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ANALYTICAL AND STRUCTURAL STUDY OF SOME FOOD EMULSIFIERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND OFF-LINE MASS SPECTROMETRY

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SUMMARY

Components of various food emulsifiers distilled monoglycerides (E 471), sodium stearylactylate (E 481), calcium stearylactylate (E 482), diacetyltartaric acid esters of mono- and diglycerides (E 472 e) and mixed acetic and tartaric acid esters of mono- and diglycerides (E 472 f), have been separated analytically and on a semi-preparative basis by high-performance liquid chromatography. The various products were identified by off-line high-resolution mass spectrometry.

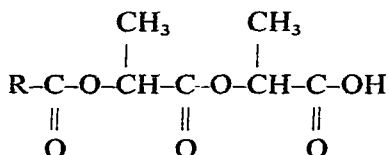
INTRODUCTION

Emulsifiers are widely used in the food industry because they facilitate the processing of food and improve the quality of the resulting products. They are derived from edible fats, which consist of molecules with both hydrophilic and lipophilic characters, thus conferring tensioactive properties upon the emulsifier. Emulsifiers may also complex with proteins (*e.g.*, anionic and polyethoxylated emulsifiers) or starch (*e.g.*, amylose forms a helical complex around the hydrocarbon chain of saturated monoglycerides), resulting in the dough strengthening or bread softening properties well known in baking¹. When these compounds possess both an anionic part and a long saturated hydrocarbon chain, *e.g.*, diacetyltartaric acid esters of mono- and diglycerides, they behave both as dough strengtheners and bread softeners².

The different emulsifiers are generally described by a generic name which refers to the major components. In fact they are mixtures of well defined chemical compounds the proportions of which vary according to the starting materials and processing conditions: temperature and reaction time. Their contents in different basic constituents^{3,4} may be specified, *e.g.*, total lactic acid in sodium stearylactylate.

Much work has been done on the separation and identification of emulsifiers in food by thin-layer chromatography (TLC)⁵ and gas-liquid chromatography (GLC)⁶. High-performance liquid chromatography (HPLC) has been used for the separation of glycerides⁷⁻¹⁰ and for fingerprinting of some food emulsifiers¹¹. In contrast, the separation and identification of the components of complex emulsifiers has been little investigated.

The present study was undertaken mainly on two emulsifiers widely used respectively in the U.S.A. and Europe in the baking industry, namely: sodium (and calcium) stearylactylate (E 481*, E 482*); diacetyltartaric acid esters of mono- and diglycerides (E 472 e*) and mixed acetic and tartaric acid esters of mono- and diglycerides (E 472 f*). The first are manufactured by reacting fatty acids with lactic acid and a suitable base^{12,13}. The main product is thought to be



because the fatty acid:lactic acid molar ratio is roughly 1:2 in the emulsifier. TLC^{6,12} or GLC⁶ has been used for their identification. The latter are very complex mixtures manufactured either in two steps^{13,14} or in one step¹³ using acetic acid (or anhydride), tartaric acid and mono- and diglycerides as starting materials. They are identified by fingerprinting TLC or after saponification^{6,15}.

In this study, HPLC and off-line mass spectrometry were used for separation and identification of various components of these two types of emulsifiers.

EXPERIMENTAL

Chromatography

Varian 5000 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a loop injector (Valco) and a Pyc Unicam LC-UV spectrophotometer detector (Pyc Unicam, Cambridge, Great Britain) at 213 nm was used. Mass spectrometry was performed on an AEI MS-9 high-resolution mass spectrometer, calibrated with perfluorokerosine (electron energy 80 eV). The columns (15 × 0.47 cm) were filled with 5- μm Partisil 5 silica gel (Whatman, Clifton, NJ, U.S.A.) or 10- μm LiChrosorb RP-18, octadecyl silica (Merck, Darmstadt, G.F.R.). The solvents hexane and acetonitrile, LC grade, were supplied by S.D.S. (Peypin, France); 2-propanol, spectroscopic grade, and perchloric acid (70%) were obtained from Prolabo (Paris, France).

