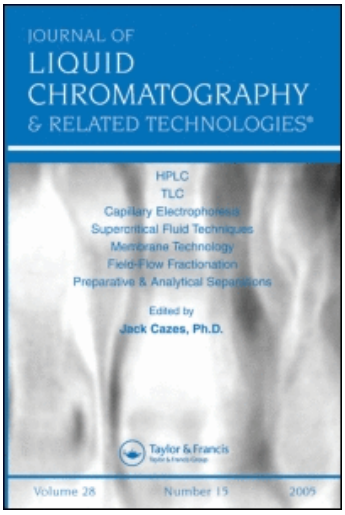


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Fractionation of Black Seed (*Nigella sativa* Linn) Proteins by Using Rotofor

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**To cite this Article** Haq, Afrozul , Remo, Nona and Al-Sedairy, Sultan T.(1996) 'Fractionation of Black Seed (*Nigella sativa* Linn) Proteins by Using Rotofor', Journal of Liquid Chromatography & Related Technologies, 19: 4, 593 — 599
**To link to this Article: DOI:** 10.1080/10826079608005522
**URL:** http://dx.doi.org/10.1080/10826079608005522

J. LIQ. CHROM. & REL. TECHNOL., 19(4), 593–599 (1996)

# FRACTIONATION OF BLACK SEED (*NIGELLA SATIVA* LINN) PROTEINS BY USING ROTOFOR

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## ABSTRACT

Many plants and their products are used as sources of pharmaceuticals and as ingredients of traditional medicines, and are of value in new drug discovery. In this study, we fractionated the whole black seed proteins into twenty fractions by using Rotofor. The pH range was recorded as (2.63 to 10.46). Protein concentration range and percent protein recovered were 0.305 to 1.01 mg/mL and 5 to 17% respectively. A single 4 hr Rotofor run at 12W in the pH 3 to 10 range yielded well separated proteins into twenty fractions. Black seed (*N.sativa*) proteins isolated range between 200 kDa and 14 kDa when these fractions were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

## INTRODUCTION

Herbal remedies form a potpourri that ranges from plants that people collect themselves and then take for health reasons to approved medicinal products. Herbal products vary from well known European herbs to less familiar Asian and South American ones and from botanicals with an excellent safety record to potentially dangerous ones, such as broom[1] and yohimbe[2]. The

World Health Organization estimates that some 20,000 species of higher plants are used medicinally throughout the world. A recent compilation on traditional medicines describes the uses of medicinal plants in countries as far apart as China, Ghana, India, Japan, Mexico, Panama, Samoa and Thailand[3].

*Nigella sativa* (*N.sativa*) commonly known as black seed which belongs to the botanical family of Ranunculaceae, has been in use in many Middle Eastern and Far Eastern countries as a natural remedy for over 2000 years. *N.sativa* seeds are commonly eaten alone or in combination with honey and in many food preparations. Abou-Basha et al,[4] and Aboul-Enein & Abou-Basha,[5] determined thymoquinone, dithymoquinone and thymol in black seed (*N.sativa*) oil by TLC and HPLC. Recently, we successfully studied the effect of water soluble *N.sativa* fractions on lymphocyte response to pooled allogeneic cells from 21 normal donors. *N.sativa* protein extract enhanced the production of interleukin-3 by human lymphocytes when cultured with pooled allogeneic cells or without any aided stimulator. Interestingly, *N.sativa* proteins increased interleukin-1ß, suggesting therefore, that it has an effect on macrophages[6].



Amino acid analysis of the black seed protein hydrolysate by gas chromatography of n-propyl, N-acetyl derivatives showed the presence of 15 amino acids including 9 essential amino acids. The seed protein is rich in arginine, glutamic acid, leucine, phenylalanine and lysine, arginine being the major amino acid[7]. Polypeptides and proteins are playing an increasing role in drug therapy. Molecules such as calcitonin, human growth hormone, interferons, colony stimulating factors have all made an important impact in the market place. A careful evaluation of the published literature leads to the conclusion that under normal circumstances only small quantities of peptides and proteins will be expected to be absorbed intact from the human gastrointestinal tract[8].

