Chemical Constituents of Seed Oils/Meals of Pakistani Umbelliferae Species as Potential Industrial Raw Material

B. KHALID, S. HAMID AND C.M. ASHRAF*
PCSIR Laboratories Complex
Ferozepur Road, Lahore

(Received 16th September, 2004, revised 13th July, 2005)

Summry: *Carum carvi, Anethum graveolens* and *Petroselinum crispum* cultivated in Pakistan contained 9.12%, 15.3.% and 12.9% fixed oils respectively and were evaluated for their fatty acid and cake composition. It has been found by GC and degradative oxidation techniques that the seed oils contain high percentages (38.2%, 48.70% and 83.1%) respectively of petroselinic acid. Other component fatty acids consisted of oleic, linoleic and palmitic acids with minor amounts of other saturated fatty acids.

The seed cakes contained 19.25%, 15.68% & 25.0% protein, 11.83%, 14.8% & 11.02% fibre, 8.24%, 9.82% & 8.03% ash, 8.19%, 8.39 & 12.39% moisture and 43.37%, 36.0% & 30.62% carbohydrates respectively of *Carum carvi, Anethum graveolens & Petroselinum crispum.*

Introduction

Fatty acid composition of the seed oils of some Pakistani cultivated umbelliferae has already been reported, in pursuance of the programme to examine the seed oils of umbelliferae available in Pakistan [1]. These plants are annual or biennial herbs and are widely cultivated in different parts of Pakistan.

This plant family is important due to sweet smelling essential oils present in the seeds and other parts of the plants [2-5]. These seeds are used in medicines, dental preparations, condiments, confectinery, pickles, soft drinks, cosmetics [6-8]. Essential oils of this family show antibacterial and antifungal activity [9]. Water and alcohol extracts are also used as antioxidants for oils and fats [10-11]. These plants are biologically active against several species of stored product insects [12]. Many members of this family are cultivated as commercial crops for the production of essential oils while a vast grow wild.

Klieman & Spencer (1982) described the physicochemical and fatty acids composition of *Carum carvi*. The seeds contained 19.4% oil and 21.5% protein. The oil showed refractive index 1.4700, iodine value 135.0 with palmitic acid 5.2%, stearic acid 1.1%, petroselinic acid 35.4%, oleic acid 24.1 % and linoleic acid 33.9% [13]. Christian & Hilditch (1929) reported fatty acid composition of *Carum carvi* as palmitic acid 3%, oleic acid 40%, linoleic acid 31% and petroselinic acid 26% [14] while VanLoon (1927) described the fatty acid composition of *Petroselinium sativum* as palmitic acid 3%, oleic acid 15%, linoleic acid 18% and petroselinic acid 70% [15]. Hilditch and Miss Jones (1927) reported the fatty acid composition of the same species as palmitic acid 3%, oleic acid 15%, linoleic acid 6% and petroselinic acid 76% [16]. Christian & Hilditch (1929) also reported the fatty acid composition of fennel as palmitic acid 4%, oleic acid 22%, linoleic acid 14% and petroselinic acid 60% [14].

El-Gendi (1988) & Fiad Seham et al (1993) investigated the minerals in the ash content of the meal (cake) of carrots. The results revealed that relatively rich amounts of aluminium, calcium, potassium and magnesium and moderate amounts of copper, iron, manganese, sodium and zinc together with appreciable amounts of carbohydrates and protein was present in the meal of carrot [17,28,29]. Vitamins are also present in appreciable amounts. Dill, Parsely and others contained vitamin E (α-tocopherole acetate) in lipids as free alcohol in lipid hydrolysate, ascorbic acid ($\leq$175-100 mg/100 g), β-carotene retinol, pantothenic acid and pyridoxin were also detected [18-21]. Dill oil 5-10% is used in preparation against corrosion for sealants and under bodies of vehicles [22].

The earlier studies indicate that little work has been carried out on the fixed oils of this family.

*To whom all correspondence should be addressed.

