Effect of Refining and Thermal Processes on Olive Oil Properties

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ABSTRACT

Olive cultivars Bladi and Arbequina were collected and their oil was extracted (cold press). Oil was refined under bleaching and deodorization conditions for 30 minutes at 55-50°C and their property was compared with the virgin olive oil. Virgin and refined oils of the cultivars were processed by fryer heating at 180°C (intervals of 0, 1, 2, 4, and 8 hours) and indexes of fatty acid, free fatty acid, peroxide, triacylglycerol, conjugated dienes and trienes, Rancimat, color, total polyphenol, tocopherol, chlorophyll, carotenoid, and oxidative stability were compared. Results showed that these oils mainly contained oleic (48.69-60.18%), palmitic (18.31-27.02%), linoleic (7.54-18.58%), palmitoleic (3.9-3.08%), stearic (1.78-2.53%), and linoleic acids (1.87-0.22%). According to the results, it was recommended to refine virgin olive oil by mild bleaching and deodorization to save bioactive compounds. Due to the heating condition, the relative amounts of unsaturated fatty acids (22.37-50.6%), polyphenols, tocopherols, chlorophyll, carotenoid, and oxidative stability decreased and the relative amounts of saturated fatty acids (14.2-17.1%), acidity, peroxide, conjugated dienes and trienes, polar compounds, and thiobarbituric acid increased significantly, but the colorant initially decreased and then increased (P<0.05). Due to polar compounds, for frying process, mild refined oil was better than extra virgin oil.

Keywords: Bioactive compounds, Bleaching and deodorization, Olive cultivars, Stability indexes, Virgin olive oil.

INTRODUCTION

Virgin Olive Oil (VOO), one of the main sources of fat in the Mediterranean diet, has been widely associated with prevention of several pathologies such as cancer, heart disease, and aging by inhibiting the oxidative stress. These useful properties are mainly attributed to its composition [3, 7, 18]. Refined Olive Oil (ROO) is obtained from VOOs by refining methods that do not change initial triacylglycerol composition of the oil [8]. Cardiovascular health effects, high nutritional value, and oxidative stability of olive oil is mainly due to its high levels of MUFA (especially oleic acid content), the MUFA contents are correlated with a bitter taste [30], a natural antioxidants such as phenols and tocopherols; low levels of Free Fatty Acids (FFAs); pigments; hydrocarbons, oxygenated compounds; and formation of fewer free radicals which are highly toxic for health [2, 10, 11, 17, 25, 32]. Another factor that makes olive oil different from other oils with high levels of MUFA such as high oleic sunflower oil is the presence of biophenols with strong antioxidant [7]. The contribution of the Fatty Acid (FA) composition in oxidative stability

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was 24% that were much lower than 51% obtained for polyphenols. These results suggest that the oils that have the same FA composition may have different resistance against oxidative degradation [35].

Frying is one of the most popular and common cooking processes and a method for preparing food at home and industry [6, 11, 13, 29]. Fried products have unique sensory and organoleptic properties such as flavor, texture, and appearance that make them largely enjoyable for the consumers [8, 11, 22, 37]. Also, this procedure significantly reduces cooking time. During frying, due to high temperatures and presence of oxygen, moisture, and light, a series of chemical reactions such as thermoxidation, hydrolysis, polymerization, isomerization, and cyclization inevitably reduces oil shelf life and directly affects the quality of the fried food [4, 11, 33, 27, 34,]. The frying process may reduce the amount of antioxidants in the oil and produce new compounds that reduce the nutritional value and quality of the oil [28]. Acrolein is formed even at low temperatures, and it has been reported that home cooking has to be considered as an indoor pollution problem [24].

The bioactive compounds are valuable and very important compounds that are volatile, and are removed during the refining process and heating at high temperatures. The oxidative stability of olive oil is higher than other vegetable oils due to the presence of high natural antioxidant compounds. The stability can be different in various cultivars. Few studies have been carried out on physicochemical properties of olive oil extracted from different olive cultivars. Considering the importance of extra virgin olive oil for the human health and its economic value, performing this study seems to be feasible. In this study, the properties of virgin and refined olive oils of two cultivars (Bladi and Arbequina) were compared.

