

## Blending of Virgin Olive Oil With Less Stable Edible Oils to Strengthen Their Antioxidative Potencies

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**Abstract:** Virgin olive oils are known to be more resistant to oxidation because of their content of more potent natural antioxidants and lower unsaturation levels. Admixing of virgin olive oil with other (less stable) edible oils leads to improvement of physico-chemical characteristics and oxidative stability. Blends (10, 20 and 40 v/v) of virgin olive oil with sunflower and soybean oils were prepared and evaluated by determining the changes in physicochemical properties such as refractive index, color index, peroxide value, free fatty acid %, iodine value, fatty acid composition. In addition, total phenolic contents as well as their antioxidation efficiency, measured by Rancimat method and DPPH scavenging activity were examined. It was concluded that the sunflower and soybean oils, admixed with cold pressed virgin olive oil at a level of 20 and 40 %, have the best quality parameters, however, 20% virgin olive oil blend is more satisfactory and superior to other blends combining both stability and economy aspects.

**Key words:** blending, oxidative stability, virgin olive oil, natural antioxidants.

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### INTRODUCTION

Because the health and nutritional aspects of edible oils in foods and food products are receiving increasing attention; it is becoming important to formulate new vegetable oil composition of improved stability and nutritional value. Lipid oxidation is one of the major factors resulting in loss of vegetable oil quality by formation of products having negative effects on flavor and nutritional value of the food. It also increases risk of cardiovascular disease (Chow, 1992). Soybean oil (SBO) and sunflower oil (SFO) have a good nutritional profile, with poor oxidative stability and is, accordingly, prone to flavor deterioration because of their high proportion of unsaturated fatty acids, especially, linolenic acid in SBO (White, 2000).

A consumer-friendly way of improving oxidative stability of edible oils is the addition of natural antioxidants. Therefore, search for finding useful source of natural antioxidants is highly desirable, because synthetic antioxidant has been questioned (Bera et al., 2004). Blending of vegetable oils and fats has emerged as an economical way of modifying the physicochemical characteristics of vegetable oils and fats besides enhancement in oxidative stability (Chu and Kung, 1997 & 1998). In our previous work, Hassanein (2010) investigated the improvement of the oxidative stability of SFO and olive oils by blending with sesame seed oil. The cold-pressed olive oil, involves neither heat nor chemical treatments, thus becoming an interesting substitute for conventional oils because of the consumer desire for natural and safe food products. Also it has better nutritive properties than refined oils, because, refining processes reduce oxidative stability of oils by removing the natural antioxidants and antioxidant precursors (EL-Mallah et al., 2011). Olive oil has a long shelf-life and can be blended with less stable vegetable oils to improve their stability and longevity. Furthermore, using olive oil can lower blood pressure and decrease the risk of heart disease (Kochhar, 2000, Psaltopoulou et al., 2004, McKeon 2005).

It was planned to admix refined bleached deodorized (RBD) SBO and SFO with cold pressed virgin olive oil (OO) to improve oxidative stability and physico-chemical properties of these oils formulations that verify the cooking purposes. Evaluation through determination of: fatty acid composition, iodine value, color index, refractive index was performed. In addition, determination of total phenolic contents as well as the evaluation of the oxidative stability by accelerated oven test, Rancimat method and free radical scavenging activity DPPH was carried out. Moreover, oxidizability and the protective factor (PF) were calculated to express susceptibility to oxidation of the investigated oils and their blends.

### MATERIALS AND METHODS

#### 2.1 Materials:

The main materials in this study were: OO which was purchased from Siwa Oasis, Jet Master); RBD SFO and SBO were purchased from Cairo for Oil and Soap Company, Cairo, Egypt.

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Authentic samples, standard fatty acids methyl esters (C16- C24, saturated and unsaturated) were purchased from Sigma Company. All reagents used were from E Merck or Sigma Aldrich.

## 2.2 Methods:

### 2.2.1 Preparation of Oil Blends:

Individual and blends of vegetable oils amounting to 250 ml were placed in 500 ml conical flask and were mixed thoroughly by using mechanical stirrer to form uniform blends. Seven different blends having various proportions were prepared as shown in Table 1.

