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Proximate Analysis and Fatty Acid Composition of *Nigella sativa* (Kalonji) Seed Oil Growing in Pakistan

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Abstract. Physical and chemical characteristics including fatty acid composition of samples of seven commercially available *Nigella sativa* oil and three freshly extracted seed oil, collected from different localities, were determined by gas liquid chromatography. The average and standard deviations found were: refractive index at 20 °C, 1.473 ± 0.0018; specific gravity at 20 °C, 0.9166 ± 0.0002; iodine value (IV, Wij's), 119.98 ± 1.8; saponification value, 201.80 ± 2.2 and unsaponifiable matter, 0.61% ± 0.05. Fatty acid (FA) profile was based on high levels of unsaturated FA like oleic acid, 24.17% ± 0.61; linoleic acid, 53.64% ± 0.799 and eicosadienoic acid, 2.3% ± 0.37. Saturated FA such as palmitic acid and stearic acid amounted to 14.82% ± 0.49 and 2.95% ± 0.37, respectively. Myristic and palmitoleic acids were also detected in minor quantity.

Keywords: *Nigella sativa* oil, oleic acid, linoleic acid, eicosadienoic acid, fatty acid composition

Introduction

Nigella sativa (black cumin) is well known in western countries, the Middle East and Western Asia due to its traditional and medicinal applications (Randhaw and Al-Ghamdi, 2002; Ghaznavi, 1996). In Pakistan and India it is cultivated as an annual herb and is called Kalonji (Randhaw and Al-Ghamdi, 2002; Nadkarni, 1976). Intake of 1g seeds orally, twice a day for two weeks, decreased human blood glucose level (Bamosa *et al.,* 1997). In other toxicity studies, low toxicity of *N. sativa* fixed oil is found to contribute to its safe application at therapeutic dose levels (Zaui, 2002). Traditionally, the fixed oil expressed from seeds of *N. sativa* is used topically for the treatment of eczema, arthritis, back pain and psoriasis. Anti-inflammatory effect of the fixed oil has also been investigated (Teuscher, 2006; Mahfouz and EI-Dakhkhany, 1960).

As far as the nutritional value of *N. sativa* oil is concerned, it contains valuable nutrients, such as fixed and volatile oils besides protein, ash, minerals, essential amino acids and some vitamins. (Takruri *et al.,* 1998). Fixed oil of *N. sativa* seeds yields triglycerides of fatty acid; the latter help to determine the biological properties of the body cells. Much recent research has confirmed that a dietary source of Omega-6 and Omega-3 fatty acids is essential for optimum tissue functions in humans. These essential fatty acids cannot be manufactured by the human body and thus must be taken through essential fatty acid containing food supplements in order to sustain health. *N sativa* oil is a rich source of linoleic fatty acid (ω6) which has the ability to boost human immune system significantly.

In the present study, the physicochemical properties and fatty acid composition of freshly extracted *N. sativa* oil (from 3 seed samples) and seven samples of commercially available seed oil have been determined through the classical and instrumental methods. Such work has not been reported previously on *N. sativa* cultivated in Pakistan.

Materials and Methods

Product selection. The seeds of *N. sativa* are tiny, sharp cornered and deep black in color. Seeds were purchased from three different localities and assigned codes: KLJ, HOC and SOS. Seven branded samples of expressed oil of *N. sativa* were also purchased from the local market and coded as ASA, BPS, SAE, DOS, BCS, VER, and STS.

Reagents and glass wares. All the chemical reagents such as iodine monochloride, potassium iodide, hydrochloric acid and some pure standards of fatty acid methyl esters used in the analytical work were purchased from E. Merck and Sigma Chemical Company. The glasswares were cleaned with 1:1 HNO_3 before use.

Apparatus. A Perkin Elmer gas chromatograph model Clarus 500 fitted with a polar capillary column SP 2330, 60x0.25x0.20 film thickness, flame ionization detector and a HP Laser jet 1300 printer was used for fatty acid quantification.

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Methods of analysis. ***Extraction of oil from seed samples.*** Seeds of *N. sativa* were cleaned and ground in an electrical grinder. One kg of three seed samples, KLJ, HOC and SOS, were pressed to yield the oil and cake. Oil from the cake and powdered seeds were obtained by soxhlet extraction method using *n*-hexane. After 6 h the Soxhlet extracts of three samples were distilled off under vacuum in a rotary evaporator.

Analysis of oil seed residue. The oil seed residue (meal) obtained after extraction of oil from seeds were analyzed for ash, protein , fibre and moisture in seeds according to the standard methods.

