

# Formulation and Stability of Healthy Vegetable Oil Blends Rich in Omega-3 Fatty Acids

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## ABSTRACT

Refined canola oil, virgin olive oil and pre-refined flaxseed oil were used to prepare 7 oil blends containing n-3 to n-6 fatty acids ratio ranged from 1: 0.83 to 9.1. A mixture of 6 ppm  $\beta$ -carotene and 100 ppm BHA (butylated hydroxy anisol) was added to the blends and packed in an opaque glass bottles in addition to control (antioxidant free). The storage stability of the oil blends was determined through 6 months of storage at room temperature ( $22 \pm 2^\circ\text{C}$ ). The results showed that oil blends containing canola oil had more red colour than other oil blends. Extending of storage period to six months caused gradual increases in colour darkening, values of FFA, PV, TBA, P-anisidine and UV-specific absorbance at 232 and 270 nm. The rate of changes in these parameters were more pronounced in blends containing flaxseed oil rich in linolenic acid and were relatively less in blends having olive oil high in oleic acid. Addition of antioxidant mixture lowered the oxidation rate of oil blends. According to PV values, the shelf life of the formulated blends free of or containing antioxidant were ranged from 3 to 6 and 5 to 6 months, respectively.

**Keywords:** stability, flaxseed oil, olive oil, canola oil, oil blends,  $\omega$ -3.

## INTRODUCTION

Since studies of Greenland Eskimos which showed low incidence of cardiovascular diseases (CHD), serum cholesterol and triacylglycerol (Dyreberg *et al.*, 1975), interest in the increase consumption of omega-3 (n-3) family of polyunsaturated fatty acids (PUFA) has been grown. The n-3 fatty acids (FAs) help to maintain total cholesterol and triacylglycerols in the normal levels in the blood (Stretenovic *et al.*, 2009), develop brain and nerve tissues in children and improve mood pattern and prevent aggression and hostility of adults (Hilbeln *et al.*, 2006).

The vegetable sources of n-3 FAs are oils of peilla, canola, soybean, flaxseed and walnut. All of which contain relatively high amount of alpha-linolenic acid (ALA). Fish are the major source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the metabolite products of n-3 alpha linolenic acid. (Stretenovic *et al.*, 2009).

The intake of n-3 FAs can be increased by encouragement the consumption of food rich in n-3 FAs, changing the FAs composition of the commonly

eaten foods by biotechnology and/or utilizing microcapsules of fish oil (Huber *et al.*, 2009).

Recently the increase concern about industrial pollution and human wastes contaminating fish led to lower from the consumption of fish oil as n-3 FAs source (Gomez-Candela *et al.*, 2011).

In this study a new approach was suggested to increase the intake of n-3 FAs. This approach was depended on formulating vegetable oil blends rich in n-3 FAs to use in food preparation. The considered factors taken during formulating such blends were their safety, stability and bioavailability. The selected oils of such blends were refined canola oil, virgin olive oil, and pre-refined flaxseed oil having ~ 41% ALA. These oils are safe for human consumption, effectively absorbed and utilized by body. The main problem of these sources especially flaxseed oil is their stabilities against oxidation. This can avoid by taking a great care during blending, storage and utilization of such source. Addition of antioxidants such as butylated hydroxy anisol (BHA) butylated hydroxy toluene (BHT) and  $\beta$ -carotene either in single or in combination to such blends will help also to improve their storage stabilities. Therefore, the storage stability of the suggested formulating oil blends backed in opaque glass bottles were determined through six months of storage at room temperature. The effect of adding mixture of  $\beta$ -carotene and BHA on retarding the oil oxidation was also investigated along with control blends.

## MATERIALS AND METHODS

### Materials:

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 flaxseed oil was pre-refined in the same day of it's pressing by degumming with 85% phosphoric acid, neutralizing with 15% sodium hydroxide and bleaching under vacuum at  $90^\circ\text{C}$  for 30 min. using Tonsil ACCFT activated bleaching earth as described by Lillard (1982). The cooled bleached oil was packed in opaque, glass

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bottles and stored at 4°C.  $\beta$ -carotene (E. Merk, Germany) and food grade butylated hydroxy anisole (BHA) under trade name TENOX were brought from Eastman Chemical products, INC, USA.

