

# Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils

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## Abstract

Three seeds of Turkish origin, flax, poppy and safflower were analyzed for their proximate, fatty acids, tocols (tocopherols and tocotrienols) and total phenolic composition, and oxidative stability of their oil. The major fatty acid in the flax oil was  $\alpha$ -linolenic acid, comprising 58.3% of total fatty acids, whereas poppy and safflower oils were rich in linoleic acid at 74.5% and 70.5% level, respectively. The amount of total tocols was 14.6 mg/100 g flax, 11.0 mg/100 g poppy and 12.1 mg/100 g safflower seed. Flax and poppy oil were rich in  $\gamma$ -tocopherol as 79.4 mg/100 g oil and 30.9 mg/100 g oil, respectively, while  $\alpha$ -tocopherol (44.1 g/100 g oil) was dominant in safflower oil. Only  $\alpha$ - and  $\gamma$ -tocotrienol were found in the oils. Oxidative stability of oils was measured at 110 °C at the rate of 20 L/h air flow rate, and poppy oil (5.56 h) was most stable oil followed by safflower oil (2.87 h) and flax oil (1.57). There were no correlation between oxidative stability and unsaturation degree of fatty acids and tocol levels of the oils. All of the seeds investigated provide a healthy oil profile and may have potential as a source of specialty oils on a commercial scale.

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**Keywords:** Flax, poppy and safflower seeds; Fatty acid; Tocol; Phenolics; Oxidative stability

## 1. Introduction

Oilseeds are major source of raw materials such as fat, protein carbohydrate with potential application as nutraceuticals and functional foods. They also might provide low-cost renewable resource of high value-added compounds such as tocopherol and phytochemicals. Seed oils are the main source of dietary ingredients related to their fatty acid composition and tocopherol content. The oil rich in unsaturated fatty acids, which are believed to be beneficial agents, and with high level of tocopherols are now added into infant formula and various food products and available as nutraceutical supplements in many countries (Oomah and Mazza, 1999; Moyad, 2005; Lampi et al., 2002). Moreover, not only oil components but also remaining meals after oil extraction are the important source with

their protein, carbohydrate and non-nutritive but bioactive compounds such as phenolics (Naczk and Shahidi, 2006).

Oxidative stability is an important parameter in evaluating the quality of oils and fats, and oxidative stability of seed oils is greatly affected by their fatty acid composition and minor components such as tocopherol and tocotrienols. The oxidation process mainly involves the degradation of polyunsaturated fatty acid (PUFA) and the generation of free radicals, which cause to loss of functional properties and nutritional value (Gordon, 2001). Both tocopherols and tocotrienols are important antioxidants in stabilizing of unsaturated fatty acids in foods and provide an effective protection against oxidative stress together with other antioxidants, such as phenolics, in the human body (Papas, 1998; Comb, 1998; Lampi et al., 1999).

Flax, poppy and safflower seeds subjected to our study are considered to be important seed crops in the oilseed market. Particularly, flaxseed (*Linum usitatissimum*) is fast becoming a new food in many diets due to health benefits

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of its oil, fiber components and phytochemicals such as lignans (Mazza, 1998). Main physiological benefits of flax oil are attributed primarily to the high  $\alpha$ -linoleic acid (ALA) content (Burdge and Calder, 2005). Safflower is the seed of *Carthamus tinctorius* L., which has been grown for a long time for oil production and for coloring purposes. Today this crop supplies oil, meal, birdseed, and foots (residue from oil processing) for the food and industrial products markets, although this crop is now primarily grown for the oil. The importance of safflower seed oil is in its linoleic acid content, which is a required product with high-PUFA claims (Smith, 2005). Poppy (*Papaver somniferum* L.) has been grown since ancient times for its oil rich seeds and the opium, which is exuded from its incised seed capsules. While alkaloids from poppy capsules and straw are widely used in the pharmaceutical industry, its seeds are used extensively in various baked products (Bernath, 1998; Singh et al., 1998).

Although proximate and fatty acid composition of three seeds and seed oils subjected to this study are well known, there were inadequate information about their oxidative stability under accelerated conditions and their minor components. Therefore, the objectives of this study were to determine the oil composition in terms of fatty acid and tocol (tocopherol and tocotrienol) contents and to evaluate oil composition–oxidative stability relationship.

