

Original Article



Quality Characteristics of Iranian Extra Virgin Flaxseed Oil and the Effect of the Refining Stages before Deodorization on its Physicochemical Properties

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Narges Esmaeilifard¹, Manochehr Bahmaei^{2*}, Yousef Ramezan³, Parvin Eshratbadi⁴

¹Department of Food Science & Technology, Pharmaceutical Science Branch, Islamic Azad University, Tehran, Iran.

²Islamic Azad University, North Tehran Branch, Tehran, Iran.

³Department of Food Science & Technology, Pharmaceutical Science Branch, Islamic Azad University, Tehran, Iran.

⁴Institute of Standard and Industrial Research, Karaj, Iran.

Abstract

Flaxseed oil is known as a functional oil because of the high content of α -linolenic acid; therefore, the aim of this research was to investigate some physicochemical properties of Iranian extra virgin flaxseed oil (EVFO) and the impact of the refining stages before deodorization on these properties. Fatty acid composition, peroxide and anisidine values, free fatty acids, Crystallization point, chlorophyll content, β -carotene content, and color were analyzed. The Iranian flaxseed oils had about 39-40% omega-3 fatty acids and a low ratio of omega-6 to omega-3 and equal to 0.44-0.47, which can be combined with oils rich in omega-6 to reach a favorite omega-6 to omega-3 ratio. In addition, the results showed that when EVFO was refined, the crystallization thermograms changed significantly. The presence of phospholipid compounds in EVFO made its onset, crystallization, endset points higher than the RFO. The amount of β -carotene and chlorophyll content of refined flaxseed oil (RFO) were 6 and 22.33 times lower than its EVFO. Also, RFO had significantly ($p < 0.05$) more L*, less tendency to yellow color, and tendency to a green color as compared to its EVFO. The results showed that flaxseed oil fatty acid content, free fatty acids, peroxide, Anisidine values, crystallization temperature, chlorophyll and β -carotene content and the color were significantly affected by the refining stages. So it is recommended that flaxseed oil not to be refined, but be used in a short time period. Also, it should be consumed in blending with omega-6 oils (in order to maintain omega-6 to omega-3 ratio).

Keywords: Flaxseed oil, functional oil, α -linolenic acid, refining stages before deodorization.

*Corresponding author: Manochehr Bahmaei. Islamic Azad University, North Tehran Branch, Tehran 19136 74711, Iran.
Tel: Tel: +98 912 1488481; fax: +98 212 2248284
E-mail address: Mbahmaie@gmail.com

Introduction

Flaxseed (*Linum usitatissimum*) belongs to the genus *Linum* and *Linaceae* species. *Linum* genus has about 230 species, which includes 19 species that are found in the Iranian flora (Rechinger, 1974). This seed, because of its high content of omega-3 fatty acids, soluble and insoluble dietary fiber, natural phenolic antioxidants, and protein are taken into consideration (Hosseinian et al., 2006; Kasote, 2013; Kajla et al., 2015; Rabetafika et al., 2011; Kaushik et al., 2016). It consists of 40 to 50% of oil, 23 to 34% of protein, 4% of ash, 5% of mucilage, and 0.9 to 3% of Lignan (Toure and Xueming, 2010). Herchi et al. (2011) found that flaxseed oil has 27 to 40% phosphatidylethanolamine, 29 to 32% phosphatidylinositol, 7 to 18% phosphatidylcholine, 8 to 21% lysophosphatidylcholine, 1 to 4% phosphatidylglycerol, and 1 to 9% phosphatidic acid. The dominant sterols in flaxseed are β -sitosterol, campesterol, and stigmasterol (Hosseinian et al., 2004). Flaxseed oil has remarkable antioxidant properties because they are rich in γ -tocopherol. Flaxseed oil contains the essential fatty acid alpha-linolenic acid (ALA), which the body converts into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Chen et al., 2002). On the other hand, its high amount of ALA causes rancidity, offensive odor, and flavor reversion.

