Research Article

Ali Ashrafi, Sodeif Azadmard-Damirchi*, and Javad Hesari

Quality of oil extracted by cold press from *Nigella sativa* seeds incorporated with rosemary extracts and pretreated by microwaves

https://doi.org/10.1515/gps-2022-8149
received December 14, 2022; accepted February 24, 2023

Abstract: Black cumin (*Nigella sativa*) seed (BS) oil has high a peroxide value (PV) and acid value (AV). In this study, BS was incorporated with different levels of rosemary extracts as a natural antioxidant source before and after pretreatment by microwaves. Based on the oil extraction yield (33%), PV (8.4 meq O_2 per kg oil), and AV (3.2 mg KOH per gram oil), the optimum condition was determined as microwave radiation for 120 s and, after that, moisturizing by the extract at 4% level. AV and PV were lower, and chlorophylls, carotenoids, polyphenols, thymoquinone, and tocopherols content were higher in oil extracted from pretreated BS than oil extracted from control BS. Reduction and loss of bioactive components occurred in oil samples during the 120 days of storage; however, it was higher in the control sample. There were no significant differences ($p < 0.05$) in the fatty acid composition of oil samples; however, oxidation and reduction of linoleic acid in the control oil sample were higher (4.2%) than the oil extracted from pretreated BS (2.5%). In conclusion, the oil extracted from the pretreated BS had higher oil extraction yield, high bioactive components, and stability; therefore, microwave radiation and incorporation of rosemary extract before oil extraction from BS is suggested.

Keywords: extraction, oil, peroxide, quality, yield

*** Corresponding author: Sodeif Azadmard-Damirchi,** Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran, e-mail: sodeifazadmard@yahoo.com, s-azadmard@tabrizu.ac.ir, tel: +98 41 33392032; fax: +98 41 33356005
Ali Ashrafi, Javad Hesari: Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

1 Introduction

Black cumin (*Nigella sativa* L.) is an herbaceous annual plant in the *Ranunculaceae* family that grows widely along the Mediterranean Sea shores. This plant has seeds with slightly bitter, peppery flavor and a crunchy texture. Many nations, including Iran, Syria, Egypt, and Tunisia, use black cumin seed (BS) for both edible and medicinal uses. BS has a high content of oil (25–40%), which has wide applications in the food, pharmaceutical, and cosmetics industries [1]. BS oil has many bioactive components with many valuable positive effects on health [2,3]. Therefore, there is growing interest in using this oil in daily diet. This oil is used in different forms, such as blended with other oils, in the formulation of various food products, or even as a supplement in the encapsulated forms [4,5].

Oil has been extracted from BS using different methods such as press (cold or hot pressing) or using solvents [3,6,7]. However, oil obtained by hot press and solvent has to be refined, which lowers the nutritional value of the oil. Therefore, using press, particularly cold press, is preferred. Generally, BS oils extracted by different methods have high peroxide value (PV) and acid value (AV), which is a serious problem and also it is as an obstacle in the BS oil applications for various purposes [3,8].

There have been different approaches and pretreatments on BS before oil extraction to overcome its low oxidative stability [9]. One method was the extraction of oil from blends of BS with other oilseeds, such as sunflower seeds [5]. Also, roasting and pretreatment by microwaves have been used to inactivate enzymes to lower the oxidation and hydrolysis of BS oil during extraction by press [8]. Extraction of oil from BS blended with rosemary leaves via cold press was another approach to obtain oil with low PV [10]. Results of these studies showed that roasting in the oven or pretreatment by microwaves and also blending with rosemary leaves could be helpful in obtaining high-quality oil by cold press from BS.