Fibre. Fibre content was determined by ISO method (ISO, 1981). Meal (2.5 g) was weighed and defatted by extraction with 20 ml *n*-hexane. The residual test portion was boiled with sulphuric acid (0.255 mol/l) followed by separation,washing and drying. The dried residue was weighed and ashed in a muffle furnace at 600 °C. Loss of weight was determined.

Ash. A test portion (2.0 g) was taken into the previously heated combustion crucible and carbonized by heating on a gas flame. The carbonized material was ashed in an electric muffle furnace at 600 °C until constant weight was achieved (AOCS, 2004a).

Protein. Protein contents were determined according to AOCS method (AOCS, 2004b). Weighed amounts of sample was digested in Kjeldahl flask after adding catalyst mixture and concentrated sulphuric acid. The contents of the flask were cooled and water was added. After further processing, the content of receiving flasks was titrated with 0.25 N, NaOH solution using 3 to 4 drops of methyl red indicator. A blank determination was also conducted in a similar way. Nitrogen percentage and protein were calculated by using conversion factor of 6.25.

Moisture %. Sample (2 g) was weighed into a moisture dish previously dried and heated with its cover. The uncovered dish was placed in a vacuum oven, the pressure was adjusted to 25 mm Hg and the contents dried to constant weight at 98±2 °C (AOCS, 2004c).

Analysis of extracted oil. Physical and chemical parameters including density, refractive index, iodine value (Wij's), saponification value and unsaponifiable matter of the extracted oil were determined by standard AOCS methods (AOCS, 2004d).

Fatty acid composition. Fatty acid methyl esters (FAME) were prepared according to standard method (2.301, IUPAC, 1987). Oxygen free nitrogen gas was used as a carrier gas at a flow rate of 2.5 ml/min. Other conditions were as follows:

Initial oven temperature: 70 °C for 5 min, ramp rate 10 °C/min upto 180 °C; final oven temperature: 220 °C, at a rate of 3 °C/min, holding for 15 min; injector temperature: 260 °C; detector temperature: 275 °C.

A sample volume of 0.5 microlitre was injected (splitless). FAME were identified by comparing their relative and absolute retention time with authentic standards. The quantification was done by a built-in data handling programme (total chrome navigator) provided by the manufacturer of gas chromatograph. The fatty acid composition was reported as a relative percentage of the total peak area and was performed in triplicate.

Results and Discussion

Table 1 shows some physical and chemical characteristics (mean ± SD; range) of the seven *N. sativa* commercial oils. The average values and ranges obtained are as follows: specific gravity at 20 °C, 0.9166 (0.9163-0.9169); refractive index (RI) at 20 °C, 1.473(1.4699-1.475); iodine value (Wij's), 119.98 (116.85-121.96); saponification value 201.80 (198.69-204.58); and unsaponifiable matter, 0.61% (0.54-0.7%). Values of R.I. and unsaponifiable matter are in good conformance with those reported by Gad *et al.* (1963) while saponification value and iodine value are higher and S.G is lower than the values determined and reported by Gad *et al.* (1963).

Table 2 indicates the oil content (average, range) of *N. sativa* seed samples (KLJ, HOC, SOS). Soxhlet extraction with *n*-hexane yielded 35-37.5 % oil. By cold pressed method 30.2-34.5 % oil was obtained. Subsequent Soxhlet extraction with *n*-hexane yielded total amount of pressed oil plus extracted pressed cake oil of 35.6-39%, agreeing closely with the oil yield obtained by straight Soxhlet extraction of the ground seed as reported by other studies (Abdel and Attia, 1993; Al, 1992).

Table 3 shows proximate analysis of oil seed residue of *N. sativa* being protein, 34.03 %, fibre, 7.16 %, moisutre, 6.04 % and ash, 4.4 %. Moisture, ash and fibre content of oil seed residue are in general agreement with the previously published data (Randhaw and Al-Ghandi 2002; Al, 1992).

High protein and oil content in the present analysis indicate high nutritional potential of Pakistani Kalonji seeds. Oil seed residue may be added to the poultry or other animals feed as a source of energy.

