The Use of Refractive Index Measurements In Fatty Acid Ester Analyses[*]

KARL F. MATTIL and HERBERT E. LONGENECKER
University of Pittsburgh, Pittsburgh, Pa.

The fractional distillation of methyl esters of mixed fatty acids from fats and oils, followed by analysis of the resultant ester fractions, has been a valuable tool in the determination of their compositions. The ester mixtures can be readily separated into fractions containing no more than two adjacent homologous components when packed columns are used (1, 2). Such fractions can then be analyzed for composition by the use of simultaneous equations based on analytical constants (3). However, this method of analysis possesses certain inherent difficulties which derive from the not too satisfactory analytical constants which must be employed.

In general, for such analyses, the determination of the saponification equivalent and the iodine value is mandatory; quite often the thiocyanogen value is also required. That these determinations are at times somewhat unsatisfactory has been the experience of many investigators. For example, composition analyses are based upon a difference of approximately 28 saponification equivalent units between any two adjacent homologous esters. Thus, in a mixture of methyl palmitate and methyl stearate, the saponification equivalent must be determined accurately to ± 0.28 units in order to calculate the composition to ± 1.0% of the true value. This represents a maximum analytical error of ± 0.1% in the determination of the saponification equivalent which corresponds to ± 0.02 ml. of N/2 acid used in the titration.

Other difficulties are often encountered. Distillations must at times be made of small samples, necessitating fractions as small as 0.1 to 0.2 grams. In addition, time is an all-important factor, thus ruling out micro-methods. Hence, we have considered the use of additional constants as checks on or as substitutes for the others, provided such constants would require only a very small quantity of sample.

Refractive index measurements have been used in other fields of chemistry as an analytical tool, but their use in fat and oil analysis has been relatively limited. Wyman and Barkenbus (4) applied a long-familiar technique to methyl esters when they used refractive indices to follow an ester distillation. They showed that the index of refraction of the esters increased with an increase in the chain length, and suggested that this constant might be used as a substitute for the saponification equivalent in analytical distillations, but they did not indicate clearly how it could be applied.

In our work it has been found that the refractive index may be used as a supplement to or a substitute for not only the saponification equivalent but also, under the proper conditions, for the iodine value and the thiocyanogen value. In order to use this constant in the analysis of mixtures of esters, it was first necessary to determine the effect of temperature upon the indices of the various highly purified esters and mixtures of esters. Then, inasmuch as some mathematical relationship between the refractive index of a mixture and its composition was essential to our purpose, a study of this relationship was conducted.

Experimental

Preparation of Methyl Esters. A series of highly purified methyl esters was prepared. The saturated esters were obtained by fractional distillation of the methyl esters prepared from fatty acids which had been purified by fractional crystallization to essentially theoretical saponification equivalents. Methyl-oleate was prepared by crystallization, according to the procedure outlined by Brown (5). The more unsaturated methyl esters, linoleate and linolenate, were prepared by debromination of tetra- and hexabromostearic acids in acidified methyl alcohol (6). They were further purified by fractional distillation.

Relationship of Refractive Index to Temperature. In order to assess the effect of the temperature at which the refractive index is taken upon any calcu-