Rosmarinus officinalis is a shrub of the *Lamiaceae* family, and the main compounds of its essential oil are 1,8-cineole and α-pinene, comprising about 26.5% and 20%, respectively [11]. Rosmarinic acid, carnosic acid, and carnosol are the main diterpenes of rosemary essential oil with a tremendous antioxidative properties [12]. Rosemary extracts and essential oils are known for their suitable antioxidative properties, which are used in different food formulations, and also in edible oils and fats [13,14]. As the PV and AV of the BS oil are high upon extraction, there should be a stabilizing approach before oil extraction or during oil extraction from BS to ensure the inhibition or reduction of the oxidation and hydrolysis reactions that cause high PV and AV, respectively. Therefore, this study aimed to incorporate the rosemary extract into BS via soaking and moisturizing the seeds before oil extraction by the press. Also, BS containing rosemary extract was exposed to microwaves to study the effect of the combination of extract incorporation and microwave pretreatment on the extracted oil quality.

2 Materials and methods

2.1 Sample

BS samples and dry rosemary leaf were obtained from the local market (Tabriz, Iran). Solvents were obtained from Merck (Darmstadt, Germany). Fatty acid methyl ester standards used in this study included 37 components ranging from C4:0 to C24:1 that were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2 Preparation of the aqueous extract

Dried and powdered rosemary leaves (100 g) were extracted with distilled water (1,000 mL) at room temperature (23°C) for 1 h according to the method described by Afonso et al. [15]. The samples were centrifuged at 15,000 rpm for 10 min. The supernatant was filtered through qualitative filter paper, and water was evaporated to reach a total volume of 50 mL.

2.3 Seed pretreatments

Design expert was used to investigate the effect of two factors: (i) rosemary extract level and (ii) microwave radiation time according to the method described by Mazaheri et al. [8]. Two procedures were applied. (1) Spraying different levels of rosemary extract into seeds in plastic bags and shaking and mixing very well and keeping the seeds for 24 h in the refrigerator for homogenous distribution of the extract on the seeds and then pretreatment with microwaves at different times. (2) Pretreatment with microwaves at different times and then spraying different levels of rosemary extract to seeds in the plastic bags and shaking and mixing very well and keeping the seeds for 24 h in the refrigerator for homogenous distribution of the extract on the seeds. The runs of the experiments are presented in Table 1.

2.4 Microwave pretreatment

For each microwave pretreatment of BS, 1,000 g of seeds were placed in an even layer in Pyrex Petri dishes (26 cm diameter) of the microwave (Model: MW2300 GF, 800 W). Samples were microwave treated at a frequency of 2,450 MHz at the radiation times presented in Table 1 according to the method described by Mazaheri et al. [8].

2.5 Oil extraction by pressing

Oil was extracted from each set of BS samples by using a screw press (Screw Press Model 85 mm, Kern Kraft,

Table 1: Runs of the experiments for conditioning black cumin seeds by rosemary extract and pretreatment by microwave radiation

Run	Conditioning	Microwave time
1	0	0
2	7.3	150
3	4.45	64.5
4	4.45	64.5
5	5.53	0
6	4.45	64.5
7	10	0
8	1.15	49.26
9	8.1	51
10	3.65	123.79
11	5.35	0
12	0	98.16
13	10	99.75
14	0	150
15	10	99.75
16	7.3	150

Germany) at temperatures below 40°C based on an original method described by Mazaheri et al. [8]. Parameters included 12 mm nozzle, 8 mm press head, and rotation speed of 50 rpm. Experiments were carried out on extracted oil samples as soon as possible after oil extraction; otherwise, extracted oil samples were stored at 4°C for a short time.

2.6 Oil extraction yield

The oil extraction yield was calculated based on the weight of obtained oil after pressing and the weight of black cumin seed as follows:

$$\text{Extraction yield}(\%) = \frac{\text{Extracted oil amount(g)}}{\text{Seed weight(g)}} \times 100 \quad (1)$$

2.7 Determination of total phenolic content (TPC)

The TPC of oil samples was determined using the Folin-Ciocalteu method according to the method of Womeni et al. [16] with brief modification. First, 1 mL of oil sample ($1\ \text{mg·mL}^{-1}$) dissolved in ethanol was mixed with 1 mL of diluted Ciocalteu reagent (1:10, v/v) and 3 mL of sodium carbonate 7%. The prepared mixtures were kept in the dark place at an ambient temperature for 30 min. The absorbance of oil mixtures was determined at 760 nm using a UV-Vis spectrophotometer. Also, pure ethanol was used as the control, and gallic acid at a concentration of $0–0.06\ \text{mg·mL}^{-1}$ was used as the standard. Eventually, the TPC of oil mixtures was expressed as milligram gallic acid equivalents (GAE)/gram samples.

