Progress in Nutrition 2019; Vol. 21, Supplement 1: 428-434 DOI: 10.23751/pn.v21i1-S.6556

Nutritional composition of the indigenous cultivar of black cumin seeds from Bangladesh

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Summary. Proximate analysis of black cumin (*Nigella sativa* L.) seeds showed a mean composition of 20.3% protein, 45.4% fat, 7.1% moisture, 7.4% ash and the rest being total carbohydrate. The fat and ash content were much higher than the value reported in the literature. Potassium, phosphorus, calcium, and magnesium were the predominant elements present. Sodium, iron, zinc, and copper were found at lower levels. Cadmium, lead, and molybdenum were also detected in trace levels. Linoleic acid (57%) followed by oleic acid (21.8%) were the major unsaturated fatty acids, while palmitic acid (13.1%) was the main saturated one. Glutamic acid (23.95%), aspartic acid (9.32%) and arginine (8.90%) were the main amino acids present while tryptophan and methionine were the minor amino acids. These results indicate the high nutritional potential of Bangladeshi black cumin seeds especially as a source of protein (20.3±0.63%) and fat (45.4±0.53%) especially unsaturated fatty acids (84%), which may be utilized as food supplements.

Key words: black cumin, *Nigella sativa* L., nutritional composition

Introduction

Black cumin (*Nigella sativa* L.) seeds are used almost entirely for edible and medicinal purposes, such as for seasoning many kinds of backer products and for treatment of some diseases. In consideration of potential utilization, detailed knowledge on the compositional of *N. sativa* seed is of major importance. Like most herbs, the composition of black cumin varies with the geographic distribution, time of harvest and agronomic practices. Scientific investigations have depicted its composition i.e. moisture, oil, proteins, ash and total carbohydrates contents in the range of 3.8–7.0%, 22.0–40.35%, 20.85–31.2%, 3.7–4.7% and 24.9–40.0%, respectively (1,2). Fatty acid compositions of black cumin were linoleic acid (40.3-70.8%) followed by oleic (15.2-28.1%), palmitic (9.47-13.34%) and stearic (2.6-3.1%) acids (3-5). They also revealed that the oleic and linoleic acids are the most abundant monounsaturated and polyunsaturated fatty acids; in all samples, respectively. Black cumin seed fixed oil contains appreciable quantities of polyunsaturated fatty acids; constitute the bulk of oil ranging from 48–70%, while monounsaturated (18–29%) and saturated fatty acids (12–25%) are in lesser proportions (1,5). Besides better fatty acid profile, it contains considerable quantities of tocopherols and allied bioactive compounds. Ramdan and Morsel (6) reported that β-sitosterol and stigmasterol were among the major components, together constituting about 65 per cent of total sterols whereas campesterol, lanosterol and D7-avenasterol were present at lower levels.

Butt and Sultan (7) in a review article reported that *N. sativa* seeds and its oils, reduced risk of various diseases due to the presence of various nutritionally essential components such as PUFA, phytosterols, antioxidants, antidiabetic, anti-tumor and other active constituents. The compounds responsible for the biological activity of *N. sativa* seeds are thymoquinone (30-48%), p-cymene (7-15%), carvacrol (6-12%),

4-terpineol (2-7%), t-anethole (1-4%) and a sesquiterpene longifolene (1-8%) (8), in which thymohydroquinone and thymol are the most presumed pharmacologically active constituents of *N. sativa* (9,10). Furthermore, thymoquinone also effective in regulating several hematological and serological functions and maintenance of body homeostasis (11).

No doubt, the nutritional composition of black cumin fixed oil is of significance. Literature data on the chemical composition of *N. sativa* L. seeds are very limited and no information is available concerning the composition of black cumin seeds cultivated in Bangladesh. Hence, the purpose of this study is to determine the proximate, mineral, fatty acid and amino acid composition of the black cumin seeds of Bangladesh origin.

Materials and Methods

Materials

Mature black cumin seeds of indigenous variety were obtained from Dhaka, Bangladesh. All the reagents (analytical and HPLC grade) and standards were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Proximal analysis

Proximal analysis for moisture, crude fat, crude protein, dietary fiber and ash was performed according to respective methods of the official Methods of Analysis of the Association of official Analytical Chemists (AOAC) (12). The carbohydrate of *N. sativa* seed was calculated by subtracting the total of protein, fat, moisture and ash from 100.

