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Some Aspects of the Fluorescence of Drying Oils[1]

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THE FLUORESCENCE of drying oils has received little attention. In 1937 the Cincinnati Paint and Varnish Club (2) related fluorescence to the bodying of linseed oil. However they estimated intensity visually, and their data are therefore qualitative. More recently Petit (7) reported the formation of a green fluorescence in linseed oil during heat treatment; the intensity of fluorescence increased with heating time.

In this laboratory fluorescence appeared to interfere with molecular weight determinations of polymerized linseed oil by the light-scattering technique, using the 3650 mercury line. Therefore a study of the fluorescence of linseed, safflower, tung, and oiticica oils was made to develop a means of measuring fluorescence intensity conveniently and to obtain information on the fluorescence of heated and unheated drying oils.

Materials and Methods

The linseed and safflower oils were alkali-refined and bleached before bodying whereas the tung and oiticica oils were heated without prior refining. The tung oil, analyzed by the method of O'Connor *et al.* (6), contained 75% α- and 5% β-eleostearic acid on a glyceride basis. Some of the oil was isomerized in the presence of a trace of iodine to 80% β-isomer. The polymerization apparatus and technique have been described previously (8).

Of the materials listed in Table I the heptane and glycerol were reagent grade chemicals from freshly opened bottles, the fatty acids and esters were distilled products, and the saturated triglycerides were Eastman Kodak White Label materials. The glyceryl monostearate, supplied by Emery Industries Inc., had an iodine value of 5.0 units. The triolein used in the fluorescence fatigue experiments was obtained from the Theodor Schuchardt Laboratories, Gorlitz, Germany.

Fluorescence intensity was measured with a Coleman Photofluorometer, model 12. The filters B1 365 mμ, B2 430 mμ, PC1 480 mμ, and PC2 $>$ 510 mμ were tested for transmittance and were found satisfactory. Intensity was reported relative to the fluorescence of a pure solvent, an unheated oil, or a standard solution of quinine sulphate (5).

All the oils had a natural fluorescence of such high intensity that the cuvettes supplied with the instrument could not be used. Therefore intensity measurements were made on solutions of oil in heptane or diisopropyl ether. Linear relations between intensity and concentration were observed for the various filter combinations with solutions containing up to 0.1 g./ml.

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To permit direct measurement of the fluorescence of undiluted oils, cuvettes of new design were constructed by fusing a Pyrex 1-mm. capillary to the bottom of a 16- x 80-mm. Pyrex test tube (Figure 1).

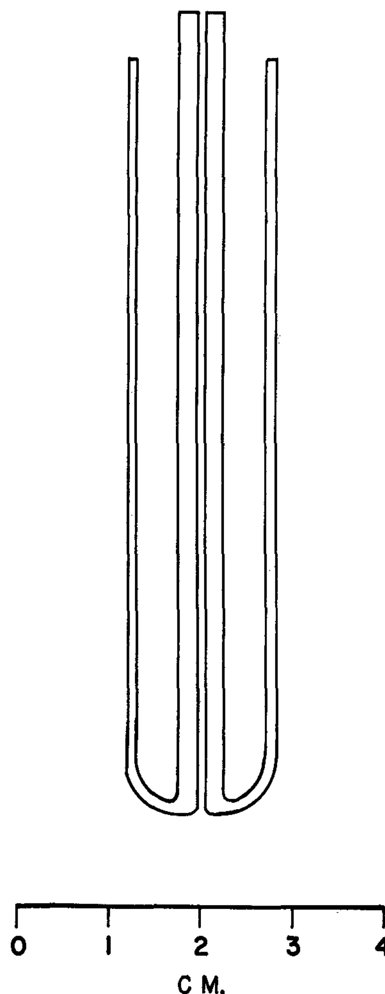

FIG. 1. Capillary cuvette.

The lower end of the capillary was plugged by a small piece of wax after the sample had been inserted, using a hypodermic syringe. The annular space between the capillary and the wall of the test tube was filled with a mixture of benzene and carbon tetrachloride of the same refractive index as Pyrex. The coefficient of variation resulting from the use of the new style cuvette in a random manner, 0.98%, was less than that of the fluorometer error, $\pm 5\%$ of the scale reading.

Because quantity and not quality of fluorescence is the main topic of the present investigation, the term

fluorescence will be used in this paper to indicate fluorescence intensity.