**MATERIALS AND METHODS**

Two olive cultivars (Bladi and Arbequina) were collected about 70 kg (each cultivar) from the Fadak Farm in Qom, Iran, from at least 30 trees by manual method that is the best method of harvesting the olive fruit. The weather in Qom is mostly warm and dry in summer and somewhat cold in winter. The average annual temperature is 20°C in warm areas and 13°C in cold areas. All solvents and reagents were prepared from the Merck, Sigma and Fluka Companies.

Three stages of crushing, malaxation, and separation of the solid and liquid phases were carried out by Oliomio extraction machine (Italy). Then, solid residues were filtered from the mixture of vegetation water and oil; then, olive oil was separated from the vegetation water using decanter and then centrifugation.

After separation of VOO, the oil samples of each cultivar were divided in two parts: one part for testing the virgin oil and the other for testing the refining process.

The purpose of refining oil i.e. deodorizing and bleaching under laboratory condition, is removing undesirable impurities. With minimal damage to natural triglycerides, tocopherols of oil and the oil loss is minimal. Bleaching earth was added to the olive oil for bleaching at the rate of 1.5% with activated carbon at the rate of 0.05% of the bleaching earth. After the vacuum forming, the temperature was increased to 50°C and oil and bleaching earth were blended at the mean speed of 30 rpm for 30 minutes. Then, the oil was twice filtered by Whatman paper No. 42 under vacuum conditions. For deodorizing, after vacuum forming, bleached oil was kept under nitrogen gas for 30 minutes at 50°C. After deodorizing, the oil temperature was immediately decreased to the room temperature.

Samples of the oil (250 mL; VOO or ROO) was separated and added to an electric domestic deep fat fryer. The fryer was adjusted at 180±5°C and every half hour its temperature was checked, and at the intervals of 0, 1, 2, 4 and 8 hours, 50-mL samples were collected from the oil. The experiments (refining and frying process) were done in one replicate.

The FA were analyzed by a Young Lin 6000 series GC (South Korea) equipped with a flame ionization detector and a BPX-70 capillary column (60 m length×0.25 mm
id=0.25 µm film thickness; SGE, Melbourne, Australia). Hydrogen with a flow rate of 1 mL min⁻¹ was used as the carrier gas. The initial column temperature was 150°C for 5 minutes which was increased to 180°C at a rate of 2 °C min⁻¹ and then held at this temperature for 40 minutes; the injector and detector temperatures were set at 250 and 280°C, respectively (IOC, 24) [20].

FFA, peroxide value, thiobarbituric acid, K₂₃₂ and K₂₇₀ extinction coefficients, total polar compounds (by column chromatography) and oil color (Lovibond) were determined according to AOCS official methods Ca 5a–40, Cd 8–53, Cd,19–90, Ch5–91, Cd, 20–91 and Cc 13e–92, respectively [5]. The induction period was estimated by a Rancimat apparatus (Metrohm Series 743) [26].

The total tocopherol content was determined according to the AOCS, No. Ce 8-89. The total phenolic content was determined according to the IOC, No. 29. Analyses were performed on a Young Lin 9000 series HPLC system chromatograph (South Korea) equipped with a gradient pump, vacuum degasser and mixer, column oven, and UV/VIS detector. A Tracer Excel 120 ODS-A (C18) (5 µm id, 150x4.6 mm) column was used [20]. Chlorophyll was determined using a UV spectrophotometer at 630, 710, and 670 nm (PG Instruments Ltd, T80, UV/VIS, England) with 1 cm of path length against air as the control [32]. Total carotenoid content was determined by a UV spectrophotometer at 454 nm (PG Instruments Ltd, T80, UV/VIS, England) according to the BS, No.684-2.20 with 1 cm of path length against cyclohexane as the control [9]. Refractive index was determined according to ISO 6320 at 20°C [21].

Statistical analysis was performed by SAS. Descriptive analysis, one-way ANOVA, LSD comparison test, and principal component analysis were used. For each sample, three determinations were done. Differences at a confidence interval of 99% were considered significant.

