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FATTY ACID COMPOSITION OF NEW VARIETY BREEDER SEEDS OF *PISUM SATIVUM*

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ABSTRACT

Fatty acids are necessary in the normal functioning of all tissues of the body. Their deficiencies exhibit the symptoms of disorders especially abnormalities in the liver and kidney. Changes in the blood, reduced growth rates, decreased immune function, depression and skin changes, including dryness and scaliness. Adequate intake of the essential fatty acids results in numerous health benefits. Prevention of atherosclerosis, reduced incidence of heart disease and stroke, and relief from the symptoms associated with ulcerative colitis and joint pain. Hence, the present study is carried out with new variety breeder seeds of Pisum sativum (*Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15, JM-6*) to find out the fatty acids composition (essential and non essential), which could be useful in reducing some ailments and helpful in supplementing human nutrition.

Key words: Immune function, Atherosclerosis, Ulcerative colitis, Fatty acids composition, Human nutrition.

INTRODUCTION

Vegetarians must adopt the foods, which contain rich omega-3 and omega 6 fatty acids in daily diet. α-Linolenic acid, a common omega-3 fatty acid, which is found in beans, pea, nuts, pulse seeds and fruits. The unsaturated fatty acids, oleic acid and linoleic acid were predominant in seed lipids[1-3]. Omega-6 fats are found in leafy vegetables, seeds, nuts, grains, and vegetable oils. The other omega-6 fatty acids, such as γ-linolenic acid (GLA), can be found in more rare oils, including black currant, borage, evening primrose, and hemp oils. Most of the diets provide adequate amounts of omega-6 fatty acids such as green leafy vegetables, legume seeds, citrus fruits and ground flaxseed fats. Legumes, in general, are rich in essential fatty acids[2,4].

Oleic acid (C 18:1) is a monounsaturated fatty acid (MUFA), in nutritional view,

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this fatty acid is beneficial to health and it is preferred over polyunsaturated fatty acid (PUFA) because of its lesser number of double bonds in its structure.

Study was performed by selecting six new variety breeder seeds (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15, JM-6) of *Pisum sativum*. All the samples are collected from Field crop unit, Jawaharlal Nehru Krishi Viswavidyalaya, Jabalpur. Care has being taken in order to select healthy matured and disease resistant varieties to analyses and study for their fatty acid composition by subjecting these, processed samples to gas chromatography [5-7] in order to obtain reliable data on their suitability for both; human nutrition and pharmaceutical purposes.

EXPERIMENTAL

In the present investigation, Perkin Elmer Gas Chromatograph (GC), using flame ionization detector, was used to analyse seeds for their fatty acids composition.

Extraction of crude lipids

Total lipids were extracted from the whole powder of the samples under investigation, in the Soxhlet apparatus for 20 hour, using petroleum ether (40-60)^{o}C fraction as a solvent, after evaporating the solvent at 60^{o}C[8].

