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Evaluation of Chemical Properties of Cold Pressed *Ficus Carica* Seed Oil

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Abstract

Free fatty acid, peroxide value , conjugated diene and triene, chlorophyll, β-carotene, fatty acid composition, triglyceride, tocol (tocopherol and tocotrienol) compositions, sterol, wax and total polymeric compound amounts of cold pressed *Ficus carica* seed oil were evaluated by using chromatographic and spectrometric methods in this study. While the % free fatty acid of cold pressed *ficus carica* seed oil was 0.76±0.06, the peroxide value was found as 1.06 ± 0.09 meqO_2/kg. It also had low content of conjugated diene and triene amounts, chlorophyll, wax and total polymeric compounds. The obtained results demonstrated that cold pressed *ficus carica* seed oil had rich linolenic and linoleic acid, and contained high amounts of Linoleic- Linolenic- Linolenic, Linolenic- Linolenic- Linolenic, Olenic- Linoleic– Linolenictriglycerides. Cold pressed *ficus carica* had a high content of β-carotene (4114.9 ppm), total tocol (1006 ppm) and sterol (7250.83 ppm). The obtained results showed that *ficus carica* seed oil is a product with superior properties due to its high nutritional value and beneficial phytochemicals. Therefore this oil can be an alternative to vegetable oils and used as a medical product.

Keywords: *Ficus carica* seed oil, Cold pressed, Fatty acid composition, Tocopherol, Triglyceride composition, Sterol, Wax, Polymeric compounds.

Introduction

Ficus Carica, which is cultivated in a wide geographical area extending from Turkey to Afghanistan is a member of the Moraceae family and contains high amounts of carbohydrates, vitamins, dietary fiber, minerals and oils [1-3]. *Ficus Carica* seeds contain fixed oil between 21.54% -28.52% [4]. These seeds have valuable essential fatty acids, tocopherols & tocotrienols and triglycerides. Edible oils are generally obtained from oilseeds using dehulling, solvent extraction, pressing and separation methods. During the production of these oils, various undesirable substances are formed due to temperature, humidity, pressure, use of solvents and the quality decreases [5-7]. Cold pressing is a method used instead of industrial applications and no heat is applied to the raw material as in the screw pressing process. Cold pressing doesn`t have a negative effect on the beneficial components of the edible oils. In addition, no organic solvents that would be chemical contaminants in the product are used in cold pressing [8,9]. Therefore, cold pressing oils contain more bioactive components such as lecithin (phospholipids), vitamins (tocopherols / vitamin E, etc.), phytosterols, lignin, squalene, organo minerals, lipoproteins [6,8, 10-12].

Fatty acid composition, triglyceride (TG), tocol (tocopherol and tocotrienol) compositions, sterol, wax, total polymeric compounds, chlorophyll, β-carotene, peroxide value, conjugated diene and triene amounts for cold pressed oils are very important parameters for nutritional value, oil quality and shelf life [5,6,13]. In many previous studies, it has been found that *Ficus Carica* seed oil is an important source of linolenic acid, tocol and sterol, which have been proven to prevent many diseases such as obesity, cardiovascular and some cancer types [14-17].

The free fatty acid (FFA), peroxide value (PV), chlorophyll, conjugated diene (CD) and triene (CT) amounts are important oil components related to oxidative stability [13,18]. The amounts of these components are important criteria used for the quality and consumability of oil [5]. The amounts of these ingredients must be within the limit values set by the Turkish Food Codex and relevant codex [19]. The goal of this study was to investigate chemical properties such as FFA, PV, CD and CT, chlorophyll, β-carotene, fatty acid composition, triglyceride (TG), tocol (tocopherol and tocotrienol) compositions, sterol, wax, total polymeric compound amounts of cold pressed *Ficus carica* seed oil in Turkey. Although there are previous reports on the fatty acid, tocol and sterol compositions of cold pressed seed *Ficus carica* oil, this is the first study that reports the identification and quantification of triglyceride, wax and total polymeric compounds.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents for analysis were of analytical grade and purchased from Merck (Darmstadt, Germany) and BDH (Poole, UK). A fatty acid methyl ester (FAME) mix (37-component FAME blend), triglyceride standards kit (TRI19-1KT,) and tocopherol (α-T, β-T, γ-T, δ-T) and tocotrienols (a-T3, β-T3, γ-T3, δ-T3) standards were purchased from Supelco (Bellefonte, PA) and Sigma Aldrich. *Ficus carica* seed was obtained from İzmir, Turkey.