#### Vegetable Oil Blends:

Seven vegetable oil blends were formulated (W/W) by mixing olive oil (O) with each of canola (C) and flaxseed (F) oils and also canola oil (C) with flaxseed oil (F) as follows: (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) 1.0 flaxseed oil, (CF-1) 19.0 canola oil :1.0 flaxseed oil, (CF-2) 9.0 canola oil :1.0 flaxseed oil and (CF-3) 1.0 canola oil : 1.3 flaxseed oil. The blending was carried out at room temperature ( $22 \pm 2^\circ\text{C}$ ) then packed in opaque glass bottles (100 g) under stream of nitrogen gas, with and without adding a mixture of 6 ppm  $\beta$ -carotene and 100 ppm BHA. The bottles were kept at room temperature ( $22 \pm 2^\circ\text{C}$ ) for 6 months.

#### Methods:

Fatty acid composition (linoleic and linolenic acid) of oil blends was determined as described by Radwan (1978) using Shimadzu gas liquid chromatograph (GC4-CMPFE). Colour of oil blends was assessed using Lovibond Tintometer, Model. E, 100000 G, USA, using 0.5 inch cell as described by Mackinery and little, 1962). Free fatty acids as % oleic acid (AOCS, 1985), peroxide value (PV) as meq  $\text{O}_2/\text{kg}$  oil (AOCS, 1985), thiobarbituric acid (TBA) value (Pokorny *et al.*, 1985), Para-anisidine value (P-AV), absorbance at 350 nm/gram oil (Egan *et al.*, 1987), specific absorbance of oil at 230 and 270 nm. (Kiritsakis, 1991) were determined monthly during the storage period of oil blends.

**Statistical analysis:** The standard deviation (SP) was calculated using the method described by Steel and Torrie (1980).

### RESULTS AND DISCUSSION

#### 1-Formulation:

The results of linoleic and linolenic acid determinations and calculations of n-3: n-6 fatty acid

ratio was reported in front of each blend in Table (1). Generally the prepared seven oil blends had n-3 to n-6 fatty acid ratio lies within the ratio recommended by World Health Organization and Health and Welfare-Canada's Scientific Review Committee, 1: 4-10 n-3 to n-6, (Kris-Etherton *et al.*, 2000 ).

#### 2-Storage stability:

**Colour:** Table (2) showed the changes in red colour values of the oil blends during storage as determined by Lovibond. As shown from the results, oil blends containing canola oil had higher red colour values than other oil blends. The intensity of the colour was depended mainly on the proportion of canola oil than other the type of oils sharing in blends. Generally, blends consisting of canola and flaxseed oils had higher red colour values than those prepared either from canola and olive oils and/or olive and flaxseed oils. Addition of the antioxidant did not cause marked changes in the red colour of oil blends. In contrast, extending storage period to six months at room temperature increased the intensity of the red colour, causing darkening of oil blends. This change may be due to the oxidation of the natural pigments in these oils. However, the rate of such changes was more pronounced in CF-3 followed by CF-1, CF-2, OC-2, OC-1, OC-3, and OF oil blends, respectively. Antioxidant addition did not reduce colour changes. The presence of  $\beta$ -carotene in the added antioxidant mixture may lead to a slight increase in the redness of the oil blends colour. According to Sharara (1998), storage for six months decreased both chlorophyll and  $\beta$ -carotene of olive oil. The lowest reduction was in oil stored in tin can followed by that kept in glass, opaque and transparent polyethylene bottles, respectively.

**Free fatty acids (FFAs):-** A gradual rise in free fatty acids (FFAs) percentage of the oil blends was occurred during storage, Table (3). The rate increment was depended upon the initial free fatty acid content, proportion and type of oils used for preparing the blend formulation.