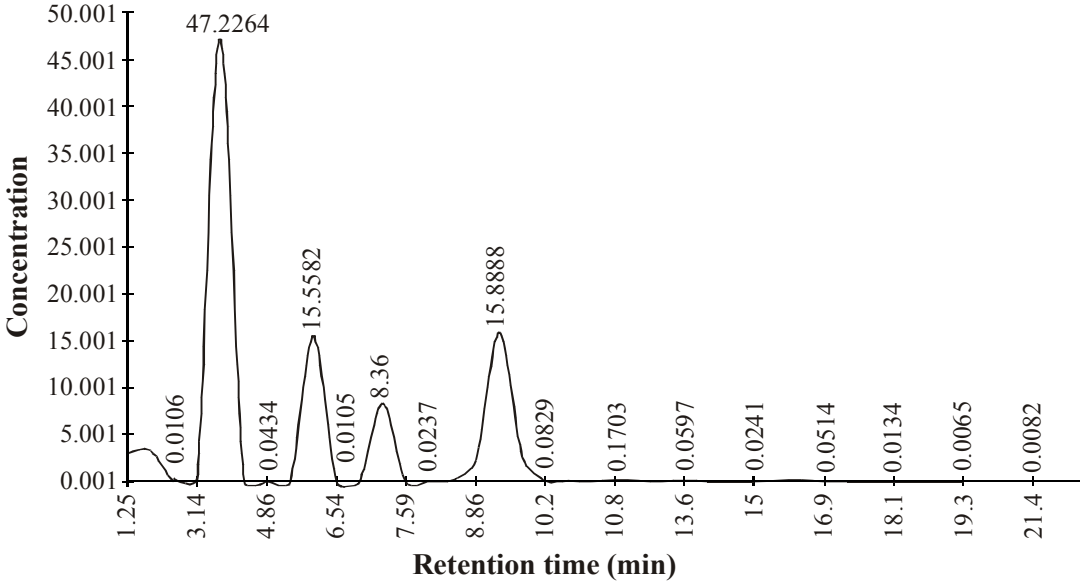

Fig. 1: Chromatogram of standard

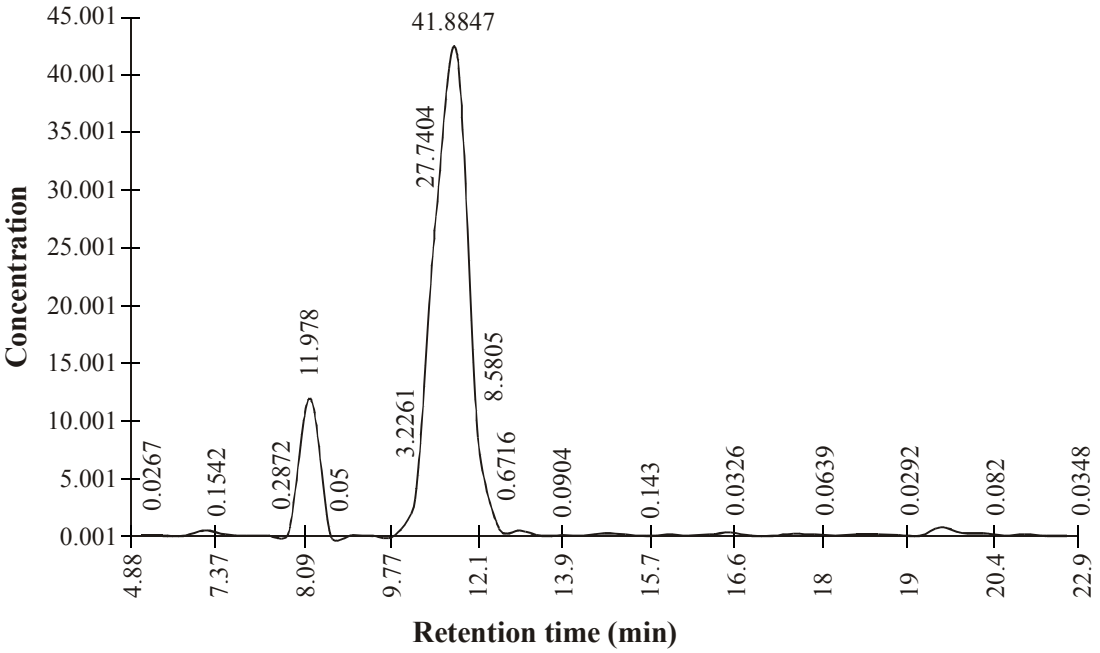

Fig. 2: Chromatogram of sample – 1

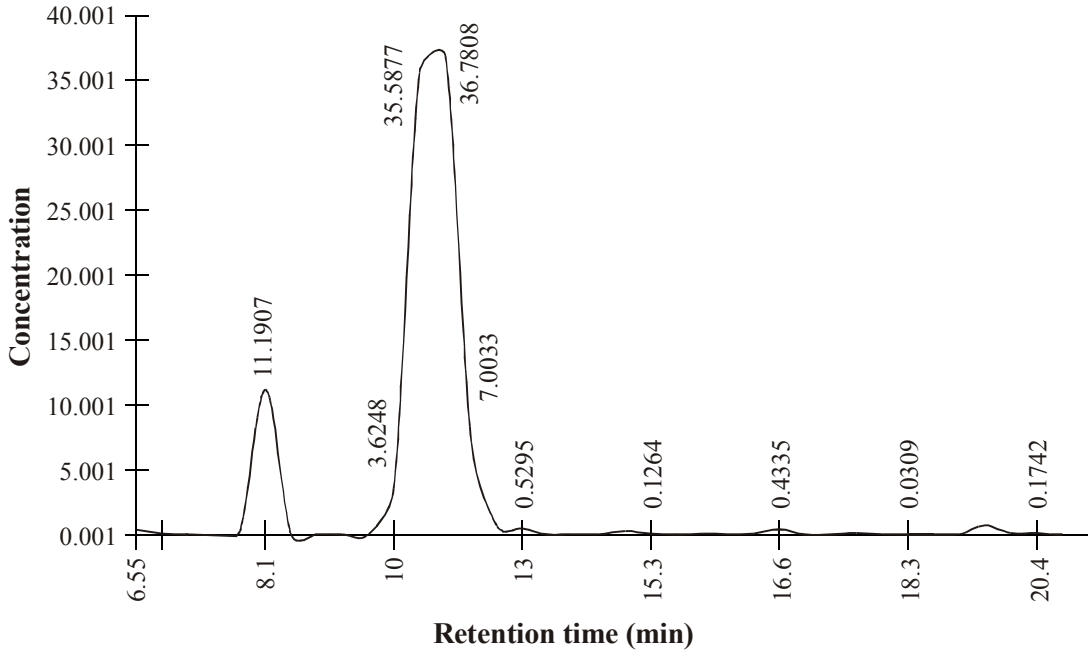

Fig. 3: Chromatogram of sample – 2

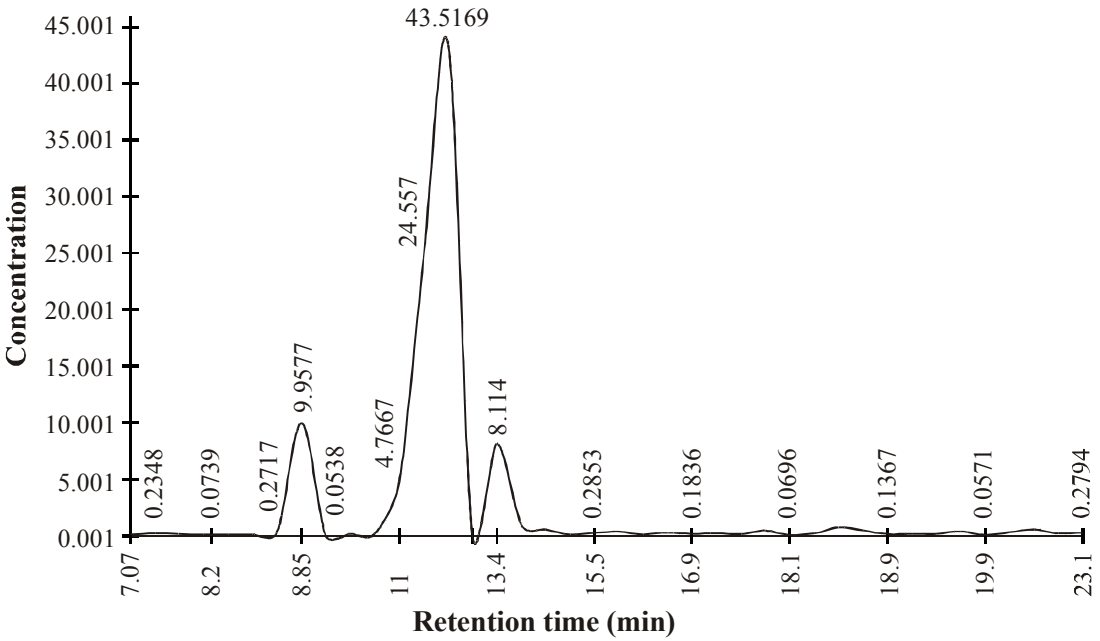

Fig. 4: Chromatogram of sample - 3

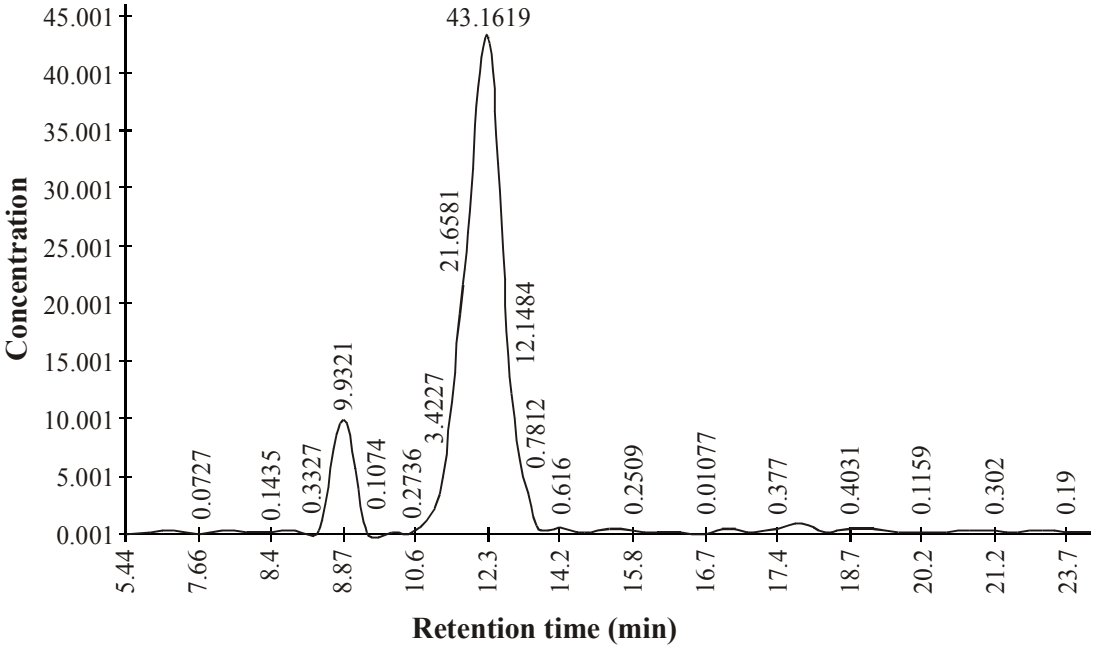

Fig. 5: Chromatogram of sample - 4

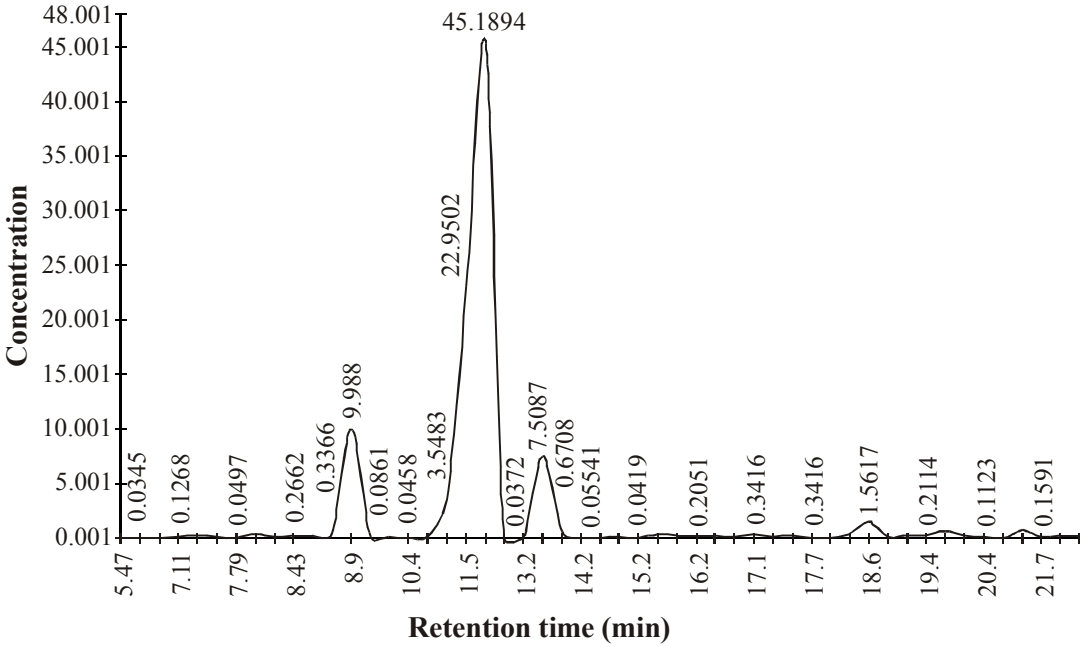

Fig. 6: Chromatogram of sample – 5

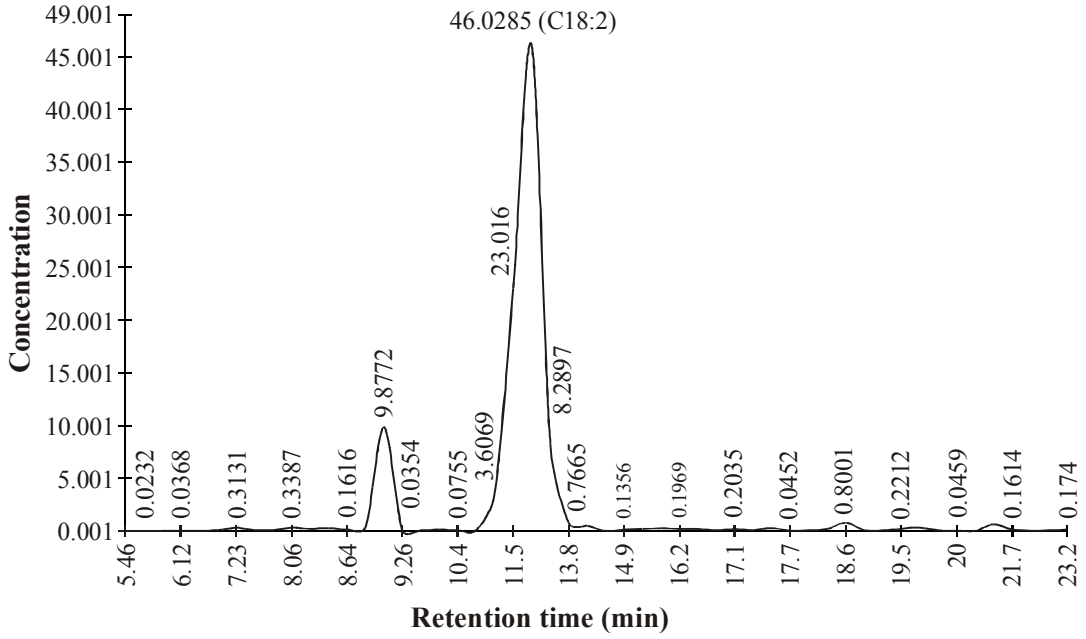

Fig. 7: Chromatogram of sample – 6

Preparation of methyl ester of fatty acids

Methyl esters of the fatty acids were prepared[9] and 0.2 g of oil was mixed with 25 mL of N/2 alcoholic KOH, 10 mL of water and 200 mL of ethanol and then refluxed for 4-6 hour. The contents were made up to 25 mL by evaporating the excess of ethanol. The residue was dissolved in sufficient water; transferred to a separating funnel and then about 15 mL