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Original Article

Effects of Different Filtration and Clarification Techniques on Palestinian Virgin Olive Oil Chemical and Microbial Quality

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ABSTRACT

Virgin olive oil filtration is a pre-treatment usually used before bottling to facilitate suspended particle removal and reduce the moisture content so that the quality and organoleptic properties are preserved during storage. In the current study, samples of virgin olive oils were subjected to different clarification and filtration techniques such as (1) commercial filtration (CF) using diatomaceous earth as a filter aid, (2) clarification by insufflating of carbon dioxide, and (3) argon gas. All treated samples as well as control unfiltered (UF) virgin olive oil (VOO) were bottled under dark conditions in diffused daylight glass bottles and kept at room temperature for six months. Basic quality indices (free acidity (FA), peroxide value (PV), and extension coefficient) and total polar phenol were determined within respected time of analysis during the storage period of six months. In addition, microbial quality was evaluated by determination of yeasts and molds, total plate count, Bacillus cereus, and clostridium perfringens. The main results showed that at the end of the storage, acidity increased in all samples with time in both storage conditions, whereas samples stored in dark more stable compared to those stored under diffused daylight. On the other hand, clarified samples with CO_2 showed significant stability compared to the rest of the samples stored under diffused day light. Peroxide values (PV) and K232 increased in all stored samples. Moreover, K270 increased with time, and showed more stability in samples stored in dark. Total polar phenols decrease in all samples, but showed no significant difference among all samples after 180 days of storage. Microbial results revealed the presence of yeasts and molds in all samples at the beginning of storage, and then disappeared after 135 days for all samples except UF. Total plate count, Bacillus cereus, and clostridium perfringens were not found in any stored VOO.

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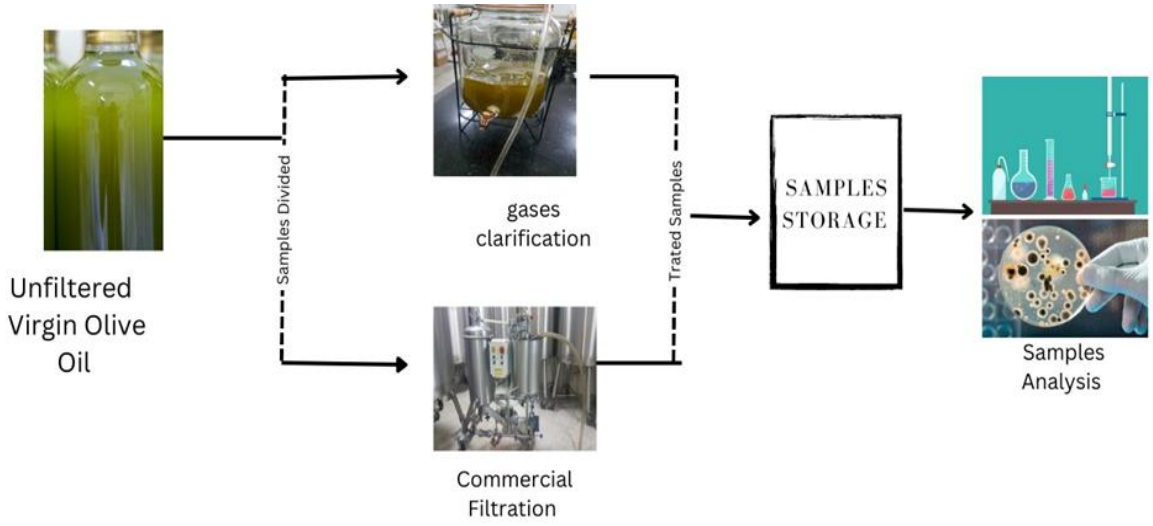
GRAPHICAL ABSTRACT



Introduction

Palestinian ecosystem is rich in biodiversity, where olive trees are considered among the most abundant trees (*Olea Europaea L.*) [1]. Olive oil is the vegetable oil obtained from olive tree fruit (*Olea europea sative)* by mechanical extraction [2].