Mineral analysis

Samples were digested by wet-ashing (12) and minerals were determined using a PE model 5000 atomic absorption spectrophotometer. In brief, A 0.5 g seed samples were digested in 5 ml of conc. HNO3 in a microwave oven at 150 oC for 2 h using high performance microwave digestion unit (Milestone Microwave Laboratory System, USA). Digestion was performed in the presence of nitric-perchloric acid mixture (HNO3:HClO4 at 2:1 ratio). Digested samples were cooled in an ice bath, diluted to 50 ml with deionized water, and filtered through Whatman No. 5B filter paper. The filtrates were used for mineral analysis. Sodium, potassium, calcium, and magnesium were determined by Shimadzu AA-6800 atomic absorption spectrophotometer, while iron, zinc, manganese, copper, cadmium, lead, chromium and molybdenum were determined by ICP-MS. Phosphorus content was determined by the calorimetric method.

Amino acid analysis

The acid hydrolysis of the seed protein was carried out in a mini-reaction vial using 0.2 g of seed and 3 ml of 6N hydrochloric acid at 110 oC for 16 hr under nitrogen atmosphere. After the hydrolysis was complete, the contents of the vial were filtered, and the filtrate made up to 10 ml. One hundred microliters of this hydrolysate, along with 2. µl of the internal standard alpha amino adipic acid was used for amino acid analysis using an amino acid analyzer JLC-500/V (JEOL Ltd., Japan) consisted of LCR-6 column (4 mm x 120 mm). Chromatographic peaks were integrated, identified and quantified using BreezeTM software version 3.20 by comparing it to the known standards (Amino acid standard Pierce and Rockford, Illinois).

Methionine and cysteine were determined from the same method of acid hydrolysis after treatment using performic acid oxidation. Tryptophan was analyzed by HPLC after alkali hydrolysis.

Extraction of fixed oil

2 g ground seeds were extracted with petroleum ether (40 – 60 oC) for 24 h in a Soxhlet apparatus. The extract was concentrated under reduced pressure. 1 ml concentrated extract was dissolved in 20 ml petroleum ether and 2 ml 2 M methanolic KOH added. The mixture was shaken for 2 min and allowed to stand for 10 min. the upper layer was removed and washed with water. The lipid extract was collected in a flask and subsequently treated with sodium sulfate to remove traces of water. The solvent residue in the oil was removed under a stream of nitrogen, capped and stored at – 20 oC until analyses. The extracted oil was stored in a dark place at room temperature.

Fatty acid composition

The fatty acid composition of the seed extract was determined by gas liquid chromatography of their methyl esters. Fatty acid methyl esters (FAMEs) were prepared from the oil samples according to the following laboratory protocol. Briefly, 150-200 mg of extracted oil was reacted with 4 ml of 0.5 M NaOH in MeOH on a steam bath for about 5 min until the lipids dissolved. Then, 10 ml of the boron trifluoride-methanol complex were added and refluxed for 2 min. and the organic phase was used for gas liquid chromatography (GLC) analysis. Gas chromatography analysis of the methyl esters was conducted with a Shimadzu GC-1700 gas chromatograph equipped with a flame-ionization detector (FID) and a Shimadzu auto sampler (Shimadzu Corporation). A fused silica capillary column DB-23 (φ0.25 mm×30 m, df. 0.25 μm) from J & W Scientific was used with helium as the carrier gas at a flow rate of 1.5 mL/min. The column temperature was held at 50 ˚C for 1 min and then increased to 170 ˚C at a rate of 10 ˚C/min to 210 ˚C at a rate of 1.2 ˚C/min. Both the injector and detector temperatures were set at 250 ˚C. A split less injection system was used. Injection volume was 1 mL. Make up gas (Helium); 80kPa. Gas pressure: Hydrogen; 60 kPa, Air; 50 kPa. Individual fatty acid methyl esters were identified by comparing their retention times with those of FAME standards, run on the same column under the same conditions. The area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids identified. Results are reported as g fatty acid/100 g total fatty acids.

