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COMPARISON OF PHYSICO-CHEMICAL AND MICROBIAL PROPERTIES OF INDIAN AND ETHIOPIAN ORIGIN BLACK CUMIN (*Nigella sativa*) SEEDS

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Abstract

Scope this study is to determine variations occur in physico-chemical and microbial properties black cumin seeds produced in different parts of the world. Hence two types of Black cumin seeds "Ethiopian and Indian" origin were taken and subjected to proximate analysis. Results revealed that moisture, crude protein, fat, fiber and carbohydrate contents of Indian and Ethiopian Nigella sativa were 8.11 & 7.06%, 23.13 & 20.57%, 33.03 & 37.03%, 6.03 & 6.90 and 32.70 & 31.16% respectively. These proximate values pertaining to the two types were significantly different to each other ($p<0.05$) except ash content. While moisture, crude protein and total carbohydrates are higher in Indian type fat and fiber are higher in Ethiopian type. With respect to microflora, both varieties Indian and Ethiopian showed less than 10 colony forming units per ml for Total Plate count and yeasts and moulds.

Key Words: Nigella sativa, Physico-chemical properties, microbial properties

1 Introduction

Nigella sativa is an annual flowering plant often called as fennel flower native to South Asia and belongs to Ranunculaceae family. Blue colour flower holds small black caraway type seeds and it grows to a maximum height about 60cm [1]. Seeds of black cumin are small in size and angular in shape with a pungent bitter taste and black cumin seeds are used as a carminative and spice due its unique volatiles. Often used in coffee, tea, casserols and salads [2]. Furthermore, as traditional sweet dishes with paste, sweetened with honey and as a condiment in bread, cheese and pickles [3]. In spite of culinary importance *Nigella sativa* is known for medicinal purposes as well as for functional cosmetics and dietary. In fact, this spicy plant has occupied a special place for its important applications in medical side. Traditionally *Nigella sativa* is used as diuretic, diaphoretic, stomachic, liver tonic and digestive remedy which is naturally occurred [4]. Extract is having protective and curative actions for many diseases. Composition of *Nigella sativa* is different from one type to another considering the quantity of each parameter. In fact, the compositions are very complex as it is containing aromatics of high volatility, oils and fats, which are easily oxidized [5]. Most of the research studies have been done to investigate black

cumin types like Tunsanian and Egyptian and only a lesser amount of information could be found on Indian and Ethiopian varieties. Therefore, the extensive focus of this study is to analyze these two black cumin types, Indian and Ethiopian origin.

2. Materials and method

2.2 Plant material

Seeds of *Nigella sativa* samples were collected from a Sri Lankan company and one sample was imported from India and other one from Ethiopia. Seeds were tested for quality parameters and they were stored in the laboratory condition in the department of food science and technology, university of Sri Jayewardenepura. Two types of black cumin samples were separately milled in a heavy grinder for 2 minutes, to pass 1mm diameter aperture while cautiously avoiding undue heat buildup of apparatus during grinding. Samples were mixed separately to avoid stratification. Samples were preserved in dry stoppered containers at -20^0C until analysis. (A.O.A.C 17th edition 2000, Official Method 920.164 Preparation of Test sample)

2.3 Analytical methods

2.3.3 General

All analytical determinations were performed according to method of Association of Official Analytical Chemists17th edition 2000, test methods for spices. Values were expressed as the mean standard deviations for triplicates. The statistical analysis of data was carried out for all experiments using ANOVA to test the significance of each variable (α=0.05) and followed by comparisons performed using the Turkey test by the statistical software MINITAB R 17.

2.3.4 Moisture content

Moisture content of grinded blackseeds was estimated according to Dean and Stark Toluene Distillation Method A.O.A.C 17th edition 2000 Official Method 986.21, Moisture in Spices. The amount of water is determined by distilling the material with an organic liquid not miscible with water and collecting the distillate in a graduated tube of Moisture Distillation Apparatus.

2.3.5 Fat content

Total fat content was determined following the Majonnier Ether Extraction method as mentioned in Association of Official Analytical Chemists (AOAC method,922.06). Oil was extracted from 2g of black seed powder in a Majonnier flask by using diethyl ether and petroleum ether. Result was expressed as a percentage of total fat in dry basis.