**Table 1:** Ratio of oil blends.

(v/v)		
OO/SBO	OO/SFO	SBO/SFO/OO
10/90	10/90	30/30/40
20/80	20/80	
40/60	40/60	

OO: olive oil, SBO: soybean oil, SFO: sunflower oil

Individual oils of olive, sunflower and soybean were taken as control oils.

### 2.2.2 Physico-Chemical Analysis of Edible Oils and Their Blends:

The physical parameters of color index (CI) and refractive index were estimated by methods described in Yoshida and Kajimoto (1989) and Mordret *et al.*, (1985) respectively. The chemical parameters of the unsaturation characters of the oils were measured according to AOCS (1998).

### 2.2.3 Determination of Fatty Acids Pattern by GLC:

The oil was converted into methyl esters via transesterification according to Chrisite (1973). The identification of the components of fatty acids methyl esters was done using gas liquid chromatograph (Hewlett Packard Model 6890 chromatograph) under the following conditions: Separation was done on an INNO wax (polyethylene glycol), capillary column (30.0 m x 530  $\mu$ m x 1.0  $\mu$ m). Column temperature was 240°C with temperature programming: Initial temperature 100°C to 240°C maximum with 10°C rising for each minute and then hold at 240°C for ten minutes. Injection temperature was 280°C; carrier gas was nitrogen with flow rate 15 ml / min. Flame ionization detector temperature 280°C. Hydrogen flow rates were 30 and 300 ml/ min, respectively.

### Calculated Oxidizability (Cox) Value:

The Cox value of the oils was calculated by applying the formula proposed by **Fatemi and Hammond** (1980):

$$\text{Cox value} = (1 [18:1\%] + 10.3[18:2\%] + 21.6 [18:3]) / 100$$

### 2.2.4 Total Phenolic Content (TPC):

The TPC was determined spectrophotometrically using Folin-Ciocalteau reagent according to the method described by Capannesi *et al.* (2000). A calibration curve of gallic acid in methanol was performed in concentrations ranging from 0.04-0.40 mg/ ml and the results expressed as gallic acid equivalent.

### 2.2.5 Oxidative Stability:

#### 2.2.5.1 Accelerated Oven Test:

Oil samples were heated in an electric oven at 60°C for 32 days. Methods such as peroxide value (PV) and free fatty acid (FFA) are then employed to measure oxidation levels in the individual oils and their blends as described in AOCS (1998).

#### 2.2.5.2 Rancimat Method:

The susceptibility of all oil samples to oxidation was studied by using the Rancimat apparatus. The test was performed on an automated Metrohm Rancimat model 679 at 110 $\pm$ 0.1°C and an air flow of 20 L/hr to determine the induction period (IP) of the individual oils and their blends (Aparicio *et al.*, 1999).

### Protective Factor (PF):

The protective factor of the susceptibility to oxidation of the investigated oils, expressed as percentage extension of the induction period, (Abramovic and Abram, 2006), was calculated as follows:

$$\text{PF} = \text{IP}_{\text{blend}} - \text{IP}_{\text{original oil}} / \text{IP}_{\text{original oil}} \times 100$$

### 2.2.5.3 Free Radical Scavenging Activity (DPPH Assay):

The determination of DPPH is based on procedure by Lee *et al.* (2007), they reported that iso-octane could dissolve both DPPH and oil samples. Four milliliters of 0.10 mM DPPH in iso-octane were mixed with each 25, 50 and 75  $\mu$ L oil samples separately in a 30-mL serum bottle and after 30 min standing in the dark, the absorbance of the sample mixture was measured at 517 nm using a UV/Vis-spectrophotometer.

## RESULTS AND DISCUSSION

### 3.1 Physico-Chemical Properties:

The concentration of natural dyes in oil is a major factor influencing its sensory, antioxidants, and health properties, as well as its overall quality and typicality (Ranalli *et al.*, 2005). Color is related to other chemical and physical properties of oils (Melgosa *et al.*, 2004). All the oil blends showed an increase in CI and were found to be generally different than the individual oils as in Table 2.

No distinct differences were observed for refractive index (RI) of the various individual oils and their blends and it ranged from 1.469 to 1.470 units in all oil blends which were seen to be different than the control (Table2).