Because of easy availability and high yield of oil content *Carum carvi*, *Anethum graveolens* and *Petroselinum crispum* have been selected for evaluations of their physicochemical contents, fatty acid profile, seed cake composition and mineral composition of ash content. These plants are not only commercially important for their industrial utility as their essential oils but also have good yields of fixed oils which contain petroselinic acid as the major acid and may be exploited as a good source of industrial chemicals.

Results and Discussion

Fixed Oils and Cakes of Carum carvi, Anethum graveolens and Petroselinum crispum

The fixed oils of Pakistani species of umbelliferae *Carum carvi*, *Anethum graveolens* and *Petroselinum crispum* were extracted and evaluated for the fat content, physicochemical properties of the oils and their fatty acid composition. The results indicate that the fat content on dry weight basis was 9.15%, 15.30% and 12.92% of *C. carvi*, *A. graveolens* and *P. Crispum* respectively (Table-1). Iodine values 125, 102 & 190.66 of C. carvi, A. graveolens and P. crispum respectively showed the presence of high degree of unsaturation. Acid values 2.96, 3.45 and 3.18 respectively of *C.carvi*, *A. graveolens* and *P. crispum* indicated the extent of hydrolysis occurring due to presence of moisture. Refractive indeces were 1.4703, 1.4649 and 1.4780 respectively of the three species. The physico-chemical values of these oils fall in the edible oils category.

The seed cakes after extraction of oil were investigated for protein, fibre, moisture, ash and carbohydrates. Results are shown in Table-2. The seed cakes of C. carvi, A. graveolens and P. crispum contained 19.25%, 15.68% & 25.0% protein respectively thus being a rich source of protein. The cakes contained carbohydrates 43.37%, 36.0% and 30.62 % respectively of the three species. The ash contents were investigated for minerals shown in Table-3. They contained high amounts of aluminium, calcium, potassium and magnesium and appreciable amounts of copper, iron, manganese sodium and zinc [17,28,29].

These cakes can be used as cattle feed as such or as diluents with other feeds, which are rich in protein and fat content but poor in carbohydrates and minerals.

Table-1: Physicochemical Evaluation of *Carum Carvi*, *Anetheum Graveolens* and *Petroselinum Crispum* Seed Oils

Sr.#	Properties	Carum carvi	Anethum graveolens	Petroselinum crispum
1.	Oil %age (on dry weight basis)	9.12	15.30	12.92
2.	Refractive Index	1.4703	1.4649	1.4780
3.	Specific gravity	0.9109	0.9167	0.9216
4.	Iodine value	125.0	102.0	140.66
5.	Acid value	2.96	3.45	3.18
6.	Unsaponifiable matter	1.30	1.19	1.24

Table-2:Chemical Composition of *Carum Carvi*, *Anetheum Graveolens* And *Petroselinum Crispum* Seed Cakes

Sr.#	Component (%)	Carum carvi	Anethum graveolens	Petroselinum crispum
1.	Protein	19.25	15.68	25.0
2.	Moisture/ volatile	8.19	8.39	12.39
3.	Ash	8.24	9.82	8.03
4.	Fibre	11.83	14.80	11.02
5.	Carbohydrate	43.37	36.00	30.62

Table-3: Mineral Contents of *Carum Carvi*, *Anetheum Graveolens* and *Petroselinum Crispum* Seed Ash

Sr.#	Percentage element on dry weight basis	Carum carvi	Anethum graveolens	Petroselinum crispum
1.	Al (mg)	113	1.24	1.31
2.	Mg (mg)	129	1.43	1.18
3.	Ca (mg)	71	0.64	0.47
4.	K (mg)	170.9	3.35	3.92
5.	Na (mg)	29.38	33.05	39.12
6.	Cu (mg)	25.23	26.12	20.85
7.	Fe (mg)	11.37	14.04	12.11
8.	Mn (mg)	0.27	0.39	0.41
9.	Zn (mg)	2.91	3.12	3.43
10.	P (mg)	0.43	0.59	0.62

Fatty acid composition of Carum carvi, Anethum graveolens and Petroselinum crispum:

