Original Research Article

Effect of Added Black Cumin Seed (*Nigella sativa L*) Oil on Oxidative Stability of Flaxseed Oil

ABSTRACT

Background: Lipid oxidation has adverse effect on food deterioration and human health. Oxidative reactions limit the shelf-life of fresh and processed foodstuffs which are of great concern in the vegetable oil and fat industry.
Aim: The antioxidant activity of black cumin (*Nigella sativa L*) oil (BCO) as a source of natural antioxidants was compared against a synthetic antioxidant (BHA). Its oxidative stability was evaluated in Flaxseed oil (as the most sensitive oil against oxidation) in concentrations of BHA (200ppm).
Study Design: Oils were analyzed for the composition of fatty acids, tocopherols and for some physicochemical properties [Peroxide (PV), P-Anisidine (AV),TOTOX values, Thiobarbituric acid, Iodine value, Oxidative stability (OS) and refractive index] to assess the extent of oil deterioration during 30th days of storage.
Place and Duration of Study: Islamic azad university of Varamin and Tehran University, Tehran, Iran, From March 2018 to April 2018.
Methodology: Optimal condition was investigated to protect edible oil like flaxseed oil from Oxidative reaction. Black cumin seeds and flaxseeds were pressed separately by cold press. Then BCO was prepared at five concentration levels 5, 10, 15, 20 and 25% (w/w) and after that added to Flaxseed oil. . In terms of antioxidant activity, they were analyzed by Gas Chromatography and compared with samples containing 200 ppm of synthetic antioxidant (BHA) during 30th days at ambient temperature (22-25° C).
Result and Conclusion: The results showed that different levels of BCO were able to slow down the lipid oxidation well, but the effect of sample contained 5% BCO was higher than other levels and has potential source of natural antioxidant for the application in food industry to prevent lipid oxidation.

Keywords: Flaxseed oil, Black Cumin Seed oil, Cold press, Oxidative stability, physicochemical properties

1. INTRODUCTION

Flaxseed oil also known as flax oil is an edible oil obtained from the flax plant (*Linum usitatissimum*) .Flaxseed oil is used as a nutritional supplement and contains the highest level of α-Linolenic acid (ALA),(51.9–55.2%) among vegetable oils [1]. Since flaxseed oil contains high amount of di- and tri-unsaturated ester, it is easily oxidized and rapidly becomes rancid, with an unpleasant odour. In order to use the nutritional benefits of this oil, antioxidants can be used to enhance its oxidative stability [2].
Lipid oxidation leads to the spread of off-flavors and undesirable tastes which result in shorter shelf life [3]. Oxidative reactions diminish the shelf-life of fresh and processed foodstuffs which are of great concern in the vegetable oil and fat industry. The highly

oxidized oils could have toxic effects on human health and, therefore, these oils are not suitable for nutritive purposes because of reaction products [4, 5]. Due to unfavorable effects on food degradation and human health, development of the exogenous antioxidants are required to prevent not only the harmful effects of free radicals on health related problems, but also the deterioration of fats and other compounds of food stuffs[6] .

Traditionally, chemically synthesized compounds, such as butylated hydroxyanisole (BHA) , butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are widely used as antioxidants in oil products [7]. Nevertheless, it has been found that these synthetic antioxidants have carcinogenic and toxic effects on human health. For example, BHT causes hepatomegaly – the enlargement of the liver [8]. Therefore, research on natural antioxidants with lower toxicity than synthetic compounds has to be carried out. Natural antioxidants are constituents of many fruits and vegetables, which have attracted a great deal of public and scientific attention due to their anti-carcinogenic potential and other health promoting effects.

