GRASAS Y ACEITES 74 (1)
January-March 2023, e493
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0908212

Oxidative stability and compositional characteristics of oil from microwave irradiated black cumin seed under accelerated oxidation condition

J. Hossen[a], M. Abbas Ali[a,✉], N. Hidayu Othman[b] and A. Md Noor[b]

[a]Department of Chemistry, Rajshahi University of Engineering & Technology, Rajshahi- 6204, Bangladesh

[b]Department of Processing Technology, Oils & Fats, Sime Darby Research Sdn. Bhd., Carey Island, Selangor, Malaysia.
✉Corresponding author: radwiya44@yahoo.com

Submitted: 29 September 2021; Accepted: 05 May 2022; Published online: 22 March 2023

SUMMARY: The present work evaluated the impact of microwave pre-treatment on the storage stability, fatty acids and triacylglycerol contents in black cumin seed oil (BCO) during storage at 62 ºC. During storage, the oxidative indicator values (free acidity, peroxide value, *p*-anisidine value, TOTOX, specific extinctions and thiobarbituric acid) for the oils increased faster in untreated oil samples than in the microwaved samples. The degradation rate of polyunsaturated fatty acids (PUFAs) and triacylglycerol species (LLL and OLL) during storage were higher in untreated samples compared to treated ones, indicating that oxidation proceeded more slowly in the treated samples. During storage, the generation of hydroperoxides, their degradation and the formation of secondary oxidation products as investigated by FTIR, were lower in the treated oils. In conclusion, microwave pre-treatment prior to oil extraction reduced the oxidative degradation of oil samples, thereby increasing the storage stability of BCO.

KEYWORDS: *Black cumin seed oil; Fatty acids; Microwave pretreatment; Oxidative stability*

RESUMEN: ***Estabilidad oxidativa y composición del aceite de semillas de comino negro, irradiadas con microondas, en condiciones de oxidación acelerada*.** En el presente trabajo se evaluó el impacto del pretratamiento de las semillas de comino negro con microondas sobre la estabilidad durante el almacenamiento, los ácidos grasos y las especies de triacilgliceroles del aceite de las semillas de comino negro (BCO) durante el almacenamiento a 62 ºC. Durante el almacenamiento de los aceites, los indicadores oxidativos (acidez libre, peróxidos, *p*-anisidina, TOTOX, extinciones específicas y ácido tiobarbitúrico) aumentaron más rápidamente en los aceites de semillas sin tratar que en los de las muestras tratadas con microondas. La degradación durante el almacenamiento de los ácidos grasos poliinsaturados (PUFA) y las especies de triacilgliceroles (LLL y OLL) fue mayor en las muestras no tratadas en comparación con las tratadas, lo que indica que la oxidación avanzó más lentamente en las muestras tratadas. Durante el almacenamiento, la generación de hidroperóxidos, su degradación y la formación de productos de oxidación secundarios investigados por FTIR, fueron menores en los aceites tratados. En conclusión, el pretratamiento con microondas de las semillas antes de la extracción del aceite redujo la degradación oxidativa de los aceites, lo que aumentó la estabilidad de almacenamiento de BCO.

PALABRAS CLAVE: *Aceite de semilla de comino negro; Ácidos grasos; Estabilidad oxidativa; Pretratamiento de microondas*

Citation/Cómo citar este artículo: Hossen J, Abbas Ali M, Hidayu Othman N, MD Noor A. 2023. Oxidative stability and compositional characteristics of oil from microwave irradiated black cumin seed under accelerated oxidation condition. *Grasas Aceites* **74** (1), e493. https://doi.org/10.3989/gya.0908212

1. INTRODUCTION

Nigella sativa Linn. is an annual herb cultivated mostly in South and Southwest Asia. The used part is the seed known as black cumin seed, which is utilized worldwide for edible and medicinal applications. The seed contains a high amount of oil which may play a significant role in health and nutrition because of its fatty acid composition (FAC), polyphenol compounds, volatile oil and other important phytochemicals (tocols, sterols and polar lipids) (Ramadan, 2013; Piras *et al.,* 2013). The black cumin seed and its oil (BCO) have medicinal and therapeutic benefits (Mazaheri *et al.,* 2021). The consumption of BCO obtained from pre-treated seeds has a wide range of possible applications in the pharmaceutical and food industries. BCO is dominated by unsaturated fatty acids, especially PUFAs (59.7%), followed by monounsaturated fatty acids (24.1%), and saturated fatty acids (16.1%) (Kiralan *et al.,* 2020). The quality of oil highly depends on its processing methods or conditions.

Several pre-treatment processes for seeds, such as freeze-thaw, infrared, UV irradiation, rapid gas decompression, ultrasonic baths, and microwave, are applied for edible seeds to enhance the extraction of bioactive phytochemicals, and accessibility of favorable nutraceuticals (Zhang *et al.,* 2020; Fathi-Achachlouei *et al.,* 2019; Kiralan *et al*., 2016). Among them microwave irradiation is used as an impressive technique in the food industry which may results in various physicochemical alterations such as oxidation stability, flavor, fatty acid concentration, tocols, antioxidative status, bioactivity, color and nutritional properties (Ali *et al.,* 2017a; Ali *et al.,* 2017b; Fathi-Achachlouei *et al.,* 2019; Karrar *et al.,* 2020). Microwave irradiation has gained in popularity, because it needs very short processing time compared to traditional heating methods (Đurđević *et al.,* 2017). The microwave irradiation of black cumin seed can affect its phytochemical composition, quality and oxidative stability, and understanding the impact of microwave irradiation on oilseeds is of great importance. To optimize BCO yield and further increase its quality and oxidative stability, optimum time and microwave power combination needs to be established. To date, few research works have been determined the oxidation degradation and physicochemical characteristics of microwave roasted BCO without applying any heat or storage treatment of the seed oil (Mazaheri *et al.,* 2019; Suri *et al.,* 2019; Bakhshabadi *et al.,* 2017). This work has therefore been considered to determine the impact of microwave irradiation prior to oil extraction on the oxidative stability, fatty acids and triacylglycerol contents in BCO under accelerated oxidation conditions.