Samples

1-Monostearine and 1-monopalmitine were purchased from Sigma (St. Louis, MO, U.S.A.); 1,2-dipalmitine, 1,3-dipalmitine, 1,2-distearine, tristearine and tripalmitine were from Fluka (Buchs, Switzerland). Monoglycerides and tartaric acid esters were dissolved in chloroform. Sodium and calcium stearylactylate (1 g) were

* European Economic Community (EEC) code number.

added to aqueous 1 M HCl (20 ml) and extracted with diethyl ether (3×7 ml). The organic layers were recombined and washed with water (7 ml), then with saturated sodium chloride solution (4 ml) (yield $\approx 75\%$).

Fatty acids analysis

Samples were saponified and esterified with boron trifluoride-methanol before injection in a Fractovap 2160 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a glass capillary column ($48 \text{ m} \times 0.2 \text{ mm I.D.}$) coated with Carbowax 20M. Carrier gas: hydrogen, flow-rate 2 ml/min. Temperature gradient: 150°C to 190°C at $2^\circ\text{C}/\text{min}$.

RESULTS

Distilled monoglycerides (emulsifier type E 471)

Analysis of these compounds by GLC^{16,17} and HPLC on a polar stationary phase¹⁰ has been described previously. Monoglycerides distilled under high vacuum contain more than 90% monoesters. Fig. 1 shows a separation obtained on a LiChrosorb RP-18 column and Table I the identified compounds from a sample of saturated distilled monoglycerides. As expected this emulsifier is mainly a mixture of monopalmitine and monostearine, and was probably manufactured from hydrogenated lard.

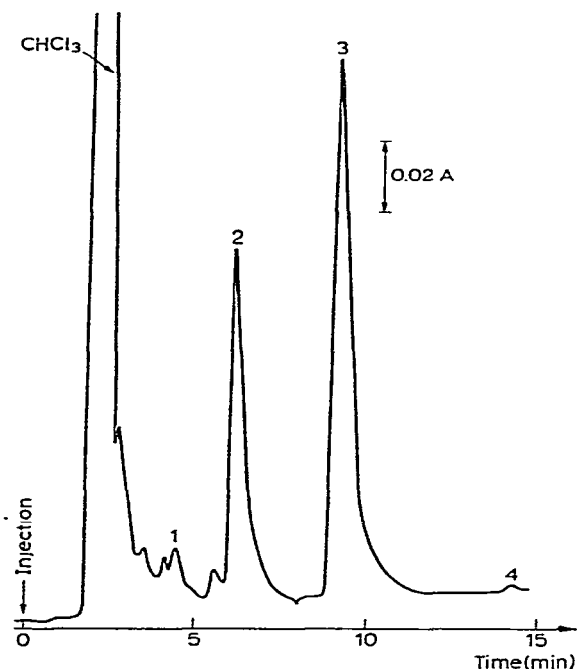


Fig. 1. Separation of saturated distilled monoglycerides by reversed-phase chromatography. Column: $15 \times 0.47 \text{ cm I.D.}$ Stationary phase: LiChrosorb RP-18, $10 \mu\text{m}$. Mobile phase: Acetonitrile; flow-rate 0.9 ml/min. UV detection at 204 nm. Peaks: 1 = monomyristine; 2 = monopalmitine; 3 = monostearine; 4 = monoarachidine.

TABLE I

IDENTIFIED COMPOUNDS IN A SAMPLE OF DISTILLED MONOGLYCERIDES (E 471)

Second column gives formula number in Appendix.

Identified compound	Formula number in Appendix
Monomyristine	1a
Monopalmitine	1b
Monostearine	1c

Calcium and sodium stearyllactylates (emulsifier types E 481, E 482)

A chromatographic study was made of four samples from two manufacturers. We observed a good resolution according to the lactoyl group number by adsorption chromatography (Fig. 2a, b) and according to the carbon number of the fatty acid radicals by reversed-phase chromatography (Fig. 3a, b). The compounds identified by off-line mass spectrometry are shown in Table II. The most typical fragmentations were at $m/e = M - \{p[\text{OCH}(\text{CH}_3)\text{CO}] + \text{OH}\}$ where $0 \leq p \leq n$ and n is the total lactoyl group number in the molecule and M the molar weight.