RESULTS AND DISCUSSION

Fatty acid composition (%) in VOO and ROO of the two cultivars is presented in Table 1. Changes in FA composition of Bladi and Arbequina oil during heating are presented in Figure 1, the FAs below the
Figure 1. Relationship between the content of individual fatty acids (four graph top) and total Unsaturated Fatty Acids (UFAs), Saturated Fatty Acids (SFAs), MonoUnsaturated Fatty Acid (MUFA), PolyUnsaturated Fatty Acid (PUFA), ratio of C18:2/C16:0 and ω3/ω6 (four graph down) before and after thermal treatment. (FA_B = % of fatty acid before thermal treatment; FA_A = % of fatty acid after 8 hours thermal treatment at 180°C; VB: Virgin Bladi; VA: Virgin Arbequina; RB: Refined Bladi, RA: Refined Arbequina).
dashed line decreased relatively, while FAs above the dashed line increased relatively during the heating. It is clear in Figure 1 that the amount of linolenic acid and then linoleic acid had the highest decrease in the virgin oil of the Bladi cultivar. This is while the lignoceric acid amount had the highest increase in the Arbequina cultivar. For refined oil samples, this was not the case. In the refined Bladi, the highest increase related to lignoceric acid and then myristic acid and the maximum decrease was related to linolenic acid and heptadecanoic acid. Also, in the refined Arbequina, lignoceric acid had the highest increase while the highest decrease was observed for linolenic acid followed by linoleic acid, myristic acid, and behenic acid. Iodine value and linolenic acid content decreased as the frying time, dielectric constant, color index, and polarization component increased [36]. It is evident from Figure 1 that there was a significant increase in the contents of SFA and decrease in PUFA, while the content of MUFA remained unaltered. The results showed that the degradation rate of FA increased by the number of double bonds. The trans isomer and content of oleic acid increased, and content of linoleic acid and linolenic acid due to degradation and formation of volatile compounds decreased.

This resulted in oxidation reactions with increasing the heating time. The results obtained in the current study were in agreement with the findings of previous studies [16].

Changes in the amount of trans FAs during the heating process are shown in Figure 2. The amount of trans isomer in all samples increased as the heating time prolonged. The amount of trans isomers increased in refined samples compared to the virgin samples before heating (Table 1). Trans FAs arise during refining of vegetable oils as well as during hydrogenation [15]. Trans isomers can be increased by an isomerization increase under thermal conditions [16] and the presence of chemical substances such as acid activated the bleaching earth. After completion of the heating process, the rate of increase of trans FA was higher for Bladi oil sample than that for Arbequina oil (93.7 and 91%, respectively). This could be due to its carotenoids level that, at high-oxygen concentration, may exhibit a pro-oxidant activity. Decrease in the trans FA content or remaining unchanged at some points could be due to measurement and detection errors. Increase in the amount of trans FAs in refined samples is less than that in the virgin samples (Figure 2). This is probably due to lack of chlorophyll and carotenoid pigments.

![Figure 2](image_url)

**Figure 2.** Changes of trans fatty acids in olive cultivars studied during the heating process. (VB: Virgin Bladi; VA: Virgin Arbequina; RB: Refined Bladi, RA: Refined Arbequina).
that act as a strong pro-oxidant, respectively, in high temperature and in the presence of oxygen; as well as the absence of tiny water droplets suspended in the refined oil due to the vacuum. Also, the rate of increase for Arbequina oil was higher than Bladi oil (80 and 45%, respectively). This could be due to its FA composition, which contains more USFA. Changes in the SFA, USFA, MUFA, PUFA contents during the heating process are shown in Figure 1. FA composition of the samples was not significantly affected by the refining process (Figure 3).

In oil analysis, C18:2/C16:0 ratio is used as a quality parameter [23]. This ratio decreased during heating due to oxidation of double bonds of FAs. It has been reported that reduction in this ratio is associated with reduction of oil quality [12].

The ω3 and ω6 are called essential fatty acids which body cannot synthesize, thus, must be obtained through diet. The ratio of ω3/ω6 decreased during heating but reduction of the ratio in Bladi oil was lower than Arbequina.