2. MATERIALS AND METHODS

2.1. Materials

Black cumin seed (2.5 kg) was bought from Rajshahi, Bangladesh. The seed was cleaned, dried in the shade at ambient temperature, and preserved at 4 °C in a refrigerator. The chemicals and solvents used were of analytical grade. Thioburbituric acid was product of HiMedia Laboratories (Mumbai, India). Acetic acid and standards were bought from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals or reagents were purchased from Merck (Darmstadt, Germany or Mumbai, India) unless otherwise stated.

2.2. Pre-treatment and oil extraction

The cumin seed samples were spread on the Pyrex petri dishes (12 cm diameter) set on a turntable plate of the microwave oven (MS3042G, LG, China). After covering the dishes, the samples were then microwaved at a frequency 2450 MHz (capable of producing 580 W power) for 1, 2, and 3 min depending on trial results. After pre-treatment, the samples were allowed to cool to 25 °C and thoroughly mixed. Oils from untreated and treated whole black cumin seeds were obtained by pressing using a locally-made mechanical pressing machine at room temperature (27 °C). The temperature of the outflowing oil was around 40 °C. After filtering to remove particles, the oils were weighed and stored in capped glass bottles at a temperature below −15 °C for analysis.

2.3. Accelerated oxidation of oil samples

The untreated or treated BCOs (75 g) were placed in 100-mL glass beakers, and beakers were put in an incubator at 62 °C for accelerating oil oxidation. The oils were withdrawn at regular intervals of 0, 7, 14, and 21 days.

2.4. Oxidative indices

The American Oil Chemists' Society (AOCS, 1987) methods were applied to estimate free fatty acids (FFA) (method Ca 5a-40), peroxide value (PV)

(method Cd 8-53), and thiobarbituric acid value (TBA) (method Cd 19-90). Following the PORIM (PORIM, 1995) test methods, specific extinctions (method p2.15) at 233 and 269 nm ($E^{1\%}_{233}$ and $E^{1\%}_{269}$) and *p*-anisidine value (*p*-AV) (method p2.4) of the oils were estimated using a spectrophotometer (T 60, PG Instruments, Leicestershire, UK). The oxidative value was determined by Holm's equation: TOTOX = 2PV + *p*-AV (Wai *et al.,* 2009).

2.5. Color development

The absorbance of a 5.0% (w/v) oil solution in chloroform was computed at 420 nm with a spectrophotometer (T 60U, PG Instruments, Leicestershire, UK) indicating an index of color formation, (Yoshida *et al.,* 1999).

2.6. Fatty acid composition (FAC)

The FAC was estimated after the preparation of methyl esters using the PORIM (PORIM, 1995) test method p3.4. A gas chromatography (Clarus 590 GC PerkinElmer, USA) equipped with a flame ionization detector was used to determine the FAC oil samples. Helium gas was passed (2 mL/min) as carrier gas. Fatty acids were separated on a 0.25 mm i.d. × 30 m × 0.25 µm capillary column (Elite-FFAP). Analysis was carried out at an initial oven temperature 120 °C which was raised to 240 °C at 4 °C/min. The injector and detector temperatures were controlled at 120 °C and 250 °C, respectively. The peaks were identified by comparison with the standards (methyl arachidate, methyl behenate, methyl decanoate, methyl *cis*-13-docosenoate, methyl dodecanoate, methyl linoleate, methyl linolenate, methyl myristate, methyl octanoate, methyl oleate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl tetracosanoate) (Sigma-Aldrich Co., St. Louis, MO, USA).

2.7. Triacylglycerol (TAG) molecular compounds

The concentrations in molecular TAG compounds present in seed oils were determined by a HPLC system (Agilent 1260 Infinity, USA) equipped with a column (50 mm x 4.6 mm i.d x 2.7 µm) packed with Poroshell 120 EC-C18 (Agilent, USA) and evaporative Light Scattering Detector (ELSD). The solvent system, acetone/acetonitrile (65:35, v/v) was used as a mobile phase at a flow rate of 1 mL/min. The concentrations in TAG species were determined by using standards (POL, OOL, POO, OLL, PLL, MOL, OOO, PLP, POP, SOO and POS, where P- palmitic, M- myristic, O- oleic, L- linoleic) (Sigma-Aldrich Co., St. Louis, MO, USA).

2.8. FT-IR spectroscopy

The FTIR spectra of oils were measured by a Fourier Transform Spectroscopy (IRAffinity- 1S, Shimadzu Corporation, Kyoto, Japan) furnished with a high-sensitivity pyroelectric detector (deuterated L-alanine doped triglycine sulphate). Samples were applied to a sodium chloride cell and periodic scans (15 scans and 4 cm^{-1} resolution) were performed in the spectral range of 850-4000 cm^{-1}. The spectra were computed as absorbance values at each data point.