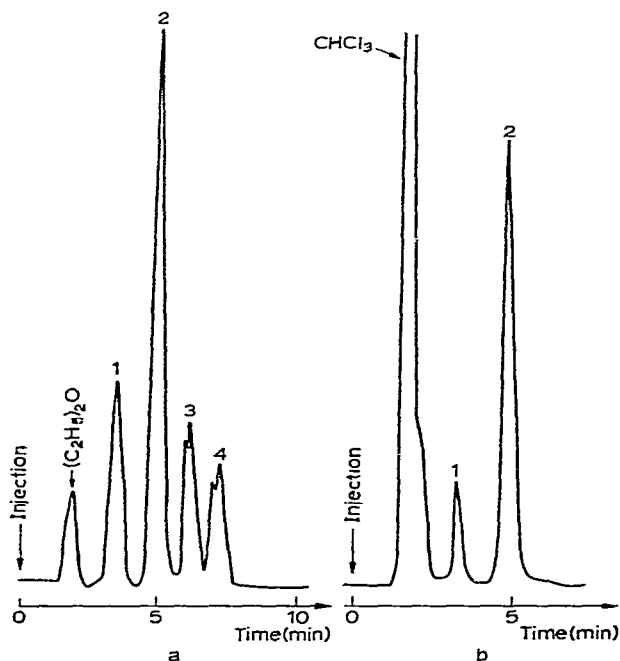


Fig. 2. Separation of calcium (a) and sodium (b) stearyllactylate by adsorption chromatography. Column: 15×0.47 cm I.D. Stationary phase: Partisil 5, $5 \mu\text{m}$. Mobile phase: hexane-2-propanol (with 2% water and 0.5% perchloric acid) (99.1:0.9 v/v). flow-rate 1.3 ml/min. UV detection at 204 nm. Peaks: 1 = fatty acids; 2 = fatty acids ester of lactic acid; 3 = fatty acid esters of lactoyl-lactic acid; 4 = fatty acid esters of di(lactoyl)lactic acid.

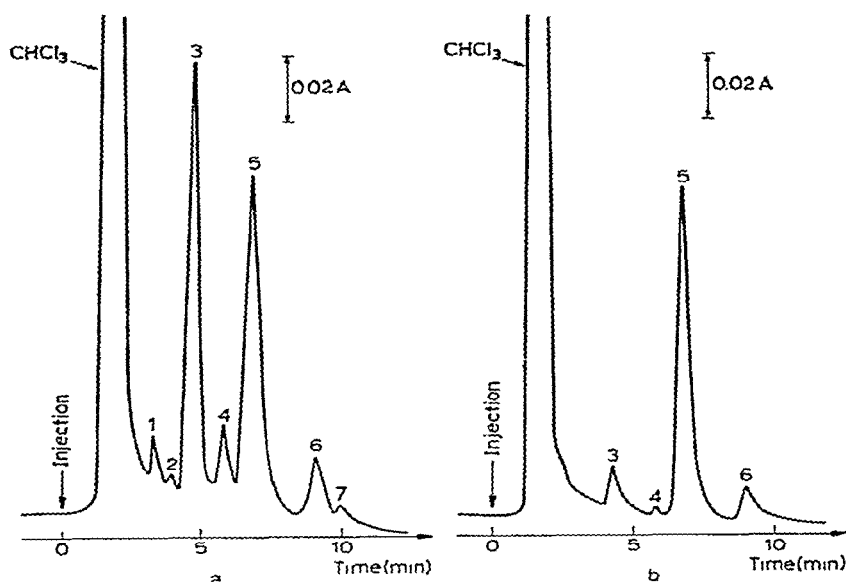


Fig. 3. Separation as in Fig. 2 but by reversed-phase chromatography. Column: 15×0.47 cm I.D. Stationary phase: LiChrosorb RP-18, $10 \mu\text{m}$. Mobile phase: acetonitrile + 10^{-4} M HClO_4 ; flow-rate 1.3 ml/min. UV detection at 204 nm. Peaks: 1 = myristoyllactic acid and myristoylpolyllactic acid; 2 = myristic acid; 3 = palmitoyllactic acid and palmitoylpolyllactic acids; 4 = palmitic acid; 5 = stearyllic acid and stearylpolyllactic acids; 6 = stearic acid; 7 = arachidoyllactic acid and arachidoylpolyllactic acids.