2.8 AV

The AV (mg KOH per gram oil) was assessed, using Cd 8-53 method devised by the American Oil Chemists Society (AOCS) [17].

2.9 PV

The PV (meq O_2 per kg oil) was assessed using, Cd 5a-40 method described by the AOCS [17].

2.10 Carotenoid and chlorophyll contents

The contents of chlorophylls and carotenoids were assessed using a spectrophotometer (UNICO, England) by a previously published method [18]. The amount of chlorophyll in the absorption spectra was evaluated at 630, 670, and 710 nm. To assess carotenoid contents, 7.5 g of the oil sample was made up to 25 mL using cyclohexane, and absorption of the contents was then measured at 470 nm. Chlorophyll and carotenoid quantities were calculated using the following equations:

$$\text{Chlorophyll} = 345.3 \times (A670 - 0.5 \times A630 - 0.5 \times A710)/L \quad (2)$$

$$\text{Carotenoid} = A470 \times 106/2{,}000 \times 100 \times L \quad (3)$$

where $A(\lambda)$ is the absorbance, and L is the thickness of the cuvette (10 mm).

2.11 Fatty acid composition

Fatty acid methyl esters (FAMEs) of the oil samples were prepared through transesterification of the oils using methanolic potassium hydroxide and then analyzed by gas chromatography (GC) (Agilent 7890 B, Agilent, USA), using the previously published method [19]. The GC device featured a flame ionization detector, split/splitless injector, and BPX70 capillary column (50 m × 0.22 mm, 0.25 μm). Helium was used as the carrier gas, and nitrogen was used as the make-up gas. Temperatures of the injector and detector were set at 230°C and 250°C, respectively. One microliter of FAME dissolved in *n*-hexane was injected in split injection mode at a split ratio of 25:1. Temperature of the oven was initially set at 158°C for 5 min and then increased to 220°C and retained for 10 min. Detection of FAMEs was carried out by comparing the retention time with that of the relevant standards and peak areas used to calculate the fatty acid percentage.

2.12 Determination of the thymoquinone content

The high-performance liquid chromatography (HPLC) equipment (Agilent 1200, Palo Alto, USA) was used for the quantification of the thymoquinone content of samples. The previously published method was used to determine the method thymoquinone content in the oil samples [20,21]. In brief, 1 g of sample was extracted in 1 mL methanol and vortexed three times for 1 min each time. It was centrifuged at 1,400 rpm for 25 min. The supernatant was separated into a falcon tube and evaporated with nitrogen gas, yielding 500 µL. A 4 mL mobile phase was added to the same falcon tube, vortexed again, then filtered, and a 20 mL aliquot was injected into the Agilent 1260 HPLC's c18 column with a UV detector set to 254 nm. At a flow rate of 1.0 $mL \cdot min^{-1}$, acetonitrile:water (60:40) was used as the mobile phase.

2.13 Tocopherols analysis

The tocopherols composition and content were determined by HPLC (Agilent, Crawford Scientific Ltd., UK) according to the previously published method [19] coupled with the LiChro CART 250–4 column packed with LiChrosphere 100 NH2 (Merck KGaA, Darmstadt, Germany) and Agilent 1260 fluorescence detector (Model: G1321B) with the mobile phase of n-heptane:tert-butyl methyl ether:tetrahydrofuran:methanol (79:20:0.98:0.02; v:v:v:v) mixture at a flow rate of 1.0 $mL \cdot min^{-1}$.

2.14 Statistical analysis

All data were conducted in three replications and provided in mean value and standard deviation. The statistical analysis was performed by one-way ANOVA and the mean difference between the data was implemented by the Duncan test at the probable level of 5% ($p < 0.05$) using SPSS software (IBM SPSS Statistics 23).

