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Investigation the fatty acid profile of commercial black cumin seed oils and seed oil capsules: Application to real samples

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Abstract: Medicinal and aromatic plants are widely used around the world. Today, Black cumin (*Nigella sativa* L.), one of the most popular medicinal plants, offers numerous benefits for human health. Black cumin oil has known to be used for the treatment of asthma, hypertension, cancer, rheumatism, and headaches over the course of many years. In this study, ten different black cumin seed oils and seed oil capsules, which have been obtained from local markets in Turkey, were investigated for their fatty acid profiles as percentages via gas chromatography and mass spectroscopy (GC-MS). According to the results obtained, the major fatty acids found in capsules and oil samples were similar with small differences in terms of amount; linoleic, oleic and palmitic acids were found as major compounds; 35.27-58.15%, 25.06-51.83%, 7.29-12.88 % in oil samples; 36.67- 56.61%, 24.93-46.71%, and 7.90-12.71% in seed oils capsule samples, respectively. This is the first study determining the fatty acid percentages of commercial black cumin seed oil and seed oil capsules.

Keywords: *Nigella sativa* L.; black cumin; fatty acid profile; seed oil; seed oil capsule; GC/MS.

1. Introduction

Black cumin (*Nigella sativa* L.), is an annual plant belonging to the Ranunculaceae family. It is cultivated in different regions of world and has been widely used since the ancient Egypt era. Some Nigella seeds were also found in the tomb of Tutankhamen [1]. Black cumin is commonly known as "Çörek otu" in Turkish and has different usage forms, such as spice in salads, breads and pastry.

Black cumin oil is obtained from the seeds of the plant; it is considered as a valuable and edible oil due to its fatty acid content, tocochromanols, phytosterols, thymoquinone and its derivatives. The oil has antioxidant, antitumor, antiinflammatory, antibacterial, antidiabetic, antiulcer, gastroprotective properties [2-5]. Recently, the popularity of black cumin oil has been increasing as nutritional supplement due to its various benefits and known usage traditionally mentioned above. Among edible oils, black cumin oil is known to be 10 to 15 times expensive than palm oil, sunflower oil, etc. Its high price lead producers to adulteration with cheaper oils such as refined corn oil, sunflower oil as well as other cheaper and low-quality seed oils. It is important to detect if there is any adulteration. This is necessary for both consumers' safety and health. Thus, several methods have been developed for detecting adulteration of fixed oils: chromatographic methods (Gas Chromatography), Mass Spectroscopy, total synchronous fluorescence spectroscopy, Chemometric Analysis, Fourier Transform Infrared (FTIR) Spectroscopy, Raman techniques [6-9].

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Reported pharmacological properties of black cumin seed oils are: anti-cancer [10,11], anti-inflammatory [12-15], cytotoxic [16-19], anti-asthmatic [20,21] , anti-microbial [22-26], antioxidant [27-32] as well as neuroprotective [33-39] and gastroprotective effects [40-47] .

Normally, before extraction, black cumin seeds have a lower Free Fatty Acid (FFA) content, which means there are long chained fatty acids [48]. Depending on the obtaining methods of oil such as extraction or pressing methods, the thymoquinone content and the fatty acid composition may negatively be altered because of high temperature and increased FFA content. Contrary to expectations, oils obtained by cold press machines (Figure 1.) may can also be affected due to being exposed to the high temperatures of the machine components.

Figure 1. (**A**) Illustration of cold press machine for the extraction of black cumin seed oil. (**B**) Heated components of a cold press machine. (Photo credit: Prof. Dr. Maksut Coşkun; used with his permission)

The aim of this study is to identify the fatty acid composition of black cumin seed oils and seed oil capsules as well as to investigate and reveal the differences between these products and also to find out if there is an adulteration or not. To the best of our knowledge, this is the first study determining the fatty acid percentage range scale of commercial black cumin oils and seed oil capsules.

2. Materials and Methods

2.1. Sample Materials

Black cumin oils and oil capsules were obtained from different local markets in Ankara, Turkey. Each sample used was from a different brand. Both oils and capsules were selected from cold- press extracted and conventional extracted. The black cumin oils and oil capsules were coded as NO and NOC, respectively.

2.2. Preparation of Fatty Acid Methyl Esters from Samples

For fatty acid methyl esterification (FAME), 150 mg of the oils were placed in 25 mL volumetric flasks saponified by adding 4 mL 0.5 N NaOH/MeOH and then heated on a boiling water bath until the fatty bubbles disappeared. Next, 4mL of BF_3/MeOH was added to each flask and they were boiled on a Bunsen burner for 2.5 minutes. When the solutions had cooled down to room temperature, they were filled up to 25 mL with saturated sodium chloride solution. The oily part then, separated by adding hexane.

