30 days in glass and polyethylene terephthalate (PET)-stored oil and after 90 days in HDPE. The loss of phenols was the largest in glass and smallest in HDPE-stored oil. Glass stored-oil lost organoleptic edible compliance before 90 days, while that in PET was virgin at 90 days and that in HDPE maintained EVOO quality 90 days. At the end of experiment, oils in all packages were not edible. In dark, all packages maintained oil in EVOO quality in terms of all indices. The loss of phenols was marginal but was the least in glass and the highest in HDPE. It was concluded that HDPE bottles conserve stored olive oil at shelf or illumination better than PET or glass, while in dark, glass was superior over plastic.

Key words: Acidity, oil oxidation, olive oil, stability indicators, storage conditions.

1. Introduction

Olive tree is one of the most important trees internationally, from which high quality olive oil is produced [1]. From more than 750 million olive trees cultivated worldwide, 95% of which, are planted in the Mediterranean region [2]. The global production of olive fruits in 2011 was around 19.9 million tons, and 115,551 tons are produced in Palestine [3], from which around 30% olive oil is normally extracted. Olive oil plays a special role among vegetable oils because of its balanced fatty acid composition [4-7], which rank this product as the best among dietary fats [8]. Olive oil is categorized according to its organoleptic properties (sensory attributes) and chemical tests into extra virgin, virgin and lampant oil in terms of decreasing its edible quality, hence its healthy and marketable values. The highest grade extra virgin olive oil (EVOO) must

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contain zero defects and greater than zero positive attributes as evaluated by a certified taste panel, and must have a free acidity percentage of less than 0.8, and peroxide value does not exceed 20 milliequivalent peroxide O_2 per kg oil and conform to all the standards listed in its category. EVOO should have clear flavor characteristics that reflect the fruit from which it is made. In relation to the complex matrix of variety, fruit maturity, growing region and extraction technique, extra virgin olive oils can be very different from one another [9].

Specific sensory characteristics including color, aroma, and taste distinguish the extra virgin olive oil from other edible vegetable oils and other grades of olive oil [10] and accounts for its nutritive and health-giving properties [11, 12]. Therefore, its excellent organoleptic and nutrient properties, together with the current tendency of consumers to choose the least-processed foods, have enhanced its presence in consumers' diets and its marketable value [13, 14]. The antioxidant effects of extra-virgin olive oil seem to be a result of the phenolic compounds [15, 16], of which content depends on the cultivar, climate and degree of ripeness of the fruit [8]. Other factors which influence the quality of the oil include the cultural and harvesting practices, the health of the drupe, and the interval between harvest and processing [17], and accordingly, only 50% of the world olive oil production is classified as grade EVOO [18]. As in other foods, the quality of olive oil decreases during storage, and is attributable to lipid oxidation mechanisms which lead to rancidity [8], and hydrolytic degradations causing the partial loss of minor constituents having health-promoting effects [19, 20]. Therefore, it would be a good practice to consume the extra virgin olive oil produced during one crop season before the following crop season [14]. It is a matter of great concern for the olive oil industry to preserve the positive attributes of oil during the time elapsing from production to bottling, and up to purchasing [13, 14]. Accordingly, variation of storage conditions during olive oil storage and transportation, affecting its quality, is common and may be attributed to natural climatic changes as well as bad storage techniques [21, 22].

During the shelf-life of bottled extra virgin olive oil, the packaging must adequately protective against autoxidation processes that cause rancidity [10]. Therefore, several types of plastic films or metal containers can be used, but glass bottles of different shape and color are the most common [14, 23]. For example, in Spain, 90% of virgin olive oil is packaged in bio-use PVC, PET and clear glass, with the latter being increasingly used for the packaging and marketing of “extra quality” olive oils [10]. Although, extra-virgin olive oil is usually packaged in glass, or plastic bottles, these packages have some disadvantages because their bottled contents may be subjected to photo-oxidation [23]. The effect of different packaging materials on the quality of olive oil is widely reported [10, 14, 23-27]. Furthermore, the non-optimal storage conditions, such as those occurring on a store shelf, may alter the qualitative characteristics of the product to the extent that they may eventually differ from those indicated on the label, which, as legally, should maintain the analytical characteristics of the oil at the time of bottling. Thus, an investigation into the type and magnitude of the alterations in oil undergoes during its shelf life by comparing the changes occurring during storage in the light and in the dark may provide useful information [14]. In real time storage of oil in super- and hyper-markets, bottled oils are may exposed to light and high temperatures (typically 28-30 °C), which are not optimum conditions of preservation for the virgin olive oil [10]. It is known that oxidative reactions are catalyzed by light and heat and are partly slowed down by compounds belonging to the unsaponifiable fraction (phenolic compounds, carotenoids and tocopherols) naturally found in olives [28-32].

Accordingly, oil producers need to pay a great deal of attention to the type of containers they place the oils after production and to the storage conditions they are

kept in before sale [14]. The influence of glass and high density polyethylene on oil quality during storage was frequently studied [23], while little information is known about the effect of high density polyethylene (HDPE). Some investigators studied the changes occurring in few quality parameters over either short periods of time [26, 27], or long time as 12 months [14, 27] as the maximum storage period considered from bottling to consumption as real time stability studies. Therefore, the aim of this study is to determine which of the standard quality indices of oil may be used as markers to predict the time when a stored bottled virgin olive oil loses its “extra” quality (acidity 0.8%, peroxide value 20 mequiv kg^{-1}, K_{232} 2.50, K_{270} 0.25, sensory score 6.5) in Glass bottles, PET plastic bottles and HDPE plastic bottles in an accelerated stability study in terms of different lighting conditions (dark, diffused day light, and extra-lighting conditions). Furthermore, we studied the effect of these selected packaging materials and lighting conditions on the loss of phenol compounds of the stored oil during six months of storage.