2.9. Statistical analysis

The data were declared as the mean and standard deviation (SD) of triplet determinations. One way analysis of variance (ANOVA) was performed, and mean values were compared at $p < 0.05$ significance level by Duncan's multiple range test using IBM SPSS 22 statistics.

3. RESULTS AND DISCUSSION

The moisture content in black cumin seed was 5.50% (DM), which reduced to 4.10, 2.60 and 1.10% (DM) with treatment times 1, 2 and 3 min respectively. The quantities of crude oil ranged from 27.65% (DM) for fresh sample to 35.54% (DM) for the 3-min microwaved one. The oil content increased with increasing pre-treatment times. Mazaheri *et al.* (2019) and Bakhshabadi *et al.* (2017) also reported that the oil yield in black cumin seed increased with increasing seed pre-treatment times. The microwave pre-treatment of seeds enhanced extraction efficiency and mass transfer coefficients of the seeds due to the severely ruptured cell membranes. A permanent pore was formed in the seed which allows oil migration through the permeable cell walls (Azadmard-Damirchi *et al.,* 2010).

3.1. Oxidative indices

The concentrations of FFA in the oil samples increased significantly ($p<0.05$) as a result of increasing accelerated storage time (Figure 1a). This

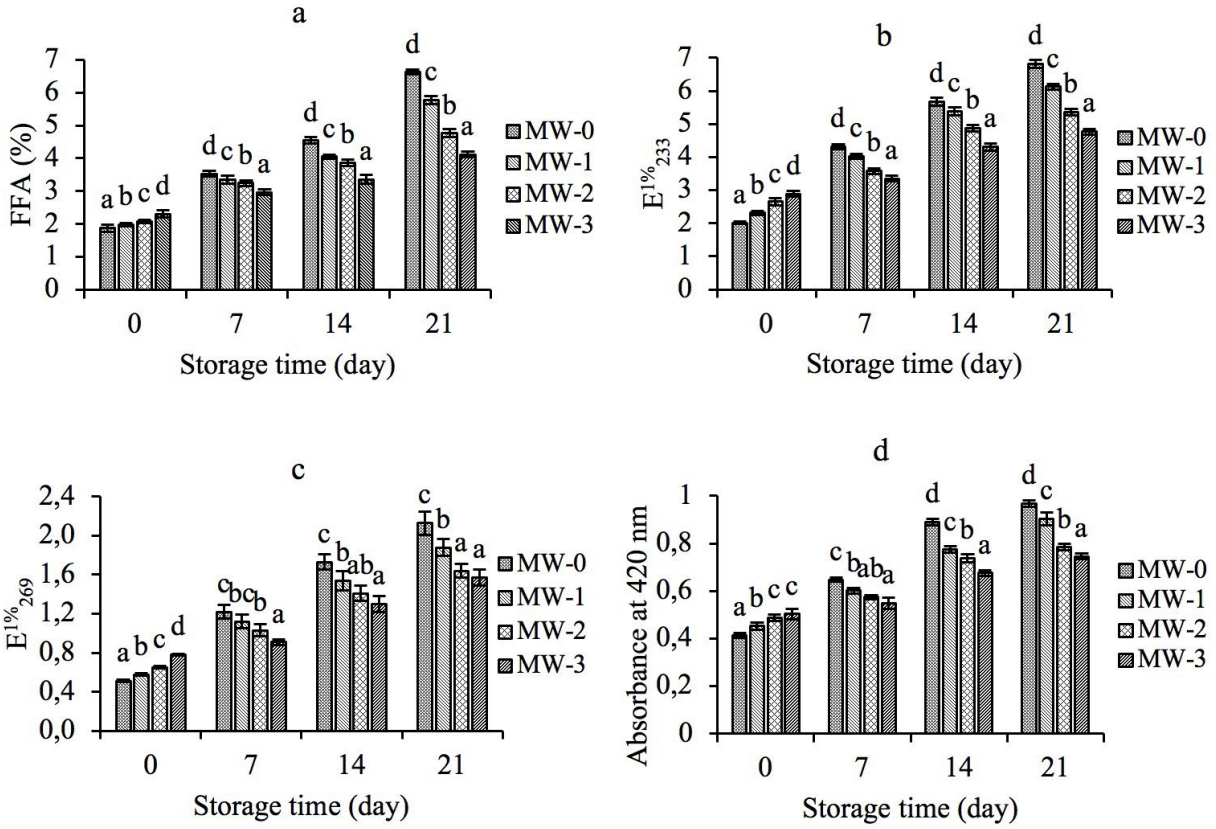

FIGURE 1. Changes in FFA (a), specific extinction at 233 nm, (b) specific extinction at 269 nm (c), and color value (d) of untreated and microwave pre-treated (MW-1, pre-treated for 1 min; MW-2, pre-treated for 2 min; and MW-3, pre-treated for 3 min) black cumin seed oils during storage. Each value is the mean ± standard deviation of triplicate determinations. Mean values were compared by Duncan's multiple range test. Values in each storage time grouped with different letters on bars are significantly different ($p < 0.5$).