Consumers have become more health conscious about the use of additives and the quality of diet they take. This has evolved the food industry to replace synthetic antioxidants with antioxidants that are safer and come from natural origins. This has led to an increase in the investigation to find resources that are rich in natural antioxidants [9]. This fact has been proven that many plants contain natural antioxidants. [10-11,12]. The antioxidant activity of Chamomile (*Matricaria chamomilla L.*) extract (as natural resources) in sunflower oil has been reported. [13]. A study has been reported the antioxidant activity of some plant extracts of the Labiatae family, stored at 75 ^{o}C [14]. The antioxidant activity of the extract of ajowan seeds in an accelerated storage test using soybean oil was studied [15]. In another research, the antioxidant activity of some selected plant extracts in rapeseed oil was investigated [9]. The antioxidant effects of thyme in rapeseed oil, at 40℃, have also been stated [16]. The antioxidant activity of sage and oregano in salad dressing was investigated [17]. In another study, the effects of heat treatment on the antioxidant activity of extracts from citrus peel was examined [18]. The antioxidant effects of some natural compounds have also been reported under microwave heating of vegetable oils [19]. In another review some characteristics of nigella (*Nigella sativa L.*) seed cultivated in Egypt and its lipid profile were studied. [20] Oxidative stability of virgin olive oil was investigated by addition of *microalga chlorella vulgaris* biomass [21].

Comment [P1]: Use uniform style for citation, author number or author date, correct for the whole paragraph

Black Cumin (*Nigella sativa L*) is an annual herbaceous plant belonging to the Ranuculacea family. It mainly contains linoleic acid, oleic acid, palmitic acid, and other minor components [22]. Aromatics include thymoquinone, dihydrothymoquinone, *p*-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene and *trans*-anethole. The seeds also contain thymoquinone. [23]. They have been used for thousands of years as a spice and food preservative. Black cumin is widely grown in the Mediterranean countries, Middle East, Eastern Europe and Western Asia. The seeds are used as seasoning for vegetables, legumes and different types of baked products.

In the Middle East, Northern Africa and India, it has been applied for centuries in order to treat asthma, cough, bronchitis, headache, rheumatism, fever, influenza and eczema and for its antihistamine, antidiabetic and anti-inflammatory activities [24].

In this study, the effect of added BCO at different levels (as a source of natural antioxidants) on Flaxseed oil (as the most sensitive oil against oxidation) was studied in order to investigate the physicochemical properties and oxidative stability.

2. MATERIAL AND METHODS

Black cumin (*Nigella sativa L*) and Flaxseed (*Linum usitatissimum*) were purchased from Pakan Bazr Co. Isfahan, Iran. Butylated Hydroxyanisole (BHA), Potassium iodate, Acetic acid (glacial), Sodium thiosulfate, Hexane and Acetonitrile HPLC Grade were purchased

from Merck Chemical Co, Germany. All others un-labeled chemicals and reagent were used analytical grade.

2.1 Oil extraction

Black cumin seeds and flaxseeds were cleaned and pressed separately (cold press, P500R, Germany) at room temperature (25℃) without any thermal treatment. Mesilla was stored for one at room temperature to separate oil phase from Mesilla then oil was filtered over anhydrous sodium thiosulphate and cotton filter using glass funnel.

2.2 Samples Preparations

After oils extraction, black cumin seed oil was prepared at five concentration levels 5, 10, 15, 20 and 25% (w/w) and then added to Flaxseed oil. Samples were stored at ambient temperature in amber glass containers until analysis. In terms of antioxidant activity, they were compared with samples containing 200 ppm of synthetic antioxidant (BHA) during thirty days (1, 10, 20 and 30th) at ambient temperature (22-25° C). In addition, the flaxseed oil was considered as control sample. (Table 1)

Table 1: Treatments examined in the study

Name of Treatment	BHA	BCO
Control sample(C)	0%	0%
B	200 PPM	0%
A1	0%	5%
A2	0%	10%
A3	0%	15%
A4	0%	20%
A5	0%	25%

2.3 Analytical Methods

2.3.1 Identification of fatty acid composition, triacylglycerol's and tocopherols of BCO

To determine the fatty acid composition, the methyl ester derivative was prepared according to AOAC standard No. 969/33. Then they were analyzed by Younglin ACME 6100 Gas Chromatography equipped with a flame ionization detector on a 100-metre (cp sill 88) capillary column in accordance with AOCS cele -91 [25]. The temperature of the injector, capillary column and detector were 240, 198°C, and 280 ° C, respectively. The carrier gas flow rate (nitrogen) was 14 mL / min , Pressure 10 psi and the injection rate was 1 μL [26]. Fatty acid composition was expressed as weight percent (%) of total fatty acid methyl ester.