2. Materials and Methods

2.1 Experimental Design

A homogeneous sample of olives (*Olea europea* L.) of the cultivar “Nabali Baladi” were handpicked with no defects and at an optimal stage of ripening (5.5 N detachment force, 3.8 pigmentation index, 57.5% water content) in late October from trees located in Salfeet district of a Mediterranean climatic region of Palestine. Olives were processed (stone mill and hydraulic press), after defoliation and washing the drupes. The initial whole oil sample was filled in two 20 L HDPE containers and directly transported to the laboratories of Al-Quds University. EVOO quality at the beginning of the experiment (November, 2008) was tested initially for its quality indexes and confirmed as extra quality virgin olive oil (peroxide value < 20, acidity < 0.8%, K_{232} < 2.5 and K_{270} < 0.25, iodine value 75-94, refractive index 1.4677-1.4700 and oil density). The 40 L extra virgin olive oil sample was divided into small subsamples (200 mL each) that were bottled in different packaging materials maintaining 2% head space in each bottle: non colored glass bottles, plastic polyethylene terephthalate (PET) and HDPE. Bottled EVOO small samples were stored under different illumination conditions at room temperature (25 °C ± 3 °C); firstly diffused day light, secondly continuous extended illumination (400 Lux white lamp) in white painted room (12 h daily), where the samples and were rearranged weakly to insure uniform exposure to light to avoid unequal spacial distribution of the bottles, and finally in dark (in a completely closed woody box having 1.5 cm wall thickness, painted with gray color from inside). The bottles (in three replicates for each treatment) of different packaging materials were randomized in a complete randomized design (CRD) in each storage condition. The effect of each of these factors (packaging materials and illumination conditions) on the stability of Palestinian extra virgin olive oil was studied in a non orthogonal design by monitoring oil quality indicators that include: acidity percent (as oleic acid), peroxide value, extinction coefficients (K_{232} and K_{270}), total phenolic contents (expressed as mg of gallic acid kg^{-1} oil), and sensory attributes (Panel test) in consequent days during six months of the experimental period (0, 30, 60, 90 and 180 days of storage).

2.2 Statistical Analysis

Three bottles of each treatment were independently analyzed in each sampling, and all of the determinations were carried out in triplicate. The results are expressed as mean ± standard deviation. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of different storage conditions and different packaging materials were carried out using the GLM procedure considering a fully randomized design, treating main factors (packaging materials and storage conditions)

separately using one-way analysis of variance. The Bonferroni procedure was employed with multiple *t*-tests in order to maintain an experiment wise of 5%.

2.3 Oil Quality Indicators

Acidity and peroxide values were performed according to the methods described in AOAC [33]. Data obtained were expressed as g oleic acid $(100\ g)^{-1}$ oil for the former and as milliequivalent $O_2\ kg^{-1}$ oil for the later. Ultraviolet light absorption K_{232} and K_{270} indexes (K_{232} and K_{270} extinction coefficients) were determined using the methods described in IOOC [34]. Total phenol compounds were extracted according to Georgios et al. [33]. The total polar phenol content was determined spectrophotometrically at 765 nm and its concentration was expressed as mg gallic acid kg^{-1} oil. Sensory evaluation test was run by taster team for sensory analysis in the Palestinian standard institution laboratory, Ramallah, Palestine. The test was performed by the analytical panel done by 13 trained technicians, working according to the method defined by the Standard IOOC/T.15/NC No 3/rev. 2. The results obtained based on the ranking based on the median of notes from the tasters. Each bottle in each treatment was analyzed monthly for each mentioned quality indicators up to six months, except the sensory evaluation which were inspected in three periods (0, 3 and 6 months).

3. Results

3.1 Storage at Diffused Normal Day Light (Shelf)

3.1.1 Effect of Different Packages on Acidity

Free acidity as an important parameter for assessment of hydrolysis of triacylglycerols in virgin olive oil (VOO) as shown in Table 1 increased significantly with increasing time of storage in all types of packaging materials under study. The increase in acidity values in glass-bottled samples was significantly higher than that stored in PET and HDPE bottles at all respective sampling dates. Comparing the effect of PET and HDPE packaging on acidity of stored oil reveals that both storage materials affected acidity in similar way until 45 days after storage, but acidity of oil stored in HDPE bottles out-yielded that of oil stored

Table 1 Evolution of stability indexes: acidity, peroxide value (PV), extinction coefficient and polar phenols for different packaging materials during the storage time at shelf (room temperature). SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± SD*	PV ± SD	K_{232} ± SD	K_{270} ± SD	Polar phenols ± SD
Glass	0	0.38 ± 0.008 e	10.49 ± 0.84 b	2.02 ± 0.01 c	0.16 ± 0.002 f	214 ± 1.46 a
	30	0.44 ± 0.005 de	9.36 ± 0.20 bc	2.12 ± 0.01 a	0.20 ± 0.005 e	197 ± 0.44 b
	45	0.48 ± 0.020 dc	12.63 ± 0.85 a	1.91 ± 0.00 d	0.21 ± 0.000 d	198 ± 10.37 b
	90	0.51 ± 0.020 c	8.42 ± 0.20 c	1.82 ± 0.00 e	0.22 ± 0.000 c	171 ± 1.87 c
	135	0.58 ± 0.020 b	8.11 ± 0.05 c	2.01 ± 0.01 c	0.23 ± 0.001 b	164 ± 0.72 c
	180	0.66 ± 0.020 a	8.23 ± 0.26 c	2.07 ± 0.01 b	0.27 ± 0.002 a	155 ± 6.25 d
PET	0	0.38 ± 0.008 c	10.49 ± 0.84 b	2.02 ± 0.01 a	0.16 ± 0.002 c	214 ± 1.46 a
	30	0.42 ± 0.009 bc	14.34 ± 0.51 a	2.01 ± 0.06 a	0.22 ± 0.010 b	200 ± 9.05 a
	45	0.41 ± 0.010 cb	14.30 ± 0.22 a	2.10 ± 0.00 a	0.23 ± 0.000 a	202 ± 0.66 ab
	90	0.43 ± 0.020 b	7.99 ± 0.51 c	2.03 ± 0.00 b	0.24 ± 0.002 a	198 ± 2.23 b
	135	0.51 ± 0.010 a	7.24 ± 0.22 c	1.85 ± 0.03 b	0.23 ± 0.003 a	184 ± 3.82 c
	180	0.52 ± 0.030 a	8.55 ± 0.26 c	1.85 ± 0.03 b	0.23 ± 0.003 a	166 ± 2.35 d
HDPE	0	0.38 ± 0.008 b	10.49 ± 0.84 b	2.02 ± 0.01 a	0.16 ± 0.002 c	214 ± 1.46 a
	30	0.43 ± 0.003 cb	9.87 ± 0.02 b	1.56 ± 0.01 c	0.15 ± 0.010 c	209 ± 1.35 b
	45	0.42 ± 0.020 cb	13.04 ± 0.50 a	1.87 ± 0.27 bc	0.19 ± 0.005 b	202 ± 0.92 c
	90	0.49 ± 0.050 b	9.42 ± 0.21 b	1.73 ± 0.08 c	0.18 ± 0.009 b	192 ± 0.33 d
	135	0.58 ± 0.020 a	9.91 ± 1.03 b	1.93 ± 0.00 b	0.22 ± 0.001 a	190 ± 0.87 d
	180	0.56 ± 0.003 a	10.84 ± 0.08 b	1.80 ± 0.01 bc	0.21 ± 0.010 a	183 ± 0.16 e

in PET bottles after 45, 135 and 180 days of storage. At the end of storage period, acidity values were higher in glass bottles, followed by HDPE bottles followed by PET, but all types of packaging materials maintained the acidity of stored olive oil in its extra virgin grade (< 0.8% as oleic acid).