Oil Extraction by Cold Press Procedure

Dry seeds (1 kg) were extracted using cold pressing machine (Ecotoner 01, TOKULLAR Agro Products Ltd. Co., Antalya, Turkey). *Ficus carica* seed oil was obtained in cold press machine at 2 mm head diameter, 70 °C temperature, 20 rpm engine speed.

Determination of Free Fatty Acid

The % FFA content of the oil sample was determined as % oleic acid using the standard AOCS Ca-5a-40 method [20]. Briefly, 1 g of sample was solved 15 mL of neutral ethyl alcohol and titrated with 0.01 N NaOH solution along with phenolphthalein indicator until a pink color was formed. The %FFA amount was calculated by reading NaOH consumption. It was performed three times for each oil sample.

Determination of Peroxide Value (PV)

The PV was determined using the standard AOCS Cd-8b-90 method [20]. The peroxide value is defined as meqO_2/kg of oil and is a measure of the hydroperoxide content. Briefly, 1 g of sample was solved in 1 mL of chloroform and 1.5 mL of acetic acid and then 0.1 mL KI was added and left in the dark for 3 minutes. After, 25 mL of distilled water and 3 drops of 1 % starch solution were added and titrated with 0.002 N adjusted $Na_2S_2O_3$ until the color became clear. It was performed in triplicate for each oil sample.

Determination of Conjugated Diene and Triene

CD and CT amounts of *ficus carica* seed oil were determined according to The European Communities official method using a double-beam path UV-visible spectrophotometer [21]. 0.05 g oil sample, which dissolved in 10 mL isooctane was filled into the sample cuvette. Absorbance values were read at 232 and 270 nm wavelengths by a Shimadzu UV-1800 spectrophotometer (Shimadzu Europe GmbH, Germany) and were used to calculate the CD and CT amounts.

Determination of Chlorophyll and β-carotene Amounts

Chlorophyll and β-carotene amounts in the *ficus carica* seed oil were determined using a dual-beam Shimadzu UV-1800 spectrophotometer (Shimadzu Europe GmbH, Germany) [22]. The oil sample dissolved in isooctane was filled into the sample cuvette without any pre-treatment and spectrum scanning was performed.

$$\text{Chlorophyl(ppm)} = \frac{\left(b - \frac{(a+c)}{2}\right) \times 1000}{0}.1$$

$$\beta - \text{carotene (ppm)} = \frac{d \times 1000}{0}.261$$

a: Absorbance at the starting point of the chlorophyll peak at 650 nm
b: Absorbance at the peak of the chlorophyll peak at 650 nm
c: Absorbance at the endpoint of the chlorophyll peak at 650 nm
d: Absorbance at the peak of β-carotene peak at 450 nm

Fatty Acid Compositions Analysis by GC-FID

0.1 g of oil was derivatized with 1 mL of 2 N methanolic KOH solution for 10 min at room temperature. Later 7 mL of n-hexane was added to extract FAME and the mixture was shaken vigorously for 1 min. The upper phase of the solution, which was centrifuged at 2000 g for 10 min, was taken and dried with 1 g of Na_2SO_4 [23-26].

Fatty acid composition of the oil was determined by using an Agilent 7890 series gas chromatography-flame ionization detector (GC-FID) onto an HP-88 column (100 m, 0.25 mm, 0.25 μm, Agilent Technologies, USA). 1 μL FAME were injected at a split ratio of 100:1. The carrier gas was hydrogen at a flow rate of 1.3 mL/min. The oven temperature was programmed as follows: initial oven temperature of 50 °C was held for 10 min and increased to 250 °C at 4 °C/min and was held at 250 ° C for 10 min. Injector and detector temperatures were kept at 250 °C. The identification of FAMEs was based on retention times compared to those of the standard FAME mix. Fatty acid analysis were performed three times and the mean values were reported as a percentage.

