Quality Assessment on the Oxidative Stability of Almond Kernels during Extensive Storage Time

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Abstract
The objective of our study is to monitor the oxidative stability of different cultivars of almonds (Australian, American and Iranian) kernels/oil during the 12th month of storage at room temperature. Several physicochemical parameters free fatty acids (FFA), peroxide value (PV), p-anisidine value (p-AV), total oxidation value (TOTOX), Fourier transforms infrared spectrophotometer (FT-IR) and Gas chromatography Mass spectrometry (GC-MS) were used to check the oxidative stability of almond kernel. According to the results, effects of room temperature in the early stages of oxidation, primary oxidation products remained stable, whereas secondary oxidation product levels continued to rise in the later stages. In general, FFA increased with increasing storage time, the range was observed (0.21-0.97 %), PV (1.31-16.23 meqO₂/kg), p-AV (2.21-19.35), TOTOX (4.83-15.81), respectively. During storage at room temperature for up to 12th months, there was no significant shifting of the spectral band in the FT-IR study. The most bounteous fatty acid in the almond oil range was observed oleic acid C18:0 (71.01-79.56 %) followed by linoleic acid C18:2 (13.13-20.65 %), palmitic acid C16:0 (4.86-5.67 %), stearic acid C18:0 (1.20-3.81 %), and palmitoleic acid C16:1(0.21-0.47 %) in all three samples during storage. These results suggest that almond oil during the 12th month of storage keeps its good chemical properties.

Keywords: Almond oil, Physiochemical properties Oxidative stability, FTIR, GC-MS

Introduction
Almonds (Prunus dulcis (Miller) D.A. Webb) are nutritious-dense vegetable food. There is an expanding interest in them as a solid staple on the grounds that numerous medical advantages have been accounted for identified with their customary utilization [1]. The almond (nuts) is utilized as shelled and stripped pieces and pressed nuts, or as an element for some food items like pastry shop and candy parlor items, and as an enhancing specialist in drinks and frozen yogurts. Almonds are generally known as a wellspring of high lipid content (48–67 %) [2]. Oleic acid was available as significant unsaturated fat, addressing 50–78 % of the complete unsaturated fat substance. Notwithstanding, C18:2, C16:0 and C18:0 are available at levels of 5-11, 1.5-4 and 10-31 %, individually.
Myristic as well as linolenic acids might be found at low focuses (less than 0.1 %). Minor parts described in almond oil incorporate sterols (around 2000 µg/g), tocopherols (around 450 µg/g oil), and squalene (95 µg/g). In addition, proteins (12-22 %) and carbohydrates (20 %) are also present in the seed [3, 5].

The oxidation reactions caused to produce undesirable flavors, rancid odors, discoloration, and other forms of spoilage. Hydroperoxide are produced as a result of the primary oxidation products (aldehydes, ketones) that have very pungent odor, taste and flavor [6]. Lipid oxidation of food is impacted by numerous components, for example, heat power, light energy, metals, photosensitizers, oxygen content, and the number of twofold bonds in unsaturated fats [7, 8]. Oxidized lipids can speed up the disintegration of tactile quality in eatable oils and decline customer acknowledgment [9]. Oxidative dependability is a significant pointer to decide oil quality and timeframe of realistic usability since low-sub-atomic load off-flavor compounds are gradually created during oxidation, making oil unsuitable to the buyer or for mechanical use as a food fixing [10]. In the oxidation cycle, the fatty oil structure goes through changes with the result of the development of an incredible number of mixtures. It has been hypothesized that as a matter of first importance, essential oxidation compounds, for example, hydroperoxides, are created. Among the optional oxidation items, it is feasible to discover alkanals, (E)-2-alkenals and (E, E)-2, 4-alkadienals, and c-oxygenated b-unsaturated aldehydes [11]. Since almonds are utilized as palatable fixings in numerous food items that are handled at rather high temperatures which eventually influences the time frame of realistic usability and nature of food [12]. Therefore, the object of the present research is to monitor the oxidative stability of most stable almond cultivars during storage upto the 12th month at room temperature. In addition, the study focuses on determining the physico-chemical composition of three different almond varieties by using several classical methods as well as instrumental techniques such as FTIR, and GC-MS.

