CHEMICAL EXAMINATION OF *HELIANTHUS ANNUS* LINN. THE COMPONENT FATTY ACIDS & THE PROBABLE GLYCERIDE STRUCTURE OF THE SEED OIL

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In this investigation we describe the component fatty acids and the glyceride structure of the seed oil of *Helianthus annus* Linn. This plant belongs to the natural order Compositæ. It is known as *Suraj-Mukhi* in Hindustani and *Suryamukhi* in Sanskrit. The different parts of the plant (flowers, roots and seeds) have been used by Ayurvedic and Unani physicians for a number of ailments.[1]

The seed used in producing the crop at the Allahabad Agricultural Institute (employed in our experiments) was the fourth generation of the Russian Sunflower (*Helianthus annus*) grown from seed imported from America.

The Sunflower seed is eaten by the Russians, as people in this country eat groundnut, and is the type that has in recent years become the primary oil crop of Argentine.

It was found at the Allahabad Agricultural Institute that the insects which pollinate the Sunflower in other countries are often scarce or completely absent in India at the time the heads are ready, and so this crop of seed was produced by hand pollination using a cloth to wipe the heads gently. Other growers who have raised this crop in some other parts of India also failed to get good natural pollination.

The yield of the oil from the seeds expressed in an ordinary village press is 15 per cent., and on extraction with petroleum ether (40°–60°), it is 52·5 per cent. The titre of the oil is 13, and it gives good soap. On blending it with other oil stocks, such as cocoanut oil, it gives very good soap for domestic use.

The seed oil of *Helianthus annus* from different parts of the world has been examined[2,3,4,5,6,7] for its component acids, but in no case the glyceride structure of the oil was attempted, nor any Indian variety of the plant was investigated. In view of this we undertook the work on the Indian variety of the plant which was cultivated in the Agricultural Institute, Naini, Allahabad. In our preliminary examination, we found that the oil differs

markedly in its constants from other oils[2, 8] of the seeds of *Helianthus annus*
so far investigated. The percentage composition of the fatty acids of the
oil from different countries, as shown in the following table, correlates the
influence of habitat of the plant on the relative unsaturation of the fatty
acids of the seed oil:—

Habitat	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
America[3] ..	3·5	2·9	34·1	58·5
Congo[4] ..	3·7	1·6	42·0	52·0
Russia[5] ..	—9·6—		36·2	54·2
„ [6] ..	—5·0—		33·2	61·8
„ [7] ..	—9·8—		32·7	57·5
India (Allahabad) present work	4·27	5·46	49·41	40·48

The percentage amount of the saturated fatty acids in our oil is the same
as that of the Russian oil, but higher than that of the American or Congo
oil. The most marked difference between our oil and those from other
sources is with regard to the relative amounts of the unsaturated acids, oleic
and linoleic acids. The proportion of oleic acid in our oil is much higher
and of linoleic acid much lower than that in other oils. The total percent-
age of the unsaturated acids is, however, about the same (90 to 94) in all
the oils.

The variation in the relative amounts of oleic and linoleic acids above
noted may be correlated with temperature of habitat of the plant and rela-
tive unsaturation of seed fats. The studies of Ivanov[20] and others have
demonstrated that a given plant species, capable of existence in different
climates, produces when grown in a cold climate more unsaturated (linoleic
and linolenic) acids in its seed oil than when it is grown in a warm climate.
Cases in support of this relationship[21] between temperature of growth and
component unsaturated fatty acids in the seed oils could be cited from Pinus
species, and from members of the Moraceæ and Celastraceæ. Again species
of oak (Fagaceæ), of Papaveraceæ, of sesame (Pedaliaceæ), of safflower
(Compositæ) and of groundnut (Leguminosæ) furnish further evidence for
the occurrence of greater proportion of the more unsaturated acids in the
seed oils of plants which inhabit cooler regions. The higher proportion
of oleic acid as compared with linoleic acid in our oil can thus be correlated
with higher climatic temperature of India (Allahabad) than that of Russia,
America and Congo. It must, however, be pointed out that there are
several well marked exceptions among the species of Rosaceæ, Euphorbiaceæ,
etc., to the abovementioned generalisation regarding the effect of climatic
temperature on the degree of general unsaturation of seed oils.

