UREA COMPLEXES : FRACTIONATION OF ACIDS FROM A TYPICAL OLEIC-RICH SAFFLOWER OIL AND PREPARATION OF OLEIC AND LINOLEIC ACIDS

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Previous studies[1-2] have indicated that the rough composition of fatty acids can be estimated through urea complex fractionation of total fatty acids of a natural oil. Furthermore, reasonably pure fatty acid and esters may also be prepared[2] by these methods. Since safflower oil (locally known as "Kusum oil" derived from the seeds of *Carthamus Tinctorius*) has good prospects in the climatic conditions of East Pakistan, a systematic approach to the studies on this oil available from the different parts of the country was made. A very typical sample of safflower seeds was procured from Savar, in western outskirts of Dacca district. The samples were collected in the latter part of two seasons for checking the results previously obtained.

Experimental

Materials.—The safflower oil seeds were crushed in a machine. The crushed seeds (4 Kg.) were extracted in a large soxlet apparatus, three times by petroleum ether (b.p., 60-80°C.) to begin with and then three times by mixed solvents of petroleum ether and ethyl ether (1:1). The combined extracts were thoroughly washed with water and dried over anhydrous sodium sulphate. On removal of the solvent under vacuum the oil was preserved under dried CO_2 gas and in vacuum in cooler at 2-3°C. for occasional experimental use. Five hundred gms. of safflower oil was saponified and acidified with HCl to free fatty acids which were washed, freed of mineral acids and dried over anhydrous sodium sulphate. The fatty acids were also stored in a filtering flask under CO_2 and vacuum at 2-3°C. The characteristics of the oil and its acid (Table 1) were also established.

Procedures.—One hundred gms. of urea was dissolved in 250 ml. ethyl alcohol (95%) by heating. Simultaneously, 250 gms. safflower oil acids and 10 ml. ethyl alcohol were also slightly warmed and gradually added to the above hot solution under constant stirring. Immediately after the addition, the mixture was boiled and stirred, until no more crystallization occurred. The crystals were filtered,

TABLE 1.—CHARACTERISTICS OF SAFFLOWER OIL AND ACIDS.

Properties	Safflower oil	Safflower oil acids
1. Per cent of oil in seeds (dry basis).	21.5	..
2. Iodine value ..	87.8	91.7
3. Saponification value.	191.2	..
4 Contents of the unsaponifiables (% of oil).	1.7	..
5 Refractive Index at 36° in sodium light.	1.4652	..
6 R. M. value ..	0.11	..
7 Polensky value ..	0.47	..
8 Specific gravity ..	0.96	0.97
9 Acid value ..	35.4	..

TABLE 2.—FRACTIONATION OF SAFFLOWER OIL ACIDS THROUGH UREA COMPLEXES

	Urea added gms.	Urea-complex gms.	Fatty Acids (found) gms.	Loss (calculated) gms.	Fatty Acids (corrected) gms.
1	100	100.3	27.5	0.5	28.0
2.	,,	141.4	35.4	1.3	36.7
3.	,.	125.3	33.1	0.6	33.7
4.	,,	129.5	33.2	1.1	34.3
5.	,,	130.5	33.7	0.7	34.4
6.	,,	125.2	32.7	1.4	34.1
7.	,,	120.5	31.0	0.4	31.4
8.	50	54.5	7.1	1.5	9.6
9.	Filtrate	Solvent removed.	5.2	2.6	7.8
	Total..	..	238.9	11.1	250.0
	Percentage	..	95.6	4.4	100.0

TABLE 3.—FRACTIONS OF SAFFLOWER ACIDS THROUGH UREA COMPLEXEX.

Fractions (same as table 2)	Percent of original Acids (corrected)	Iodine value (I. V.)	Major Acids present in the group
1. ..	11.3	59.1	A: Oleic acid with saturates
2. ..	14.7	71.3	
3. ..	13.4	84.5	
4. ..	13.7	90.7	B: Oleic acid with Linoleic Acid (minor amounts)
5. ..	13.7	93.3	
6. ..	13.7	93.5	
7. ..	12.5	128.8	C: Linoleic acid with Oleic acid
8. ..	3.8	143.9	
9. ..	3.2	153.9	