3 Results and discussion

3.1 Extraction yield

Rosemary is a rich source of phenolic compounds, which has strong antioxidative properties [13,14]. It has been shown that using a high amount (higher than 5%) of rosemary leaves with BS during oil extraction by the cold press can have an adverse effect on oil stability and play a peroxidative role [10]. In this study, phenolic compounds were extracted from the dry rosemary leaves, and their TPC was 15 mg GAE per gram leaf which was slightly lower than the data reported previously (16.6 mg GAE per gram leaf) [15].

According to the preliminary experiments, it was decided to make an aqueous extract from rosemary leaves (100 g) by using distilled water (1,000 mL) and then the obtained extract was evaporated and concentrated to 50 mL. Addition of this extract to the BS before or after microwave pretreatment was carried out to monitor the effectiveness of its antioxidative properties on the BS oil. Also, these pretreatments effect on the oil extraction yield by the cold press and the quality of the extracted oil were investigated.

Microwave radiation has been used for different types of oilseeds such as BS, canola, milk thistle, and pomegranate seeds [8,22–24]. It has been shown that this pretreatment could be helpful to oil quality and oil extraction yield. It has been reported that microwave radiation of oilseeds for a long period is not suitable as it can cause smoking and spoil the oil quality. Also, the effect of moisturizing on the extraction yield of the BS oil has been shown that pressing and extracting of oil is challenging and gives a very low yield if moisture content higher than 10% in BS [8]. Therefore, in this study, microwave radiation and moisture level were set at 150 min and 10%, respectively (Table 1).

One of the essential parameters in oil extraction from oilseeds is oil extraction yield. The oil extraction yield in the cold-press extraction method is affected mainly by the moisture content of the seed [8,22,25]. Pretreatments before oil extraction should not reduce the oil extraction yield, which can affect the whole procedure economically. Obtained results showed that conditioning and incorporation of the rosemary extract after microwave pretreatment could generally increase at least 10% the oil extraction yield compared to conditioning and incorporation of rosemary extract before the microwave pretreatment (Table 2). Microwave pretreatment likely causes changes such as partial protein denaturation, which prevents from absorption of moisture to the seed structure. This phenomenon can have adverse effect on the emulsification nature of the proteins, which prevents the release of oil from the BS cake and reduces the oil extraction yield.

Obtained results showed that lower and higher extract (moisture) levels could lower the extraction yield (Table 2). However, the radiation period could compensate and reduce the negative effect of the higher moisture content of the seeds on the oil extraction yield. Very low moisture content of the seeds can reduce the flexibility of the seeds and make more frictions which can destroy the pressing equipment and affect the oil extraction yield. Also, seeds get a pasty form in a very high level of moisture, which prevents from the separation of oil from seed cake, resulting a very low oil extraction yield. Therefore, according to the obtained

Table 2: Effects of different levels of rosemary extract and microwave pretreatment of black cumin seeds on the oil extraction yield

Run	Extraction yield	
	Conditioning-microwave	Microwave-conditioning
1	28.8	28.8
2	25.3	27.5
3	27.8	30.2
4	27.8	30.2
5	24	24.0
6	27.8	30.2
7	19.2	19.2
8	29.5	33.1
9	27.5	30.7
10	30	33.0
11	24	24
12	30.5	30.5
13	25.0	26.8
14	27.8	27.8
15	25.4	28.0
16	26.3	27.5

Table 3: Effects of different levels of rosemary extract and microwave pretreatment of black cumin seeds on the AV (mg KOH per kg oil) of extracted oil

Run	Conditioning-microwave	Microwave-conditioning
1	5.6	5.6
2	5.5	5.1
3	5.6	4.9
4	5.6	4.9
5	5.9	5.9
6	5.6	4.9
7	8.6	8.6
8	4.1	3.7
9	5.0	4.9
10	3.2	3.5
11	5.9	5.9
12	2.5	2.4
13	4.5	4.7
14	2.6	2.6
15	4.5	4.9
16	5.5	5.1

results, 3–5% of moisturizing and 50–120 s of radiation give better oil extraction yields (Table 2). It has been reported that the untreated seeds had an oil extraction yield of 21.9%, which is lower than the results obtained in this study which can be due to seed variety and also extraction conditions. These results are in agreement with the data reported for BS pretreated with microwaves [8].

