

# CHEMICAL EXAMINATION OF THE SEEDS OF *CARTHAMUS OXYCANTHA*

## Part I. The Component Fatty Acids and the Glyceride Structure of the Seed Oil

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THE following investigation describes the component fatty acids and the glyceride structure of the seed oil of *Carthamus oxycantha*. The plant belongs to the natural order compositæ.

It is distributed throughout the Punjab, Baluchistan, Afghanistan and westwards towards Caucasus. It is called Wild Safflower in English and Poli or Polian in Hindustani. The seeds contain a large quantity (27%) of oil which is used as a medicine in the Punjab (Stewart),<sup>1</sup> and is the chief ingredient of Afridi Wax Works.

Crossley and Le Sueur<sup>2</sup> also have done some work and they report that the oil from seeds is used for edible purposes but add a note of caution: "It is reported that the plant is a troublesome weed in India and this should be carefully considered before introducing it in other countries."

The plant seems to have been discovered very recently as it has not been mentioned in any of the old works and as a result of this very little work has been done on it, though many other plants of this family have been worked out thoroughly, one of them, *Helianthus annus*,<sup>3</sup> having been investigated by us very recently.

Under these circumstances the work was undertaken and the seed oil of this plant thoroughly examined. The oil contains five acids, Myristic, Palmitic, Stearic, Oleic and Linoleic and seven glycerides, though the maximum number of glycerides obtainable from five acids and the glyceryl radical,  $\text{CH}_2\text{-CH-CH}_2$ , is seventy-five.

Vidyarthi<sup>4</sup> has examined the seed oil of *Carthamus tinctorius* and his results are given here for the comparative study of these two plants. He obtained Myristic acid (with Lauric and lower acids) 1.5%, Palmitic 3%, Stearic 1%, Arachidic (with a trace of Lignoceric) 0.5%, Oleic 33% and

Linoleic (with a trace of Linolenic) 61.0%; and the glycerides were Myristo-oleo-linolein 2%, Myristo-di-linolein 1%, Palmito-oleo-linolein 7%, Palmito-di-linolein 4%, Stearo-oleo-linolein 2%, Stearo-di-linolein 1%, Di-oleo-linolein 15%, Oleo-di-linolein 63% and Tri-linolein 3%. The glycerides of higher acids than Stearic have been considered with Stearic acid and those lower than Myristic with Myristic acid.

On comparing these two oils we see that both of them contain the same component acids as well as the same solid acid contents, but while in *Carthamus oxycantha* the major acid amongst the liquid acids is Oleic acid, in *Carthamus tictorius* it is Linoleic.

#### EXPERIMENTAL

A preliminary examination of the seeds of *Carthamus oxycantha* collected near Lyallpur through the kindness of Dr. J. C. Luthra, Principal, Agricultural College, Lyallpur, gave the following results:

Yield of the oil on extraction ..	..	27.0%
Average weight of a seed ..	..	0.0095 gm.
Ash content of the seeds ..	..	2.88%
Water-soluble in the ash ..	..	32.32%
Water-insoluble in the ash ..	..	67.68%

The qualitative analysis of the ash gave:

Water-soluble: Sodium, Potassium, Carbonate and Chloride.

Water-insoluble: Aluminium, Calcium, Carbonate and Sulphate.

Fifty gms. of the seeds were subjected to extraction by different solvents in succession in a Soxhlet apparatus and the result was as follows:

1. Petroleum-ether ..	..	A light yellow oil. Yield 27.0%
(40°-60°)		
2. Absolute ether ..	..	.. .. 1.7%
3. Chloroform ..	..	Light yellow semi-solid. Yield 0.7%
4. Ethyl acetate ..	..	.. .. 1.7%
5. Absolute alcohol ..	..	Pale yellow semi-solid. Did not give any reaction for sugar or glucoside. Yield 0.9%
6. 70% Alcohol ..	..	.. .. 0.7%

The expressed and the extracted oils were purified by animal charcoal and Fuller's earth and the physical and chemical constants determined. These results together with those of Crossley and Le Sueur<sup>2</sup> and Barnes and Single<sup>5</sup> are given in Table I below;