3.1.2 Effect of Different Packages on Peroxide Values

Evolution of peroxide value which indicates the state of primary oxidation products in EVOO stored in glass increased significantly after 45 days of storage, decreased significantly compared to the initial value after 90 days of storage and stayed stable until the end of the storage time. In PET bottles, peroxide values increased significantly after 30 days of storage, stayed at the highest level at 45 days of storage then was reduced significantly compared to the initial value after 90 days of storage and this reduced value was maintained until the end of the experiment. Peroxide values in olive oil stored in HDPE increased significantly after 90 days, then was reduced to values not significantly different from the initial value at the rest period of storage. Comparing different packages, the peroxide value increment was reported in PET bottles and was significantly higher than that in glass and HDPE. At the end of the experiment, peroxide values in oil stored in glass and PET were similar but were significantly lower than that in HDPE, and none of samples exceeded the official limit of extra virgin olive oil (20 meq O_2 kg^{-1} oil).

3.1.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

Spectroscopic values of K_{232} and K_{270} extinction coefficients in ultraviolet indicate the level of oxidation to produce primary and secondary products incurred during production and/or storage. Inspection of the results reveals differences within different packaging materials during storage at shelf (Table 1). It was clearly observed that K_{232} values in EVOO stored in glass fluctuated with increasing time of storage without a clear trend, while the values of this quality indicator in olive oil stored in plastic bottles (PET and HDPE) decreased marginally but significantly with increasing time of storage. After six months, none of the packaging materials under investigation exceeded the official limit in terms of extinction coefficient K_{232} < 2.5, these results highlighted that K_{232} was correlated with PV not only at zero time but also during storage for different types of bottles. Extinction coefficient K_{270} increased significantly during storage in all types of bottles used for storage and exceeded the official limits of the EVOO grade (< 0.22) in glass and PET, while HDPE marginally reached the critical limit after 135 day of storage then decreased to below the critical limit at the end of storage period. K_{270} of oil samples stored in glass showed higher values at the end of storage period compared to plastic bottles (PET and HDPE) and exceeded the limits for even virgin olive oil quality (0.25). The least values of K_{270} were found in oil stored in HDPE compared to glass and PET at all respective testing dates during storage period. This indicates that HDPE protects EVOO better than glass and PET when K_{270} was used as quality indicator. Furthermore, the PET bottles provide more protection for EVOO in the presence of light than glass in terms of mentioned coefficients. Glass was found to be the worst storage packaging material at shelf in terms of K_{232} and K_{270} since glass is permeable to light more than the other materials under study.

3.1.4 Effect of Different Packages on Phenol Compounds

Total polar phenolic compounds which are considered as natural antioxidants in EVOO decreased during storage time at shelf in all types of packaging materials under study (Table 1). In particular for EVOO stored in glass bottles which showed dramatic decrease during storage period and their values were the least compared to EVOO stored in PET and HDPE at all respective testing dates. The loss of polar phenols was the largest and more rapid in oil stored in glass (from 214 mg to 166 mg gallic acid kg^{-1} of olive oil), while plastic bottles maintained these antioxidants

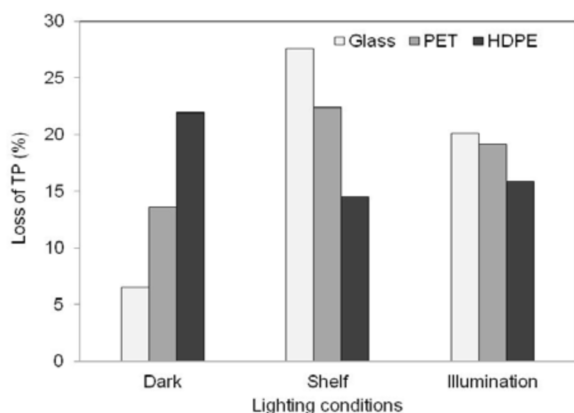

Fig. 1 Loss of total phenols as affected by different means of packaging conditions and lighting storage conditions (percentage lost after 6 months of storage compared to the initial values at the beginning).

better than glass. The reduction of this quality indicator was more sharp (from 214 mg to 166 mg Gallic acid kg^{-1} of olive oil) in PET compared to HDPE (from 214 to 183 mg Gallic acid/kg of olive oil). At the end of the storage period, the loss of phenolic compounds concentration in stored EVOO was higher in glass, followed by PET followed by HDPE (Fig. 1).

3.1.5 Effect of Different Packages on Sensory Evaluation

Olive oil legislations refer to four groups of off-flavors: fusty, mustiness-humidity, winey-vinegary, and rancid. The three first groups are related to olive quality whereas the last one, rancid, develops in storage. Sensory evaluation of olive oil under investigation (Table 2 and Fig. 2) showed that samples stored in glass bottles maintained their extra virgin category in the first three months of storage, while become virgin after this time of storage and quit from the virgin grade at the end of storage period. For samples, stored in PET bottles, the oil quit the EVOO grade after 30 days and stayed in the VOO category till the end of storage period, while HDPE maintained the extra virgin quality of stored oil for 90 days, and the oil stayed as virgin till the end of the storage period. These results indicated that sensory evaluation test correlates with the results of K_{270} which was also failed out of extra virgin category for EVOO stored in glass bottles.