of petroleum ether was also added to the separating funnel, shaking gently. After the formation of two separate layers (petroleum ether layer and aqueous layer), the petroleum ether layer (as it contains un-saponified matter) was removed. The saponified material was then hydrolyzed by adding 1:2 dilute HCl acid (turns blue litmus paper to red). The free fatty acids were then extracted with petroleum ether; this extraction process was carried out with petroleum ether four times, in order to ensure the complete extraction of fatty acids. These petroleum ether extracts were transferred into dried round bottom flask To this round bottom flask, anhydrous Na_2SO_4 powder was added and kept on the water bath to dryness. The dry fraction was fatty acids. To the dry fraction, 25 mL of 2% methanolic H_2SO_4 acid (1.5 mL of H_2SO_4 in 98.5 mL of CH_3OH) was added and then refluxed for 4 hour over a water bath. Excess of methanol was removed by rotary evaporation. Sufficient distilled water and about 15 mL petroleum ether were added to the refluxed material and then it was transferred to separating funnel. This mixture was then shaken in separating funnel with 10 mL of 1% KOH and then the aqueous layer was removed. The resultant petroleum ether extract contains the methyl esters of fatty acids. The excess amount of ether was evaporated on a water bath.

The residue, which was the methyl esters of fatty acids and then with the help of micro syringe, 1.0 µL of extracted ester was injected into a gas chromatograph (GC) and various chromatograms were obtained. These chromatograms were compared with the set of standards fatty acid methyl esters (FAME'S) and analyzed under similar conditions for quantification.

Method for analysis of methyl esters of fatty acids on gas chromatography

Instrument	:	Perkin Elmer Gas Chromatograph
Model	:	Auto system XL
Make	:	Perkin Elmer