EXPERIMENTAL

The material employed for this investigation consisted of authenticated seeds of *Helianthus annus* Linn. grown at Naini, Allahabad. The preliminary examination of the seed was conducted and the following results obtained:

Average weight of a seed .. .. .. 0·052 gm.
Ash content of the seeds .. .. .. 5·0%
Water-insoluble ash .. .. .. 53·8%
Water-soluble ash .. .. .. 46·2%

The qualitative composition of ash is as below:

Water-insoluble .. .. Aluminium, Magnesium, Carbonate;
Water-soluble .. .. Sodium, Potassium, Carbonate, Chloride.

Fifty gms. of the crushed seeds were extracted in a Soxhlet apparatus with various solvents in succession as given below:

1. Petroleum Ether (40°–60°) A clean yellow oil. Yield 52·5%.
2. Ether .. .. A clean yellow oil. Yield 2·7%.
3. Chloroform .. .. A pasty mass. Yield 0·8%.
4. Ethyl acetate .. .. A pasty mass. Yield 0·8%.
5. Absolute alcohol .. A pasty mass. Did not give any reaction of glucoside. Yield 1·2%.
6. 70% Alcohol .. .. A pasty mass. Did not give any reaction of sugar. Yield 0·7%.

EXAMINATION OF FATTY OIL

The expressed oil was purified with animal charcoal and Fuller's earth, dried over fused calcium chloride and filtered and the constants of the oil found out as given below in Table I:

TABLE I

Constants		Expressed Oil	Extracted Oil
Specific gravity (35° C.)	..	0·9147	0·9062
Refractive Index (25°)	..	1·4732	1·4715
Acid value	..	6·44	1·08
Saponification value	..	194·7	191·2
Iodine value (Hanus)	..	111·6	111·0
* Thiocyanogen value (24 hours)	..	79·12	75·41
Acetyl value	..	3·62	3·53
Hehner value	..	91·60%	91·20%
R. M. value	..	0·51	0·56
Unsaponifiable matter	..	0·55%	..

800 gm. of the oil were saponified with alcoholic caustic soda and the unsaponifiable matter extracted with ether.   The soap solution was decomposed with dilute sulphuric acid, when the mixed fatty acids having the following constants were obtained:

TABLE II

Iodine value (Hanus) ..	119·2
Thiocyanogen value (24 hours)	81·91
Saponification equivalent ··	280·1

The mixed acids were then separated into ' Liquid ' and ' Solid ' constituents by the Twitchell's Lead-salt-alcohol process[9] and the constants found out are given in Table III:

TABLE III

Constants		Solid Acids	Liquid Acids
Percentage	..	10·96	89·04
Iodine value (Hanus)	..	8·94	126·6
Thiocyanogen value (24 hours)	..	4·84	87·81
Saponification equivalent	..	270·9	281·1

EXAMINATION OF LIQUID ACIDS

The quantitative examination[10] of the liquid acids was done by the method of Eibner and Mugganthalor[11] modified by Jamieson and Baughman.[12]   The results of the experiments were as follows:—

TABLE IV

Weight of acids brominated ..	2·502 gm.
Weight of linoleic acid tetrabromide ··	1·009 gm.
M.P. of linoleic acid tetrabromide ··	113–114° C.
Weight of residue (Di- and tetrabromide) ..	3·262 gm.
Bromine content of residue ..	42·11%
Weight of linoleic acid tetrabromide in residue	1·126 gm.
Total weight of tetrabromide ..	2·135 gm.
Weight of oleic acid dibromide in residue ..	2·136 gm.
Weight of linoleic acid ··	1·139 gm.
Weight of oleic acid ..	1·363 gm.
Percentage of linoleic acid in liquid acids ··	45·51
Percentage of oleic acid in liquid acids ..	54·49

Five grams of the mixed acids were oxidised by Hazura method[13] and 10·04% of solid acids were obtained.

The percentage of oleic, linoleic and solid acids as calculated by different methods is given below in Table V:

TABLE V

		Linoleic	Oleic	Solid
		%	%	%
1	SCN of mixed acids	41·16	49·61	9·23
2	SCN of liquid and solid acids	39·38	49·56	11·06
3	Bromination of liquid acids	40·91	48·98	··
4	Bertram's method	··	··	10·04
5	Average 1 to 4	40·48	49·41	10·11
	From glyceride structure	40·85	49·22	9·93

EXAMINATION OF SOLID ACIDS

18 gm. of methyl esters of solid acids were fractionally distilled at 10 mm. pressure and the result of distillation and calculation[14] are as follows:

TABLE VI

S_1	··	165–170° C.	2·82 gm.
S_2	··	170–175° C.	3·70
S_3	··	175–180° C.	3·03
S_4	··	180–185° C.	4·45
S_5	··	Residue	3·78
Loss	··	··	0·22