3.2 Storage under Extended Fluorescent Light

3.2.1 Effect of Different Packages on Acidity

Acidity of EVOO stored under extended illumination increased significantly during storage in all types of packaging materials under study (Table 3). At the end of storage period of 180 days, glass bottles showed significantly higher acidity in stored oil compared to PET and HDPE. Furthermore, acidity of stored oil was significantly higher in PET compared to HDPE at the end of storage period. All packaging materials under study maintained stored oil in its EVOO grade (< 0.8%) at all testing intervals during time of storage.

3.2.2 Effect of Different Packages on Peroxide Values

Peroxide values of EVOO stored in glass and PET decreased with increasing storage time at extended illumination conditions, while that of oil stored in HDPE was marginally and insignificantly reduced (Table 3). At the end of storage period, peroxide value

Table 2 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials on shelf.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
Glass	0	0.0	4.9	EVOO
	90	0.0	2.0	VOO
	180	0.8	1.0	Not VOO
PET	0	0.0	4.9	EVOO
	90	0.0	3.0	VOO
	180	0.0	2.55	VOO
HDPE	0	0.0	4.90	EVOO
	90	0.0	1.19	VOO
	180	1.85	1.30	VOO

EVOO: extra virgin olive oil; VOO: virgin olive oil.

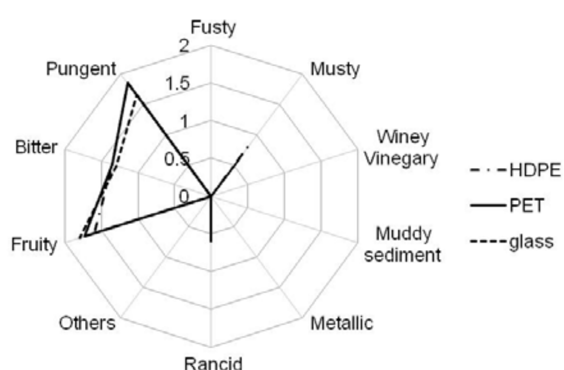

Fig. 2 Evaluation of sensory attributes for EVOO stored in different packaging materials at shelf (normal diffused day light) after six months of storage.

of oil stored in glass bottles was not significantly different from that of oil stored in PET bottles, while that of oil stored in HDPE bottles was maintained in a significant higher value compared with the other two types of packaging materials under investigation.

3.2.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

The extinction coefficients K_{232} of olive oil samples stored in glass bottle under florescent light increased significantly at the end of the storage period compared to that at the beginning of the experiment, but values fluctuated within the time borders of the experiment (Table 3). Although K_{232} of oil stored in glass increased slightly at the end of storage period, the unclear trend between the beginning and the end of the storage period was also the case in terms of K_{232} extinction coefficient values for oil stored in both types of plastic packages (PET and HDPE). All types of packaging materials maintained the oil in its extra virgin quality in terms of $K_{232} < 2.5$. The extinction coefficient K_{270} increased significantly with increasing time of storage in all types of packaging materials under study. Oil stored in glass and PET quit the EVOO grade (< 0.2) after a period of less than 30 days, while HDPE maintained the oil in its extra virgin quality for more than 90 days under accelerated stability study in terms of extra light condition. At the end of the experiment, oil stored in glass showed the highest K_{270} value.

3.2.4 Effect of Different Packages on Phenol Compounds

Total polar phenols decreased significantly with increasing time of storage under florescent illumination (Table 3). The loss of polar phenols was faster in oil stored in glass compared to that stored in PET and HDPE

Table 3 evolution of stability indexes: acidity, peroxide value (PV), extinction coefficient and polar phenols for different packaging materials during the storage time under florescent light illumination (400 Lux). SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± S.D*	PV ± S.D	K_{232} ± S.D	K_{270} ± S.D	Polar phenols ± SD
Glass	0	0.38 ± 0.008 b	10.49±0.84 a	2.02±0.01 c	0.16±0.002 c	214±1.46 a
	30	0.38 ± 0.030 b	8.56±0.18 ab	2.10±0.01 b	0.23±0.004 b	184±2.71 b
	45	0.50 ± 0.030 b	9.34±1.18 ab	1.97±0.04 d	0.23±0.010 b	182±0.91 b
	90	0.50 ± 0.000 a	9.30±0.05 ab	1.92±0.01 e	0.23±0.000 b	176±0.49 c
	135	0.57 ± 0.006 a	8.96±1.00 ab	2.01±0.01 c	0.26±0.002 a	172±0.49 d
	180	0.58 ± 0.040 a	8.18±0.32 b	2.17±0.00 a	0.28±0.010 a	171±0.16 d
PET	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.01 bc	0.16±0.002 d	214±1.46 a
	30	0.40 ± 0.008 c	9.64±0.49 a	1.99±0.02 ba	0.24±0.010 c	204±0.49 b
	45	0.46 ± 0.020 b	7.71±0.26 b	2.08±0.02 a	0.23±0.010 c	189±0.49 c
	90	0.50 ± 0.001ab	7.52±0.53 b	1.95±0.01 c	0.23±0.035 c	176±0.00 d
	135	0.51 ± 0.020 a	8.06±0.22 b	1.99±0.00 bc	0.25±0.002 b	175±0.33 d
	180	0.53 ± 0.000 a	7.67±0.69 b	2.02±0.02 ab	0.26±0.001 a	173±0.30 e
HDPE	0	0.38 ± 0.008 d	10.49±0.84 a	2.02±0.01 bc	0.16±0.002 e	214±1.46 a
	30	0.40 ± 0.020 cd	8.94±0.33 b	1.99±0.02 ab	0.18±0.010 d	193±1.35 b
	45	0.45 ± 0.008 bc	8.49±0.12 ab	2.08±0.02 a	0.22±0.010 c	187±0.44 c
	90	0.47 ± 0.000 ba	8.79±0.09 ab	1.95±0.01 c	0.21±0.020 c	184±1.43 c
	135	0.53 ± 0.003 a	8.84±0.32 ab	1.99±0.00 bc	0.23±0.002 b	183±0.72 c
	180	0.51 ± 0.020 a	9.49±1.10 ab	2.02±0.02 ab	0.27±.002 a	180±0.82 d

Table 4 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials under florescent light.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
	0	0	4.9	EVOO
Glass	90	2.56	2	Not VOO
	180	2.55	1.65	Not VOO
	0	0	4.9	EVOO
PET	90	0	2.3	VOO
	180	2.3	0.65	Not VOO
	0	0	4.9	EVOO
HDPE	90	0	2.6	EVOO
	180	1.9	1.9	Not VOO

EVOO: Extra virgin olive oil; VOO: virgin olive oil.

bottles in the first 45 days of storage. After 45 days of storage, total polar phenols of oil stored in glass were reduced in the same scale as that stored in PET bottles, while HDPE bottles maintained higher total polar phenols at all testing times throughout the storage period. At the end of the experiment, total polar phenols were maintained in larger contents in oil preserved in HDPE followed by that stored in PET bottles, and the least was found in oil stored in glass (Fig. 1).