Fatty acid composition of the seed oils of the three species is given in Table-4. The results showed that the seed oils contained high amounts of unsaturated fatty acids 91.1%, 82.55% & 97.26% in *Carum carvi*, *Anethum graveolens* and *Petroselium crispum* respectively. These high values of unsaturated fatty acids correspond to the high iodine values (125, 102 & 140, which is also an indication of unsaturation of the oils. $C_{18:1}$ monoenoic acid was the most abundant fatty acid found in these species. Linoleic acid ($C_{18:2}$) and palmitic acid ($C_{16:0}$) were the second and third major fatty acids. Their quantities

Table-4:Fatty Acid Composition of *Carum Carvi, Anetheum Graveolens* And *Petroselinum Crispum* Seed Oils

Sr.#	Species	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$ & higher
1.	Carum carvi	-	Traces	0.3	5.4	1.29	38.2	22.7	30.2	-	1.96
2.	Anethum graveolens	1.10	2.10	0.95	6.16	0.78	48.70	8.5	23.5	1.85	6.36
3.	Petroselinum cripsum	-	-	0.44	0.79	0.88	83.1	4.41	9.50	0.25	0.63

were nearly the same in *Carum carvi* and *Anethum graveolens* but very low in *Petroselium crispum*. Linolenic acid ($C_{18:3}$) was not found in Carum carvi but present in very small amount in Apium graveolens and Petroselium crispum. Among lower fatty acids $C_{10:0}$ and $C_{12:0}$ (lauric acid) were present only in Anethum graveolens, while C14:0 was present in all the three species with a very small quantity $C_{20:0}$ and higher fatty acids were present in small amounts in *Carum carvi* and *Petroselium crispum* while appreciable amount was found in *Anethum graveolens*.

The methyl esters of short chain fatty acids nonanoic and dodecanoic acids obtained after oxidation of $C_{18:1}$ were also analysed by GC and their percentages were found as petroselinic acid (PA) and oleic acid (OA). Table-4 showed that PA (C18:1 Δ^6) in C. carvi, A. graveolens and P. crispum was 38.2%, 48.7% & 83.1% respectively, while OA ($C_{18:1}$ Δ^9) was 22.7%, 8.5% and 4.41% respectively. The results indicate that *Petroselinum crispum* contained the highest amount of petroselinic acid as compared to the other species. The high percentage of PA in comparison to OA is in agreement with the general characteristics of fatty acid pattern of this family [13,30]. The fatty acid profile of the fixed oils of these species is almost similar to the other cultivated as well as wild species of this family. The little variations in the composition of fatty acids may be due to variations of climatic conditions and soil composition. In general high percentage of petroselinic acid in all the mentioned species make them useful for the industrial purpose. Petroselinic acid is an isomer of oleic acid and is general characteristic of seed oil of Umbelliferae family. Oxidation products of P.A are lauric and adipic acid. Both the products are commercially important for their industrial application in soap/cosmetics as well as polyester/nylon manufacturing respectively. From the present data evaluated and the earlier studies revealed that the different members of this family may find their extensive use as industrial products as well as in food and feed for animals.

Experimental

Fresh seeds of Carium carvi, *Anethum graveolens* and *Petroselinium crispum* were obtained from the market in the months of April and May and other reagents used were prepared according to the standard methods (AOAC).

Extraction of Oils:

The seeds of *Carum carvi, Anethum graveolens* and *Petroselinum crispum* after extraction of essential oils were dried in an oven at 105°C and crushed into fine powder. The oils were extracted with hexane using soxhlet apparatus. The oils thus obtained were filtered through anhydrous sodium Sulphate and the solvent was removed under reduced pressure. The oil percentages of *Carum carvi, Anethum graveolens* and *Petroselinum* were determined on dry weight basis and the oils were kept in an inert atmosphere.

Composition of seed cakes and physicochemical evaluation of the oils:

Physicochemical values of oils such as saponification value, acid value and iodine value of the oils were determined by standard methods AOAC (Association of Official Agriculture Chemist) [23] and refractive index with Atago 4T refractometer.

The seed cakes, obtained after oil extraction, were freed from the solvent at 70°C under vacuum and were then subjected to protein, moisture, ash, fibre and carbohydrate content analysis according to standard methods (AOAC) [23]. Carbohydrates were determined using subtraction method The ash contents were analysed for mineral composition using a Perkin Elmer Model 1272 atomic absorption spectrometer [24].

