• TECHNICAL

Separation and Evaluation of Components of Lactylated Emulsifiers

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Abstract

The composition of glycerol lactopalmitates was determined by analysis of fractions obtained from chromatographic separation on silicic acid. These fractions, when incorporated into shortenings and test baked in white cakes, showed that the most active components are those containing 2 free hydroxyl groups. Further breakdown of individual fractions indicates that, when these hydroxyl groups are located on the lactic acid portion of the molecule rather than on the glycerol portion, they are more effective in emulsification.

Introduction

RECENTLY, lactylated emulsifiers have been used extensively in shortenings. They have supplemented and, to an extent, replaced monoglycerides which had been the chief emulsifiers in shortenings. The composition of these lactylated emulsifiers, consisting chiefly of glycerol lactopalmitates (GLP) and glycerol lactostearates (GLS), may vary considerably depending on the manufacturing process and on the ratios of fatty acid:glycerol:lactic acid employed. Because lactic acid has a reactive hydroxyl group, the composition of the emulsifier can be very complex. It would be of value to know the composition of these lactylated products and to know which of the components are the most effective emulsifiers.

Column chromatography has been used for the separation of mono, di, and triglycerides (2). Quinlin and Weiser (3), showed that, on a silica gel column, using adsorption chromatography, the number of hydroxyl groups in the glyceride determined the tenacity of adsorption. This procedure was followed essentially in the separation of the components of GLP. The hydroxyl of the lactic acid behaves similarly to the glycerol hydroxyl as far as adsorption is concerned.

Separation by this method does not give fractions containing a single type molecule. To further characterize each fraction, saponification values, lactic acid content, and monoglyceride content (where applicable) were determined.

Experimental

Four GLP samples were analyzed for (a) water-soluble content, (b) saponification value, (c) lactic acid content, (d) alpha monoglyceride content, (e) total fatty acid content, (f) average molecular weight of fatty acids, and (g) total glycerine content.

Analyses (b), (c), and (d) were conducted on both the original GLP and on the product after water-washing, whereas analyses (e), (f), and (g) were conducted only on the washed product.

The water soluble content was determined by extracting a chloroform solution of the product 3 times with distilled water and determining the weight of the combined extracts after evaporation of the water. Saponification values, monoglycerides, total fatty acids, and glycerine were determined by standard AOCS procedures. Lactic acid was determined, after conversion to acetaldehyde, by its color reaction with p-phenyl phenol (1).

The 4 samples were each washed 3 times with hot water to remove water soluble components. After drying over anhydrous sodium sulfate, the emulsifiers were separated chromatographically into 4 fractions.

One gram of the emulsifier was accurately weighed into a 50 ml beaker and dissolved in 15 ml of A.R. grade chloroform. The solution was placed on a column prepared as follows:

Column Preparation. Twenty-nine grams of silicic acid (Mallinckrodt's 2847) were thoroughly mixed with one ml of distilled water and slurried in low boiling petroleum ether. The slurry was transferred to a chromatographic column 400 mm long by 30 mm I.D. The silicic acid was allowed to settle, and a filter paper disc having the circumference of the inside of the column was placed on top of the silicic acid, after which the contents were forced down with a plunger. The petroleum ether was forced through the column with air pressure until within 2 mm of the top of the column.

Fractionation. The 15 ml of sample solution was poured onto the top of the column and allowed to pass into the column without application of pressure. The sample beaker was rinsed with 5 ml of chloroform which was added to the column with a pipet, washing down the sides of the column during addition. The washings were allowed to pass into the column. After 5 min, a small amount of pressure was applied to the top of the column.

When nearly all of the solvent had passed into the column, development was begun with 150 ml A.R. grade benzene. A tared 250 ml Soxhlet flask was used as receiver. During development, air pressure, applied to the top of the column, was adjusted to give an effluent flow of 1–2 drops per second. Three more 150 ml portions of eluting solvents were added to the column in the following order: 10% U.S.P. grade ethyl ether in benzene; ethyl ether; and 1:1 methanol:ethyl ether. Receivers were changed each time a new solvent was introduced.