In the present study, we fractionated the whole black seed (*N.sativa*) by using Rotofor fractionation. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to determine the molecular weight of *N.sativa* proteins.

## MATERIALS AND METHODS

### Chemicals

Acrylamide, bisacrylamide, TEMED, Biolyte (ampholyte) (Bio-Rad, Richmond, CA, USA). SDS, ammonium persulphate, glycine, Tris (Pharmacia

Biotech, Uppsala, Sweden). NaOH and $H_3$ $PO_4$ (Fisher Scientific Co. Pittsburgh, PA. USA) were used.

## Protein Extraction

The black seed (*N.sativa* Linn) proteins were defatted by using Soxhlet apparatus. The defatted *N.sativa* was air dried for 24 hr at room temperature and then powdered. Protein concentrate was made according to the method of Mattil[9].

Defatted black seed powder was dissolved in phosphate buffer saline (PBS) in a ratio of 1:10 (w/v) for 1 hr with continuous stirring at room temperature and then centrifuged at 10,000 rpm for 30 min. The supernatant was collected, and protein concentration was determined[10]. Whole black seed proteins were first dialyzed (overnight at 4°C) against 0.01M Tris using 3,500 MW cut off dialysing bags. Following dialysis, carrier ampholyte (Bio-Rad , Bio-Lyte), pH range 3-10, 2% V/V was added and the total volume was brought to 60mL with PBS (pH7.1). Constant power (12 W) was applied to Rotofor for 4 hr with a system cooled to 4°C. Runs were terminated when the voltage had stabilized (1500V) for about 30min.



Twenty Rotofor fractions were collected. Eighteen of these fractions were then analyzed by SDS-PAGE. pH gradient, % protein recovery and total protein concentration in each sample was determined. Removal of ampholyte was accomplished by dialyzing all fractions overnight against 100 volumes of 0.1M Tris, pH 7.4 containing 0.25 mM NaCl and 0.5 mM MgCl2.

## Preparative SDS-PAGE

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) was performed in 12% gels according to Laemmli[11]. Protein samples were added to sample buffer and then denatured by boiling for 5-10 mins. Low molecular weight and high molecular weight calibration kits (Bio-Rad, Richmond, USA) containing myosin (200 kDa), ß-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21 kDa) and lysosyme (14 kDa) were used as marker proteins. Staining of gels was done with R250 coomassie brilliant blue (0.1%).

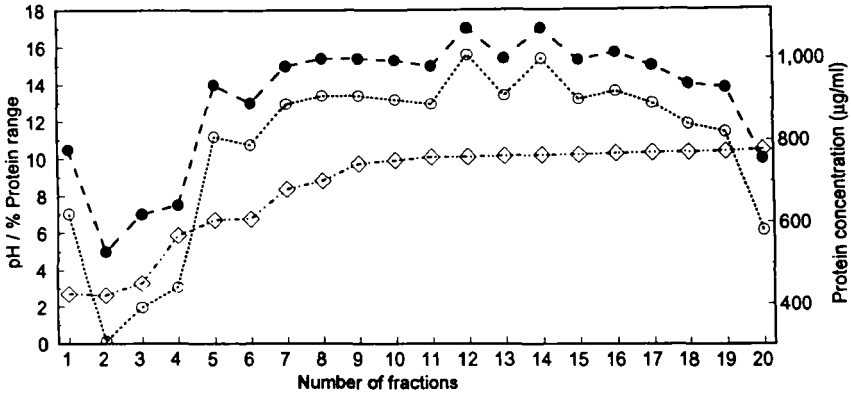


**Figure 1.** Analysis of 20 Rotofor fractions by pH gradient, percent protein recovered and protein concentration (µg/mL). X-axis represents number of fractions, Y axis represents pH gradient, % of protein range and protein recovery (µg/mL). (◇)pH gradient; (○)% Protein recovery); (●) Protein concentration (µg/mL). X axis - represents number of fractions. Y axis - represents pH gradient/% of protein range and protein recovery (µg/mL).