Protein Determination:

Crushed defatted seeds of each species 1.5 g were subjected to digestion in separate Kjeldahl flasks by adding 1.5 g K_2SO_4, 50 mg of HgO and 20 ml H_2SO_4. The materials were digested for 4 hours at vigorous boil with acid condensing into neck of flask. Cooled, added minimum quantity of H_2O to dissolve solids and placed thin film of Vaseline on lip of flasks. Transferred digests to distillation apparatus one by one conical flask containing 10 ml 4% H_3BO_3 solution and 4 drops of methyl red Methylene

indicator was placed under condenser with tip below surface. Added 10 ml of $NaOH\text{-}Na_2S_2O_3$ reagent to still and steam distilled until about 20 ml distillate was collected. Diluted contents of receiver to 50 ml with distilled water and titrated NH_3 with 0.02 N HCl till red colour appeared. Blank determination is also carried out under the same conditions and calculated % nitrogen in the sample by equation:

$$\frac{(\text{ml HCl in determination} - \text{ml blank}) \times \text{normality of HCl} \times \text{equivalent wt of N} \times 100}{\text{Weight of sample}} = \% \text{ Nitrogen}$$

Thus % nitrogen obtained is multiplied by factor 6.25 to obtain % protein.

$$\% \text{ protein} = \% \text{ nitrogen} \times 6.25$$

Carbohydrate Determination:

Total carbohydrates were determined by subtraction method. Oil, moisture, fibre, Protein, and ash % contents were determined, sum of these contents was subtracted from 100, to get total carbohydrate percentage.

Chromatographic analysis:

The seed oils were saponified with 0.5 N alcoholic potassium hydroxide and then extracted with petroleum ether to reserve non-saponifiable matters, fatty acids were then regenerated from the soaps by acidification with 4 N sulphuric acid. Methyl esters of the liberated fatty acids were prepared according to P.R. Kumar & S.T. Sunoda (1978) and the esters were analysed by GC and thin layer chromatography [25].

The esters of monoenoic acids were separated on glass plates (20 x 20 cm) coated with 0.5 mm thick layer of 20% silver nitrate ($AgNO_3$) in silica gel using 50:50 benzene/chloroform mixture as the developing solvent. The ester bands, scraped from the plates, were recovered from the adsorbent by extraction with petroleum ether. The solvent was removed on a hot water bath by a rotary evaproator and the esters were oxidized by modified Von Rudloff's method [26]. The short chain fatty acids thus liberated were separated by TLC and found to be nonanoic and dodecanoic acids by GC analysis [27]. Gas chromatography was carried out with a GC-14A Gas chromatograph (Shimadzu) fitted with hydrogen flame ionization detectors and data processor. A PEG Capillary column (25 m x 0.2 mm i.d.) was used and the column temperature was maintained at 180°C for all fatty acid methyl esters and for the adipic and lauric acid esters, the column was operated with temperature programming from 150°C to 180°C. The injection and detector temperatures were maintained at 250°C and 300°C, respectively. The flow rate of nitrogen gas (carrier) was 20 ml/min at split ratio of 1:50. Identification of components was based on their retention times as compared with those obtained for standard methyl esters analysed under the same conditions.

