The Estimation of Lactic Acid in Lactylated Monoglycerides and Shortenings Containing Lactylated Monoglycerides

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Abstract

A titrimetric method has been presented for the determination of lactic acid in lactylated monoglycerides and shortenings containing these products, when the only water soluble acid present is lactic acid. Analyses by this method: of knowns, samples from a material balance test, and commercial products, indicate that it is applicable to these types of product. Comparison of analyses of the material balance samples and commercial products by the titrimetric method and the colorimetric p-phenylphenol method indicated that the latter gave lower and more variable results.

Introduction

THE INTRODUCTION of new compounds into food products is accompanied by demands for methods of analysis. When lactylated monoglycerides were produced for use in shortening it was important to analyze these products for total, free, and combined lactic acid. Analysis for lactic acid involves two steps: 1) separation of the total or free lactic acid from the sample; 2) analysis for the separated lactic acid.

Procedures and Data

The lactic acid may be determined colorimetrically using ferric chloride (1) or p-phenylphenol (2,3) or by titration if no other water soluble acids are present. Of the colorimetric methods the p-phenylphenol appeared to be the better and has been applied to glycerol lactopalmitates (4). Difficulties were encountered with this method in obtaining consistent reproducible color development. Triplicate blanks usually would vary only 2 or 3% in transmittance but occasionally one of the three, for no explainable cause, would differ from two of the group by 8 to 10%. Similar variability was encountered in the color development of the standards used for establishing the calibration curve. Because of these difficulties the possibilities of a titrimetric method were investigated and a suitable procedure was developed.

Knowns were prepared and analyzed before and after the addition of lactic acid. The free lactic acid content found by analysis and the amount present are given in Table I. Table II shows the results of applying the method for total lactic acid to known mixtures. Combined lactic acid is determined by subtracting the free lactic acid from the total lactic acid.

As a further check on these methods a material balance was run on a lactylated monoglyceride produced in the laboratory by heating lactic acid and monoglyceride together for 4 hr at 185C. The loss of some of the reaction products in the first batch required production of a second batch. In the latter no losses occurred, and the weight of the reaction products equalled the weight of the ingredients.

TABLE I

Analysis of Known Samples for Free Lactic Acid

Product	% Free lactic acid	
	Found by analysis	Present
Lactylated monoglyceride "A"	0.04	.....
Lactylated monoglyceride "A" plus 0.31% lactic acid	0.36	0.35
Shortening "B"	0.00	.....
Shortening "B" plus 3.04% lactic acid	2.92	3.04

Reaction ingredients and reaction products were analyzed by both the titrimetric method and the p-phenylphenol method. In the p-phenylphenol method the free lactic acid was washed from a 0.5 g sample. The combined lactic acid was split out by saponification, extracted away from the fatty acids, and tested colorimetrically using p-phenylphenol. The results of the analyses by the two methods are given in Table III. In the second batch, 99.2% of the lactic acid was accounted for in the reaction products, when using the titrimetric method. This indicates that the titrimetric method can be relied upon to give accurate results when applied to lactylated monoglycerides.

Analyses of commercial lactylated monoglycerides by the titrimetric method and the p-phenylphenol method are given in Table IV, and analyses of shortenings containing lactylated monoglyceride are given in Table V. The variability of results obtained with the p-phenylphenol method is clearly evident in the comparison of results in Table IV at the 10% to 25% level, but less evident, Table V, at low levels of approximately 1.0%, as would be expected. In our hands the p-phenylphenol method gave results that were generally lower than the titrimetric method, but occasionally a higher value was obtained.

Method for Total Lactic Acid

APPARATUS

Flask, Erlenmeyer, glass stoppered, 250 ml with 24/40 standard taper joint.
Condenser, air, 650 mm with 24/40 standard taper ground glass joint, inner type.
Pipets, 25 and 50 ml.
Buret, 50 ml.
Funnel, separatory, 500 ml.
Cylinder, graduated, 100 ml.

REAGENTS

Potassium hydroxide, A.C.S. Grade.
Hydrochloric acid, sp. gr. 1.19, A.C.S. Grade.
Sodium hydroxide, A.C.S. Grade.
Ethyl alcohol, 95% (U.S.S.D. Formula 30 or 3A permitted).

TABLE II

Analysis of Known Samples for Total Lactic Acid

Product	% Total lactic acid	
	Found by analysis	Present
Reagent blank	0.0	.....
Shortening	0.0	.....
Shortening plus lactic acid, 20.7%	20.7	20.7
Lactylated monoglyceride	22.6	.....
Shortening plus lactylated monoglyceride (equivalent to 2.26% lactic acid)	2.2	2.26
Shortening plus lactylated monoglyceride (equivalent to 0.9% lactic acid)	1.0	0.9