Table 4 represents the fatty acid composition (mean ± SD range) of the *N. sativa* oils; the total amount of saturated fatty

Table 1. Physical and chemical characteristics of commercial *N. sativa* oil samples

Sample code	Density at 20°C[a] (g/ml)	Refractive index at 20°C[b]	Iodine Value (Wij's)[a]	Saponificaton value[a] (mg of KOH/g oil)	Unsaponifiable matter (%)[a]
ASA	0.9164	1.4751	120.8	203.49	0.54
BPS	0.9163	1.4699	119.84	198.69	0.70
SAE	0.9167	1.472	121.96	200.98	0.62
DOS	0.9168	1.473	116.85	202.4	0.59
BCS	0.9166	1.475	118.48	199.25	0.66
VER	0.9165	1.472	121.87	204.58	0.58
STS	0.9169	1.474	120.08	203.26	0.60
Av.	0.9166	1.473	119.98	201.80	0.61
Range	0.9163-0.9169	1.4699-1.475	116.85-121.96	198.69-204.58	0.54-0.7
SD	0.0002	0.0018	1.8	2.2	0.05

[a] = average of two determinations; [b] = average of three determinations; SD = standard deviation

Table 2. Oil content in *N. sativa* seeds (%)

Extraction method	Sample KLJ	Sample HOC	Sample SOS
Cold press	34.5	30.2	32.6
Soxhlet extraction of pressed cake	4.5	5.4	6.2
Total of 1+2 (*[1])	39.0	35.6	38.8
Soxhlet extraction of ground seed (*[2])	36.8	34.9	37.5
Average of *[1]	37.8		
Average of *[2]	36.40		
Range of *[1]	35.6-39.0		
Range of *[2]	34.9-37.5		

Table 3. Analysis of oil seed residue

Components	Sample KLJ	Sample HOC	Sample SOS	Average (%)	Range	SD
Protein (%)	34	33.6	34.5	34.03	33.6 – 34.5	0.45
Fiber (%)	7.2	6.9	7.4	7.16	6.9 – 7.4	0.25
Moisture (%)	6.03	5.6	6.5	6.04	5.6 – 6.5	0.45
Ash (%)	4.45	3.98	4.8	4.41	3.98 – 4.8	0.41

Table 4. Fatty acid composition of commercial *N.sativa* seed oil (% of total fatty acids)

Sample	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosadienoic acid
ASA	0.36	14.9	0.17	2.8	23.5	54.5	0.29	2.4
BPS	0.32	14.2	0.2	3.4	23.8	53.6	0.24	2.9
SAE	0.39	15.3	0.21	3.0	24.7	52.9	0.31	2.6
DOS	0.28	14.8	0.13	2.5	24.1	54.9	0.25	1.9
BCS	0.37	14.4	0.15	3.5	24.6	52.7	0.32	2.3
VER	0.24	15.6	0.18	2.6	23.7	53.5	0.30	1.9
STS	0.28	14.6	0.16	2.9	24.8	53.4	0.28	2.1
Av.	0.32	14.82	0.17	2.95	24.17	53.64	0.28	2.3
Range	0.24-0.39	14.2-15.6	0.13-0.21	2.5-3.5	23.5-24.8	52.7-54.9	0.24-0.32	1.9-2.9
SD	0.05	0.49	0.01	0.37	0.61	0.799	0.03	0.37

Table 5. Fatty acid composition of *Nigella sativa* seed oil extracted by two methods (% of total fatty acid)[a]

	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosadienoic acid
Soxhlet extracted oil from ground seeds	0.25	14.2	0.15	3.6	24.4	54.3	0.37	1.7
Cold pressed oil from ground seeds	0.23	13.2	0.10	3.1	24.2	55.4	0.27	2.4
Soxhlet extracted oil from pressed cake	0.28	12.8	0.08	2.9	23.4	55.8	0.20	3.1
Av	0.25	13.4	0.11	3.2	24.0	55.16	0.28	2.4
Range	0.23-0.28	12.8-14/2	0.08-0.15	2.9-3.6	23.4-24.4	54.3-55.8	0.2-0.37	1.7-3.1

[a] = average of three determinations.

acids, palmitic and stearic acid, is 17.77% i.e, 14.82% palmitic acid and 2.95% stearic acid.

Monounsaturated fatty acid, oleic acid was 24.17%, whereas, polyunsaturated fatty acid (PUFA), linoleic acid (ω6) was identified as the major component, being 53.64 % (Fig.1). An unusual fatty acid, eicosadienoic acid (2.3 %) was also found to be present in *N. sativa* seed oil; this acid was first reported by Houghton *et al.* (1995) and makes a major contribution in the total oil due to its anti-eicosanoid and antioxidant activity. Pharmacological properties support the use of *N. sativa* fixed oil or its derived products for the treatment of rheumatism and related inflammatory diseases. The present investigation revealed that there is no noticeable variation in fatty acid composition of seven commercial oils and freshly extracted seed

