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Formulation and characterization of vegetable oil blends rich in omega –3 fatty acids

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Abstract

Seven blends of vegetable oils were formulated from flaxseed, olive and canola oils differing in their content of n–6 to n–3 and n–9 to n–6 to n–3 fatty acids (FAs) ratio. FA composition, oil classes, triacylglycerol fractions and identity characteristics of the original three oils and their seven blends were determined. Results showed marked differences in the FAs composition between the three oils used for preparing the seven oil blends. The prepared blends had n–3 to n–6 FAs ratio which varied from 1.2: 1 to 1: 9.1 with the highest ratio of n–3 to n–6 FAs in flaxseed – canola oil blends. Increasing the level of canola oil in canola - olive oil blends increased from the linolenic acid and lowered from linoleic acid. Meanwhile increasing the proportion of olive oil in the vegetable oil blends was associated with a decrease in iodine value, linolenic acid, n–3 to n–6 and saturated to unsaturated FAs ratio. The opposite trend was noticed with increasing flaxseed and/or canola oil in such blend. Slight differences were observed in the specific gravity, refractive index and saponification values. Among the formulated blends, triacylglycerols were the major fractions of three oils and their seven blends. Also, the triacylglycerols of flaxseed and canola oil blends were fractionated into 9 groups rich in unsaturated triacylglycerols.

Key words: Fatty acids, Lipid classes, Oil blends, Omega-3, Triacylglycerols

Introduction

Nearly 85% of the Egypt's needs for oils and fats are obtained from imports. More than 70% of such amount is palm oil followed by sunflower, animal fats, soybean, cottonseed and corn oils in addition to limited quantity of canola and olive oils (FAO, 2005). Except soybean oil, the other's major imported oils are rich either in saturated fatty acids (Palm oil) and/or n–6 fatty acids, and very poor in n–3 fatty acids. Also, the rise in the consumption rate of Western diets in Egypt in the last few years has caused a significant increase (nearly 3 fold) of fat intake. This is accompanied by a significant rise in death due to cardiovascular disease (CVD) and the blood cholesterol (Miladi, 1999). Usually, PUFA and olive oil have been considered as healthy lipids because they reduce the incidence of CVD (Barzi et al., 2003; Mozaffarian et al., 2007).

Nutritionists recommend 30% level or lower of calories as fat with the intake of saturated fat not to exceed 10% of total energy. The remaining can be divided equally between monounsaturated and polyunsaturated fats (Simopoulos, 1996). World Health Organization (WHO) recommends 4–10:1 ratio of n–6 to n–3 fatty acids (FAs) (Richter, 2001). Eating the proper ratio of n–6 to n–3 FAs from the right edible oil sources will help in enhancing thermogenesis, building muscles, and hormones like eicosanoids, reducing risk factors of CVD, high levels of blood pressure, triglycerides, lipoprotein-a, fibrinogen, clot formation and inflammation, muscle breakdown, improving brain functions, mood, intelligence, behavior and vision (Fan and Chapkin, 1998; Rodriguez–Leyva et al., 2010).

The main sources of n–3 FAs are fish and vegetable oils which are low in both saturated and n–6 FAs such as flaxseed oil (Consisting of 60% α -linolenic acid), canola oil, walnut oil and olive oil. Both olive and canola oils contain generous amount of monounsaturated FAs. Virgin olive oil is also rich in antioxidants and a substance called squalene that has anti-inflammatory properties, slows blood clot formation, lowers cholesterol and increases the amount of n–3 FAs taken up by body cells (Trichopoulou et al., 1995). Munoz et al. (2009)

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found that the adherence to a Mediterranean dietary pattern rich in fruits and vegetables, fish and olive oil, was associated with high scoring for self-perceived health.

The aim of this study was to formulate vegetable oil blends differing in their content of n-6 to n-3 and n-9 to n-6 to n-3 FAs ratio using pre-refined flaxseed, virgin olive and refined canola oils. The characteristics and structure of these blends compared to their original oil sources was also determined.