2.3. Determination of FAMEs with Gas Chromatography and Mass Spectroscopy

The obtained FAMEs were dissolved in 2 mL *n*-hexane and 1µL of the samples was injected and analysed by Gas Chromatography-Mass Spectroscopy (GC/MS). Chromatographic analysis was

performed using an Agilent 6890N-Network Gas Chromatography system which is combined with an Agilent 5973-Network Mass Detector (GC-MS). HP Innowax Capillary (60.0m × 0.25mm × 0.25µm) was used as a capillary column. The carrier gas was helium with 3.3 mL/min flowrate and the injection volume was 1µl. Column temperature was at 100 ºC initially for 1 min after injection, gradually increased to 170 ºC with 10 ºC/min and increased to 215 ºC with 5 ºC/min for 5 min. Then, the finally column temperature was increased to 240 ºC with 10 ºC / min for 10.5 min. Split mode (20:1) was used for the injection at 270 ºC. Temperatures of detector and injector were 280°C and 250 °C, respectively. The run time was 35 min [49].

3. Results and Discussion

For this study, fatty acid methyl esters (FAMEs) of commercially available black seed oils and black seed oil capsules of various brands on the market were formed and GC-MS and fatty acid profiles were obtained, which are summarized in Table 1 and Table 2, respectively. Black cumin oil samples are classified as NO and black cumin oil capsules are encoded as NOC.

The results as the percentages of the fatty acid content reveal that the major fatty acids found in capsules and oil samples were similar with small differences in terms of amount; linoleic acid (LA), oleic acid (OA) and palmitic acid (PA) were found as major compounds; 35.27-58.15%, 25.06- 51.83%, 7.29-12.88 in oil samples ; 36.67-56.61%, 24.93-46.71%, and 7.90-12.71% in oil capsule samples, respectively.

Kaskoos [50] reported that the major fatty acids of black seed oil were LA (42.76%), OA (16.59%) and PA (8.51%).

Lutterodt et al [51] studied the fatty acid profile of extracted seed oil and the major fatty acids found were LA (58.8-61.2%), OA (22.6-24.5%) and PA (13-13.3%) .

Babayan et al,[52] reported that the major fatty acids of black seed oil were LA (56%), OA (24.6%) and PA (12%).

In their study, Ramadan and Morsel [53] found the major fatty acids of black cumin seed oil to be LA (57.3%), OA (24.1%) and PA (13%).

Tulukcu [54] collected black cumin seed oils from 8 different regions and studied their fatty acid profiles. As a result of his study, the major fatty acids of seeds were found to be La (54-70%), OA (15-24%) and PA (8.2-13.3).

Also a result of the study conducted by Atta [55], the major fatty acids of black seed oils were determined to be LA (47.5-49%), OA (18.9-20.1%) and PA (9.9-12.1%).

According to Turkish Pharmacopoeia [56], black cumin oil should contain 40-60% linoleic acid, 20-30% oleic acid and 10-20% palmitic acid. When we compare the results with Turkish Pharmacopoeia, it is clearly that NO2 and NOC2 has lower linoleic acid percentages (35.27% and 36.67%, respectively); NO2, NO3, NO4, NOC2, NOC4 and NOC8 has lower palmitic acid percentages (8.06, 7.29, 8.18, 8.89, 7.90, 8.34%, respectively) when compared to the pharmacopoeia values but the values are very close to the limitations. Oleic acid percentages of all samples are consistent with the ranges of Turkish Pharmacopoeia.

Among the oils studied, the major fatty acids of black seed oils and seed oil capsules were determined as linoleic acid (36-56%), oleic acid (25-46%) and palmitic acid (7-13%). These results are consistent with the results of previous studies. High content of oleic acid supports its usage .

The term of “specialty oils” has usually been used for some oils which has special dietary or functional properties with the help of specific fatty acid they contain. Especially oleic and linoleic acids are two main members of these specific fatty acids. They have beneficial and prevention effects on cancer, cardiovascular, autoimmune and inflammatory diseases [57-59]. According to these findings it may be concluded that 18 of 20 samples, could help on prevention of cardiovascular, autoimmune and inflammatory diseases.

The results of our study on the fatty acid profile of black cumin seed oil and the results reported in previous literature research have been combined and approximately indicated in Table 3.