Materials and Methods

Reagents and Sample Collection

All chemicals and reagents were used in the present study were obtained from E-Merck Darmstadt, Germany, such as glacial acetic acid, n-hexane, p-anisidine, methanol, potassium hydroxide, sodium hydroxide, sodium thiosulphate pentahydrate, sodium-thiosulphate anhydrous, potassium iodide, phenolphthalein, carbon tetrachloride, starch, bromophenol blue, hydrochloric acid, iodine trichloride, ethyl alcohol, petroleum ether, anhydrous sodium sulphate, and sulphuric acid.

The almond kernels of different varieties (Australian, American, and Iranian, were collected in July 2019 from the local market of District Hyderabad (Longitude: N, 25.1827° and Latitude: E, 69.1924°) province of Sindh, Pakistan.

Physiochemical Parameters

All physicochemical parameters such as FFA, PV, pH/AV, and TOTOX of almond kernel oil samples were assessed using an official AOCS methods.

Free Fatty Acid (FFA)

FFA as a (%) of oleic acid was determined by using an Official American Oil Chemists’ Society (AOCS) standard procedure (Aa 6-38) through the titration [13]. The extracted almond kernel oil was dissolved in warm neutral ethanol (C2H5OH), which is
then titrated with NaOH (0.01N) along with indicator phenolphthalein.

**Peroxide Value (PV)**

The official standard method AOCS was used to determine the PV of almond kernel oil [13]. 2 g of oil (each variety) was taken precisely in a conical flask with (10 mL) of chloroform (CHCl₃). Then added 15 mL of acetic acid (CH₃COOH) and 1 mL of potassium iodide (KI), the solution was mixed for 1 min. After that prepared solution (sample) was kept at room temperature in the dark for 5 min. Subsequent to the (75 mL) of de-ionized water and 2 to 3 drops of starch indicator (1 %), ethanol added to the sample solution. The last solution was titrated against sodium thiosulphate (Na₂S₂O₃) the blurred blue color solution of the oil sample turned colorless.

**p-Anisidine Value (p-AV)**

The p-AV was measured using the official AOCS standard method (Cd 8-53) [13]. In this procedure, 2 g of extracted almond kernels oil were measure into 25 mL (volumetric flask) then diluted by isooctane. After that, the absorbance of the solution was recorded with a UV-Visible spectrophotometer at 350 nm, where the reference solvent isooctane used as blank. Finally 5 mL of oil sample was added with 1mL of p-anisidine solution, the mixture was then kept for 10 min and then absorbance recorded. p-anisidine solution was used as a blank in the reference cell [14].

**Total Oxidative Value (TOTOX)**

For the assurance oxidative deterioration value of oil samples, the absolute oxidative value was utilized, which is the summation of PV and p-AV [13].

\[
\text{TOTOX value} = 2\text{PV} + p-\text{AV}
\]

**Fourier Transform Infrared Spectrophotometer (FT-IR)**

The extracted almond kernel oil was performed on a sensitive analytical instrument, (Thermo Nicolet FTIR-5700 Madison, WI). 50 μL of extracted oil (almond) was put on the crystal made of zinc selenide for spectra recording. The preceding crystal was cleaned carefully after the taken each spectrum with n-hexane by means of soft tissue to remove the contamination of the various sample to gets next to the new spectra of other samples followed by the same procedure. It is controlled by OMNIC software 7.2 versions. The entire spectrum was recorded in the mid IR range (650 to 4000 cm⁻¹) at room temperature 25 °C with 4 cm⁻¹ resolution and 16 scans/sample removable ZnSe SB-ATR sampling accessory. The spectrum of various prepared samples was obtained against the background of the air spectrum.