Materials and Methods

Materials

Vegetable oils

Thirty kilograms of each of the following three vegetable oils were used in this study, (i) an imported Netherland extra refined canola oil from a big supermarket at Alexandria city Egypt, (ii) cold press extra virgin olive oil from Wadifood company, Egypt, and (iii) fresh cold press crude flaxseed oil from a private commercial flaxseed press mill at Alexandria city, Egypt. The crude oil was pre-refined in the same day of its pressing in lab by degumming with 85% phosphoric acid, neutralizing with 15% sodium hydroxide and bleaching under vacuum at 90°C for 30 min. using Tonsil ACCFT activated bleaching earth as described by Lillard (1982). The cooled bleached oil was packed in brown glass bottles and stored at 4°C.

Vegetable oil blends

Seven vegetable oil blends were formulated as shown in Table 1 by mixing olive oil (O) with each of canola (C) and flaxseed (F) oils and also canola oil (C) with flaxseed oil (F).

Table 1. Vegetable oil blends.

Vegetable oil blends	Blending ratio (w:w)
OC-1	5.7 olive oil : 1.0 canola oil
OC-2	4.0 olive oil : 1.0 canola oil
OC-3	3.0 olive oil : 1.0 canola oil
OF	19.0 olive oil : 1.0 flaxseed oil
CF-1	19.0 canola oil : 1.0 flaxseed oil
CF-2	9.0 canola oil : 1.0 flaxseed oil
FC	1.0 canola oil : 1.3 flaxseed oil

The blending was carried out at room temperature ($22 \pm 2^\circ\text{C}$) in brown glass bottles under a stream of nitrogen gas. The bottles with oil blends were kept at 4°C until analysis.

Methods

Color of oils was assessed using Lovibond Tintometer, Model E, 100000 G, USA, using 0.5

inch cell as described by Mackinery and Little (1962). Specific gravity at 25°C (28.003 and 28.004), refractive index at 25°C (28.007), iodine number (28.019 and 28.020) were determined according to AOCS (1985) and saponification value was estimated as mentioned by Egan et al. (1987). Oils were fractionated into different classes as described by Mangold and Malins, (1960) using (20 × 20 cm) TLC plates coated with 0.25 mm thickness silica gel (Merk G, type 60), developing solvent system consisted of petroleum ether (40–60°C): diethyl ether: glacial acetic acid (70: 30: 2, v/v/v) and exposing to iodine vapors in a closed jar for visualization. Triacylglycerols were separated on chromatoplates coated with a slurry of silica gel (Merk G, type 60) in an aqueous silver nitrate 20% solution according to Gegiou and Georouli (1983) method using developing solvent consisting of toluene : diethyl ether (96: 4 v/v). Visualization was carried out by charring at 180°C for 10 min. Fatty acid methyl esters of oils were prepared as described by Radwan (1978) in a screw cap vial using 1% H₂SO₄ in methanol under a stream of nitrogen gas. The closed vials' were heated in an oven at 90°C for 90 min. Analysis of fatty acids was carried out by Shimadzu Gas Chromatography (GC-4 CM, PYE) using, SP-216 packing material, 130–190°C (2°C/min rise) as a column temperature, flame ionization detector at 250°C, 5 mm/min. sheet speed, 0.5 ml/min air flow rate, 1 ml/min. H₂ flow rate and 30 ml/min nitrogen flow rate (Radwan, 1978). The n-6: n-3 and n-3: n-6: N-9 fatty acid ratios of flaxseed, canola and olive oils and their blends were calculated according to the determined values of linolenic, linoleic and oleic acids.

Statistical analysis

The standard deviation was calculated using the method described by Steel and Torrie (1980).

Results and Discussion

Fatty acids composition, classes, triacylglycerols and identity characteristics in addition to the calculation of n-3 to n-6 and n-3: n-6: n-9 fatty acids of the original three oils and their seven blends were determined.

