Lactones in Butter, Butteroil, and Margarine[1]

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ABSTRACT

The γ- and Δ-lactones of butter, butteroil, and margarine samples were determined by a recently developed extraction and chromatographic method. A chromatographic column is made of three layers consisting of 6 g aluminum oxide upon which is placed 35 g sodium sulfate and then a fat-Celite mixture obtained by grinding 10 g sample with 35 g Celite 545. The column was eluted with acetonitrile, and the extract was concentrated and lactones determined by gas chromatography. γ-Dodecalactone, Δ-decalactone, Δ-dodecalactone, and Δ-tetradecalactone were recovered quantitatively. Freshly made butter had only 7 ppm of these lactones, whereas commercial samples ranged from 12-30 ppm. Two of eight commercial margarine samples had as much as 10 ppm of Δ-decalactone and Δ-dodecalactone. Comparison of unheated butteroil with butteroil heated at various temperatures showed a correlation of lactone content with time and temperature of heating. Butteroil heated at 65 or 100 C showed increasing levels of lactones up to 8 hr, whereas butteroil heated at 145 or 125 C showed a maximum production at ca. 2 and 4 hr, respectively.

INTRODUCTION

Lactones are compounds important in the flavor of butter and other dairy products and are added to some margarines to simulate butter flavor (1).

The detection of these compounds in fat, however, has been a problem. The method primarily used in determining lactones involve isotope dilution, silicic acid adsorption chromatography, or steam distillation (1-3). These methods are often time consuming and tedious. In addition, since the lactone precursors are heat labile, methods involving the use of heat determine total lactone value which is a combination of free and potential or "bound" lactones. Consequently, in many applications these methods are of limited value.

We recently reported a simple column extraction technique followed by gas chromatography that quantitatively recovers the γ- and Δ-lactones (4). The method does not involve the use of heat and measures only the free lactones or those present at the time of analysis. In the study reported here, we used this method to examine the lactone content of butter, heated butteroil, and margarine.

EXPERIMENTAL PROCEDURES

Materials

Ten samples of margarines representing eight different brands were purchased locally during September and October 1973. Two of the brands were re-examined in February 1974. Six of the samples were soft margarines in plastic containers, and four were regular margarines packaged four sticks to 1 lb.

Three samples of butter representing different brands were bought in local markets in September 1973. The butter designated DFNL, was prepared in our laboratory from fresh raw cream and analyzed for lactones 16 hr after preparation.

Butteroil was prepared in the laboratory from fresh raw cream. The cream was churned, and the butter was washed with water at 40 C and centrifuged. This procedure was repeated twice. The butter was dissolved in redistilled petroleum ether, and the aqueous layer was separated and discarded. The ether then was removed on a rotating evaporator at 40 C. The butteroil, containing ca. 2% water, was flushed with nitrogen and sealed in glass ampoules. The samples then were heated at 65, 100, 125, and 145 C for various time intervals.

Authentic lactones were obtained from C&A Aromatic Corp., Floral Park, N.Y. The lactones were at least 95% pure as determined by gas chromatography.

Acid aluminum oxide was obtained from the J.T. Baker Co., Phillipsburg, N.J. The alumina was heated at 170 C for 16 hr and then equilibrated 24 hr with 8% of 5N H_2SO4.

Lactones Determination

Procedure for determination of lactones was essentially that of Wong, et al. (4). Margarine, butter, or butteroil (10 g each) were mixed with 35 g Celite 545 in a mortar and ground until homogeneous. A chromatographic column was made of 3 layers consisting of: (A) 6 g treated aluminum oxide, (B) 35 g anhydrous sodium sulfate, and (C) the ground margarine, butter, or butteroil-Celite mixture which was added in three equal portions with tamping after each addition. The column was eluted with redistilled acetonitrile until 14 ml were collected. The solvent extract of margarine or butter was evaporated carefully under a fine stream of nitrogen to 0.2 ml. The residue was re-extracted with acetonitrile and passed over a small column containing 0.8 g treated aluminum oxide. For analyses of butteroil, the extract was reduced to ca. 1.5 ml under nitrogen and then transfered to a 2 ml graduated centrifuge tube and further reduced to 1 ml. The extract was allowed to stand 30 min for the residual fat to separate (30-40 mg). The acetonitrile fraction was then carefully pipetted off and the fat residue re-extracted with 1 ml fresh acetonitrile which was combined with the original acetonitrile extract. The volume was reduced to 0.5 ml and passed over the small alumina column as described for margarine and butter. The final extracts were reduced to 0.5 ml, and 4 μliter aliquots were gas chromatographed.