Table 2 shows the effect of blending on iodine value (IV) of individual oil and their blends. The IV of SBO, SFO and OO were 129.6, 126.0 and 83.5 respectively. The addition of OO to each of SBO and SFO at ratios of 10, 20 and 40% resulted in decline of IV to 124.0, 117.0 and 108.0 for SBO mixtures and to 120.5, 118.9 and 110.9 for SFO mixtures. On the other side, the reduction % in IV was 4.3, 9.7, 16.2% for SBO and 4.36, 5.6, 12.0% for SFO. The measurement of IV of blended oils revealed that every 40% addition of OO to each of SBO and SFO reduced their IV's by 16.2, 12.0% respectively. On the other side, the decrease in IV of the ternary blend SBO: SFO: OO (30: 30: 40) was 15.7%.

**Table 2:** Physico-chemical characteristics of individual oils and their blends.

Pure oil and their mixtures	Refractive index	Color index	Iodine value		TPC mg/100g	Rancimat	
			IV	% decrease in IV		IP hr	PF %
SBO	1.47	25.6	129.6	-	10.5	9.63	-
SFO	1.47	11.38	126.0	-	20.5	10.1	-
OO	1.454	194.2	83.5	-	117.0	31.1	-
SBO:OO 90:10	1.469	47.92	124.0	4.3	18.5	9.67	0.4
SBO:OO 80:20	1.4683	58.53	117.0	9.7	23.5	11.2	16.3
SBO:OO 60:40	1.4671	76.5	108.0	16.2	51.0	16.3	69.2
SFO:OO 90:10	1.469	31.78	120.5	4.36	27.5	11.8	1.6
SFO:OO 80:20	1.4683	46.17	118.9	5.6	31.0	12.8	2.6
SFO:OO 60:40	1.4672	83.81	110.9	12.0	69.5	18.1	7.8
OO :SBO: SFO 40:30:30	1.4671	78.53	109.2	15.7	57.0	18.0	86.92

### 3.2 Fatty Acid Profiles:

Fatty acid composition of investigated oils and their blends are presented in Table 3. The lowest percentage of polyunsaturated fatty acids (PUFA) was observed in OO (14.8%). From the results in Table 3, Cox value was greater in SBO, followed by the SFO and OO, while these values decreased (enhanced) when OO blended with SBO and SFO. Whereas, PUFA/SFA was SFO> SBO> OO (3.97> 3.8> 0.71). The blends that may achieve a nearer balance of fatty acid proportion 1: 1: 1 is recommended by the International Organizations. In the ternary blend, namely, OO: SBO: SFO (40: 30: 30%) generally, noticeable alteration in saturated and unsaturated fatty acids were observed. It is well known that the PUFA/SFA ratio and Cox value are usually taken as a measure of tendency of oils to undergo oxidation (Fatemi and Hammond 1980, Mendez *et al.*, 1996). Blending of OO with SBO or SFO resulted in increases in the palmitic and oleic acid contents which have a positive influence on oxidative stability. For unsaturated fatty acids, a decrease in the contents of linoleic and linolenic acids was observed, (linolenic acid is more susceptible for oxidation than lionoleic).

### 3.3 Total Polyphenolic Compounds (TPC):

It was of interest to evaluate TPC using Folin-Ciocalteu's method. It can be noticed from Table 2 that the polyphenol content of the individual OO was the highest in quantity (117.0 mg/100g) than the other investigated oils; therefore, it increases the antioxidation potency of other oils when it is blended with it. Accordingly,