Triglyceride Composition Analysis by HPLC

The triglyceride composition of the oils was determined by an Agilent 1200 series HPLC system consisted of a UV detector set at wavelengths of 215 nm. 1 g of oil was dissolved in 10 mL acetone and injected into the ACE 5 C18 column (250 x 4 mm, 5 μm particle size, Advanced Chromatography Technologies, Aberdeen, Scotland). An isocratic elution system with acetone-acetonitrile (1:1) mixture was used at a flow rate of 1.5 mL/min [20,26].

Tocols (Tocopherols and Tocotrienols) Analysis by HPLC

The tocols were analyzed by an Agilent 1200 series HPLC system consisted of FLD detector adjusting 295 nm for excitation and 320 nm for emission. 1 g of oil was

dissolved in 10 mL of hexane and injected into the LICHROSPHER (5 µm Si 100 250x4.0 mm) column. An isocratic elution system with n-hexan/isopropanol (96:4) mixture was used at a flow rate of 1.0 mL/min [6]. The tocols were measured by the linear calibration curve with respect to peak areas compared to external standards.

Sterol Composition Analysis by GC-FID

Sterol analysis was carried out by the International Olive Oil Council (COI) official method [27]. Briefly, 500 µL of α-cholestanol (100 mg/L) used as internal standard was placed in a 50 mL flask and the solvent was removed. After that, 5 g of oil sample and 50 mL of 1 N ethanolic KOH were added flask. The saponification was carried out by boiling at 90 °C for 1 h under reflux. The saponified part was extracted with 100 mL of water and 100 mL of diethyl ether and, the separated organic phase was washed 3 times with 50 mL of water. At the end of the washing process, the solvent of the organic phase separated from the aqueous phase was removed. The sample, whose solvent was removed, was re-dissolved in 1 mL of chloroform. 200 µL was taken from the dissolved part and solvent was removed. 250 µL anhydrous pyridine and 250 µL BSTFA silylation reagent were added on it. The mixture was subjected to silylation at room temperature for 5 min and at 60 °C for 20 min.

Sterols in the oil were determined using an Agilent 7890 series gas chromatography-flame ionization detector (GC-FID) onto HP-5MS (30 m x 0.25 mm x 0.25 µm, Agilent Technologies, USA) column. The silylated sterols were injected at a flow rate of 1 µL. The carrier gas was hydrogen at 1 mL/min. The oven temperature was programmed as follows: initial oven temperature of 100 °C was held for 0 min and increased to 300 °C at 5 °C / min and was held at 300 °C for 20 min. Injector and detector temperatures were kept at 250 °C and 325 °C, respectively.

Wax Composition Analysis by GC-FID

Wax analysis was carried out by the International Olive Oil Council (COI) official method [27]. Briefly, 15 g of silica gel, that was slurried in hexane, was filled into the glass column and was cleaned by passing 30 mL of hexane through the column. A mixture of 500 mg oil sample, 500 mL lauryl arachidate used as internal standard and 100 µL of 1% Sudan-I used as indicator was dissolved in 2 mL hexane. The prepared mix solution was transferred to the column and was eluted with the hexane/ethyl ether (99:1) mobile phase in ~ 10-15 drops in 10 s. The elution process was continued until the red colored Sudan-I indicator was seen at the end of the column. The eluted part was collected in a beaker. The sample, whose solvent was removed, was re-dissolved with 2 mL of n-heptane.

Waxes in the oil were determined using an Agilent 7890 series gas chromatography-flame ionization detector (GC-FID) onto RTX -5 (15 m x 0.18 mm x 0.20 µm) column. The waxes were injected at 1 µL. The carrier gas was helium at a flow rate of 2.4 mL/min. The oven temperature was programmed as follows: initial oven temperature 80 °C was held for 0 min, increased to 200 °C at 28 °C/minute for 1 min, was kept at 340 °C at 2.8 °C/min and was held at 340 °C for 25 min. The injector temperature was programmed as follows: on-column inlet temperature was raised to 80 °C for 0 min and increased to 320 °C at 40 °C/min. The detector temperature was kept at 350 °C. The flow rates of dry air and hydrogen gas were 300 and 30 mL/min, respectively.

Total Polymeric Compounds Analysis by HPSEC

The total polymeric compounds were analyzed by using an Agilent 1200 series HPLC system consisted of a refractive index (35 °C) detector. 1 g of oil sample was dissolved in 10 mL of tetrahydrofuran (THF) and injected into guard HPSEC column (PL-Gel 100 A°) (5 cm x 7.6 mm id, 5 μm) and HPSEC column (PL-Gel 100 A°) (30 cm x 7.6 mm id, 5 μm). An isocratic elution system with tetrahydrofuran was used at a flow rate of 1 mL/min. Results were reported as a percentage [28].