TABLE I

Constants	Extracted oil	Expressed oil	Crossley and Le Sueur	Barnes and Single
Saponification Value ..	193.6	195.0	189.4	174.2
Iodine Value (Hanus) ..	112.0	112.8	135.5	167.4
Thiocyanogen Value (24 hrs.) ..	79.6	80.2	..	..
Acid Value ..	1.2	1.34	..	..
Acetyl Value ..	2.75	2.98	..	..
Hehner Value ..	94.8%	93.8%	..	..
Unsaponifiable matter ..	..	0.42%	..	..
Specific Gravity (35° C.) ..	0.9131	0.9175	..	0.9270
Refractive Index (30° C.) ..	1.4697	1.4715	..	(15.5° C.) 1.4755
R. M. Value ..	0.69	0.84	..	(20° C.) 0.60

One kg. of the expressed oil was saponified with alcoholic caustic soda, unsaponifiable matter removed with ether and the mixed fatty acids liberated. The chemical constants of the mixed acids were determined which are given in Table II below:

TABLE II

Constants	Result
Saponification Value ..	200.6
Iodine Value (Hanus) ..	113.7
Thiocyanogen Value (24 hrs.) ..	84.5

The mixed fatty acids were separated into their solid and liquid constituents by Hilditch's modification of Twitchell's lead-salt-alcohol process<sup>6</sup>. The various chemical constants of solid and liquid fractions were found out and are given in Table III below:

TABLE III

Constants	Solid acids	Liquid acids
Percentage ..	8.05	91.95
Saponification Value ..	208.4	199.8
Iodine value (Hanus) ..	4.8	126.0
Thiocyanogen Value (24 hrs.) ..	2.9	90.1

EXAMINATION OF LIQUID ACIDS

2.59 gm. of liquid acids were brominated<sup>7, 8</sup> at -10° C. in dry ether and kept for two hours. No solid separated showing the absence of linolenic

acid. The excess of bromine was destroyed with a solution of sodium thio-sulphate, washed with distilled water, dried and the solvent distilled off. Dissolved the residue in 500 c.c. of light petroleum and kept in refrigerator overnight. Some solid separated which was filtered on a weighed Gooch. The filtrate was concentrated to half its bulk and kept again in refrigerator. Some more solid separated which also was transferred to the same Gooch. Solvent from the filtrate was distilled off and the bromine content of the residue determined. The results of the experiment and the calculations are given below in Table IV.

TABLE IV

Weight of acid brominated	..	2.59 gm.
Weight of linoleic acid tetrabromide	..	1.992 "
M. P. of tetrabromide	..	113.0° C.
Weight of residue (di- and tetrabromide)	..	2.659 "
Bromine content of the residue	..	37.55%
Weight of linoleic acid tetrabromide in residue	..	0.207 "
Total weight of tetrabromide	..	2.199 "
Weight of oleic acid dibromide in residue	..	2.452 "
Weight of linoleic acid	..	1.026 "
Weight of oleic acid	..	1.564 "
Percentage of linoleic acid in liquid acids	..	39.6
Percentage of oleic acid in liquid acids	..	60.4

Here below in Table V we have given the amounts of saturated, oleic and linoleic acids as obtained from different experiments. We find that they are in close agreement with each other, thus proving the accuracy of our experiments:

TABLE V

Method	Solid acids	Oleic acid	Linoleic acid
	%	%	%
1 I. V. and SCN. V. of mixed acids	.. 6.31	55.94	37.75
2 I. V. and SCN. V. of solid and liquid acids	.. 7.89	55.51	36.60
3 Bromination of liquid acids	.. ..	56.11	36.67
4 Bertram's method	.. 7.98	..	..
5 Average of 1 to 4	.. 7.39	55.80	36.81
6 From glyceride structure	.. 7.26	54.98	37.76

## EXAMINATION OF SOLID ACIDS

The solid acids were converted into methyl esters and 70.88 gm. of the esters were fractionally distilled at reduced pressure. The results of the experiment and the calculations<sup>9</sup> are given in Tables VI and VII below.