hydrolytic degradation was noted to be biggest in the untreated sample (6.62%) with the lowest in the 3 min microwaved sample (4.12%) at the end of 21 days of storage. It also indicated that the higher initial FFA content in the sample microwaved for 3 min, did not affect the hydrolytic degradation of BCO during storage at 62 °C. Microwave pre-treatment had a significant impact on ultraviolet absorptions at 233 ($E^{1\%}_{233}$) and 269 nm ($E^{1\%}_{269}$) in the oils (Figures 1b and 1c). The absorptions were significantly ($p<0.05$) enhanced for all the oils throughout the storage treatments. At the end of 21 days of storage, the concentrations in conjugated dienes and trienes were the greatest in the untreated samples, with the lowest found for the 3-min microwaved samples. The lower values for absorptions indicate better storage stability of treated samples compared to untreated samples. In addition, the concentration of conjugated diene was higher than triene in all oils expressed by the biggest value for $E^{1\%}_{233}$ at 233 nm. Ali *et al.* (2017a) also followed a similar trend for pumpkin seed oil. Pre-treatment and storage conditions employed in this research affected the formation of color in the oils (Figure 1d). Browning substances were generated during microwave irradiation and storage treatment which resulted in a significant ($p < 0.05$) increment in absorbance at 420 nm. The absorbance values limited from 0.41 to 0.50 at 420 nm, were enhanced markedly ($p <0.05$) during incubation and these increments were detected to be higher in the untreated oils. The formation of Maillard reaction products at the storage temperature might be responsible for the color increments in the oils during storage. However, longer seed pre-treatment time had a bigger impact on the further reduction in oxidative stability. Thus, the present findings support the earlier work done by Ali *et al.* (2017b), where they indicated that oil color increased with increasing seed pre-treatment time or heating time of a groundnut oil sample.

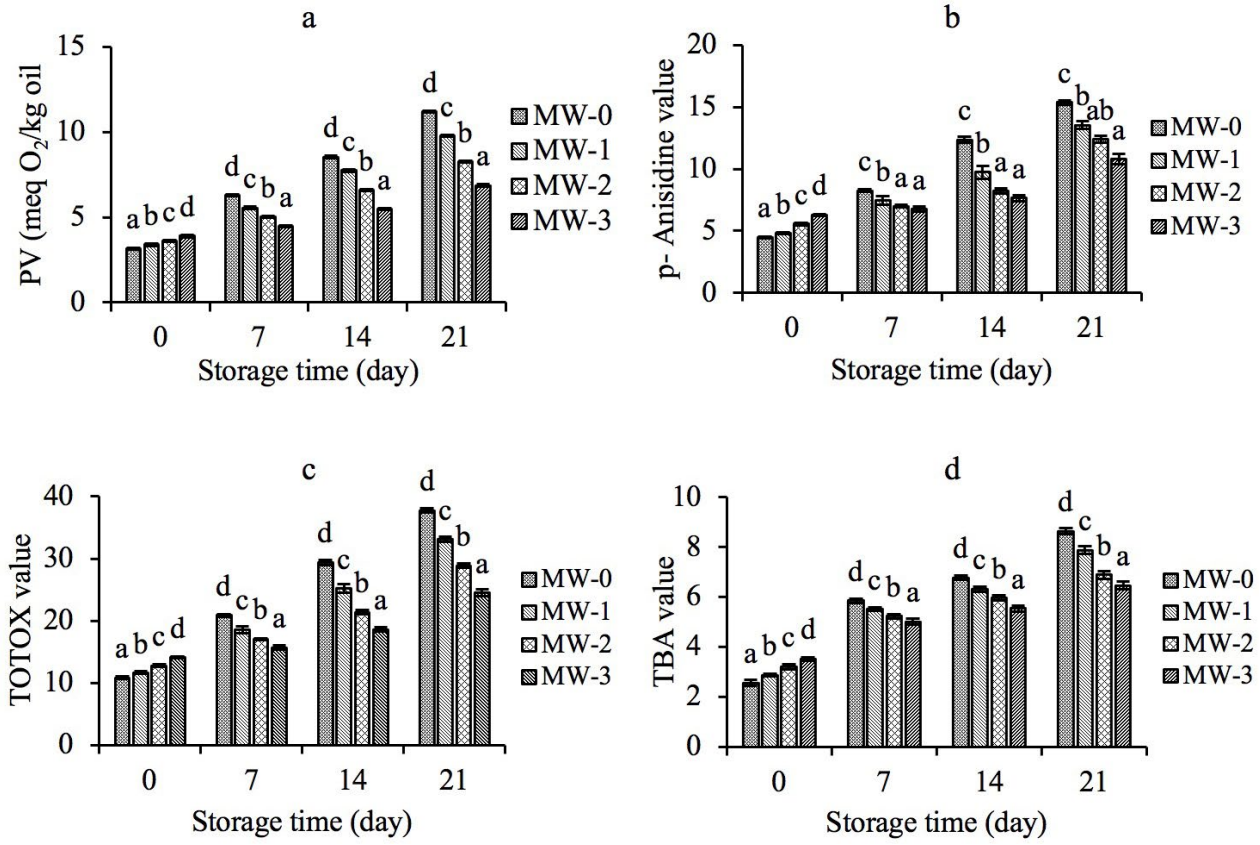

FIGURE 2. Changes in peroxide value (a), *p*-Anisidine value (b), TOTOX value (c), and TBA value (d) of untreated and microwave pre-treated (MW-1, pre-treated for 1 min; MW-2, pre-treated for 2 min; and MW-3, pre-treated for 3 min) black cumin seed oils during storage. Each value is the mean ± standard deviation of triplicate determinations. Mean values were compared by Duncan's multiple range test. Values in each storage time grouped with different letters on bars are significantly different ($p < 0.05$).