TABLE III

Material Balance on Production of Lactylated Monoglycerides

Sample	Grams	Analysis	% Lactic acid		Grams lactic acid	
			Titrimetric method	P-Phenylphenol method	Titrimetric method	P-Phenylphenol method
Test 1						
Reaction ingredients						
Lactic acid	575	Total	87.7	.....	504.3	.....
Monoglyceride	1,500	Total	0.36	0.45	5.4	6.8
Total	2,075	.....	.....	.....	509.7 [a]	.....
Reaction products						
Lactylated monoglyceride	1,865	Combined	24.0	23.0	447.6	429.0
		Free	0.02	0.02 [b]	0.4	0.4
Distillate	189	Combined	3.7	4.0	7.2	7.6
		Free	12.9	12.9 [b]	24.4	24.4
Total	2,054	.....	.....	.....	479.6	461.4
Accounted for	99.3%	.....	.....	.....	93.5%	90.1%
			Laboratory	Laboratory	Laborotary	Laboratory
Test 2			A B	A B	A B	A B
Reaction ingredients						
Lactic acid	573	Total	86.7 87.6	....	495.6 501.9	....
Monoglyceride	1,507	Total	0.4 0.4	0.45 0.50	6.0 6.0	6.8 7.5
Total	2,080	.....	....	....	501.6 [a] 507.9 [a]	....
Reaction products						
Lactylated monoglyceride	1,942	Combined	21.30 22.15	10.4 23.85	413.7 430.1	203.0 464.1
		Free	3.60 3.65	3.60 [b] 3.65 [b]	69.9 70.9	69.9 70.9
Reaction distillate	138	Combined	0.2 0.2	0.4 0.5 [b]	0.3 0.3	0.6 0.7
		Free	1.5 1.8	1.5 [b] 1.8 [b]	2.1 2.5	2.1 2.5
Total	2,080	.....	....	....	486.0 503.8	275.6 538.2
Accounted for	100.0%	.....	....	....	96.9% 99.2%	54.9% 106.0%

[a] Used in calculating % lactic acid accounted for.
[b] Free lactic acid from the tritrimetric method used with the combined lactic acid by the p-phenylphenol method in calculating lactic acid accounted for.

Petroleum ether, Skellysolve F. or equivalent.
Phenolphthalein, A.C.S. Grade.

SOLUTIONS

Alcoholic potassium hydroxide. Dissolve 40 g of potassium hydroxide in one liter of 95% alcohol.
Hydrochloric acid, 0.5*N* approximately.
Sodium hydroxide, 0.5*N* accurately standardized.
Phenolphthalein solution, 1% in 95% alcohol.

PROCEDURE

Weigh 5 ± 0.1 g of sample into a 250 ml glass stoppered flask.
Pipet 50 ml of alcoholic KOH into the flask. Prepare and conduct 2 blank determinations with each group of samples using 50 ml of KOH.
Attach an air condenser and place flask on steam bath and boil gently for 30 min, or until completely saponified.
Remove flask from bath. Immediately remove air condenser and cool, but not enough for sample to jell. Add 75 ml of 0.5*N* HCl from a pipet and mix.
Transfer the solution to a 500 ml separatory funnel, washing with two 15 ml portions of water, adding washings to funnel.
Cool to at least 35C and add 100 ml of petroleum ether from a graduated cylinder; stopper and shake thoroughly.
Allow water layer to separate and draw off into a 500-ml separatory funnel.
Wash the PE with 20 ml of water, and add water wash to water in separatory funnel. Discard the PE solution.
Extract water solution with 100 ml PE, allow the water to separate, and draw off into a 500-ml Erlenmeyer flask.
Wash the PE with 20 ml water. Allow water to separate and draw off into the flask.
Add 1 ml of phenolphthalein solution and titrate with 0.5*N* NaOH until a slight pink color appears.

CALCULATION

$$\% \text{ Total Lactic Acid} = \frac{(S - B) \times N \times 9.008}{W}$$

S = Titration of sample.
B = Titration of blank.
N = Normality of NaOH solution.
W = Weight of sample.

Method for Free Lactic Acid

APPARATUS

Graduated cylinders, 500- and 1000-ml, glass stoppered.
Erlenmeyer flasks, 500-ml.
Pipet, 100-ml.

REAGENTS

Chloroform, U.S.P. grade or equivalent.
Sodium hydroxide, A.C.S. grade.
Ethyl alcohol, U.S.S.D. No. 30.

SOLUTIONS

Phenolphthalein solution, 1.0% in 95% alcohol.
Sodium hydroxide 0.5*N* accurately standardized.