It is believed that regular consumption of flaxseed oil decreases cardiovascular diseases, cancer, atherosclerosis, immune system disorders, and diabetes (Patade et al., 2008; Eilati et al., 2013; Xu et al., 2012; Mason et al., 2010; Jangale et al., 2013). Vijaimohan et al. (2006) reported that flaxseed oil reduced hepatic lipids in hyperlipidemia. Karminskas et al. (1992) reported that flaxseed oil increased High-Density Lipoprotein (HDL) and slightly decreased total cholesterol in diabetic patients. Flaxseed oil contains both omega-3 and omega-6 fatty acids, which are essential for being healthy. The ratio of omega-6 to omega-3 in the Western diet is about 15-16 to 1, and in Iran is about 11-30 to 1. Today, it shows a lack of omega-3 fatty acids and the presence of high amounts of omega-6 fatty acids in all society's diet, which promotes diseases such as heart disease, cancer, inflammatory and autoimmune diseases. The high content of omega-3 fatty acids or less omega-6 to the omega-3 ratio in diet can avoid these diseases (Simopoulos,

2002; Iranian National Standard organization, 2009). FAO/WHO reported that ALA would protect against heart disease and recommends the use of flaxseed, canola, and soybean oils in the daily diet (Kris-Etherton et al., 2002). In a study, Xiao et al. (2012) compared the effects of both cold pressed and refined flaxseed oils (RFO) enriched with vitamin E in mice diet. They found that cold pressed flaxseed oil significantly ($P < 0.05$) decreased plasma triglyceride, total cholesterol, and low-density lipoprotein (LDL) by 12, 21 and 18%.

To avoid rapid rancidity of flaxseed oil, it is enriched with vitamin E and stored in dark bottle (Lukaszewicz et al., 2004). Unfortunately, there is no specific standard for the flaxseed oil quality. For virgin oils and cold pressed fats and oils are used Codex Alimentarius Commission (1999) and Iranian National Standard organization (No. 13392, 2012) standards. Also, New Zealand Food Regulation (1984) is used for edible and virgin fats and oils. The aim of this paper was to analyze the physicochemical and quality characteristics of Iranian flaxseed oil and evaluate the effect of refining stages on its physicochemical properties.

Materials and Methods

Materials

Flaxseed oil was extracted from the seeds by the cold pressing method. All the chemicals and solvents used were of analytical or gas chromatography (GC) grade.

Sample preparation

The refining of flaxseed oil
Flaxseed oil was heated to about 50°C, thereafter 85% phosphoric acid (in terms of 0.1 of the weight of the oil) was added to the oil. The content was mixed in such a way that in 20 min, the temperature increased from 50°C to about 70 to 80°C. Then, 4 N sodium hydroxide was added to the oil and was swiftly mixed for 1 min. After removing the soap formed on the surface, the remaining oil was put in the centrifuge within 15 min at a speed of 4500 rpm ($\times 360g$). Thereafter, the content was washed three times with 90 to 100°C distilled water. Once more, the remaining oil was centrifuged for 15 min at a speed of 4500 rpm ($\times 360g$). Afterward, oil was put under vacuum and heated, the bleaching earth (the amount of used bleaching earth should be about 1% W/W), activated with acid, was added. The temperature was increased

from 60°C to 90 to 100°C in 30 min.

Fatty acid composition by gas chromatography (GC)

The component of fatty acids was determined using gas liquid chromatography. Fatty acids were converted into methyl esters using transesterification methods of oils with sodium methoxide as a catalyst according to AOCS method (AOCS, 1995a). Agilent-technologies 6890 N gas chromatograph was equipped with a flame ionization detector and a capillary column (120 m × 25 mm ID-BPX 700. 250) (USA). To determine the fatty acid composition, a capillary column with temperature programming was used according to the AOCS method (AOCS, 1995b). Helium was used as the carrier gas.

Standard chemical analyses

Free fatty acids (FFA), peroxide (PV) and Anisidine (AV) values of the samples were determined according to the AOCS method (AOCS, 1992).

Differential scanning calorimeter (DSC) analysis

To evaluate the crystallization temperature, an American Mettler Toledo differential scanning calorimeter (DSC) was used. To calibrate the device, indium and n-dodecane were used.

Calibration was done using the pass of nitrogen gas from the empty pan and the appearance of a baseline. The device temperature was reduced from 30 to -40°C at the rate of 5°C/min.