**Table 1. Content of linolenic, linoleic acids and the calculated n-3 : n-6 fatty acid ratio of Formulated vegetable oil blends**

Oil blend	Linolenic acid (%)	Linoleic acid (%)	n-3 : n-6 Fatty acid ratio
CF-3	26.99	22.42	1.0 : 0.83
CF-1	12.97	24.77	1.0 : 1.88
CF-2	11.42	24.69	1.0 : 2.16
OC-1	2.47	18.76	1.0 : 7.60
OC-2	2.94	19.15	1.0 : 6.50
OF	2.65	17.39	1.0 : 6.56
OC-3	2.01	18.58	1.0 : 9.10

**Table 2. Changes in red colour value of oil blends during storage at room temperature (22±2°C)**

Oil blend	Storage period (months)					
	Zero time		3		6	
	A	B	A	B	A	B
CF-3	1.85	1.90	2.29	2.33	2.69	2.71
CF-1	2.22	2.23	2.31	2.32	2.60	2.62
CF-2	2.26	2.28	2.30	2.31	2.60	2.61
OC-1	1.10	1.12	1.30	1.31	1.80	1.81
OC-2	1.17	1.18	1.32	1.33	1.81	1.82
OF	0.83	0.83	1.07	1.07	1.61	1.62
OC-3	0.92	0.93	1.09	1.10	1.71	1.72

A - without antioxidants.

B - with antioxidants.

Oil blends consisting of flaxseed and canola oils had higher FFAs than those formulated from flaxseed and olive oils and/or olive and canola oils. Generally, addition of antioxidant reduced the rate of the FFA formation during storage. The high rate of FFAs formation was noticed of blends containing flaxseed oil rich in linolenic, (CF-3, CF-1, CF-2), then canola, (OC-1, OC-2, OC-3) and olive oils rich in oleic acid, (OF). However such increment in all oil blends did not reach to the FFA value at which oil should be rejected, 2% as oleic acid. This confirms the good stability of such oil blends against hydrolysis especially in the presence of antioxidants.

Malcolmson *et al.* (2001) reported that flaxseed oil rich in linolenic acid is easily damaged when exposed to light, heat and air. Therefore, this oil should never be cooked at high temperature. It is best to eat this oil in raw state and storage in unopened dark containers under 20°C to extend its shelf life. Alpha-linolenic acid in a free form or as a component in oil is susceptible to autoxidation and polymerization when exposing to air or high temperature (Chen *et al.*, 1994). Sharara (1998) found that the value of FFAs was higher in olive oil stored for 3 months at 40°C than at room temperature (22±2°C) and in oil packed in transparent polyethylene bottles than in opaque one.

**Peroxide value (PV):-** The results in Table (4) indicated that:

- 1- PV increased gradually with extending the storage period of the oil blends. The formation of PV was due to the oil oxidation by the air trapped during filling and left in the head space after of the closing containers. Addition of antioxidant reduced the formation rate of PV in oil blends during storage.

Generally the rate of PV formation depends on type, content and the saturation degree of the fatty acids of the oil blends in addition to other factors which promote or accelerate the oxidation process such as oxygen availability, prooxidants, light ...etc. (Huber *et al.*, 2009).

- 2- Blends containing both flaxseed and canola oil (CF-3, CF-1, and CF-2) and OF had higher PV values compared with other blends. Such increase in PV reduced the shelf life of such oil blends. PV value reached more than 10 meq O<sub>2</sub>/kg oil in CF-3, CF-1, CF-2 and OF oil blends free from and that containing antioxidant after 3 and 5; 3 and 5; 4 and 6; 6 and 6 months of storage in glass bottles at room temperature, respectively. On the other side, the stability of other oil blends, OC-1, OC-2 and OC-3, was continued to the end of storage period. The Canadian Standards stated that canola oil considers acceptable as edible oil when its peroxide value was not more than 10 meq peroxide oxygen per kilogram of oil (Genser and Eskin, 1982).