Column	:	BP-225 Capillary column (moderate polar) (25 meters x 0.25 µm x 0.22 mm)
Carrier gas	:	Nitrogen
Detector	:	Flame ionization detector

Injection temperature	:	220°C
Oven temperature	:	100°C for 5 minutes, Rate 10°C/min to 220°C (hold for 20 minutes)
Detector temperature	:	250°C
Injection volume	:	1.0 µL
Split ratio	:	1:100.

RESULTS AND DISCUSSION

The data were obtained from the calculation of the peak value divided by 100 and then multiplied by the total extracted lipid. The total lipids per 100 g of Arkel is 2.0727 g, Pusa pragati is 2.1518 g, IPF-99-25 is 1.1885 g, JP-885 is 1.1609 g, MM-15 is 1.2265 g and JM-6 is 1.1309 g. The saturated fatty acid composition and unsaturated fatty acid composition (g/100 g) of seeds were calculated and mentioned in the Tables 1 and 2.

Table 1: Saturated fatty acid composition of leguminous seeds (g/100 g)

Sample	Saturated fatty acids					Total saturated fatty acids
	Palmitic acid $C_{16:0}$	Stearic acid $C_{18:0}$	Arachidic acid $C_{20:0}$	Behenic acid $C_{22:0}$	Lignoceric acid $C_{24:0}$	
Arkel	0.24	0.066	0.014	0.006	0.007	0.333
Pusa pragati	0.24	0.077	0.011	0.003	0.009	0.34
IPF-99-25	0.118	0.06	0.011	0.004	0.006	0.199
JP-885	0.115	0.041	0.009	0.006	0.002	0.173
MM 15	0.123	0.044	0.008	0.003	0.004	0.182
JM-6	0.11	0.04	0.009	0.003	0.003	0.165

The results of saturated fatty acids consisting of palmitic acid was found to be high in Pusa pragati and Arkel with 0.24 % and remaining varieties were found to have 0.123% to 0.11 %. Stearic acid was also found in the range of 0.077% to 0.044 % in almost all the varieties having a uniform quantity. Arachidic acid was detected 0.014% in Arkel, Pusa pragati and IPF-99-25, with 0.11% remaining varieties were found to have less quantity, about 0.009% to 0.008%. Behenic acid was found 0.006 % to 0.003 %. Similarly lignoceric acid was also found to be from 0.009% to 0.002%.

