Quantitative Determination of Sodium Stearoyl-2-Lactylate in Soy-Fortified Wheat-Flour Blends

E. L. WHEELER, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710[1]

ABSTRACT

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Sodium stearoyl-2-lactylate (SSL) was determined in flour and flour blends by extracting with chloroform, separating from other lipids by thin-layer chromatography, and estimating its content using a colorimetric procedure involving an SSL hydroxamic acid derivative/Fe^{+++} complex.

Sodium stearoyl-2-lactylate (SSL) is the only dough conditioner now approved by the U.S. Department of Agriculture (1972) for use in soy-fortified wheat-flour blends used in PL-480 Food for Peace programs. Studies done at this laboratory (Bean et al 1977, Mecham et al 1976) showed that the baking quality of soy-fortified flours with or without added SSL deteriorated when the blends (13% moisture) were subjected to prolonged storage at 100° F. Also fresh SSL added at mixing did not restore baking quality to deteriorated blends, whereas ethoxylated monoglycerides and sucrose esters did. Monitoring the SSL content of blends during storage seemed desirable in order to determine if SSL content might be used as an index of baking quality. However, no quantitative method for SSL determination could be found in the general literature, only a lengthy, indirect gas-liquid chromatographic procedure submitted to the Food and Drug Administration (Barry 1972). This article reports a quantitative method for direct determination of SSL in wheat-flour and flour-soy blends. In addition, the amount of SSL extractable from wheat-soy blends is shown to decrease upon storage.

[1] Reference to a company or product is for purposes of information only and does not imply approval or recommendation by the USDA to the exclusion of others that may also be suitable.

MATERIALS AND METHODS

Commercial SSL (Emplex, C. J. Patterson Co., Kansas City, MO) was found to be a complex mixture of compounds. Thus pure SSL used in this work was synthesized in the laboratory from lactide (a lactic acid dimer) by a method devised by C. A. Elliger (*unpublished*) (Fig. 1). It was estimated to be greater than 95% pure SSL by thin-layer chromatography (TLC). It had mp, 62–66° C. Analysis for $C_{24}H_{44}O_6$: calculated, C, 67.26%; and H, 10.35%. Found: C, 67.5% and H, 10.3%.

Wheat flour and soy blends were described previously (Bean et al 1977, Mecham et al 1976).

A Delux tube mixer (Scientific Products Co., Menlo Park, CA) and a Varian (Palo Alto, CA) Techtron series 634 UV-visible spectrophotometer were used in the ester determinations. Precoated TLC plates, obtained from Kontes (San Leandro, CA), were a neutral pH, low polarity silica gel type (designated LQD), 250 μ thick, and equipped with a preabsorbant sample spotting area.

Ferric perchlorate was prepared by dissolving 0.50 g of iron in 80 ml of 70% perchloric acid by gentle warming on a hot plate, then diluting to 250 ml with water.

Hydroxylamine hydrochloride solution and ethanolic sodium hydroxide were prepared by dissolving 5.0 g of hydroxylamine hydrochloride and sodium hydroxide, respectively, in 5.0 ml of water, then diluting each to 100 ml with absolute ethanol. These stock solutions last several months when stored in a refrigerator.

TLC developing solvent was a 100:100:1 mixture of light petroleum ether (Skelly-F)/diethyl ether/acetic acid. Various acid-base indicators were appropriate for visualizing SSL on the TLC plates; in this work, a mixture of 0.03% bromphenol blue-0.01% bromthymol green in 95% ethanol was used.

Determination of SSL

Extraction. With a mechanical shaker, 1.00 g of flour containing 0–0.5% SSL was extracted with about 10 ml of chloroform and 1 drop of concentrated hydrochloric acid (to covert SSL to the free acid) in a 25-ml Erlenmeyer flask for 45 min and was filtered quantitatively (E&D grade 509) through anhydrous sodium sulfate into a test tube. Solvent was removed with a stream of air in a 35–40° C water bath. The sides of the tube were washed down with a few milliliters of chloroform and were dried by evaporation.

