• TECHNICAL

Evaluation of the Internal Standard Method for the Quantitative Estimation of Oil Polymer Content by Gas Chromatography

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Abstract

The use of an internal standard to quantitatively estimate the polymer content of fatty acid ester samples has been described and evaluated. Standard samples of known composition have been prepared and analyzed. The mean of the deviations from actual polymer values was found to be less than one per cent. The application of this approach to the analysis of partially polymerized oils is discussed.

Introduction

NATURALLY OCCURRING vegetable and marine oils used in the preparation of organic coatings by the paint industry are quite often subjected to industrial processes which desirably increase their viscosity. Such processes include air blowing, liming and heat-bodying treatments. In instances where reactive chemicals (maleic anhydride, cyclo- and dicyclopentadiene, etc.) are not also added during processing to form Diels-Alder-type addition products, the polymer moiety of the processed oil consists of polymerized fatty acids.

When polymerized or partially polymerized oils in such coatings are analyzed by gas chromatography, diminished peak areas for the polyunsaturated fatty acids are recorded on the chromatogram. One approach at estimating the percentage of oil polymer acids in such a sample by comparison of the total peak area of the sample with the total peak area of a standard sample of identical injection size has been attempted with fair success (5). However, precise replicate injections are often erroneous due both to inaccuracies in reproducing sample volumes in a microliter syringe and variable sample losses which sometimes occur upon injection. The use of a suitable internal standard, preweighed into the isolated oil sample obviates the dependency upon sample injection volumes. This paper describes the accuracy of such an approach and outlines a procedure which couples the use of an internal standard and simple equations for the quantitative estimation of the polymer content in oil samples. Other workers have reported the use of an internal standard in the analysis of milk fatty acids (1) and of tall oil fatty acids (3). The approach described herein could likewise be taken to estimate the non-chromatographable portion of any sample analyzed by gas chromatography.

Experimental

Apparatus. Gas chromatographic analyses were carried out on a Perkin-Elmer 154-C vapor fractometer equipped with a hot-wire filament detector. The usual instrument operating conditions for this study were as follows: column temp, 199C; injection block temp, 260C; filament current, 205 ma (W-2 tungsten 1 mil wire, Gow-Mac Instrument Co.); helium flow rate of 40 ml/min measured at the column outlet. The column was 6 ft x 0.25 in. OD stainless steel tubing packed with 20 wt per cent polyethylene glycol succinate on 60–80-mesh Chromosorb P. Detector signals were attenuated to a 0–1 mv recorder and peak area measurements were made with a Perkin-Elmer model 194 printing integrator.

Reagents. Methyl myristate was obtained from the Eastman Kodak Co., margaric acid (n-heptadecanoic acid) from Fisher Scientific Co., and methyl palmitate and stearate from Applied Science Labs. The polymer standard was a trimer acid sample (Emery 3162-D), obtained from Emery Labs., Inc. Methyl ester preparations of the polymer acid sample and of margaric acid were made by allowing each to stand overnight, under nitrogen, in the presence of equimolar amounts of methanol and acetone dimethyl acetal and 1 or 2 drops of concn HCL (4). The last traces of solvent were removed from the ester preparations by placement in a 50C vacuum oven for 20 min.

Procedure. An ester aliquot of either myristate, palmitate or stearate was added to a tared 15 ml centrifuge tube on an analytical balance. This was followed by an addition of the polymer ester preparation, and the wt per cent polymer in the sample was calculated. Methyl margarate was then added, and the wt per cent methyl margarate added was calculated. The tube was then filled with nitrogen, stoppered and alternately rotated under a hot water tap and held on a Vortex, Jr., Mixer (Scientific Industries, Inc.) for several min until the solution appeared homogeneous. This was validated with successive gas chromatographic analyses. Care was taken during weighing that none of the ester additions was deposited on the inside walls of the tube.

The purity of the ester standards was determined by gas chromatography and was found to be 99.0% for methyl myristate, 100.0% for methyl palmitate, 100.0% for methyl stearate, and 99.7% for methyl margarate. Corrected peak areas of analyzed samples were obtained by dividing an ester peak area by its respective per cent purity. Esters were individually chromatogrammed to determine the location of ester impurities in the chromatogram. In no samples analyzed did ester impurities contribute to the major peak areas measured, hence no area correction for this was necessary.

The polymer ester standard was found to contain 98.1% polymer, and the fatty acid ester standards, no polymer, by the procedure reported in this paper.