## 2. Methods

### 2.1. Materials

Commercial flax, poppy and safflower seeds were supplied from a local producer in Central Anatolia (Eskisehir and Konya) and kept below  $-20\text{ }^{\circ}\text{C}$  until used. All reagents (Analytical and HPLC grade) used were E. Merck or Sigma Aldrich.

### 2.2. Analysis of seed samples

The seeds were ground by using a coffee grinder. Moisture content and ash value were determined according to AOCS Methods (Af 2-54) (AOCS, 1993). Crude protein ( $N \times 6.25$ ) was determined using LECO FP-428 Nitrogen and Food Protein Determinator. Carbohydrate content was estimated by difference of the other components. Oil from seeds was extracted using *n*-hexane for 5 h followed by solvent removal under vacuum at  $40\text{ }^{\circ}\text{C}$ . Tocols were obtained from the seeds according to slightly modified method of Oomah et al. (1997). The grinded seed was homogenized in HPLC grade methanol and then the samples were centrifuged. The supernatant was removed and residue resuspended in methanol, and the homogenization and centrifugation steps were repeated. The supernatants were combined and methanol was removed under nitrogen. The dried residue was redissolved in hexane, and then placed in a 2 ml ambercript vial and stored at  $-20\text{ }^{\circ}\text{C}$  until analysis. Free phenolics from defatted plant material (3 g)

was extracted with 40 ml of 70% aqueous methanol in a shaker bath set at  $40\text{ }^{\circ}\text{C}$  for 30 min and filtered. Extraction procedure was repeated 3 times. The filtrates were combined and methanol was evaporated at  $40\text{ }^{\circ}\text{C}$  by rotavapor until dryness. Free phenolics extracted with ethyl acetate. Ethyl acetate phases were evaporated under vacuum at  $40\text{ }^{\circ}\text{C}$  until dryness. In order to obtain esterified phenolic compounds from defatted seeds, samples (5 g) and 150 ml of 1.2 M HCl in 50% aqueous methanol (v/v) were mixed. These were carefully mixed and shaken at  $80\text{ }^{\circ}\text{C}$  for 1 h in a shaker water bath (Lab-Line Shaker Bath 3540, USA). The extract was cooled, filtered and methanol was evaporated. The water phase was extracted with 75 ml of ethyl acetate three times. Ethyl acetate phases were evaporated under vacuum at  $40\text{ }^{\circ}\text{C}$  until dryness.

Total phenolic content was determined by spectrophotometrically using Folin–Ciocalteu reagent after addition of 20%  $\text{Na}_2\text{CO}_3$  solution at 765 nm against blank. Results were expressed as gallic acid equivalent in dry matter (Hoff and Singleton, 1977).

### 2.3. Analysis of extracted oils

**Fatty acid composition:** Fatty acid content of oil samples was determined according to the method described by Bozan and Temelli (2002). The fatty acid methyl esters (FAME) were then analyzed by gas chromatography (Varian 3600 GC, Mississauga, ON). The system was equipped with an auto sampler (Model 8200, Varian) and a flame ionization detector. The data were processed by a computer using Class-VP data processor (Shimadzu Corporation, Columbia, MD). Helium was used as the carrier gas. The FAMES were separated on a fused silica capillary column (50 m  $\times$  0.32 mm, BPx-70, SGE Column, Pty. Ltd, Victoria, Australia) with the film thickness of 0.25  $\mu\text{m}$ . The detector temperature was set at  $230\text{ }^{\circ}\text{C}$ . Initial injector temperature was held  $70\text{ }^{\circ}\text{C}$  for 3 min, then increased at  $150\text{ }^{\circ}\text{C}/\text{min}$  to  $230\text{ }^{\circ}\text{C}$  and held for 17 min. Initial column temperature was  $50\text{ }^{\circ}\text{C}$  for 0.1 min and increased to  $170\text{ }^{\circ}\text{C}$  at the rate of  $25\text{ }^{\circ}\text{C}/\text{min}$ , held at  $170\text{ }^{\circ}\text{C}$  for 1 min, then increased to  $180\text{ }^{\circ}\text{C}$  at the rate of  $2\text{ }^{\circ}\text{C}/\text{min}$ , and then increased to  $230\text{ }^{\circ}\text{C}$  at the rate of  $10\text{ }^{\circ}\text{C}/\text{min}$  and held for 3 min.