References

1. S. Waheed, B. Khalid and C.M. Ashraf, *J.Chem.Soc.Pak.*, **22**, 133 (2000).
2. K.R. Kritkar and B.D. Basu, Indian Medicinal Plants, Edit. Lalit Mohani, M.B. Basu Allahabad, Vol. **11,** P-1227 (1984).
3. E. Nasir and S.I. Ali, Flora of West Pakistan, Stewart Herbarium, Gorden College, Rawalpindi, No.20 (1972).
4. A.K. Nadkarni, Indian Materia Medica, 3rd Ed. P. 936 (1954).
5. W. Dymock, Pharmacographia Indica Published by the Journal of Institute of Health and Tibbi (Medicinal Research) Vol. **25**, Pakistan (1972)
6. Atlle Burpee, W. Co. Seed Growers. Burpee (Philadophic 32, P.A. Clinton Lowa, Riverside, California) P. 69, (1969).
7. K.M. Nadkarni, Indian Materia Medica, Dhoota Paeshawar Prak Shan Ltd. Panel Kalaka and the Popular Book Depot, Bombay, 3rd Edition, P. 119, (1954).
8. R.N. Chopra, Indigenous Drugs of India, U.N. Dhar Private Ltd., Calcutta, P. 495, 556, 595, (1958).
9. M.K. Bhatty, M. Ashraf, Rafi Ahmad, Abdul Waheed Sabir, Bushra Asghar and Shahid Mahmud. Final Research Report P. 374, (1977).
10. I.N. Demidove, I.A. Danilova, L.A. Chernova, V.F. Gladkaya, L.L. Rdionova, V.A. Dementy, (Khar'k, Politekh). Inst., Ukraine). Izu, Vyssh. Uchem. Zaved, Pishch. Tekhnol, (3-4), 30, (1992).
11. S.V. Baranov, L.P. Zhukovskaya, N.V. Mizha, (Moscow Inst of People's Economy) USSR. Sul, 413, 127 (Cl. C11B5/00 30 July 1988, Appl. 4, 035\7047, 435, 05 Mar. 1986 from Olkrytiya, Izobret, (28), 106, (1988).

12. Su, Helen C.F.; Horvat, Robert, *J. Agric. Food Chem.*, **36**(4), 752 (1988).
13. R. Klieman and G.F. Spencer, *Journal of American Oil Chemist Society*, **59**, 29 (1982).
14. T.P. Hilditch and P.N. Williams The Chemical Constitution of Natural Fats (B.C. Christian and T.P. Hilditch, *Biochem, J.*, **23**, 327, 1929) 4th Edition, John Wiley & Sons, Inc., New York, NY, (1964).
15. T.P. Hilditch and P.N. Williams The Chemical Constitution of Natural Fats (T.P. Hilditch and (Miss) E.E. Jones, *J. Soc. Chem. Ind.* 1927), 4th Edition, John Wiley & Sons, Inc., New York, NY, (1964)
16. T.P. Hilditch and P.N. Williams The Chemical Constitution of Natural Fats (J. VanLoon, Rec. Trav. Chim. 46, 492 1927) 4th Edition, John Wiley & Sons, Inc., New York, NY, (1964)
17. S.H. EL Gendi "Chemical evaluation of carrot seed" Vol. 39, Fase 6, 343 (1988).
18. Fiad Seham El-Hamidi, Mona (Univ. California Berkeley Ca 94720 USA), Seifen, Olec, Fette, Wachre Vitamin E and trace elements, **119**(1), 25-6, 28, (1993).
19. Imer, Filiz; Koseoglu, Ayten; Sefer, *Edmen Marmara Univ. Eczacilik Derg* **5**(2), 115, (1989).
20. C.A. Vol. 111, 1989, 171169p
21. C.A. Vol. 110, 211229j 1989.
22. Kalmar Nagy, *Imre Hung C.A.* **110**, 175262d, 1989
23. Official Methods of Analysis of Association of Official Agriculture Chemist. 12th Ed. (Washington 4, D.C.) 1975.
24. A.R. Chowdhury; R. Banerji, G. Misra and S.K. Nigma, *J.Am.Oil chemist Soc.*, **60**, 1893 (1983).
25. P.K. Kumar and S.T. Sunoda, *Journal of American Oil Chemists Society (JAOCS)*, **55**, 320 (1978).
26. E. Von Rudloff, *Journal of American Oil Chemists Society (JAOCS)*, **33**, 129 (1956).
27. R.J. Hamilton & M.Y. Raie, *J.Chem.Educ.*, **49** (1972).
28. R.G. Schamschula, E. Sugar, P.S.H. Un, J.L. Duppenthaler, K. Toth, D.E. Barmes, *Acta Physiol. Hung.*, **72**(2) 237 (1988).
29. R.B. Volgborlo, *Food Chem.*, **48**(3), 317 (1993).
30. J.F. Mallet; E.M. Gaydou and A. Archavlis, *J. Am. Oil. Chem. Soc.*, **67**, (10) 607 (1990).