Filtration means removal of humidity and suspended solids from VOO before bottling [3]. Tri-glycerides hydrolyzed because of lipolytic enzymes (lipases) that cause the release of free fatty acids (FFA) and mono-glycerides or di-glycerides. These lipases are present naturally in olive, and they are hydrophilic. Thus, oil filtration is crucial to remove partial amounts of water and suspended solids and cells residues [4-7].

Fregapane *et al.* [8] and Lozano-Sánchez *et al.* [9] reported findings with no significant changes in FFA for filtered VOO samples. On the other hand, Valli *et al.* (2019) found that % FA values were significantly higher in unfiltered EVOO than in clarified and filtered samples [10].

Free fatty acidity increase in unfiltered samples could be attributed to the presence of molds and microorganisms in precipitate which produce large amounts of very active lipases [4]. Moreover, Valli et al., 2019; Bendini et al., 2013; Sanchez et al., 2012, and Yun et al., 2012 observed that both clarification and filtration treatments were very effective in reducing the water content, and consequently, the rate of hydrolysis [11].

Both clarification and filtration treatments were very effective in reducing the water content and in removing the suspended solids enriched with polyphenols [10].

Commercial filtration (CF) is a common filtration, achieved using filter aids such as diatomaceous earth (diatomite and extracted from the fossil of microscopic algae) of different particle sizes and different permeabilities. It is normally mixed in various steps with oil to get a filter membrane, which results from the aggregation of the filter aid with the suspended solids during the filtration process. Filtration is considered complete when the maximum partial pressure (insert a number of this pressure in psi or bar) is reached [3].

Inert gas clarification is another method that was developed recently to extract moisture and suspended solids from virgin olive oils. It is based on the insufflation of flow of inert gases (nitrogen or argon) directly into the middle of the virgin olive oil mass from the bottom of filtration tank containing the cloudy virgin olive oil. As far as carbon dioxide clarification is concerned, to our knowledge, there are no studies describing this clarification technique.

The existence of microorganisms in oil could be attributed to prolonged storage of the olive fruits [12]. It should also be noted that olive oil is not free from moisture, which plays an important role in microbial growth [13].

The presence of microorganisms in olive oil could lower its quality and could make it harmful to human health [14].

In the current study, different clarification and filtration techniques were applied to virgin olive oil samples such as (1) commercial filtration (CF) using diatomaceous earth as a filter aid, (2) clarification by insufflating of carbon dioxide, and (3) argon gas.

Materials and Methods

Samples

VOO was extracted from olives of 'nabali balasi' cultivar from Ramallah region (Palestine) in November 2019, and then divided into four parts. An aliquot of this extract was subjected to a commercial filtration system using diatomaceous earth as a filter aid to produce filtered VOO sample. A second and a third aliquots of cloudy VOO were clarified by directly injecting inert gases; namely, carbon dioxide or argon (at a flow rate of 12 L/min for 2 hours), into the center of VOO mass using a pilot clarification system, that was developed and patented by the University of Bologna and Sapio [15]. In this system, CO_2 gas was directly injected into the veiled VOO bulk mass (pressure = 1 bar for 2 hours) to produce CO_2-clarified VOO. Another part of the veiled VOO was injected. The remaining VOO was kept unfiltered. Clarification and filtration were conducted at room temperature.

Storage simulation

All VOO samples were filled in 150 mL dark and clear 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 or under artificial and diffused day light. The samples were stored for 6 months (December 8th, 2019 to June 6th, 2020) at room temperature.

The samples were analyzed for microbial and chemical quality parameters at time zero and after 45, 90, 135, and 180 days of storage. Analysis included basic quality parameters (acidity percentage, peroxide value, and extinction coefficients), and total phenols to evaluate the oxidation stability of such treated VOO during storage under different conditions. Two bottles of each stored sample were removed from the storage room at each respective time of analysis, and aliquots of the oil (drawn from the geometrical center of each bottle) analyzed in triplicates, two replicates were obtained from the same bottle, while the third one was obtained from a separate bottle of the olive oil.

Quality chemical parameters and microbial analysis

Stored samples were analyzed for, peroxides value, free acidity (expressed as mill-equivalents of oxygen per kg of oil meq O_2/kg), and percentage of oleic acid (%), respectively. The UV absorption coefficients (K_{232} and K_{270}) were also measured according to the official methods described in European Union council regulations EEC. Reg 2568/91 of the European Commission.

