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Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia

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Proximate analysis of black cumin seeds showed a composition of 20·85% protein, 38·20% fat, 4·64% moisture, 4·37% ash, 7·94% crude fibre and 31·94% total carbohydrates. Potassium, phosphorus, sodium and iron were the predominant elements present. Zinc, calcium, magnesium, manganese and copper were found at lower levels. However, lead, cadmium and arsenic were not detected in the seeds. Linoleic and oleic acids were the major unsaturated fatty acids while palmitic acid was the main saturated one. Glutamic acid, arginine and aspartic acid were the main amino acids present while cystine and methionine were the minor amino acids. These results indicate the high nutritional potential of Saudi black cumin seeds especially as a source of protein and fat. The total aerobic bacterial count was 7×10^7 cfu/g and the yeast and mould counts were 4×10^2 cfu/g. The low numbers observed for *Staphylococcus aureus* and *Bacillus cereus* make black cumin seeds acceptable, without any associated health hazard.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is a member of the Ranunclaceae family and it is widely distributed in countries bordering the Mediterranean Sea, Middle Europe and western Asia (Hashim & Elkiey, 1962). The plant is also cultivated in middle and western regions of Saudi Arabia. The seeds of black cumin are small, black and possess aromatic odour and taste (Salama, 1973).

The seeds have been used as a spice from early times. Janson (1981) reported that whole or crushed seeds were used in or on bread in India, Sri Lanka, Egypt, Turkey and the USSR. They are used as a flavouring agent in vinegar, as a constituent of 'curry' or as a substitute for pepper in cooking. Babayan *et al.* (1978) mentioned that these seeds are used by Egyptians as a flavouring agent, by Syrians for cheese flavouring and by Americans for flavouring bakery products.

The *Nigella* seeds have been reported to have many medical properties. The seeds are digestive stimulants as well as carminative, aromatic, diuretic, diaphonetic, stomachic, anthelmintic, asthmatic. (Hashim & Elkiey, 1962; Salama, 1973; Babayan *et al.*, 1978; Agarwal *et al.*, 1979). Therefore, this research was initiated to investigate the properties of Saudi Arabian black cumin seeds. The studies include proximate analysis, mineral contents, fatty acids, amino acid composition and the microflora of black cumin.

MATERIALS AND METHODS

The material comprised samples of black cumin seeds obtained from Riyadh, Saudi Arabia.

Chemical composition

Proximate analysis for moisture, crude fat, crude protein, crude fibre and ash was performed in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1984). A sample size of 2·5 g of seed was used for analysis.

Dry matter content was estimated by difference (100% − % moisture). Total carbohydrates were estimated by difference: 100 − (% moisture + % crude protein + % crude fat + % ash content), while the nitrogen-free extract (NFL content) was determined as: % total carbohydrate − % crude fibre.

Samples were digested by wet-ashing (AOAC, 1984) and minerals were determined using a PE model 5000 atomic absorption spectrophotometer.

Protein was hydrolysed with 6N HCl and analysed using an LKB amino acid analyser (Model 4151 ALPHA) (LKB, Bromma, Sweden. 1983).

Fatty acid composition of the lipid extract

The fatty acid composition of the oil was determined by gas chromatography, according to the procedure reported by Metcalfe *et al.* (1966). Fatty acid methyl esters were identified on a GC-14A Shimadzu with C-R4A Chromatopac integrator.

Hydrogen, air and nitrogen flow rates were 30, 310 and 35 ml/min, respectively. A sample of 1 μl was injected on a 200 cm × 2 mm column which was packed with GP 10% SP–2330 on 100/120 Chromosorb WAW. The injector temperature was 250°C. The initial column temperature was 130°C programmed by 11·30°C/min until 200°C. The temperature of the flame ionization detector was 250°C. A comparison between the retention times of the samples with those of authentic standards, run on the same column under the same conditions, was made to facilitate identification.

Microbiological tests

Black cumin samples were powdered aseptically and 1 g of each was weighed and placed in a 9 ml sterile 0·1% (w/w) peptone solution. After shaking, serial dilutions were prepared and used for the microbiological tests. The total aerobic bacterial count was determined on Standard Plate Count Agar (Difco, Detroit, MI, USA) incubated at 30°C for 48 h. The total aerobic sporeformers count was determined on CASO Agar (Merck, Darmstadt, Germany) incubated at 30°C for 48 h. The suspension was pasteurized at 80°C for 20 min before plating. The *Bacillus cereus* count was enumerated on *B. cereus* Selective Agar (Merck) after pasteurization of the black cumin suspension, incubated at 30°C for 48 h. *Staphylococcus aureus* was detected on Baird-Parker agar (Difco) incubated at 30°C for 48 h. Enterabacteriaceae were determined on Brilliant Green Phenol Red lactose agar with plate incubation at 37°C for 24 h. Enumeration of yeast and moulds was carried out using Potato-Dextrose Agar (Difco) incubated at 22°C for 5 days.