Table 1. Fatty acid percentages of black cumin oils

Fatty Acid	NO1	NO 2	NO 3	NO 4	NO 5	NO6	NO 7	NO 8	NO 9	NO10
(C14:0)	-	-	-	-	0.15 ± 0.01	-	-	0.14 ± 0.02	0.16 ± 0.02	-
(C16:0)	10.55 ± 0.03	8.06 ± 0.24	7.29 ± 0.12	8.18 ± 0,19	12.41 ± 0.04	-	12.88 ± 0.1	12.12 ± 0.17	12.62 ± 0.25	-
(C16:1)	-	-	-	-	0.16 ± 0.02	-	-	0.17 ± 0.01	0.17 ± 0.02	-
(C18:0)	3.02 ± 0.07	0.89 ± 0.01	3.27 ± 0.08	3.64 ± 0.17	3.07 ± 0.09	-	2.97 ± 0.08	3.40 ± 0.23	3.34 ± 0.09	-
(C18:1)	33.08 ± 1.63	51.83 ± 0.20	41.06 ± 0.12	28.94 ± 0.31	25.31 ± 0.34	-	25.98 ± 0.09	25.31 ± 0.37	25.06 ± 0.11	-
(C18:2)	52.12 ± 1.86	35.27 ± 0.37	48.36 ± 0.14	56.97 ± 0.20	55.73 ± 0.62	-	58.15 ± 0.25	55.47 ± 0.15	55.61 ± 0.33	-
(C18:3)	0.56 ± 0.12	3.93 ± 0.07	-	0.56 ± 0.06	0.20 ± 0.01	-	-	0.34 ± 0.06	0.21 ± 0.02	-
(C20:0)	-	-	-	0.21 ± 0.03	0.25 ± 0.04	-	-	0.21 ± 0.1	-	-
(C20:2)	0.65 ± 0.26	-	-	0.75 ± 0.01	2.68 ± 0.29	-	-	2.80 ± 0.13	2..95 ± 0.03	-
(C22:2)	-	-	-	0.71 ± 0.07	-	-	-	-	-	-

Table 2. Fatty acid percentages of black cumin oil capsules

Fatty Acid	NOC 1	NOC 2	NOC 3	NOC 4	NOC 5	NOC 6	NOC 7	NOC 8	NOC 9	NOC 10
(C14:0)	0.13 ± 0.04	-	-	0.08 ± 0.02	0.13 ± 0.01	0.16 ± 0.01	0.07 ± 0.01	-	0.12 ± 0.01	0.13 ± 0.01
(C16:0)	11.34 ± 0.06	8.89 ± 0.11	12.04 ± 0.05	7.90 ± 0.09	11.28 ± 0.03	12.71 ± 0.03	10.66 ± 0.25	8.34 ± 0.07	12.34 ± 0.47	11.99 ± 0.13
(C16:1)	-	-	-	-	-	0.15 ± 0.01	0.13 ± 0.09	-	0.17 ± 0.02	-
(C18:0)	3.90 ± 0.07	3.14 ± 0.01	3.53 ± 0.04	3.98 ± 0.08	3.89 ± 0.06	3.66 ± 0.23	5.42 ± 0.05	3.90 ± 0.29	3.87 ± 0.11	3.93 ± 0.03
(C18:1)	27.38 ± 0.11	46.71 ± 0.07	24.93 ± 0.06	34.35 ±0.39	27.34 ± 0.04	26.39 ± 0.47	35.08 ± 0.18	31.13 ± 0.17	26.34 ± 0.18	27.19 ± 0.28
(C18:2)	50.61 ± 0.17	36.67 ± 0.1	56.45 ± 0.40	52.84 ± 0.06	50.31 ± 0.03	53.84 ± 1.28	45.15 ± 0.20	56.61 ± 0.46	54.38 ± 0.44	53.63 ± 0.56
(C18:3)	0.59 ± 0.05	2.94 ± 0.04	-	-	0.62 ± 0.07	-	1.68 ± 0.07	-	-	-
(C20:0)	-	-	-	-	-	-	0.52 ± 0.09	-	-	-
(C20:1)	1.14 ± 0.04	-	-	-	1.23 ± 0.04	-	-	-	-	-
(C20:2)	2.73 ± 0.01	1.62 ± 0.03	3.05 ± 0.09	0.85 ± 0.05	2.69 ± 0.1	2.76 ± 0.11	1.25 ± 0.04	-	2.74 ± 0.06	3.10 ± 0.14
(C22:1)	2.15 ± 0.05	-	-	-	2.47 ± 0.05	-	-	-	-	-
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Table 3. Expected ranges of fatty acid percentages in standardized black cumin seed oils

Fatty Acid Profile	Percentage
C14:0	Up to 0.2%
C16:0	7-13%
C16:1	Up to 0.2%
C18:0	2-4%
C18:1	20-30%
C18:2	42-60%
C18:3	Up to 1%
C20:0	Up to 0.5 %
C20:1	Up to 1.2%
C20:2	Up to 3%
C22:1	2-3 %
C22:2	Up to 1 %

In conclusion, it may be stated that the fatty acid profile of a standardized seed oil is expected to be within the ranges given in Table 3 according to our results and literature survey mentioned above. This is the first study in the literature that has compared the fatty acid profiles of different branded black cumin oils and oil capsules. In our previous study [60], we investigated the thymoquinone contents of these products and when we compare these two study results, it is clear that the fatty acid profiles are not related with the amount of thymoquinone. The fatty acid profiles of NO6 and NO10 couldn't be determined although they revealed different thymoquinone percentages calculated previously. In spite of the fact that these two samples were extracted and analysed at the same time as the other samples, it may be stated that these two products are in fact counterfeit products. Consequently, this study may be a global reference for further studies in the related fields for more contributions to science.

Conflict of Interest

The authors declare no conflicts of interest.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/journal-of-chemical-metrology

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