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Changes in qualitative properties of cold press extracted oil from *Nigella sativa L.* seed pre-treated with ethanol solution during storage

Haghgouy, R.[1], Azadmard-Damirchi, S.[2*], Torbati, M. A.[3], Khakbaz Heshmati, M.[4]

1- M.Sc. Graduated Student, Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

2- Professor, Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

3- Associate Professor, Department of Food Science and Technology, Faculty of Nutrition and Food Science, Tabriz University of Medical Science, Tabriz, Iran

4- Assistant Professor, Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

ABSTRACT

Many scientific reports have been published about the health effects of black cumin seed (*Nigella sativa*) oil such as anti-cancer, anti-diabetic, anti-microbial, anti-inflammatory and antioxidant properties. However, one of the main problems in extracting oil from black cumin seed (BS) is the low yield and also the low quality of the extracted oil. Various methods such as microwave treatment, roasting and soaking have been performed to improve the extraction conditions and quality of BS oil before cold pressing. In this study, BS was pre-treated with ethanol (70%) at 0 (control sample), 1, 3 and 6% and then oil extracted by cold press. Extracted oil qualitative characteristics (acid value, peroxide value, chlorophyll content, carotenoid content and total phenolic content) were assessed in production day and every 30 days during 90 days of storage. Also, oxidative stability of BS oil was measured by Schaal test. The results demonstrated that by increasing the level of ethanol in the pre-treatment of BS, acid value and peroxide value significantly ($p < 0.05$) decreased and oxidative stability, chlorophylls, carotenoids and phenolic contents of extracted oil significantly ($p < 0.05$) increased. Acid value and peroxide value increased and chlorophyll and carotenoid contents due to decomposition of their contents decreased during storage. Oil extracted from BS pre-treated with highest level of ethanol had the lowest acid value (4.61 mg NaOH/g oil) and peroxide value (7.03 meq O_2/Kg oil) and the highest chlorophyll (21.21 mg/Kg oil), carotenoid (3.86 mg/Kg oil) and total phenolic (496.3 mg caffeic acid/Kg of oil) contents at the end of the storage. In conclusion, the results showed that pretreatment of BS by ethanol before cold pressing can be used to improve qualitative properties and stabilize the extracted oil.

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*Corresponding Author E-Mail:
sodeifazadmard@yahoo.com

1- Introduction

Black seed, a one-year plant with the scientific name (*Nigella sativa L.*) From the Alale family[1] Is. This plant is native to Southwest Asia, North Africa and Southern Europe. It is also cultivated in many Mediterranean and Middle Eastern countries including Iran, India, Pakistan, Saudi Arabia, Turkey and Syria [1].

Black seed oil is an excellent source of phytosterols, tocopherols, polyphenols, essential fatty acids, polyunsaturated fatty acids and phospholipids. The therapeutic and nutritional properties of black seed oil are due to the presence of bioactive compounds such as polar lipids, phospholipids and some essential fatty acids such as linolenic acid (omega 3) and linoleic acid (omega 6), sterols and other compounds [2]. Black seed oil is widely used to treat many diseases including asthma, bronchitis, rheumatism, paralysis, jaundice, indigestion and nervous disorders. It also has beneficial properties such as blood pressure lowering, antimicrobial, anti-inflammatory, anti-diabetic, anti-tumor, pain-relieving, diuretic, anti-cancer, antioxidant and stimulating effects on the immune system. In addition, black seed is believed to have antihistamine-like properties that are suitable for treating congestion [3].

The percentage of oil extracted from black seeds varies from 28 to 39 percent based on the type of extraction method (press method or solvent extraction) [1 and 4]. Also, the oil extraction method is effective on its quality and nutritional properties. Cold press oil extraction has better nutritional value and natural properties compared to solvent oil extraction due to the lack of chemicals and purification, and it is easier to perform due to fewer steps compared to solvent oil extraction.[1، 7-5].