Assessment of β -carotene and chlorophyll content

Evaluation of chlorophyll content was determined by PFX 995 Tintometer Lovibond (1977) in accordance with AOCS Cc 13d-55 (AOCS, 1993) and β -carotene content, in accordance with BS684 Section 2.20.

Color

The oil color "Lab" was measured by PFX 995 Tintometer Lovibond (made in Germany). L^* (ranges from 0 to 100) is a value of the lightness. The positive or negative a^* values are related to the redness and greenness, respectively. The positive and negative b^* values refer to the yellowness and blueness, respectively.

Statistical analysis

All examinations for each sample were performed three times. Statistical Package for Social Sciences (SPSS) software, version 23 (IBM, New York, USA) was used for the statistical analysis. To confirm or reject the hypotheses, a paired sample t-test ($p < 0.05$) was used for all samples.

Table 1. The composition of extra virgin and refined flaxseed oils fatty acids.

factor/ type of oil	EVFO	RFO	Sig (P<0.05)
C16: 0	7.64 ± 0.003	6.65 ± 0.01	0.005
C18: 0	6 ± 0.03	7.04 ± 0.01	0.006
C18: 1	25.84 ± 0.03	28.24 ± 0.03	0.001
C18: 2	18.89 ± 0.02	17.49 ± 0.02	0.002
C18: 3	40.25 ± 0.01	39.92 ± 0.01	0.039
Others	1.35 ± 0.04	0.66 ± 0.02	0.018
TUFA	84.99 ± 0.03	85.65 ± 0.01	0.024
PUFA	59.14 ± 0.01	57.41 ± 0.03	0.007
MUFA	25.84 ± 0.03	28.24 ± 0.03	0.001
TSFA	13.64 ± 0.03	13.69 ± 0.01	0.170
n-6 / n-3 ratio	0.47 ± 0.001	0.44 ± 0.002	0.007
PV (meq/Kg)	3.60 ± 0.21	3.10 ± 0.07	0.179
AV (unit)	0.26 ± 0.03	2.30 ± 0.03	0.001
TV	7.76 ± 0.74	8.62 ± 0.17	0.140
FFA (%)	0.74 ± 0.02	0.07 ± 0.003	0.002

All data are reported as mean ± SD. The significant difference ($p < 0.05$) between extra virgin and refined flaxseed oils observed except the TSFA, PV and TV. TUFA: Total of unsaturated fatty acid, PUFA: Polyunsaturated fatty acid, MUFA: Monounsaturated fatty acid, TSFA: Total of saturated fatty acid, PV: Peroxide value, AV: Anisidine value, TV: Totox value, FFA: Free fatty acids.

Results and discussion

Comparison of extra virgin and refined flaxseed oil fatty acid composition

Flaxseed oil is a functional oil, because of the high content of ALA. Flaxseed was purchased from Golestan Province in the North of Iran. Flaxseed oil when extracted by the cold pressing method contained 40.25% ALA, 18.89% linoleic acid, 25.84% oleic acid, 6% stearic acid, and 7.64% palmitic acid. When compared with other studies on flaxseed species from India, Europe, Canada, Argentina and England, the Iranian flaxseed oil was found to have higher oleic acid and lower ALA (Berg et al., 2003; El-Beltagi et al., 2007; Hassan Zadeh et al., 2008; Madhusudhan and Singh, 1985; Popa et al., 2012). The Iranian extra virgin flaxseed oil (EVFO) had a low ratio of omega-6 to omega-3 fatty acid and equal to 0.47, which can combine with other oils (rich in omega-6 fatty acids) to reach a favorite omega-6 to omega-3 ratio.

According to the Codex Alimentarius standard, the free fatty acid value of extra virgin oil has to be less than 0.8 g/100 g (in terms of oleic acid). Meanwhile, its peroxide value should be less than 15 meq/kg. In this research, the Iranian EVFO had free fatty acid and peroxide values of 0.74% and 3.6 meq/kg, respectively, which were lower than the amount, set by the Codex standard.