TLC of the Extract. The contents of the tube were dissolved in exactly 1.0 ml of $CHCl_3$, with the tube tightly capped, and the TLC plate was spotted with aliquots of solution estimated to contain 0.1–0.7 mg of SSL (normally 10–100 μl). For flour extracts containing low concentrations of SSL, 0.5 ml of chloroform may be used to save time in spotting the TLC plates. The TLC tank was pre-equilibrated with developing solvent, and the solvent was allowed to run 15 cm up the plate. After drying, SSL can be visualized with the bromphenol blue spray reagent, which gives yellow spots slowly turning blue on a purple background. The natural lipids are not visible with this reagent but can be seen after exposure to iodine vapor.

Quantitation. The SSL band was scraped from the TLC plate into a 12-ml centrifuge tube. It was not necessary to elute SSL from the gel, and neither the bromophenol spray reagent nor the iodine visualization interferred with the test. A 1:1 mixture of the stock ethanolic sodium hydroxide and stock hydroxylamine solutions was prepared, and 0.50 ml of the mixture was added to the gel in the centrifuge tube. The mixture was covered and mixed vigorously with a mechanical test tube mixer two or three times over a 5-min period. The stock ferric perchlorate solution was diluted 1:10 with absolute ethanol, and 5.0 ml was added to the mixture in the tube. Again, the mixture was covered and mixed vigorously until the silica gel in the bottom of the tube no longer appeared redder than the solution. After centrifugation for 3–5 min, the color of the supernate was read against a reagent blank at 530 nm. The color developed immediately and was stable for at least several hours.

A standard curve of absorbance vs. SSL concentration was obtained following the above procedure, which included having the SSL adsorbed to silica gel during reaction with the color reagent. The standard solution of SSL either was added to some gel already in a tube or was first developed on the TLC plate and scraped into a tube. Both procedures gave identical results. Three different solutions of SSL were used to obtain 36 data points over a period of several weeks. The curve obtained had a straight line correlation of 0.996 and went through the origin (0–0.7 mg range of SSL concentration). The scatter of the data points was so small that the line drawn through the points freehand was the same as the computer generated line.

Calculations. The amount of SSL, in milligrams, in each aliquot scraped from the TLC plate was obtained using an absorptivity constant of 0.690 (the slope of the gel-adsorbed SSL standard curve), so that absorbance/0.690 = mg SSL (assuming a 1-cm cell path length and the 5.5-ml final volume described above). The amount of SSL in the original flour blend was then obtained from a consideration of the size of the aliquot taken from the 1.0-ml total volume of flour extract before spotting the plate.

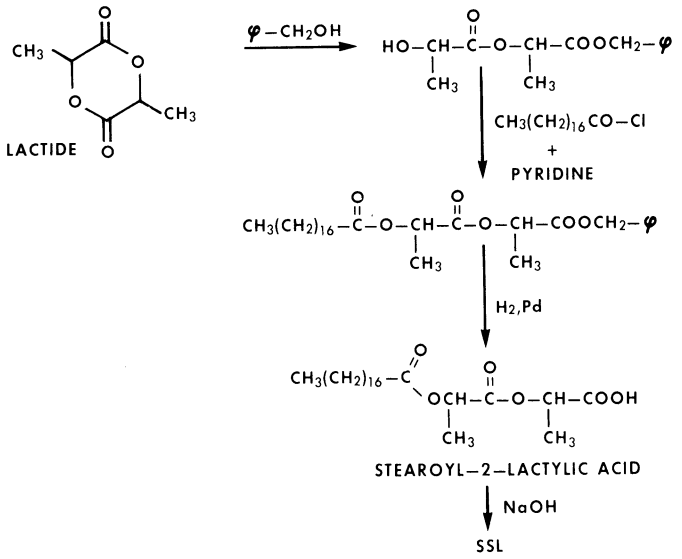

Fig. 1. Outline of the C. A. Elliger procedure for the synthesis of sodium stearoyl-2-lactylate.