TABLE VI

Fraction No.	Pressure	Temperature range	Weight in grams
S <sub>1</sub>	.. 10 m.m.	170°-175°	4.91
S <sub>2</sub>	.. do	175°-180°	4.95
S <sub>3</sub>	.. do	180°-185°	4.51
S <sub>4</sub>	.. do	185°-190°	7.34
S <sub>5</sub>	.. do	190°-195°	6.80
S <sub>6</sub>	.. do	195°-200°	5.17
S <sub>7</sub>	.. do	200°-205°	7.79
S <sub>8</sub>	.. do	205°-210°	3.75
S <sub>9</sub>	.. do	210°-215°	5.51
S <sub>10</sub> (residue)	.. do	..	17.15
Loss	..		0.56
Total	..		70.88

Fractions S<sub>1</sub> & S<sub>2</sub>—On repeated crystallisation of the liberated acids from dilute acetone, Myristic acid, m.p. 56° C. and Palmitic acid, m.p. 61°-62° C. were obtained. The melting points were not depressed by adding authentic sample of the respective acids.

Fractions S<sub>3</sub>, S<sub>4</sub> & S<sub>5</sub>—On a similar treatment gave Palmitic acid, m.p., 61°-62° C., which was not depressed by its authentic sample.

Fractions S<sub>6</sub>, S<sub>7</sub> & S<sub>8</sub>—On a similar treatment gave Palmitic acid, m.p., 61°-62° C. and Stearic acid, m.p., 68°-69° C., which were not depressed by the authentic samples of the respective acids.

Fractions S<sub>9</sub> & S<sub>10</sub>—Similarly gave Stearic acid, m.p., 68°-69° C., which was not depressed by an authentic sample of the acid.

TABLE VII

Fraction	I.V.	S.E.	Myristic acid		Palmitic acid		Stearic acid		Liquid acids	
			%	gm.	%	gm.	%	gm.	%	gm.
S <sub>1</sub>	.. 1.03	253.6	56.67	2.753	37.58	1.846	..	..	0.82	0.040
S <sub>2</sub>	.. 1.08	257.5	43.03	2.130	40.65	2.507	..	..	0.86	0.043
S <sub>3</sub>	.. 1.27	267.5	8.99	0.405	84.76	3.823	..	..	1.00	0.044
S <sub>4</sub>	.. 1.28	268.9	4.66	0.342	89.08	6.539	..	..	1.0	0.073
S <sub>5</sub>	.. 1.15	270.1	..	..	93.83	6.533	..	..	0.91	0.062
S <sub>6</sub>	.. 1.21	283.7	..	..	48.26	2.496	45.82	2.369	0.96	0.050
S <sub>7</sub>	.. 1.61	289.2	..	..	29.06	2.263	64.82	5.049	1.28	0.100
S <sub>8</sub>	.. 1.64	289.8	..	..	27.71	1.040	66.15	2.480	1.30	0.049
S <sub>9</sub>	.. 2.49	298.2	..	..	..	..	93.33	5.142	1.98	0.109
S <sub>10</sub>	.. 2.24	298.9	..	..	..	..	93.53	16.440	1.78	0.313
				5.630		27.047		31.480		0.883

On solid acids | On total acids

	%	%
Myristic acid	.. 9.02	0.66
Palmitic acid	.. 41.93	3.11
Stearic acid	.. 49.05	3.62

Five grams of the mixed acids were oxidised by Bertram's method<sup>10</sup> and 7.98% of the solid acids were obtained.

Thus the seed oil of *Carthamus oxycantha* contains Myristic, Palmitic, Stearic, Oleic and Linoleic acids. The percentages are given in Table VIII:

TABLE VIII

		%
Myristic acid	..	0.66
Palmitic acid	..	3.21
Stearic acid	..	3.62
Oleic acid	..	55.80
Linoleic acid	..	36.81

#### EXAMINATION OF UNSAPONIFIABLE MATTER

The unsaponifiable matter after recrystallisation from absolute alcohol was found to melt at 135°. The acetyl derivative melted at 121°–122°. The substance gave all the colour reactions of Phytosterol, hence it is Sitosterol present in almost all the vegetable oils.

#### GLYCERIDE STRUCTURE

The purified oil was neutralised with sodium carbonate, dried over fused calcium chloride and filtered.