All plates were made in duplicate according to the methods described by Speck (1984).

RESULTS AND DISCUSSION

The proximate and mineral compositions of Saudi black cumin seeds are presented in Table 1, along with the value obtained by Babayan *et al.* (1978). As seen from this table the crude fat represents the major component in the black cumin seed, followed by total carbohydrate, then crude protein. These results differed slightly in moisture, protein, ash and dry matter from those reported by Babayan *et al.* (1978). However, numerical differences were found in crude fat, crude fibre, total carbohydrate and NFL. These differences may be due to variations in the environmental factors in the areas where the black cumin seeds were grown. The low moisture content of black cumin seeds resulted in a high dry matter content (95·64%). The crude fibre content of 7·94% in black cumin seeds makes it a source of dietary fibre which could be helpful in reducing gastrointestinal disorders (Spiller *et al.*, 1978; Mercurio & Behm, 1981). The high levels of fat (38·20%) and protein (20·9%) render Saudi black cumin seeds a good source of fat and protein.

Table 1. Proximate and mineral compositions of Saudi black cumin seeds

	Present results[a]	Babayan *et al.* (1978)
	(%)	(%)
Component		
Moisture	4·6 ± 0·45	5·52
Crude protein (N × 6·25)	20·9 ± 1·35	21·26
Crude fat	38·2 ± 2·20	35·49
Crude fibre	7·9 ± 0·93	5·50
Ash	4·4 ± 0·32	3·77
Total carbohydrate	31·9 ± 2·56	33·96
Dry matter	95·6 ± 1·91	94·48
NFL	24·0 ± 2·83	28·46
Mineral element	(mg/100 g)	
Potassium	7·6 ± 0·42	
Phosphorus	1·8 ± 0·23	
Sodium	0·75 ± 0·10	
Iron	0·15 ± 0·04	
Zinc	0·06 ± 0·015	
Calcium	0·04 ± 0·008	
Magnesium	0·03 ± 0·005	
Manganese	0·02 ± 0·003	
Copper	0·02 ± 0·002	
Cadmium	—	
Lead	—	
Arsenic	—	

[a] Mean of 10 determinations ± SD on fresh weight basis.

Table 1 also shows the mineral content of black cumin seeds essential in human nutrition. Potassium was the predominant element in the seeds followed by phosphorus, then sodium and iron. However, zinc, calcium, magnesium, manganese and copper were present in low amounts and thus another source is necessary for supplying some of these elements. The heavy metals, cadmium, lead and arsenic were absent in black cumin seeds. Based on the recommended daily intake (Cuthbertson, 1989) and on the values obtained in Table 1, it is obvious, assuming high in-vitro bioavailabilities, that the Saudi black cumin seeds would be im-

Table 2. Fatty acid composition of Saudi black cumin seeds

Fatty acid		(%)[a]	Babayan *et al.* (1978)(%)
Myristic	C14 : 0	0·90 ± 0·08	0·16
Myristoleic	C14 : 1	0·18 ± 0·03	—
Palmitic	C16 : 0	11·90 ± 0·18	12·08
Palmitoleic	C16 : 1	0·30 ± 0·01	—
Stearic	C18 : 0	2·28 ± 0·11	3·11
Oleic	C18 : 1	23·58 ± 1·03	24·64
Linoleic	C18 : 2	59·34 ± 1·96	56·12
Arachidic	C20 : 0	0·14 ± 0·02	—
Linolenic	C18 : 3	0·30 ± 0·12	0·70
Eicosadienoic	C20 : 2	—	2·53
Lignoceric	C24 : 0	1·08 ± 0·012	—
Saturated fatty acids		16·30	15·35
Unsaturated fatty acids		83·70	84·65

[a] Mean of five determinations ± SD.

portant in contributing, partially, to the overall daily dietary intake of the elements.