Also, the EVFO fatty acid content was significantly ($p < 0.05$) affected by the refining stages before deodorization. In addition, only total saturated fatty acid was not affected by the refining stage before deodorization. During the refining stages before deodorization, the Anisidine value increased, indicating the failure of hydroperoxides and production of secondary oxidation products (aldehydes and ketones). The deodorization stage not only did eliminate these compounds, but also increased the removal of flaxseed oil phenolic compounds, tocopherols, and sterols. This process also reduced the shelf life of the product. The free fatty acid value was evaluated after the refining process. In this research, the free fatty acid value was significantly ($p < 0.05$) reduced by the refining stages before deodorization. This is because of the removal of free fatty acids in the neutralization stage.

Choo et al. (2007) reported the physicochemical and quality characteristics of cold pressed flaxseed oil in a few different brands available in the New Zealand market. On this basis, the

content of saturated omega-6 and omega-9 fatty acids of Iranian flaxseed oil was more than those of different brands of cold-pressed flaxseed oil in the Romanian and New Zealand markets. In addition, the New Zealand and Romanian counterparts had higher ALA content.

Also, New Zealand flaxseed oil was found to contain 0.75% free fatty acid value, 2.04 meq/Kg peroxide value and 0.52 units of Anisidine value (Teh and Birch, 2013), while the Iranian flaxseed oil were 0.74%, 3.6 meq/kg, and 0.26 units, respectively. In other words, the Iranian flaxseed oil had higher peroxide value and lower Anisidine value as compared to its New Zealand counterpart. It showed that the antioxidant properties of Iranian flaxseed oil were extremely high because it prevented the breaking of hydroperoxides into secondary oxidation products, such as aldehydes and ketones. According to the Iranian standard, the peroxide value had to be less than 15 meq/kg. Therefore, on the basis of this paper, the Iranian extra virgin flaxseed oil had a significantly ($P < 0.05$) lower peroxide value compared to the Iranian standard. Meanwhile, according to the New Zealand standard, the recommended maximum Anisidine value for oils is 2 units, but in this research, the studied Anisidine was lower than 2 units. Interestingly, the recommended maximum totox value is 4 units (New Zealand Food Regulation, 1984). The finding of the present research showed that the totox value of Iranian extra virgin flaxseed oil was more than 4 units.

Crystallization point

Differential scanning calorimetry (DSC) monitors the change of physicochemical characteristics of a sample as a function of temperature by finding the heat changes related to phase transition (such as crystallization and glass transition). Oils have 96-99% triacylglycerol. Each edible oil has a particular DSC curve, which is related to fatty acid composition and polar compounds (Zhang et al., 2014). In order to study the crystallization point of EVFO and RFO were used by DSC and shown in Figure 1 (a and b). The Iranian EVFO had an onset point significantly ($p < 0.05$) higher than RFO, because of the presence of phospholipid compounds in EVFO. In other words, other phospholipids present in the crude flaxseed oil increased the crystallization temperature by 3°C when compared with the RFO. As shown, there was no glass transition temperature in any flaxseed oil. The onset and crystallization points