Fatty acids composition

Fatty acid composition is depicted in Table 2:

1- Marked difference in the fatty acids composition between the three oils used for preparing the seven oil blends. Flaxseed oil was the highest source of linolenic acid (n-3 FA) while olive oil was the richest in oleic acid (n-9 FA), palmitic acid and nearly free from linolenic acid

among the three oil sources. Linoleic acid (n-6 FA) was found in the highest value in canola oil followed by flaxseed and olive oils, respectively. Generally, the unsaturated FAs represented 90.63, 90.46 and 81.87% of the total fatty acids of flaxseed, canola and olive oils, respectively. Therefore, the ratio between saturated to unsaturated FAs was nearly similar in flaxseed and canola oil, (1:9.5) and (1:9.67), respectively, in spite of the clear variation in their FAs composition, and lower in olive oil (1:4.5).

2- The FAs composition of the prepared seven oil blends was differed according to the type and proportion of the used oil in their formulation. Generally, they had lower linolenic and stearic, higher linoleic, oleic and palmitic acids than flaxseed oil. Also, they contained less amount of oleic and palmitic acids, higher value of linolenic, linoleic and stearic acids than olive oil.

3- Among the prepared seven oil blends, FC-blend was the highest in linolenic acid (n-3 FA), the lowest in oleic acid (n-9 FA), and medium in linoleic acid (n-6 FA). The ratio of saturated to unsaturated FAs of this blend was similar (1:9.56) with those of flaxseed and canola oils in addition to both CF-1 and CF-2 oil blends in spite of the obvious variation in their FAs composition.

4- Slight differences in FAs composition were observed between CF-1 and CF-2 oil blends. Both contained medium amount of linolenic and oleic

acids and highest amount of linoleic acid among the prepared seven oil blends. Comparing with canola oil, they had more linolenic acid.

5- Except linolenic acid content, slight differences in FAs composition among OC-1; OC-2; OC-3 and OF oil blends were noticed. Comparing with olive oil, the four blends had higher levels of linolenic acid. The ratio of saturated to unsaturated FAs of these blends was ranged from 1:4.6 to 1:5.3.

The results of the fatty acids analysis of flaxseed, canola and olive oils in this study were in agreement with those stated in literatures. Linseed oil composed of 10.5% total saturated fatty acids, 20.5% oleic, 17.5% linoleic and 51% linolenic acids. Meanwhile, the oil of low-linolenic version of linseed consisted of 11% total saturated fatty acids, 16.2% oleic, 70% linoleic and 2.2% linolenic acids (Tomkrawczyk, 1999).

Canola oil is characterized by a relatively high concentration of oleic acid (55.8%), a low concentration of saturated fatty acids (13%) and a moderate level of PUFAs (20%) of which linolenic acid contributes between 25 and 30% (Mc-Donald et al., 1989; Lawson, 1995). Petukhov et al. (1999) found that the 18:1, 18:2 and 18:3 FA levels were 56.5, 22.3 and 10.8% in canola oil, 58.2, 27.9 and 3.74% in low linolenic canola oil, 75.2, 8.0 and 5.5% in high oleic canola oil, respectively.

Table 2. Fatty acids composition of flaxseed; canola and olive oils and their blends.

Type of oils**	Fatty acids* (%)								Saturated : Unsaturated fatty acids ratio
	Saturated				Unsaturated				
	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{16:1}	C _{18:1}	C _{18:2}	C _{18:3}	
F	0.21	0.50	4.06	4.60	1.07	28.18	20.37	41.01	1 : 9.67
C	0.23	0.39	5.54	3.38	0.92	54.76	24.92	9.86	1 : 9.50
O	0.39	0.39	14.21	3.14	0.28	63.73	17.23	0.63	1 : 4.50
FC	0.26	0.44	4.73	4.04	0.99	40.13	22.42	26.99	1 : 9.56
CF-1	0.23	0.40	5.39	3.50	0.94	52.10	24.77	12.97	1 : 9.50
CF-2	0.23	0.41	5.46	3.44	0.92	53.43	24.69	11.42	1 : 9.50
OC-1	0.36	0.39	12.47	3.19	0.41	61.95	18.76	2.47	1 : 5.09
OC-2	0.34	0.38	12.05	3.21	0.44	61.49	19.15	2.94	1 : 5.30
OF	0.38	0.40	13.70	3.21	0.32	61.95	17.39	2.65	1 : 4.60
OC-3	0.36	0.39	12.92	3.18	0.38	62.38	18.38	2.01	1 : 4.93

*Values are means of duplicates.