**Gas Chromatography Mass Spectrometry (GC-MS)**

Standard IUPAC method 2.301 was used for the preparation of fatty acid methyl ester (FAME) for different varieties of almond kernels oil samples. The FAME was analyzed and identified by gas chromatography mass spectrometry (GC-MS). The high sensitive instrument (Agilent 6890 N gas chromatography GC) coupled with (mass selective detector) Agilent Technologies (MS-5975 inert XL). Separation of the FA components achieved on a 5 % phenyl methyl siloxane containing HP–5MS capillary column. The column measurements were 30 m in length, 0.25 mm i.d x 0.25 μm thickness of its film (Agilent Technologies, Palo Alto, CA, USA). A sample solution of 1.0 μL methyl ester was injected on a capillary column (HP-5MS) throughout a sampler (7683-B). As a carrier gas, Helium (He) was used with 1.0
mLmin\(^{-1}\) flow rate; detector and injector temperatures were set in the range of 150 °C and 270 °C; the split ratio was 1:35. Initially, the oven temperature was set at 150 °C for 2 min then the temperature was elevated to 270 °C at the rate of 10 °C min\(^{-1}\) (4 min hold). The total analysis time of sample running was 45 min. In the examiner range of 50-550 m/z, the mass spectrometer instrument was operated in the electron impact (EI) mode at 70 eV, respectively.

**Statistical analysis**

Three different varieties of almond kernels (Australian, American and Iranian) were collected from the local market of Hyderabad city and all the data presented was statistically assessed by using software Minitab 16.

**Results and Discussion**

**Free Fatty Acid (FFA)**

The hydrolysis of oils and fats produces FFAs. In terms of oil quality, the FFA assessment of oil is a vital qualitative parameter because fats and oils contain some amount FFAs. Their acidity will steadily rise over time as they are transported and stored [15]. The initial FFA values of Australian almond oil 0.24 %, American almond oil 0.21 %, Iranian almond oil 0.20 %, respectively. Table 1 represents the FFA values of all three samples studied from 1\(^{st}\) month to 12\(^{th}\) month. During storage, FFA values increased gradually throughout the study. The more significant FFA influence was found in Australian almond oil than in Iranian almond oil and American almond oil, respectively. According to the Iranian National Standardization Organization (INSO), When the FFA content of fryer oil reaches 1%, it should be discarded [16]. Above 36% of our discarded oil samples shows FFA levels higher than 1%. The maximum FFA levels in frying oil recommended by the Agriculture Department of the United States, as well as regulatory strategies in various European states, ranged from 1.0 to 2.5 %, depending on the regulations of each country [17, 18].

**Peroxide Value (PV)**

The PV is surveying the number of peroxides and hydroperoxides created in the oils and fats because of oxidation. The PV assurance is mostly utilized to estimate oxidative rancidity of oils and fats [19-21]. The PV measures the initial (essential) oxidation of any oil and is the main marker. In the current examination, the underlying PV of Australian almond oil was acquired at 1.31 meqO\(_2\)/kg, American almond oil at 1.37 meqO\(_2\)/kg, and Iranian almond oil at 1.35 meqO\(_2\)/kg. At the point when PVs of three almond oil tests were contrasted and the fundamental qualities, the expansion in PV was found till the twelfth month of capacity. Be that as it may, a higher estimation of PV was seen in Iranian almond oil, trailed by American almond oil and Australian almond oil. Table 1 shows the PV of each of the three almond bit oil tests. PV under 5.0 meqO\(_2\)/kg in almond oil is considered as a marker of fresh almond accounted for conveyed by Buransompob et al., [22]. In contrast with the current investigation, the entire piece and ground almonds bundled (under vacuum and CO\(_2\)) at an encompassing and refrigeration temperature had at any rate 10 months of the timeframe of realistic usability thought about new. The whole parts and ground almond at surrounding temperature presented to air stayed new for 8, 9, and 7 months separately, while the examples put away at the refrigeration temperature stayed new for over 10 months. In a comparable report, Mexis et al., [23] show that the PVs of crude almond pressed in low-density polyethylene (LDPE) with the oxygen maintain after twelfth long periods of capacity at (20 °C and 4 °C) in obscurity and the worth was noticed 1.62, 0.99 and 1.15 meqO\(_2\)/kg separately. Their
outcomes were identified with the PV of ground almond under \( \text{CO}_2 \) in the current examination following 10 months at encompassing and refrigeration temperatures.