3.2.5 Effect of Different Packages on Sensory Evaluation

Sensory evaluation of olive oil stored in different packaging materials under study shows a great effect of light in the deterioration of sensory attributes of olive oil (Table 4, Fig. 3). Extended artificial illumination largely affected the organoleptic properties of oil stored in glass bottles more than that stored in PET and HDPE bottles. Oil stored in glass under this extreme condition lost its compliance as edible oil before 90 days and become not virgin olive oil, while oil stored in PET was found virgin after 90 day of storage and that stored in HDPE maintained its extra virgin quality. At the end of the storage period, oil stored in all packaging materials under study lost its virginity and hence its compliance as edible oil.

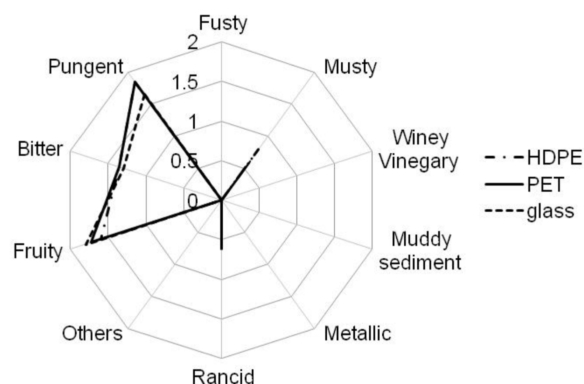

Fig. 3 Evaluation of sensory attributes for EVOO stored in different packaging materials at extended illumination after six months of storage.

3.3 Storage in Dark Conditions

3.3.1 Effect of Different Packages on Acidity

The acidity of oil stored in all packaging materials under study at dark conditions increased significantly with increasing time of storage (Table 5). The significant increase in acidity began after 90 days of storage in oil stored in glass while significant increase of this indicator began after 30 days of storage in oil stored in plastic bottles (PET and HDPE). At the end of the experiment, oil stored in PET bottles showed the lowest acidity value, followed by oil bottled in glass, and the highest acidity was found in oil stored in HDPE bottles. Oil stored in all packaging material under investigation did not exceed the limits for the extra virgin quality (< 0.8%).

3.3.2 Effect of Different Packages on Peroxide Values

Peroxide values responded in different ways among different packaging materials under study (Table 5). Peroxide value of oil stored in both glass and PET bottles began to decrease significantly after 45 days of

Table 5 Evolution of stability indexes: acidity, peroxide value, extinction coefficient and polar phenols for different packaging materials during the storage time indark. SD: standard deviation.

Source of variation	Storage time (days)	Acidity % ± SD*	Peroxide Value ± SD	K_{232} ± SD	K_{270} ± SD	Polar phenol ± SD
Glass	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.010 a	0.16±0.002 b	214±1.46 a
	30	0.42 ± 0.020 bc	9.88±0.52 a	1.77±0.004 d	0.16±0.002 b	203±2.80 b
	45	0.42 ± 0.010 bc	7.36±0.06 b	1.74±0.010 d	0.18±0.010 a	203±2.80 b
	90	0.43 ± 0.020 b	8.23±0.32 b	1.85±0.030 c	0.16±0.000 b	196±0.16 d
	135	0.52 ± 0.020 a	8.38±0.10 b	1.90±0.005 bc	0.16±0.010 b	201±1.15 bc
	180	0.53 ± 0.003 a	8.42±0.36 b	1.96±0.050 ba	0.16±0.001 b	200±0.82 c
PET	0	0.38 ± 0.008 c	10.49±0.84 a	2.02±0.010 a	0.16±0.002 c	214±1.46 a
	30	0.42 ± 0.020 b	10.40±0.40 a	2.03±0.020 a	0.19±0.004 a	206±3.97 b
	45	0.42 ± 0.010 b	8.25±0.01 b	2.01±0.000 ab	0.19±0.000 a	202±0.81 bc
	90	0.49 ± 0.002 a	8.46±0.14 b	2.02±0.013 a	0.19±0.010 a	199±3.21 bc
	135	0.50 ± 0.002 a	8.62±0.01 b	1.98±0.014 bc	0.18±0.006 b	195±3.61 c
	180	0.51 ± 0.020 a	8.67±0.36 b	1.96±0.010 c	0.18±0.010 b	185±2.83 d
HDPE	0	0.38 ± 0.008 e	10.49±0.84 a	2.02±0.010 ba	0.16±0.002 c	214±1.46 a
	30	0.43 ± 0.010 d	8.68±1.14 a	2.02±0.010 ba	0.18±0.010 ba	208±1.45 b
	45	0.50 ± 0.020 c	8.64±0.08 a	2.03±0.014 a	0.20±0.010 a	202±0.42 c
	90	0.53 ± 0.004 b	9.37±0.93 a	2.08±0.005 c	0.15±0.010 cd	183±1.32 d
	135	0.53 ± 0.004 b	9.22±0.10 a	1.75±0.140 ba	0.15±0.004 cd	192±0.57 e
	180	0.57 ± 0.004 a	10.04±0.13 a	1.95±0.005 bc	0.14±0.002 d	167±0.28 f

storage, while that of oil stored in HDPE did not change significantly within storage time. At the end of the storage period, peroxide value in oil stored in glass and PET bottles share similar values, while that of oil stored in HDPE was higher significantly. Peroxide values of oil samples bottled in all types of packaging materials under study did not exceed the limit of the extra virgin grade of olive oil during the storage period (20 meq O_2 kg^{-1}).

3.3.3 Effect of Different Packages on Extinction Coefficients (K_{232} and K_{270})

There was no clear trend in the response of K_{232} in oil stored different types of packaging materials under investigation, as the values of this extinction coefficient fluctuated with storage time (Table 5) within a very narrow range and no oil sample exceeded the limit of extra virgin quality (2.5). The extinction coefficient K_{270} of oil stored in glass bottles increased significantly after 45 days of storage then returned to its initial value till the end of storage period, the same response was observed in oil stored in both PET and HDPE bottles. The values of this indicator were sustained below the limit for the extra virgin grade of olive oil and all oil samples stored in all packaging materials were sustained under the critical limit of extra virgin olive oil (0.22).