Results and Discussion

Proximal analysis and mineral composition of the black cumin seeds are presented in Table 1. The results indicated that it contains 7.12 ± 0.22, 20.3 ± 0.63, 45.4 ± 0.53, 7.39 ± 0.27, and 19.7± 0.44% of moisture, proteins, fat, ash, total carbohydrate contents, respectively, while dry matter was found to be 92.9 ± 0.22%. As shown in Table 1, the crude fat represents the major

Table 1. Proximal and mineral composition of black cumin seeds

Proximal Composition (%)	**Present study**[a]	**Literature value**[b]	
		1	**2**
Moisture	7.12 ± 0.22	5.89 ± 0.05	6.46 ± 0.17
Crude Protein (N x 6.25)	20.3 ± 0.63	22.1 ± 0.25	22.8 ± 0.60
Crude fat	45.4 ±0.53	31.7 ± 0.68	31.2 ± 0.82
Ash	7.39 ±0.27	5.07 ± 0.07	6.03 ± 0.16
Total carbohydrate	19.7 ±0.44	25.47 ± 0.62	nd
Dry matter	92.9 ±0.22	nd	nd
Mineral	**(mg/100g)**	**(%)**	**(mg/100g)**
Potassium	1498.3 ± 25.0	0.83 ± 0.01	808 ± 6.61
Phosphorus	481.5 ± 47.4	0.57 ± 0.01	543 ± 10.0
Sodium	44.8 ± 3.7	0.35 ± 0.02	17.6 ± 2.21
Calcium	366.7 ± 20.1	9.13 ± 0.16	570 ± 21.5
Magnesium	355.2 ± 2.3	10.2 ± 0.09	265 ± 4.87
Zinc	6.7 ± 0.1	0.05 ± 0.00	6.23 ± 0.21
Iron	42.6 ± 2.5	0.26 ± 0.02	9.70 ± 0.65
Manganese	3.1 ± 0.1	0.05 ± 0.00	8.53 ± 0.11
Copper	1.5 ± 0.03	0.03 ± 0.00	2.60 ± 0.03
Lead	0.13 ± 0.02	0.06 ± 0.00	nd
Cadmium	0.006 ± 0.001	nd	nd
Molybdenum	0.03 ± 0.002	nd	nd

[a] Mean± SD of 5 determinations. [b](1) Iqbal et al. (2011); (2) Sultan et al. (2009); nd = not determined.

component in the black cumin seed, followed by crude protein, then total carbohydrate. A comparison of these results with the literature values indicates that the major differences are in the amount of fat and ash. The fat and ash content were much higher than the value reported by Iqbal et al. (13) and Sultan et al. (14), whereas carbohydrate was relatively lower than their value (Table 1). The fat content of Bangladeshi black cumin seeds was close to the amounts reported for Iranian variety (40.4%) by Cheikh-Rouhou et al. (5). High levels of fat (45.4%) and protein (20.3%) render Bangladeshi black cumin seeds a good source of fat and protein.

The percent composition of mineral in black cumin seeds showed that the potassium (1498.3 ± 25.0 mg/100g) was the predominant element in the seeds followed by phosphorus (481.5 ± 47.4 mg/100g), then calcium (366.7 ± 20.1 mg/100g) and magnesium (355.2 ± 2.3 mg/100g) (Table 1). Moreover, considerable amounts of zinc, manganese and copper were present in the indigenous Bangladeshi variety of black cumin seeds. These results are similar to those reported by others (2,14, 15), although they reported a much lower amount of potassium in their seeds. In addition, Iqbal et al. (13) and Sultan et al. (14) did not detect the heavy metals cadmium and molybdenum, we detected them in our black cumin seeds, although very low amount. We also detected lead, which was also detected by Iqbal et al. (13), but not by Sultan et al. (14). Black cumin seeds contained significant amounts of important mineral elements. However, the nutritional role of these minerals cannot be predicted from the small quantity of black cumin consumed (2).