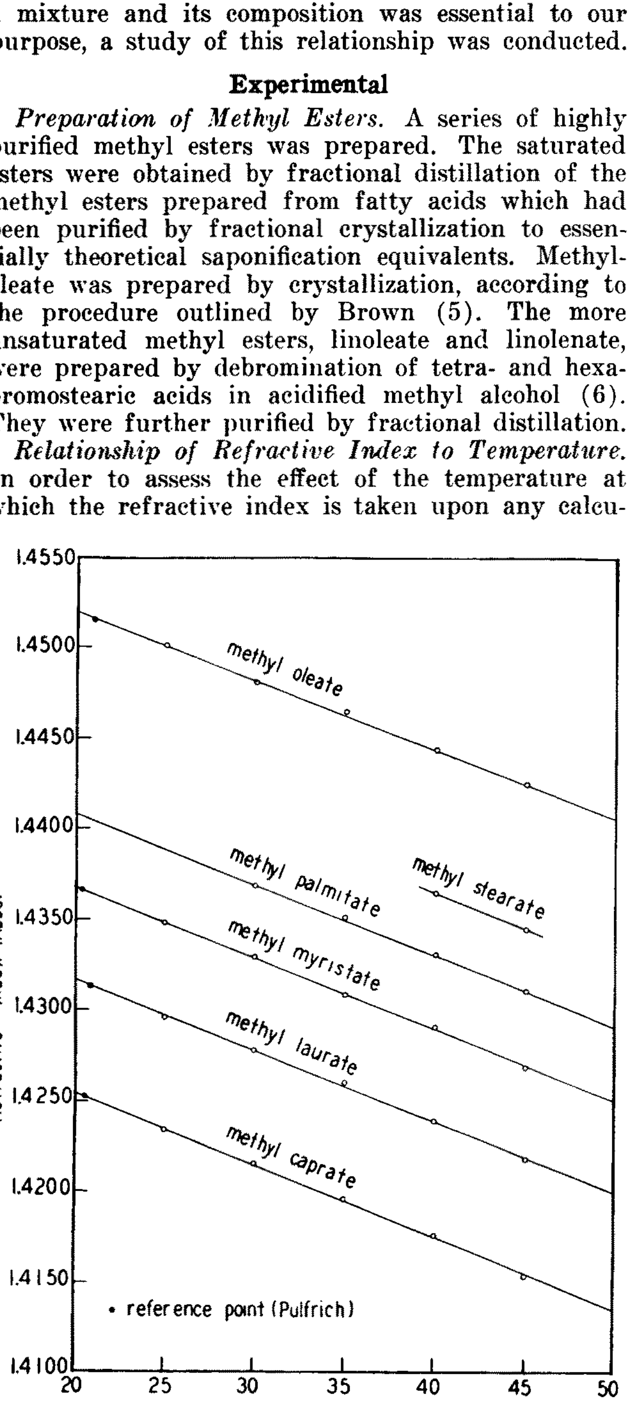

FIG. I. The relationship of refractive index to temperature for highly purified methyl esters.

---

[*] This work was aided by grants from the Buhl Foundation and Swift and Company. A portion of the material was reported at the 1942 Fall Meeting of the American Oil Chemists' Society held in Chicago.

Contribution No. 499 from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania.

lation which may be made from that reading, the indices of the esters were determined over a temperature range from 20 to 45° C., insofar as the melting points of the esters allowed (Fig. I). The readings from 25 to 45° C. were made with an Abbé refractometer, which can be read to ± 0.0001 index units. All temperatures reported for Abbé readings were controlled to ± 0.1° C. From the Abbé data the equation for the best straight line relating index to temperature for each ester was calculated by the method of least squares as follows:

Methyl caprate: $R.I._T = -0.00040\,T + 1.4334$
Methyl laurate: $R.I._T = -0.00039\,T + 1.4395$
Methyl myristate: $R.I._T = -0.00039\,T + 1.4446$
Methyl palmitate: $R.I._T = -0.00039\,T + 1.4486$
Methyl oleate: $R.I._T = -0.00038\,T + 1.4595$
where
$R.I._T$ represents the refractive index of the ester at temperature T,
and
T represents temperature reading in centigrade degrees.

From these equations were drawn the lines shown in Fig. I. The calculated slopes of the best lines indicate that the indices of the several esters decrease, in the range examined, at the rate of 3.8-4.0 fourth-place units per degree Centigrade rise in temperature. This fact had been previously indicated by Wyman and Barkenbus (1). It is interesting to note that this rate of change is comparable with that found regularly in this laboratory for series of synthetic glycerides. For methyl stearate, methyl linoleate, and methyl linolenate insufficient points were determined from which to calculate an equation for a line. Those points determined were:

	45° C.	40° C.	25° C.
Stearate	1.4345	1.4365	
Linoleate			1.4597
Linolenate			1.4692

For those esters with sufficiently low melting points, absolute indices were measured using a Pulfrich refractometer, which may be read to the fifth decimal. Inasmuch as there was no convenient means of controlling the temperature of the Pulfrich instrument used, the readings were obtained as follows:

Compound	Temperature °C.	Refractive Index
Methyl caprate	20.6	1.42515
Methyl laurate	20.9	1.43136
Methyl myristate	20.4	1.43667
Methyl oleate	21.0	1.45157

It will be noted that when these absolute reference points are plotted (cf. Fig. I), they fall close enough to the extended Abbé line to be within the experimental error of the latter instrument. The deviations from the best line are, in the order of compounds listed above, —0.8, +0.4, +0.3, and —0.4 units of the fourth place.