2.3.6 Protein content

Micro kjeldhal method was utilized to determine protein content in Ethiopian and Indian *Nigella* seeds. According to the AOAC method 960.52, sample of 30 mg of blackseed powder was digested in Kjeldhal kit (VELP SCIENTIFICA – UDK 129) for nearly 3 hours. After neutralization in the Kjeldhal instrument, released condensed fume was trapped in to 4% Boric

acid solution which was used then to titrate against with 1 M HCl. Protein content was calculated using nitrogen conversion factor 6.25 (Al-Gaby, 1998) and data were expressed as percent of dry weight.

2.3.7 Fiber

I.S Specification No I.S 1797 – 1985 Methods of Test for Spices and Condiments was followed to determine fiber content in both Indian and Ethiopian *Nigella* seeds which were defatted by using soxhelt extraction. Then the defatted samples were digested by boiling Sulphuric acid and sodium hydroxide solutions in a flask connected to a water-cooled reflux condenser.

After washing the digested samples, they were kept in the oven at 105±1°C for 3 hours for drying and incinerated in a muffle furnace at 550±25° C until all carbonaceous matter is burnt. Final weight difference was used to calculate fiber content in which expressed as percent of dry weight.

2.3.8 Ash and mineral content

Organic matter in the sample was removed by igniting and then incinerating powdered 2g of samples placed in the muffle furnace (Wise Therm) at 550^0C for 3-4 hours. Ash content was determined as mentioned in A.O.A.C Method 941.12 and mineral constituents Ca, Na, Mg, K, Fe, Mn, Zn, Cu, Al, Ni, Pb were determined using atomic absorption spectrophotometer.

2.3.9 Carbohydrate content

Carbohydrate content was estimated by difference of mean values: 100 – (Total fat + Crude protein + Crude Fibre + Total ash).

2.4 Microbiological analysis

Total plate count (TPC) and yeast & mould count were determined in both Ethiopian and Indian origin blackseeds. Powdered samples of *Nigella* seeds were taken for the preparation of dilution series. 1g of seed powder was added into test tube containing 9 ml of sterile peptone water in preparing the series. Sterilized buffered peptone water was prepared for both testing. Plate count agar was used as the culture media to determine total plate count and Chloramphenicol Yeast Glucose Agar was used as the culture for yeast and moulds. Sterilization of glassware, peptone water and culture media were sterilized at 121 ^{0}C and 15 psi for 20 minutes. Then inoculation was followed by pipetting 1ml into a marked, sterile petri dish. After adding sterilized medium dishes were thoroughly mixed evenly by alternate rotation and back-and- forth motion of petri dishes. Media was allowed to solidify and the solidified petri dishes were inverted and incubated quickly for 3-5 days at 25 ± 1^0C. Finally, colony forming units (CFU) calculated.

3 Results and discussion

3.2 Proximate analysis

Both Indian and Ethiopian origin black cumin seeds were analyzed for their proximate compositions and mineral content. Moisture, crude protein, fat, crude fiber, carbohydrate & ash contents along with the minerals are given in table 3.1 and 3.2

Table 1: Proximate and mineral composition of* Nigella sativa *seeds of Indian and Ethiopian origin

Characteristics	Indian (%)	Ethiopian (%)
Moisture content	8.13 ± 0.11^{a}	7.06 ± 0.15^{b}
Crude protein	23.13 ± 0.25^{a}	20.57 ± 0.67^{b}
Fat	33.03 ± 0.36^{a}	37.03 ± 0.15^{b}
Fiber	6.03 ± 0.15^{a}	6.90 ± 0.10^{b}
Total carbohydrate	32.70 ± 0.20^{a}	31.16 ± 0.25^{b}
Ash	4.00 ± 0.17^{a}	4.10 ± 0.20^{a}
Dry matter content	98.89	99.76

Data presented as mean values for triplicates ± S.D (n=3) in same raw are significantly different at ($p < 0.05$) level.