The fatty acids composition of the *N. sativa* seed is presented in Table 2. The fatty acids consist of 57.0% linoleic, 21.8% oleic, 13.1% palmitic, 2.91% eicosadienoic, 2.47% stearic and 1.18% cis-vaccenic acid. The fatty

Table 2. Fatty acid compositions of black cumin seeds

Fatty acids (%)	Present study	Literature value[b]	
		1	2
Myristic acid (14:0)	0.23	0.16	0.40 ± 0.03
Palmitic acid (16:0)	13.1	8.51	14.8 ± 0.47
Palmitoleic acid (16:1)	0.28	0.16	0.28 ± 0.05
Stearic acid (18:0)	2.47	2.22	2.90 ± 0.01
Oleic acid (18:1)	21.8	16.6	19.2 ± 0.13
Cis-vaccenic acid (18:1)	1.18	nd	nd
Linoleic acid (18:2)	57.0	42.8	58.1 ± 0.54
Linolenic acid (18:3)	0.46	0.25	0.41 ± 0.06
Arachidic acid (20:0)	0.18	nd	1.05 ± 0.43
Eicosenoic acid (20:1)	0.39	0.16	nd
Eicosadienoic acid (20:2)	2.91	1.94	nd
Eicosatrienoic acid (20:3)	nd	4.71	nd
Eicosapentaenoic acid (20:5)	nd	5.98	nd
Behenic acid (22:0)	nd	nd	2.89 ± 0.34
Docosahexaenoic acid (22:6)	nd	2.97	nd
Lignoceric acid (24:0)	nd	3.60	nd
Nervonic acid (24:1)	nd	2.76	nd
Saturated acids	16.0	15.1	22.0 ± 0.60
Unsaturated acids	84.0	79.9	19.5 ± 0.20[b]
			58.5 ± 0.64[c]
Unsaturated/Saturated	5.25	5.29	nd

[a] (1) Kaskoos (2011); (2) Bourgou et al. (2010); nd = not detected; [b]MUFA; [c]PUFA.

acid composition determined in the present study is similar to some previously reported values (5,14,16,17) and different from other reported values which differ themselves considerably (1,18). The linoleic acid was the dominating fatty acid in the indigenous Bangladeshi black cumin seeds, which have been supported by others (4,5,14,19,20). It is well known that dietary fats, rich in linoleic acid, prevent cardiovascular disorders such as coronary heart diseases, atherosclerosis, and high blood pressure. Also, it was reported that the nutritional value of linoleic acid is because of its metabolism at the tissue levels, which produces the long-chain polyunsaturated fatty acids and prostaglandins (21). In the present study, unsaturated fatty acids, linoleic and oleic acids were the dominant fatty acids and could be a good source of essential fatty acids for human nutrition. They account for more than 78% of the total fatty acids which is higher than that reported previously for seeds originated from other countries (1,5,18), but in good agreement with the reports of Bourgou et al. (16).

It has been reported that composition or proportion of fatty acids in black cumin seeds depends on the country of origin. For example, Egypt origin seeds contain oleic and linoleic acids at relatively high levels (18.9-20.1 and 47.5-49.0%, respectively) in comparison to Tunisian (25.0 and 50.3, respectively) and Iranian (23.7 and 49.2, respectively) origins (1,16,17). In the present study, we found that the linoleic and oleic acids contents of Bangladeshi origin seeds were higher than that of the contents of Egypt, Tunisia or Iran origins. The ratio of linoleic acid (57%) to oleic acid (21.8%) was more than 2.5:1, which agreed with the value reported for black cumin seeds of Yemen origin (22) The differences in fatty acids composition may be ascribed to genetic (plant cultivar, variety grown), seed quality (maturity, harvesting-caused damage and handling/ storage conditions), oil processing variables, or accuracy of detection as well as lipid extraction method and quantitative techniques (6).

The black cumin seeds contain a comparatively lower level of saturated fatty acids such as palmitic (13.1%) and stearic (2.47%) acids. The amount of unsaturated fatty acids was 84% of the total fatty acid content of lipid extract, which is in good agreement with Kaskoos (18) and Sultan et al. (14). The total unsaturated fatty acids are more and saturated fatty acid less than the values reported in literature (1,5,20). The ratio of un-saturated to saturated fatty acids (U/S%) in Bangladeshi seeds was much higher (5.25%) than that reported for black cumin seeds from Egyptian (2.4 and 3.0%), Iranian (2.9%), and Tunisian (3.4%) origins (1,16,17), but same as in Iraqi seeds (5.29%) reported by Kaskoos (18) (Table 2).