Despite the many healing and nutritional properties of black seed oil, this oil has low stability and has a high acid value and peroxide value, which limits its use.[1, 4 and 6]. For this reason, due to the low stability and problems in the oil trade, no international standard has been formulated. To overcome these problems, various solutions have been proposed to reduce the acid number and peroxide number of this oil. Pressing a mixture of black seeds with other oil seeds at the same time is one of these solutions[7 and 8]. Other solutions include the use of various pretreatments such as ultrasound, microwave, roasting and humidification to improve the extraction conditions and bioactive compounds from black seeds.[4، 12-9]. Considering the high acid number and peroxide value and low oxidative stability of black seed oil, the aim of this study was to investigate the possibility of using ethanol for the pretreatment of black seed before cold pressing and to investigate the quality characteristics of oil extracted from it during storage.

2- Materials and methods

2-1- Raw materials

Black seed was purchased from the market (Tabriz, East Azerbaijan). All chemicals used in this research were obtained from Sigma company with laboratory grade purity.

2-2- To qualify the samples

After sieving and cleaning from possible impurities, the black seed was dried in an oven at 70 degrees Celsius.[13]. Black seed samples by sprayingPercentages of zero (control sample), 1, 3 and 6 percentEthanol (70%) were pretreated. The treated seeds were stored in polyethylene bags at 4°C for 24 hours in the refrigerator.

2-3- Oil extraction

[1] 1- *Ranunculaceae*

from black seedby screw press (modelmm 85، Core strength, Germany) at a temperature below 40 degrees Celsius, degreasing was performed [14]. Extracted oil samples were filtered with Whatman No. 2 strainer, and due to the absorption of ethanol solution into the cake and seed flour during the pressing process, it was not possible to identify and measure ethanol in the extracted oil. Then the oil samples were transferred to the laboratory in dark glasses and kept in the dark for 90 days. Qualitative tests of acid number, peroxide number, chlorophyll amount, carotenoid amount, and phenolic compounds amount were performed on days 1, 30, 60, and 90, and oxidative stability test was performed on the extracted oil during 10 days.

2-4- acid number

To measure the acid value, 3-4 drops of phenolphthalein reagent were added to weighed oil and dissolved in 100 milliliters of a mixture of ethanol and chloroform (1:1) and titrated with 0.1 sodium hydroxide solution [15].

5-2- Peroxide number

To measure the peroxide value, 0.5 milliliters of saturated potassium iodide was added to 5 grams of oil dissolved in 30 milliliters of acetic acid and chloroform (2:3) and kept in the dark for 1 minute, and after adding 30 milliliters of distilled water and 0.5 ml of starch solution was titrated with 0.1 normal sodium thiosulfate solution [15].

6-2- The amount of chlorophyll

The amount of chlorophyll in the oil sample with a spectrophotometer(Unique, UK) measured at three wavelengths of 630, 670 and 710 nm and calculated using the following formula:

$$\text{Chlorophyll (mg/kg)} = 345.5 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})/L$$

A: Absorption at the mentioned wavelengths (nm) andL: The thickness of the tube is spectrophotometer (mm).[16].

2-7- The amount of carotenoids

5.7 grams of oil sample was weighed in a 25 ml volumetric flask and made up to volume with cyclohexane and absorbed at 470 nm with a spectrophotometer.(Unique, UK) was measured. The amount of carotenoid (mg/kg of oil) was calculated with the following formula:

$$\text{Carotenoid (mg/kg)} = (A_{470} \times 10^6)/2000 \times 100 \times d$$

2000 = extinction coefficient (AND_0) for lutein (the main compound of the carotenoid group) andd Also, the thickness of the tube is (1 cm) [17].

8-2- Amount of phenolic compounds

The amount of phenolic compounds in the oil sample was determined by Folin-Ciocaltiu colorimetric method according to the method of Kepansi et al. [18].

2-9- Shawl test

To determine the oxidative stability using the Shawl test, the oil sample was kept in a glass bottle in an oven at a temperature of 65 degrees Celsius for 10 days and its peroxide number was measured every 24 hours.[19].

2-10- Statistical analysis

In this research, a completely random statistical design was used. All tests were performed in three repetitions and the average was calculated. The resulting information using analysis of variance (ANOVA(Analysis and in order to determine the difference between the average samples from Duncan's multiple range test at the 5% level)$p < 0.05$) Was used. softwareSPSS 22 It was used for this purpose.