## RESULTS AND DISCUSSION

Rotofor IEF cell provides a rapid and efficient means of purifying proteins without subjecting them to ionic detergents. After 4 hr run at 12 W constant power at 4°C, 20 fractions were collected. Figure 1 shows the pH profile, % of protein recovered and total protein concentration for each fraction. A total of 160 mg (27mL) of *N.sativa* protein was applied to the Rotofor cell and the recovery range was 5 to 17%. Figure 2 shows the results of SDS-PAGE electrophoresis of 18 fractions obtained from Rotofor. Lane A and lane B represent high and low molecular weight marker proteins respectively.

The purpose of fractionating black seed proteins is to use these preparations to raise antibodies in experimental animals *in vivo* and antioxidant defense mechanism *in vitro* that can be classified as a promoter of positive health by combatting free radicals and reactive oxygen species mediated degenerative changes. Although the exact physiological role of these black seed proteins remains uncertain, they all have been shown *in vitro* experiments to affect lymphocytes, macrophages and monocytes[6]. Chromatographic and spectroscopic techniques of isolation, purification and structure determination are highly sensitive and there has never before been a time when a combination of chemical, biological and molecular biological techniques could be used for

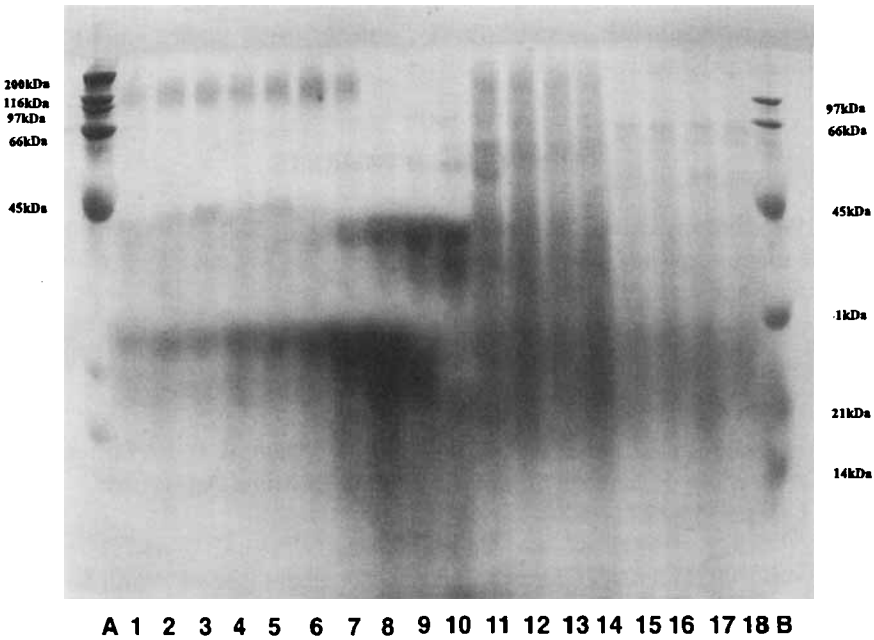


**Figure 2.** Rotofor compartment fractions 1-18 containing black seed (*N.sativa*) proteins analyzed by SDS-PAGE. Lane A and lane B represent high and low molecular weight markers respectively.



the isolation and production of novel drugs. Such studies offer a logical approach for new drug discovery and for the identification of template molecules for the design of new drug molecules. To our knowledge, we present the first data about the fractionation of black seed proteins. Studies are underway in our lab to isolate and purify the specific protein/peptide molecule(s) from *N.sativa* which may be used as an immunomodulator. On the other hand a number of studies have been conducted with black seed oil *in vivo* and showed that intravenous administration of volatile oil of black seed to rats decreased the arterial blood pressure and the heart rate in dose dependent manner[12]. These authors also found a dose-dependent increase in the respiratory rate and intratracheal pressure when black seed volatile oil was injected (I.V.) to guinea pigs[13].