Tocopherols ($\alpha, \beta, \gamma \; and \; \delta$ tocopherols) were analyzed by high-performance liquid chromatography (HPLC) with UV –visible detection in accordance with AOCS 8–89 (1993). For this purpose, a 5-60 SI column with a diameter of 4.5 × 250 mm and a particle size of 5 µm was used with fluorescence detector. The mobile phase consisted of acetonitrile/ distilled water (95:5, v/v) at a flow rate of 0.6 mL/min and the injection volume 20μL. Tocopherols were identified and quantified using Calbiochem tocopherols (Merck, Darmstadt, Germany) as external standards. The amount of tocopherols in the oils was calculated as mg tocopherols per kg oil.

2.3.2 Determination of physical and chemical properties of Samples

Iodine value was evaluated by *Hanus method.* Reflective index (RI) measured using Abee´ Refractometer at 20 ℃ and conducted according to AOCS Cc 7-25 (AOCS, 2005)

2.3.2.1 Oxidation stability tests

Peroxide values (PV) of oil samples were according to AOCS Cd 8-53 (AOCS, 2005). P-Anisidine value assay was carried out according to AOCS Cd 18-90 (AOCS, 2005). Total oxidation (TOTOX) values of oil samples were determined using the following equation [27]

$$\text{Total oxidation (TOTOX) value} = 2 \times PV + AV$$

2.3.2.2 Oxidative Stability (OS)

In addition, Oxidation stability was measured with a Rancimat 743 apparatus (Metrohm Co., Basilea, Switzerland) according to AOCS Official Method (1997) Cd 12b-92 to determine the induction time for samples. The temperature was set at 110 ℃ and 20 L/h air flow, the oil sample was 2.5 g and the stability was expressed as oxidation induction time (h). Measurement of Thiobarbituric acid was carried out using butanol as a mobile solvent coupled with the Thiobarbituric acid reagent at a wavelength of 530 nm in compliance with AOCS Cd 19-90.(AOCS,2005)

2.4 Statistical analysis

The results of all tests were analyzed by comparison of mean values using a factorial model at $p < 0.01$ with SPSS software (version 21). Duncan's multiple range tests were conducted to determine the statistical differences among different means. In addition, all charts were drawn using Excel software. All tests were performed in three replications.

3. RESULTS AND DISCUSSION

3.1 Fatty acids, tocopherols' composition of BCO

Table 2 reports the results of BCO fatty acid composition. According to the consequences the major fatty acids in BCO were Palmitic (C16:0, Behenic (C22: 0) and Stearic (C18:0) as saturated fatty acids, while oleic (C18: 1) and linoleic (C18: 2n-6) were the main unsaturated fatty acids. Likewise, measurable amounts of myristic and arachidic (C20:0) as saturated fatty acids as well as palmitoleic (C16:1) as mono-unsaturated fatty acids and Linoleic acid as a polyunsaturated fatty acid were detected. These results are in agreement with previously published data [28, 29, and 30] .The total saturated and unsaturated fatty acids in BCO extracted by cold press were 19.14 and 80.73%, respectively.