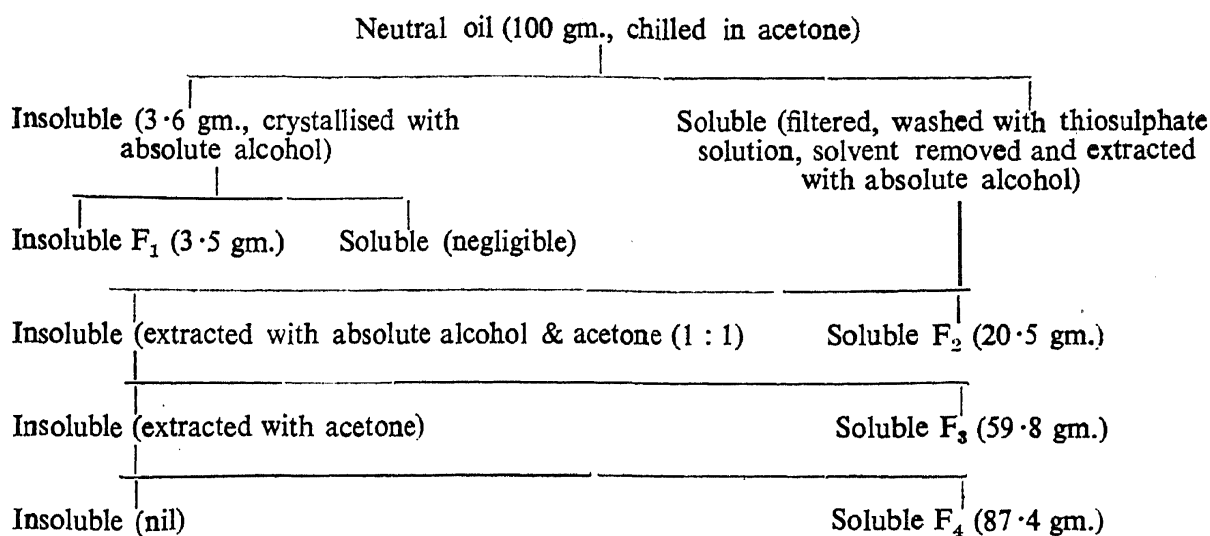
Fifty gm. of the above oil was chilled<sup>11, 12</sup> in the refrigerator for a week with six times its weight of pure and dry acetone. No solid separated showing the absence of tri-saturated and di-saturated-mono-unsaturated glycerides.

The absence of tri-saturated glycerides was further confirmed by oxidising the oil, dissolved in ten times its weight of pure and dry acetone, with four times its weight of powdered potassium permanganate.<sup>13</sup>

One hundred gm. of the oil was brominated<sup>14, 15</sup> in ten times its weight of dry light petroleum at – 5° till the brown colour persisted. 3.6 gm. of the solid (F<sub>1</sub>) separated which on recrystallisation from absolute alcohol gave a solid, m.p. 78°–79°, and bromine content 51.62%. Therefore F<sub>1</sub> is an impure sample of tetra-bromo linoleic glyceride.

The filtrate after filtering F<sub>1</sub> was treated with sodium thiosulphate solution to destroy excess of bromine, washed and the solvent distilled off. The viscous mass was extracted with absolute alcohol, alcohol and acetone (1:1) and acetone in succession. The scheme of separation is given below;

**SCHEME**



The fractions F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub> were debrominated by taking them in dry methyl alcohol, adding an equivalent amount of zinc dust, saturating with dry hydrochloric acid gas and refluxing for about eight hours. The debrominated fractions were saponified, the unsaponifiable matter removed and the fatty acids liberated. The Iodine, Thiocyanogen and Saponification values of the mixed acids determined. The mixed acids were oxidised with alkaline potassium permanganate<sup>16</sup> and the saponification value of the solid acids thus obtained was also determined. The glyceride structure was calculated with these data as given in Tables IX–XIII below.

