Determination of Total and Water-Insoluble-Combined Lactic Acid in Lactic Acid Modified Fatty Glycerides

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Abstract

Analytical methods for the determination of total and water insoluble combined lactic acid in lactic acid modified fatty glycerides are described. The acids are liberated from their esters by saponification. Lactic acid is determined by simple acid-base titration after removal of fatty acids via liquid-liquid extraction. Water soluble constituents are extracted from a dichloromethane solution of the esters with 5% aqueous sodium sulfate prior to determination of water insoluble lactic acid. Methods are rapid, simple, and suitable for use in a plant control laboratory. Extension of the procedure provides for the simultaneous determination of fatty acid and glycerine on a single sample.

Introduction

LACTIC ACID modified monoglyceride mixtures have recently gained commercial importance as emulsifiers in shortening used in cake-mix. These mixtures represent mixed glycerol esters containing varying amounts of lactic and fatty acids. The effectiveness of these emulsifiers depends upon their chemical composition. It is alleged that water soluble lactic acid present as mono-, di-, and trilactin and/or free lactic acid does not contribute to the emulsification properties of these products. Therefore it is important to have rapid, simple, and reliable analytical methods to measure both the total lactic acid and the water insoluble combined lactic acid, (WICLA).

The methods most commonly used for the determination of lactic acid involve conversion of the acid to acetaldehyde and subsequent colorimetric measurement of the aldehyde. An excellent review of the pertinent literature on these methods is given by Fett (1). The colorimetric method of Barker and Summerson (2) employing p-phenyl phenol was modified and adapted to the determination of lactic acid in shortenings by Fett (1). Friedemann and Graeser (3) measured the lactic acid content of biological samples by oxidizing the acid to acetaldehyde with manganese dioxide, distilling the aldehyde into bisulfite, and titrating the bisulfite complex iodometrically. Partition chromatographic separation of organic acids, lactic acid included, is reported by Marvel and Rands (4).

The methods mentioned above are all tedious and time consuming. Special precautions must be taken to avoid: (1) loss of acetaldehyde (bp 20.2C) by evaporation, and (2) contamination from lactic acid on the skin. The colorimetric methods are designed specifically for measuring lactic acid in the μg range and are therefore not easily adapted to the analysis of lactated glycerides containing 10–20% lactic acid. The methods presented in this paper involving saponification of the esters, separation of the fatty and lactic acids by liquid-liquid extraction, and titration of the lactic acid with standard alkali are simple, rapid, and suitable for use in a plant control laboratory.

Experimental

To determine the total lactic acid content of lactated monoglyceride mixtures, the esters are saponified by refluxing with an accurately measured volume of a standard alcoholic potassium hydroxide solution to yield glycerol and the potassium salts of lactic and fatty acids. The soap solution is converted to an aqueous solution by the addition of water and evaporation of the alcohol. The volume of mineral acid required to just neutralize the potassium hydroxide is determined by titrating reagent blanks with hydrochloric acid to the methyl red end-point. Addition of the same volume of acid required to neutralize the reagent blank to the saponified sample solution liberates lactic and fatty acids from their respective salts and converts the potassium to potassium chloride. Extraction with hexane removes the liberated fatty acids, leaving the lactic acid in the aqueous salt solution. Quantitative determination of lactic acid is accomplished by simply titrating the aqueous solution after hexane extraction with a standard alkali to the phenolphthalein end-point. The effect of any acidic or basic impurities in the solvents and reagents is cancelled out by a similar titration of the hexane extracted reagent blank.

Water soluble glycerides are removed by extracting a dichloromethane solution of the sample with a 5% aqueous sodium sulfate solution. After removal of the dichloromethane by evaporation, the WICLA content of the sample is determined by the same procedure as used for the determination of total lactic acid.

Analysis Procedures

Apparatus. Erlenmeyer flasks—250 ml—alkali-resistant glass. Separatory funnels—250 ml—Teflon stopcock.

Reagents. Alcoholic KOH—approx 0.5N—dissolve 96 g of KOH in 1000 ml of 95% ethanol. Allow solution to stand overnight. Filter to remove precipitated carbonates. Dilute to 3000 ml with 95% ethanol. Solution should remain clear.

Sodium sulfate solution—5% w/v—dissolve 50 g of Na_2SO_4 in 1000 ml of distilled water.

All other reagents and apparatus are common laboratory items.