Figure 2a indicates the formation of unstable oxidative substances determined by peroxide value (PV) which was found to be faster in the raw sample than the treated samples during the storage oxidation. Oils from pre-treated samples (6.88 meq O_2/kg) had the lowest concentrationa in hydroperoxide than that of the raw one (11.20 meq O_2/kg) at the end of 21 days incubation. Ali *et al.* (2017b) also found a slower increment in PV for treated groundnut seed oil compared to the unroasted sample during thermal oxidation. In addition, at the initial phase of the storage period, samples from the microwave pre-treated oils had slightly higher PV (3.40-3.90 meq O_2/kg); while in the untreated oils it was relatively low (3.17 meq O_2/kg). The higher value could be the result of the exposure time at an elevated temperature during the microwave pre-treatment of the seed samples. Microwave pre-treatment itself was reported to cause slight oil oxidation during prolonged heating (Anjum *et al.,* 2006). The PV and *p*-AV are normally used to determine the degree of lipid oxidation. The pre-treatment of seeds decreased the *p*-AV significantly ($p < 0.05$) in BCO compared to the raw samples under accelerated oxidation conditions (Figure 2b). Ali *et al.* (2017b) reported similar results after 9 h heating of oil extracted from microwaved groundnut seed. In the present work, the *p*-anisidine values (*p*-AVs) from the lowest to the highest (10.77 to 15.36), were followed in oils pre-treated at 3, 2, 1 and 0 min after 21 days of accelerated storage. This indicates an extended shelf-life of oils produced from microwaved seeds, probably through the formation of Maillard reaction products (MRPs). Figure 2c shows the marked differences in total oxidation values (TOTOX) in the BCOs under storage conditions. The oil samples of untreated seeds displayed the highest TOTOX values, which revealed that the microwave irradiation reduced the formation of oxidative products during storage at 62 °C. The changes in the thiobarbituric acid (TBA) level of treated BCOs were significantly lower ($p<0.05$)

than that of the untreated seed oil (Figure 2d). Before the storage of oils under accelerated conditions, the TBA values at 0, 1, 2 and 3 min for pre-treated samples were 2.56, 2.87, 3.21 and 3.52, respectively, and after 21 days, the TBA values were increased to 8.64, 7.88 6.89 and 6.47, respectively. This reveals that the oil samples from untreated seeds were more susceptible to oxidation at storage temperature than the oil samples from treated seeds. In addition, a sharp increase in TBA values was detected at an earlier stage of storage followed by a decrease. This can be due to the volatilization of secondary oxidation products or their breakdown.

3.2. Fatty acid composition

The changes in fatty acid composition (FAC) in the oil may indicate its stability, physical properties and nutritional attributes. The dominant fatty acids in BCO were mainly oleic, linoleic and palmitic acids with percentages of 23.25, 57.94 and 13.43, respectively, and myristic, palmitoleic, stearic, linolenic, behenic and lignoceric present in concentrations of less than 1% (Table 1). Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in fresh BCO amounted to 15.01, 23.46 and 58.51% of the total fatty acids, respectively. The FAC suffered small changes upon microwave pre-treatment. During pre-treatment, the concentration in C18:2 decreased slightly; whereas the concentrations of C16:0 and C18:0 increased slightly. The PUFA degradation may cause this trend, and Ali *et al.* (2017a) saw a similar trend in the case of pumpkin seed oil during thermal oxidation. As can be seen in Table 2, the relative content in PUFA was reduced; while that of SFA or MUFA increased in BCOs during storage treatment. Ali *et al.* (2017b) followed a similar trend in groundnut oil during heating at 170 °C. However, the changes in fatty acid concentrations were lower in microwaved samples than in untreated ones during storage at 62 °C. Suri *et al*. (2019) also reported that dry air roasting slightly influenced the FAC of BCO. The greatest reduction in PUFA was recorded for fresh BCO (7.90%) and the lowest for 3-min microwaved sample (3.20%) after 21 days of storage. The present data indicate that the change in FAC in the pre-treated samples was smaller compared to the raw sample; this indicates the higher tendency of fresh sample towards the generation of oxidation products and polymerized compounds by oxidation degradation of PUFAs. In addition, the ratio of PUFA to SFA (P/S) of all samples decreases with increasing storage time, which serves to realize the status of oxidative tendency of lipids (Lee *et al.,* 2007). In this regard, the pre-treated samples showed the lowest P/S ratio change (decrease) which indicates that oxidative reactions progressed more rapidly in raw samples than in microwaved samples during storage.

Table 1. Fatty acid composition (%) of untreated and microwave pre-treated black cumin seed oils before storage

	Pre-treatment time (min)			
Fatty acids	**0**	**1**	**2**	**3**
Myristic acid (C14:0)	0.17±0.01^{a}	0.17±0.02^{a}	0.16±0.01^{a}	0.16±0.01^{a}
Palmitic acid (C16:0)	13.43±0.21^{c}	13.03±0.15^{b}	12.52±0.19^{a}	14.19±0.18^{d}
Palmitoleic acid (C16:1)	0.21±0.02^{c}	0.22±0.02^{c}	0.18±0.01^{b}	0.15±0.01^{a}
Stearic acid (C18:0)	0.50±0.03^{ab}	1.20±0.10^{c}	0.47±0.05^{a}	0.61±0.04^{b}
Oleic acid (C18:1)	23.25±0.14^{a}	23.36±0.18^{a}	23.39±0.22^{a}	24.31±0.43^{b}
Linoleic acid (C18:2)	57.94±0.39^{b}	57.24±0.42^{b}	55.92±0.37^{a}	56.09±0.32^{a}
Linolenic acid (C18:3)	0.57±0.01^{b}	0.55±0.02^{ab}	1.45±0.03^{c}	0.51±0.04^{a}
Behenic acid (C22:0)	0.57±0.02^{a}	0.58±0.03^{a}	1.29±0.02^{b}	0.56±0.09^{a}
Lignoceric acid (C24:0)	0.34±0.02^{a}	0.42±0.02^{b}	1.91±0.03^{c}	0.46±0.02^{b}
∑Saturated fatty acids	15.01	15.40	16.35	15.98
∑Monounsaturated fatty acids	23.46	23.57	23.57	24.46
∑Polyunsaturated fatty acids	58.51	57.79	57.37	56.69