The saponification equivalent and the iodine value of the different fractions were determined and different acids in different fractions calculated[14]:

TABLE VII

Fraction	I.V.	S. E.	Myristic		Palmitic		Stearic		Liquid	
			%	gm.	%	gm.	%	gm.	%	gm.
S_1	1·15	263·7	—	0·62	—	2·03	—	—	—	·026
S_2	1·06	279·0	—	—	—	2·43	—	1·05	—	·033
S_3	2·49	282·1	—	—	—	1·68	—	1·14	—	·06
S_4	2·76	292·6	—	—	—	0·85	—	3·29	—	·097
S_5	4·73	300·2	—	—	—	—	—	3·46	—	·141
				0·62		6·99		8·94		0·357

TABLE VIII

		In solid acids	In liquid acids
Myristic	··	3·75%	0·38%
Palmitic	··	42·25%	4·27%
Stearic	··	54·01%	5·46%

Fraction S_1.—After repeated crystallisation with dilute acetone of the acids liberated after saponification gave myristic acid, m.p. 49° C., which was not depressed on adding pure sample of myristic acid.

Fraction S_2 *and* S_3.—After repeated crystallization with dilute acetone of the acids liberated after saponification gave palmitic acid, m.p. 61–62° C., which was not depressed by adding pure sample of palmitic acid.

Fraction S_4.—After repeated crystallization with dilute acetone of the acids liberated after saponification gave stearic acid, m.p. 69° C., which was not depressed by adding pure sample of stearic acid.

Fraction S_5.—This was saponified, acids liberated and extracted with petroleum ether (40–60°). The acids thus obtained were crystallized from dilute acetone giving stearic acid, m.p. 68° C., which was not depressed by pure stearic acid.

The fatty acids of the oil from the seeds of *H. annus* therefore consist of oleic, linoleic, myristic, palmitic and stearic acids. The percentage of each is given in Table IX:

TABLE IX

		%
Oleic	..	49·41
Linoleic	..	40·48
Myristic	..	0·38
Palmitic	..	4·27
Stearic	..	5·46

EXAMINATION OF UNSAPONIFIABLE MATTER

The unsaponifiable matter obtained gives the colour reactions of a phytosterol. Its melting point is 133–35° C. It is probably sitosterol present in most vegetable oils, but its amount being too small no further investigation could be done.
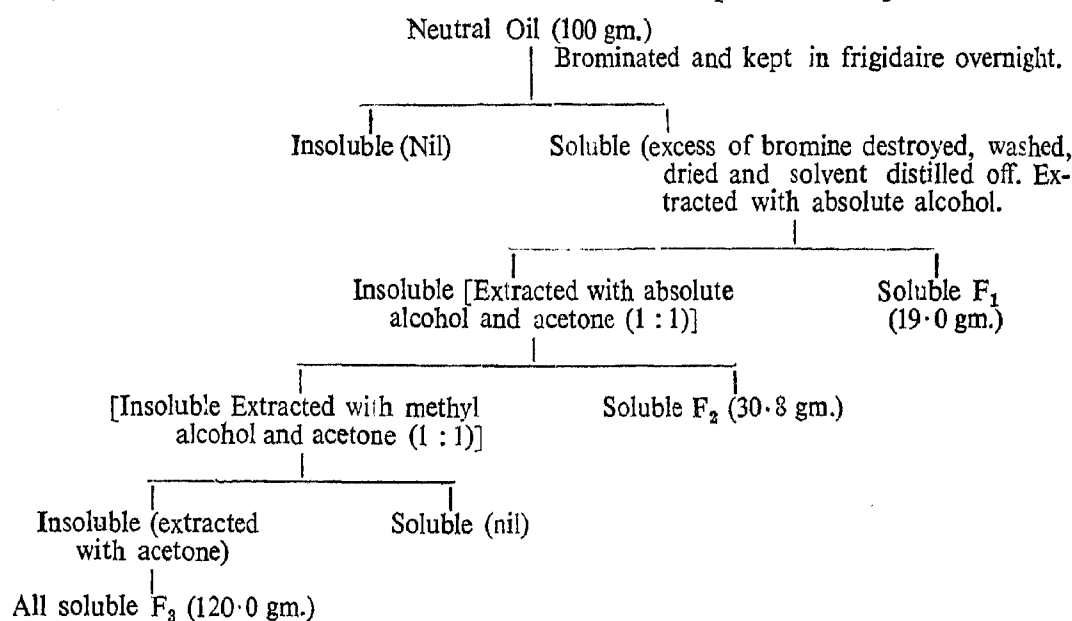
PROBABLE GLYCERIDE STRUCTURE

The purified oil was neutralized with sodium carbonate and filtered.