Table 2: Fatty acid composition of BCO as a□ected by cold press

Fatty acids	Percentage (%)
C14:0	0.07
C16:0	10.44
C18:0	3.56
C20:0	0.44
C22:0	4.63
C20:1	0.63
C16:1	0.36
C18:1	24.56
C18:2c	54.27
C18:2t	0.43
C18:3	0.48

The different tocopherol isomers, that is, alpha (α), beta (β), gamma (γ), and delta (δ), are distinguished by the locations of methyl groups on the chromanol ring. Tocopherol content of BCO is given in Table 3. The highest content was refereed to γ -tocopherol with levels 241.72 ppm. However, β -tocopherol was the lowest tocopherol isomer found in BCO (6.29 ppm). This result was lower than those reported by another study earlier.[31]

Table 3: Levels of tocopherols (mg/kg) in BCO

$\boldsymbol{\alpha}$ Tocopherol	16.4
$\boldsymbol{\beta}$ Tocopherol	6.29
$\boldsymbol{\gamma}$ Tocopherol	241.72
$\boldsymbol{\delta}$ Tocopherol	9
Total tocopherols	273.41

3.2 Physical and chemical properties of Samples

3.2.1 *Refractive Index (RI)*

Mean values of data demonstrated that the highest reduction in Refractive Index over time was occurred in the control sample (C) which reached from 1.479 in the first day to 1.473 in the 30th day. As can be seen in Fig 1, when synthetic antioxidant was added in flaxseed oil, the change in refractive index was lesser than control sample. However, the minimum reduction in the refractive index was observed in the treatment containing 5% BCO during the storage period.($p< 0.01$)

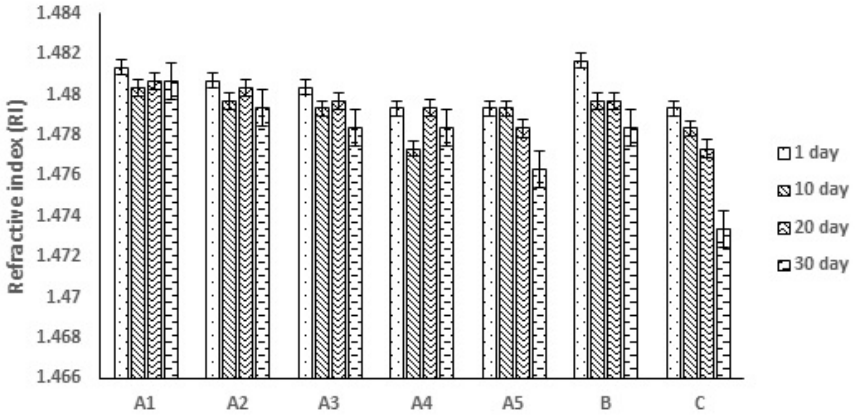

Fig 1: Changes in Refractive Index (RI) of treated Flaxseed oil samples at different storage time (1, 10, 20 and 30th days)

3.2.2 Iodine value

Iodine values are often used to determine the amount of unsaturation in fatty acids. According to the results, along with the increase in the amount of BCO in flaxseed oil from 5 to 25%, iodine number decreased over time. However, the least reductions in iodine value occurred in sample containing 5% BCO on 30th day. Besides, the highest reduction in iodine number was observed in the treatment contained 25% BCO.

3.2.3 Impact of extraction method and storage on Oxidative stability (OS) of flaxseed oil containing BCO

According to the result, induction periods of different samples were in the range of 2.56 to 1.01 h. The results of induction period showed that sample containing 5% BCO was the highest (2.56 h) and control sample was the lowest (1.01 h). However, the use of 5 to 10% of BCO in flaxseed oil had a greater effect on the induction periods compared with treatment containing of synthetic antioxidant (BHA). (Fig 2)