Changes of the physicochemical properties in VOO and ROO from Arbequina and Bladi cultivars during heating at 180 ºC are presented in Table 2. As presented in Table 2, FFA content increased in all samples during the heating process (P< 0.05). Increase in FFA content can be caused by hydrolysis of triacylglycerols as well as formation of secondary oxidation products for high temperature during the oxidation process, which occurs during heating. An increase in the FFA content during the heating process was higher in the Arbequina samples. This seems to be due to the high amount of USFAs in the Arbequina oil compared to the Bladi oil.

The peroxide value increased and then decreased during the heating process (P< 0.05). Double bonds of the USFAs present in the oil can absorb oxygen that produces peroxide. Peroxide is very active and will accelerate degradation reactions, which can be measured by the iodine titration [15]. So, reduced peroxide value during heating is caused by the formation of secondary products. Peroxides quickly decompose or form cross links, create polymeric or dimeric triglycerides, and increase the viscosity and oil color, and then reduce the quality of oil [16]. Peroxide levels in refined samples had a significant decrease compared to the virgin samples. The reduction can be related to refining processes, which affected the amount of peroxide.

TBA increased during the refining and

Figure 3. Relationship between the content of total Unsaturated Fatty Acids (UFAs), Saturated Fatty Acids (SFAs), Mono Unsaturated Fatty Acid (MUFA), Poly Unsaturated Fatty Acid (PUFA) before and after the refining process. (FA<sub>A</sub>= % of fatty acid before the refining process; FA<sub>B</sub>= % of fatty acid after the refining process; B: Bladi sample, A: Arbequina sample).
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Time</th>
<th>FFA% (Oleic acid)</th>
<th>PV (mg Eq Oi kg⁻¹)</th>
<th>TBA (mg malondialdehyde kg⁻¹ oil)</th>
<th>K₁₃₂</th>
<th>K₂₇₀</th>
<th>ΔK</th>
<th>IP (h)</th>
<th>Red (Lovibond)</th>
<th>Yellow (Lovibond)</th>
</tr>
</thead>
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<tr>
<td>Virgin olive oil</td>
<td>0</td>
<td>0.406±0.04</td>
<td>12.66±0.76</td>
<td>8.19±0.00</td>
<td>2.59±0.006</td>
<td>0.2±0.00</td>
<td>-0.003±0.006</td>
<td>12.03±0.23</td>
<td>1.35±0.05</td>
<td>35.40±0.20</td>
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<td>21.28±0.00</td>
<td>2.74±0.003</td>
<td>1.15±0.00</td>
<td>0.06±0.00</td>
<td>12.73±0.58</td>
<td>0.45±0.05</td>
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<tr>
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<td>11.33±0.76</td>
<td>17.44±0.01</td>
<td>2.8±0.001</td>
<td>2.08±0.001</td>
<td>0.11±0.00</td>
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<tr>
<td>4</td>
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<td>5.5±0.00</td>
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<tr>
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<td>2.83±0.57</td>
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<td>0.17±0.01</td>
<td>-0.01±0.00</td>
<td>14.34±0.33</td>
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<td>12.44±0.87</td>
<td>0.60±0.00</td>
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<tr>
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<td>0.2±0.1</td>
<td>0.99±0.14</td>
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<tr>
<td>8</td>
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<td>2.89±0.01</td>
<td>2.38±0.00</td>
<td>0.06±0.02</td>
<td>0.26±0.00</td>
<td>1.35±0.05</td>
<td>8.75±0.05</td>
<td></td>
</tr>
</tbody>
</table>

- **FFA**: Free Fatty Acid; **PV**: Peroxid Value; **TPC**: Total Polar Compounds; **TBA**: ThioBarbituric Acid value; **K**: Extinction coefficients; **IP**: Induction Period. Each value is the mean of three determinations±standard deviation. Letters within columns designate statically significant differences (P<0.05).
heating processes. The increase is due to the high temperature in frying and decomposition of primary products such as hydroperoxides, conjugated dienes, and trienes into secondary oxidation products such as aldehydes. K_{232} and K_{270} in Bladi were higher than Arbequina oil and good correlations between conjugated dienes and peroxide value have been found [15]. Conjugated dienes decreased after the refining process because bleaching earth decompose the primary products such as peroxides. Then, the amount of the primary products decreased, but the content of conjugated trienes, which shows secondary products of oxidation, increased according to the measurement as 268-270 nm (P<0.05).