Relationship of Refractive Index to Composition of Mixed Methyl Esters. Several series of binary mixtures of the purified methyl esters were prepared. For each series the refractive indices of the several mixtures were plotted against their respective known compositions. The first question that arose was whether the refractive index was more likely to be related to molar per cent composition or to weight per cent composition. The data were plotted both ways, and it was found that in any given binary series there exists a linear relationship between the refractive index and the per cent composition by weight; no such relationship obtains with molar composition.

A large variety of series was prepared, of which several of the more significant are presented (Fig. II). For each series the points were plotted and the best straight line calculated and drawn as indicated in the figure. Again, all data were obtained with an Abbé refractometer, the temperature being controlled at 30.0 ± 0.1° C. From the figure it can be seen that the ranges of indices between the intercepts of the various systems (i.e., difference between the refractive indices of the two pure components) are: myristate-palmitate, 0.0040; palmitate-oleate, 0.0112; oleate-linoleate, 0.0094; and linoleate-linolenate, 0.0097. The average deviations of the various individual points from the best lines were, respectively, 0.3, 0.8, 0.5, and 0.1 units of the fourth place. It is apparent that these errors are within the limit of accuracy of the Abbé readings, with only the palmitate-oleate curve showing consistently large discrepancies.

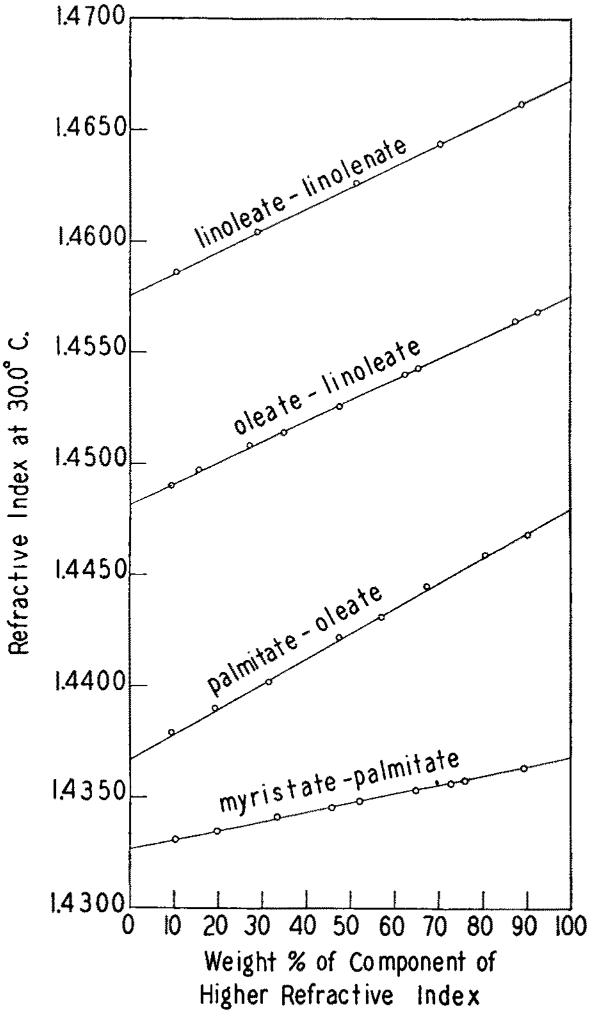

FIG. II. The relationship of refractive index to weight percentage composition of binary mixtures of highly purified methyl esters.

From the above data, it is obvious that the chief limitation to the use of the refractive index in ester analysis is the refractometer itself. For example, according to the evidence presented by the myristate-palmitate curve (where the range of indices is but 0.0040), it is impossible to estimate the composition of a mixture of these two esters to greater precision than ± 1.5% with Abbé readings. However, there are available refractometers with which the fifth decimal can be estimated, making possible calculations which increase the precision by a factor of three to five. With such an instrument, ester compositions could be analysed with at least the same and probably greater accuracy than may be obtained from saponification equivalents, even with the low range of only 40 fourth-place units shown by the myristate-palmitate curve.

It is apparent that in the other three series the ranges are such as to make it possible to increase the precision of a composition analysis to ± 0.5% even with an Abbé refractometer. Use of a more refined instrument would reduce this even more. The significant increase in refractive index effected by additional double bonds makes possible a more precise determination of mixtures containing unsaturated components.