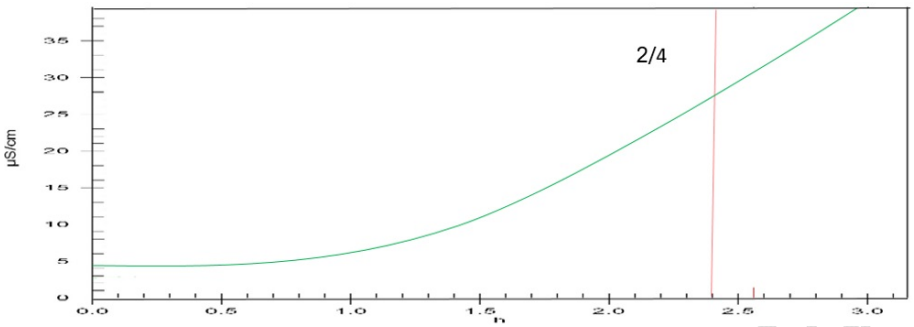

Fig 2: Rancimat test for treatment contained 5% of BCO

3.2.4 Peroxide value (PV)

According to the hypothesis of this study, BCO was considered as a natural antioxidant to slow down the lipid oxidation. Hydroperoxide is the primary product of lipid oxidation; therefore, determination of PV can be used as oxidative index during the early stage of lipid oxidation [32, 33]. The PV calculated for samples are shown in Fig. 3..All samples showed more antioxidant activity in comparison with control sample after 1, 10, 20 and 30th days ($p<0.01$). However, the peroxide value of the treatment contained BCO (5 %) in the whole of storage time was lesser than other samples ($p<0.01$). The results also revealed that, with increase of amount of BCO in flaxseed oil from 5 to 25 %, the total amount of peroxide value was regularly expanded. This can be attributed to the amount of unsaturated fatty acids in BCO. The total increase in PV was followed in descending order by Control sample> $A5 > A4 > A3 > B > A2 > A1$ with maximum *4*.38, *3*.79, 2.43, *2*.14, 1.99, *1.34* and *0*.6 meq O_2 / kg of oil respectively in *30*th day ($p< 0.01$). However, the treatment contained 5% of BCO has had a superior activity in terms of delaying the hydroperoxides formation comparing to the sample contained BHA. (Fig 3). As it could be seen results show a considerable effect made by BCO, approving the hypothesis of this research.

Comment [P2]: The p values have to be calculated to show existence of statistically significant or insignificant observation. Hypothesis testing is necessary.

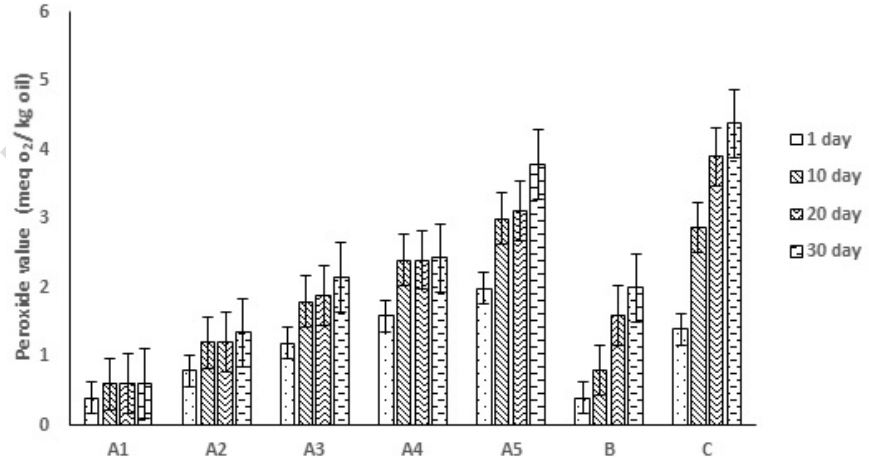

Fig 3: Changes in Peroxide value (PV) of treated Flaxseed oil samples at different storage time (1, 10, 20 and 30th days)

3.2.5 P-Anisidine values (AV)

Generally, The AV of all the samples increased in a regular pattern over the storage time (Fig 4). Under accelerated storage for 30th days, the total increased of AVs were in the following sequence: $A3 > A4 > A5 > C > B > A2 > A1$ with maximum values of 3.73, 3.72, 3.56, 3.49, 3.22, 2.98 and 2.64 respectively in 30th day. This could be explained that A1 and A2 treatments were more effective than B sample in retarding the formation of secondary oxidation products compared to primary oxidation products. The AV of all the samples raised in a regular pattern over the storage time (Fig *4*).

