thousand g of carbon powder were added to the oil before processing.

The butyro index for palladium processing gives a different correlation with I.N. than that obtained from nickel processing, due to the higher amount of *trans* isomers in the palladium product. Although much less selective conditions were used in the plant, i.e., higher pressure and lower temperature, these were offset by a much lower agitation, so that the product at 210 min was only slightly less selective than those obtained in pilot plant processing. Despite the slightly higher solid content of this product at 36C than is generally desirable for margarine stocks, it is not waxy, due probably to its *trans* isomers which have, of course, much lower melting points than the saturated glycerides present. The catalyst at the end of plant processing had lost about 15% of its initial activity, as determined by re-use in a laboratory hydrogenation of soybean oil.

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Estimating the Average Carbon Chain Length of Saturated Fatty Acid Esters by Infrared Spectroscopy[1,2]

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Abstract

The average carbon chain length of saturated fatty acid esters can be determined by comparing absorption intensities in the 3.3 and 5.75 μ infrared regions. Data are presented for triglycerides, monoglycerides, and methyl esters. The method was used to follow the fractionation of hydrogenated milk fat from acetone, and the average values for fatty acid chain length were in good agreement with those obtained by gas chromatographic methyl ester analysis. The I.R. method is particularly applicable when only a few mg of sample is available and the material being fractionated contains both long and short chain saturated fatty acids.

Introduction

INFRARED ANALYSIS is extensively employed in lipid research, especially now that small, relatively inexpensive instruments are available and are becoming commonplace in research laboratories. A great amount of information is obtained quickly, only a few mg of material is needed, and the analysis is nondestructive.

An interpretation of I.R. data that has apparently been overlooked concerns the measurement of the average carbon chain length of certain saturated fatty acid esters by comparing the intensity of the $C-H$ stretching vibrations of CH_2 and CH_3 groupings in the region of 3.3 μ with the $C=O$ stretching of the COOR ester group in the region of 5.75 μ. Surprisingly, a literature search did not uncover any mention of this use of I.R. data. The intent of this report is to describe a method whereby an estimate can be made of the average carbon chain length of saturated triglycerides, methyl esters, and monoglycerides. To demonstrate the utility of the method data are presented for hydrogenated milk fat fractionated by low temperature crystallization from acetone.

Experimental Procedure

Infrared Analysis. A Perkin-Elmer Infracord Model 137 equipped with a 0.025 mm NaCl prism was used. A reference line was established on the chart by running a tracing in the 3.3 and 5.75 μ region with pure solvent in both the reference and sample cells. Then a solution of the test material was placed in the sample cell and the absorption traced at 3.0–3.5 μ and 5.5–6.0 μ. As many as 6 sample spectra can be traced on a single sheet of paper by repositioning the paper, clockwise, about the cylindrical drum after each test run. Only 3 or 4 min are needed for flushing and filling the cell, and for running the spectrum.

For determining the average carbon chain length of a fatty ester the absorbance units in the 3.3 μ region are divided by the absorbance units in the 5.75 μ region to establish the CH_2/COOR ratio. The value for this ratio is referred to a previously prepared plot of similar values for an homologous series of the esters to obtain the average chain length of the unknown sample.

Source and Purification of Esters. Triglycerides, 1-monoglycerides, and methyl esters were obtained from Distillation Products Industries, Rochester 3, New York. Purity of triglycerides and methyl esters were determined by I.R. spectroscopy and gas chromatographic methyl ester analysis. Contaminated samples were purified either by distillation or by crystallization from solvents. Gas chromatography revealed the monoglyceride samples to be at least 90% pure in regard to fatty acid content, but no attempt

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was made to determine if diglycerides and triglycerides were present, nor was positional isomerism measured. Monoglycerides were used without further purification.

Butteroil Fractionation. Ten g of hydrogenated butteroil (I.V. 5), dissolved in 100 ml of acetone, was held 12 hr at 26C. The precipitate was recovered by filtration and washed with 10 ml of 26C acetone. Then the filtrate and washings were combined and held at 9C for 12 hr, after which the filtering and washing steps were repeated. This procedure was followed for the −35 and −50C crystallizations except that the solutions were held only 1 hr prior to recovering the precipitates. Gas chromatography (2) was used to measure the concentration of each fatty acid except butyric, which was determined by a silicic acid method (1). The average carbon chain length of each triglyceride fraction was calculated using these data.

Results and Discussion

Figure 1 shows that the ratios obtained for the members of an homologous series of saturated triglycerides, as well as for a similar series of methyl esters, plot essentially as straight line functions of the carbon chain length. The I.R. data were obtained from 5% solutions of the ester material dissolved in CCl_4. Concentration greater than 5 or 6% should be avoided because high molecular weight compounds, such as tripalmitin and tristearin, may crystallize from solution in the I.R. sample cell, and if this occurs the $CH_2/COOR$ ratio will be affected. Furthermore, representative ratios cannot be obtained for samples rich in low mol wt triglycerides if the concentration exceeds the recommended level, since excessive absorption will be recorded in the 5.75 μ region.