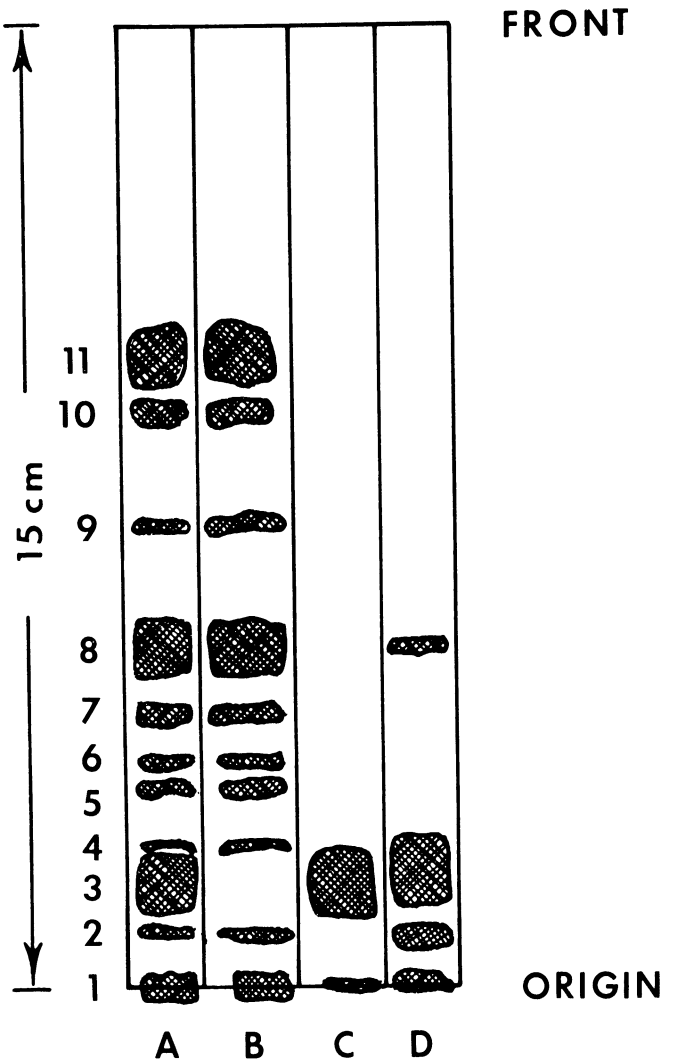

Fig. 2. Thin-layer chromatograms of chloroform extracts of: **A,** soy blend + 0.5% pure sodium stearoyl-2-lactylate (SSL); **B,** soy blend containing no SSL; **C,** pure SSL; **D,** commercial SSL. Spots visualized by first spraying with bromophenol blue-bromothymol green solution, then immersing the thin-layer chromatography plate in I_2 vapor for 5 min.

RESULTS AND DISCUSSION

Esters react with hydroxylamine under basic conditions to form hydroxamic acids that, in turn, form reddish complexes with ferric ion. Feigl et al (1934) first used the reaction as a spot test, but it has since been quantitated by other workers (Goddu et al 1955, Vioque and Holman 1962) using numerous variations and modifications. Individual esters and classes of esters behave quite differently in this reaction with respect to optimum conditions of reagent concentrations, temperature of reaction, and adsorption maximum of the Fe^{+3} complex. The method presented here differs from others in that the reaction proceeds at room temperature, the ester is adsorbed to a gel during the reaction, and color develops immediately upon addition of Fe^{+3} to the hydroxamic acid derivative. In early experiments to determine optimum conditions for reaction, a family of standard curves of different slopes (but all with good straight line correlation) was obtained; their slopes depended on reaction conditions. The procedure therefore should not be altered if an absorptivity constant of 0.690 is to be used to calculate SSL concentration. That includes using a low polarity silica gel of the type described in the Methods section. SSL apparently is too strongly adsorbed to standard type silica gels to react quantitatively with hydroxylamine and is not completely eluted from such a gel, even with a polar solvent such as methanol. Commercial SSL cannot be used as a standard because it contains varying amounts (20–40% in samples tested) of other esters, stearic acid, and lactic acid, depending on its storage history (SSL should be stored dry at 0° C to inhibit hydrolysis). A chromatogram of a typical commercial sample of SSL is shown in Fig. 2, column D. Obviously, if commercial SSL is added to flour at 0.5%, a determination by this method, based as it is on pure SSL, will give a value lower than 0.5% depending on the actual amount of pure SSL in the sample.

Figure 2 also demonstrates that the natural lipids in the flour blend, with the possible exception of spot 4, separate completely from SSL using the solvent system described. Spot 4 by itself does not give a positive ester test and can be ignored.

To determine the percent recovery of SSL from flours, exact amounts of pure SSL were added to fresh 12% soy blends and wheat flour. The SSL values obtained indicate that about 92–96% recovery of SSL from flours can be expected, even with a low (0.13%) initial SSL concentration. (Table I).