\textit{p-Anisidine Value (p-AV)}

In oil or fat, \( p \)-anisidine test is utilized to assess the optional oxidation, which is for the most part imputable to aldehydes and ketones and is in this manner skilled to express the oxidation "history" of oil and fats [24]. In the current investigation, the underlying \( p \)-AV of almond oil tests was discovered to be 2.21, 2.33, and 2.28, separately for Australian, American, and Iranian, almonds. All through the capacity of almond, parts expanding request was seen from the first month to the most recent month of capacity on the whole examples as demonstrated in Table 1. The most elevated \( p \)-AV was seen in Iranian almonds, followed by American almond and Australian almond parts. As per the norm of the Iranian National Standardization Organization (INSO), a significant \( p \)-AV of fresh oil in the reach from 4.0 to 6.0, the oil would be profoundly oxidized when \( p \)-AV is higher than 6.0 value [25, 26].

\textit{Total Oxidative Value (TOTOX)}

The oxidative disintegration of lipids is habitually used to rough by figuring the estimation of TOTOX. Since it is the mix of essential (hydroperoxides) and auxiliary (alkenals and alkadienals) all out oxidation items in lipids [27]. As indicated by the detailed investigation by The German Society for Fat Sciences, the suggested estimation of TOTOX ought to be more modest than 20 for eatable oil [28]. Table 1 shows the TOTOX value in almond oil from three assortments at room temperature for the twelfth month's oxidation period. It clearly showed that TOTOX increases progressively from the first month to the twelfth month. The underlying TOTOX evaluation of Australian almond oil was noticed 4.83, American almond oil 5.07, and Iranian almond oil 4.98 individually. Then again, most reduced to most noteworthy TOTOX esteems were gone from (4.83-51.81) taking all things together with three assortments. The expanding pattern of TOTOX in Australian almond oil is much slower contrasted with American and Iranian almond oil tests that at last address the higher dependability of Australian almond oil towards oxidative harm. The more prominent soundness of oil and fat mirrors the lower substance of TOTOX revealed by Wai et al., [29].

\textit{FT-IR Characterization of Almond Oil}

FTIR is a powerful instrumental technique used for quantitative and qualitative analysis of edible oils and fats due to the functional group information (\( i.e., \) strong signals for polar groups such as \( \text{O}–\text{H}, \text{C}–\text{H} \), and \( \text{C}–\text{O} \) contained within an IR spectrum. As a result, FTIR has been used to investigate oil oxidation by recording the spectra of samples taken over time from oils that have been exposed to oxidative stress [30]. Figure 1 portrays the FTIR spectra recorded for Australian, American and Iranian almond oil for the twelfth month's store up under room temperature. The analyzed FTIR spectra of investigated oil samples contain characteristic and fundamental band frequencies of several functional groups with different intensities.

The FTIR spectra of Australian, American, and Iranian almond portion oil were practically comparable however, different contrasts could be noticed for distinguishing proof purposes. As the oxidation cycle advances, a few changes occur in the FTIR spectra of almond kernel oil. The essential changes to the spectra appeared in the accompanying districts:
Table 1. Comparative results of (FFA, PV, p/AV, TOTOX) during 12th month's storage of (Almond) kernels/oil.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Almond (Kernel)</th>
<th>PV (AV)</th>
<th>p/AV</th>
<th>TOTOX</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Mean±S.D.</td>
<td>Mean±S.D.</td>
<td>Mean±S.D.</td>
<td>Mean±S.D.</td>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>FFA (%, O.A.)</td>
<td>0.24±0.00</td>
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<td>0.28±0.02</td>
<td>0.30±0.02</td>
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<td>5</td>
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<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Australian</td>
<td>0.21±0.02</td>
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<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Indonesian</td>
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<td>8</td>
</tr>
<tr>
<td>Australian</td>
<td>1.33±0.04</td>
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<tr>
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<td>1.57±0.05</td>
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<tr>
<td>Australian</td>
<td>2.21±0.02</td>
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<tr>
<td>Indonesian</td>
<td>2.31±0.02</td>
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<tr>
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<td>3.91±0.01</td>
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<td>3.95±0.01</td>
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<td>8</td>
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<tr>
<td>Indonesian</td>
<td>4.03±0.02</td>
<td>4.05±0.02</td>
<td>4.07±0.02</td>
<td>4.09±0.02</td>
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<tr>
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<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Australian</td>
<td>5.97±0.03</td>
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<td>6.03±0.03</td>
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<td>8</td>
</tr>
<tr>
<td>Indonesian</td>
<td>6.08±0.05</td>
<td>6.11±0.05</td>
<td>6.14±0.05</td>
<td>6.17±0.05</td>
</tr>
</tbody>
</table>