Yeasts and molds were enumerated in malt extract agar medium, *clostridium perfringens* were enumerated in Sulfite Polymyxin Sulfadiazine (SPS) agar, *Bacillus cereus* were enumerated in Bacara agar, and total plate count, all of which were determined for each sample at each respective time of analysis according to the methods described in FDA Bacteriological analytical manual FDA- BAM. The results were expressed as colony forming unit of microorganism per ml of sample (CFU/ ml).

Results and Discussion

Changes in quality parameters

Basic quality indicators (Free fatty acid, peroxide value, K_{232}, and K_{270}) of the unfiltered, commercial filtered, carbon dioxide, and argon gas clarified VOO samples were evaluated before

storage and at different time intervals after storage. Two storage conditions were studied; namely, dark and diffused day light. As listed in Tables 1 and 2, the acidity of filtered and clarified VOO were lower than unfiltered. In filtered VOO samples stored exposed to diffusive light, % FA followed the following order Ar <CF<CO_2<UF. In the samples stored under dark conditions, the trend was quite different compared to those observed in samples stored exposed to daylight. The highest % acidity was observed in oil samples subjected to CO_2 clarification, the second highest was measured in the UF samples whereas the lowest % acidity was found in the oil samples subjected to Ar clarification.

Also, for peroxide value (PV), the results indicated that there was no significant difference among all samples stored in dark. A higher PV values were found in samples stored under the light compared to those measured in samples stored under dark conditions. The data showed higher peroxide values in samples subjected to Ar clarification and stored under the light than other samples.

Nevertheless, as indicated in Tables 1 and 2, all stored VOO samples yielded PV values of ≤ 20%, and were within the limits of regulations (for example, EU 1348/2013).

These results agreed with those reported by Valli *et al.* (2019) who found that PV of EVOO during storage was relatively unchanged [10].

As shown in Tables 1 and 2, there was no significant difference in K_{232} among VOO samples stored in dark, as well as for VOO samples stored in daylight except, for Ar clarified VOO samples, where K_{232} values increased significantly after the end of storage (6 months).

This increase in K_{232} values might be attributed to the progress in oxidation reaction during storage. As a comparison, Brenes *et al.* (2001) reported higher K_{232} values of for UF oil due to more rapid oxidation during storage [16].

These changes in PV values might be explained by the presence of oxidative enzymes in the suspended solids, as suggested by Georgalaki *et al.* (1998) and Valgimigli, *et al.* (2001) [17, 18].

However, upon evaluating K_{270} results for all treated samples as shown in Tables 1 and 2, higher K270 values were observed for VOO samples stored under diffused daylight compared to that found in VOO samples stored in dark conditions. However, no significant difference was found between the results of the filtered and clarified samples. In contrast, a significant increase in the K270 values measured in the unfiltered samples at the end of storage was observed.

Change in phenolic compounds

As shown in Tables 1 and 2, there was no significant difference in the measured phenolic compounds among clarified/filtered and unfiltered VOO samples in both storage conditions, in dark and under diffused daylight after the end of storage time (6 months). However, the UF VOO samples stored in Dark conditions had higher phenolic compounds values and less reduction than what was found in samples stored under diffused daylight.

Phenolic compounds react as an antioxidant, as they prevent lipids autoxidation via trapping intermediate peroxyl radical, as a result the total phenol decrease [19]. Moreover, as a consequence of light, the action of photo oxidation is higher compared to auto-oxidation process [20].

As a comparison, Ciafardini *et al.* (2002a) reported values of polyphenols lower in the filtered olive oil than what was observed in the unfiltered VOO samples [21]. Okogeri *et al.* (2002) reported that the decrease of total phenol measured in samples stored light was less than that measured in samples stored in dark [22].

On the other hand, Bendini *et al.* (2013) found that there was no clear difference in the observed total phenols for inert gas clarified VOO and non-clarified samples [3]. Valli *et al.* (2019) reported that phenolic compounds decreased significantly after the end of storage of virgin olive oil, a trend similar to what was observed in the study [10].