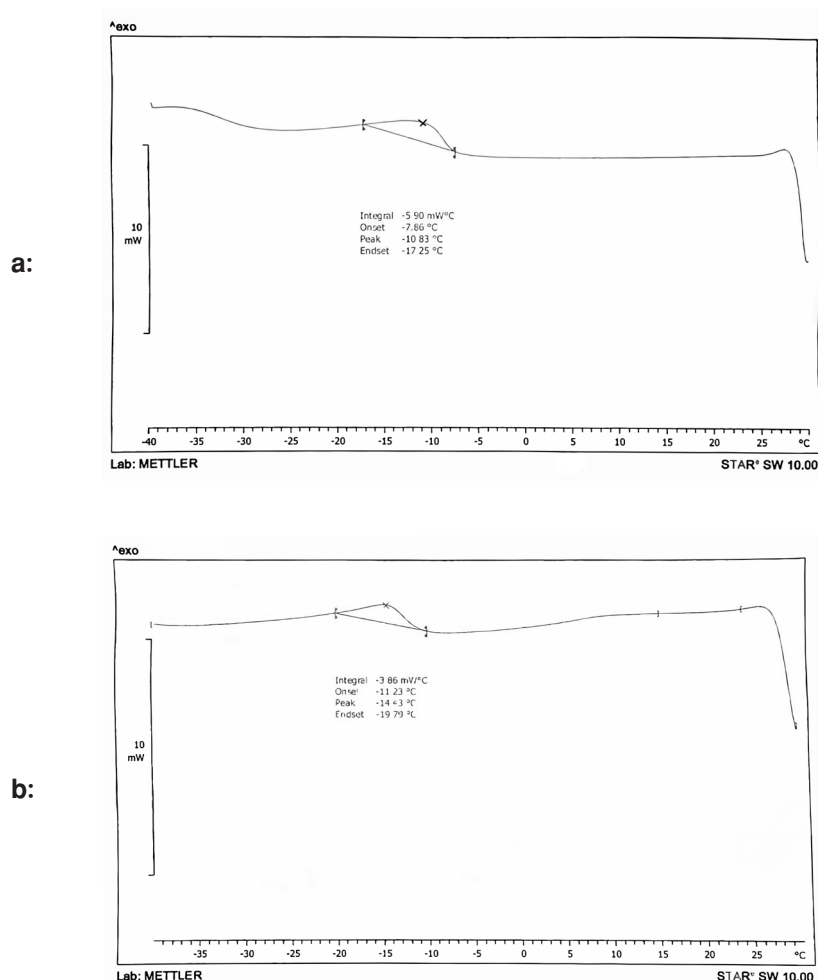


Figure 1: (a) DSC profile of extra virgin flaxseed oil. (b) DSC profile of refined flaxseed oil.

of EVFO were significantly ($p < 0.05$) affected by the refining process. Actually, the presence of phospholipid compounds in EVFO made its onset and crystallization points higher than the RFO. However, these compounds would be removed from the refined oil during the process of degumming.

Comparison of the content of chlorophyll and β -carotene in oils

The chlorophyll and β -carotene content of EVFO were, respectively, 1.34% and 48.35 mg/kg. According to a study by Rafatowski et al. (2008), the amount of β -carotene in cold pressed flaxseed oil from Poland was estimated to be 150.1 mg/kg. However, in this study, β -carotene of Iranian cold pressed flaxseed oil was lower than that of Poland. Hence, the Iranian cold pressed flaxseed oil was more resistant to autoxidation. Another finding of

this research was that the refining stages before deodorization were significantly ($p < 0.05$) affected by β -carotene and chlorophyll flaxseed oil content so that the amount of β -carotene and chlorophyll content reduced by 6 and 22.33 times. Chlorophyll in photo-oxidation phenomenon played the role of sensitization and caused the formation of hydroperoxides (Tautorus & Low, 1994). In addition, chlorophyll content could be reduced by refining, especially by the bleaching stage. Kreps et al. (2014) concluded comparable observation.

Also, β -carotene operated as a 'quencher' in the photo-oxidation phenomenon because of its conjugated and unsaturated structure and avoided the formation of hydroperoxides. On the other hand, it participated as a peroxidized in autoxidation and promoted the formation of hydroperoxides in this phenomenon. It can be removed from oil in the bleaching stages.

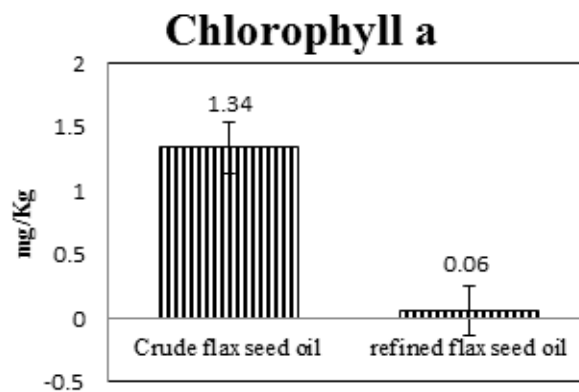


Figure 2: Comparison content of chlorophyll extra virgin and refined flaxseed oils. Refining before deodorization significantly ($P < 0.05$) effects on flaxseed oil chlorophyll content.