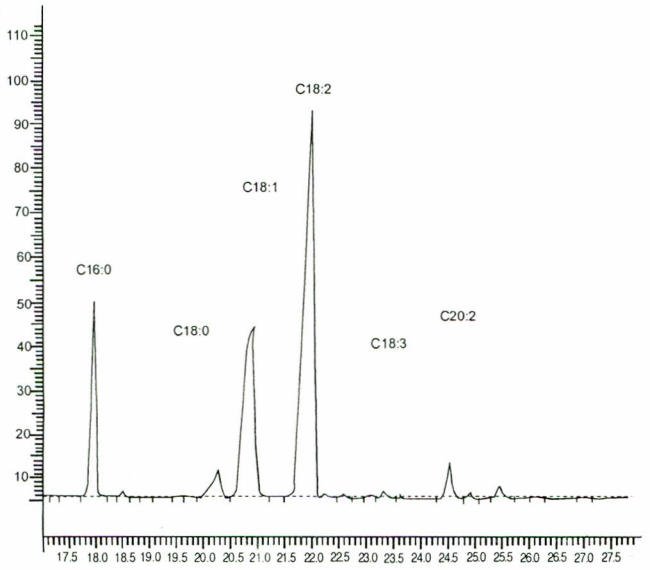

Fig. 1. Chromatogram of methyl esters of *Nigella sativa* seed oil.

oils by two modes of extraction (Table 5). which are very similar to each other and the data also agrees with the published GLC data of *N. sativa* fixed oil (Babayan *et al.*, 1978).

References

Abdel-Aal, E.S.M., Attia, R.S. 1993. Characterization of black cumin *(Nigella sativa)* seeds. 1.Chemical composition and lipids. *Alex. Sci. Exch.* **14 :** 467-82.

Al, J.M.S. 1992. Chemical composition and microflora of black cumin *(Nigella sativa)* seeds growing in Saudi Arabia. *Food Chem.* **45:** 239-242.

AOCS. 2004a. *Official and Recommended Practices of the American Oil Chemists Society, Ash.* Ba 5a-49, 5th edition, US FDA, Washington, DC.,USA.

AOCS. 2004b.*Official and Recommended Practices of the American Oil Chemists Society, Nitrogen-Ammonia Protein,* Ba 4d-90, 5th edition, US FDA, Washington, DC., USA.

AOCS. 2004c. *Official and Recommended Practices of the American Oil Chemists Society. Moisture and Volatile Matter,* Ba 2a-38, 5th edition, US FDA, Washington, DC., USA.

AOCS. 2004d. *Official and Recommended Practices of the American Oil Chemists Society*, 5th edition, AOCS, Champaign, Illinoise, USA.

Babayan, V.K., Koottungal, D., Halaby, G.A. 1978. Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* L seed. *J. Food Sci.* **43:** 1314-1315.

Bamosa, A.O., Ali , BA., Sowayan , S.A. 1997. Effect of oral ingestion of *Nigella sativa* seeds on some blood parameters. *Saudi Pharm. J.* **5:** 126-129.

Gad, A.M., El-Dakhakhany, M., Hassan, M.M. 1963. Studies on the chemical constitution of Egyptian *Nigella sativa* L.oil. *Planta Med.* **11:** 134-138.

Ghaznavi, K. 1996. *Tibbe Nabvi aur Jadeed Science*,

pp. 46-254, Al-Faisel Publishers, Lahore, Pakistan.

Houghton, P.J., Zarka, R., de las Heras, B., Hoult, J.R. 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* **61:** 33-36.

IUPAC. 1987. *Standard Methods for the Analysis of Oils, Fats and Derivatives,* 7th revised and enlarged edition, C. Paquot, A. Hautfenne, (eds.), Blackwell Scientific Publications, London , UK.

ISO 1981. *Agricultural Food Products, Determination of Crude Fibre Content. General Method,* ISO-5498, Geneva, Switzerland.

Mahfouz, M., EI-Dakhkhany, M. 1960. Some chemical and pharmacological properties of the new antihistaminic drug "Nigellone". *Egypt Pharm. Bull.* **42:** 411-242.

Nadkarni, A.K.1976. *Indian Materia Medica,* Popular Parkashan Pvt. Ltd., Bombay, India.

Randhawa, M.A., Al-Ghamdi, M.S. 2002. A review of the pharmacotherapeutic effects of *Nigella sativa. Pak. J. Med.Res.* **41:** 77-83.

Takruri, H.R.H., Dameh , MAF. 1998. Study of the nutritional value of black cumin seeds *(Nigella sativa). J. Sci. Food Agric.* **76:** 404-410.

Teuscher, E. 2006. *Medicinal Spices: A Hand Book of Culinary Herbs, Spices, Spice Mixtures and their Essential Oils*, pp. 83-87, Medpharm Scientific Publisher, Stuttgart, Germany.

Zaoui, A., Cherrah, Y., Mahassini, N., Alaoui , K., Amarouch, H ., Hassar, M. 2002. Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine* **9:** 69-74.