A Hewlett-Packard 7620A gas chromatograph with dual flame ionization detectors was used. The column was 8 ft x 1/8 in. stainless steel containing 7.5% ethylene glycol adipate + 2% H_3PO_4 on 80-90 mesh Anakrom ABS. The gas chromatograph was temperature programed from 130-180 C at 2 C/min. The peak sizes were determined by an Infotronics CSR 208 automatic digital integrator. The area counts of the lactones were plotted against μg of the lactones, and a straight line relationship was obtained.

The identification of the lactones was made from retention times and mass spectral data. The mass spectral data of the authentic lactones and lactones from products were obtained on an LKB model 9000 combination gas chromatograph-mass spectrometer.

The efficiency of the extraction procedure was determined by adding authentic γ-dodecalactone (γ-C_{12}), Δ-decalactone (Δ-C_{10}), Δ-dodecalactone (Δ-C_{12}), and Δ-tetradecalactone (Δ-C_{14}) to the products and extracting with acetonitrile. Recoveries of added lactones ranged from 92-100%.

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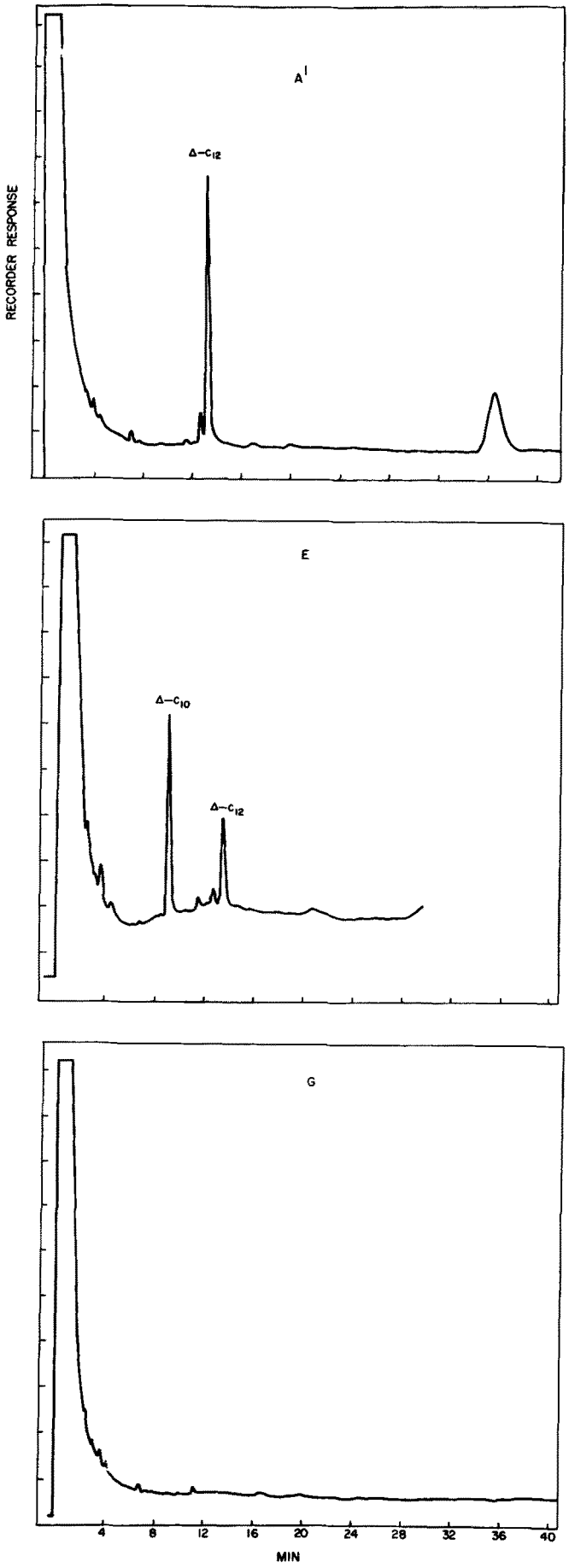

FIG. 1. Gas chromatograms of lactone fractions from margarines.