**Thiobarbituric acid value (TBA):-** This test measures malonaldehyde. Such aldehyde gives the obnoxious odour and flavour characteristics of oxidative rancidity in oil. According to the data in Table (5), TBA value differed according to source of used oil and its proportion in oil blends, storage period and the interaction between these factors. Generally, a gradual rise in TBA values was observed with extending the storage period of oils. The highest TBA values were recorded in the blends containing flaxseed oil with canola one, CF-3, CF-1, CF-2, then that with olive oil, OF. As in the case of PV, the lowest values of TBA were in blends, OC-1, OC-2 and OC-3. This may be due

**Table 3. Changes in free fatty acids (as % oleic acid<sup>3</sup>) of oil blends during storage at room temperature (22±2°C)**

Oil blend	Zero time	Storage period (Months)											
		1		2		3		4		5		6	
		A	B	A	B	A	B	A	B	A	B	A	B
CF-3	0.36±0.00	0.50±0.01	0.41±0.03	0.65±0.07	0.49±0.01	0.71±0.00	0.51±0.01	0.76±0.01	0.60±0.06	1.07±0.05	0.73±0.01	1.10±0.01	0.80±0.01
CF-1	0.35±0.02	0.49±0.01	0.38±0.01	0.58±0.07	0.49±0.01	0.70±0.00	0.50±0.00	0.75±0.00	0.58±0.10	0.76±0.01	0.72±0.02	1.01±0.01	0.77±0.01
CF-2	0.34±0.04	0.48±0.02	0.36±0.01	0.50±0.01	0.49±0.01	0.67±0.01	0.50±0.01	0.73±0.00	0.53±0.02	0.75±0.00	0.66±0.01	0.96±0.02	0.75±0.00
OC-1	0.23±0.02	0.24±0.05	0.24±0.00	0.46±0.01	0.35±0.02	0.51±0.05	0.49±0.02	0.57±0.07	0.50±0.03	0.74±0.02	0.65±0.09	0.78±0.03	0.74±0.03
OC-2	0.24±0.00	0.35±0.03	0.24±0.02	0.47±0.03	0.39±0.04	0.53±0.01	0.50±0.01	0.58±0.07	0.51±0.01	0.76±0.04	0.66±0.01	0.77±0.02	0.74±0.01
OF	0.23±0.02	0.36±0.01	0.32±0.01	0.47±0.04	0.43±0.03	0.49±0.01	0.48±0.02	0.50±0.00	0.49±0.03	0.63±0.01	0.50±0.00	0.76±0.01	0.71±0.04
OC-3	0.30±0.07	0.37±0.01	0.34±0.04	0.48±0.02	0.45±0.05	0.49±0.01	0.49±0.00	0.54±0.02	0.49±0.03	0.70±0.04	0.55±0.05	0.77±0.01	0.74±0.01

\* Mean ± SD

A - Without antioxidants

B - with antioxidants.

**Table 4. Changes in peroxide value (as meq O<sub>2</sub> / Kg oil<sup>3</sup>) of oil blends during storage at room temperature (22±2°C)**

Oil blend	Zero time	Storage period (months)											
		1		2		3		4		5		6	
		A	B	A	B	A	B	A	B	A	B	A	B
CF-3	2.89±0.05	6.63±0.16	6.28±0.52	7.36±0.92	6.67±0.07	10.79±0.05	6.92±0.05	12.10±1.03	7.81±0.04	12.71±0.01	8.58±0.03	13.04±0.26	12.89±0.25
CF-1	2.25±0.03	6.45±0.02	4.02±0.06	6.54±0.03	5.59±0.02	10.08±0.14	6.86±0.17	11.25±0.02	7.20±0.08	12.18±0.02	7.95±0.86	12.59±0.05	10.86±0.04
CF-2	2.17±0.07	4.79±0.04	3.99±0.05	5.19±0.01	5.03±0.07	7.46±0.03	6.44±0.03	10.46±0.05	7.06±0.11	10.49±0.06	7.52±0.02	11.42±0.19	10.30±0.02
OC-1	2.07±0.12	3.81±0.02	2.75±0.02	4.29±0.14	3.90±0.09	5.76±0.04	5.32±0.04	6.08±0.10	5.85±0.03	7.02±0.09	6.93±0.02	9.11±0.14	7.71±0.07
OC-2	2.07±0.24	3.87±0.14	2.91±0.04	4.52±0.04	4.42±0.05	6.15±0.04	5.57±0.24	6.27±0.34	6.00±0.07	7.55±0.26	6.96±0.02	9.82±0.03	8.42±0.02
OF	2.12±0.04	4.41±0.12	3.67±0.03	5.13±0.05	4.86±0.02	6.41±0.52	6.28±0.05	6.82±0.18	6.17±0.03	8.90±0.04	7.40±0.10	10.82±0.03	10.03±0.45
OC-3	2.06±0.11	2.90±0.02	2.50±0.06	4.05±0.09	3.86±0.40	5.48±0.13	4.89±0.03	5.90±0.74	5.29±0.12	6.33±0.11	5.98±0.17	8.49±0.18	7.49±0.06