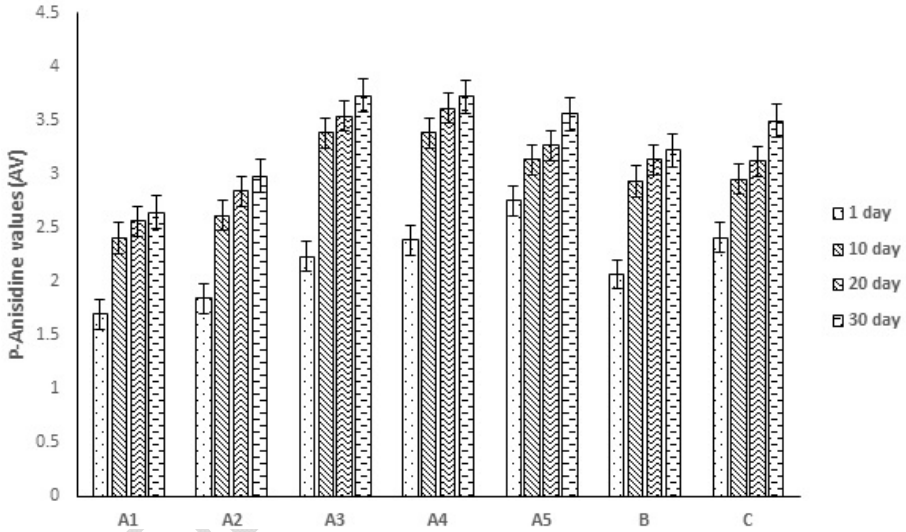

Fig 4: Changes in P-Anisidine values (AV) of treated Flaxseed oil samples at different storage time (1, 10, 20 and 30th days)

3.2.6 Total oxidation (TOTOX) values

As can be seen in Figure 5, the TOTOX values of all the samples increased in a regular pattern over the storage time. On the first day, the least TOTOX values was related to sample containing 5% BCO with a value of 2.48 and the highest was referred to treatment contained 25% BCO with a value of 6.72%. Over the time, the TOTOX value increased in all treatments. Under accelerated storage for 30th days, the total increased of TOTOX values were in the following sequence: Control sample> $A5 > A4 > A3 > B > A2 > A1$ with maximum *12*.26, *11*.13, *8*.58, *8*.01, *7*.21, *5*.66, and 3.84 respectively in 30th day. The results showed that, in samples with lower levels of BCO (samples A1, A2), the TOTOX value was increased slower than the other treatments. However, optimum concentration of BCO

(sample contained 5% BCO) had better preventing effects on retarding oil degradation compared with synthetic antioxidant. ($p< 0.01$)

Comment [P3]: Statistical analysis is required to make this satement true

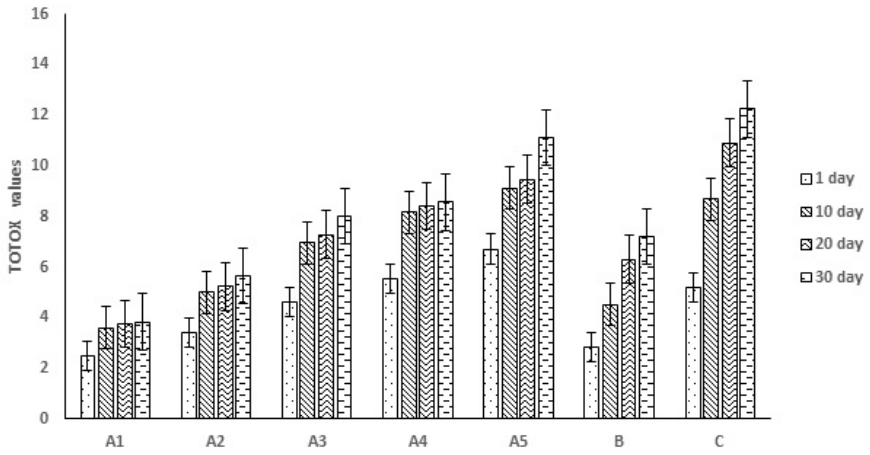

Fig 5: Changes in Total oxidation (TOTOX) values of treated Flaxseed oil samples at different storage time (1, 10, 20 and 30th days)