**Table 3:** Fatty acid profiles of individual oils and their blends.

Fatty acid	OO	SBO	SFO	OO: SBO			OO: SFO			OO :SBO: SFO
				10: 90	20:80	50: 50	10: 90	20:80	40:60	40: 30: 30
C16:0	16.5	11.5	6.8	12.3	12.2	13.1	8.5	8.9	10.8	13.5
C16:1	2.5	-	-	0.3	0.1	1.1	0.3	0.5	1.0	0.3
C18:0	4.2	4.4	7.3	3.9	4.2	3.5	6.5	6.4	5.8	4.9
C18:1	62.0	22.0	29.0	26.6	31.3	39.0	32.3	35.7	42.3	40.2
C18:2	14.0	55.3	56.7	51.9	48.2	40.0	52.3	48.3	39.8	39.1
C18:3	0.8	6.0	-	5.0	4.0	3.3	0.1	0.2	0.3	2.0
C20:0	-	0.2	0.2							
C20:1	-	0.6	-							
SFA	20.7	16.1	14.3	16.2	16.4	16.6	15.0	15.3	16.6	18.4
MUFA	64.5	22.6	29.0	26.9	31.4	40.1	32.6	36.2	43.3	40.5
PUFA	14.8	61.3	56.7	56.9	52.2	43.3	52.4	48.5	40.1	41.1
PUFA/ SFA	0.71	3.8	3.97	3.52	3.2	2.61	4.49	3.169	2.415	2.233
Cox value	2.2348	7.2119	6.1301	6.692	6.142	5.222	5.731	5.3751	4.5872	4.8614

moderate polyphenol contents were observed when OO was added to SBO or SFO. In case of ternary blend, the total phenolics contents amounts to 57.0. Phenolics compounds have much influence on the stability, sensory and nutritional characteristics of the product and may prevent deterioration through quenching of radical reactions responsible for lipid oxidation (Koski *et al.*, 2003).

### 3.4 Oxidative Stability:

#### 3.4.1 Accelerated Oven Test:

From Fig 1 (a, b and c) a general trend of increase in FFA, in SBO, SFO, OO and their blends stored in oven at 60°C for 32 days, can be noticed. However the rate of increase was lower in blends of SBO or SFO with olive oil than individual SBO and SFO. Generally, the addition of OO to SBO or SFO reduces the FFA formation during storage at 60°C for 32 days. Formation of FFA resulted partly from hydrolysis and from further oxidation of the secondary products formed during heating (Kun, 1990; Abou-Gharbia *et al.*, 2000; Wanasundara *et al.*, 2001).

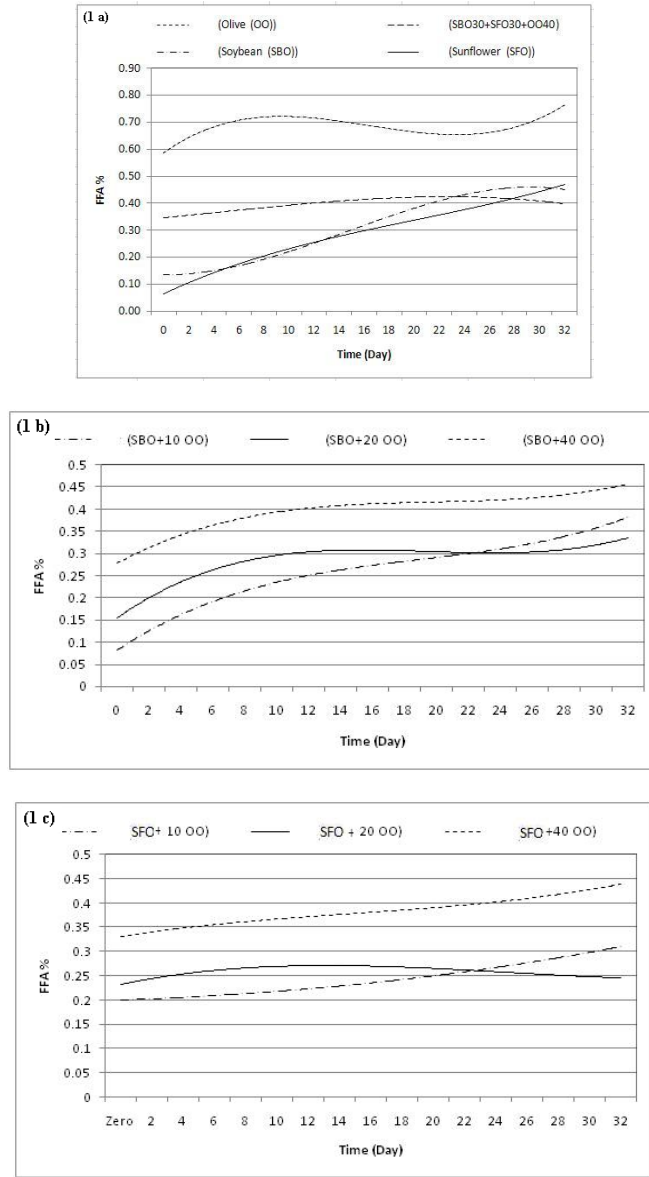
The effect of blending on PV during accelerated oven test storage for 32 days at 60°C registered a progressive increase with the storage period as shown in Fig 2 (a, b and c). Results in Figure 2a, shows that the oxidative stability of SBO, SFO and OO oil at 60°C, based on PV, was generally increased by time. In SBO and SFO, there was a steady increase in PV reaching the maximum values at 20 days, then decreasing thereafter. This not the case with OO in which the change in PV was limited all over the heating period. Increasing the ratio of OO in the blends caused improvement in the antioxidation potency and accordingly delays the increase in PV. The course of hydroperoxide formation in blends with OO showed moderate behavior between higher stable OO and less stable SBO or SFO during storage time of 32 day at 60°C. In fig 2a, the blends of SBO: SFO: OO, had lowest PV in comparison with other individual SBO or SFO. The rate of formation and degradation of hydroperoxides seem to be higher in RBD oils than in case of OO and their blends. In fact, the increase in the amount of monounsaturated fatty acid (after admixing) helps increase in the oxidative stability of oil blends. [The presence of natural antioxidants (as a positive effect) and the polyunsaturated fatty acid content (as a negative effect) are more important factors of the oxidative stability of oils (Chu and Kung 1998)].