Quantity of microorganism

As presented in Table 3, Y&M microbes were found in all VOO stored samples (filtered, clarified, and unfiltered) while total plate count, clostridium perfringens, and bacillus cereus were not found in any stored VOO samples. As illustrated in Table 3, Y & M in CF and clarified VOO samples decreased and reached to undetectable level at the end of storage time (i.e. 6 months) whereas Y & M in UF VOO samples

Table 1: Values of FA (g oleic acid 100 g^{-1} oil), PV (meq O_2 kg^{-1} oil), K_{232}, K_{270}, and TPP registered during storage of different VOO samples stored in dark condition at room temperature

Samples	Storage time (days)	FA	PV	K_{232}	K_{270}	TPP
UF	0	1.0075 ± 0.00 b	8.23 ± 0.29 b	2.271 ± 0.04 c	0.159 ± 0.01 c	156.013 ± 12 a
	45	1.0075 ± 0.00 b	8.60 ± 0.35 b	2.061 ± 0.10 d	0.192 ± 0.02 b	116.526 ± 04 c
	90	1.0478 ± 0.04 a	9.13 ± 0.12 a	2.700 ± 0.12 a	0.167 ± 0.00 c	147.620 ± 05 a
	135	1.0344 ± 0.02 ab	9.30 ± 0.14 a	2.475 ± 0.10 b	0.168 ± 0.02 c	140.714 ± 05 ab
	180	1.0478 ± 0.00 a	9.33 ± 0.12 a	2.734 ± 0.08 a	0.242 ± 0.01 a	127.460 ± 12 bc
CF	0	0.9269 ± 0.00 b	7.33 ± 0.12 d	2.151 ± 0.13 c	0.151 ± 0.01 b	152.679 ± 07 a
	45	0.9672 ± 0.00 a	7.67 ± 0.42 cd	1.978 ± 0.11 c	0.156 ± 0.01 ab	139.154 ± 04 ab
	90	0.9538 ± 0.02 ab	8.80 ± 0.20 b	2.678 ± 0.23 ab	0.171 ± 0.02 ab	125.000 ± 12 bc
	135	0.9806 ± 0.02 a	8.13 ± 0.46 bc	2.487 ± 0.00 b	0.167 ± 0.00 ab	125.000 ± 08 bc
	180	0.9672 ± 0.00 a	10.07 ± 0.70 a	2.843 ± 0.17 a	0.177 ± 0.01 a	114.120 ± 18 c
CO_2	0	1.0075 ± 0.00 b	7.26 ± 0.23 c	2.070 ± 0.01 c	0.141 ± 0.01 b	155.025 ± 16 c
	45	1.0207 ± 0.02 b	7.80 ± 0.35 c	1.864 ± 0.00 d	0.153 ± 0.01 b	143.705 ± 12 c
	90	1.0344 ± 0.02 b	8.93 ± 0.12 b	2.582 ± 0.18 ab	0.172 ± 0.01 a	159.762 ± 12 b
	135	1.0344 ± 0.02 b	8.53 ± 0.50 b	2.437 ± 0.05 b	0.157 ± 0.00 ab	123.810 ±12 b
	180	1.0747 ± 0.02 a	10.00 ± 0.00 a	2.740 ± 0.16 a	0.173 ± 0.01a	120.000 ± 04 a
Ar	0	0.9808 ± 0.02 a	7.46 ± 0.12 c	2.173 ± 0.05 c	0.156 ± 0.01 b	142.808 ± 11 ab
	45	0.9538 ± 0.05 a	7.47 ± 0.12 c	1.924 ±0.10 d	0.165 ± 0.00 ab	139.346 ± 04 abc
	90	0.9672 ± 0.04 a	8.20 ± 0.20 b	2.421 ± 0.18 b	0.160 ± 0.01 ab	144.762 ± 08 a
	135	0.9672 ± 0.04 a	8.60 ± 0.40 b	2.471 ± 0.10 b	0.163 ± 0.01 ab	128.413 ± 08 bc
	180	0.9538 ± 0.02 a	9.46 ± 0.76 a	3.120 ± 0.06 a	0.175 ± 0.01 a	124.286 ± 11 c