Data on 9% chloroform solutions of an incomplete series of monoglycerides are also included. Although the ratios increase as the chain length increases, the plots do not approach as consistent a straight line as was the case for the triglycerides and the methyl esters. It is believed that the inconsistent results were caused by impurities in the samples.

Table I and Table II are included to demonstrate the practical applications of the I.R. method. The data for Table I were obtained by preparing various combinations of triglycerides. The average carbon chain length for each mixture was estimated by applying the I.R. ratio to the graph prepared using the homologous series of triglycerides. Table II shows a similar comparison made on a sample of hydrogenated milk fat that had been fractionated by low temperature crystallization from acetone. As can be seen, the fatty acid chain length values obtained by I.R. analysis agree quite well with those calculated from known fatty acid content as in Table I, and by chromatography as in Table II.

TABLE I

A Comparison of the Carbon Chain Length Values Obtained by the Infrared Method with Calculated Values of Known Mixtures of Triglycerides

Triglyceride mixture	Average fatty acid carbon chain length	
	Calculated	Infrared
Tri-C_{14}, Tri-C_6	8.78	9.15
Tri-C_{18}, Tri-C_6	9.83	10.10
Tri-C_{16}, Tri-C_8	10.95	10.62
Tri-C_{14}, Tri-C_{12}	12.90	12.70
Tri-C_{16}, Tri-C_{14}	14.92	15.00
Tri-C_{18}, Tri-C_{16}	16.95	16.50
Tri-C_{18}, Tri-C_{12}, Tri-C_4	10.15	10.42
Tri-C_{16}, Tri-C_{10}, Tri-C_{12}	11.65	11.50

TABLE II

A Comparison of the Carbon Chain Length Values of Fractionated Hydrogenated Milk Fat as Measured by the Infrared Method with Values Calculated from Chromatographic Data

Milk fat fraction	Recovered from 10 g fat	Average fatty acid carbon chain length	
		Calculated	Infrared
26C Precipitate	3.8	16.6	16.8
9C Precipitate	2.9	14.0	13.7
−35C Precipitate	2.9	12.9	13.4
−50C Precipitate	0.2	11.5	12.0
−50C Filtrate residue	0.2	11.2	11.6

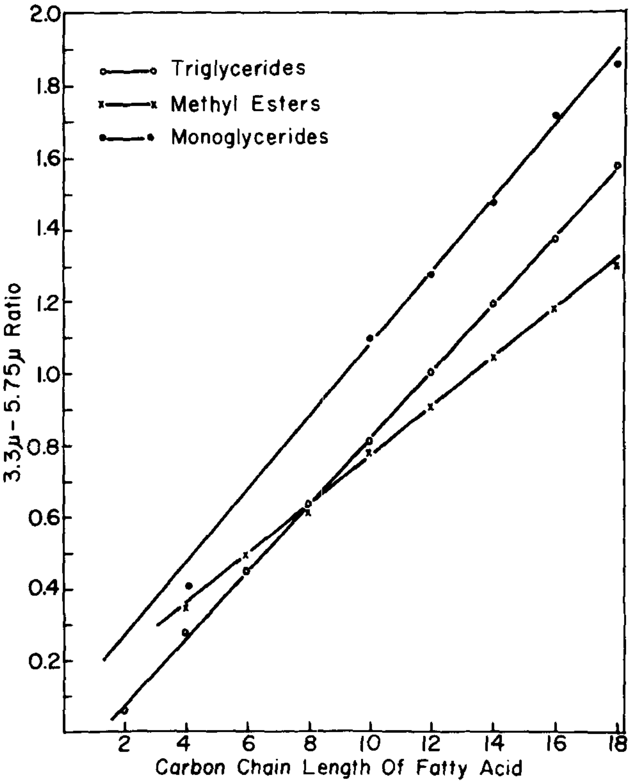

Fig. 1. The influence of the carbon chain length of fatty acid esters on the intensity of absorption at 3.3 μ compared to the absorption at 5.75 μ.

The values obtained for a sample are, of course, influenced by the accuracy and reproducibility of the I.R. instrument. A standard deviation of ± 0.04 ratio units was calculated, based upon data collected on the simple triglycerides used in this study. It can be predicted that the average carbon chain length can be measured within ± 0.4 carbons with a reasonable degree of assurance. Admittedly, the I.R. method may not have the precision of saponification equivalent determinations when sufficient material is available for the latter type of analysis. However, often only a mg of lipid is available in a fraction, which precludes the use of saponification equivalents. Fortunately, this very small sample size requirement presents no problem when the I.R. method is used. Furthermore, only a few minutes are needed for an analysis and the lipid can then be recovered and used for other purposes if needed.

As expected, unsaturated acids have a marked effect on the I.R. absorption intensity in the 3.3 μ region and the method is limited to saturated systems unless something is known about the unsaturated fatty acid content so that appropriate corrections can be made.

The I.R. method can be applied best to lipid materials that have appreciable quantities of both long and short chain acids such as milk fat, coconut fat, and acetoglycerides.

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