**Tocol content:** Tocols (tocopherols and tocotrienols) were analyzed by high performance liquid chromatography (HPLC). The Varian 9010 HPLC system (Varian, Mississauga, ON) was equipped with HP 1050 series auto injector. The detector used was a Shimadzu-RF 535 fluorescence detector (Shimadzu, Corporation, Columbia, MD) with wavelengths set at 330 nm for emission and 298 nm for extinction. Tocols were separated on a normal phase column (Supelcosil-LC-Diol, 25 cm  $\times$  4.6 mm ID, 5 mm particle size, Supelco, Oakville, ON) with the mobile phase flow rate at 1 mL/min. The mobile phase was a mixture of *n*-hexane:isopropanol (99.4:0.6, v:v). The data were integrated and analyzed using Shimadzu Class-VP Chromatography Laboratory Automated Software system (Shimadzu Corporation, Columbia, MD). Standards of tocopherol  $\alpha$ ,  $\beta$ ,

$\gamma$  and  $\delta$  isomers (Sigma Chemical Co., St. Louis, MO) and tocotrienol  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  isomers (Merck, Darmstadt, Germany) were dissolved in hexane and used for identification and quantification of peaks. The amount of tocopherols in the extract samples was calculated as mg tocopherols per 100 g seed and oil samples using external calibration curves, which were obtained for each tocopherol isomer standard.

#### 2.4. Oxidative stability

An automated Methrom Rancimat model A 743 was used for the determination of oxidative stability of seed oils. Oil samples (3 g) were weighted into each of the six reaction vessels and analyzed simultaneously. The conductometric cells were filled with deionized water up to a volume of 60 ml. The heating temperature was 110 °C, and filtered, dried air was allowed to bubble through the hot oil at the rate of 20 L/h. Processing and evaluation of the experimental data were performed from the break points of the plotted curves, and induction periods of the samples were recorded automatically (Louli et al., 2004).

All extraction processes and analysis of samples were carried out in duplicate and triplicate, and means with standard deviations were reported.

### 3. Results and discussion

#### 3.1. Proximate composition of seeds

The proximate composition of the oilseeds analyzed is presented in Table 1. All seeds with low moisture content (5.28–6.43%) levels could store for a long time since higher moisture content could cause decomposition of fatty acids by microbial action. The ash and carbohydrate content were important parameters in terms of the suitability of seed cake for compounding of animal feeds. With 1.91% ash and 51.87% carbohydrate content, safflower seed was suitable for this purpose among the seeds studied. Protein content was 12.6%, 17.9% and 21.6% in safflower, flax and poppy seeds, respectively. The highest oil content was found in poppy seed as 50% followed by 33.6% in flax and 27.5% in safflower seeds.

#### 3.2. Free and esterified phenolic content of seeds

Phenolic compounds are associated with nutritional and sensory quality of seeds. While at low concentration

phenolics may play a role of protection of seeds from oxidative deterioration, at high concentrations they might contribute to dark color, bitter taste and off-flavor of some oilseeds (Shahidi, 2000). Not only seeds but also seed oils contain considerable amounts of phenolic compounds that have a great effect on the stability of oil (Tovar et al., 2001).

Free, esterified form and total phenolic content of seed samples are shown in Table 2. Total free and esterified phenolic content of flax seed was 1670 mg/100 g, followed by safflower seed (1526 mg/100 g) and poppy seed (930 mg/100 g). Esterified phenolics were the predominant form of phenolics in flax and poppy seeds. Flaxseed and poppy seed contained 77% and 75% of esterified phenolics of total phenolics, respectively, while safflower seed was rich in free phenolics (63%). Esterified and total phenolic contents were reported that ranged from 469 to 975 mg/100 g, and 537 g/100 g to 1815 g/100 g, respectively, in different cultivars of whole flaxseed (Oomah and Mazza, 1997). No report was found on phenolic content of poppy and safflower seeds.