3.2.6 Thiobarbituric acid

The consequences showed that the amount of thiobarbituric acid in all treatments increased over time. The control sample, which did not contain any antioxidants, had the highest escalation in the value of Thiobarbituric acid. Among the different concentrations of BCO, the sample containing 5% (A1) exhibited the highest reduction in oxidation rate which was followed by sample containing 200 ppm BHA (B). As a result of the decomposition of the hydroperoxides formed in the early days and converting them into aldehydes and ketones, the value of thiobarbituric acid increased over time. The results also showed that the effect of treatment, time and interactions of treatment and time on the value of thiobarbituric acid was significant ($p < 0.01$). These results were accordance with another study. [34]

4. Conclusion

The results of our study showed that the treatment function containing 5% BCO in terms of oxidation prevention was equal with the sample containing synthetic antioxidant (BHA). However, the sample which contained 5% BCO had a better antioxidant performance over time. According to the outcomes attained from this study, higher concentrations of BCO not only decrease the antioxidant activity but also have a Pro-oxidants effect. Therefore, A1 (5% BCO) treatment is considered as the superior treatment in this study. As a final point, BCO as a source of natural antioxidants has ability to react with free radicals resulting from lipid oxidation and it can discontinue chain reactions, oxidation and finally slow down the autoxidation rate rather than synthetic antioxidant (BHA). Consequently this oil could be introduced as an alternative to synthetic antioxidants which are commonly used in food.

References

1. Vereshagin ,A. G. and Novitskaya, G. V. (1965) The triglyceride composition of Flaxseed oil. Journal of the American Oil Chemists' Society 42, 970-974
2. Berab. D.; Lahirib. D. & Naga .A (2006). "Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants". Journal of Food Engineering. 74 (4): 542–545.
3. Sikwese F., DuoduK. G., (2007). Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. Food Chemistry, 104(1), 324-331
4. Farag R., Badei A., El Baroty G.,(1989). Influence of thyme and clove essential oils on cottonseed oil oxidation. Journal of the American Oil Chemists Society, 66(6), 800-804.
5. Kazuhisa Y., (2001). Oils and fats. Reito. 76, 405-409.
6. Potterat O., (1997). Antioxidants and free radical scavengers of natural origin. Current organic chemistry, 1(4), 415-440.
7. Laguerre M., Lecomte J., Villeneuve P., (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in Lipid Research, 46(5), 244-282.
8. Zuo Y., Wang C., Zhan J., (2002). Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC-MS. Journal of agricultural and food chemistry, 50(13), 3789-3794.
9. Bandoniene D., Pukalskas A., Venskutonis P., (2000). Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. Food Research International, 33(9), 785-791.
10. Peterson D. M.,(2001). Oat antioxidants. Journal of cereal science, 33(2), 115-129.
11. Gheldof N., Engeseth N. J., (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. Journal of Agricultural and Food chemistry, 50(10), 3050-3055.
12. Pezzuto J. M., Park E. J., (2002). Autoxidation and antioxidants. Encyclopedia of pharmaceuticals technology, 1, 97-113.
13. Sazegar, M. R., Baakar, A., Bahrami, N., Bahrami, A., Baghbani, m., Nematolahi, P.& Mottaghi, M. (2010). The antioxidant activity of Chamomile (Matricaria chamomilla L.) extract in sunflower oil. World Applied sciences Journal,9(8),873-878.
14. Economou K. D., Oreopoulou V., Thomopoulos C. D., (1991). Antioxidant activity of some plant extracts of the family Labiateae. Journal of American Oil Chemistry. 68, 109-113.
15. Mehta R. L., Zayas J. F., Yang S. S.,(1994). Ajowan as a source of natural lipid antioxidant. Journal of agricultural and food chemistry, 42(7), 1420-1422.
16. Wyen D., Takacsova M., Jakubik T., Dang M., (2000). Antioxidant effects of thyme in rape-seed oil. Biologia (Bratislava), 55(3), 277-281
17. Abdalla A. E., Roozen J. P.,(2001). The effects of stabilized extracts of sage and oregano on the oxidation of salad dressings. European Food Research and Technology, 212(5), 551-560.
18. Jeong S. M., Kim S. Y., Kim D. R., Jo S. C., Nam K., Ahn D., Lee S. C., (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. Journal of agricultural and food chemistry, 52(11), 3389-3393
19. Yoshida H., Takagi S., (1999). Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating. Journal of the Science of Food and Agriculture, 79(2), 220-226
20. Atta, M.B., (2003). Some characteristics of nigella (Nigella sativa L.) seed cultivated in Egypt and its lipid profile. Food Chem. 83, 63–68.