Each value is the mean ± standard deviation of triplicate determinations. Mean values were compared by Duncan's multiple range test. Values within a row with the same letters are not significantly different at $p < 0.05$

Table 2. Saturated, monounsaturated, and polyunsaturated fatty acids of untreated and microwave pre-treated black cumin seed oils during storage

Pre-treatment time (min)	Storage time (days)	Fatty acid composition (%)			
		Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	P/S
0	0	15.01 (100)	23.46 (100)	58.51 (100)	3.9
	7	15.19 (101.2)	24.01 (102.3)	57.91 (99.00)	3.8
	14	19.24 (128.2)	23.53 (100.3)	57.23 (97.8)	3.0
	21	19.71 (131.3)	23.56 (100.4)	53.87 (92.1)	2.7
1	0	15.40 (100)	23.58 (100)	57.79 (100)	3.8
	7	15.41 (100.1)	26.73 (113.4)	55.28 (95.7)	3.6
	14	15.78 (102.5)	25.30 (107.3)	55.23 (95.6)	3.5
	21	18.26 (118.6)	23.74 (100.7)	55.03 (95.2)	3.0
2	0	16.35 (100)	23.57 (100)	57.37 (100)	3.5
	7	17.82 (109.0)	23.63 (100.3)	55.68 (97.1)	3.1
	14	18.76 (114.7)	23.71 (100.6)	55.41 (96.6)	3.0
	21	19.02 (116.3)	23.54 (99.9)	55.16 (96.1)	2.9
3	0	15.98 (100)	24.46 (100)	56.60 (100)	3.5
	7	16.14 (101.0)	25.72 (105.2)	55.13 (97.4)	3.4
	14	16.36 (102.4)	26.67 (109.0)	54.74 (96.7)	3.3
	21	17.37 (108.7)	26.68 (105.0)	54.48 (96.8)	3.1

Each value is the mean of triplicate determinations. Number in parenthesis is relative % of saturated, monounsaturated, and polyunsaturated fatty acids based on the initial saturated, monounsaturated, and polyunsaturated fatty acid content before oxidation. P/S- ratio of polyunsaturated to saturated fatty acids.

3.3. Triacylglycerol (TAG) composition

During the accelerated oxidation test, the changes in concentration in TAG species (P, palmitic; M, myristic; O, oleic; L, linoleic) from BCO determined by HPLC are given in Figures 3 and 4. The major TAG species were LLL (21.89%), PLL (18.75%), OLL (17.22%), POL (13.22%), OOL (9.21 %) and PLP (5.06%). The species OOO, POO, and LOS were present as minor components (< 4%). However, microwave pre-treatment did not inflict changes (with few exceptions) in the TAG species of BCOs because only a few species possessing more than four double bonds were present in the TAGs. The percentages of LLL, OLL, PLL, OOL and POL in BCO decreased whilst, in most case, the percentages of PLP, OOO, and POO remained unaltered or slightly increased with increasing storage time. At the end of 21 days of storage, the changes in concentration in those TAG species were significantly lower ($p < 0.05$) in 3-min pre-treated samples than untreated ones. The changes in POL were not significant during storage. The most significant reduction was found in LLL (Figure 3a) among all the species. The percentage of this TAG in untreated and 3-min treated samples reduced from 21.89 to 15.57% and from 20.38 to 19.41%, respectively, after 21 days of storage at 62 °C. This reduction might be due to a decrease in the concentration of C18:2 by the oxidative process. In this work, good agreement between the fatty acid and TAG compositions was also noted.

3.4. Evaluation by FT-IR

The most significant spectral changes occurring in BCOs during accelerated storage conditions are shown in Figure 5. The detected functional groups responsible for the IR absorption peak (Ali *et al.,* 2017b; Lerma-Garcia *et al.,* 2010; Guillen *et al.,* 1997): 3008 (C–H stretching vibration of cis-double bond); 2928 and 2854 (Asymmetric and symmetric stretching vibration of CH_2, resp.); 1745 (C=O stretching vibration); 1465 (bending vibrations of the CH_2 and CH_3); and 1163 (C–O stretching vibration). The absorbance of *cis*-double bond at 3008 cm^{-1} (shoulder) suffers a slow shifting toward higher values during storage.