\* Mean ± SD

A - without antioxidants.

B - with antioxidants.

**Table 5. Changes in TBA value (as absorbance/g oil<sup>3</sup>) of oil blends during storage at room temperature. (22±2°C)**

Oil blend	Storage period (months)											
	Zero time		1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B	A	B
CF-3	0.044±0.00	0.017±0.00	0.057±0.00	0.198±0.00	0.197±0.00	0.504±0.01	0.333±0.01	0.662±0.00	0.475±0.01	0.759±0.01	0.669±0.05	0.978±0.01
CF-1	0.032±0.00	0.077±0.00	0.055±0.00	0.095±0.00	0.066±0.01	0.453±0.00	0.188±0.00	0.486±0.00	0.441±0.02	0.589±0.01	0.525±0.38	0.888±0.01
CF-2	0.031±0.00	0.067±0.00	0.050±0.00	0.084±0.00	0.065±0.00	0.253±0.00	0.166±0.01	0.293±0.01	0.284±0.00	0.486±0.01	0.487±0.01	0.753±0.01
OC-1	0.016±0.00	0.042±0.00	0.028±0.00	0.055±0.00	0.039±0.00	0.078±0.00	0.041±0.00	0.135±0.01	0.071±0.00	0.246±0.01	0.228±0.01	0.465±0.00
OC-2	0.016±0.00	0.043±0.00	0.036±0.00	0.060±0.00	0.041±0.00	0.108±0.00	0.050±0.00	0.157±0.01	0.072±0.01	0.269±0.29	0.254±0.01	0.459±0.01
OF	0.012±0.00	0.065±0.00	0.047±0.00	0.081±0.00	0.060±0.00	0.185±0.01	0.104±0.00	0.248±0.01	0.233±0.01	0.465±0.00	0.382±0.00	0.720±0.01
OC-3	0.014±0.01	0.035±0.00	0.026±0.00	0.053±0.00	0.030±0.01	0.049±0.01	0.032±0.00	0.087±0.01	0.051±0.00	0.220±0.01	0.122±0.00	0.347±0.00

\* Mean ± SD

A - without antioxidants.

B - with antioxidants.

**Table 6. Changes in P-anisidine value (as absorbance/g oil<sup>3</sup>) of oil blends during storage at room temperature (22±2°C)**

Oil blend	Storage period (months)											
	Zero time		1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B	A	B
CF-3	2.65±0.01	4.96±0.01	4.91±0.02	8.36±0.02	5.03±0.06	10.37±0.04	5.43±0.02	10.95±0.01	7.99±0.09	15.52±0.07	11.75±0.02	17.66±0.02
CF-1	1.53±0.02	4.38±0.03	4.32±0.09	5.04±0.06	4.72±0.10	8.98±0.05	4.38±0.02	10.54±0.05	5.07±0.10	14.93±0.07	9.38±0.81	17.56±0.02
CF-2	1.30±0.02	4.03±0.05	2.41±0.02	4.17±0.02	3.00±0.06	5.25±0.02	4.17±0.05	5.46±0.03	4.20±0.08	8.33±0.06	6.04±0.07	13.00±0.02
OC-1	0.826±0.01	1.54±0.01	0.982±0.01	2.14±0.01	1.03±0.05	2.35±0.05	1.51±0.08	2.97±0.24	1.82±0.01	4.50±0.04	2.93±0.02	5.12±0.01
OC-2	0.756±0.03	1.88±0.00	1.26±0.02	2.16±0.18	1.10±0.07	2.89±0.19	1.76±0.05	3.21±0.05	2.24±0.01	4.51±0.09	2.93±0.04	5.49±0.48
OF	0.923±0.01	2.67±0.05	1.70±0.04	3.57±0.08	2.13±0.00	4.35±0.07	3.57±0.08	4.47±0.07	3.75±0.04	6.73±0.08	5.51±0.06	6.99±0.01
OC-3	0.799±0.02	1.48±0.01	0.850±0.01	1.26±0.01	0.957±0.01	1.48±0.13	1.26±0.08	2.24±0.05	1.82±0.11	3.86±0.06	2.42±0.06	4.21±0.12