Data Analysis

All experiments were replicated at least three times. The statistical evaluation of the results was carried out using Microsoft Excel, 2007. Statistical significance was declared at $P< 0.05$.

Results and Discussion

Ficus carica seed was found to contain 26.44 % fixed oil. The obtained result was in agreement with the previous report by Hssaini *et al.*, [4].

FFA and PV are accepted as important quality indicators in the production, storage and consumption of vegetable oils. FFA and PV contents were % 0.76 ± 0.06 oleic acid and 1.06 ± 0.09 megO_2/kg oil, respectively. In the edible oils communiqué published by the Turkish Standards Institute, peroxide values should be maximum of 15 meqO_2/kg oil for extra virgin and cold pressed oils [19]. When the obtained FFA and PV contents were examined, it was seen that these values were appropriate in terms of the consumability of the oil.

CD and CT amounts of *ficus carica* seed oil were found to be 0.02 and 0.03, respectively. When the obtained results were evaluated, it has been observed that values were compatible with PV analysis and very low.

Chlorophyll and carotene contents are among the most important parameters affecting the shelf life of oils. The amount of chlorophyll and β-carotene in *ficus carica* seed oil was determined as a result of spectrum scanning between 200-800 nm and found to be 25 ppm and 4114.9 ppm, respectively. The results showed that the amount of β-carotene was high and the amount of chlorophyll was low in cold pressed *ficus carica* seed oil.

The fatty acid composition of the cold pressed *ficus carica* seed oil was shown in Table 1. A total of 13 different fatty acids were determined in cold pressed *ficus carica* seed oil. The obtained results showed that cold pressed *ficus carica* seed oil was a rich source of polyunsaturated fatty acids. The linoleic (33.74%) and linolenic acid (34.05%) were major fatty acids in *ficus carica* seed oil, which were similar to previous reported by Güven *et al.,* [29] and Hssaini *et al.,* [4]. Oleic acid (C18:1), a member of monounsaturated fatty acids, was found to be 19.61% in *ficus carica* seed oil, which was in agreement with previous reports [3, 4, 29]. It also contained saturated fatty acids such as palmitic acid (8.0 %) and stearic acid (3.72%).

The results of the triglyceride composition analysis for cold pressed *ficus carica* seed oil was given in Table 1. Triglycerides are the most concentrated energy source in the diet and serve as carriers for fat-soluble vitamins such as A, D, E and K [25]. The most abundant triglycerides in cold pressed *ficus carica* seed oil were LLnLn, LnLnLn, OLLn, LLLn and PLLn. The LLnLn (20.44 %) was found to be a major triglyceride in *ficus carica* seed oil. LnLnLn, LLLn, LLL,

OLLn and PLLn containing linolenic and linoleic acid were determined for 17.59, 13.57, 4.84, 16.63 and 9.02%, respectively, of the total triglycerides.

Although studies on *ficus carica* seed oil have been comprehensively searched in the literature, no studies have been found about triglyceride composition analysis. Therefore, this study is the first for the triglyceride composition of *ficus carica* seed oil. For this reason, our study gives more information about the composition of triglycerides in *ficus carica* seed oil compared to the reported many studies [3, 4, 29].

Table 1. **Fatty acid and triglyceride compositions of cold pressed *ficus carica* seed oil.**