#### 3.4.2 Rancimat Method:

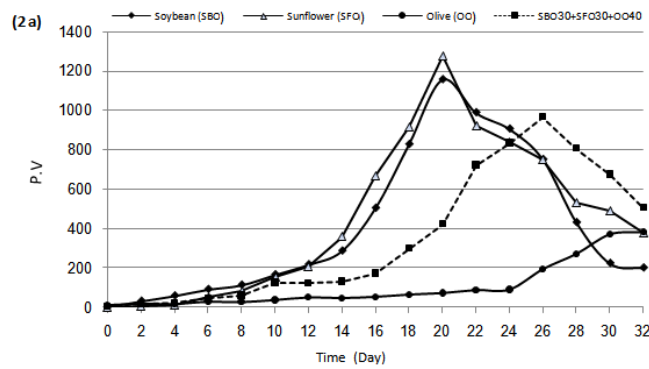
The susceptibility of the studied oils and their blends to oxidation was also measured by the Rancimat test. The end point of the Rancimat test can be determined by IP to the inflection point in the oxidation curve (Mendez *et al.*, 1997). The length of the IP is considered a relative measure of the stability of oils. The results of oxidative stability, in terms of measurement of IP of RBD SBO, SFO and their blends with virgin OO are shown in Table 2. It was found that OO had the highest IP. The results also, showed that the IP of the blends increased following the order SFO 60: OO 40 > SBO 30: SFO 30: OO 40 > SBO 60: OO 40 > SFO 80: OO 20 > SFO 90: OO 10 > SBO 80: OO 20 > SBO 90: OO 10. The protective factor (PF) expresses the susceptibility to oxidation of oils, expressed as percentage extension of the IP. The highest PF was found to be of the OO 40: SBO 30: SFO 30 blend followed by SBO 60: OO 40> SBO 80: OO 20> SFO 60: OO 40> SFO 80: OO 20 > SFO 90: OO 10> SBO 90: OO 10.

#### 3.4.3 Free Radical Scavenging Activity (DPPH Assay):

The use of DPPH method provides an easy and rapid way to evaluate antioxidants. Differences in DPPH scavenging activity were found between vegetable edible oils and their blends as shown in Fig 3. From the figure (at concentrate 75µl oil) it was found that OO had the highest DPPH scavenging activity with an estimate value of (95.74%) followed by blends of 60 SBO: 40 OO (78.59%) and 60 SFO: 40 OO (70.85%) whereas the oil blend SBO: OO (90:10%) had the lowest DPPH scavenging activity with an estimated value of 55.74%.