(a) Determination of Total Lactic Acid

Weigh accurately a sample containing 140–170 mg of lactic acid into a 250 ml alkali-resistant Erlenmeyer flask. Pipette 20 ml of 0.5N alcoholic KOH solution into the flask and reflux for 30 min to saponify the sample. Add 20 ml. of distilled water and concentrate the soap solution to approx 20 ml by evaporation on a steam bath or hot plate. Run re-

TABLE I

Accuracy of Method

Sample	Lactic acid present, mg	Lactic acid found, mg	Recovery, %
Triglyceride	None	None	.....
USP lactic acid			
(1)	158.3	155.2	98.0
(2)	131.4	128.9	98.1
(3)	175.0	172.1	98.3
Mixture No. 1—Triglyceride + lactic acid	162.4	160.5	98.8
Mixture No. 2—triglyceride + lactic acid	132.8	131.2	98.8
Mixture No. 3—triglyceride + lactic acid	173.3	172.1	99.3

agent blanks, omitting only the sample, in the manner described above. Cool sample and blanks to approximately 40C. To the blanks only, add 10 drops of methyl red indicator solution, and titrate to the indicator end-point with 0.5N HCl. Record volume of HCl required. To the saponified sample, add the same volume of 0.5N HCl as required to neutralize the blank solution. Swirl flask during addition of acid to disperse the liberated fatty acids. Add 50 ml of hexane to both sample and reagent blanks. Swirl sample flask vigorously to dissolve fatty acids. Transfer solutions to 250 ml separatory funnels and agitate for approx 30 sec. Withdraw the aqueous phase into a 300 ml Erlenmeyer flask. Wash the hexane solution with 50 ml of distilled water and combine aqueous wash solution with the original aqueous phase in the Erlenmeyer flask. Titrate sample and reagent blank solutions to phenolphthalein end-point with standard 0.1N KOH. Use the difference between the blank, Vb, and the sample, Vs, to calculate the lactic acid content of the sample.

(b) Determination of Water Insoluble Combined Lactic Acid (WICLA)

Weigh accurately a sample containing 140–170 mg of WICLA into a 100 ml beaker. Dissolve sample, heating if necessary, in 25 ml of dichloromethane. Transfer sample solution to a 250 ml separatory funnel using 25 ml of dichloromethane to rinse the sample beaker in order to obtain quantitative transfer. Add 50 ml of 5%, w/v, aqueous sodium sulfate solution to the separatory funnel. Agitate the separatory funnel for approx 30 sec and allow the two phases to separate. Withdraw the lower dichloromethane phase into a second 250 ml separatory funnel and transfer the aqueous phase to a 250 ml beaker. Repeat the extraction of the dichloromethane solution with two additional 25 ml portions of 5% aqueous sodium sulfate solution in the same manner described above. Combine the aqueous extracts and wash by shaking in a separatory funnel with 50 ml of dichloromethane. Combine the dichloromethane solutions and transfer to a 250 ml alkali-resistant Erlenmeyer flask. Evaporate the dichloromethane solution on a steam bath under a stream of nitrogen until no odor of solvent remains. Add 50 ml. of 95% ethanol to the flask and evaporate under a stream of nitrogen until no odor of solvent remains. Repeat ethanol treatment a second time. (Ethanol is used to "chase" the last traces of dichloromethane since the latter will consume alkali and consequently be measured as lactic acid.) Run reagent blanks as described above, omitting only the sample.

Determine the lactic acid content of the dichloromethane residue using the procedure described under (a) above, starting with: Pipette 20 ml. of 0.5N alcoholic KOH . . . . Report results as water insoluble combined lactic acid (WICLA).

TABLE II

Precision of Total Lactic Acid and WICLA Determinations
Samples—Commercial Lactated Monoglyceride

Total lactic acid found g lactic acid/100 g sample	WICLA found g lactic acid/100 g sample
15.7	13.1
15.7	13.0
15.9	13.5
15.8	13.2
16.0	13.3
16.0	13.4
15.8	13.5
15.7	13.4
15.8	13.4
15.8	13.6
Av value 15.8	13.3
Stand dev ±0.12	±0.2

Results and Discussion

To prove the accuracy of the method for the determination of total lactic acid, it was necessary to prepare synthetic mixtures since a standard sample of lactated monoglyceride was not available. Known mixtures of a fatty triglyceride and USP lactic acid were prepared and the lactic acid content determined by the proposed procedure. Each component, i.e., the triglyceride and lactic acid, was also subjected to the analysis procedure. The results are shown in Table I.

The precision of the method was evaluated by running 10 determinations on a commercial lactated monoglyceride sample. The results are shown in Table II. Several months' experience with the method in analyzing commercial lactated monoglycerides has shown that duplicate analyses usually agree within ± 1.5% (relative).

Several solvent systems, including the commonly employed chloroform : water system, were investigated as possible systems for the extraction of water soluble lactic acid and lactic acid esters prior to the determination of WICLA. The system dichloromethane : 5% aqueous sodium sulfate was found to be the most satisfactory. Practically no emulsion difficulties are encountered using this system.

A large sample of a commercial lactated monoglyceride was carefully washed with warm water to remove all water soluble components. Saponification analysis of the water insoluble esters indicated that the sample contained 13.6 g/100 g WICLA. Ten determinations of WICLA were run on this sample using the proposed procedure. The results are shown in Table II. Experience has shown that duplicate determinations of WICLA usually agree within ± 2% (relative).

