Modified Color Stability Test for Fatty Acids

The standard color stability test for fatty acids, AOCS Method Td 3a-64, (ASTM D 1981-61) requires approximately 45 g of material. In our experimental and development work, this amount of acid was often more than could be spared for the color stability test.

To alleviate this problem, a smaller size tube, 0.63 in. (1.6 cm) x 5.9 in. (15 cm), was used in place of the standard size tube, 1 in. x 9.5 in. Only 5 g of fatty acid was required per test with this size tube.

TABLE I

Heat Stability of Tall Oil Products

Material [a]	Rosin acid	Gardner (1933) Color		
		Initial	AOCS stab. method	Modified stab. method
	%			
1. Distilled tall oil	29	4—	5—	5—
2. Fatty acid	4.0	7+	9	9
3. Fatty acid	3.8	5—	7	7
4. Fatty acid	1.3	3+	5+	5+
5. Fatty acid	0.6	4—	6+	7—
6. Fatty acid	2.0	3	5—	4+
7. Fatty acid	3.7	3+	4	4
8. Fatty acid	0.7	1—	2—	2—
9. Fatty acid	0.3	1—	1—	1—
10. Fatty acid	0.9	3+	6	6
11. Fatty acid	0.5	1—	4—	4—
12. Stearic acid	None	5	10	10

[a] Samples 1 to 11 heated for 1 hr. Sample 12 heated for 2 hr.

To correlate this method (small tube) with the AOCS Method, the procedure was checked over several months by making comparisons using both sizes of tubes. These acids gave a range of heat stability colors from 1— to 10, the normal range used in our work. A few examples are shown in Table I.

Heating was conducted according to the AOCS Method L 15a-58 (1 hr at 205C if iodine value (I.V.) greater than 15, 2 hr at 205C if I.V. less than 15. All samples were blanketed with nitrogen).

There was excellent agreement between both methods in all the fatty acids that were checked. The difference in samples 5 (6+, 7−) and 6 (5−, 4+) is within the normal reproducibility limits.

Aside from the obvious advantage of small sample requirement, this method permits more tubes to be placed in a given size oil bath. Furthermore, the temperature of the bath is easier to maintain within the prescribed limits.

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Detection of Adulteration of Plant Oils with Special Reference to Olive Oil

Standard methods of testing plant oils for adulteration by cheaper fatty materials are still based either on (*a*) color reactions (Bellier, Halphen, Baudouin, etc.) specific for a type of seed oil, but negative in oils heated above 200C in the presence of oxidants, or (*b*) the determination of iodine value, refractive index, fatty acid pattern, etc. Since physical and chemical constants have a wide range of variation for each species of plant oil, adulterations up to 25–30% may not be detected even when the adulterated oil is very different from the adulterant as in the case of seed oils added to olive oil, a fruit coat oil (see Table I). The difficulty arises from the fact that the constants depend on the corresponding additive properties of the fatty acid mixtures which consist almost exclusively of the same C_{16} and C_{18} fatty acids in various proportions. This is also the reason why glyceride analysis by GLC does not give useful information in this case, in contrast to the case of animal and milk fats where various proportions of triglycerides exist in the range C_{22} to C_{54} (or even to C_{60}). Many papers have been published dealing with the adulteration of oils, but the problem has not been satisfactorily resolved (see the Reports of the Literature Review Committee, *JAOCS*, 1958 through 1964).

Triglycerides of plant oils are almost exclusively of the types C_{54}, C_{52}, C_{50}. However, each plant oil contains characteristic triglycerides that can serve as "fingerprints" (Fig. 1) when classified as SSO, SOL, LLLn, etc., where S and U are saturated and unsaturated fatty acids; O, L, Ln are oleic, linoleic, and linolenic acids and designations of SSO, etc., are without regard to position of the fatty acids. Detection of adulteration does not require complete separation and quantitation of the types of glycerides. It is sufficient to isolate a fraction enriched with a specific type of triglyceride and to determine its fatty acid composition. A paper chromatographic method based on an incomplete separation of triglycerides was proposed for this purpose by Kaufmann and Aparicio (1). These authors did not examine extreme cases of each species, i.e., samples containing very high or very low

TABLE I

	Refractive index (40C)	Iodine value	Linoleic acid (%)
Range for olive oils	1.4608–1.4630	78–90	4–20
Typical olive oil "A"	1.4612	82	6
Typical cottonseed oil "B"	1.4660	104	45
Olive oil "A" adulterated with cottonseed oil "B" up to 30%	1.4626	88.6	17.5

TABLE II

Oil tested	Fraction I		Fraction II	
	Oleic acid (%)	Linoleic acid (%)	Linoleic acid	
			(%)	(% of U)
Pure olive oils of linoleic acid contents 6–16%	71–74	3–4.5	0.5–2.0	0.9–3.0
Olive oil *plus* 6% cottonseed oil	67	10	6	10
Olive oil *plus* 10% soybean oil	62	14	12	19