**F= flaxseed oil, C = canola oil, O = olive oil, FC = 1 canola oil: 1.3 flaxseed oil, CF-1 = 19 canola oil: flaxseed oil, CF-2 = 9 canola oil: 1 Flaxseed oil, OC - 1= 5.7:1 canola oil, OC-2 = 4 olive oil: 1 canola oil, OC - 3 = 3 olive oil: 1 canola oil, OF = 19 olive oil: 1 flaxseed oil

Table 3. Calculated ratio of n-6: n-3 and n-3: n-6: n-9 fatty acids of flaxseed, canola and olive oils and their blends.

Type of oils	n-6 : n-3 ratio	n-3 : n-6 : n-9
F	1.00: 2.01	2.01:1.0:1.38
C	2.50: 1.00	1.00:2.50 :5.55
O	27.3 : 1.00	1.00: 27.30 :101.16
FC	1.00: 1.20	1.20:1.00: 1.79
CF-1	1.88 : 1.00	1.00:1.88 :4.02
CF-2	2.16 : 1.00	1.00:2.16 :4.68
OC-1	7.60: 1.00	1.00:7.60:25.08
OC-2	6.50 :1.00	1.00:6.50:54.93
OF	6.56: 1.00	1.00:6.56 :23.38
OC-3	9.10:1.00	1.00:9.10:31.04

Olive oil is composed of oleic acid (55.0–83.0%), palmitic acid (7.5–20.0%), linoleic acid (3.521.0%), stearic acid (0.5–5.0%) and linolenic acid (0.0–1.5%) (Wessels and Walking, 1981; Cuellar, 1990). Also, Teresa-Sature et al. (1995); Sharara (1998) indicated that the major fatty acids in olive oil were oleic (65–79%), palmitic (9–15%), linoleic (5–14%) and stearic (2–4%).

The results of n-3 to n-6 and n-3 to n-6 to n-9 FAs ratios of the flaxseed, canola and olive oils and their blends were reported in Table 3. Generally, the n-3 to n-6 FAs ratio reveals the presence of the essential polyunsaturated fatty acids and their proportions in oil source. The height ratio of n-3 to n-6 fatty acids helps in establish and maintain the human health (Fan and Chapkin, 1998; Krauss et al., 2000). The highest value of n-3 to n-6 fatty acids ratio was found in flaxseed oil (2.01:1) rich in linolenic acid and the lowest one was in olive oil (1:27.3) rich in oleic and poor in linolenic acids. On the other hand, canola oil had 1:2.5 n-3 to n-6 fatty acids ratio. According to World Health Organization, the ratio of n-3 to n-6 fatty acid should be ranged from 1:4–10 (Kris-Etherton et al., 2000). Therefore, both flaxseed and canola oils are considered from the main sources of n-3 fatty acids among vegetable oils. But both oils, especially flaxseed oil, are subjected for high rate of autoxidation and thermal polymerization (Malcolmson et al., 2001). The prepared seven oil blends had n-3 to n-6 fatty acids ratio varied from 1.2:1 to 1:9.1. The highest ratio of n-3 to n-6 fatty acids was in FC oil blend (1.2:1) followed by CF-1, CF-2, OC-2, OF, OC-1 and OC-3, respectively. This ratio was much closed in both CF-1 (1:1.9) and CF-2 (1:2.1) and also in both OC-2 (1:6.5) and OF (1:6.56) oil blends. Generally, the prepared seven oil blends had n-3 to n-6 fatty acids ratio lies within the ratio recommended by World Health