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TABLE I

Relative Detector Response Values

Methyl ester	Relative response
Myristate	1.160
Palmitate	1.091
Margarate	1.000
Stearate	1.037
Oleate	1.041
Linoleate	0.993
Arachidate	1.133
Linolenate	0.864

An additional adjustment of peak areas was necessary to compensate for nonlinearity in the detector response for the different fatty acid ester standards. Instrument detector responses relative to methyl margarate show in Table I. These values are not absolute and will vary from one detector to another. Adjusted peak areas were obtained by dividing the peak areas by their appropriate relative response values. Following this adjustment, the per cent methyl margarate in the chromatogram was calculated. From this figure, together with the value for the per cent methyl margarate actually added to the sample and the peak area for methyl margarate from the chromatogram, the per cent polymer in the sample was calculated from Equations 3 and 4 listed below.

Equations. The wt per cent methyl margarate added to the sample may be expressed by

$$\%MM_{SAMP} = MM/(MM + F + P) \times 100 \quad [1]$$

where: MM is the wt of methyl margarate added to the sample
F is the wt of non-polymerized fatty acid esters in the sample
P is the wt of polymer in the sample

Since the peak areas in a chromatogram (adjusted for detector response differences) are equivalent to respective component wt in the injected sample; and since

$$\%MM_{CHROM} = MM/(MM + F) \times 100 \quad [2]$$

Equations 1 and 2 may be combined to give

$$P = (\%MM_{CHROM} - \%MM_{SAMP})MM/\%MM_{SAMP}(\%MM_{CHROM}) \times 100 \quad [3]$$

where: MM is the peak area of methyl margarate from the chromatogram
P is the hypothetical polymer "peak area"

And the per cent polymer is determined by

$$\%P = P/(\Sigma \text{ total peak areas}) - MM + P \times 100 \quad [4]$$

Alternately, one may substitute the wt of methyl margarate added to the sample, for MM in Equation 3, and P will then be determined as the wt of polymer in the sample. The per cent sample polymer in this case, would be calculated as

$$\%P = P/\text{sample wt} \times 100 \quad [5]$$

which results in a value identical with that obtainable from Equation 4.

Results and Discussion

The use of myristic, palmitic or stearic acid methyl esters in the sample served to represent the non-polymerized fatty acid portion (F in the equations) of a hypothetical oil sample. These acids were chosen because they are stable to oxidative polymerization (and hence free of polymer) and are found in naturally occurring vegetable and marine oils. The sample resulting from the addition of polymer to a tube containing one of the above esters was intended to similate a partially bodied oil.

TABLE II

Determination of Sample Polymer Content

Ester (F)	Per cent polymer added	Per cent polymer found
Myristate	0.0	0.0
Myristate	10.4	11.0
Myristate	21.9	21.6
Myristate	33.6	34.6
Myristate	57.1	58.4
Myristate	66.7	67.1
Myristate	75.6	76.3
Myristate	82.0	82.7
Myristate	92.8	93.2
Palmitate	0.0	0.4
Palmitate	45.6	45.9
Palmitate	65.8	66.8
Stearate	0.0	0.0
Stearate	21.9	22.6
Stearate	44.9	44.8
Stearate	65.8	64.3
None	100.0	98.1

Samples containing varying amounts of polymer were prepared, and the data and results of analyses show in Table II. It was noted that agreement between per cent polymer actually added to the sample and the per cent polymer found by the internal standard procedure was excellent, and usually deviations were in the order of 1% or less. The mean of all deviations listed was 0.66%. The accuracy apparently is not affected by the amount of polymer present in the sample, nor by the interchange of esters representing F in the sample. In applying the procedure to actual oil samples, it would be necessary to know the relative detector response values for all fatty acids present in the oil in order that adjusted peak areas could be determined prior to the calculation of oil polymer. Appropriate correction would also be required for samples containing an eluted component which would contribute to the peak area of methyl margarate. Instrument operating conditions should be adjusted to give sufficient peak resolutions between the palmitate, margarate and stearate peaks.

The polymer content of several oil samples analyzed by the internal standard procedure shows in Table III. Calculations have shown standard ester mixtures free of polymer, and the calculated composition of such standard mixtures in excellent agreement with the actual ester wt percentages in the mixture. Polymer absence was also noted for pure soya oil. Dehydrated castor and the linseed-oiticica oil samples on the other hand, revealed high polymer contents. This was not too unexpected realizing the high level of polyunsaturated and conjugated acids initially present in such samples.

It is of interest to compare the values for the soya alkyd samples shown in Table III. The ester values obtained solely on a chromatogram basis indicate the characteristics of a soybean oil fatty acid distribution, but do not reveal the true level of linoleate in a sample as indicated in the same samples when analyzed by the internal standard method.