3.3.4 Effect of Different Packages on Phenol Compounds

Total polar phenols decreased significantly during storage at dark conditions in oil stored in all packaging materials under study (Table 5). The loss of polar phenols at the end of storage period (Fig. 1) was more pronounced in oil stored in HDPE (22% reduction) followed by PET (13.6% reduction) followed by glass (6.5% reduction).

3.3.5 Effect of Different Packages on Sensory Evaluation

Olive oil stored in all types of packaging materials was maintained their extra virgin category without any sensory defects (Table 6, Fig. 4).

4. Discussion

One of the most fundamental reactions in lipid chemistry is oxidation, in which a series of compounds are formed, causing off-flavors and rancidity, loss of nutritional value and finally consumer rejection of the

Table 6 Sensory evaluation and other stability indexes for olive oil samples stored in different packaging materials in dark.

Source of variation	Storage time (Days)	Sensory evaluation (Defects)	Sensory evaluation (Fruity)	Olive oil grade
	0	0	4.9	EVOO
Glass	90	0	2.5	EVOO
	180	0	1.65	EVOO
	0	0	4.9	EVOO
PET	90	0	2.3	EVOO
	180	0	0.65	EVOO
	0	0	4.9	EVOO
HDPE	90	0	2.6	EVOO
	180	0	1.6	EVOO

EVOO: extra virgin olive oil.

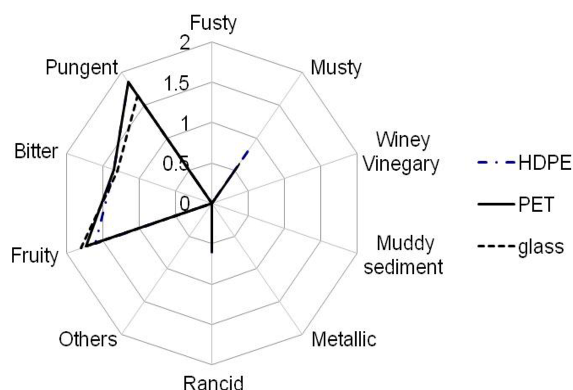

Fig. 4 Evaluation of sensory attributes for EVOO stored in different packaging materials in dark after six months of storage.

food product. Auto-oxidation-occurs in the absence of air by reactive oxygen species or "free radicals" is temporarily prevented by the natural antioxidants in the oil that absorb these free radicals. When the antioxidants are used up, the oil ages quickly. Studies of the autoxidation of oleic acid process date back to 1943 [34]. Autoxidation is therefore the main cause of olive oil quality deterioration and its reaction rate determines the shelf-life of this product [35]. In the case of virgin olive oil, upper limit values for different oxidation indexes were established (peroxide value: 20 meq kg^{-1}, K_{232}: 2.50 and K_{270}: 0.20) which could be employed as end points for its shelf-life [36].

4.1 Acidity

Comparing the influence of previously mentioned packaging materials in terms of their effect on acidity of olive oil stored in dark, glass showed the least (best results) acidity values, where in contrast to plastic material, glass is not permeable to oxygen and humidity which could increase the acidity of the oil through increasing the rate of hydrolysis of triglyceride to liberate free fatty acids. At the end of storage period of six months, none of the samples stored at these conditions exceeded the critical limit of extra virgin olive oil category in terms of acidity (0.8%) according to the international standards. Our results are in accordance with what was reported previously [37, 38] which found that acidity did not increase significantly with increasing time when samples were stored in dark.

In agreement with our findings, it was previously documented that free acidity was higher in oil stored in light compared to that stored in dark because light negatively affects olive oil quality with increasing storage time [8, 39-41]. The increase in acidity throughout storage time as affected by light can be explained by its effect on the activation of triglycerides hydrolysis resulting in the liberation of free fatty acids [10, 41-44] and the subsequent development of oil rancidity [41, 42].

The increase of acidity of oil stored in glass in the presence of light (at shelf and extended illumination) is because the transparency of glass to light, therefore negatively affecting olive oil quality in terms of acidity as a stability indicator [39]. A significant increase in acidity was also observed in oil samples stored in plastic packages (both polyethylene terephthalate (PET)

and high density polyethylene (HDPE)) as time of storage increased. This can be explained by their diffusivity to oxygen which negatively affects olive oil quality by enhancing the oxidative deterioration of oil. Acidity of oil stored in plastic containers (PET and HDPE) was maintained in lower levels compared to that stored in glass, which can be attributed to the fact that plastic has barrier properties to light more than glass [23]. The increase of acidity as increasing storage time found in our study is reported by previous investigators [39, 45-47].

Comparing previously mentioned packaging material in terms of their influence on acidity of olive oil subjected to artificial illumination; our findings showed that glass was the most affected by light followed by HDPE followed PET. PET was found to be more protective in terms of light diffusion where it prevents wave length less than 300 nm to pass through it compared to glass [23]. HDPE bottles gave better results (less increase in acidity %) since these packages are colored and relatively prevent light form passage to the oil more than transparent PET bottles. Our finding are in agreement with the results of many researchers [8, 45] who found that acidity was affected by illumination and increased within time of storage in glass and plastic containers.

4.2 Peroxide Value

In agreement with our results, the peroxide values of oil stored at shelf in all studied packaging materials underwent an initial increase at the beginning of storage period, and then it marginally decreased with increasing storage period [8, 39, 47]. This because the newly formed oxidation products (we left a bottle headspace) are further converted to secondary products [39]. Oil samples stored in the dark showed higher peroxide values compared to that subjected to light (shelf or extended lighting) at each respective storage time [8, 26, 39, 47] which indicated greater primary oxidation, while the samples exposed to light exhibited a lower peroxide value, which could be ascribed to evolution from primary to secondary oxidation [14]. The lesser formation of secondary products in samples stored in the dark may explain the higher peroxide values obtained for oil stored at this condition in this study [8]. In the same line with our findings, peroxide values of oils stored in glass at illumination showed a linear decrease with storage time [10]. The decrease in the PV with increasing time can be explained by the degradation of primary oxidation products (peroxides) to form secondary oxidation products which can be detected by K_{270} value. The results of PV was correlated with that obtained by K_{232} which was observed to be decreased or stay stable during the storage period [10, 40, 44, 47]. The oil samples packed in HDPE and exposed to light presented higher peroxide values compared to those packed in glass containers. These results are similar with other findings and point to the probable intrusion of oxygen through HDPE as a consequence of its permeability to oxygen and its less light penetration ability. Peroxide values in oil stored in PET was similar to that stored in glass as affected to increasing storage time at extended illumination due to the combined effects of the permeability of PET to oxygen and at the same time its transmittance to light [48].