Uf: unfiltered EVOO sample, CF: commercial filtered VOO sample, CO_2: carbon dioxide clarified VOO sample, Ar: argon clarified VOO sample, FA: Free Acidity, PV: Peroxide value, and TPP: total polar phenol.
** The same letters (a-d) denote that there is no significant difference during storage within the same sample.*

Table 2: Values of FA (g oleic acid 100 g^{-1} oil), PV (meq O2 kg^{-1} oil), K232, K270, and TPP registered during storage of different VOO samples stored exposed to diffusive light at room

Samples	Storage time (days)	FA	PV	K_{232}	K_{270}	TPP
UF	0	1.0075 ± 0.00 a	08.23 ± 0.29 c	2.271 ± 0.04 a	0.159 ± 0.01 b	156.013 ± 12 a
	45	1.0478 ± 0.00 a	09.50 ± 0.42 b	1.808 ± 0.11 d	0.153 ± 0.02 b	107.551 ± 11 b
	90	1.0478 ± 0.12 a	11.47 ± 0.12 a	2.117 ± 0.05 bc	0.169 ± 0.00 b	110.477 ± 02 b
	135	1.0612 ± 0.02 a	12.20 ± 0.85 a	2.047 ± 0.03 c	0.157 ± 0.01 b	110.317 ± 11 b
	180	1.1150 ± 0.06 a	12.13 ± 0.58 a	2.231 ± 0.10 ab	0.204 ± 0.01 a	112.452 ± 01 b
CF	0	0.9269 ± 0.00 c	07.33 ± 0.12 d	2.151 ± 0.13 b	0.151 ± 0.01 b	152.679 ± 07 a
	45	1.0881 ± 0.00 a	08.70 ± 0.14 c	1.922 ± 0.00 c	0.150 ± 0.01 b	124.347 ± 15 b
	90	0.9672 ± 0.00 c	10.80 ± 0.35 b	2.762 ± 0.13 a	0.205 ± 0.02 a	114.048 ± 04 bc
	135	0.9538 ± 0.02 c	10.27 ± 0.95 b	2.136 ± 0.04 b	0.168 ± 0.01 b	108.254 ± 05 c
	180	1.0344 ± 0.06 b	12.20 ± 0.20 a	2.330 ± 0.18 b	0.210 ± 0.01 a	107.936 ± 02 c
CO_2	0	1.0075 ± 0.00 ab	07.26 ± 0.23 d	2.070 ± 0.01 c	0.141 ± 0.01 d	155.025 ± 16 a
	45	1.0075 ± 0.00 ab	08.90 ± 0.79 c	1.799 ± 0.00 d	0.149 ± 0.01 cd	126.654 ± 20 b
	90	1.0344 ± 0.05 a	11.53 ± 0.90 ab	2.350 ± 0.12 a	0.181 ± 0.01 b	103.572 ± 02 bc
	135	0.9806 ± 0.05 b	10.40 ± 0.40 b	2.120 ± 0.15 bc	0.157 ± 0.00 c	099.207 ± 09 c

	180	1.0478 ± 0.00 a	12.60 ± 0.72 a	2.232 ± 0.06 ab	0.199 ± 0.01 a	117.238 ± 16 bc
Ar	0	0.9808 ± 0.02 a	07.46 ± 0.12 e	2.173 ± 0.05 d	0.156 ± 0.01 c	142.808 ± 11 a
	45	0.9806 ± 0.02 a	14.73 ± 0.31 a	1.797 ± 0.00 e	0.182 ± 0.01 b	112.231 ± 11 b
	90	1.0075 ± 0.07 a	09.60 ± 0.87 d	2.520 ± 0.17 c	0.208 ± 0.01 a	107.143 ± 05 b
	135	0.9806 ± 0.02 a	11.70 ± 0.14 c	2.738 ± 0.13 b	0.173 ± 0.10 b	110.238 ± 13 b
	180	0.9672 ± 0.04 a	14.00 ± 0.00 b	2.812 ± 0.54 a	0.198 ± 0.00 a	103.651 ± 10 b

Uf: unfiltered EVOO sample, CF: commercial filtered VOO sample, CO_2: carbon dioxide clarified VOO sample, Ar: argon clarified VOO sample, FA: Free Acidity, PV: Peroxide value, and TPP: total polar phenol.