Phenolic acids and their derivatives and flavanoids are dominant phenolic compounds in seeds rich oil. The antioxidant capacities of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule. Several studies indicated that phenolic acids esterified with a group such as sugar enhance antioxidant potency of the molecule (Liyana-Pathirana and Shahidi, 2006; Amarowicz et al., 2003). Therefore higher amount esterified phenolics may play role to protect of seeds from oxidative deterioration.

#### 3.3. Fatty acid and tocol composition of flax, poppy and safflower oils

Main fatty acid composition of flax, poppy and safflower seed oils are presented in Table 3. Results were in agreement with previous reports (Bernath, 1998; Singh et al., 1998; Lee et al., 2004). Flax oil contained 88.4% unsaturated oil made up mainly linolenic acid (58.3%), whereas poppy and safflower oil contained 88.2% and 83.6% unsaturated oil with 74.5% and 70.5% linoleic acid, respectively. Although total percentages of saturated (SAT) and unsaturated fatty acids did not vary, there were wide variations in the content of individual fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids in the oil studied. While palmitic acid was dominant (84.0% of total SAT) in poppy oil, stearic acid was higher (40% of

Table 1  
Proximate composition of flax, poppy and safflower seed flours<sup>a</sup>

| Assay                | Flax seed  | Poppy seed | Safflower seed |
|----------------------|------------|------------|----------------|
| Crude fat (%)        | 33.6 ± 2.8 | 49.9 ± 0.7 | 27.5 ± 2.6     |
| Crude protein (%)    | 17.9 ± 0.1 | 21.6 ± 0.3 | 12.6 ± 0.3     |
| Moisture (%)         | 6.4 ± 0.1  | 5.3 ± 0.01 | 6.1 ± 0.01     |
| Ash                  | 3.9 ± 0.7  | 5.9 ± 0.02 | 1.9 ± 0.03     |
| Carbohydrate + fiber | 38.1 ± 0.1 | 18.3 ± 0.1 | 51.9 ± 0.2     |

<sup>a</sup> Values are mean ± standard deviation of triplicate determinations.

Table 2  
Content (mg/100 g seed) of free and esterified phenolics in seeds<sup>a</sup>

|           | Free     | Esterified | Total |
|-----------|----------|------------|-------|
| Flax      | 383 ± 18 | 1287 ± 115 | 1670  |
| Poppy     | 229 ± 16 | 701 ± 86   | 930   |
| Safflower | 559 ± 52 | 967 ± 81   | 1526  |

<sup>a</sup> Values are mean ± standard deviation of triplicate determinations.

Table 3  
Fatty acid composition (% GC area) of seed oils<sup>a</sup>

| Fatty acid   | Flax oil | Poppy oil | Safflower oil |
|--------------|----------|-----------|---------------|
| C14:0        | nd       | nd        | 0.16          |
| C15:1        | nd       | nd        | 0.17          |
| C16:0        | 6.86     | 9.79      | 7.59          |
| C16:1w-7     | 0.14     | 0.13      | 0.28          |
| C18:0        | 4.59     | 1.93      | 2.42          |
| C18:1-w9-cis | 15.07    | 11.94     | 11.04         |
| C18:1-w7-cis | 0.87     | 1.09      | 0.86          |
| C18:2-w6-cis | 13.96    | 74.47     | 70.46         |
| C18:3-w3     | 58.31    | 0.60      | nd            |
| C20:0        | nd       | nd        | 0.36          |
| C20:1-w9     | nd       | nd        | 0.13          |
| C22:0        | nd       | nd        | 0.27          |
| C22:2        | nd       | nd        | 0.32          |
| C24:1        | nd       | nd        | 0.15          |
| C22:5        | nd       | nd        | 0.27          |

<sup>a</sup> Values are means of duplicate determinations, nd (not detectable): <0.1%.

total SAT) in flax oil than that of poppy oil (16.5% of total SAT) and safflower oil (22.4% of total SAT). Oleic acid content did not vary significantly in poppy (11.9%) and safflower (11.0%) oils. Linoleic acid content was dominant in poppy and safflower oils; its level was 14.0% in flax oil. Only safflower oil contained long-chain fatty acids (C<sub>20</sub>–C<sub>22</sub>) with the minor amount (1.2% of total fatty acid).