The solvent was evaporated from each of the fractions, which were then dried and weighed to obtain weight percentage of each of the fractions. Figure 1 illustrates the separation of a commercial GLP in which 25 fractions of 25 ml each were removed from the column. Using 150 ml of each eluting solvent assured complete separation of fractions.

Each of the first 3 fractions was analyzed for saponification value and lactic acid content. Monoglycerides were determined only on the third fraction, after it had been established that there were none present in the first 2 fractions. Since there was only sufficient material in a given fraction to perform one of the above analyses, 3 separations were

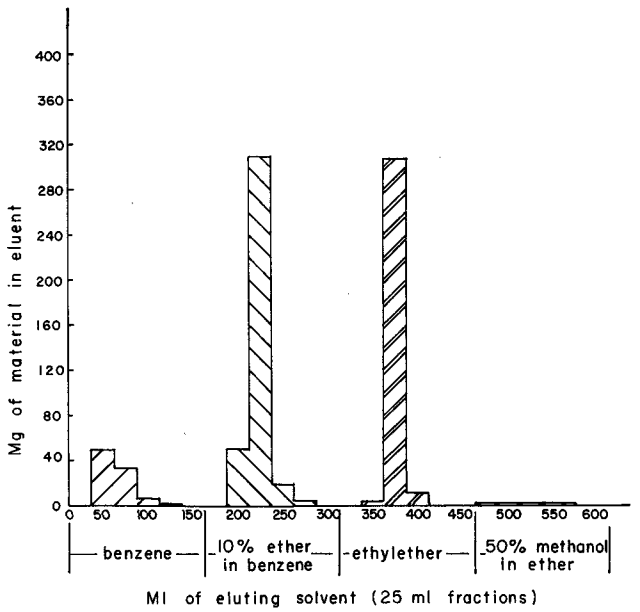

FIG. 1. Separation of a commercial GLP by silicic acid absorption chromatography.

made of each emulsifier. Replicate separations of a given sample checked within ± 1% on a weight basis for any given fraction.

Results and Discussion

Separation and Analysis of GLP Components. The results of the analyses on the 4 emulsifiers are shown in Table I. Table II shows the analyses on the fractions of these emulsifiers obtained from the column separation.

From the saponification values and lactic acid contents of the 3 major fractions, it can be established that separation on the column is based on polarity and, as in the case of separation or mono, di, and triglycerides, takes place in accordance with the number of hydroxyl groups present in the molecule. Although the lactic acid hydroxyl is somewhat more polar than the glycerol hydroxyl, it was not found possible (using solvents of intermediate polarity) to separate, e.g., a molecule containing only one hydroxyl group located on the glycerol portion of the molecule from one containing only one hydroxyl group located on the lactic acid portion of the molecule.

Components of the 4 fractions obtained could consist of the following: (It was not established whether or not all possibilities existed.)

Fraction 1. Eluted with benzene—Components consist of a glyceride molecule containing 3 fatty acid groups and from 0 to 3 lactic acid groups—e.g., tripalmitin or 1-0-palmitoyl lactyl 2, 3, dipalmitin.

Fraction 2. Eluted with 10% ether in benzene—Components consist of a glyceride molecule containing 2 fatty acid groups and from 0 to 3 lactic acid groups.

Fraction 3. Eluted with ethyl ether—Components consist of a glyceride molecule containing one fatty acid group and from 0 to 3 lactic acid groups.

Fraction 4. Eluted with 1:1 methanol:ethyl ether solution. Identity of this fraction was not established. In each of the emulsifiers analyzed, this fraction constituted less than 2% of the total washed product.

To obtain more complete knowledge of the composition of the individual fractions, further work would be indicated in separating components of a given fraction.

Bake Tests on GLP Components. Fractions 2 and 3 of sample 4, Table II, were each incorporated into shortenings which were used in white layer cakes. (This sample was chosen because it had the lowest monoglyceride content of the 4 samples.) A commercial shortening containing GLP was used as a control for the bake tests. Results of these bakes, given in Table III, show that effective emulsifying components in GLP are those esters which contain 2 free hydroxyl groups, at least one of which is located on a lactic acid portion of the molecule.