Experimental and Results

Fluorescence Fatigue. To determine whether the fluorescence of fatty substances varied with time of exposure to incident light, relative fluorescence was determined after exposure to light of wavelength of 365 mμ for periods ranging from 1 sec. to 30 min. and to daylight for periods of up to 30 days. The fluorescence of quinine sulphate solutions and of synthetic triolein did not decrease on extended irradiation. However linseed and tung oils, bodied and unbodied, did display fluorescence fatigue (Figure 2). As fluorescence decreased with duration of irradiation, all subsequent samples were measured with an exposure time of 2 sec. or less. When samples of bodied linseed oil (0 to 2.5 hrs. at 310°C.) were kept in clear glass bottles in the laboratory, the fluorescence decreased with time. After 25 days of exposure the intensity was reduced to about half the original value. When unisomerized tung oil was exposed to 365 mμ light, its fluorescence decreased and then increased.

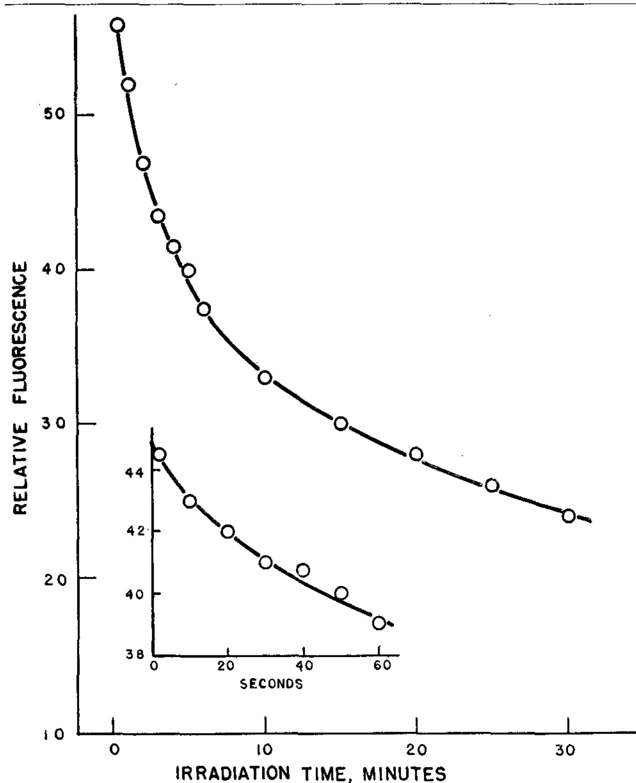

FIG. 2. The relation of fluorescence to duration of irradiation at 365 mμ; linseed oil heated at 310°C. for 3 hrs.

Fluorescence of Unheated Fatty Material. The fluorescence of myristic and stearic acids, their methyl esters and triglycerides, was measured relative to n-heptane (Table I). The data showed that methyl esters were more fluorescent than the fatty acids and that the presence of glycerol in the molecule appeared to increase intensity of fluorescence.

To determine whether the non-glyceride components of a refined and bleached vegetable oil could contribute to its fluorescence, two samples of linseed oil were heated at 310°C. in parallel experiments (Figure 3). With unstripped oil the fluorescence decreased by about 25% during the first two hours of heating and then rose slowly to its original value after five hours. When the oil was stripped of its volatile material by repeated cycling on a centrifugal molecular still, the fluorescence increased linearly with time from a lower initial value. This observation suggested that some of the fluorescent material could be removed by molecular distillation and that the fluorescent ability of unstripped oil was decreased by heating. In addition, incident light of wavelength 365 mμ produced much more fluorescence than light of 430 mμ wavelength. The fluorescence, resulting from irradiation by the 365 mμ line, had peak transmission at about 480 mμ. The native fluorescence of safflower oil was also found to decrease during the first few hours of heating and then to increase with further heating.