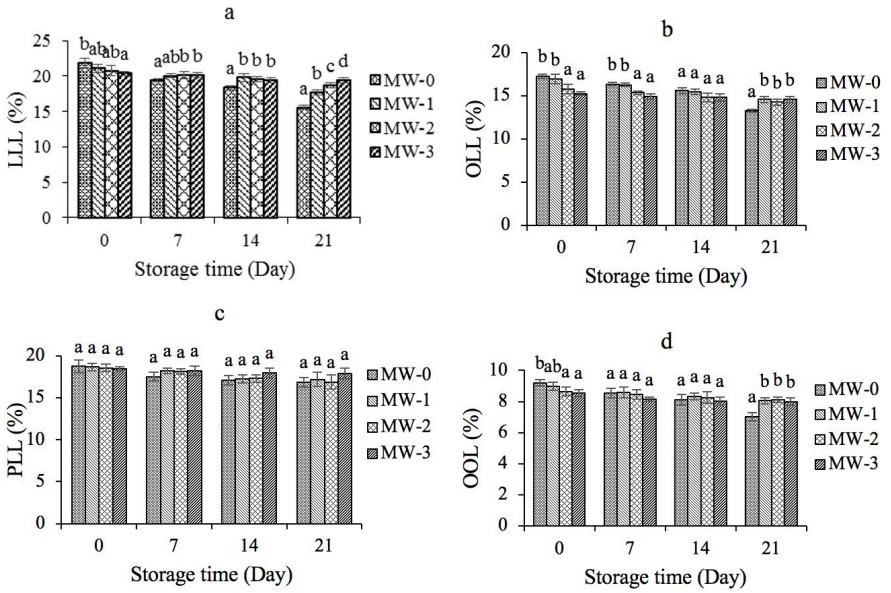

FIGURE 3. Changes in triacylglycerol composition of untreated (MW-0) and pre-treated (MW-1, pre-treated for 1 min; MW-2, pre-treated for 2 min; and MW-3, pre-treated for 3 min) black cumin seed oils during storage. (a) LLL, (b) OLL, (c) PLL, and (d) OOL. Each value is the mean ± standard deviation of triplicate determinations. Mean values were compared by Duncan's multiple range test. Values in each storage time grouped with different letters on bars are significantly different ($p < 0.05$).

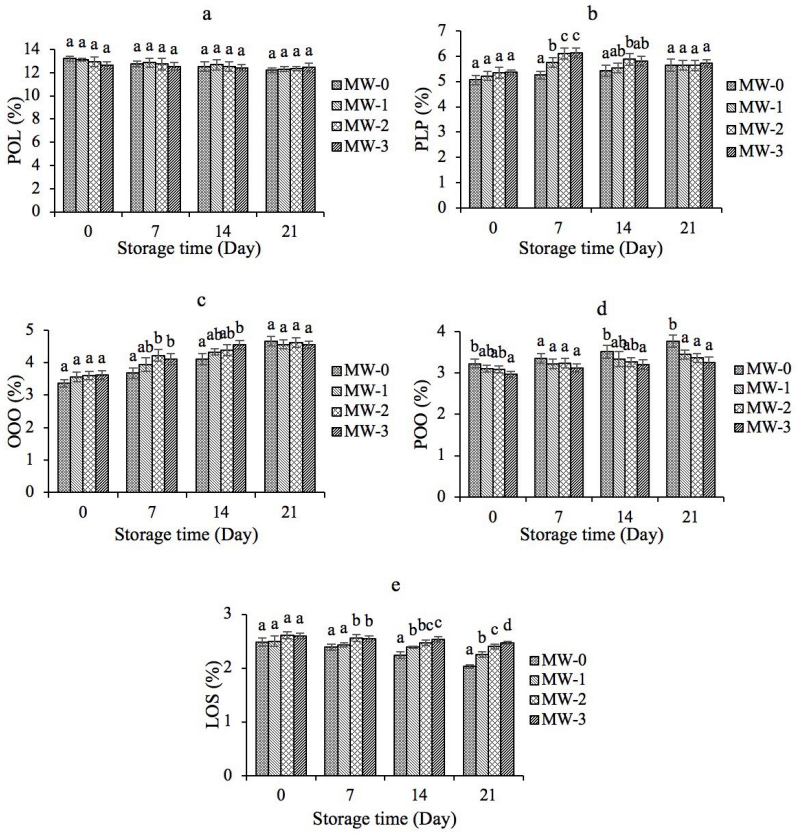

FIGURE 4. Changes in triacylglycerol composition of untreated (MW-0) and pre-treated (MW-1, pre-treated for 1 min; MW-2, pre-treated for 2 min; and MW-3, pre-treated for 3 min) black cumin seed oils during storage. (a) POL, (b) PLP, (c) OOO, (d) POO, and (e) LOS. Each value is the mean ± standard deviation of triplicate determinations. Mean values were compared by Duncan's multiple range test. Values in each storage time grouped with different letters on bars are significantly different ($p < 0.05$).