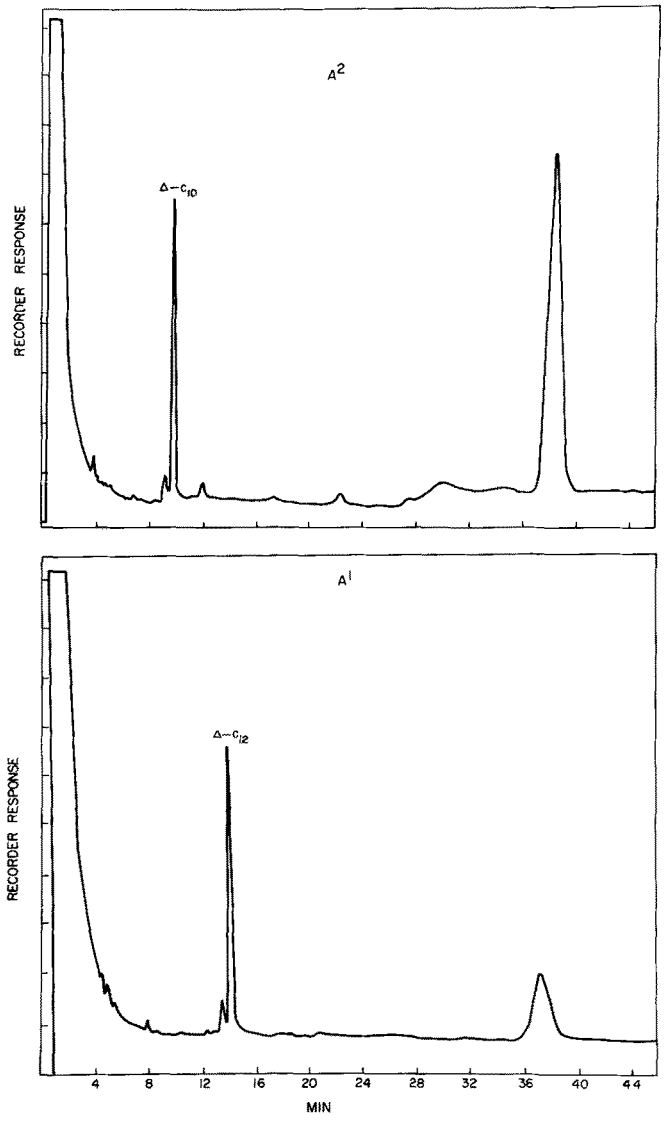

FIG. 2. Gas chromatograms of lactone fractions from different samples of the same brand of margarine.

RESULTS AND DISCUSSION

Lactone Composition of Margarine

Typical gas chromatograms of lactone fractions from margarines are shown in Figure 1. Chromatogram C is typical of six brands of margarine in which lactones were not detected. Chromatogram E is from a margarine in which two lactones were detected. The peak at 10 min is Δ-C_{10} lactone and 14 min Δ-C_{12} lactone. Chromatogram A^1 is from a margarine in which only one lactone was detected, the peak at 14 min being Δ-C_{12} lactone.

An inconsistency was observed in the lactones detected in one brand of margarine. The gas chromatograms of different samples of this brand of margarine are shown in Figure 2. Six samples of this brand of margarine were examined over a period of 5 months. Chromatogram A^1 is typical of the lactone fraction from four of the six samples, while the other two samples are represented by chromatogram A^2. The peak at 14 min in chromatogram A^1 is Δ-C_{12} lactone and the peak in A^2 at 10 min is Δ-C_{10} lactone. The large peak at 37 min could not be identified, although the mass spectrum indicated that it was not a γ- or Δ-lactone. The size of the unidentified peak also varied considerably in different samples of this brand of margarine.