The values provided in the Table 1 are the mean values of triplicate analysis with standard deviation. Means followed by different superscripts in the same column differ significantly (Tukey's test at 0.05 p-Level the population are significantly different).
Figure 1. FT-IR spectra (A) Australian (B) American and (C) Iranian almond kernel oil during oxidation process (12th month storage period)

The FTIR spectra of Australian, American, and Iranian almond portion oil were practically comparable however, different contrasts could be noticed for distinguishing proof purposes. As the oxidation cycle advances, a few changes occur in the FTIR spectra of almond kernel oil. The essential changes to the spectra appeared in the accompanying districts:

[1]. 3,700–3,150 cm⁻¹: This region remained unchanged for the first 6 months. Following this phase, the region's profile began to shift, that the sample was exposed to the oxidation conditions. The frequency of this band gradually progressed from (3,470 to 3,466 cm⁻¹). The reduction in frequency values was because of the cover with the first band (3,470 cm⁻¹) because of the suggestion of the glyceride ester carbonyl absorption and new retentions caused by hydroperoxides or essential oxidation items produced in the oxidation procedure [31].

[2]. 3,010–2,999 cm⁻¹: The band appeared nearly at 3006 cm⁻¹ was due to CH stretching vibration of the cis olefinic group. The absorbance was noted under the oxidative period to remain unaltered for the first 5 months of treatment while it has been started to change after 6th months. The lower values of absorbance show the oil was going to oxidized and the double bonds in fatty acid undergo the isomerization process of cis to trans [32].

[3]. 1,800–1,600 cm⁻¹: The major band noticed at 1743 cm⁻¹ was due to the carbonyl group of triglycerides [33]. An asymmetric modification was observed in the selected band whilst the oxidative study progressed. However, this band initially remained
unaltered for 6th month of the oxidation study, while the frequency of this band started slightly to decrease from 1,743 to 1,738 cm⁻¹. The change might be due to the band of secondary oxidation product whose absorbance appeared at 1728 cm⁻¹ overlap by the band for ester group or could be appeared owing to the occurrence of aldehyde functional group (saturated). Consequently, this overlapping caused the increase in the width of the observed band when oxidation undergoes from 6th to 12th months.

[4]. 1,000–900 cm⁻¹: The absorbance at 967 cm⁻¹ was noticed due to the isolated trans-olefins CH functional groups bending vibrations. It started to increase at different rates depends on the nature of oil. Throughout the oxidative period, this band reveals information about the ongoing cis to trans isomerization. These outcomes are in acceptable concurrence with those recently announced by BeltranSanahuja et al [34] and Guillen and Goicoechea [35].

Fatty Acid Composition of Almond Oil Samples

The GC-MS instrument was used to detect the level of unsaturated fatty acids present in Australian, American and Iranian almond kernel respectively. The five major fatty acids were recognized in almond kernel oil. Among them, two saturated fatty acids (C16:0 and C18:0) and three unsaturated fatty acids (C16:1, C18:1, and C18:2) were found. Among saturated fatty acid the C16:0 was seen in the scope of 4.86-5.67 %, C16:1 (0.21-0.47 %), C18:0 (1.20-3.81 %), C18:1 71.01-79.56 %, and C18:2 (13.13-20.65 %) taking all things together with three assortments. The consequences of unsaturated fatty acid organization of Australian, American, and Iranian almond part oil during the capacity time of twelfth months as impacted by different convergences of almond oil are surrendered (Table 2). The most elevated grouping of C16:0 was found in Australian almond oil and the least found in Iranian almond oil. Nonetheless, the C16:1 was discovered higher in American almond oil and lower in Australian almond oil. The centralization of stearic acid was noticed higher in Iranian almond oil and lower in Australian almond oil. C18:1 was major unsaturated fatty acid in almond oil. The most noteworthy convergence of oleic acid was found in American almond oil and the least found in Iranian almond oil. Among polyunsaturated fatty acids, linoleic acid was predominant on the whole three assortments. Linoleic acid was discovered higher in Iranian almond oil and lower in American almond oil. Likewise, A. Beltran sanahuja et al., [34], completed the work on various almond oils put away at 0, 11, and 20 days to see the effect on the primary unsaturated fats profile like the substance of C16:0, C16:1, C18:0, C18:1, and C18:2, acids. As per their exploration, oleic acid was the most copious unsaturated fatty acid in almond oil (57–74 %) as for linoleic acid (16–28 %). The C16:0, C18:0 and C16:1 was additionally present in limited quantities, for example, (6–7 %), (1.7–2.7 %), and (0.37–0.54 %) individually.