In addition to the above, bake tests were made on

TABLE I

Comparison of Composition of Four Commercial GLPs

Type of analysis	Product 1		Product 2		Product 3		Product 4	
	As received	After washing	As received	After washing	As received	After washing	As received	After washing
% Water-soluble	4.3	.....	2.3	.....	5.4	.....	0.8	.....
Saponification value	254.0	249.1	251.6	248.3	250.4	239.2	256.5	253.5
% Lactic acid	15.7	12.0	13.7	13.0	15.3	12.4	.....	14.6
% Alpha monoglycerides[a]	9.9	10.6	12.0	12.2	15.6	16.2	5.1	5.3
% Total fatty acids	72.7	76.0	.....	76.2	.....	74.3	.....	75.2
Avg mol wt acids	.....	257	.....	258	.....	258	.....	259
% Total glycerine	.....	17.7	.....	16.9	.....	17.9	.....	14.6
Mole ratio FA : LA : G	.....	1.5/0.7/1.0	.....	1.6/0.8/1.0	.....	1.5/0.7/1.0	.....	1.8/1.0/1.0

[a] Calculation based on avg mol wt of fatty acids.

TABLE II

Comparison of Compositions of Column Separated Fractions of Four Washed Commercial GLPs

Fractions analyzed and analyses	Product numbers			
	1	2	3	4
1. No free hydroxyls				
Wt % of total	12.5	12.1	9.7	18.9
Saponification value	194	226	226	222
% Lactic acid	1.5	3.9	1.4	2.3
2. One free hydroxyl				
Wt % of total	46.8	49.6	40.4	44.6
Saponification value	236	245	236	245
% Lactic acid	7.3	9.1	7.5	11.0
3. Two free hydroxyls				
Wt % of total	39.8	36.4	46.4	33.3
Saponification value	278	271	262	296
% Lactic acid	21.8	20.4	17.3	26.6
% Alpha monoglycerides	25.6	34.1	34.0	14.0
4. Fraction eluted with 50% methanol				
Wt % of total	1.3	0.9	1.2	1.8
Total wt % recovered from column	100.4	99.0	97.7	98.6

TABLE III

Results of Bake Tests on GLP Fractions from Product No. 4 in White Layer Cake[a]

Fraction No.	% of fraction in shortening	Specific gravity of cake batter	Cake volume (cc)
2	10	0.99	1050
3	3	0.76	1125
3	6	0.67	1195
Control shortening	....	0.82	1085

[a] The alpha monoglyceride content of each shortening was 4.5%.

shortenings containing 5 laboratory-prepared products obtained from Armour Industrial Chemical Co.

TABLE IV

Results of Bake Tests on White Cakes Containing Shortenings into Which Varying Percentages of Laboratory-Prepared Lactylated Glycerides Were Incorporated

Component	% Used in shortening	Specific gravity of cake batter	Cake volume (cc)
1-mono-0-palmitoyllactin	4.5	1.09	880
1-mono-0-palmitoyllactin [a]	5.0	1.02	1030
1 palmitoyl, 2 lactin	4.0	0.84	1100
1 palmitoyl, 2 lactin	6.0	0.79	1170
1-0-palmitoyl lactyl, 2 lactin	4.0	0.88	1100
1-0-palmitoyl lactyl, 2 lactin	6.0	0.84	1145
1 palmitoyl, 2, 3, dilactin	4.0	0.91	1190
1 palmitoyl, 2, 3, dilactin	6.0	0.90	1160
1-0-palmitoyl lactyl, 2, 3, dilactin	4.0	0.95	1230
1-0-palmitoyl lactyl, 2, 3, dilactin	6.0	0.92	1200

[a] With the exception of this sample which contained a total of 7.8% alpha monoglycerides, all shortenings contained 4.5% alpha monoglycerides.

Each of these products was a glyceride containing one fatty acid group and one or more lactic acid groups. Results of these bakes are shown in Table IV.

Based on the results obtained on pure samples, the conclusion reached is that those molecules which contain 2 free hydroxyl groups, both of which are located on the lactic acid portions of the molecule, are the best emulsifying agents in a GLP. This being the case, the best GLP would then be one that was richest in these components.