3.2 AV

AV shows the amount of free fatty acids in the oils and fats, which can be increased by the thermal stresses and lipolytic enzyme's reaction in the presence of water. As the presence of a higher amount of free fatty acid can have several adverse effects on the quality of fats and oils such as reducing the smoke point, rapid oxidation, and also limitation in the different food and non-food applications, there is a maximum level of 4 mg KOH per gram oil) for this quality parameter in the Codex Alimentarius standards [26]. The obtained results showed that the conditioning sequence and moisturizing before and after the microwave pretreatment of the BS did not affect the AV of the extracted oil (Table 3). Oil extracted from the BS pretreated by microwave and without moisturizing had the lowest AV among the oil samples and compared to the control sample (without any pretreatments). Also, results revealed that moisturizing, particularly at a high level without microwave pretreatment of the BS could increase the AV of the extracted oil, even oil extracted from the BS with 10% of moisturizing had AV higher than the oil of the control sample (Table 3). Hydrolytic reactions and lipolysis by enzymes such as lipase need enough moisture to be more noticeable. This can be due to the preparation of the favorable condition for the enzymatic activity for lipase to hydrolyze the triacylglycerols and the formation of the free fatty acids. Previously published reports indicated that microwave pretreatment of oilseeds could inactivate the lipases and lower the formation of free fatty acids, and consequently AV [8]. The optimum condition to produce oil from BS with low free fatty acid is using a moisturizing extract of around 5%.

Results also indicated that conditioning with rosemary extract did not reduce the AV in the extracted oil. Formation of the free fatty acid in the BS happens mainly by the enzymatic activity, particularly by lipase. It has been reported that lipase activity in the BS is so intensive, and it is active even in intact seeds and during storage [27]. Also, it has been reported that extracted oil by cold press from BS with rosemary leaves powder did not affect the free fatty acid content.

3.3 PV

BS is a unique seed that has valuable oil with different applications, but it is susceptible to oxidation and hydrolytic reactions even upon extraction. This oil has very high PV and acidity even on production day and increases rapidly during storage in room conditions. Microwave pretreatment can affect the enzymatic activity in the oil seeds and reduce their adverse effects on the extracted oil. Also, the incorporation of antioxidative compounds into oilseeds in addition to microwave pretreatment before extraction can help to prevent effectively different oxidation and hydrolysis reactions.

There are different pathways for the occurrence of oxidation in vegetable oils. Autooxidation, photooxidation, and enzymatic oxidation are the three oxidation pathways that can deteriorate the quality of oils and fats by producing peroxides (primary oxidation products), which can further decompose to the carbonyls (secondary oxidation products). Oxidation products have adverse effects on the oil quality and on its nutritional value. Therefore, there is maximum level for the PV in the standards for oil and fats and also even in the food product containing oils and fats. The maximum PV limit for cold press vegetable oils is 14 meq O_2 per kg oil according to the Codex Alimentarius standards [26]. The obtained results showed that the oil extracted from the BS samples treated for more than 90 s by the microwaves has a lower PV than the maximum PV limit (Table 4). Therefore, it is advisable to use microwave pretreatments as a common way to obtain high-quality BS oil. Previously similar results have also been reported for pretreatment by roasting and microwave; however, microwave pretreatment was favorable due to its efficiency in lowering of the PV in less time [8]. Results also showed that oil extracted from BS soaked with a relatively low amount of rosemary extract, even without microwave pretreatment has lower PV compared to the control sample (without any pretreatments). It shows that a higher amount of rosemary extract can have a prooxidative effect (Table 4). There are several studies claiming the peroxidative effect of high concentrations of antioxidative components such as phenolic compounds, carotenoids, and tocopherols in vegetable oils [28].