One hundred gm. of the oil in six times its weight of dry and pure acetone were chilled[15] in frigidaire for a week. No solid separated showing the absence of trisaturated and disaturated mono-unsaturated glycerides. The absence of trisaturated glyceride was further established by oxidizing the oil dissolved in ten times its weight of pure and dry acetone with powdered potassium permanganate.[16]

One hundred gm. of the oil were brominated[17, 18] in ten times its weight of dry petroleum ether (40–60° C.) at − 5° C. till the brown colour persisted.

It was kept in frigidaire overnight. No solid separated. Excess of bromine was destroyed with sodium thiosulphate solution, washed, dried over fused calcium chloride and the solvent distilled off. The residue was extracted with absolute alcohol, absolute alcohol + acetone (1 : 1), methyl alcohol + acetone (1 : 1) and acetone in succession. The scheme of separation is given below:

Neutral Oil (100 gm.)
Brominated and kept in frigidaire overnight.

Insoluble (Nil)   Soluble (excess of bromine destroyed, washed, dried and solvent distilled off. Extracted with absolute alcohol.

Insoluble [Extracted with absolute alcohol and acetone (1 : 1)]   Soluble F_1 (19·0 gm.)

[Insoluble Extracted with methyl alcohol and acetone (1 : 1)]   Soluble F_2 (30·8 gm.)

Insoluble (extracted with acetone)   Soluble (nil)

All soluble F_3 (120·0 gm.)

The fractions F_1, F_2 and F_3 were debrominated by taking them in methyl alcohol, adding zinc dust, saturating with dry hydrochloric acid gas and refluxing for several hours. The debrominated products were saponified, the unsaponifiable matter removed and the mixed fatty acids liberated. The iodine, thiocyanogen and saponification values of these mixed acids determined and then the quantity of individual acids in the fractions calculated. The mixed fatty acids were then oxidized with alkaline potassium permanganate[19] and the solid acids extracted with petroleum ether (40–60° C.). The saponification value of these solid acids also was determined and the probable glyceride structure calculated from these data as given in Tables X–XIII:

TABLE X

	F_1	F_2	F_3
Weight of brominated product in gm.	19·0	30·8	120·0
Weight of unsaponifiable matter	0·241	0·106	0·055
Weight of debrominated glyceride in gm.	11·48	18·52	68·98
Weight of debrominated glyceride free from unsaponifiable matter	11·24	18·41	68·93
Weight percentage of glyceride free from unsaponifiable matter	11·39	18·65	69·96
Molecular percentage of liberated acids	11·54	18·87	69·59
Iodine value of liberated acids	109·0	110·1	122·8
SCN of liberated acids	74·87	75·0	84·1
Mean molecular weight of liberated acids	275·2	275·8	280·6
Mean molecular weight of solid acids	259·8	270·7	276·7

TABLE XI. *Molecular percentage of acids in each fraction*

		F_1	F_2	F_3
Linoleic	..	37·29	38·64	42·84
Oleic	..	44·52	43·94	50·28
Saturated	..	18·19	17·42	6·88

TABLE XII. *Molecular per cent. of acids in total acids*

		F_1	F_2	F_3	Mean	
		11·54	18·87	69·59	100	
Linoleic	..	4·30	7·29	29·82	41·41	(40·48)
Oleic	..	5·14	8·29	34·99	48·42	(49·07)
Saturated	..	2·10	3·29	4·78	10·17	(10·45)

N.B.—Figures in brackets are from direct determination in mixed acids.

TABLE XIII. *Probable glyceride structure*

			F_1	F_2	F_3	Mean
			11·54	18·87	69·59	100
1.	Trisaturated glyceride	..	Nil	Nil	Nil	—
2.	Disaturated-monounsaturated glyceride	..	Nil	Nil	Nil	—
3.	Monosaturated-diunsaturated glyceride	..				
	(a) Monosaturated oleolinolein	..	6·30	9·87	14·34	30·51
4.	Trisaturated glyceride	..				
	(a) Oleodilinolein	..	1·36	3·00	19·87	24·23
	(b) Dioleolinolein	..	3·88	6·00	35·38	45·26

1. By oxidation of neutral oil with potassium permanganate in acetone.
2. By chilling the neutral oil in acetone at 0° C.
3 & 4. By calculating from the component fatty acids of the brominated glycerides in the oil.