That the above relationship of refractive index to weight percentage composition, established for binary mixtures, is independent of temperature was established by determining the refractive indices of several series at 45° C. The curves plotted from these points were replicas of their counterparts plotted at 30° C. except for their intercepts.

In addition to the binary mixtures there were prepared some three and four component systems. We have attempted to simulate all the various types of mixtures one is likely to encounter in fractional distillation of mixed esters. For each known mixture the experimentally determined index of refraction was compared with the theoretical index calculated from the equation:

$$\text{R.I.}_{\text{total}} \times 100 = (\text{R.I.}_{\text{a}} \times \text{Wt.\%}_{\text{a}}) + (\text{R.I.}_{\text{b}} \times \text{Wt.\%}_{\text{b}}) + \cdots$$

where,

$\text{R.I.}_{\text{total}}$ represents the theoretical index of total mixture,

R.I._{a} and R.I._{b} represent the indices of components a and b, and

$\text{Wt.\%}_{\text{a}}$ and $\text{Wt.\%}_{\text{b}}$ represent the weight percentage of components a and b in the mixture.

The composition of the mixtures and their experimental and calculated refractive indices are shown in Table 1. This table contains examples which were chosen as representative. An extra figure, not to be regarded as a significant figure, has been added to each calculated refractive index in order to show that those errors which do exist are undoubtedly due to the limitations of the Abbé instrument.

Discussion

In order to illustrate the usefulness of refractive index measurements in fatty acid analysis by the ester distillation procedure, the following example is cited. A particular methyl ester fraction obtained during a recent analysis had the following analytical constants: saponification equivalent, 291.3; iodine value, 77.5; refractive index, 1.4453 at 35.0° C. There was some question concerning the validity of the saponification equivalent of this particular fraction. Methyl linoleate and methyl linolenate were determined spectrophotometrically, and the ester composition of the mixture was calculated by the customary procedure to be:

	Weight per cent
Methyl palmitate	20.0
Methyl stearate	4.5
Methyl oleate	61.5
Methyl linoleate	13.2
Methyl linolenate	0.8

Using the equation given above, together with the refractive indices for the respective esters, the calculated refractive index was 1.44525, which agrees with the determined index within experimental error, thereby establishing the accuracy of the calculated composition for the fraction.

It has been shown that a linear relationship exists between the composition and the refractive index of a mixture of methyl esters, which makes it possible to use the index as a tool in the calculation of the composition of unknown mixtures. It has the advantage of being an extremely simple and fool-proof determination, and may be made immediately on every fraction cut in a distillation, thus providing information which can often simplify and otherwise facilitate either an analytical or preparative distillation. Another advantage is the very small quantity of sample required, which can be reclaimed if desirable.

In applying the method, certain limitations must be clearly recognized. First, the worker is limited by his instrument; however, that is not serious if a five-place refractometer is available. Second, the temper-

TABLE 1

Refractive Indices of Mixtures of Three and Four Different Methyl Esters

Composition of mixtures studied (percentage by weight)								Refractive Index (30.0°C.)	
Caprate	Laurate	Myristate	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Calculated	Determined Abbé
11.6	73.6	14.8						1.42780	1.4278
33.0	31.9	35.1						1.42724	1.4273
	15.0	73.7	11.3					1.43229	1.4323
	31.4	40.3	28.3					1.43226	1.4323
		15.9	68.3	15.9				1.43664	1.4367
		34.8	30.5	34.7				1.43622	1.4363
			24.9		24.0	27.0	24.2	1.45246	1.4525
			52.4		23.5	12.8	11.3	1.44566	1.4455
					9.4	81.5	9.1	1.45757	1.4576
					37.4	21.8	40.9	1.45789	1.4579

ature must be carefully controlled. For anything approaching precision work the temperature must be controlled, or at least known, to a few hundredths of a degree. Any temperature may be used, provided an accurate index-temperature curve is at hand. Third, for precise results each operator should calibrate his own instrument and his own technique against a pure sample. Most people will read a refractometer consistently the same, but one person may read it consistently different from another. For this reason we have preferred not to regard the above reported indices as absolute values. It is clear, however, that once the calibrations are obtained, the refractive index provides an extremely simple and direct tool. The calibration reported above may be used for many purposes where the highest precision is not required.