The lactone determinations of eight brands of margarine are shown in Table I. Only two of the brands analyzed, A

TABLE I

Lactone Composition of Margarines

Type	Brand	Lactones (μg/g) Δ-C_{10}	Δ-C_{12}
Hard	A[1]	---	9.6
	A	9.5	---
	B,C,D,I,J	---	---
	E	7.2	3.4
Soft	E	5.8	2.9
	B,G	---	---

TABLE II

Lactone Composition of Butter Lactones (μg/g)

Brand	Δ-C_{10}	γ-C_{12}	Δ-C_{12}	Δ-C_{14}	Total
DFNL[a]	1.0	---	2.1	3.9	7.0
A	2.7	0.4	4.0	5.0	12.1
B	5.4	0.7	9.9	14.0	30.0
C	4.0	0.7	7.1	8.4	20.2

[a]Designates butter prepared in our laboratory from fresh raw cream.

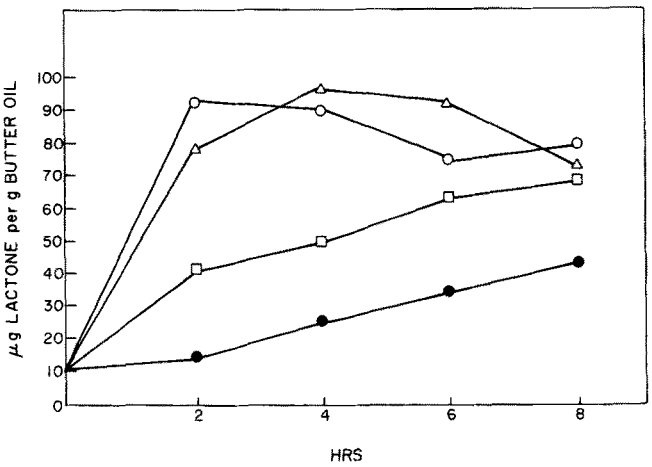

FIG. 3. Effect of heat upon the lactone content of butteroil. ○—○ = 145 C, Δ—Δ = 125 C, □—□ = 100 C, and ●—● = 65 C.

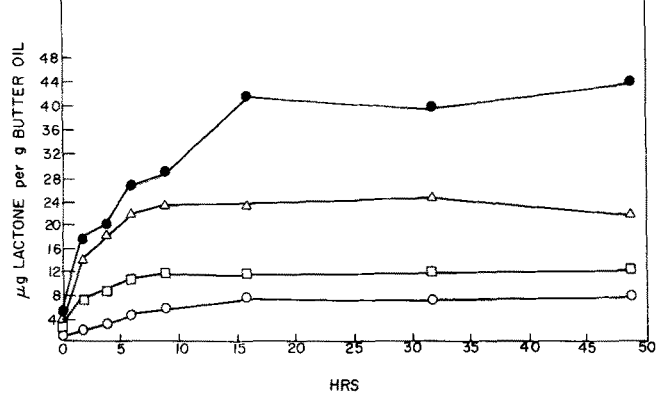

FIG. 4. Effect of heat (100 C) upon individual lactones of butteroil. ●—● = Δ-C_{14}, Δ—Δ = Δ-C_{12}, □—□ = ΔC_{10} and ○—○ = γ-C_{12}.

and E, had any detectable lactones. The average lactone value of 4 of 6 samples of Brand A was 9.6 μg/g Δ-C_{12}, whereas the average lactone value for the other 2 samples was 9.5 μg/g of Δ-C_{10}. Although the lactone detected shifted from Δ-C_{10} to Δ-C_{12} in different samples of brand

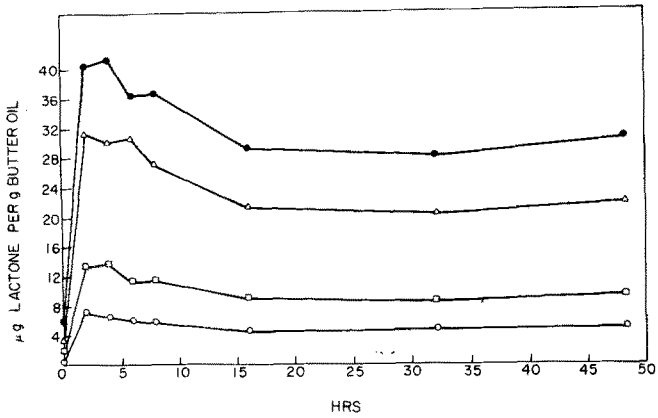

FIG. 5. Effect of heat (145 C) upon individual lactones of butteroil. ●—● = ΔC_{14}, Δ—Δ = ΔC_{12}, □—□ = ΔC_{10}, and ○—○ = γC_{12}.