\*Mean ± SD

A - without antioxidants.

B - with antioxidants.

**Table 7. Changes in the specific absorbance values\* ( $K_{232}$ ) of oil blends during storage at room temperature (22±2°C)**

Oil blend	Storage period (months)											
	Zero time		1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B	A	B
CF-3	0.75±0.12	1.34±0.00	1.30±0.00	2.00±0.07	1.57±0.07	2.22±0.07	1.72±0.06	2.49±0.04	2.22±0.07	3.40±0.15	2.42±0.01	3.92±0.04
CF-1	0.54±0.02	1.32±0.01	1.02±0.01	1.40±0.03	1.38±0.03	1.75±0.02	1.52±0.04	2.42±0.05	1.72±0.06	2.79±0.17	2.01±0.02	3.20±0.06
CF-2	0.52±0.01	1.18±0.05	0.93±0.02	1.26±0.03	1.19±0.03	1.65±0.00	1.29±0.04	2.26±0.08	1.33±0.01	2.51±0.01	1.80±0.13	2.91±0.01
OC-1	0.51±0.00	0.79±0.01	0.60±0.04	0.89±0.00	0.84±0.00	1.16±0.12	1.00±0.03	1.39±0.00	1.16±0.06	1.71±0.02	1.34±0.06	1.95±0.07
OC-2	0.51±0.03	0.84±0.00	0.71±0.02	0.89±0.02	0.94±0.02	1.22±0.05	1.09±0.15	1.46±0.04	1.30±0.25	1.82±0.17	1.48±0.07	2.02±0.01
OF	0.60±0.03	0.91±0.01	0.84±0.01	1.15±0.01	1.09±0.01	1.49±0.17	1.16±0.04	1.70±0.04	1.33±0.06	2.06±0.00	1.69±0.06	2.78±0.04
OC-3	0.51±0.02	0.78±0.01	0.59±0.01	0.88±0.01	0.84±0.01	1.04±0.04	0.99±0.03	1.32±0.02	1.14±0.00	1.65±0.04	1.33±0.09	1.91±0.02

\*Mean ± SD A - without antioxidants.

B - with antioxidants.

**Table 8. Changes in the specific absorbance values\* ( $K_{270}$ ) of oil blends during storage at room temperature (22±2°C)**

Oil blend	Storage period (months)											
	Zero time		1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B	A	B
CF-3	0.054±0.00	0.121±0.00	0.072±0.00	0.173±0.00	0.121±0.00	0.209±0.01	0.132±0.00	0.250±0.03	0.134±0.01	0.289±0.00	0.241±0.02	0.349±0.02
CF-1	0.030±0.00	0.050±0.00	0.050±0.00	0.066±0.01	0.061±0.00	0.119±0.00	0.069±0.01	0.152±0.01	0.073±0.02	0.164±0.00	0.094±0.00	0.297±0.00
CF-2	0.028±0.00	0.049±0.00	0.049±0.00	0.060±0.00	0.060±0.00	0.105±0.00	0.062±0.00	0.125±0.00	0.073±0.00	0.137±0.00	0.085±0.00	0.251±0.00
OC-1	0.026±0.00	0.041±0.00	0.038±0.00	0.053±0.00	0.047±0.00	0.055±0.00	0.051±0.01	0.071±0.01	0.055±0.00	0.086±0.00	0.057±0.01	0.100±0.00
OC-2	0.026±0.00	0.042±0.00	0.039±0.00	0.053±0.00	0.047±0.00	0.060±0.02	0.055±0.01	0.080±0.00	0.063±0.01	0.095±0.01	0.075±0.00	0.120±0.00
OF	0.029±0.00	0.048±0.00	0.048±0.00	0.055±0.00	0.050±0.01	0.088±0.01	0.061±0.01	0.100±0.02	0.068±0.01	0.116±0.01	0.079±0.00	0.239±0.02
OC-3	0.026±0.00	0.040±0.00	0.033±0.01	0.040±0.00	0.044±0.01	0.055±0.00	0.047±0.00	0.059±0.00	0.052±0.00	0.081±0.11	0.056±0.00	0.097±0.01