	***Fatty Acid Composition* (%)**				***Triglyceride Composition* (%)**	
	Present Study	***Nakilcioğlu-Taş [3]***	***Hssaini et al. [4]***	***Güven et al. [29]***		***Present Study***
C16:0	8.00 ± 0.02	3.58-7.40	8.539-9.05	5.0-9.0	LnLnLn	17.59 ± 0.73
C16:1	0.05 ± 0.01	0.05-0.06	0.059-0.162	-	LLnLn	20.44 ± 0.70
C17:0	0.07 ± 0.00	0.06	0.036-0.053	-	LLLn	13.57 ± 0.39
C18:0	3.72 ± 0.01	2.97-3.73	2.594-3.302	2.0-4.0	LLL	4.84 ± 0.13
C18:1 cis	19.61 ± 0.01	16.82-17.79	13.438-15.642	14.0-24.0	OLLn	16.63 ± 0.50
C18:2 cis	33.74 ± 0.06	31.80-37.95	28.905-33.866	20.0-35.0	PLLn	9.02 ± 2.98
C20:0	0.44 ± 0.00	0.02-0.03	0.066-0.099	Max 0.7	OLL	6.62 ± 0.18
C18:3n3	34.05 ± 0.01	37.87-41.80	38.436-43.753	32.0-50.0	PLL	5.21 ± 2.48
C20:2	0.05 ± 0.01	-	-	-	OOL	2.93 ± 0.62
C22:0+C20:3n6	0.12 ± 0.00	-	-	Max 0.5	POL+StLL	3.26 ± 0.61
C23:0	0.03 ± 0.03	-	-	-	PPL	0.06 ± 0.10
C24:1	0.10 ± 0.01	-	-	-	OOO	1.28 ± 0.04
C22:6	0.01 ± 0.02	-	-	-	StOL	0.04 ± 0.03

Table 2. Tocol and sterol compositions of cold pressed *ficus carica* seed oil.

	Tocopherol and tocotrienol (ppm)				**Sterol (ppm)**	
	Present Study	***Güven et al. [29]***	***Baygeldi et al. [31]***		***Present Study***	***Güven et al. [29]***
α-T	23.87±0.35	157	460	Campesterol	51.47±1.16	194.71
β- T	ND	-	-	Stigmasterol	3660.42±3.01	141.97
γ- T	955.4±4.36	4267	3918.9	β- Sitosterol	2398.00±2.31	4326.05
δ- T	20.73±4.05	147	76.5	5-Avenesterol	834.80±0.83	1312.21
α-T3	2.79±0.16	--	-	Stigmastadien	54.54±1.23	83.35
β- T3	3.24±0.46	-	-	Total	7250.83±8.59	6516.20
γ- T3	ND	-	-			
δ- T3	ND	-	-			
Total T	1000±2.23	4571	4014.4			
Total T3	6.03±0.60	-	-			
Total Tocol	1006±0.60	4571	4014.4			

*ND: Not detected

Tocopherols and tocotrienols (Tocol) found naturally in vegetable oils are important antioxidants. The oxidative stability of oils is directly related to presence of these compounds [30]. Tocol values for cold pressed *ficus carica* seed oil sample were given in Table 2. Cold pressed *ficus carica* seed oil had γ- tocopherol with 955.4 ppm as

the basic component. The α-tocopherol (23.87 ppm) and δ-tocopherol (20.73 ppm) were prominent tocols in cold pressed *ficus carica* seed oil. Cold pressed *ficus carica* seed oil also contained small amounts of α-tocotrienol (2.79 ppm) and β-tocotrienol (3.24 ppm). Total tocol content in cold pressed ficus carica seed oil (1006 ppm) was found lower than that reported by Güven et al. [29] (4571 ppm) and by Baygeldi *et al.*, [31] (4041.4 ppm). β-tocopherol, γ-tocotrienol and δ- tocotrienol were not detected in cold pressed *ficus carica* seed oil.

Sterol composition values for cold pressed *ficus carica* seed oil were given in Table 2. Cold pressed *ficus carica* seed oil contained stigmasterol with 3660.42 ppm as the major sterol. It has been determined that the β-sitosterol content, which is the most found in vegetable oils, was 2398 ppm in oil. Cold pressed *ficus carica* seed oil also contained 5-avenesterol (834.80 ppm), stigmastadien (54.54 ppm) and campesterol (51.47 ppm). When the obtained sterol composition results were examined, the total sterol amount was 7250.83 ppm, which was similar to previously reported by Güven *et al.*, [29] (6516.20 ppm).

Waxes (generally C36-C50) formed by esterification of long-chain fatty acids and alcohols are known to reduce the freezing point of oils, create a crystalline structure, and reduce their light transmission and solubility [32]. Wax composition chromatogram and values for cold pressed *ficus carica* seed oil were given in Fig.1 and Table 3, respectively. Cold pressed *ficus carica* seed oil contained C_{40} with 76,56 ppm and C_{42} 213,83 ppm. C_{44} and C_{46} waxes were absent in cold pressed *ficus carica* seed oil. When the results of the wax composition obtained were examined, the total amount of wax in cold pressed *ficus carica* seed oil was 290.39 ppm. Although studies on *ficus carica* seed oil have been comprehensively searched in the literature, no studies have been found about wax composition analysis. Therefore, this study is the first for the wax composition of *ficus carica* seed oil.