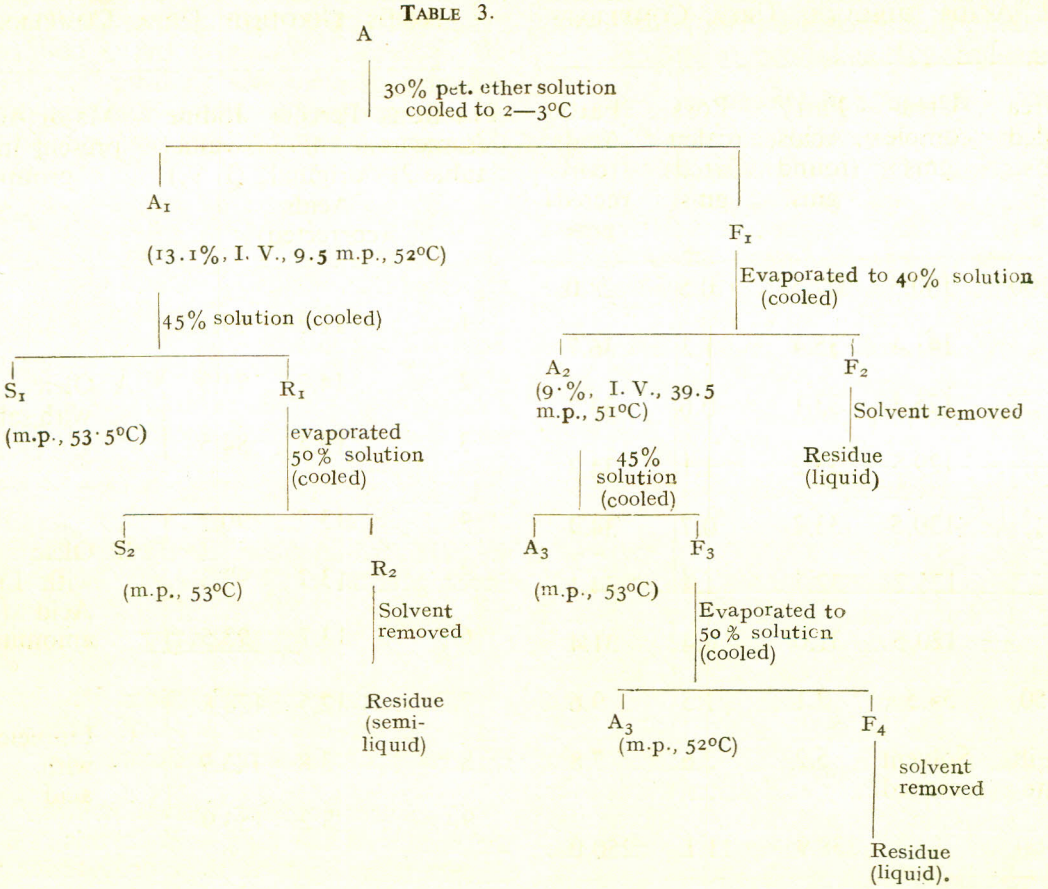
TABLE 4.—REFRACTIONATION OF GROUP B LIQUID ACIDS THROUGH UREA COMPLEXES

Fractions	Percent of B (quantitative)	I. V.	Major Acids
B_1 ..	20.5	89.08	Oleic acid with probable trace impurity of linoleic
B_2 ..	29.7	89.9	,,
B_3 ..	21.7	90.7	,,
B_4 .. (filtrate residue)	28.1	95.8	Oleic Acid with a little more linoleic acid

TABLE 5.—REFRACTIONATION OF GROUP C ACIDS THROUGH UREA COMPLEXES

Fractions	Percent of C (quantitative)	I. V.	Major Acids
C_1 ..	70.4	124.6	Linoleic with oleic acid
C_2 ..	12.0	151.4	Linoleic with minor amounts of Oleic acid
C_3 (filtrate residue)	17.6	159.4	Linoleic with still less Oleic acid

CHART 1 CRYSTALLIZATION OF A OF TABLE 3.



washed first by a small amount of ethyl alcohol and then thoroughly by ethyl ether. This filtrate, freed of ether, was added to the main alcohol filtrate and heated to boiling and the processes were repeated by adding urea successively for next fractions as shown in Table 2. The last fraction is obtained as residue from the last filtrate by removal of the solvent involved. The urea complexes devoid of all solvents, were treated with hot water, traces of HCl acids and NaCl. The resulting oil layers were separated, washed and dried over anhydrous sodium sulphate. The recovery (Table 2) was made, almost quantitatively, through the help of a mixed solvent (petroleum ether, m.p. 40-60°C. and ethyl ether, 1 : 1). The loss in the case of each fraction was calculated on the basis of discrepancy through manual processes and adsorption in driers (liberated in water solution).