Table 4: Effects of different levels of rosemary extract and microwave pretreatment of black cumin seeds on the PV (meq O_2 per kg oil) of extracted oil

Run	Conditioning-microwave	Microwave-conditioning
1	19.5	19.5
2	8.9	10.1
3	19.3	17.4
4	19.3	17.4
5	16	16
6	19.3	17.4
7	25.9	25.9
8	18	15.3
9	19	17.5
10	8.5	9.7
11	16	16
12	18	16.0
13	7	6.5
14	6.8	6.8
15	7	6.5
16	8.9	10.1

3.4 Changes in the BS oil quality during storage

According to the obtained results for extraction yield, which is important from the economical point of view, and PV and AV, which have the maximum limit in the standards and are important from qualitative and nutritional points of view, the optimum conditions for the oil extraction from BS were pretreatment by microwave for 125 s and then moisturizing by the rosemary extract at the level of 4%. This condition gives a higher extraction yield (33%), and a lower PV and AV. Oil extracted from the BS at optimum conditions was analyzed for PV, AV, fatty acid composition, and bioactive components (tocopherols, carotenoids, and thymoquinone) and compared with the oil extracted from the control BS (without any pretreatment) during the storage for 120 days at room condition. The obtained results showed that AV and PV increased in both oil samples. Still, the increment rate was slow in the pretreated sample as it had rosemary extract and was pretreated with microwave, which can increase the antioxidative properties of the extracted oil and inactivate the deteriorating enzymes, respectively (Table 5). However, the AV and PV of the oil extracted from the control sample were higher than the Codex Alimentarius limits even on the production day, but the oil extracted from pretreated seeds was after 30 and 60 days, respectively (Table 5). It is advisable to store the extracted oil at a lower temperature, for example, at a refrigerator condition (<4°C), to extend its shelf life and preserve its quality for a longer time. There have been many reports indicating that the AV and PV of the extracted oil from the BS are higher than the other seed oils even on the production day. The rate of hydrolysis and oxidation was also reported to be higher in the BS oil compared with other fats and oils [4,8,10].

3.4.1 Chlorophyll and carotenoids

Chlorophyll is responsible for photooxidation as it is a sensitizer and can convert the triplet oxygen to singlet oxygen, the very active species of oxygen which can

Table 5: Changes in some qualitative properties of the oil extracted from control and pretreated black cumin seed during storage

Properties	Day									
	1		30		60		90		120	
	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS
AV (mg KOH per gram oil)	5.6^{f*}	3.2^{g}	7.4^{e}	3.8^{g}	15.1^{c}	8.1^{e}	22.3^{b}	10.4^{d}	28.0^{a}	15.7^{c}
PV (meq O_2 per kg oil)	19.5^{f}	8.4^{g}	32.0^{d}	9.1^{g}	38.9^{c}	12.7	43.7^{b}	24.0^{e}	68.3^{a}	39.6^{c}
Chlorophyll (mg·kg^{-1})	12.1^{c}	14.7^{a}	11.0^{d}	14.5^{a}	9.8^{e}	13.7^{b}	9.2^{e}	11.2^{d}	7.0^{f}	8.4^{ef}
Carotenoids (mg·kg^{-1})	3.3^{b}	4.4^{a}	3.0^{c}	3.2^{b}	2.5^{cd}	3.0^{bc}	2.4^{cd}	3.8^{a}	2.5^{cd}	2.9^{c}
Total phenol (mg GAE per kg oil)	220^{e}	317^{c}	218^{e}	352^{a}	234	338^{b}	270^{d}	340^{ab}	251^{a}	329^{bc}

CS – oil extracted from control (black cumin seed without any pretreatment) sample, PS – oil extracted from pretreated black cumin seed.
*Different letters indicate a significant difference in the probability level of 5%.

attack the unsaturated fatty acids and produce peroxides. However, in the dark condition, it is not a prooxidant and even can act as an antioxidant. BS oil had 12–14 mg per kg oil chlorophylls which were higher in the oil extracted from the pretreated seeds as microwave radiation can destroy the cell structure, and its components can be extracted and leached at a higher rate to the oil. This amount of chlorophyll is comparable with most vegetable oils, but it is lower compared with virgin olive and canola oils [29]. Chlorophyll content was reduced by about 30–40% during the 120 days of storage which is predictable as it was also reported previously that this pigment could be decomposed and decreased during the storage of vegetable oils [8,30]. One way to increase the shelf life and oxidative stability of BS oil is to store this oil in the dark condition or perform bleaching to prevent photooxidation as it has a relatively high content of chlorophyll.