The data obtained from the study were in line with the previous studies conducted by Atta [6], Nergiz [7] and Sultan [8]. The fat represents the major component in both type of black cumin seed, followed by total carbohydrate and crude protein as shown in the table 3.1. Proximate analysis of Indian origin *Nigella* seeds show that moisture content, crude protein and total carbohydrate were considerably higher than that of Ethiopian blackseeds whereas fat and fiber contents were higher in the Ethiopian origin. According to the statistical analysis at 95% confidence level these values were significantly different to each other except the ash content ($p<0.05$).

Reasons for these differences may be due to the geographical variations and climatic conditions which are predominantly contrast as one type is grown in India and other is in Ethiopia where a dry climatic condition is prevailed. Atta [6] also reported environmental factors such as soil condition, storage condition and even maturity stage contribute in variation of physico-chemical properties of black cumin seeds excluding genetic variation which is not identical in these two types in order to cause a significance variation. Further, as these two varieties were imported to Sri Lanka not at the same conditions and even at different times, those factors may also cause for the variations. However, same conditions were maintained during the analysis for physic-chemical properties. The moisture contents of two types of black cumin seeds resemble with Egyptian origin (7-8% [6]) but compared to Saudi Arabic type (4%, [9]) it's slightly higher. It may due to climatic condition variations in these regions.

Nevertheless, the high levels of fat (37.0%) and protein (20-24%) render both varieties of black cumin seeds as a good source of fat and Ethiopian origin is more significant in this regard. The crude fibre content of 6-7% in both origins of black cumin seeds makes it's a potential source of dietary fibre which could be helpful in reducing gastro intestinal disorders [10].

Table 2: Mineral content of black cumin seeds of Indian and Ethiopian origin types

Mineral	Indian	Ethiopian
K[a]	75.27 ± 1.18	57.95±0.85
Na[a]	93.60 ± 1.03	116.86±0.16
Mg[a]	198.36 ± 1.41	206.05±1.41
Fe[a]	7.91±1.49	6.85±0.73
Ca[a]	111.70±3.60	126.11±4.19
Zn[b]	18.40±0.63	15.91±0.74
Cu[a]	0.77±0.01	0.90±0.02
Mn[a]	1.67±0.13	1.67±0.007
Cr[a]	1.91±0.41	1.43±0.03
Pb[a]	0.04±0.003	0.31±0.23
Ni[a]	1.12±0.02	2.40±0.04
Al[a]	9.15±0.04	4.71±0.15

Data presented as mean values for triplicates ± S.D (n=3). a and b letters in same raw are significantly different at ($p < 0.05$) level; a in mg/100g and b in µg/100g

According to table 3.2, a significant mineral content can be observed in both Indian and Ethiopian black cumin seeds. Magnesium and the Calcium are the predominant elements in the seeds followed by potassium and sodium. However, iron and aluminummetals were presented in low quantity in both varieties. Heavymetals like lead were presented in slightest amount in both varieties. Metals like Cr, Mn and Pb in both types are not significantly different whereas important minerals are significantly different at 95% confidence level.

3.3 Determination of Microflora of black cumin

The microbial load of black cumin was analyzed as it is a primary parameter when taking into account of quality of a product. Total plate count and the yeast and moulds of Indian and Ethiopian origin black cumin were analyzed and results are given in the table 3.3

Table 3: Microflora of Indian and Ethiopian black cumin

Type of organism	**Microorganism (cfu/ml)**	
	Ethiopian origin *Nigella sativa*	Indian origin *Nigella sativa*
Total plate count	<10	<10
Yeast and moulds	<10	<10

In both types observed, microorganisms yeast and moulds and the total plate count were less than 10. According to the microbiological criteria of species, which have been recommended by the International Commission on Microbiological Specification for Foods (1974), many species are considered unacceptable in quality when the bacterial count exceeds 10^6/g and when the number of moulds are higher than 10^4/g. under these criteria, black cumin can be considered as a quality species as results are less than the accepted specifications.

4 Conclusion

This study has revealed that both types of black are rich source of nutrients such as fat and protein. Metals like Magnesium and the Calcium are the predominant elements in the seeds and this study provides evidence in important health impacts of *Nigella sativa* that can be utilized in food and pharmaceutical industry.

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