**TABLE IX**

	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
Wt. of brominated product in gm. ..	3.5	20.5	59.8	87.4
Wt. of debrominated product in gm. ..	1.69	11.55	3.29	51.57
Wt. of unsaponifiable matter in gm. ..	..	0.05	0.13	0.15
Wt. of debrominated product free from unsaponifiable matter	1.69	11.50	35.16	51.42
Mol. % of mixed acids ..	1.69	11.53	35.24	51.54
I. V. of mixed acids ..	..	128.6	115.2	115.21
SCNV. of mixed acids ..	..	80.4	86.2	82.4
S. E. do ..	..	279.6	280.1	282.0
S. E. of solid acids ..	..	260.1	270.5	275.3

**TABLE X**

*Weight per cent. of acids in each fraction*

	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
Linoleic acid ..	100.0	53.20	32.01	36.21
Oleic acid ..	..	35.81	63.60	55.16
Solid acids ..	..	10.99	4.39	8.63

TABLE XI

*Weight per cent. of acids on total acids*

	F <sub>1</sub> 1.69	F <sub>2</sub> 11.53	F <sub>3</sub> 35.24	F <sub>4</sub> 51.54	Mean 100.0
Linoleic acid ..	1.69	6.13	11.28	18.66	37.76
Oleic acid ..	..	4.13	22.42	28.43	54.98
Solid acids ..	..	1.27	1.54	4.45	7.26

TABLE XII

*Mol. per cent. of acids on total acids*

	F <sub>1</sub> 1.69	F <sub>2</sub> 11.53	F <sub>3</sub> 35.24	F <sub>4</sub> 51.54	Mean 100.0
Linoleic acid ..	1.69	6.09	11.32	18.70	36.80
Oleic acid ..	..	4.08	22.32	28.30	55.70
Solid acids ..	..	1.36	1.60	4.54	7.50

TABLE XIII

*Probable glyceride structure*

	F <sub>1</sub> 1.69	F <sub>2</sub> 11.53	F <sub>3</sub> 35.24	F <sub>4</sub> 51.54	Mean 100.0
1 Tri-saturated glycerides ..	nil	nil	nil	nil	
2 Di-saturated mono-unsaturated glyceride ..	nil	nil	nil	nil	
3 Mono-saturated di-unsaturated (a) Do oleo-linolein ..	nil	4.08	4.80	13.62	22.50
4 Tri-unsaturated glyceride					
(a) Oleo-di-linolein ..	nil	6.74	nil	4.56	11.30
(b) Di-oleo-linolein ..	nil	0.71	29.16	33.36	63.23
(c) Tri-olein ..	nil	nil	1.28	nil	1.28
(d) Tri-linolein ..	1.69	nil	nil	nil	1.69

N.B.—1. By oxidation of the 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 the above calculations. According to the law of even distribution we are justified in assuming that the greater portion of the glycerides exist as mono-saturated oleo-linolein rather than mono-saturated di-olein and mono-saturated di-linolein. Further it has been assumed that the solid acids are proportionately distributed in different glycerides. From the above considerations



the probable glyceride structure may be given as Myristo-oleo-linolein 2.05%, Palmito-oleo-linolein 9.82%, Stearo-oleo-linolein 10.33%, Oleo-di-linolein 11.3%, Di-oleo-linolein 62.23%, Tri-olein 1.28% and Tri-linolein 1.69%.

#### SUMMARY

The fatty oil from the seeds of *Carthamus oxycantha* has been examined and found to have Sp. Gr. (35° C.) 0.9175, Refractive index (30° C.) 1.4715, Acid value 1.34, Sap. val. 195.0, Acetyl val. 2.98, Hehner val. 93.8%, unsaponifiable matter 0.42%, R.M. val. 0.84, Iodine val. (Hanus) 112.8, Thiocyanogen val. (24 hrs.) 80.2. The component fatty acids of the oil are Myristic 0.66%, Palmitic 3.11%, Stearic 3.62%, Oleic 55.80% and Linoleic 36.81%. The unsaponifiable matter is Sitosterol, m.p. 135° C. The component glycerides of the oil are Myristo-oleo-linolein 2.05%, Palmito-oleo-linolein 9.82%, Stearo-oleo-linolein 10.33%, Oleo-dilinolein 11.30%, Di-oleo-linolein 62.23%, Tri-olein 1.28% and Tri-linolein 1.69%.

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