**Fig. 1:** Periodical changes of FFA of individual oils and their blends heated at 60°C, for 32 days.



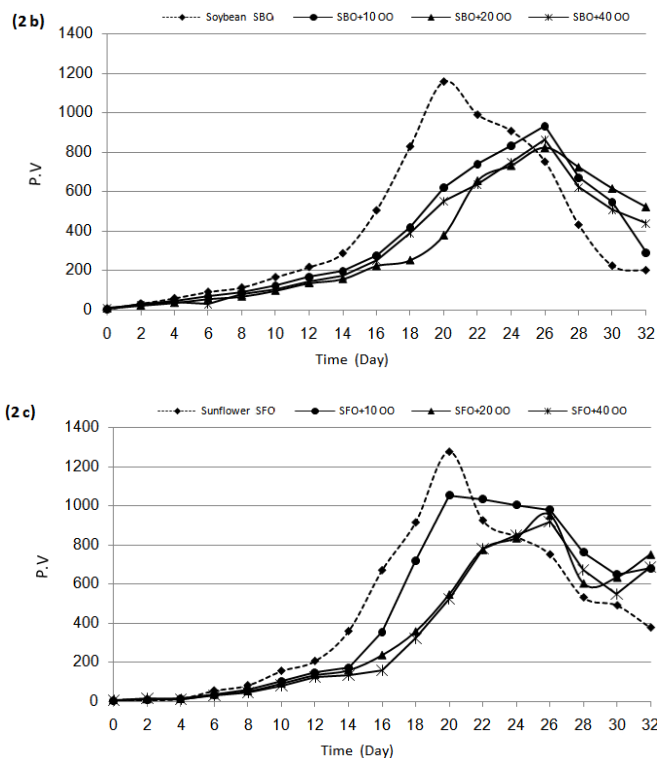


Fig. 2: Periodical changes of PV of individual oils and their blends heated at 60°C, for 32 days.

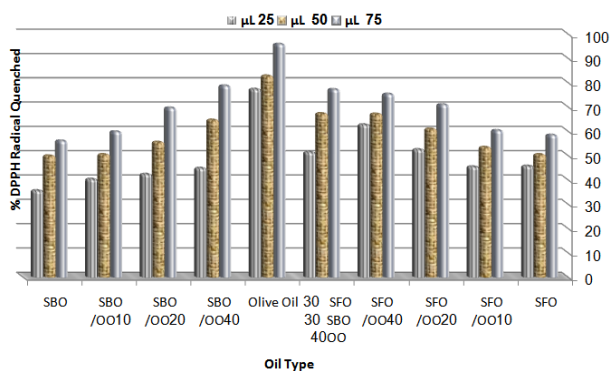


Fig. 3: Scavenging activity of oils and their blends on DPPH.

From the data obtained, it was found that the TPC and DPPH scavenging activity of individual OO and its blends with other oils exhibited higher values than those of RBD vegetable oils.

#### 4. Conclusion:

It is well known that, cold-pressed virgin olive oil contains natural high potent antioxidants, especially polyphenols as well as tocopherols with high content of monounsaturated fatty acids. These compounds may play a potential role as antioxidation protective factors as well as health promoting. Mixing different proportions (10, 20 and 40% v/v) of OO with SBO or SFO provides improvement in antioxidative potency of these edible oils. Considering the merits and demerits of single oil as cooking medium, blended oils seem to be just as or even more suitable than single oil for culinary purposes. Meanwhile, the blends achieving fatty acid composition with a nearer balance of fatty acid proportion 1: 1: 1 are recommended from nutritional point of view. There is also an important change in antioxidants types that can synergistically strengthen oxidative stability of oil blends. Subsequently, the oil blends would have longer shelf-life stability, more nutritional value as well as it is easy and relatively economic. The blending does not only stabilizes the edible oils but also serves to improve and enhances the nutritional and functional qualities of the oils by combining the good attributes of the two oils into one. It was concluded that the SFO and SBO, admixed with cold pressed OO at a level of 20 and 40 %,

have the best quality parameters, however, 20% virgin olive oil blend is more satisfactory and superior to other blends combining both stability and economy aspects.

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