Organization, Health and Welfare-Canada's Scientific Review Committee (1:4–10 n-3 to n-6 fatty acid ratio) (Kris-Etherton et al., 2000). Calculation of n-3: n-6: n-9 fatty acids ratio reveals the variation in the unsaturated fatty acids and their proportion in oil source. It is known that the rate of oil oxidation increases with the increase in the number of double bonds in the fatty acids of the oil. Therefore, it is expected that the increasing of monounsaturated fatty acids (n-9 fatty acids) in oil with keeping the proper ratio of n-3 to n-6 fatty acids will lower from its exposure to oxidation. As seen from Table 2 both olive and canola oils contain high content of monounsaturated fatty acid, oleic acid. Therefore, both oils carry a lower risk of peroxidation than flaxseed oil. According to Viola and Audisio (1987) virgin olive oil contains α -tocopherol, as a natural antioxidant, squalene and rich in monounsaturated fatty acid. Squalene increases the amount of n-3 fatty acid taken up by the cells of body. The highest ratio of n-3: n-6: n-9 was in flaxseed oil followed by FC and CF-1 blends, canola oil, CF-2, OC-2, OF, OC-1, OC-3 oil blends and olive oil, respectively (Table 3). This sequence may reveal the degree of the peroxidation stability of these oil sources during preparation, storage and utilization.

Identity characteristics

Table 4 shows some identity characteristics of flaxseed, canola and olive oils in addition to their oil blends. Generally, no much difference in specific gravity, refractive index, and saponification value were observed between flaxseed, canola and olive oils and their blends. Specific gravity, refractive index and saponification value ranged from 0.911–0.915, 1.467–1.481 and 186.25–192.87 among the three oils and their blends respectively. Meanwhile, marked variation was noticed in iodine value between the three oils and their oil blends. Iodine value increased

with the increase of unsaturation degree or the numbers of double bonds in oils and fats. Therefore, the highest value of iodine value was in flaxseed oil rich in linolenic acid and the lowest one was in olive oil rich in oleic acid. The other oils can be arranged into three classes according to their iodine value. The class one includes FC oil blend with 153.7 iodine value and 1:9.56 saturated to unsaturated fatty acid ratio. The second class contains canola oil, CF-1 and CF-2 oil blends with 119.52 to 125.94 iodine value and 1:9.5 saturated to unsaturated fatty acid ratio. The third class includes the other vegetable oil blends, OC-1, OC-2, OF and OC-3, with 89.81 to 92.89 iodine value and 1:4.6–5.3 saturated to unsaturated fatty acids ratio. The variations in iodine value among these oil sources may be affected their peroxidation stability.

According to Schoene et al. (1998) flaxseed oil had 0.931–0.936 specific gravity at 15.5°C, 1.477–1.482 refractive index at 25°C, 189–195

saponification numbers and 165–204 iodine value (Wijs).

Canadian standards stated that canola oil should has a relative density at 20°C not less than 0.914 and not more than 0.920, a refractive index at 40°C not less than 1.465 and not more than 1.467, a saponification value not less than 182 and not more than 193, an iodine value (Wijs) not less than 110 and not more than 126 and an unsaponifiable matter content not more than 20 grams per kilogram (Genser and Eskin, 1982).

The data in literatures showed that virgin and refined olive oils had 1.4689–1.4700 and 1.4700–1.4702 refractive index at 20°C (Sonntag, 1979), 0.9090–0.9131 and 0.910–0.916 specific gravity at 20°C (Firestone et al., 1985), 184–196 and 184–196 saponification value, mg KOH/g (Cuellar, 1990) and 75–94 and 75–94 (Wijs) iodine value, respectively (Rahmani and Saari–Csallany, 1991).

Table 4. Some identity characteristics of flaxseed, canola, olive oils and their blends*.