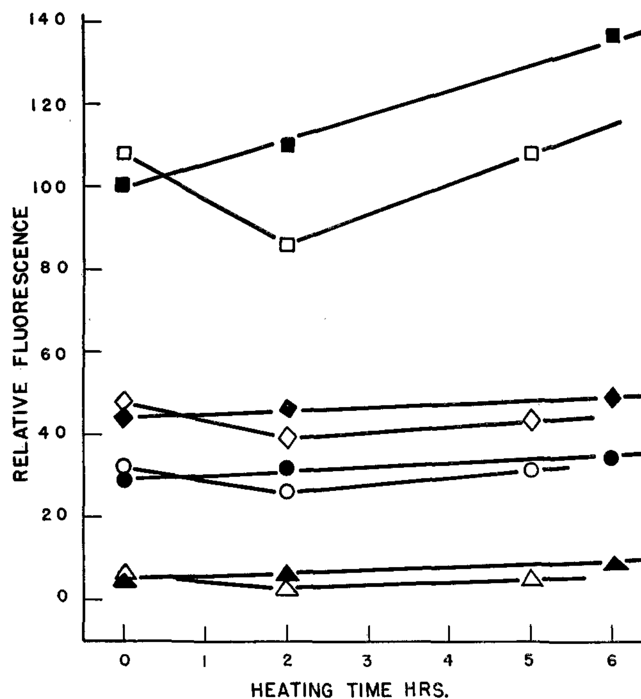

FIG. 3. Relative fluorescence of stripped and unstripped linseed oil; 0.03 gm. oil/ml. heptane.
□ = filter combination 365/480
◇ = filter combination 430/>510
○ = filter combination 365/430
△ = filter combination 365/>510
Closed symbols = stripped oil; open symbols = unstripped oil.

The fluorescence of native tung oil was found to be less than that of isomerized tung oil at all stages of the bodying process. Extrapolation of these data from 3.0 to 0 min. heating time gave an initial fluoresence ratio of 0.79.

TABLE I

Relative Fluorescence of Various Fatty Materials

Material	Concn. moles/l. × 100	Relative fluorescence			
		B1			B2
		PC1	B2	PC2	PC2
Heptane	......	18	10	1.0	1.0
Myristic acid	3.74	26	13	1.7	2.0
Me. myristate	3.19	25	14	1.0	1.0
Trimyristin	0.39	35	15	4.6	5.6
Me. stearate	2.22	40	17	3.5	25
Glyc. monostearate	2.34	*	76	46	67
Tristearin	0.33	*	33	27	48
Glycerol	......	*	76	25	38

* = off scale.

Fluorescence of Heated Materials. To determine whether decomposition products formed during bodying affected the fluorescence, the following experiments were made. A sample of linseed oil, polymerized at 220°C. for 642.7 hrs., was stripped of its volatile components on a centrifugal molecular still at 90°C. and 180°C. The fluorescence of the stripped oil was greater than that of the unstripped oil. In another experiment the fluorescence of two samples of linseed oil heated at 310°C. was measured. One series of samples was polymerized in sealed tubes where the decomposition products accumulated, whereas the other samples were taken from a nitrogen-swept reactor. The data

TABLE II
Reduction of Fluorescence by Decomposition Products

Time	N_2 Swept		Sealed Tubes	
	V30 (stks)	F[a]	V30 (stks)	F[a]
0.0	0.38	7.6	0.37	8.5
0.5	0.65	11.3	0.59	9.6
1.0	1.09	15.7	0.93	10.7
1.5	1.82	19.2	1.49	11.8
2.0	3.01	22.9	2.31	12.9
2.5	4.83	26.6	3.38	13.4
3.0	6.50	30.1	4.51	14.6

[a] Fluorescence, equivalents of quinine sulphate, γ/ml.

(Table II) show that the fluorescence of heated oil is reduced by the decomposition products formed.

During attempts to measure the weight average molecular weight of polymerized vegetable oils by light scattering, nitromethane (5% V/V) reduced the fluorescence of linseed oil solutions tenfold. Later, when capillary tubes were used, it was shown by filling the annular space with nitromethane that inhibition could be effected in this way also, indicating an inner filter action rather than genuine quenching.

In Figure 4 fluorescence is related to heating time, specific refraction, viscosity, and % polymeric glycerides. With conjugated oils the relations were essentially linear. With non-conjugated oils however a logarithmic relation was approximated, but when log. fluorescence was the dependent variable, linear plots were not obtained.