As having an important role for protection against oxidative deterioration of polysaturated fatty acids in plant material, tocol (tocopherol + tocotrienol) level in seeds and seed oils are extremely important. Table 4 presents the level of tocol per 100 g seeds and oils studied.  $\gamma$ -Tocopherol was the main tocol in flax seed (13.93 mg/100 g) and poppy seed (8.70 mg/100 g), while  $\alpha$ -tocopherol was abundant in safflower seed (10.21 mg/100 g). As presented in Table 4 only  $\alpha$ -tocotrienol and  $\gamma$ -tocotrienol were detected in the seeds with minor amounts.

Flax oil was richest in tocol content among the oils (Table 4), followed by safflower and poppy oil.  $\gamma$ -Tocopherol was dominant in flax oil representing 95% of total tocol. Flax oil also contained  $\alpha$ -tocopherol (0.59 g/100 g oil),  $\alpha$ -tocotrienol (0.63 g/100 g oil),  $\gamma$ -tocotrienol (0.83 g/100 g oil) and  $\delta$ -tocopherol (1.68 g/100 g oil). Poppy oil contained mainly  $\gamma$ -tocopherol (21.74 mg/100 g oil) and  $\alpha$ -tocopherol

(5.5 mg/100 g oil), whereas  $\alpha$ -tocopherol was the major tocopherol (44.1 mg/100 g oil) in safflower oil.

Although the level of tocopherol was cultivar specific and depend on environmental conditions, tocol content of flax and safflower seeds and oils investigated in this study were in accordance with previously reported results. Oomah et al. (1997) reported that  $\gamma$ -tocopherol (8.45–9.72 mg/100 g seed) was the main tocol in flaxseed, followed by  $\delta$ -tocopherol (0.17–0.22 mg/100 g) and  $\alpha$ -tocopherol (0.02–0.1 mg/100 g).  $\gamma$ -Tocopherol amount was reported as 57.3 mg in 100 g flax oil (Syvaaja et al., 1986). Safflower seed oil contained 44.9 mg/100 g of  $\alpha$ -tocopherol, followed by  $\gamma$ -tocopherol (2.6 mg/100 g oil),  $\beta$ -tocopherol (1.2 mg/100 g oil) and  $\delta$ -tocopherol (0.6 mg/100 g oil) (Shahidi and Shukla, 1996). When compared tocol content with seed oils,  $\gamma$ -tocopherol was major tocopherol in soybean (72.7 mg/100 g oil), canola oil (36.9 mg/100 g oil) and rice bran oil (4.35 mg/100 g oil) as in flaxseed oil and poppy oil, whereas  $\alpha$ -tocopherol was abundant in sunflower (61.3 mg/100 g oil) as in safflower oil. The content of total tocopherol in canola oil was 56.5 mg/100 g oil, in soybean oil 112.8 mg/100 g oil, in sunflower oil 63.2 mg/100 g oil and 13.7 mg/100 g oil in rice bran oil (Normand et al., 2001; Perretti et al., 2004; Zigoneanu et al., 2008).

It is reported that there might be positive relation between unsaturation and amount of tocols in the oil and oil seeds (Shahidi and Shukla, 1996). Although a weak positive association between tocopherol and oil content in flax seed was reported (Oomah et al., 1997), no relationship was observed in this study. Concerning to the tocol content and total unsaturated fatty acids (MUFA +

Table 5  
Total tocopherol (mg/100 g),  $\alpha$ -tocopherol (mg/100 g),  $\gamma$ -tocopherol (mg/100 g), polyunsaturated fatty acids (PUFA, %) and oxidative stability values of seed oils

|               | Total tocol | $\alpha$ -T | $\gamma$ -T | PUFA  | Induction time <sup>a</sup> (h) |
|---------------|-------------|-------------|-------------|-------|---------------------------------|
| Flax oil      | 79.41       | 0.59        | 75.67       | 72.27 | 1.57 ± 0.20                     |
| Poppy oil     | 30.94       | 5.53        | 21.74       | 75.07 | 5.56 ± 0.80                     |
| Safflower oil | 53.20       | 44.09       | nd          | 71.05 | 2.87 ± 0.50                     |

nd: not detected.

<sup>a</sup> Values are mean ± standard deviation of duplicate determinations.