Type of oils	Specific gravity at 25°C	Refractive index at 25°C	Iodine value (Wijs)	Saponification value (mg KOH/ g oil)
F	0.913±0.001	1.481±0.001	182.58±1.12	192.87±0.97
C	0.914±0.001	1.467±0.000	119.52±0.98	189.21±0.81
O	0.911±0.001	1.462±0.001	84.63±0.87	191.09±0.92
FC	0.913±0.002	1.474±0.001	153.71±0.96	191.22±0.84
CF-1	0.914±0.001	1.468±0.001	125.94±0.85	189.58±0.90
CF-2	0.915±0.001	1.468±0.000	123.06±0.80	188.99±1.10
OC-1	0.912±0.002	1.464±0.001	91.45±0.76	190.86±0.86
OC-2	0.912±0.002	1.464±0.001	92.89±0.84	190.79±0.70
OF	0.911±0.001	1.463±0.000	89.85±0.79	190.81±0.82
OC-3	0.911±0.001	1.463±0.001	89.81±0.83	186.25±0.93

*Values are means of triplicates ± standard deviation

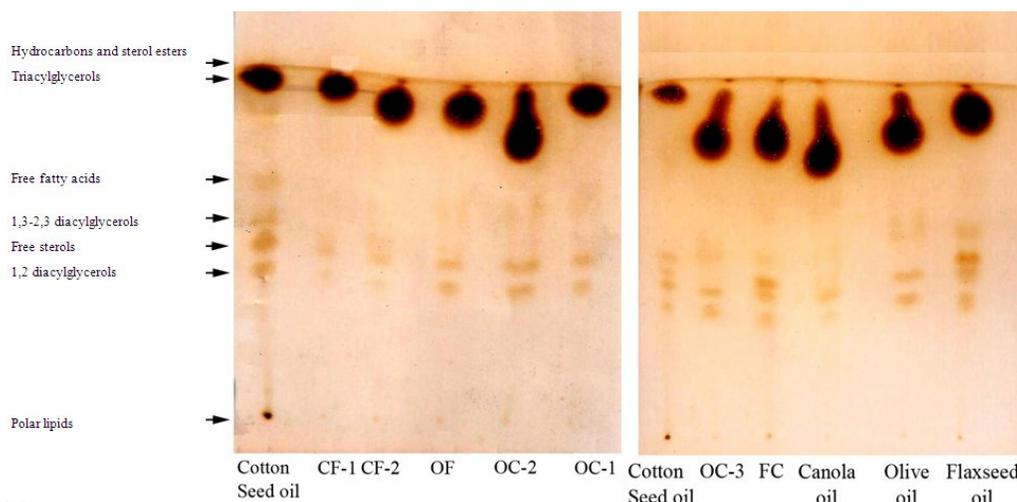


Figure 1. TLC separation of classes of flaxseed, canola, olive oils and their blends.

Lipid classes

Figure 1 illustrates the fractionation of the classes of three oil sources and their blends. Because refined flaxseed and canola oils in addition to the fresh virgin olive oil were used in this study, the main class of these oils and their blends was the triacylglycerols. The other classes which detected in small quantity were polar lipids, 1, 2–diacylglycerols, free sterols, hydrocarbons, esters and traces from 2, 3–diacylglycerols and free fatty acids. The 2, 3–diacylglycerols were clearly appeared in FC, olive oil and flaxseed oil. The free fatty acids band was more clearly appeared in flaxseed oil.

According to Gamel (1995) most of the fatty acids of olive oil are present as triacylglycerols and the non–glycerides fraction of olive oil contain non–glyceride fatty acids esters, hydrocarbons, sterols, phospholipids, chlorophylls and flavor compounds