The data in Figure 4 shows the samples were significantly different with regard to the total polar compounds (P< 0.05). The content of Polar Compounds (PC) increased during heating and decreased during oil bleaching. Percentage of PC reduction by the refining process for Bladi and Arbequina oils was 24 and 40%, respectively. The fatty acid composition, initial quality, and technology used may be changed by decrease or increase in the PC content of oils during the refining process [14]. Increase in PCs and their intensity in Arbequina oil was more than those in the Bladi oil, because of its thermal degradation compounds such as high levels of FFAs. The increased rates of PC for virgin Bladi and Arbequina oils were, respectively, 90 and 91%, and for refined Bladi and Arbequina the corresponding increases were 88 and 90%. In addition, mono-and diacylglycerols comprise a portion of the PCs in the oil and the amount of USFAs was higher in the Arbequina oil. In other words, unsaturated fatty acids are more prone to produce polar compounds during heating processes. According to a German standard for frying oil quality, the oil should be discarded if it contains > 27% TPCs. In the current study, all samples were at the desired range after 4 hours of being heated. Also, refined samples were in the range less than 27% after 8 hours of heating.

According to the results obtained (Table 2), the induction period decreased during heating for all the samples. Reduction in the Bladi sample was gradual, but this occurred in the Arbequina sample very quickly and suddenly. Arbequina oil contained more USFAs than Bladi oil. The amounts of USFA in Arbequina and Bladi oils were 77.15 and 74.23%, respectively. An increase in the PUFA level leads to oxidation susceptibility [31]. High levels of oleic acid have a high positive correlation with the stability of olive oil [19]. Before heating, the MUFA/PUFA ratio in Arbequina oil (3.9) was higher than that in Bladi oil (2.67). This is while the ratio increased during the heating, such that after 8 hours of heating the ratios were 4.63 and 8.24 in Bladi and Arbequina oils, respectively. Before heating, the ratio in refined samples was not significantly different from the virgin samples, while after 8 hours of heating, the increase observed for refined samples were less than than in the virgin samples. It is expected that oils with higher MUFA/PUFA ratio have higher stability, and the ratio is considered as an indicator of resistance against oil oxidation reactions.

The amount of yellow and red colors decreased in both samples after the refining process (Table 2). The major color pigments in edible oil are chlorophyll (green) and carotenoids (yellow to orange). During refining and bleaching, the amount of carotenoids decreased. The percentage of reduction in yellow and red colors following the refining process was 95.74 and 95% in Arbequina oil and 93.36 and 92.59%, in Bladi oil, respectively. The reduction observed was partly because of adsorption and also heat breakdown. During the heating process, the amounts of yellow and red colors initially decreased by degradation and breakdown of the pigments because of oxidation and high temperature.

Changes in the amount of total tocopherols (mg kg-1) in olive cultivars studied during the heating process are shown in Figure 4.
Figure 4. Changes of total polar compounds (%), total tocopherols (mg kg\(^{-1}\)), total polyphenols (mg kg\(^{-1}\)), chlorophyll content (mg kg\(^{-1}\)), carotenoid content (mg beta carotene kg\(^{-1}\) oil) and refractive index in olive cultivars studied during the heating process (180°C). (VB: Virgin Bladi; VA: Virgin Arbequina; RB: Refined Bladi, RA: Refined Arbequina).
Total tocopherol contents of fresh olive oil of Bladi and Arbequina cultivars were 775.29 and 366.9 mg kg\(^{-1}\), respectively. In olive oil, tocopherols are mainly represented by the α-tocopherol, which has biological effect; for instance, involvement in vitaminic action and showing antioxidant properties [1, 3, 11, 15]. Alpha-tocopherol levels in all samples decreased after heating, and the content in some samples reached zero. The levels of tocopherols, especially alpha-tocopherol, also decreased during the refining process. Percentage of total tocopherol reduction by refining process for Bladi oil and Arbequina oil was 43 and 32%, respectively. Some minor components were removed during refining.