Table 4  
Tocol content of seeds<sup>a</sup> and seed<sup>b</sup> oils<sup>c</sup>

|                | $\alpha$ -T  | $\alpha$ -T3 | $\beta$ -T  | $\gamma$ -T  | $\gamma$ -T3 | $\delta$ -T3 | Total |
|----------------|--------------|--------------|-------------|--------------|--------------|--------------|-------|
| Flax seed      | 0.10 ± 0.01  | 0.12 ± 0.01  | nd          | 13.93 ± 0.10 | 0.16 ± 0.02  | 0.31 ± 0.02  | 14.6  |
| Poppy seed     | 1.40 ± 0.02  | nd           | 0.53 ± 0.03 | 8.70 ± 0.20  | 0.21 ± 0.08  | 0.16 ± 0.01  | 11.0  |
| Safflower seed | 10.21 ± 0.40 | 0.11 ± 0.02  | 1.19 ± 0.32 | nd           | 0.13 ± 0.06  | 0.38 ± 0.05  | 12.0  |
| Flax oil       | 0.59 ± 0.01  | 0.63 ± 0.04  | nd          | 75.67 ± 1.30 | 0.83 ± 0.14  | 1.68 ± 0.08  | 79.4  |
| Poppy oil      | 5.53 ± 0.20  | nd           | 1.67 ± 0.21 | 21.74 ± 1.20 | 1.47 ± 0.01  | 0.58 ± 0.04  | 30.9  |
| Safflower oil  | 44.09 ± 2.10 | 0.70 ± 0.08  | 7.23 ± 0.30 | nd           | 1.18 ± 0.07  | nd           | 53.2  |

<sup>a</sup> mg/100 g seed.

<sup>b</sup> mg/100 g oil, T: Tocopherol, T3: Tocotrienol, nd: not detected.

<sup>c</sup> Values are mean ± standard deviation of triplicate determinations.

PUFA), flax seed and oil was richest in total tocol among the oil; however, its unsaturated fatty acid content was similar to the poppy oil which has lowest tocol content (Table 5). This might be explained that unsaturation degree of fatty acids due to the level of  $\alpha$ -linolenic acid was responsible for the higher tocol content in flaxseed and oil. Although poppy and safflower oils have similar fatty acid content in terms of linoleic acid and total polyunsaturated fatty acid, safflower seed and oil contained higher tocol content than that of poppy seed as well as its oil. This might be resulted by minor level of long-chain fatty acids present in safflower oil.

### 3.4. Oxidative stability of oils

Tocol content together with unsaturation degree of oil have dramatic impact on its oxidative stability. Antioxidant activity of tocol depends on their chemical nature and concentration. In general, the antioxidant activity of tocopherol was reported in the order of  $\alpha$ - >  $\beta$ -  $\geq$   $\gamma$ - >  $\delta$ -tocopherol (Shahidi and Shukla, 1996). According to this order,  $\alpha$ -tocopherol is most active one; however, it can lose efficiency in stabilizing fatty acids at elevated concentrations. Therefore, generally adding  $\alpha$ -tocopherol to vegetable oil does not improve their oxidative stability and tocopherol level of the oils might be enough to stabilize polyunsaturated fatty acids (Lampi et al., 1999; Burton et al., 1985). Other tocopherols and tocotrienols are reported not to act as prooxidant (Huang et al., 1994; Soldeen and Soldeen, 2005). Table 5 shows  $\alpha$ -,  $\gamma$ -, and total tocol and polyunsaturated fatty acid contents in the oils examined and their oxidative stability values. There was no correlation between oxidative stability and tocol content. Induction period (IP, h) was in order: flax oil (1.57 h) < safflower oil (2.87 h) < poppy oil (5.56 h). Although flax oil had a significant amount of tocol, its large degree of polyunsaturated regarding to linolenic acid reduced its stability. Safflower oil had almost 2/3 times more tocol level than that of poppy oil; however, its oxidative stability value (IP) was half of poppy oil induction period. This might be resulted by ever small amount polyunsaturates and long-chain fatty acids present in safflower oil negatively impacted the oxidative stability.

Although phenolic compounds are well known to contribute to the overall antioxidant capacity of oils, no correlation was found in this study between total phenolic content of seeds and oxidative stability of the oils. This results might be ascribed to antioxidant activity not only depend on total phenolic content but also type of the phenolic compounds present (Tovar et al., 2001).

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