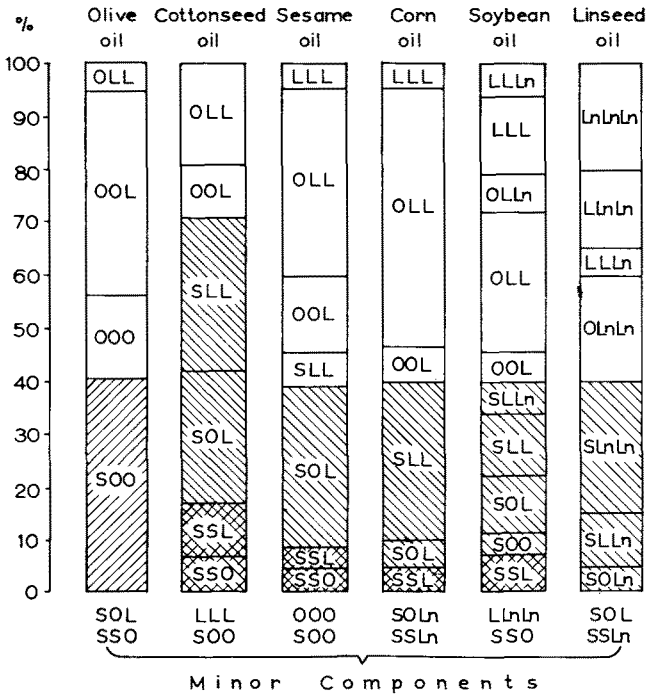

FIG. 1. Range of triglyceride types of some typical samples of plant oils, presented downwards in order of decreasing solubility in organic solvents. The percentage of each type is schematically given by the relative height of its strip. Single-shaded: SUU; Cross-shaded: SSU.

amounts of the various fatty acids.

While conventional low temperature crystallization can be used to detect adulteration, it is a tedious procedure. We overcame the disadvantages of the procedure by simplifying the technique. All crystallizations were carried out at 0C to 1C for at least 8–10 hours and, instead of modifying the temperature, various acetone-methanol mixtures of increasing polarity were used. Adulteration of olive oil is the most important case. Therefore, we devised the method described below permitting the certain identification of seed oils in olive oil even in cases of adulteration below 5%. Similar methods can obviously be worked out for other cases.

The oil (2 ± 0.1 g) is placed in a cylinder containing 25 ml of methanol-acetone (1:4) and kept in the refrigerator overnight. The supernatant solution is decanted. The insoluble fraction is dissolved in 12 ml of methanol-acetone (1:7) and kept at 0C for at least 8 hours. The supernatant solution is removed as completely as possible by decantion. The crystallized material (Fraction I) is dissolved in 8 ml of methanol-acetone (1:9), an aliquot is removed for gas-liquid chromatographic (GLC) analysis, and the rest is kept at 0C for 8–10 hours. Then, the supernatant solution is removed by decantion, while the crystallized material (Fraction II) is analyzed for fatty acids.

The fatty material crystallizing from olive and cottonseed oils is plotted against the composition of solvents in Figure 2. GLC analysis of fatty acids of the fractions thus isolated indicated that, although incomplete fractionation was achieved by this procedure, each branch of the curves thus formed corresponds mainly to one type of triglyceride. For example, as the methnol concentration is increased from 0 to 7%, the solubility of SSU of cottonseed oil decreases to a much greater extent than the solubility of SUU, thus resulting in a change of the slope of the curve at the point corresponding to methanol 7% because under

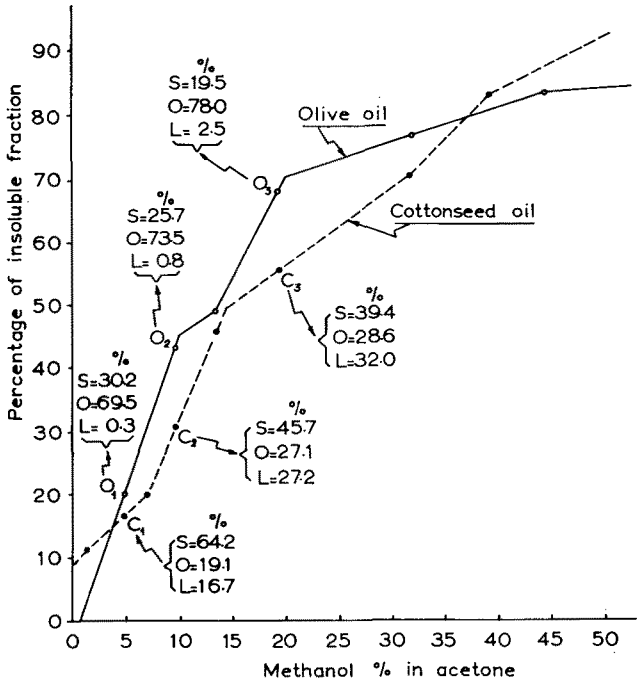

FIG. 2. Solubility data of olive oil and cottonseed oil in 9-fold amounts of several methanol-acetone mixtures at 0C. Fatty acid compositions of some characteristic fractions are included.

these conditions (9 volumes of solvent, 0C) almost all SSU crystallizes out. This is indicated by the fatty acid pattern of fraction C_1 of cottonseed oil (Fig. 2) from which a content of at least 92.5% of SSU can be assumed for the fraction.

In Table II some results of such analyses are given. Fraction I of pure olive oil contains mainly SOO accompanied by small amounts of OOO, OOL, etc. Consequently, its oleic acid content is always above 70% (usually 72–74%), while the linoleic acid content of this fraction never exceeds 5%. If olive oil is adulterated by an admixture of seed oils, the presence of SSL, SOL, SLL (SSO) lowers the oleic acid content of this fraction below 67%, while its linoleic acid content is increased above 10% for an adulteration of 5% only (Table II). On the other hand, the linoleic acid content of Fraction II was found below 2% for pure olive oils containing small or large amounts of linoleic acid, while in adulterated olive oils the linoleic acid content of the respective fraction is always higher than 5%. The examination of Fraction II is especially helpful for the detection of adulterations lower than 5%.

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1. Kaufmann, H. P., and M. Aparicio, Fette-Seifen-Anstrichmittel *61*, 768–770 (1959).

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• *Addendum*

"Determination of the Weight of Bulk Oil Shipments," JAOCS, February, 1965. On page 156, second column and second paragraph "1. Shore Tank Calibrations," delete the last two sentences, beginning "Further, this error" and ending with "the last compensation will be."