Table 2: Unsaturated fatty acid composition of leguminous seed (g/100 g)

Sample	Unsaturated fatty acids							Total unsaturated fatty acids
	Palmitoleic acid $C_{16:1}$	Oleic acid $C_{18:1}$	Linoleic acid $C_{18:2}$	Linolenic acid $C_{18:3}$	Eicosenic acid $C_{20:1}$	Total MUFA	Total PUFA	
Arkel	ND	0.575	0.868	0.178	0.011	0.586	1.046	1.632
Pusa pragati	ND	0.766	0.791	0.153	0.012	0.778	0.944	1.722
IPF-99-25	ND	0.30	0.517	0.10	0.011	0.311	0.617	0.928
JP-885	ND	0.25	0.50	0.14	0.007	0.257	0.64	0.897
MM 15	ND	0.28	0.554	0.092	0.007	0.287	0.646	0.933
JM-6	ND	0.26	0.52	0.093	0.006	0.266	0.613	0.879

MUFA = Mono Unsaturated Fatty Acid **PUFA** = Poly Unsaturated Fatty Acid **ND** = Not Detectable

The unsaturated fatty acid composition was also found to be in good proportion. Palmitoleic acid was not detected. Oleic acid was in good quantity with 0.766% in Pusa pragati . Arkel containing 0.575%; remaining varieties have 0.3% to 0.25% in proportion. High quantity of linoleic acid were found in Arkel and Pusa pragati with 0.868% and 0.791 %. IPF-99-25 with 0.517%, while remaining ranged from 0.55% to 0.51%. Linoleic acid was found 0.178% to 0.092 %, less amount was reported in MM-15 with 0.092%. Eicosenic acid was also reported in lesser amount; 0.012 % to 0.006%.

It was found that the total saturated fatty acids were found high with 0.34% in Pusa pragati , Arkel with 0.333%, IPF-99-25 with 0.199% with considerably higher side than the remaining three varieties having 0.182% to 0.165%

Total MUFA was found to be high in Arkel with 0.778%. Pusa pragati was found to have 0.586%, IPF-99-25 with 0.311% with appreciably good quantity of MUFA in remaining varieties - 0.287% to 0.257%.

Total PUFA was found considerably high in Arkel with 1.046%, Pusa pragati with 0.944 % and remaining varieties were 0.646% to 0.613%.

The total unsaturated fatty acids were found high in Pusa pragati with 1.722%, Arkel with 1.632 % and the remaining were found to be 0.933% to 0.879%.

From above the mentioned data, Pusa pragati stands high with total unsaturated fatty acid with 1.722% and saturated fatty acids with 0.34 % besides, Arkel and IPF-99-25 were having good quantity of saturated and unsaturated fatty acids. The remaining varieties may not be under estimated since they also contain considerable amount of total unsaturated fatty acids and saturated fatty acids, which could be compatible enough for nutritional supplementation in wide varieties of food products.

REFERENCES

1. Omega3: Informationsheet VegetarianSocietyhttp: //_www.veg.soc.org/info/ omega3 (2006).
2. V**.** Khader**,** Text Book of Food Science and Technology, India, (2001) pp. 138–151.
3. B. Lee, Omega-3 Oils in Grain, http://www.csiro.au/resourses/pff5 (2006).
4. N. Ali, in Processing and Utilization of Legumes, Takashi Tajima (Ed.) Tokyo, (2003) pp. 117-145.

5. IUPAC Standard Methods for the Analysis of Oils Fats and Derivatives, Blackwell Scientific Publication, 7th Edition (1987).

6. J. L. Bear Rogers and A. Dieffenbacher, Pure Appl. Chem., **62(4)**, 795 (1990).

7. A. Dieffenbacher and P. Dysseler, Pure Appl. Chem., **69(8)**, 1828 (1997).

8. S. Colowick and N. O. Kaplan**,** Methods in Enzymology III, Academic Press Inc, New York, (1957) p. 85.

9. S. P. Chowdhari, R. Banerjee, G. Mishra and S. K. Nigam, JAOCS. **61,** 1023 (1984).

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