Notwithstanding, the examination detailed by Paloma Sanchez Bel et al., [36] depicted just five saturated/unsaturated fatty acid, for example, C16:0, C16:1, C18:0, C18:1, and C18:2 in almond oil. Following unsaturated fatty acids were found as fundamental constituents in almond oil C18:1 (66.69–70.03 %) trailed by C18:2 (19.17–21.94 %). What are lesser measures of C16:0 (6.29–6.58 %), C18:0 (2.20–2.48 %) and C16:1 (0.10–0.44 %) were noticed. There isn't any huge change were seen in the lipid synthesis during the 5 months of capacity, which was adjusted at the particular snapshot of the conduct or following, yet stayed consistent during the whole oxidation time frame.
Table 2. Composition of fatty acid (Australian, American and Iranian) almond Kernel oil during storage at room temperature

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>0.29764649%</th>
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</thead>
<tbody>
<tr>
<td>AUSTRALIAN OIL</td>
<td>5.30464649%</td>
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<td>5.30464649%</td>
<td>5.30464649%</td>
<td>5.30464649%</td>
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<tr>
<td>AMERICAN OIL</td>
<td>5.30464649%</td>
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<tr>
<td>IRANIAN OIL</td>
<td>5.30464649%</td>
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</table>

The Values provided in the Table 2 are the mean values of triplicate analysis with standard deviation. Means followed by different superscripts in the same column differ significantly (Tukey’s test at 0.05 p-Level the population are significantly different).
As indicated by Solomon Giwa et al., [37] the unsaturated fatty acid organization of almond oil examined and the reach was noticed C16:0 (9.3 %), C16:1 (0.3 %), C18:0 (1.8 %), C18:1 (69.7 %), and C18:2 (18.2 %) Of the six FAs, the most common was C18:0; representing 69.7 %, and C16:0 (9.3 %) was the transcendent immersed. Another research S.K Sathe et al., [38] dissected the unsaturated fatty acid creation of various almond cultivars the most bountiful unsaturated fatty acid in the almond oil range was noticed C18:1 (59.52–73.80 %) trailed by C18:2 (19.49–33.29 %). There were additionally more modest measures of C16:0 (5.15–6.65 %), C18:0 (0.24–1.66 %) and C16:1 (0.31–0.57 %) acids.

Conclusion

It is concluded during the storage period of the 12th month of almond kernel, the Iranian cultivar was found to be the most stable variety among American and Australian oil samples. However, the Iranian almond oil acid value was remarkably inferior to the other cultivars. Additionally, the FFA level was found to be lower than the maximum permissible acid level of oil and fat 4 mg KOH/g. The level of degradation due to oxidation on the PV, AV, and TOTOX increased linearly during the storage time. FTIR analysis of almond cultivars reveals the effects of oxidation that can be best seen in the following regions. The peak shifting was observed from i.e., from 3700-3150 cm⁻¹, 3010-2999 cm⁻¹, 1800-1600 cm⁻¹ and, 1000-900 cm⁻¹ C18:1 was found the most abundant fatty acid followed by C16:0, C16:1, C18:0 and C18:2, and acids measured by GC-MS. Fatty Acid components from major (C18:0) to minor (C16:0) significantly affected by the condition of oxidation.

Conflict of Interest

The authors declare no conflict of interest.

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