Carotenoids give vegetable oils yellow color, and they are effective antioxidants and precursor of vitamin A. BS oil had 3–4 mg carotenoids per kg oil which are comparable to many vegetable oils and lower than palm oil [29]. Carotenoids act as quenchers of oxygen and can also be decomposed to some extent during storage. The amount of these pigments was reduced by 10–20% during the 120 days of storage (Table 5).

3.4.2 Polyphenols

BS oil is a rich source of polyphenols, and it is one of the unique oils among the vegetable oils containing a relatively high content of polyphenols comparable to virgin olive oils [2,4,31]. There are many reports on the antioxidative and health-effective properties of these compounds [32,33]. Oil extracted from the pretreated BS had higher content of phenolic compounds as it was soaked and moisturized with the rosemary extract, which is a rich source of phenolic compounds. This higher content of phenolic compounds can have a profound effect on the oxidative stability and nutritional properties of the BS oil [4]. BS oils had 220–317 mg polyphenols per kg oil on the extraction day; however, it was increased by about 10% until the 90 days of storage due to the decomposition of complex polyphenols and formation of simple phenolic compounds, which reacted with Folin-Ciocalteu reagent and were measured by the spectrophotometer. There were also reports on the increase in phenolic compounds content during the storage due to the mentioned reasons in the oil extracted from different oilseeds [25,30]. However, the phenolic compounds amount in BS oils decreased after 90 days of storage. It is advisable to extract and produce high-quality BS oil with low AV and PV to avoid the refining process, because the phenolic compounds, as an advantage in BS oil, can be reduced and lost during the different refining steps.

3.4.3 Thymoquinone

Thymoquinone is an essential bioactive component of BS oil, and many of its positive health effects are related to this compound [3,21]. There were no significant differences between the oil extracted from the control and pretreated BSs. BS oil samples had higher than 1,600 mg thymoquinone per kg oil), which was reduced by about 20–30% during the 120 days of storage (Table 6). The reduction in thymoquinone in the oil extracted from control BS was higher compared with the oil extracted from the pretreated BS (Table 6). Thymoquinone may be decomposed and reduced, which can be delayed by the antioxidative properties of the phenolic compounds [3,21]. The results of this study show that using the rosemary extract

Table 6: Changes in tocopherols and thymoquinone contents (mg·kg^{-1}) of the oil extracted from control and pretreated black cumin seed during storage

Compound	Day			
	1		120	
	CS	PS	CS	PS
Thymoquinone	1,628^{b*}	1,663^{a}	1,150^{d}	1,270^{c}
α-Tocopherol	45^{b}	57^{a}	31^{d}	38^{c}
β-Tocopherol	1.0^{b}	1.8^{a}	1.0^{b}	1.5^{ab}
γ-Tocopherol	214^{b}	238^{a}	158^{d}	190^{c}
δ-Tocopherol	1.2^{b}	2.5^{a}	1.0^{b}	2.3^{a}

CS – oil extracted from control (black cumin seed without any pretreatment) sample, PS – oil extracted from pretreated black cumin seed.
*Different letters indicate a significant difference in the probability level of 5%.