Triacylglycerols

Figure 2 shows the triacylglycerols fractions of the three oil sources and their blends. Triacylglycerols of flaxseed oil was fractionated into 7 groups with 1, 4, 5, 6, 7, 8 and 9 double bonds. These groups can arranged according to their colour intensity which reveal their concentration in this oil as following, triacylglycerols with 8 double bonds then with 7, 4, 5, 6, 1 and 9 double bonds, respectively. Hui (1996) isolated the following triacylglycerols of linseed oil using counter–current distribution technique, trilinolenin (18.2%), linoleodilinolenin (12.3%), oleodilinolenin (19.5%) and dilinoleolinolenin (4.17%). Triacylglycerols of canola oil were also fractionated into 7 groups with 1, 2, 3, 4, 5, 6 and 7 double bonds. The sequence of these groups according to their colour intensity in TLC plate was

the group with 3 double bonds followed by those with 2, 1, 4, 5, 6, and 7 double bonds, respectively. According to Prevot et al. (1990) canola oil had the following triacylglycerols LLnL, LLLn, OLnLn, LLL, OLLn, PLLn, OLL, OOLn, PLL, POLn, OOL, POL, OLGn, OOO, StOL, POO, PPO and StOO. Olive oil was fractionated into 7 triacylglycerol groups with 1, 2, 3, 4, 5, 6 and 7 double bonds. These groups can be arranged according to their color intensity in the following decreasing order, triacylglycerols with 3 double bonds followed by those with 2, 4, 5, 1, 6, and 7 double bonds, respectively. Sharara (1998) fractionated triacylglycerols into 8 groups. These groups included, 5.1% LLO, 0.2% LLP, 10.7% LOO, 5.2% LOP, 48.2% OOO, 22.8% OOP, 2.8% POO and 3.9% OOS. The oil blends which formulated from both flaxseed and canola oils, OF, CF–1 and CF–2, were fractionated into 9 triacylglycerols with 1, 2, 3, 4, 5, 6, 7, 8 and 9 double bonds. The color intensity of the fractionated groups was more obvious in triacylglycerol with 3 double bonds followed by those with 4, 5, 6, 7, 8, 1, 2 and 9 double bonds, respectively.

In case of oil blends which made of olive and flaxseed oils, OF blend, the triacylglycerol were separated into 9 groups with 1, 2, 3, 4, 5, 6, 7, 8 and 9 double bonds. Among these groups, the color intensity of triacylglycerol with 3 double bonds was deeper than other groups. It was followed by those with 4, 2, 5, 1, 6, 7, 8 and 9 double bonds, respectively. The triacylglycerol of canola–olive oil blends were fractionated into 7 groups with 1, 2, 3, 4, 5, 6 and 7 double bonds. The main group was that of 3 double bonds followed by those with 4, 2, 5, 1, 6 and 7 double bonds,

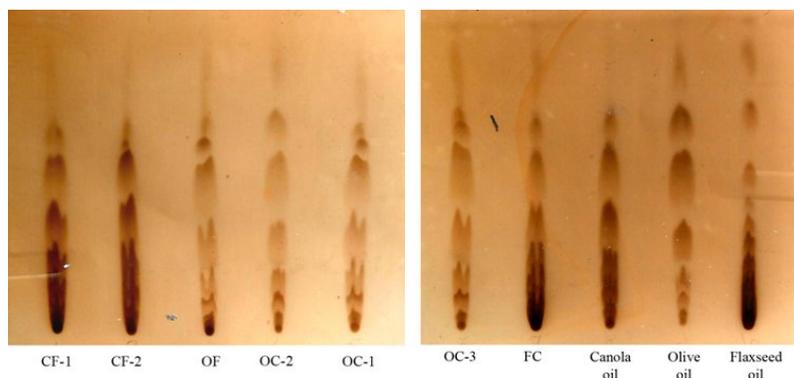


Figure 2. Ag⁺–TLC separation of triacylglycerols of flaxseed, canola and olive oils and their blends.

Conclusion

The identity characteristics, fatty acids composition and triacylglycerol fractions of the vegetable oil blends were based on type and level of their oils raw materials. The n-6 to n-3 FAs ration in the 7 prepared oil blends conforms to that recommended by WHO, 4-10:1. Therefore these blends are important for both nutrition specialists and oil manufacturers. Nutritionists will be interested to be sure from the availability and presence of n-3 FAs in proper concentration and their beneficial effects on human health. Moreover, the development of such blends and their successful utilization will be of great interest to food oil companies.

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