3. Results and Discussion

3-1- Acid number

One of the important factors in checking the quality of edible oils is acid number. A high acid value indicates the hydrolysis of triacylglycerols and the production of free fatty acids. A lot of free fatty acid in the oil can reduce the smoke point and reduce the stability of the oil against oxidation. Lipase enzyme activity, oil storage in inappropriate conditions, and oil heating in the presence of moisture are factors influencing the increase in oil acid number.[20]. Examining the results of the changes in the acid number values on the day of production of black seed oil samples showed that the acid number values of oil samples extracted from black seed oil pretreated with ethanol were lower compared to the control oil sample, and with the increase in the percentage of ethanol in the pretreatment of black seed oil before oil extraction, Cold press method, the acid value of oil samples significantly ($p < 0.05$) Decreased. The decrease in the acid value of the oil can be attributed to the inactivation of lipase due to the destruction of the cell structure. In the research results of oil extracted from black seed pre-treated with heat, a decrease in the acid number of its oil has been reported, which is consistent with the results of this study.[9 and 10].

Also, the results showed that during storage, the acid value of the oil samples significantly ($p < 0.05$) increased and this increase in oil samples extracted from black seed pretreated with ethanol compared to the control sample (mg NaOH/g oil 09/7-4/95) was less (Table 1). The lowest amount of acid number after 90 days of storage (mg NaOH/g oil 4/61) was related to the sample of oil extracted from black seed pretreated with the highest percentage of ethanol. The increase in acid number with the passage of time can be attributed to the increase in the formation of free fatty acids from the hydrolysis of triacylglycerols in black seed oil.[12]. The results of previous studies also indicated an increase in the amount of acid number during 90 days of storage[7، 8 and 21].

Table 1 Effect of ethanolic pre-treatment of seeds on acid value (mg NaOH/g oil) of the black cumin seed oil

Sample*	Storage time (Day)			
	1	30	60	90
Control	4.95 ± 0.02^{and}	5.61 ± 0.02^{dB}	6.47 ± 0.00^{dC}	7.09 ± 0.01^{dD}
1% Ethanol	3.87 ± 0.01^{that}	4.40 ± 0.01^{cB}	5.01 ± 0.04^{cC}	5.23 ± 0.02^{cD}
3% Ethanol	3.85 ± 0.02^{not}	4.24 ± 0.02^{bB}	4.72 ± 0.03^{bC}	4.93 ± 0.02^{bD}
6% Ethanol	3.8 ± 0.00^{aA}	4.02 ± 0.01^{aB}	4.41 ± 0.01^{BC}	4.61 ± 0.01^{aD}

*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol

Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

2-3- Peroxide number

Peroxide number is a measure of oil's resistance to oxidation. The oil with high stability against oxidation will have a low peroxide number and will also have a lower increase during storage or heating.[22 and 23]. Despite the many therapeutic and nutritional properties of black seed oil, this oil has little stability and has a high peroxide value. The peroxide value of this oil is significantly different from other oils extracted from oil seeds. Usually, this oil has a higher peroxide value than other vegetable oils from the moment of extraction. Therefore, it requires the use of different methods in order to control the increase of the peroxide value from the moment of extraction. However, this oil has various antioxidant compounds, the most important of which is a compound called thymoquinone, which has It also has various therapeutic properties [20, 21, 24 and 25].

The results of the peroxide value of the oil samples on the first day of extraction showed that the highest amount of peroxide value was related to the control oil samplewas, while increasing the percentage of ethanol in the pretreatment of black seedBefore cold pressing, the peroxide value of the oil samples significantly($p < 0.05$) decreased (Table 2). Therefore, it can be concluded that ethanol increases the transfer of bioactive compounds and antioxidant substances from seed to oil by destroying the cell structure of black seed. Study results has shownthat pretreatment of black seed with heat before extracting the oil with cold press has been effective in preventing oxidation and reducing the peroxide of extracted oil due to the increase of antioxidant compounds of black seed oil [4, 9 and 10].