The fatty acid composition of the lipids extracted from the Saudi black cumin is presented in Table 2 along with the values reported by Babayan *et al.* (1978). Ten fatty acids were detected. The dominating fatty acid was linoleic acid which accounted for more than 59% of the total fatty acids. This is nutritionally desirable. Aurand *et al.* (1987) have mentioned that the nutritional value of linoleic acid is due to its metabolism at tissue levels which produces the hormone-like prostaglandins. The activity of these includes lowering of blood pressure and constriction of smooth muscle; relief of nasal congestion and asthma; and prevention of gastric ulcers (Bloch, 1963; Bergstrom *et al.*, 1968; Samuelsson, 1972). The second major fatty acid was oleic acid (23·58%). The ratio of linoleic acid to oleic acid was more than 2 : 1. This result agreed that those reported in soybean oil (C18 : 2 = 52%, C18 : 1 = 25%) and in corn oil (C18 : 2 = 58·7%, C18 : 1 = 26·6%). The contents of the saturated fatty acids, palmitic (11·9%) and stearic (2·3%), are comparatively lower. The other six fatty acids were less then 3% of the total fatty acid content. However, the unsaturated fatty acids amounted to more than 83% of the total fatty acid content of the lipid extract, and all of the unsaturation was due to C18 acids. The results of this investigation agree with those reported by Babayan *et al.* (1978). However, there were negligible amounts of fatty acids detected in the present authors' results, e.g. C14 : 1 = 0·18%, C16 : 1 = 0·30%, C20 : 0 = 0·14% and C24 : 0 = 1·08, which were not reported by Babayan *et al.* (1978), while the fatty acid C20 : 2 = 2·53% was detected by Babayan *et al.* (1978) but was absent in this study. 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. Thus, it is necessary to investigate the lipid composition of individual plant sources.

Table 3. Amino acid composition of Saudi black cumin seed protein

Amino acids	Content (mg/100 g)[a] protein	% Contribution to protein content
Essential amino acids (E)		
Leucine	665 ± 3·51	5·82
Valine	527 ± 3·28	4·61
Lysine	462 ± 4·28	4·04
Threonine	417 ± 3·31	3·65
Phenylalanine	413 ± 2·67	3·61
Isoleucine	395 ± 2·11	3·46
Histidine	383 ± 1·64	3·35
Methionine	188 ± 0·37	1·65
Total essential amino acids	3450	30·19
Non-essential amino acids (N)		
Glutamic acid	2829 ± 19·34	24·74
Arginine	1051 ± 10·39	9·19
Aspartic acid	1022 ± 9·80	8·94
Glycine	642 ± 4·42	5·61
Proline	560 ± 3·91	4·90
Serine	493 ± 4·11	4·31
Alanine	427 ± 3·35	3·73
Tryosine	411 ± 2·95	3·59
Ammonium	325 ± 2·21	2·84
Cystine	224 ± 1·82	1·96
Total non-essential amino acids	7984	69·81
Total amino acids	11434	100
E/N	0·43	

[a] Mean of five determinations in duplicate of each ± SD.

The amino acid profile of Saudi black cumin seeds is shown in Table 3, listing the concentrations of 17 amino acids. These amino acids are arranged in decreasing order. Among these amino acids, eight essential amino acids were found. The major amino acids in black cumin seeds were glutamic acid followed by arginine, aspartic acid, leucine and glycine. These major acids constituted more than 54% of the total amino acids present in the protein of black cumin seeds. Cystine and methionine were the minor amino acids in black cumin seeds, while tryptophan was absent. Total non-essential amino acids (*N*) present in the black cumin seeds are much higher than their corresponding essential amino acids (*E*). Differences were observed between the authors' results and those of Babayan *et al.* (1978), regarding the amino acid profile of black cumin seeds, which may be due to genotypic and/or environmental variations.

The microbial load of Saudi black cumin seeds is given in Table 4. According to the microbiological criteria of spices, which have been recommended by the International Commission on Microbiological Specification for Foods (1974), many spices are considered unacceptable in quality when the bacterial count exceeds 10^6/g, and when the number of moulds are higher

Table 4. Microflora of Saudi black cumin seeds

Type of organism	Microorganisms (cfu/g)
Total aerobic bacteria	7×10^7
Total aerobic sporeformers	4×10^4
Bacillus cereus	$<10^2$
Staphylococcus aureus	<10
Yeast and moulds	4×10^2

than 10^4/g. Under these criteria, the black cumin seeds had a high total aerobic bacterial count (7×10^7) which is unacceptable, while the yeast and moulds of black cumin seeds are acceptable at 4×10^2. The low numbers of *S. aureus* and *B. cereus* in black cumin seeds (Table 4) mean that there was no potential health hazard associated with black cumin seeds.

CONCLUSION

The results obtained in this study demonstrated that Saudi black cumin seeds (*Nigella sativa* L.) are an important source of protein, essential fatty acids, amino acids, and crude fibre, which help in maintenance of healthy lower intestines. Some further study is needed to investigate the possibility of using Saudi black cumin seeds as a potential source for commercial production of oil.

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