Generally, during the beginning of storage, PV in different packaging materials increased as a consequence of the action of both, diluted and headspace oxygen in the containers and additionally, the light induce a rapid deterioration of oil in terms of PV. After a period of storage, the PV progressively decrease because of the degradation of primary products into secondary products, which is more obvious in the samples packed in glass containers and less in those packed in plastic bottles. This could be explained as the evolution of photo oxidation [49].

4.3 Extinction Coefficients

It was documented that the shelf-life of virgin olive oil is determined by the increase in the K_{232} absorption coefficient as a quality parameter [50], or by means of

the time required to reach the upper legal limit of K_{270} absorption coefficient [10, 51]. Primary oxidation products in olive oil (fatty acid hydroperoxides and oxidized triacylglycerols) are measured as peroxide value (PV) and K_{232} absorption coefficient, while secondary oxidation products (fatty acid hydroperoxides decomposition products such as aldehydes, alcohols, ketones and hydrocarbons) are detected by K_{270} absorption coefficient [51]. Hydroperoxides are the initial products of oxidation—very sensitive and comparatively unstable—and used as indicator of the early stages of oxidative deterioration at the beginning of the oxidation process [39, 49, 52], while the K_{270} index is used to study the behavior of the secondary oxidation products by the formation of dimers and polymers of triacylglycerides [51]. Because of the significant variation of the K_{270} value during olive oil storage as a response to oil oxidation, and is easily measured, this parameter may be of capital importance to control the quality of stored virgin olive oils in terms of determining the time at which they will lose their "extra" category [10].

It is well known that light affects olive oil quality, making possible an increase in the triene formation, measured by K_{270} [29, 53], more than in the diene measured as K_{232} [8]. In agreement with our findings, K_{270} values were affected by the exposure conditions, with higher values reported in the samples stored in the light than in those kept in the dark [8, 38, 54] probably because of the presence of chlorophylls in the oil acts as an antioxidant in the dark [47], while pigments of the olive oil (chlorophylls and pheophytins) in presence of light have an oxidizing effect through acceleration of photo oxidation [8] increasing triene containing secondary products of oxidation and thus K_{270} increased more than K_{232}. In contrary with our findings, one researcher reported higher values of K_{232} in the samples stored in dark compared to those kept in light because of conjugate dienes as the oxidation products present in greater amounts in dark [26], while concerning our results, the opposite was found and may be discussed by the high rate of production of primary and secondary oxidation products as affected by light, this indicates that the rate of secondary oxidation is not higher than that of primary oxidation. The value of K_{270} remained almost unchanged at dark condition. By contrast, in the samples exposed to light both K_{232} and K_{270} were significantly higher than the values found in oils kept in the dark. This indicated that in the light, degradation of primary oxidation compounds was facilitated and peroxides underwent breakdown reactions more rapidly. Our findings are in agreement with other researchers [23]. Furthermore, after six months of storage, the value of K_{270} of the oils exposed to light exceeded the limits for virgin olive oils and agreed with results of other researchers [14]. In this investigation, K_{232} values were maintained under the limit of 2.5 units for oil stored in light (at shelf and at extended lighting) and dark in all packaging materials under study while K_{270} values exceeded the limit of 0.20 units during the six months of storage in both light intensities (at shelf and extended illumination) in all packaging means and the same was previously reported [39].

Our findings are in agreement with results previously [55] which found that oil samples stored in PET and glass under light were associated mainly with secondary oxidation products. It was found that for oil samples stored in glass bottles under illumination, K_{232} increased while the samples stored in dark K_{232} remain constant, while K_{270} showed a sharp increase in samples stored under illumination and exceed the limit value for EVOO after three months of storage [10]. The action of light on olive oil samples stored in plastic bottles resulted from the effect of light through enhancing photo-oxidation and the permeability of plastic packaging material to oxygen and humidity. A group of investigators showed that for samples stored in glass in dark K_{232} increased from 1.96 to 2.015 after 9 months [38] while others [10] showed that for oil samples stored in glass bottle in dark K_{232} and K_{270}

remain constant throughout the storage period. In contrary, other findings showed that both UV absorption coefficients for olive oil samples stored in glass in dark increased throughout the storage time and exceed the established limit by legislation [26, 56, 57]. Glass acts as a barrier to oxygen, avoiding the loss of certain components that deteriorate under oxygen presence but glass allows the direct action of light on the stored olive oil and this could promote oxidative rancidity as a consequence of its sensibility to photo-oxidation [39]

4.4 Total Phenols

In agreement with previous reports [10], total polyphenol (TP) contents of extra virgin olive oil decreased during storage in all means of storage conditions and packaging materials under study; due to degradation of these compounds that was well fitted to first order kinetics. Although, phenolic compounds (Tables 1, 3 and 5) constantly decreased during storage; samples stored in the dark revealed a significantly higher values than those stored in the light [8, 14, 32]. Phenolic compounds act as natural antioxidants in oil and their reduction during storage is a result of oil oxidation [41, 58, 59], where phenolic antioxidants inhibit autoxidation of lipids (RH) by trapping intermediate peroxyl radicals [60]. The loss of phenolic compounds of olive oil during storage is mainly due to the action of photo oxidation as a result of light that initiate oxidation process which occur by photochemical hemolytic cleavage of RH bond to produce free radicals [61]. Photo-oxidation processes occurred in parallel with auto-oxidation [14] and consequently reduce phenol contents in stored oil.

Compared with other vegetable oils, virgin olive oil is more stable against oxidation due to multiple factors such as the relatively low content of polyunsaturated fatty acids, the high level of monounsaturated fatty acids (mainly oleic acid) and the presence of some natural antioxidants (tocopherols, carotenes and phenolic aglycons, based on the molecules of tyrosol and hydroxytyrosol, deriving from phenolic glycosides). The stability of virgin olive oil also depends on the presence of pro-oxidant substances as well as on factors linked to the storage conditions, namely the presence of oxygen, the temperature and above all light exposure, therefore, the level of degradation of an oil results from a balance of all these factors [14].