The values of peroxide value of oil samples extracted from black seed during storage significantly($p < 0.05$(increased that this increase in the control sample)meqO_2/Kg oil 84/9-10/5) It was higher than other samples, while this increase in peroxide value in oil samples during storage occurred with an increase in the percentage of ethanol in the pretreatment of black seed before cold pressing with a lower slope, so that after 90 days of storage of oil samples, the lowest The amount of peroxide number corresponding to the oil sample extracted from black seed pretreated with the highest percentage of ethanol (meqO_2/Kg oil 03/7) Was. Also, the results showed that the pretreatment of black seed with ethanol before extracting the oil by cold press can be effective in the oxidation stability and shelf life of this oil, which is consistent with the results of the Shawl test. The increasing process of peroxide value can be caused by more oxygen absorption by unsaturated fatty acids and oxidation [26 and 27]. Results consistent with this research have been reported in relation to the increase in peroxide value with the increase in the storage time of black seed oil [7, 8 and 21].

Table 2 Effect of ethanolic pre-treatment of seeds on peroxide value (meq O_2/Kg oil) of the black cumin seed oil

Sample*	Storage time (Day)			
	1	30	60	90
Control	5.10 ± 0.03^{and}	7.37 ± 0.01^{dB}	8.96 ± 0.03^{dC}	9.84 ± 0.01^{dD}
1% Ethanol	4.02 ± 0.02^{that}	5.98 ± 0.03^{cB}	7.37 ± 0.02^{cC}	8.16 ± 0.04^{cD}
3% Ethanol	3.86 ± 0.04^{not}	5.78 ± 0.02^{bB}	7.06 ± 0.01^{bC}	7.74 ± 0.01^{bD}
6% Ethanol	3.50 ± 0.01^{aA}	5.37 ± 0.02^{aB}	6.49 ± 0.01^{BC}	7.03 ± 0.02^{aD}

*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol

Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

3-3- The amount of chlorophyll

Chlorophyll in edible oils in the light causes photooxidation and reduces the stability of the oil to oxidation[28]. The results of the amount of chlorophyll on the day of production showed that by increasing the percentage of ethanol in the pretreatment of black seed before extracting the oil with cold press, the amount of chlorophyll in the oil samples significantly increased.($p < 0.05$) It was found that this increase can be caused by the destruction of the cell structure of nigral cells. Chlorophyll did not have much effect on oxidation due to keeping the oil in dark conditions and also increasing the antioxidant compounds of oil extracted from black seed pretreated with ethanol. The results of previous studies have also shown that the increase in the amount of chlorophyll in black seed oil is associated with the destruction of the cell structure[4 and 9]. Also, the results showed that the amount of chlorophyll in all oil samples extracted from black seed pre-treated with ethanol decreased significantly with the passage of time.($p < 0.05$) found (Table 3). The highest amount of chlorophyll on the 90th day corresponds to the sample of oil extracted from black seed pretreated with the highest percentage of ethanol (mg/Kg oil 21/21) was The reason for the decrease in the amount of chlorophyll during storage can be attributed to its decomposition and destruction during storage. Similar results of the reduction of chlorophyll content of black seed oil during storage have been reported[7 and 8].

Table 3 Effect of ethanolic pre-treatment of seeds on chlorophyll content (mg/Kg oil) of the black cumin seed oil

Sample*	Storage time (Day)			
	1	30	60	90
Control	14.29 ± 0.01^{aD}	14.06 ± 0.02^{BC}	13.75 ± 0.01^{aB}	13.31 ± 0.01^{aA}
1% Ethanol	18.47 ± 0.00^{bD}	18.15 ± 0.01^{bC}	17.71 ± 0.01^{bB}	17.08 ± 0.02^{not}
3% Ethanol	20.31 ± 0.02^{cD}	19.98 ± 0.01^{cC}	19.32 ± 0.01^{cB}	19.10 ± 0.01^{that}
6% Ethanol	22.19 ± 0.01^{dD}	22.54 ± 0.02^{dC}	21.44 ± 0.01^{dB}	21.21 ± 0.00^{and}

*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol

Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

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3-4- The amount of carotenoids

Carotenoids affect oil color as well as oil stability against oxidation and nutritional properties of vegetable oils[29]. The results of the carotenoid content on the day of production of the oil samples showed that the increase in the percentage of ethanol in the pre-treatment of black seed increased the amount of carotenoid in the oil samples compared to the oil sample extracted from untreated black seed.mg/Kg oil 3/30) significantly($p < 0.05$) increased, which is probably due to the destruction of the cell structure of the black seed by ethanol and the possibility of transferring more carotenoid pigment from the seed to the extracted oil. The research results have shown that the increase in the amount of carotenoids in the black seed oil samples was caused by the destruction of the cell structure, which is consistent with the results of this study.[4 and 9].