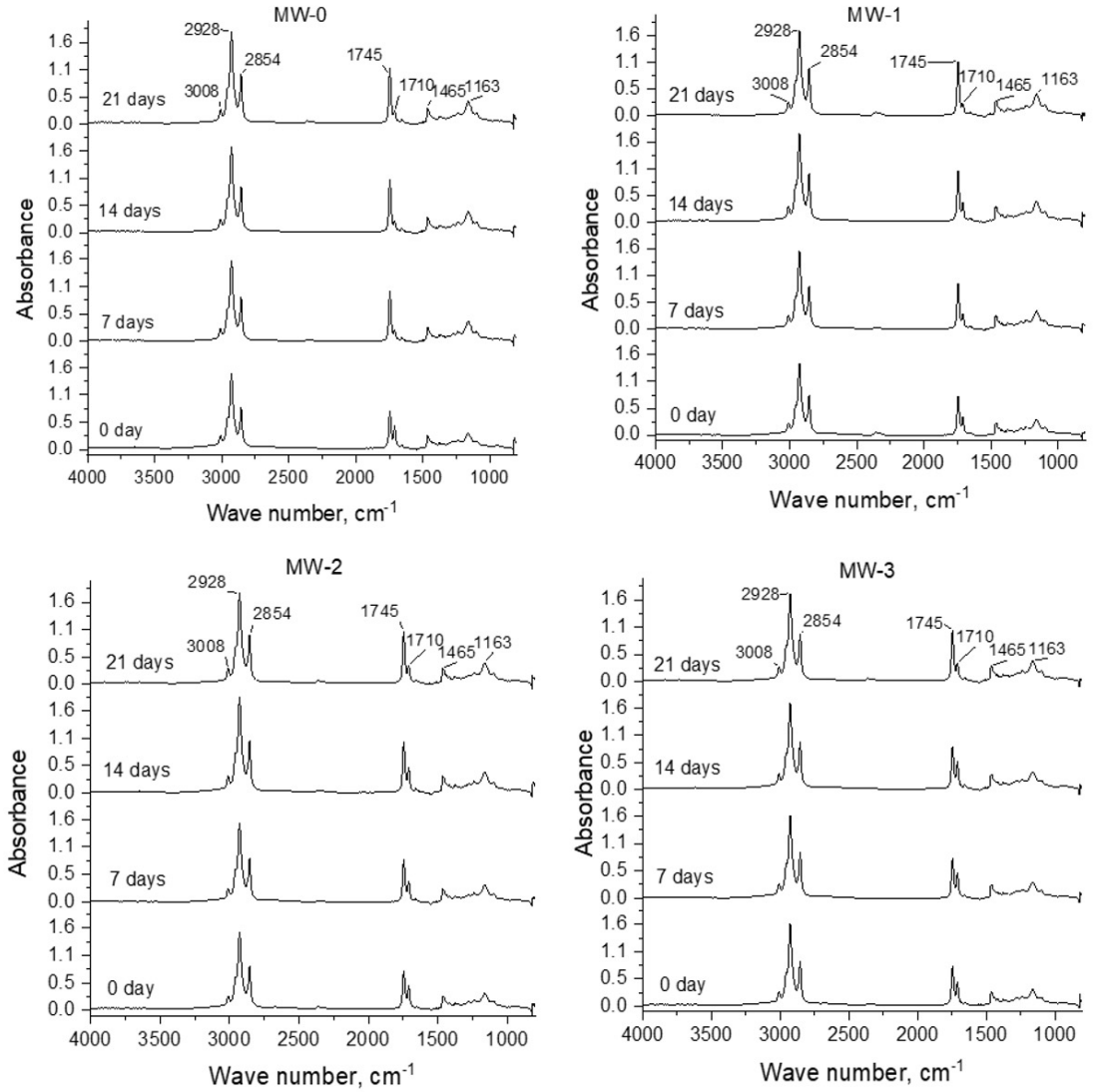

FIGURE 5. Changes in FT-IR spectra of black cumin seed oils extracted from untreated and pre-treated black cumin seeds during storage. a) 0 days of storage b) 7 days of storage c) 14 days of storage, and d) 21 days of storage.

This increment can be attributed to the formation of free radicals under accelerated oxidation conditions which initiate a primary oxidation reaction in unsaturated fatty acids (Moharam *et al.,* 2010*;* Belitz and Grosch*,* 1999). This interpretation agrees with that reported in the literature (Farag *et al.,* 1992). The bands at 2928 and 2854 cm^{-1} enhanced their intensity (absorbance) because of the surrounding chemical changes taking place due to the oxidation process. The vital peak at 1745 cm^{-1} corresponds to the carbonyl substances generated from the decomposition of hydroperoxide during accelerated oxidation (Smith *et al.,* 2007); the absorbance of it increased with oxidation time. The intensity of a weak peak near 1465 cm^{-1} increased with the oxidation treatment. The intensity of the peak at 1163 cm^{-1} related to the proportion in the sample of saturated acyl groups (Guillen *et al.,* 1997), showed similar alterations under storage conditions, and increased its intensity. A similar trend was followed for the peaks at 2927, 2854, 1745, 1465 and 1161 cm^{-1} by Valdés *et al.* (2015) for almonds during storage at 62 °C. In this research, the peak intensities of raw samples were greatly shifted compared to microwaved seed oils during storage, which indicates a clear impact of pre-treatment of BCOs. During storage, the intensities of absorbance of almost all peaks increased and these increments were bigger in fresh oils, which is attributed to the oxidative reactions

proceeding more rapidly in the fresh oils than in the microwaved ones. Similar results from IR data were reported by Jan *et al*. (2019) upon pan and microwave roasting of black cumin seeds. The results from the change in FTIR spectra are also in accordance with those shown in the changes in oxidative indices.

4. CONCLUSIONS

The present data reflects the promising impacts of microwave irradiation on the oxidation stability and compositional changes in black cumin seed oil. Oxidative indices indicate higher tendency to generate volatile and non-volatile oxidation products in the untreated oil samples compared to microwaved oil samples during storage. The exposure of black cumin seed to microwaves caused no major change in the concentration in fatty acids in the oils. During the oxidation treatment at 62 °C, both microwaved and untreated seed oils become oxidized with the decomposition of PUFA and generation of some unexpected and harmful substances. However, the slower degradation rate of PUFA in microwaved samples during treatment probably provided protection against the oil oxidation process. In conclusion, the changes in oxidation parameters, FAC, TAGs and FTIR spectral data at 21 days of storage were more evident for untreated black cumin seeds, which indicate a higher extent of oxidative compounds compared to those found in microwaved samples. Finally, the difference in quality or stability may illustrate the importance of Maillard reaction products generated by the action of microwave in seed samples.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare there are no conflicts of interest.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

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