ACKNOWLEDGMENT

Production of pure lactylated esters used in the bake tests by S. H. Shapiro and A. Mais.

REFERENCES

1. Fett, H. M., JAOCS, *38*, 447 (1961).
2. Papariello, G. J., S. Chulkaralana, T. Higuchi, J. E. Martin, and V. P. Kuceski, *Ibid.*, *37*, 396 (1960).
3. Quinlin, P., and H. J. Weiser, Jr., *Ibid.*, *35*, 325 (1958).

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Fatty Acids of Lard. A. Identification by Gas-Liquid Chromatography[1]

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Abstract

A fresh commercially rendered lard was separated into its component fatty acids by subjecting the methyl esters to gas-liquid chromatography on nonpolar and polar columns. The lard methyl esters were first chromatographed on a silicone column. This column achieved separation of the component esters principally on the basis of chain length, and fractions which were represented by a single peak or series of peaks were collected. The collected fractions were then rechromatographed on an ethylene glycol succinate polyester column to separate and identify the saturated and the unsaturated esters. Qualitative evidence was obtained for the presence of 29 fatty acids ranging in chain length from 10 to 20 carbon atoms. Included were the esters of the following: Saturated acids (10,12,14,15,16,17,18,19,20, and 22 C-atoms), monounsaturated acids (14,16,17,18,19, 20, and 22 C-atoms), and polyunsaturated acids (18:2, 20:2, 22:2, 18:3, 20:3, 22:3, 20:4, 22:4, 20:5, and 22:5).[3] Peaks for several additional trace components were also observed.

Introduction

THE IDENTITY of the principal and some of the minor fatty acids of lard has been known for at least two decades. They include lauric, myristic, myristoleic, palmitic, pamitoleic, stearic, oleic, linoleic, linolenic, arachidic, and arachidonic acids. Later pentaenoic acid of undetermined chain length (4) was added to the list.

In previous work from this laboratory (3), gas-liquid chromatography (GLC) was applied directly to the total methyl esters of lard. Evidence for presence of arachidonate or pentaenoate could not be seen on the chromatogram, although ultraviolet spectrophotometric analysis showed they were present in concentrations of 0.3–0.5 and 0.1–0.2%, respectively. The chromatogram, however, suggested the presence of small amounts of esters of lower molecular weight than myristate and also an unidentified ester having greater retention than linolenate but which did not agree with the retention of a tetraenoic or pentaenoic ester. Craig and Murty (1) have indicated that even-numbered carbon acids from C_{10} to C_{20} were present in lard along with a number of unsaturated acids in each group.

In the present work, principal emphasis was given to identification of the minor and trace fatty acids of lard. A GLC column with a nonpolar stationary phase (silicone) was employed first to separate lard methyl esters primarily on the basis of chain length. Fractions collected from this operation were then chromatographed on a polar polyester (EGS) column in order to separate saturated and unsaturated esters. This procedure enabled identification of minor and trace fatty acids to be made with greater certainty. A number of fatty acids were identified which hitherto had escaped detection. Normal saturated and monoethenoid acids having 15, 17, and 19 carbon atoms were found but none with branched chains. A series of C_{20} and C_{22} acids having from 0–5 double bonds was detected. Some of these have not been reported previously.

Experimental

A sample of commercial steam rendered lard, obtained on the same day it was produced, was promptly converted to methyl esters by methanolysis (6) and separated by GLC on a nonpolar column. Six fractions were collected and each rechromatographed on a polar (EGS) column, before and after hydrogenation.

Gas-Liquid Chromatography. The apparatus was a conventional type with thermal conductivity detector and was described previously (3). The separation on

[1] Presented in part at the AOCS meeting, St. Louis, Mo., 1961.
[2] Eastern Utiliz. Res. & Dev. Div., ARS, U.S.D.A.
[3] A "shorthand" designation of structure of each fatty acid is used throughout this paper: 18:0 = saturated C_{18} acid; 18:2 = C_{18} with 2 double bonds; 18:0 br = branched chain C_{18} acid, etc.