Table 7: Changes in the fatty acid composition (%) of oil extracted from control and pretreated black cumin seed during storage

Fatty acid	Nomenclature	Day			
		1		120	
		CS	PS	CS	PS
C16:0	Palmitic acid	12.1^{c*}	12.2^{c}	14.1^{a}	12.8^{b}
C18:0	Stearic acid	1.9^{d}	2.8^{bc}	2.5^{c}	3.5a
C18:1	Oleic acid	22.9^{b}	23.5^{b}	25.0^{a}	24.5^{a}
C18:2	Linoleic acid	58.4^{a}	57.8^{a}	54.2^{c}	55.3^{b}
C20:2	Eicosadienoic acid	2.4^{a}	2.5^{a}	2.3^{ab}	2.2^{b}

CS – oil extracted from control (black cumin seed without any pretreatment) sample, PS – oil extracted from pretreated black cumin seed.
*Different letters indicate a significant difference in the probability level of 5%.

can delay and reduce thymoquinone oxidation and decomposition during storage. Therefore, pretreatment of BS can produce a more stable, health effective oil with higher nutritional value.

3.4.4 Tocopherols

Analyzing the tocopherol composition of BS oils showed that this oil has a high content of γ-tocopherol followed by α-tocopherol, while other tocopherol isomers were detected in meager amounts. The obtained results are in agreement with the previously published data [34–36]. Oil extracted from pretreated BS had a higher content of tocopherols, it has also been reported that microwave pretreatment of different oilseeds such as canola, milk thistle, BS, and common ash (*Fraxinus excelsior* L.) could increase the bioactive components such as tocopherols and sterols in the extracted oils due to cell membrane rupture and easy release of bioactive components to the oil phase [30]. Tocopherols have vitamin E activity and many positive health effects. These compounds can act as a natural antioxidant and delay vegetable oil oxidation; however, they can also decompose and, to some extent, reduce during storage, as observed in this study (Table 6).

3.4.5 Fatty acids

Fatty acid composition of fats and oils is a very vital index in the evaluation of its nutritional quality, stability to oxidation, and its application. BS oil had a high content of linoleic acid (58%), followed by oleic and palmitic acids with other fatty acids in minor amounts (Table 7). Results showed that there were no significant differences ($p < 0.05$) in the fatty acid composition of oil extracted from control or pretreated BS samples. However, linoleic acid as a polyunsaturated fatty acid was oxidized in higher amount in the oil extracted from control BS due to its lower antioxidant components, such as phenolic compounds and carotenoids. Linoleic acid was reduced from 58.4% to 54.2% and from 57.8% to 55.3% in the oil extracted from the control sample, and pretreated sample, respectively (Table 7). It is well known that polyunsaturated fatty acids are susceptible to oxidation during the storage of vegetable oils, and there is a need to add antioxidants to retard and reduce their loss and oxidation.

The main nutritional drawback of BS oil is its trace amount of linolenic acid, which is an ω3 essential fatty acid, according to the fatty acid composition (Table 7), as there should be a suitable balance in the intake of ω3 and ω6 essential fatty acids. There could be a suggestion to blend this oil with oils high in linolenic acid, such as canola, soybean, and flaxseed oils, to enrich its ω3 fatty acid content to extend its use [1,37,38].

4 Conclusion

BS has a high content of oil, which can be extracted at a high rate by pretreating with microwave at an optimum moisture content. Results showed that oil with good stability and higher content of antioxidative compounds could be obtained by microwave pretreatment for 120 s and a 4% of rosemary extract level. Oil extracted from the untreated BS was not consumable as an edible oil without refining according to the Codex Alimentarius limits for AV and PV, but oil extracted from treated BS has a suitable quality for 60 days of storage at room condition. The reduction in bioactive components was also lower in the oil extracted from pretreated Bs compared to the control one. It is advisable to do further research on the storage of the oil extracted from treated BS in lower temperatures and under a blanket of N2 to extend its shelf life and preserve its quality during storage.

Acknowledgements: The authors appreciate the support of University of Tabriz to accomplish this research.

Funding information: This study was funded by University of Tabriz, Tabriz, Iran, Grant number of 22139-1.-P.

Author contributions: Ali Ashrafi: formal analysis, writing – original draft, and investigation; Sodeif Azadmard-Damirchi: conceptualization, investigation, supervision, and methodology; Javad Hesari: supervision, writing – review and editing, and conceptualization.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: Data are contained within the article.

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