A, the lactone contents on a per g margarine basis are almost identical. The reason for the shift in lactones is unknown. The results on brand E indicated that Δ-C_{10} and Δ-C_{12} were in ratio of 2 to 1 for both hard and soft margarine. The results also indicate that the total lactone content of brand A and brand E on a per g fat basis is in the same range. The analysis of both hard and soft samples of brands B, C, D, I, G, and J did not reveal any lactones.

Lactone Composition of Butter

The lactone composition of butter is shown in Table II. Butter prepared in our laboratory and analyzed after 16 hr had a total of only 7 μg/g of free Δ-lactones, whereas commercial samples ranged from 12-30 μg/g these lactones. Although the history of the commercial samples is unknown, the higher levels of lactones can be attributed, at least in part, to the age of the butter. It has been shown that lactones are formed in butterfat and dried whole milk under refrigeration storage (3) and that there are smaller quantities of free lactones in fresh butter than older butter (1,3). In addition, the Dairy Foods Nutrition Laboratory butter received only minimal heat treatment since it was made from raw cream and was heated to 40 C to melt the fat. γ-C_{12} lactone was not detected in freshly made butter. We have observed similar results with freshly made cheese (4). In addition, the lactone content of fresh butter and freshly made cheese on a per g fat basis is almost identical. Δ-hexadecalactone was identified in butter and butteroil but was not measured in this study.

Lactone Composition of Butteroil

The effect of heat upon the combined lactone values of γ-C_{12} Δ-C_{10}, Δ-C_{12}, and Δ-C_{14} of butteroil is shown in Figure 3. Comparison of unheated butteroil and butteroil heated at various temperatures shows a correlation of lactone content with time and temperature. Butteroil heated at 65 or 100 C showed increasing levels of lactones up to 8 hr, whereas butteroil heated at 125 and 145 C reached a maximum lactone content at 4 and 2 hr, respectively. Boldingh and Taylor (5) observed similar results. They reported a nearly 100% formation of lactones from synthetic triglycerides containing Δ-hydroxy decanoic acid when they were heated at 145 C for ca. 85 min. Although Jurriens and Oele (2) reported that bound lactones constitute one-third the total lactone content in butter, our data indicate a much higher percentage of bound lactones. In the study reported here, the differences between the lactone values of unheated butteroil and butteroil heated at 145 C for 2 hr shows that bound lactones constitute ca. 85% of the total lactones of fresh butteroil.

The effect of heating at 100 C for 48 hr on the

production of individual lactone is shown in Figure 4. The data indicate the lactone values for γ-C_{12}, Δ-C_{10}, and Δ-C_{12} reached a maximum after 8 hr and then leveled off, whereas Δ-C_{14} continued to increase up to 16 hr before leveling off. However, when the butteroil was heated at 145 C, as shown in Figure 5, the lactone values of γ-C_{12}, Δ-C_{10}, Δ-C_{12}, and Δ-C_{14} appeared to have reached a maximum at 2 hr and decreased up to 16 hr before leveling off. Lactone values of γ-C_{12}, Δ-C_{10}, and Δ-C_{12} at the leveling off point were in the range of the corresponding lactone values at the maximum at 8 hr when the oil was heated at 100 C (Figs. 4 and 5). The highest lactone values obtained when butteroil was heated at 145 C were in the range of those reported in the literature for unheated butteroil when silicic acid adsorption chromatography or steam distillation method was used to isolate the lactones from the fat (3). This would indicate that these two methods determine both free and bound lactones, whereas the method used in our study measures only free lactones.

The acetonitrile procedure is an analytical method that can be used for detecting free lactones in margarines, butter, and butteroil. One advantage of this method lies in its ability to detect only free lactones or those present at the time of analysis. This feature would (1) appear necessary in the study of lactones in foods since their contribution to flavor occurs only as free lactones and (2) serve to present a clearer picture of the effect of heat on their production.

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