21. Alavi, N ., Keramat M , Golmakani MT ,Shekarforoush S , Nowtoozi M. (2016). Oxidative stability of virgin olive oil by addition of microalga vulgaris biomass, Iranian Journal of Nutrition Sciences & Food Technology vol. 10, No. 4
22. Bharat B Aggarwal. (2009). Molecular Targets and Therapeutic Uses of Spices. Google Books. p. 259.
23. Gharby S, Harhar H, Guillaume D, Roudani A, Boulbaroud S, Ibrahimi M, Ahmad M, Sultana S, BenHaddah T, Chafchaouni-Moussaouii I, Charroufa Z (2015). "Chemical investigation of *Nigella sativa L.* seed oil". Journal of the Saudi Society of Agricultural Sciences. 14 (2): 172–177.
24. Burits, M. and Bucar, F. (2000) . Antioxidant Activity of *Nigella sativa* Essential Oil. Phytotherapy Research, 14, 323-328.
25. American Oil Chemists Society (1993). O□cial methods and recommended practices of the American oil chemist's society (4th ed.). Champaign, IL: AOCS Press.
26. Firestone D., (1998). Official methods and recommended practices of the American Oil Chemists' Society (4thed.). Champaign: American Oil Chemist Society Press.
27. Shahidi, Bhanger M., (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chemistry, 100(1), 246-254.
28. Abdel-Aal, E. S. M., & Attia, R. S. (1993). Characterization of black cumin (*Nigella sativa*) seeds. 2- Proteins. Alex. Sci. Exch., 14, 483–496.
29. Babayan, V. K., Koottungal, D., & Halaby, G. A. (1978). Proximate analysis, fatty acid and amino acid composition of *Nigella sativa L.* Seeds. Journal of Food Science, 43, 1315–1319.
30. Gad, A. M., El-Dakhakhny, M., & Hassan, M. M. (1963). Studies on the chemical composition of Egyptian *Nigella sativa L.* oil. Planta Medica, 11, 134–138.
31. Ramadan, M.F., Wahdan, K.M.M., 2012. Blending of corn oil with black cumin (*Nigella sativa*) and coriander (*Coriandrum sativum*) seed oils: impact on functionality, stability and radical scavenging activity. Food Chem. 132, 873–879.
32. Ramadan, M. F., & Moersel, J.-T. (2004). Oxidative stability of black cumin (*Nigella sativa L.*), coriander (Coriandrum sativum L.) and niger (*Guizotia abyssinica Cass*.) upon stripping. European Journal of Lipid Sciences and Technology, 106, 35–43.
33. Mohdaly, A. A. A., Sarhan, M. A., Mahmoud, A., Ramadan, M. F., & Smetanska, I. (2010). Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. Food Chemistry, 123, 1019–1026
34. Yasoubi, P., Barzegar, M., Sahari, M. A. & Azizi, M. H. (2007). Total phenolic a. Contents and antioxidant activity of pomegranate (*Punica granatum L.*) peel Extracts.J. Agric. Sci. Technol, 9, 35-42.