Table 3: Average (± SD, n=3) quantity of micro-organisms (CFU/mL) for all VOO samples at different storage conditions either in dark or exposed to diffusive light at room temperature

Species	Y&M ± SD CFU/mL (n=3) At time zero	Y&M ± SD CFU/mL (n=3) After 45 days	Y&M ± SD CFU/mL (n=3) After 90 days	Y&M ± SD CFU/mL (n=3) After 135 days	Y&M ± SD CFU/mL (n=3) After 180 days
		Dark			
UF	17.67 ± 0.58	17.50 ± 3.54	Not detected	1.00 ± 0.00	5.00 ± 1.41
CF	20.50 ± 2.00	25.00 ± 5	Not detected	Not detected	Not detected
CO_2	2.33 ± 0.58	Not detected	2.00 ± 0.00	Not detected	Not detected
Ar	9.67 ± 0.58	Not detected	1.00 ± 0.00	Not detected	Not detected
		Light			
UF		21.67 ± 5.69	Not detected	32.00 ± 0.00	2.00 ± 0.00
CF		14 .00 ± 0.00	Not detected	Not detected	Not detected
CO_2		Not detected	Not detected	Not detected	Not detected
Ar		Not detected	4 ± 1.41	Not detected	Not detected

Uf: unfiltered EVOO sample, CF: commercial filtered VOO sample, CO_2: carbon dioxide clarified VOO sample, Ar: argon clarified VOO sample, Y&M: yeast and molds, and SD: standard division.

remained detectable (present) even at storage at the end of 6 months.

The presence of droplet water in oil (oil-water emulsions) played an important role in microorganism's growth [23]. Likewise, as Ciafardini *et al.* (2002a) reported that fewer types of yeast in the filtered olive oil were found; this might be due to the mechanical removal of the suspended solids [21]. Microorganisms that might be present in water droplets and sediments particles of the fruit will be removed upon subjecting the oil to filtration treatment. Hence, filtration and clarification are considered as effective methods for lowering microorganism levels. Many studies reported the effectiveness of filtration and clarification in reducing the levels of bacteria in VOO (Valli et al., 2019; Bendini, et al., 2013; Lozano-Sa´ nchez et al., 2012; Ciafardini, et al., 2002a; Peri, 2014) [3, 9, 10, 21, 24].

In addition, the phenolic compounds could have antimicrobial activity against microorganism, as suggested by Karaosmanoglu *et al.* [25].

Conclusion

This study showed that clarification and filtration treatments might have be beneficial effects in filtered VOO compared to UF VOO in terms of reducing the precipitate, decreasing microbial count, preserving the chemical quality of VOO. Hydrolytic degradation and evaluated by FA levels were more obvious in UF VOO than those in filtered and clarified VOO at the end of the storage (6 months). For total polar phenol measured at the end of the 6-month storage, no significant difference was observed between all VOO samples. Similar trend was observed for the peroxide values, where no significant difference was also observed for all stored VOO samples after 6-month storage. It should be noted that the lowest peroxide value was found in the VOO

clarified with argon gas. As far as K_{232} and K_{270} results are concerned, the lowest values measured were those for VOO samples clarified with CO_2. Nevertheless, no significant difference was observed in the K_{270} values measure in clarified and filtered samples after 6-month storage. It was evident that filtration is mostly necessary as a final step to eliminate moisture or suspended solids and to increase the brilliance of olive oil for consumer admission. Moreover, the VOO clarification resulted in eliminating most of Yeasts and Molds. Moreover, clarification and filtration might enhance the VOO quality during storage as well. In addition, clarification seemed to be a good treatment to avoid potential interaction between VOO and filter aid. Generally speaking, and upon inspecting all quality indices, storage under dark conditions was better and yielded better quality of VOO compared to what was observed in the oil samples stored in day light.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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