TABLE IV

Analysis of Commercial Lactylated Monoglycerides

Lot	% Lactic acid by			
	Titrimetric method			P-Phenylphenol method. Combined lactic acid
	Total	Free	Combined	
1	12.1	0.6	11.5	7.6
2	12.4	0.5	11.9	9.6
3	11.9	0.7	11.2	7.8
4	11.8	0.06	11.7	12.2
				11.6
5	13.2	0.05	13.1	9.8
6	13.5	0.05	13.4	14.5
7	22.6	0.05	22.5	21.8
Avg.			13.6	11.7

TABLE V

Analysis of Shortening Containing Lactylated Monoglycerides

Lot	% Lactic aacid by			
	Titrimetric method			P-Phenylphenol method. Combined lactic acid
	Total	Free	Combined	
36	1.02	0.05	0.97	0.90
37	0.97	0.05	0.92	0.68
				0.42
				0.50
38	1.03	0.05	0.98	0.98
39	1.12	0.04	1.08	1.15
40	1.14	0.04	1.10	0.96
41	0.93	0.04	0.89	0.90
42	1.02	0.05	0.97	0.80
				0.76
Avg.			0.99	0.89

PROCEDURE

Weigh 15.0 ± 0.1 g of sample into a beaker, dissolve in chloroform, and transfer to 500-ml glass stoppered graduated cylinder.

Rinse beaker thoroughly with chloroform and add rinsing to the graduated cylinder. Add chloroform to cylinder until volume is 200 ml.

Add 200 ml of water, stopper and shake vigorously for one minute. Set aside until phases separate.

When 125 ml or more of the water phase has separated pipet 100 ml of the aqueous phase into an Erlenmeyer flask.

Add 1 ml of phenolphthalein indicator and titrate with 0.5*N* NaOH to end-point.

CALCULATIONS

$$\% \text{ Free Lactic Acid} = \frac{S \times N \times 9.008}{W}$$

S = Titration of sample.
N = Normality of NaOH solution.
W = 7.5 when 15.0 g is weighed for analysis.

Precision

A series of 15 samples of lactylated monoglycerides (10–25% lactic acid) and a series of 12 samples of shortening containing lactylated monoglyceride (approximately 1% lactic acid) were analyzed in two laboratories for total lactic acid by the proposed method.

The 95% probability limits for the difference between single analyses in the two laboratories were 0.36% for the low level and 1.46% for the high level. The standard deviation was 0.13% for the low level and 0.51% for the high level.

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Caloric Availability and Digestibility of New-Type Fats

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Abstract

Digestibility and caloric availability of new-type fats were determined by feeding these products to young rats while on a restricted caloric intake. Amylose stearate, amylose palmitate, amylose oleate, distearin adipate, and glycerol adipate were low in both categories. Diolein fumarate was completely digested but poorly utilized. These materials were compared with common oils and fatty acids. These new-type fats have potential use as pan greases and surface coatings for foods.

Introduction

ALTERED FAT PRODUCTS including amylose esters (1), diglyceride esters of succinic, fumaric, and adipic acids (2,3,4), and polymeric fats from stearic, oleic, and short-chain dibasic acids (5), have been prepared at the Southern Regional Research Laboratory. The amylose-containing esters may be useful as dip-type coatings on foods. The dibasic acid containing polyesters and diglyceride esters are also potentially edible fats and may be used as pan greases, slab dressings, or surface coatings for foods.

Little work has been done to determine the extent to which these products are digested and utilized in the animal body. Shull et al. (6) have reported that two types of adipic acid esters of glycerides have high digestibility coefficients and that the rate of oxidation of the stearic acid is greater when fed as the diglyceride adipate than as the polyester. In the present report, use has been made of the caloric availability assay devised by Rice et al. (7) to measure digestibility and utilizability of several new-type fats.

Experimental

Preparation and properties of the modified fats used in this work have been described previously (1–5). Cottonseed oil, corn oil, glucose, amylose, and palmitic, stearic, oleic, and adipic acids were included for comparative purposes.

The biological evaluation described by Rice et al. (7) measures the available energy of a test substance in terms of 1-week gains in weight of young rats fed calorically restricted diets under carefully controlled conditions. Young albino rats of either sex were housed in individual wire-bottom cages and fed a complete diet, the daily intake of which was restricted so that very little gain in weight was possible unless a supplement (caloric source) was added. The basal diet consisted of the following ingredients in per cent: sucrose 50, crude casein 40, cystine 0.3, salts USP XIV 4, corn oil 3, and a complete vitamin mixture 2.7. After an adjustment period of 5–7 days on 5 or 5.5 g of diet per day, all rats weighing 75-85 g were divided into uniform groups of 5. Then for a period of 7 days each rat received daily the basal diet or the basal diet plus a test supplement in an amount shown in Table I. A test group's gain in weight over that of the control group is a measure of the caloric availability of the supplement.

Since a number of the fat derivatives were found to be poorly digested, a modification of the Rice procedure was introduced in order to adjust the 7-day weight gain for 1) undigested intestinal residues of the supplement fed, and 2) abnormal hydration of either the body tissues or the intestinal contents (8). This modification was accomplished by changing all rats to the basal diet for a period of 48 hr at the end of the 7-day test period. During the 48-hr period, any loss in weight greater than that of the control group was interpreted as being a false-positive gain, for the reasons cited above, and was deducted from the 7-day weight gain in order to arrive at the actual gain from utilization of the supplement.

A sheet of filter paper (9 × 11 in.) backed by a thin plastic sheet was placed under each cage in order to collect the urine and feces. Collection was started the second day of the test period and continued to the

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