*N.sativa* proteins have great potential as functional agents in modulating the immune responses and in many food preparations perhaps as supplements in the diet as antioxidant. However, *N.sativa* proteins can not be recommended

until other studies including nutritional properties, toxicities, antinutritional components, determine its suitability for humans, such studies are underway and will be published separately.

## ACKNOWLEDGEMENTS

We sincerely thank Dr. Hassan Y. Aboul-Enein for his critical evaluation of the manuscript and Ms Jennifer M. Almazan for her execellent secretarial assistance and typing the manuscript.

## REFERENCES

1. J. H. Galloway, K. Farmer, G. R. Weeks, I. D. Marsh, A. R. W. Forrest, "Potentially Hazardous Compound in a Herbal Slimming Remedy," Lancet, 340-179 (1992).

2. P. A. O.M. De Smet, O. S. N. M. Smeets, "Potential Risk of Health Food Products Containing Yohimbe Extracts," B.M.J. **309**,958 (1994).

3. H. Wagner, N. R. Fransworth, (eds.); **Economic and Medicinal Plant Research**, vol. 4. Plants and Traditional Medicine, London, Academic Press (1990).

4. L. I. Abou-Basha, M. S. Rashed, H. Y. Aboul-Enein, "TLC Assay of Thymoquinone in Black Seed Oil (*Nigella Sativa* Linn) and Identification of Dithymoquinone and Thymol.," J. Liq. Chromatogr. **18(1)**, 105-115 (1995).

5. H.Y. Aboul-Enein, L. I. Abou-Basha, "Simple HPLC Method for the Determination of Thymoquinone in Black Seed Oil (*Nigella Sativa* Linn)," J. Liq. Chromatogr. **18(5)**, 895-902 (1995).

6. A. Haq, M. Abdullatif, P. I. Lobo, K. S. A. Khabar, K. V. Sheth, S. T. Al-Sedairy, "*Nigella sativa*: Effect on Human Lymphocyte and Polymorphonuclear Leukocyte Phagocytic Activity," Immunopharmacol., In press (1995).

7. V. K. Babayan, D. Kootungal, G. A. Halaby, "Proximate Analysis, Fatty Acid and Amino Acid Composition of Nigella Sativa L. Seeds," Inst. Food Technol., 1314-1319 (1978).

8. B. Testa, E. Kyburz, W. Fuhres, R, Giger, (Eds.); In Perspectives in Medicinal Chemistry. (S. S. Davis), **Oral Delivery Systems for Peptides and Proteins**, Verlag Helvetica Chimica Acta, Basel, pp. 533-544 (1993).

9. K. F. Mattil, "Compositional Nutritional and Functional Properties and Quality Criteria of Soy Protein Concentrates and Soy Protein Isolates," J. Am. Oil Chem. Soc., **15**, 18A (1974).

10. M. M. Bradford, "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizling the Principle of Protein Dye-Binding," Anal. Biochem., **72**,248-254 (1976).

11. U.K. Laemmli, "Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage $T_4$," Nature **227**,680-685 (1970).

12. K. E. H. El-Tahir, M.M. S. Ashour, M. M. Al-Harbi, "The Cardiovascular Actions of the Volatile Oil of the Black Seed (*Nigella Sativa*) in Rats: Elucidation of the Mechanism of Action," Gen. Pharm. **24**, 1123-1131 (1993).

13. K. E. H. El-Tahir, M. M. S. Ashour, M. M. Al-Harbi, "The Respiratory Effects of the Volatile Oil of the Black Seed (*Nigella Sativa*) in Guinea Pigs: Elucidation of the Mechanism(s) of Action," Gen. Pharm., **24**, 1115-1122 (1993).

Received July 31, 1995
Accepted August 22, 1995
Manuscript 3944