Determination of Monoglycerides in Crude Oils and Fats

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ABSTRACT

The periodate oxidation method was adapted for the determination of small amounts of monoglycerides present in natural oils and fats. The modification consisted in enriching the monoglycerides by extraction with 90% acetic acid saturated with boric acid in order to eliminate interfering substances prior to the reaction with periodate. A multifold concentration of monoglycerides originally present was achieved. This reduced the error of the standard periodate method to a minimum. Crude oils, such as soybean, rice bran, coconut, and palm, analyzed by the modified method showed monoglyceride contents considerably lower than those obtained by direct reaction of periodate with these oils.

INTRODUCTION

The time-honored Malaprade method employing the reaction between periodates and adjacent hydroxy groups (1) was applied to α-monoglycerides in 1945 (2,3) and forms the basis of AOCS Official Method Cd 11-57 (4) for the determination of these compounds in oils and fats. The AOCS method indicates that the procedure is applicable to monoglyceride content as low as 3% and less, and the well-known monograph of Mehlenbacher (5) shows results of monoglyceride determinations by periodate in crude soybean, cotton, and peanut oils, all below 0.5%. However, as far back as 1950, Kummerow and Daubert (6) demonstrated that the periodate method, when applied to monoglycerides present in small quantities, has limitations, and some of their results for refined edible oils were higher than those quoted by Mehlenbacher for crude oils. An investigation carried out by Bharucha and Gunstone (7) revealed that these limitations are even more pronounced than could be expected. Thus, pure oleic acid and methyl linoleate showed apparent dihydroxy-acid contents of 3.8-3.9%. The monoglyceride content of crude oils and fats is usually low. It appears, therefore, that the periodate method, while suitable for the analysis of commercial monoglyceride preparations, cannot be applied in its usual form to the determination of monoglycerides in natural oils and fats.

In the last two decades, several chromatographic and spectrophotometric methods have been developed for the estimation of monoglycerides. One of the most recent is that of Halvarson and Qvist (8), intended to determine monoglycerides in crude oils and to follow their changes during the refining of edible oils. The method, denoted by its authors as simple, consists in an enrichment of monoglycerides by extraction with acetonitrile, silylation of the residue obtained after the evaporation of the solvent, and gas chromatographic (GC) analysis of the silylation products using silylated cholesterol as internal standard. In the case of coconut and palmkernel oils, the extraction with acetonitrile is followed by thin layer chromatography to separate the monoglycerides from short chain diglycerides which could interfere with the GC analysis, whereupon the monoglyceride zone is cut out, subjected to silylation, and gas chromatographed.

There is no doubt that the idea of enrichment of monoglycerides present in small quantities, before proceeding with their determination, is sound. However, the method as proposed by Halvarson and Qvist can hardly be described as simple, particularly in the case of oils such as coconut and palmkernel, and the use of the toxic acetonitrile solvent is open to objection. The method applied in our laboratory to a number of oils and fats gave satisfactory results, especially when pyridine used as solvent during silylation was removed prior to GC (9). However, it seems to be more suitable for research work than for practical application in an edible oil refinery, where the composition of monoglycerides obtained by GC of silylated compounds is of little interest. Accordingly, a less involved procedure will be described in the present communication, whereby the monoglycerides after enrichment are determined by periodate oxidation. It can be carried out in any industrial laboratory using standard reagents and equipment.

Development of Analytical Method

The proposed method is based on the enrichment of monoglycerides present in crude oils by repeated extraction of the oil dissolved in hexane with 90% acetic acid saturated with boric acid. Any free glycerol which would interfere with the monoglyceride determination is removed beforehand by washing with 5% acetic acid.

Ninety percent acetic acid dissolves monoglycerides, leaving nonpolar glycerides in the hexane phase. The use of boric acid reduces the solubility of free fatty acids and diglycerides in aqueous acetic acid but, if anything, facilitates the dissolving of α-monoglycerides. It is thus possible to obtain monoglycerides sufficiently concentrated to subject them to periodate oxidation. The proposed solvent is at least as efficient as acetonitrile employed by Halvarson and Qvist (8), is nontoxic, and does not need to be removed before the addition of the periodic acid reagent.

EXPERIMENTAL PROCEDURE

Materials

Samples of crude soybean, palm, rice bran, Mbocayá palm, and coconut oils with various free fatty acid contents and refined peanut oil were used.

Monolaurin, monopalmitin, monoolein, and monolinolein were prepared by esterification of the corresponding fatty acids with isopropylidene glycerol and scission with boric acid (10). The purity of the saturated monoglycerides was ca. 99% and of the unsaturated ones > 95%.

All reagents employed were of the highest purity available.

Procedure

Reagents: Glacial acetic acid 5%, H_2O 95% v/v; and glacial acetic acid 90%, H_2O 10% v/v, containing 1.2% of boric acid w/v.

Periodic acid reagent: 2.7g of periodic acid is dissolved in 50 ml of water, followed by the addition of 950 ml of glacial acetic acid.

Potassium iodide: aqueous solution 15% w/v.

0.1 N sodium thiosulphate solution accurately standardized.

Starch indicator.