Although the method, with the exception of the ester test, can be modified in many ways for convenience, it is important to have a ratio (milliliter of chloroform used for extraction: milligram of SSL extracted) similar to that used in this procedure. When 2-g samples (instead of 1 g) of blend containing 0.5% SSL were extracted with 10 ml of chloroform, only 80–90% recovery of SSL was obtained. It may be that SSL has a polar attraction to proteins that makes it difficult to extract. Ether extraction gave only 70–80% yields, and heating the extractant to 40–50° C actually lowered the yield in some cases, probably by promoting hydrolysis.

Finally, the method was used on 12% defatted soy-flour blends, originally containing 0.5% commercial SSL by weight (which corresponds to 0.4% SSL) that had been stored at 100° F at 13% moisture for 0–5 months in polyethylene bags. In baking tests on the flours, loaf volume dropped to about 60% of control in five months (Bean et al 1977). Analyses of these samples by the new procedure showed that the amount of SSL also decreased during storage (Fig. 3). The deterioration in baking quality of soy-fortified flours under conditions of high temperature and moisture is not due solely to a loss of SSL, because addition of fresh SSL does not restore baking quality (Bean et al 1977, Mecham et al 1976). As Fig. 3 demonstrates, however, loss of SSL during storage can be used as an index of baking quality.

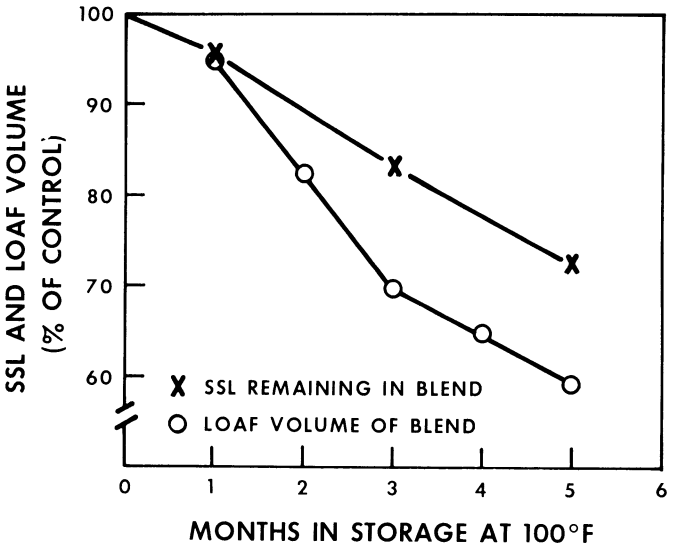

Fig. 3. Loss of commercial sodium stearoyl-2-lactylate correlated with loss in loaf volume in soy blend stored at 100° F for 0–5 months at 13% moisture.

TABLE I
Recovery of Sodium Stearoyl-2-Lactylate (SSL) (Laboratory Synthesized) from Flour and Flour-Soy Blends

Extract	Extract Applied per Spot (μl)	Theoretical SSL/Spot (mg)	SSL[a] Recovered (mg)	% Recovery
10.0 mg pure SSL (no flour)	15	0.150	0.143	95
	30	0.300	0.291	97
	45	0.450	0.430	96
0.980 g wheat flour at 5.00 mg SSL/g	20	0.098	0.090	92
	40	0.196	0.189	96
	60	0.294	0.282	96
1.00 g flour-soy blend at 5.00 mg SSL/g	20	0.100	0.091	91
	40	0.200	0.193	97
	60	0.300	0.283	94
1.00 g blend at 2.64 mg SSL/g	25[b]	0.132	0.122	92
	50[b]	0.264	0.242	92
1.00 g blend at 1.32 mg SSL/g	50[b]	0.132	0.125	95
	100[b]	0.264	0.246	93
1.00 g blend at 0.00 mg SSL/g	60	0	<0.01 [c]	...

[a] Obtained by dividing the absorbance of the Fe^{+3}-hydroxamic acid of SSL complex by 0.690.
[b] Extract dissolved in 0.5 ml chloroform. All others dissolved in 1.0 ml.
[c] Obtained by running the color reaction on the gel scraped from the region where SSL would be if it were present.

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