BIBLIOGRAPHY

1. Longenecker, H. E., J. Soc. Chem. Ind., *56*, 199T (1937).
2. Whitekamp, A. W., and Brunstrum, L. C., Oil & Soap, *18*, 47 (1941).
3. Longenecker, H. E., Oil & Soap, *17*, 53 (1940)
4. Wyman, F. W., and C. Barkenbus, Ind. and Eng. Chem., Anal. Ed., *12*, 658 (1940).
5. Brown, J. B., Chem. Reviews, *29*, 333 (1941).
6. Rollett, A., Z. Physiol. Chem., *62*, 410 (1909).

Valencia Orange-Seed Oil

G. R. VAN ATTA and W. C. DIETRICH

Western Regional Research Laboratory, Albany, California
Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration,
U. S. Department of Agriculture

Processing residues from various crops have been utilized from time to time as minor sources of oils. In Europe oils have been produced from tomato and grape seeds; raisin-seed, apricot-kernel, walnut, and avocado oils are now being manufactured and sold in this country. Expansion in the citrus industry in recent years calls attention to citrus seeds as a potential though very minor oil source. It has been estimated that the seed oil corresponding to the 1940 pack of canned orange products would amount to about 264 tons. This figure would become only 700 tons if based on the total oranges harvested but not sold as fresh fruit (11). Except for a small plant in Florida producing annually about 45 tons of grapefruit-seed oil no commercial production of citrus-seed oils has been reported thus far (13).

The work reported here was undertaken in 1941 at the Western Regional Research Laboratory in Albany, California. It had as its purposes a brief examination of some aspects of the recovery and processing of orange-seed oil and also an examination of the oil in somewhat greater detail than had been previously reported (9).

Experimental material. The seeds used were drawn from a lot of air-dry Valencia orange seeds obtained from a Southern California processing plant in the fall of 1940. They had been stored several months in burlap bags and then transferred to steel drums. The drums had been filled by strata and each layer had been lightly sprinkled with chloroform to kill insects. A grab sample showed that about half the seeds were somewhat discolored with mold.

Whole seeds contained 70.8 per cent of kernels by weight. Skin and pulp fragments reduced the proportion of kernel in the bulk sample to 62 per cent. Uncleaned air-dry seeds contained 9.2 per cent moisture and 31 per cent oil. On the dry basis the oil content of uncleaned seeds was 34.2 per cent. Dry, clean kernels contained 55 per cent of oil.

Decortication. A coffee mill equipped with granulating burrs set far apart was used to crack the hulls and loosen the kernels. Although most of the kernels were merely split in half in the process, the coffee mill produced some kernel dust which tended to cling to the hulls. Air-dry seeds and seeds that had been oven-dried to a moisture content of 1.5 per cent were decorticated in this manner. Oven drying rendered the hulls more brittle but increased kernel breakage. Hulls separated from air-dry seeds by fanning and screening were less contaminated with kernel dust after decortication than those from oven-dried seeds. Table I shows the degree of separation achieved when oven-dried seeds were decorticated.

TABLE I

Separation Accomplished by Decorticating Valencia Orange Seeds

Fraction	Weight of fraction as per cent of weight of whole seeds	Oil content of fraction in per cent	Oil in fraction as per cent of total oil in seeds
Cleaned seeds before decortication	100	35.9	100
Hulls	41.3	15.2	17.6
Kernels	58.7	50.4	82.4

Optimum moisture content for hydraulic pressing. Tests on cooked kernel meal performed under comparable conditions showed the optimum moisture content to lie between 7.5 and 8.0 per cent on the moisture-free basis, which level is within the known optimum range for most seed meals. The relationship between oil yield and moisture content is shown in Figure 1.

Oil recovery by continuous pressing. Only a few tests of continuous pressing were made. They were sufficient, however, to indicate that orange seeds would present no unusual problems in this type of process. A midget Anderson Duo Expeller was used in the tests.

Preliminary trials showed that kernel meal did not press as well alone as when mixed with shredded hulls. Ground whole seeds appeared to press satisfactorily. Two test runs differing only in moisture content were made with a mixture comprised of 85 per cent kernel meal and 15 per cent shredded hulls. The meal was preheated to 90°-95° C. in a steam-jacketed kettle and the expeller tempering trough set to maintain a wall temperature of 110° C. throughout the runs. While