Other water soluble acidic or basic compounds, free or combined, will interfere with the method. Such compounds are normally not present in commercial lactated monoglyceride mixtures.

The proposed procedure can be extended to include the quantitative determination of lactic acid, fatty acid, and glycerine contents of a lactated monoglyceride mixture using only a single sample. Fatty acids are recovered by evaporation of the hexane extract of the acidified soap solution. Quantitation of the fatty acid moiety can be achieved either by weighing the hexane residue or redissolving the residue in alcohol and titrating the acids with standard alkali solution. The acid number of the fatty acids is a prerequisite if the latter technique is used. The glycerine content, if desired, can be obtained by periodic acid oxidation of the aqueous phase after titra-

tion of the lactic acid. Sample size is adjusted by diluting the titrated aqueous phase to known volume with distilled water and removing an aliquot portion for periodate oxidation. Lactic acid does not interfere with the periodic acid reaction if the reaction is carried out at room temp. Fatty acid and glycerine contents determined in this manner agree well with the values obtained using the standard AOCS Methods G-4-40 and Ca-14-56, respectively.

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Separation of Fatty Ester-Mercuric Acetate Adducts of Alumina[1,2]

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Abstract

A chromatographic procedure is described for the elution separation on alumina for 100-mg samples of methyl palmitate and fatty ester-mercuric acetate adducts. Oleate and linoleate were obtainable in pure form; however, the linolenate adduct did not separate completely from the linoleate adduct. For quantitative results the use of alumina of proper activity and basicity is essential. Ether with small amounts of acetic acid is a suitable eluant. Since the original esters can be regenerated easily the ability to isolate individually the oleate and the linoleate adducts in high yield can be useful in radiobiochemical work.

Introduction

ATTEMPTED separations of the fatty acids from small samples (ca. 100 mg) have employed three general methods: (a) direct separation of the acids or their esters by physical means (1,2,3,4); (b) controlled oxidation of unsaturates, with subsequent fractionation (5,6,7,8); and (c) the formation of derivatives or adducts to magnify differences in properties before fractionation. Disadvantages of direct separation and oxidation procedures are that the former generally are incomplete and the latter do not permit recovery of the intact unsaturated acids. Some derivatives may promote separation but fail in the requirement for fatty acid regeneration (9). Mercuric acetate adducts are potentially suitable in both respects. They have been used to separate saturated from unsaturated fatty acids on Florisil (10) and on silica gel (11) columns. Recently, partial separations of the adducts of individual unsaturated components have been reported on silica gel (12).

The present report concerns the adaptability of alumina as an adsorbent in separation procedures which employ mercuric acetate adducts.

Materials and Methods

Samples of methyl palmitate and oleate of high purity were obtained from the Hormel Institute. Linoleate of 98% minimum purity with oleate as the contaminant was isolated by urea adduct fractionation from the mixed methyl esters of safflower oil (13). Pure linolenate was extracted as its mercuric acetate adduct from linseed oil methyl esters (14).

Basic and neutral aluminas of different degrees of activity (adsorbing capacity) were prepared by supplying known amounts of deionized water to the anhydrous material. The degree of deactivation was proportional to the amount of water added. Activities I, II, III, and IV refer to the different grades of alumina established for standardization purposes by Brockmann and Schodder using a number of azo dyes (15). These grades were obtained through the addition of 0%, 3%, 6%, and 10% water, respectively, on a w/w basis to the dry metal oxide. No attempt was made to remove moisture that may have been present on the alumina before the deactivation treatment.

A sample of methyl ester weighing about 100 mg and of known composition was dissolved in 1 ml of methanol and heated at 80C for 30 min in the presence of mercuric acetate, 20% in excess of the theoretical amount needed to react with the double bonds. The reaction mixture was cooled to room temp and transferred in 10 ml of ethyl ether to a previously prepared column of 15 g of alumina, 11.5 cm high by 1.3 cm wide, covered with ether. A water jacket at 15C surrounded the column. Eluting solvents with increasing proportions of acetic acid in ether and corresponding increasing eluting power were passed through the column in stepwise manner. The flow rate was between four and five ml/min.

Solvent was removed from the eluates with a rotary evaporator until a residual vol of approx 10–20 ml remained. Following decomposition of the adducts by a 10-min treatment at room temp with 100 ml of methanol-ether (1:1, v/v) and 10 ml of concentrated hydrochloric acid, the homogeneous solution was transferred to a separatory funnel with 100 ml of hexane. After the epiphase was extracted with water and dried on a cellulose column, the solvent was removed and the residue transferred with methanol to a 50-ml volumetric flask and made to vol.

A simplified spectrophotometric determination for ester groups (16) was used on an aliquot of the methanolic solution. A further aliquot was analyzed for ester composition by gas liquid partition chromatography under conditions described previously (17).

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