It is worthy of note that in oils containing palmitoleic acid (marine oils), the palmitoleate peak does not resolve completely from margarate and palmitate, and peak area overlap corrections are necessary. In addition, it should be pointed out that any non-volatile portion of an ester preparation from an oil sample would be included in the per cent polymer value. This should be of particular concern in the analysis of tall oil samples containing high amounts of rosin. This could be alleviated to a large extent

TABLE III

Analysis of Oils and Fatty Acid Esters

	Per cent in sample							Per cent on chromatogram basis		
	Known ester[1] mixture		Pure soya oil[2]	Dehydrated castor oil[2]	Linseed-oiticica[3,5]	Soya alkyd No. 1[3]	Soya alkyd No. 2[3]	Soybean oil[6]	Soya alkyd No. 1	Soya alkyd No. 2
	Actual, %	Found, %								
Palmitate	15.4	15.3	9.9	0.8	3.9	7.1	8.0	13.5	11.5	13.3
Stearate	15.3	15.4	3.8	0.9	4.0	2.7	3.4	3.9	4.4	5.6
Oleate	15.5	15.5	23.5	4.0	9.7	17.6	19.2	22.2	28.4	30.7
Linoleate	22.6	22.6	49.7	24.4	5.2	29.2	26.6	51.4	47.1	42.8
Arachidate	15.7	15.9	.....	.....	.....	.....	.....	.....	.....	.....
Linolenate	15.5	15.5	9.3	.....	6.0	5.3	4.6	8.9	8.6	7.0
Other	.....	.....	4.4	20.0[4]	.....	.....	.4	.....	.....	.6
Polymer	0.0	0.0	0.0	49.9	71.2	38.1	37.7	N/A	N/A	N/A
Total	100.0	100.2	100.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Hormel Foundation. "Found, %" obtained using methyl margarate as internal standard.
[2] Oils.
[3] Extracted from paint.
[4] Includes conjugated linoleates.
[5] No licanate peak on the chromatogram.
[6] Reference (5).

by esterification of fatty acid and rosin acids alike with diazomethane followed by gas chromatography of the resultant ester mixtures (2), provided appropriate rosin ester detector response factors could be ascertained. However, the internal standard procedure reported herein should have a direct application to quantitative estimations of polymer content in heat-bodied oils.

REFERENCES

1. Gehrke, C. W., D. F. Goerlitz, C. O. Richardson, and H. D. Johnson, J. Dairy Sci. *43*, 839 (1960).
2. Hudy, J. A., Anal. Chem. *31*, 1754–1756 (1959).
3. Iden, R. B., and E. J. Kahler, JAOCS *39*, 171–173 (1962).
4. Lorette, N. B., and J. H. Brown, Jr., J. Org. Chem. *24*, 261–262 (1959).
5. Zielinski, W. L., Jr., W. V. Moseley, Jr., R. C. Bricker, Offic. Dig. *33*, 622–634 (1961).

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Studies on the Changes in Fatty Acid Composition in Developing Seeds. I. *Recinus communis*

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Abstract

Oils from castor seeds at different stages of ripening have been studied. The fatty acid composition has been determined by paper chromatography. The ratio of the weight of the kernel to the weight of the seed coat changes from 1.0: 1.24 (14 days) to 1.0:0.48 (45 days) and the oil content of the seed coat is negligible. Amounts of the individual fatty acids in 1 g of kernel as well as in a single seed have been shown. The amounts of ricinoleic, linoleic and stearic acids gradually increase with the ripening of the seeds whereas the amounts of oleic and palmitic acid after an initial increase upto 28 days gradually decrease towards the later stages of growth when the amounts are calculated on the basis of a single seed.

Introduction

IN ORDER TO understand the mechanism of biosynthesis of fatty acids in plants, various investigations have been made of fats obtained from seeds at different stages of ripening, or grown at different climatic conditions. Recently Simmons and Quackenbush (1) published the results of their investigation with soybeans at different stages of ripening and Franzke (2) published similar investigations on sunflower seeds. In these reports the proportions of the individual unsaturated fatty acids and total saturated fatty acids, as well as their amounts in one g of the seed, were shown. But the proportions or amounts of the individual saturated fatty acids were not determined, probably because of the inadequacy of the method of analysis.

Burr and Miller (3) studied the formation of oil in castor beans and established its relation with the respiratory quotient. According to their observations, on the 7th day from the date of blossoming, about 3–6% oil in dry seeds was present. During the next 15 days, the respiratory quotient gradually increased with very little increase in the amount of the oil. It was only when the respiratory quotient was more than 1.0 that rapid oil formation took place. Appreciable increase in oil content was, however, noted after the respiratory quotient had again fallen below 1.0 or even 0.8. Fat metabolism in germinating castor beans also received the attention of Yamada (4).

There is no reference in the literature about the changes in fatty acid composition of ripening castor seeds. Hence, an investigation was undertaken as it was considered to be of interest because of the presence of hydroxy acids in the oil.

Experimental

Preparation of Samples

Flowers of castor plants grow in bunches and all the buds in a bunch do not blossom on the same day. Also, a proportion of flowers which bloom may not be fertilized. Hence a large number of flowers were tagged on the day of blossoming and the rest of the