The unsaturation was determined for each fraction through half-an-hour Hanus method of iodine value.[4] On the basis of distribution of unsaturation, (I.V., Table 3), three groups, A, B, C of acids were obtained. The group A acids were subjected to fractional crystallization at cooler temperatures (2-3°C.), a shown in Chart I. The distribution of certain saturated fatty acids was, thus, obtained. In order to have the distribution of fatty acids as shown in tables 4-6, the group B and C acids were refractionated through urea-complex formation. The oleic acid fractions B_1-B_3 in Table 4 were subjected to urea-complex formation to obtain a sample of oleic acid of reasonable purity (I.V., 89.6; neutralization equivalent, 282.4; and yield on the basis original oil acids, 26.4%). Similar refractionation of C_2 - C_3 in Table 5 gave linoleic acid (I.V., 164.5; and yield on the original oil acids, 4.8%). By assuming the

presence of two acids[5] only in fractions (table 3-5), the approximate composition was calculated as usual and shown in Table 6.

Results and Discussion

Table 1 shows the characteristics of the oil and its acids. The iodine value seems to be unusually low[6] and acid values of the oil quite high.[6] Tables 2-3 indicate the distribution of different acids in fractions. Savar samples of safflower oil irrevocably reproduced the oleic-rich composition in all cases. Table 6 shows the approximate composition. The

TABLE 6.—APPROXIMATE COMPOSITION OF FATTY ACIDS OF EAST PAKISTAN SAFFLOWER OIL

Saturated (Palmitic, major) ..	12.5
Oleic Acid	71.8
Linoleic Acid	15.7

analytical determinations have been repeated ehree times in each case and the worker's tfficiency has been checked through analyses of a known sesame oil sample. It is hard to elucidate the intricate natural biological mechanisms, but it is generally gathered from the topography in fatty acid distribution over different climatic belts that the soil, climate and environments have something to do with the extent of unsaturation in the oils.[7]

As to saturated acids in the oil, Chart I establishes the predominance of one acid *viz.*, palmitic. Table 4 and 5 confirm the presence of oleic acid in larger and linoleic acid in smaller quantities and demonstrate the possibilities of their isolation in pure states.

The present investigations open up some new aspects of safflower oil of one particular region of East Pakistan, rich in oleic acid that has been prepared in reasonably pure states. Such oleic acid with minor traces of impurity is good for many research purposes. Similarly, linoleic acid has also been prepared for rough uses in biological experiments involving essential fatty acids. Similar unexpected composition has also been found in one variety of linseed oil of local origin. These results will be published later and should stimulate further investigation .

SUMMARY

The urea-complex fractionation has been employed for the evaluation of Safflower oil with respect to its fatty acid contents.

The urea-complex formation has revealed the nature of the fatty acid in this oil and thrown some light on their prospective uses.

References

1. N.A. Khan, Pakistan J.Sc. Research, in press; N.A. Khan, Pakistan J. Biol and Agr. Sc., **1**, 124 (1958).

2. S. Adhikari, and N.A. Khan, Pakistan J.Sc. Ind. Research, **1**, 221 (1958).

3. Hilditch, T.P. *Industrial Chemistry of Fats and Waxes* (Bailliere, Tindal and Cox, London 1945), 2nd Edn., p. 165.

4. T.P. Hilditch, *ibid*, p. 47.

5. Official Methods, American Oil Chemists Soc., Cd-2-38 (p. 5, 1946); H.J. Cannon, J.C. Drummond, Analyst **49**, 311 (1924); T.P. Hilditch, *The constitution of Natural Fats* (Chapman and Hall, London, 1949), 2nd Ed., p. 465.

6. Ref. 3 p. 129 (1945).

7. E.W. Eckey, *Vegetable Oils*, (Reinhold Publishing Corporation, New York, 1959) ; Ref. 3, p. 102 (1945).