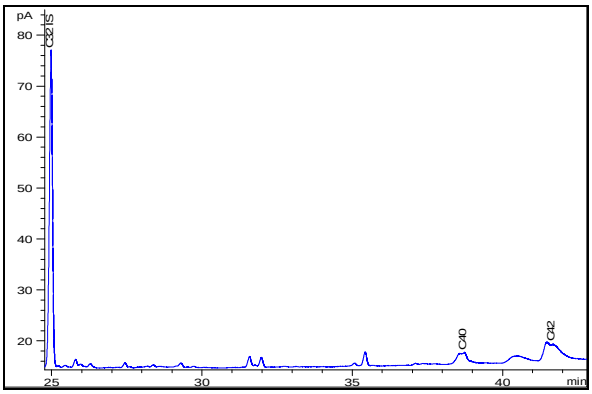

Figure 1. **Wax composition chromatogram of *ficus carica* seed oil**

Table 3. **Polymeric substance and wax compositions of cold pressed *ficus carica* seed oil.**

Wax Composition (ppm)		*Polymeric Substance (%)*	
C_{40}	76.56±12.54	Polymeric	0.01±0.01
C_{42}	213.83±34.51	Triacylglycerol	96.92±0.18
C_{44}	ND	Diacylglycerol	1.62±0.24
C_{46}	ND	Monoacylglycerol	0.52±0.03
Total	290.39±48.13	FFA	0.94±0.04

*ND: Not detected

In order to determine the total polymeric compound content of cold pressed *ficus carica* seed oil sample, analysis was carried out using HPSEC columns in the HPLC device. The polymeric and non-polymeric substances in the oil were allowed to exit the column based on size exclusion. The total polymeric compound chromatogram and amount for cold pressed *ficus carica* seed oil sample was given in Fig.2 and Table 3, respectively. The obtained results showed that cold pressed *ficus carica* seed oil had trace amount of polymeric compound with 0.01%, diacylglycerol with 1.62%, monoacylglycerol with 0.52% and FFA with 0.94%. Although studies on *ficus carica* seed oil have been comprehensively searched in the literature, no

studies have been found about polymeric compound analysis. Therefore, this study is the first for polymeric compound of *ficus carica* seed oil.

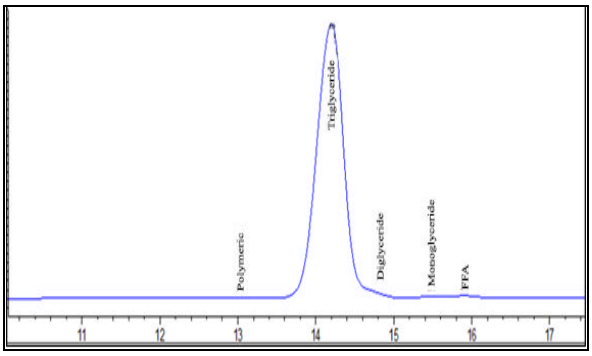

Figure 2. **Total Polymeric Substance Chromatogram for Ficus Carica Seed Oil**

Conclusion

Free fatty acid , peroxide value , conjugated diene and triene amounts, chlorophyll, β-carotene, fatty acid composition, triglyceride, tocol (tocopherol and tocotrienol) compositions, sterol, wax and total polymeric compounds are important parameters for determination of chemical properties of edible seed oils. The current results show that cold pressed *ficus carica* seed oil has a low content of free fatty acid, peroxide value, conjugated diene and triene, chlorophyll, wax and total polymeric compounds. In contrast, it has a high content of linolonic acid, β-carotene, tocol (tocopherol and tocotrienol) and sterol with high nutritional value and beneficial phytochemicals. It is also good source of LLnLn, LnLnLn, OLLn, LLLn and PLLn containing linolenic and linoleic acid which are polyunsaturated fatty acids. In conclusion, this study was showed that *ficus carica* seed oil could be used as a vegetable and medical oil with superior properties.

Conflict of Interest

The authors have no conflict of interest.

Acknowledgment

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