Five to ten grams of fat is transferred with 30 ml of hexane to a separating funnel and extracted 3 times with 10 ml portions of 5% acetic acid to remove any free glycerol present. In the aqueous extract, free glycerol may be estimated by periodate oxidation, if so desired. Otherwise, this extract is discarded.

Monoglycerides are extracted by shaking the hexane solution of the fat 3 times with 20 ml of 90% acetic acid

TABLE I

Monoglyceride Contents of Various Oils and Fats Determined by the Modified Periodate, Gas Chromatographic, and Official AOCS Methods

Oil	FFA (%)	Monoglyceride (%)		
		Modified periodate procedure	Gas chromatographic method	Official AOCS method Cd 11-57 (4)
Crude soybean	0.45	0.08	0.06	1.48
Crude palm	8.46	0.82	0.77	1.70
Crude Mbocayá palm	18.12	1.09		2.32
Crude coconut	5.15	1.18		2.69
Crude rice bran No. 1	1.73	0.48	0.39	1.95
Crude rice bran No. 2	3.65	0.66		2.74
Crude rice bran No. 3	8.63	1.22	1.30	2.15
Refined peanut	0.03	0.04		0.89

saturated with boric acid (60 ml total). To the combined extracts collected in a 250 ml Erlenmeyer flask is added 25 ml of period acid reagent by pipet. After gentle mixing, the flask is left covered for 30 min in the dark. Ten ml of 15% potassium iodide solution is added, followed by 50 ml of water and the liberated iodine titrated with 0.1 N sodium thiosulphate solution. A blank using 60 ml of 90% acetic acid is run simultaneously.

$$\% \text{ Monoglyceride} = (B - S) \cdot N \cdot 17.927/W$$

where B and S denote milliliters of sodium thiosulphate solution used for the titration of the blank and sample, N the normality of sodium thiosulphate, 17.927 the mol wt of monostearin divided by 20, and W the weight in grams of the sample. In the case of coconut oil, the factor for monolaurin (13.719) may be used instead of the factor for monostearin. If the expected monoglyceride content is small, the sample weight may be increased to 20 g, the procedure remaining otherwise unchanged.

RESULTS AND DISCUSSION

To test the extraction efficiency of 90% acetic acid saturated with boric acid in respect to monoglycerides, 50 mg samples of the four available monoglycerides were dissolved in 5 g lots of refined peanut oil, if necessary with gentle heating, and the mixture was analyzed as previously described. The results showed recoveries of 95% for monolaurin, 92% for monopalmitin, and 88% for monoolein and monolinolein. As could be expected, the recovery of monoglycerides diminished with their increasing mol wt. A corresponding correction of the results obtained for crude oils may be applied, but considering the small quantity of monoglycerides present in these oils, the error resulting from incomplete extraction is usually of little significance.

The monoglyceride contents of various crude oils and fats are shown in Table I. They vary with the type of the oils and their free acidity, some of them being considerably higher than those found by Halvarson and Qvist, who reported values below 0.05% for most crude fats and up to 0.5% for coconut, palmkernel, and palm oils. This may be due to the fact that they examined oils with low acidity. Rice bran oil which was not included in their investigation usually contains appreciable amounts of monoglycerides due to the pronounced lipolytic activity of the rice bran, but a monoglyceride content of 1.4% has been reported also for crude Congo palm oil (11).

The results for monoglycerides shown in Table I were confirmed in the case of soybean, palm, and rice bran oils by GC following the procedure of Halvarson and Qvist modified according to an earlier publication (9). It was also verified that the extraction of palm oil with 90% acetic acid saturated with boric acid produced a more than 100-fold concentration of monoglycerides, the solute containing ca. 85% of monoester. Thus, the error due to the reaction of periodic acid with components other than monoglycerides can be disregarded.

On the other hand, the application of the periodate method to the original oils without previous enrichment of monoglycerides produced inordinately high results, as may be seen in Table I. Mineral acids also affect the results. Periodic acid gives higher apparent monoglyceride contents than sodium periodate, and the addition of small amounts of perchloric acid, sufficiently diluted to prevent its isomerizing effect, increases the results even more. Some crude oils react even with iodic acid, producing apparent monoglyceride values. Thus, the interaction between periodic acid, reduced by the addition of glycerol to iodate, and soybean oil and rice bran oil No. 2 gave apparent monoglyceride contents of 0.72% and 1.68%, respectively.

The GC analysis of monoglycerides can be performed using a fraction of a milligram of the substance. The amount required by the periodate method is obviously much larger; however, this does not affect its practical application. Taking a difference of 0.5 ml of 0.1 N sodium thiosulphate solution between the titration of blank and sample as a limit of accuracy, one could determine 9 mg of monoglyceride or 0.09% in a fat sample of 10 g. On doubling the sample size, monoglyceride contets as low as 0.05% could be determined with some degree of accuracy. Quantities of this order are not likely to exert any significant effect on the behavior of oils during the refining process.

Periodic acid reacts only with α-monoglycerides. β-Monoglycerides do exist in crude oils, but their quantity is so small that no appreciable error results from disregarding their presence.

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