Carotenoid content of oil samples extracted from black seed pretreated with ethanol during 90 days of storage significantly($p < 0.05$) decreased, which could be due to the

oxidation and decomposition of this compound (Table 4). The highest and lowest amount of carotenoids on the 90th day, respectively, correspond to the sample of oil extracted from black seed pretreated with the highest percentage of ethanol (mg/Kg oil 3/86) and oil sample extracted from untreated black seed (mg/Kg oil 3/06) was Results consistent with the current research have been reported regarding the reduction of carotenoids during 90 days of storage of black seed oil at room temperature.[7 and 8].

Table 4 Effect of ethanolic pre-treatment of seeds on carotenoid content (mg/Kg oil) of the black cumin seed oil

Sample*	Storage time (Day)			
	1	30	60	90
Control	3.30 ± 0.01^{aD}	3.16 ± 0.01^{BC}	3.13 ± 0.01^{aB}	3.06 ± 0.00^{aA}
1% Ethanol	4.08 ± 0.01^{bD}	3.75 ± 0.01^{bC}	3.68 ± 0.00^{bB}	3.60 ± 0.01^{not}
3% Ethanol	4.27 ± 0.01^{cD}	3.87 ± 0.02^{cC}	3.77 ± 0.01^{cB}	3.69 ± 0.00^{that}
6% Ethanol	4.62 ± 0.01^{dD}	4.09 ± 0.01^{dC}	3.93 ± 0.01^{dB}	3.86 ± 0.01^{and}

*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol

Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

5-3- Amount of phenolic compounds

Phenolic compounds are known to be an important factor in the evaluation of oil quality; Because it shows nutritional, organoleptic and antioxidant properties in oil[30 and 31]. Because black seed oil is known as a rich source of antioxidant compounds Like phenolic compounds and tocopherols, it has been used to improve the oxidative stability of other vegetable oils[32].

Examining the results of changes in the amount of phenolic compounds on the day of production of black seed oil samples showed that with the increase in the percentage of ethanol in black seed pretreatment, their phenolic components significantly($p < 0.05$) increased Oil samples extracted from black seed pretreated with ethanol have higher phenolic compounds than the control oil sample (mg caffeic acid/Kg of oil 156/01) had that it could be due to the polarity of phenolic compounds and the denaturation of the proteins attached to them, which facilitates their extraction from black seed into its oil. The results of previous studies showed that heat pretreatment of black seed before cold pressing increased the phenolic compounds of the extracted oil, which is consistent with the results of this study.[4 and 9]. Also, according to the results (Table 5), the phenolic compounds of the oil samples increased significantly during the storage period($p < 0.05$) had that it can be caused by the decomposition of complex phenolic compounds and the production of simple phenolic compounds that can react with folin and show more absorption in spectrophotometry. The highest amount of phenolic compounds on day 90 (mg caffeic acid/Kg of oil 496/30) was related to the oil sample extracted from black seed pretreated with the highest percentage of ethanol. Similar results have been reported regarding the increase in the amount of phenolic compounds with increasing storage time of black seed oil [7].

Table 5 Effect of ethanolic pre-treatment of seeds on total phenolic content (mg caffeic acid/Kg of oil) of the black cumin seed oil

Sample*	Storage time (Day)			
	1	30	60	90

Control	156.01±0.01^{aA}	170.72±0.01^{aB}	184.23±0.01^{BC}	200.07±0.01^{aD}
1% Ethanol	400.02±0.01^{not}	416.03±0.01^{bB}	432.96±0.01^{bC}	450.16±0.01^{bD}
3% Ethanol	406.33±0.01^{that}	424.54±0.01^{cB}	441.63±0.01^{cC}	469.54±0.01^{cD}
6% Ethanol	413.93±0.01^{and}	434.24±0.01^{dB}	454.83±0.01^{dC}	496.30±0.02^{dD}

*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol

Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

3-6- Shawl test

Oxidative stability refers to the resistance of edible oils and fats to oxidative stress and the resulting quality loss. Resistance to oxidative stress is an indicator of performance and shelf life of lipid products. Shawl test shows the stability of oil oxidation during storage and can provide a good prediction of oil stability during heat treatment of oils. The oxidative stability of vegetable oils is affected by various factors such as the composition of fatty acids, antioxidants and minor compounds[7].