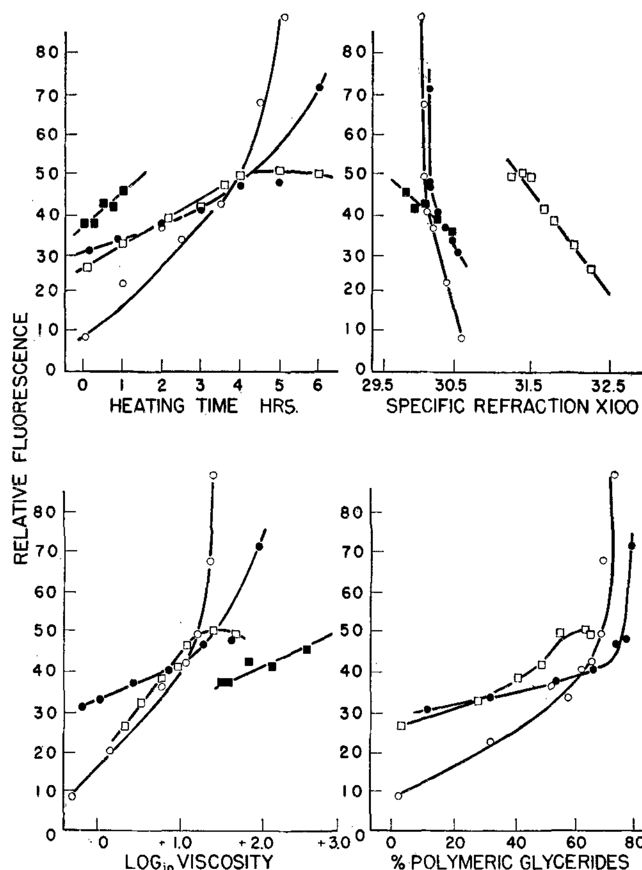

FIG. 4. The relation of fluorescence to: A, heating time; B, specific refraction; C, log. viscosity; D, % polymeric glycerides.
○ = linseed oil
● = safflower oil
□ = tung oil
■ = oiticica oil

Discussion

The fluorescence of unheated vegetable oils seems to be due to material that can be removed by molecular distillation and to the triglycerides themselves. The native fluorescence of various oils may therefore be a function of the efficacy of refining as well as glyceride composition.

Fluorescence can be related empirically to a variety of physical changes in a heated oil. However, because the various oils studied displayed different fluorescence relations, fluorescence cannot be used to compare extents of polymerization in different types of oil. Except with tung oil, where the fluorescence appeared to reach a maximum value, intensity of fluorescence increased with bodying time. This suggests the possibility of using fluorescence for automatic process control in the bodying of vegetable oils.

Hirshberg *et al.* (4) related fluorescence intensity to the degree of resonance in the molecule. They found that in *cis-trans* isomers resonance was inhibited sterically and that molecules in the *trans-trans* configuration had the greater fluorescence. Because heating is known to isomerize unsaturated fatty acyl groups to the *trans* configuration, part of the fluorescence of heated oils may be the result of thermal isomerization. Moreover isomerized tung oil fluoresced more strongly than native tung oil.

Fluorescence "fatigue," noted in this investigation, has also been observed in other substances (1, 3, 4). Hirshberg *et al.* (4) found that extended ultraviolet irradiation caused a decrease in fluorescence and attributed this phenomenon to stereoisomerization. The fluorescence "fatigue" encountered with heated vegetable oils may possibly be explained on this basis.

Acknowledgments

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Summary

The fluorescence of vegetable oils may arise from both the non-glyceride and the glyceride components of the oil. When linseed and safflower oils are heated, fluorescence decreases at first and then increases as polymerization progresses. The fluorescence of heated vegetable oils is masked by decomposition products and by nitromethane. Relating fluorescence to other characteristics of heated oils shows different functional relations for linseed, safflower, tung, and oiticica oils, and a general similarity of behavior between

the two non-conjugated oils and also between the conjugated oils.

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Studies on Some Seed Fats of Cucurbitaceae Family

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A STUDY of the fixed oils obtained from the seeds of the plant family *Cucurbitaceae* is of interest because in some genera the component fatty acid composition is more or less simple while in others diene, triene, and tetraene acids have been found (1). The trends are similar in the seed fats of the allied families, *Rosaceae* and *Euphorbiaceae.*

In the present investigation the recent spectrophotometric technique introduced by Hilditch *et al.* (2) coupled with the methods of low temperature fractionation of fatty acids from organic solvents have been employed. Therefore the compositions of the seed fats reported are expected to be more accurate than those published previously (5-12).