\*Mean ± SD A - without antioxidants.

B - with antioxidants.

to the natural antioxidant substances such as polyphenols and tocopherols found in virgin olive oil. These substances inhibit the formation of PV and its decomposition into malonaldehyde, the TBA reactant (Sharara, 1998). Addition of antioxidants lowered the formation of this component.

**Para-anisidine value (P-AV):-** This test indicates the oil rancidity due to the formation of the secondary oxidation products, aldehydes and ketones. According to the data in Table (6), the P-anisidine values were paralleled with those of TBA values. Oil blends containing flaxseed oil had higher P-anisidine values than those blended made from the other two oil sources. Generally, extending storage of the oil blends was associated with an increase in P-anisidine value. Meanwhile, the addition of antioxidant lowered the rate of the formation of the secondary oxidation products and sequentially the values of this test.

**Ultraviolet specific absorption ( $K_{232}$  and  $K_{270}$ ):-** The ultraviolet absorbance of oil estimates the conjugated hydroperoxides absorbed at 232 nm and the secondary oxidation products (aldehydes and ketones) absorbed at 270 nm. As seen from the results in Tables (7) and (8), oil blends containing flaxseed oil had the highest levels of absorbance at 232 and 270 nm followed by those containing olive and canola oils. Extending the storage period of these oils at room temperature increased their UV-absorbance values. The rate of this increase was more pronounced in blends having flaxseed oil due to their higher levels of both trienoic (linolenic) and dienoic (linoleic) fatty acids compared with blends containing olive oil rich in oleic acid (monoenoic acid). As in the case of PV, TBA and P-anisidine, addition of antioxidants slowed down from the oxidation rate and sequentially the values of the specific absorbance at  $K_{232}$  and  $K_{270}$ . This indicated that the hydrolysed products of PV were responsible for the rise of TBA, P-anisidine and UV-absorbance. According to Vieira and Regitano d' Arce (2001), the formation of secondary oxidation products of oil was correlated with the decrease of peroxide value.

Borneo *et al.*, (2007) formulated a filling for sandwich cookies containing 400 mg of eicosapentaenoic acid, 20:5, n-3 (EPA) and docosahexaenoic acid, 22:6, n-3 (DHA) encapsulated in a matrix of starch and gelatin. They found that no significant loss of EPA and DHA was observed during storage such cookies at 18 and 23°C under both atmospheric and vacuum packed for 28 days

### CONCLUSION

The above results confirmed the importance of using olive oil for preparing oil blends containing a proper n-

3 to n-6 fatty acids ratio. Blending olive oil with either flaxseed and/or canola oils could be improved their storage stability due to the increase of monoenoic fatty acid (oleic acid) in blends. The n-3 to n-6 fatty acids ratio of the oil blends containing olive oil ranged from 1 to 6.5-9.1. This range lies within the recommended range stated by World Health Organization (1:4-10) n-3 to n-6 fatty acids. Gomes-Candela *et al.*, (2011) stated that the recommended ratio of n-3 to n-6 fatty acids is 1:3-5 and should not exceeding 1:10 to maintain good health. The formulated oil blends in this study had n-3: n-6 ratio less than 1:10. Teneja and Singh (2012) showed that omega-3 fatty acids can not be synthesized in the organism but have to introduced through diet to lower the risk of chronic diseases.

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