The peroxide value of the samples extracted from black seed had a significant trend of increasing, so that after 10 days of heating at 65 degrees Celsius, the value of the peroxide number of oil samples extracted from black seed pretreated with ethanol had a higher stability than the control sample and with increasing The percentage of ethanol in black seed pretreatment increased the oxidation stability of extracted oil (Figure 1), which may be due to the increase of antioxidant compounds and inactivation of oxidative enzymes such as lipase, peroxidase, and lipooxygenase [4, 32]. The high amount of peroxide number on the first day compared to the second day can be due to the unstable nature of some natural compounds in black seed oil that showed themselves instead of peroxide. The results showed that the peroxide value of oil sample extracted from black seed pretreated with 6% ethanol (meqO_2/Kg oil 3/6-69/67) during 10 days of heating, the oil sample was the lowest and also the most stable among all samples, while the control oil sample (meqO_2/Kg oil 78/10-46/5) had significantly the lowest stability among all samples. In a research, an increase in the peroxide value of black seed oil during 10 days of heating at 65 degrees Celsius (shawl test) has been reported, which is consistent with the results of this research [7].

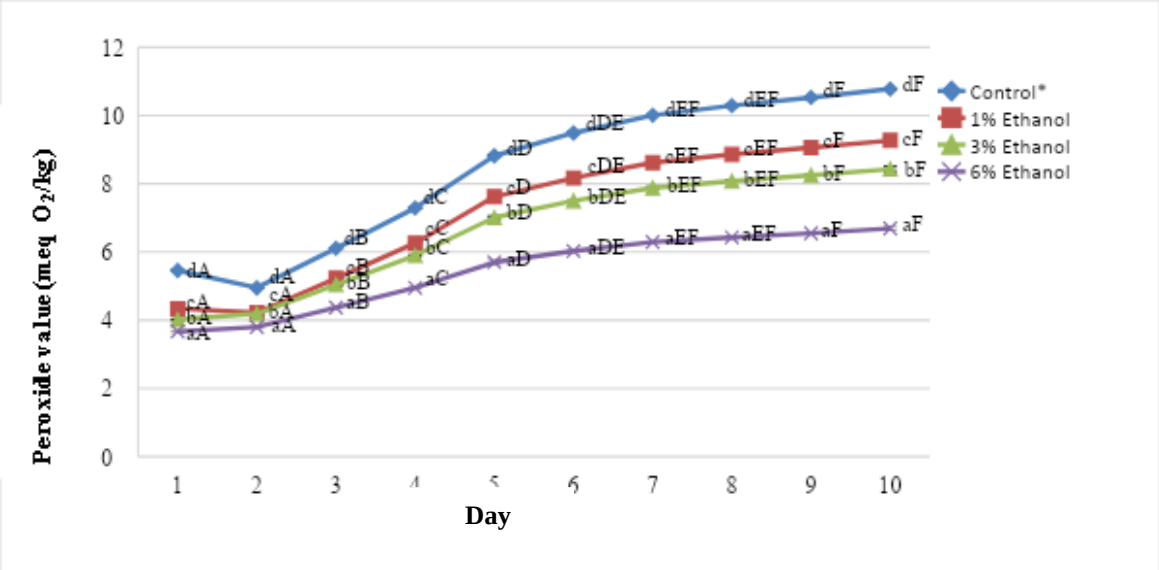

Figure 1 Effect of ethanolic pre-treatment of seeds on the peroxide value (meq O_2/Kg oil) of the black seed oil during Schaal test (10 days at 65 °C).
*Oil extracted from seeds without pretreatment (control sample) and from seeds pretreated with different levels (%) of ethanol
Different small and capital letters shows significantly differences ($p < 0.05$) in columns and rows, respectively.

4 - Conclusion

Despite the many uses of black seed oil in the food, pharmaceutical and cosmetic industries, this oil does not have a good stability and immediately after extraction, it has a high acid value and peroxide value. In this study, it was possible to use ethanol for the pretreatment of black seed before extracting the oil by cold press method in order to control the acid number and peroxide number and increase the stability of the extracted oil. Of course, more studies need to be done on the amount of bioactive and antioxidant compounds extracted from black seed to its oil and the durability of these compounds during storage.