However, for the first time, a considerable amount of cis-vaccenic acid (18:1, n-7) was detected in black cum-in seeds and the presence of cis-vaccenic acid was not reported in the literature. While the presence of myris-toleic, dihomolionolenic, arachidonic, behenic acids and eicosatrienoic, eicosapentaenoic, docosahexaenoic and lignoceric acids were reported by Sultan et al. (14) and Kaskoos (18) in their seeds, but we did not detect these acids in our seeds. Behenic acid was also detected by Bourgou et al. (16) and Hamrouni-Sellami et al. (20), which was not detected in our seeds. Only one study conducted by Cheikh-Rouhou et al. (5) detected minute amounts of margaric (C17:0) and margaroleic (C17:1) acids in seeds of both Tunisian and Iranian *N. sativa* L. In our study, both of these fatty acids were not detected even in traces, which make our study in agreement with the majority of previous works. A negligible amount of eicosenoic acid (0.39%) was detected and was in accordance with that reported by Cheikh-Rouhou et al. (5) and Ramadan and Morsel (6) but contrary to the reports of Bourgou et al. (16). On the other hand, few amounts of myristoleic (C14:1) = 0.18% and lignoceric (C24:0) = 1.08% acids were detected by Al-Jassir (15) in *N. sativa* seeds from Saudi Arabia. These two fatty acids were also detected in trace amounts by Atta (1) in Egyptian *N. sativa* seeds. These observations underline the fact that, although plants are usually recommended as a source of unsaturated fats, the contents vary widely in types and amounts among different sources. Large variations in chemical composition of *N. sativa* seeds may be related to the origin of the plant (4,5), maturation of seeds (23) and agronomic practices (24-26). It may also be due to geographical and climatic differences where Nigella seeds had been grown (1). Thus, it is necessary to investigate the lipid composition of individual plant sources.

The amino acid composition of the seed protein, determined by the amino acid analyzer is presented in Table 3. The protein consists of 18 amino acids in-

Table 3. The amino acid composition of black cumin seed protein

Amino acids (%)	Present study	Literature value[a]	
Essential amino acids (E)			
Leucine	6.00	5.82	10.88
Valine	4.77	4.61	3.06
Lysine	3.86	4.04	7.62
Threonine	3.86	3.65	1.23
Phenylalanine	3.70	3.61	7.93
Isoleucine	3.69	3.46	4.03
Histidine	2.79	3.35	-----
Methionine[1]	1.88	1.65	6.16
Tryptophan[2]	1.50	-----	-----
Total essential amino acids	32.05	30.19	40.91
Non-essential amino acids (N)			
Glutamic acid	23.95	24.74	13.21
Aspartic acid	9.32	8.94	5.02
Arginine	8.90	9.19	19.52
Glycine	6.11	5.61	4.17
Proline	4.88	4.90	5.34
Serine	4.50	4.31	1.98
Alanine	4.39	3.73	3.77
Tyrosine	3.43	3.59	6.08
Cystine[1]	2.47	1.96	-----
Total non-essential amino acids	67.95	66.97	59.09
E/N	0.47	0.45	0.69

[a]Al-Jassir. (1992) and Babayan et al. (1978); [1]Determined after oxidation treatment with performic acid, hydrolyzed by HCl. [2]Determined by HPLC method after alkaline hydrolysis.

cluding 9 essential amino acids. The seed protein is rich in glutamic acid (23.95%), aspartic acid (9.32%), arginine (8.9%), glycine (6.11%) and leucine (6.0%); glutamic acid being the major amino acid. These major acids constitute more than 54% of the total amino acids present in the protein of black cumin seeds. Al-Jassir (15) also reported that the glutamic acid was the most abundant amino acid in the Saudi black cumin seed, but not arginine as reported by Babayan et al. (27). The essential amino acids, methionine (1.88%) and tryptophan (1.5%) were the minor amino acids. Similar to our finding, Al-gaby (28) also found the lowest amount of amino acid is tryptophan in black cumin cake protein, although Al-Jassir (15) and Babayan et al. (27) did not found tryptophan in black cumin seeds. Total non-essential amino acids present in the Bangladeshi black cumin seeds are much higher than their corresponding essential amino acids, also reported previously (15,27). There was almost no difference observed in the amino acid profile of Saudi and Bangladeshi black cumin seeds, but differs with Babayan's reports, which may be due to genotypic and/or environmental variations. From the results of this study, it could be concluded that black cumin contains appreciable amounts of nutrients which can be used as food supplements and may serve a beneficial health sources.

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