In this part of the present series of investigations the seed fat composition of a) *Cucurbita Maxima* (Hubbard squash or gourd), b) *Lagenaria Vulgaris* (bottle gourd), c) *Citrullus Vulgaris* (watermelon), and d) *Benincasa Cerifera* (pumpkin) were investigated. The composition of these have been previously reported in the literature (5-12).

Experimental

The seeds were obtained from crops grown in the state of West Bengal, India. In each case the oil was extracted from ripe and dried seeds by petroleum ether (B.P. 40°-60°C.). The characteristics of the oils and seeds are presented in Table I. The contents of unsaponifiables were determined according to the methods recommended by the American Oil Chemists' Society (3). The mixed fatty acids of the oils were obtained by saponifying the latter with alcoholic caustic potash solution and then evaporating off the alcohol, extracting the unsaponifiable matters with sulphuric ether, subsequently decomposing the solution of the soap in water by HCl, extracting the liberated fatty acids with sulphuric ether, and recovering the same from this solution after washing it free from HCl.

In the case of *Cucurbita Maxima* seed fat, the mixed fatty acids of the oil were separated into different fractions of mean unsaturation by low-temperature crystallization from acetone and ether at —55°C. and —20°C., respectively (see crystallization chart). Three fractions, designated A, B, C in the increasing order of iodine value, were obtained. Their proportions are given in Table II. These fractions and the mixed fatty acids separated from the seed fat were analyzed spectrophotometrically (Table III).

TABLE I
Characteristics of Oils and Seeds

Seeds	(a) *C. Maxima*	(b) *L. Vulgaris*	(c) *C. Vulgaris*	(d) *B. Cerifera*
% Shell	32	60.1	54.1	52.3
% Kernel	68	39.9	45.9	47.7
% Oil (on kernel)	48	45.1	69.4	48.3
Oils				
Saponification Equivalent	300.7	301.6	298.2	301.3
Iodine Value (Wij's 30 min.)	98.3	126.5	116.2	126.8
Unsaponified and unsaponifiable matter, %	0.9	0.7	0.8	0.8
Free Fatty Acid (as Oleic) %	0.8	0.5	0.9	0.3
Refractive Index (at 40°C.)	1.4683	1.4711	1.4689	1.4715

MIXED FATTY ACIDS FROM *CUCURBITA MAXIMA* SEED FAT.
LOW-TEMPERATURE CRYSTALLIZATION CHART

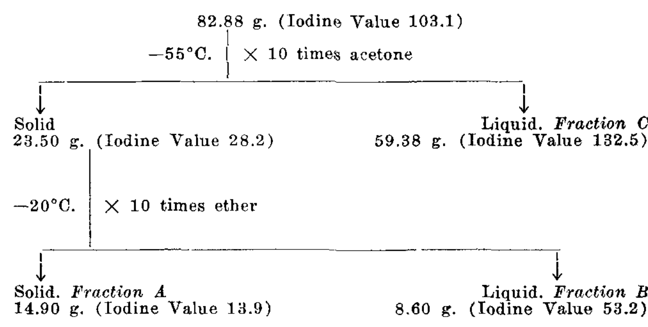

TABLE II
Fractions Obtained by Low-Temperature Crystallization of *C. Maxima* Mixed Fatty Acids (Iodine Value 103.1)

	Weight (g.)	% of Total	Iodine Value	Fraction
Soluble in acetone at —55°C.	59.38	71.6	132.5	C
Soluble in ether at —20°C.	8.60	10.4	53.2	B
Insoluble in ether at —20°C.	14.90	18.0	13.9	A
Total	82.88	100.0		

TABLE III
Spectrophotometric Data

	Iodine Value	$E^{1\%}_{1cm.}$ 268 mμ[b] 170°/15 min.	$E^{1\%}_{1cm.}$ 234 mμ 180°/60 min.
C. Maxima			
Mixed fatty acid[a]	103.1	3	396
Fraction A	13.9	nil	32
Fraction B	53.2	nil	146
Fraction C	132.5	4	532
L. Vulgaris			
Mixed fatty acid[a]	132.5	5	580
C. Vulgaris			
Mixed fatty acid[a]	123.0	5	531
B. Cerifera			
Mixed fatty acid[a]	132.6	5	564

[a] Excluding non-saponifiable matters.
[b] E values at 268 mμ being lower than 10 were neglected [see Hilditch and Shrivastava (4)].