All the saturated acids have been considered as one acid in these calculations. According to the laws of even distribution we are justified in assuming that the greatest possible proportion of glycerides exists as mono-saturated oleolinolein rather than monosaturated diolein or monosaturated dilinolein. Further it has been assumed that the solid acids are proportionately divided in different glycerides. From the above considerations the probable glyceride structure may be given as: Palmito-oleolinolein 14·88%; Stearo-oleolinolein 15·63%; oleodilinolein 24·23%; and dioleolinolein 45·26%. The amount of myristic acid being small it has been considered together with palmitic acid in palmito-oleolinolein.

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SUMMARY

The fatty oil from seeds of *Helianthus annus* Linn. has been examined and found to have the following values of the constants:—Sp. Gr. (35° C.) 0·9147, Refractive index (25° C.) 1·4732; Acid value 6·44; Saponification value 194·7; Acetyl value 3·62; Hehner value 91·6; Unsaponifiable matter 0·55%; R.M. value 0·51; Iodine value (Hanus) 111·6; Thiocyanogen value (24 hours) 79·12. The component fatty acids of the oil are myristic 0·38%; Palmitic 4·27%; Stearic 5·46%; Oleic 49·41% and Linoleic 40·48%. The component glycerides of the oil are Palmito-oleolinolein 14·88%; Stearo-oleolinolein 15·63; Oleodilinolein 24·23% and Dioleolinolein 45·26%. The effect of climatic temperature on the general unsaturation of seed oils is also discussed.

REFERENCES

1. Kirtikar and Basu .. *Indian Medicinal Plants,* 2nd edition, Vol. 2, p. 1370.
2. Hilditch, T. P., and Riley, J. P. .. *J. Soc. hem. CInd.,* 1945, **64,** 204.
3. Baughman, W. F., and Jamieson, G. S. .. *J. Amer. Chem. Soc.,* 1922, **44,** 2952.
4. Pieraerts, J. .. *Mat. Grasses,* 1925, **17,** 7280, 7340.
5. Eibner, A. .. *Farbe u. Lack,* 1926, **31,** 463, 472.
6. Juchnovski, G. .. *Masloh. Shir. Delo,* 1931, No. 6–7, 36.
7. Rankoff, G. .. *Fette u. Seifen,* 1937, **44,** 465.
8. Brodie, N. .. *Bull. Ind. Industries Res.,* 1938, **10,** 28.
9. Hilditch, T. P. .. *Chemical Constitution of Natural Fats,* 1941 edn., p. 371.
10. Singh, B. K., and Kumar, A. .. *Proc. Ind. Acad. Sci.,* 1945, **22,** 310–19.
11. Eibner, A., and Muggenthalor .. *Chem. Tech. of Oils, etc.,* 5th edn., Vol. 1, p. 513.
12. Jamieson, G. S., & Baughman, W. F. .. *J. Amer. Chem. Soc.,* 1920, **42,** 1197.
13. Hazura-Lewkowitsch .. *Chem. Tech. and Analysis of Oils, Waxes and Fats,* 6th edn., Vol. 1, p. 575.
14. Jamieson, G. S., & Baughman, W. F. .. *J. Amer. Chem. Soc.,* 1920, **42,** 42.
15. Hilditch, T. P. .. *Chemical Constitution of Natural Fats,* 1941 edition, pp. 412–13.
16. Hilditch, T. P., & Lea, C. H. .. *Jour. Chem. Soc.,* 1927, 3106.
17. Hilditch, T. P. .. *Chemical Constitution of Natural Fats,* 1941 edn. Chapter VI.
18. Vidyarthi, N. L., & Mallya, M. V. *J. Ind. Chem. Soc.,* 1940, **17,** 87.
19. Lapworth and Mottram .. *Jour. Chem. Soc.,* 1925, **127,** 1628.
20. Ivanov, S. .. *Ber. D. Botan. Ges.,* 1926, **44,** 31; *Z. angew. Chem.,* 1929, **42,** 292; *Chem. Umschan,* 1931, **38,** 96.
    Juschkivitsch, S .. *Fettchem. Umschan,* 1933, **40,** 197.
21. Hilditch, T. P. .. *Chemical Constitution of Natural Fats,* 1941 edn., p. 130–31.

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