5- References

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تغییرات در ویژگی‌های کیفی روغن استخراج شده با پرس سرد از سیاه‌دانه پیش تیمار شده با محلول اتانولی در طی نگهداری

روشنک حق‌گوی[۱]، صدیف آزادمرد دمیرچی[۲*]، محمدعلی تربتی[۳]، مریم خاکباز حشمتی[۴]

۱- دانش آموخته کارشناسی ارشد، گروه علوم و صنایع غذایی، دانشکده کشاورزی دانشگاه تبریز، تبریز، ایران

۲- استاد، گروه علوم و صنایع غذایی، دانشکده کشاورزی دانشگاه تبریز، تبریز، ایران

۳- دانشیار، گروه علوم و صنایع غذایی، دانشکده تغذیه و علوم غذایی، دانشگاه علوم پزشکی و خدمات بهداشتی- درمانی تبریز، تبریز، ایران

۴- استادیار، گروه علوم و صنایع غذایی، دانشکده کشاورزی دانشگاه تبریز، تبریز، ایران

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* مسئول مکاتبات:

sodeifazadmard@yahoo.com

چکیده

گزارش‌های علمی فراوانی در مورد اثرات سلامت بخشی همچون ضد سرطانی، ضد دیابتی، ضد میکروبی، ضد التهابی و خاصیت آنتی‌اکسیدانی برای روغن سیاه‌دانه منتشر شده است. هر چند، یکی از مشکلات اساسی در استخراج روغن از سیاه‌دانه، بازده پایین و هم‌چنین کیفیت پایین روغن استخراجی است. روش‌های مختلفی همچون تیمار با مایکروویو، برشته‌کردن و رطوبت‌دهی برای بهبود شرایط استخراج و کیفیت روغن سیاه‌دانه قبل پرس سرد انجام شده است. در این مطالعه، سیاه‌دانه با اتانول (۷۰ درصد) در درصدهای صفر (نمونه کنترل)، ۱، ۳ و ۶ درصد پیش تیمار و سپس روغن با پرس سرد استخراج شد. ویژگی‌های کیفی روغن‌های استخراجی (عدد اسیدی، عدد پروکسید، مقدار کلروفیل، مقدار کاروتنوئید و مقدار ترکیبات فنلی) در روز تولید و هر ۳۰ روز در طی ۹۰ روز نگهداری بررسی شد. همچنین پایداری اکسیداتیو روغن سیاه‌دانه با آزمون شال اندازه‌گیری شد. نتایج نشان داد که با افزایش درصد اتانول در پیش تیمار سیاه‌دانه، عدد اسیدی و عدد پروکسید به طور معنی-داری ($p < 0.05$) کاهش و پایداری اکسیداتیو، مقدار کلروفیل، مقدار کاروتنوئید و مقدار ترکیبات فنلی روغن استخراجی به طور معنی‌داری ($p < 0.05$) افزایش یافت. در طی نگهداری، عدد اسیدی و عدد پروکسید نمونه‌های روغن افزایش و مقدار کلروفیل و مقدار کاروتنوئید به دلیل تجزیه مقادیر آن‌ها کاهش یافت. در پایان نگهداری، روغن استخراجی از سیاه‌دانه پیش تیمار شده با بالاترین درصد اتانول، کمترین مقدار عدد اسیدی (۴/۶۱ mg NaOH/g oil) و عدد پروکسید (۷/۰۳ meq O_2/Kg oil) و بیشترین مقدار کلروفیل (۲۱/۲۱ mg/Kg oil)، مقدار کاروتنوئید (۳/۸۶ mg/Kg oil) و مقدار ترکیبات فنلی (۴۹۶/۳۰ caffeic acid/Kg of oil) و پایداری اکسیداتیو را در بین تمام نمونه‌های روغن داشت. در کل نتایج نشان داد که می‌توان برای بهبود ویژگی‌های کیفی و پایدارسازی روغن از سیاه‌دانه پیش تیمار شده با اتانول قبل پرس سرد استفاده کرد.