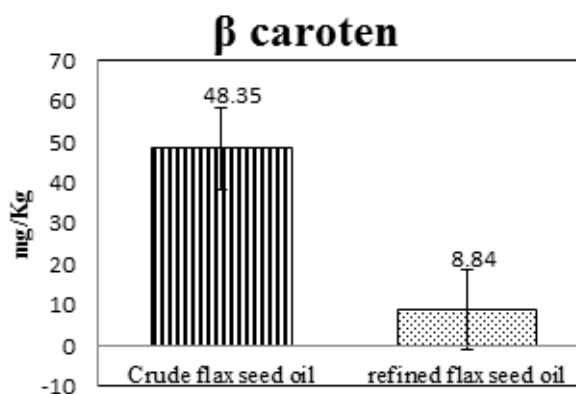


Figure 3: Comparison content of β-carotene extra virgin and refined flaxseed oils. Refining before deodorization effects significantly ($P < 0.05$) on flaxseed oil β-carotene content.

A comparison between the EVFO and RFO showed that the EVFO significantly ($p < 0.05$) contained chlorophyll and β-carotene, more than the RFO. This emphasized that refining before the deodorization process was done correctly. In fact, these compounds were removed during the bleaching stage. Teh and Birch (2013) claimed that the chlorophyll content of New Zealand cold pressed flaxseed oil was 6.78 mg/kg, while the chlorophyll content of Iranian EVFO was 1.34 mg/kg. So, Iranian EVFO was more resistant to photo-oxidation.

Color

The lightness “ L^* ”, the tendency to red color “ a^* ”, and tendency to yellow color “ b^* ”, Iranian EVFO, respectively, were 52.86, 15, and 87.19, while these factors in the Iranian RFO were

87.95, -9.23, and 55.14. In other words, RFO had significantly ($p < 0.05$) more lightness, less tendency to yellow color, no tendency to red color, and tendency to green color as compared to its EVFO. In extra virgin flaxseed oil, a Maillard reaction happened between the aldehyde resulted by oil oxidation and amino agents of phospholipids. This reaction leads to its highest redness (a^*) than RFO. According to the reports by Augustin et al. (2006) and Esmailifard et al. (2016), Maillard browning reaction and the formation of Maillard pigments decreased lightness (L^* value) and increased redness and yellowness (a^* and b^* values). Also, Bicanic et al. (2010) showed that high concentration of β-carotene in mango led to a decrease and an increase in L^* and a^* values, respectively.

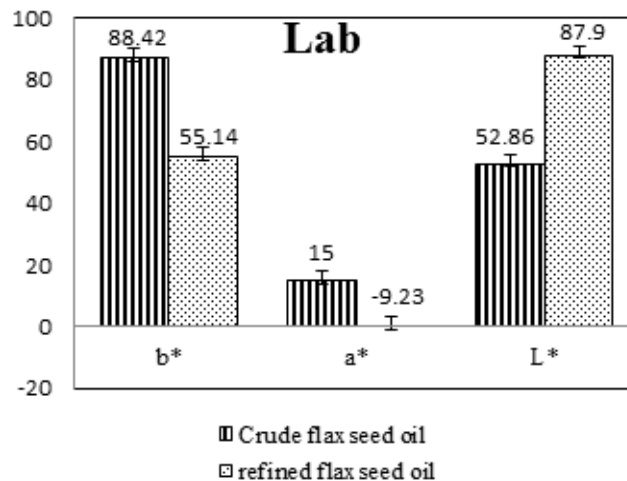


Figure 4: Comparison color of extra virgin and refined flaxseed oils. L* : Lightness, a* : redness, -a* : greenness, b* : yellowness, -b* : blueness.

Conclusion

Today, communities need omega-3 fatty acids more than before. Therefore, flaxseed oil can be used as a source of omega-3 fatty acids in a diet. As shown in this study, Iranian flaxseed oil has about 39 to 40% omega-3 fatty acids. As a result, this oil is considered as a drying oil and promotes rancidity and flavor reversion. This research showed that Iranian crude flaxseed oil has less than 0.8% free fatty acid value and 15 meq/kg peroxide value. This condition makes it an extra virgin oil. In addition, the refining stages before deodorization had significant ($p < 0.05$) effects on the fatty acid content of extra virgin flaxseed oil, free fatty acids, peroxide, and Anisidine values. Also, it reduced the content of flaxseed oil β -carotene and chlorophyll. During the refining process, lightness and tendency to yellow color increased significantly ($p < 0.05$), and had the tendency to become green, and was found to be yellow-green in color. Furthermore, DSC thermograms show that crystallization point increased significantly ($p < 0.05$) by the refining stages before deodorization.

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