The different trend observed in terms of the reduction of phenolic substances in different lighting conditions may be attributed to their specific mechanisms of action as antioxidants. The phenolic compounds act by giving an electron so that they can interrupt the radical reaction occurring with oxidation [62]. The carotenoids act as electron acceptors, quenching the singlet oxygen [63]. Finally, tocopherols act both as electron donors, slowing down the oxidative reaction, and as electron acceptors, determining the quenching or the scavenging of singlet oxygen, with consequent inhibition of the photooxidation of lipids [27]. Nonetheless, the singlet oxygen formed in the photo-oxidative reaction (in presence of light) is 1,000-1,500 times more reactive than the triplet oxygen taking part in the reaction of auto-oxidation which take place in dark [62]. This means that photooxidation takes place faster than auto-oxidation and implies a greater decrease in tocopherols in the samples exposed to light. This suggests that in presence of light oil is protected from oxidation mainly by tocopherols and carotenoids, and those phenolic substances have a secondary role, in the dark, instead, the main reaction is auto-oxidation and the phenolic substances seem to be involved more than the other antioxidants in the protection of the oil from oxidation [14].

At the beginning of storage time, olive oil contained 214 mg kg^{-1} ± 1.46 mg kg^{-1} oil of total phenolic compounds, and this value was in consistent with the data (121-410 mg kg^{-1}) reported previously [62]. Afterwards, the total content of phenols decreased as a function of time, with various degree of reduction among the storage containers, and the decrease was

more pronounced under light conditions. Fig. 1 showed that the lowest range between the initial and final antiradical activity (percentage loss of total phenols) at dark condition was in glass bottle (6.5%), then PET and HDPE (13.5% and 22%, respectively) showing the low ability of plastic containers to keep the quality of olive oil through maintaining its activity to scavenge the free radicals when stored in dark [44]. In addition, at dark condition, glass containers kept more phenolic compounds than plastic containers (PET more than HDPE), which agreed with that reported previously [42] where olive oils samples exhibited insignificant loss of their total phenols during storage at condition away from light in glass bottles. The reduction of antioxidants in plastic containers could be due to its permeability to oxygen and the migration of active compounds between oil and packaging material [45]. In the presence of light (at shelf and extended lighting), the opposite response was found. Both plastic containers retained phenolic compounds (PET more than HDPE) more than glass containers. The loss of phenolic compounds at shelf was highest in oil stored in glass (27.6%) followed by PET (22.4%) followed by HDPE (14.5%), the same response was found under extended illumination but the loss of total phenols was larger in oil stored in glass and PET bottles (20.1%, 19.2% and 15.9% for glass, PET, and HDPE, respectively). This can be discussed by the effect of light on the photo-oxidation of oil and the consequent reduction of antioxidant compounds including total phenols and the more light transparency of glass than PET followed by HDPE in light of the stated above it was cleat that phenolic compound loss intensity during storage is directly propotional to the attitude and degree of oxidation occurred.

4.5 Sensory Analysis

The descriptive sensory analysis of olive oil stored at the three types of packaging materials during storage in different lighting conditions is shown in Tables 2, 4 and 6. It can be seen that samples stored at dark condition had the lowest changes in sensory values in all studied packaging materials maintaining the stored oil in its extra quality during the period of the experiment. In the presence of light (at shelf, and extended lighting), HDPE was found the best in maintaining the stored oil with the lowest defects at the end of the storage period followed by PET, and the worst was glass containers where oil lost its virginity before 90 days of storage at extended lighting condition and before 180 days at shelf. In contrary with our findings, it was reported that samples stored in the glass container at shelf had the lowest changes in positive sensory attributes, and was considered the best material followed by plastic bottle [41]. This was due to the argument that EVOO samples in glass containers had the highest values of color, taste, flavor, and odor retention followed by those in plastic containers. The reduction of sensory attributes could be due to that the physical characteristics of the packaging material may affect the final quality of the oil, depending on the extent of the deteriorative interactions [64].

The pigments content in olive oil correlate with the shelf life of stored oil and, in particular, its resistance to oxidation. The green color of olive oil faded off as the oil ages, which might be caused by the conversion of chlorophyll to alternative yellow and brown pigments, i.e., pheophytins (PP) and pyropheophytins (PPP). The rancid flavor development in olive oil could be due to oxidation; the decomposition of the hydroperoxides formed and the consequent formation of newly generated volatile compounds [64]. The volatile aldehydes and vinyl ketones are known to be mainly responsible for potent off-flavors, because their odor threshold levels are very low [59, 65] demonstrated that as free fatty acids concentration increased, undesirable sensory properties occurred. It was demonstrated that the negative sensory attributes in olive oil can be associated with volatile compounds, which are mainly formed by chemical oxidation of oil [21, 66]. Our results show that EVOO placed in the glass container had the highest acidity followed by

those in the plastic containers when they were stored at shelf and extended lighting conditions, and as time increased from 0 to 180 days the total phenolic compounds decreased, which could be caused by oil oxidation during storage. In addition, the oil in the glass containers kept less phenolic compounds than that in the plastic container when they were subjected to light (at shelf and extended light) [59].

5. Conclusions

Finally, as a consequence of the results reported herein, the packaging material should ensure protection from storage conditions in order to maintain the olive oil quality, especially when the oil is stored under the studied commercial conditions in terms of different lighting conditions. This study has reaffirmed that HDPE bottles, stored at shelf and at extended illumination conserve the oil much better providing higher protection from oxidation compared to PET and glass containers. At both normal and extended lighting storage conditions, glass bottles were not able to protect stored EVOO, and the oil quit from extra virgin grade in the former and from edible compliance in the later during six months of storage. In the other hand glass bottles showed superiority over plastic containers in conserving oil when they were stored at dark condition but the three types of packaging material conserve oil and maintained the extra virgin quality during six months. The extinction coefficient K_{270} is the quality index that was showed tight correlation with the sensory evaluation test more than acidity, peroxide value and K_{232}. Therefore, the storage of extra virgin olive oil in HDPE bottle, could be suggested the most appropriate mean for maintaining the quality of the extra virgin olive oil.

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