Changes in total polyphenol compounds during the heating process are shown in Figure 4. The ROO do not contain phenolic compounds, because these PCs are removed by water during the refining process. Percentage of total polyphenols reduction by the refining process for Bladi oil was higher than Arbequina oil (51.8 and 35%, respectively). Also, the amount of phenolic compounds was decreased during heating due to destruction or removal of these compounds from the medium by heating at high temperatures.

Changes of chlorophyll content in olive cultivars studied during the heating process are shown in Figure 4. The level of pigment decreased at high temperatures. So, after 8 hours of heating, in both samples the amount of this pigment reached zero. Discoloration of chlorophyll can be caused by photo-oxidation and destruction of the protective lipids of chlorophyll. There was no chlorophyll pigment in the refined samples that could be the result of its complete absorption by acid activated bleaching clay.

The changes in total carotenoid content (mg β-carotene kg\(^{-1}\) oil) of olive cultivars studied during the heating process are shown in Figure 4. They have powerful antioxidant activity against both autoxidation and photo-oxidation. However, at high-oxygen concentration, they may exhibit a pro-oxidant activity. Carotenoid elimination normally occurs in hydrogenation and deodorizing. Sometimes, this takes place in the bleaching called the heat bleaching effect as these carotenoids are destroyed during heating. The antioxidant activity of β-carotene is diminished when exposed to high oxygen concentrations, because it leads to the formation of carotenoid peroxo radicals as the conjugated double bonds of carotenoid molecules are very susceptible [15].

Changes of refractive index in olive cultivars studied at 20ºC and during the heating process are shown in Figure 4. Refractive index of Bladi oil was higher than that in Arbequina oil and this index increased during the heating process. The refractive index of Arbequina oil greatly increased, both for the virgin and refined samples, which was higher than that observed in Bladi oil. The amount of PUFAs in Arbequina oil was lower than that in Bladi oil (15.72 vs. 20.19%). This is while the amounts of long-chain SFAs such as C24:0 and C22:0 were, respectively, 0.13 and 0.22%, in Bladi oil, and 0.06 and 0.12% in Arbequina oil. The refractive index value for different oils generally varies between 1.447 and 1.482. Refractive indices increased with an increase in the carbon chain length and number of double bonds. The values are higher for mono glycerides than that of triglycerides.

**CONCLUSIONS**

In the present study, a strong inverse relationship was found between the amount of PUFAs and oxidative resistance. In this regard, the amount of PUFAs in Arbequina oil (15.72%) was lower than the Bladi oil (20.19%). In the present study, a direct relationship was found between the amount of MUFAs and oxidative stability. The amount of MUFAs of Arbequina oil (61.43%) was higher than that in Bladi oil
(54.04%). So, the undesirable quality of the original Bladi oil was effective in the low oxidation resistance of this oil. It was observed that the amount of total polyphenols and tocopherols was higher in Bladi oil, while the oxidative stability was lower in Bladi oil (for higher PUFA), which can be attributed to a gradual decrease in oil stability. Instability of the fractions of the vitamin E during heating is related to a series of reactions such as hydrolysis, oxidation, and polymerization of the oil that mainly depend on the PUFA, polyphenols, and pro-oxidant contents of the oil. According to the results obtained in the study, it can be concluded that oxidative stability is not dependent on a factor; rather, it is influenced by different factors such as: (a) Composition of fatty acids, (b) Presence or absence of antioxidants and pro-oxidants, and (c) Indicators related to the initial quality of oils such as the peroxide values, FFAs, and conjugated dienes and trienes. A mild refining conditions (30 minutes at 55-50 ºC) not only can be less damaging (than the industrial refining) to the oil but it also causes less destructive effects of thermal processes in refined olive oil than in the virgin olive oil.

REFERENCES


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