TABLE II

IDENTIFIED COMPOUNDS IN SAMPLES OF CALCIUM AND SODIUM STEAROYL-LACTYLATES (E 481, E 482)

Identified compound	Formula number in Appendix
Fatty acids	2a, b, c
Fatty acid esters of lactic acid	3a, b, c, d
Fatty acid esters of lactoyllactic acid	4a, b, c, d
Fatty acid esters of di(lactoyl)lactic acid	5a, b, c

In each sample, the main components are fatty acid esters of lactic acid, and not, as generally thought (except in Regula's TLC work¹²) fatty acid esters of lactoyllactic acid. In three samples, fatty acid esters of polymeric lactic acid were found.

A semi-quantitative analysis shows that about half of the total lactic acid content of the emulsifier is esterified with fatty acids.

Acetylated tartaric acid esters of mono- and diglycerides (emulsifier types E 472 e, E 472 f)

The study was done by adsorption liquid chromatography on ten samples

from four manufacturers; although identification by mass spectrometry was carried out only on four samples, the results obtained may be extended to all ten samples.

Most of the components were separated in a semi-preparative way with four separate isocratic runs using mobile phases of decreasing elution strength. In the first run the more polar products were isolated and the less retained ones were recovered as a mixture. In the second run, with a weaker mobile phase, the mixture previously recovered was chromatographed; the more retained products were isolated and the less retained ones were again recovered as a mixture. In this way (and with two other runs), most of the components were isolated.

The chromatographic separation of the more polar compounds from two

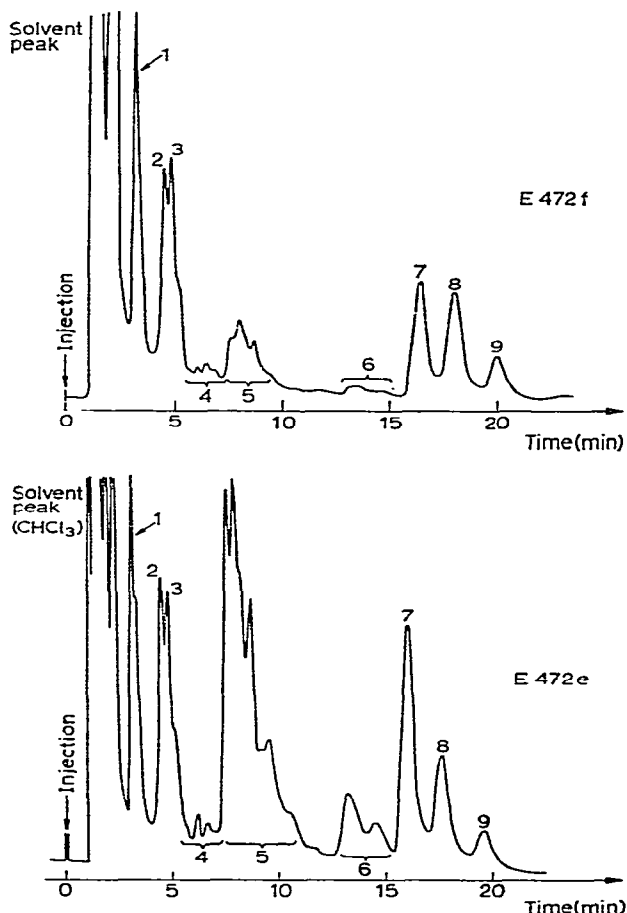


Fig. 4. Separation of tartaric acid esters by adsorption chromatography. Column: 15×0.47 cm I.D. Stationary phase: Partisil 5, $5 \mu\text{m}$. Mobile phase: hexane-2-propanol [2% water and 0.2% perchloric acid (70%)] (92:8, v/v); flow-rate 1.5 ml/min. UV detection at 213 nm. Peaks: 1 = acetic acid and acetylated monoglycerides; 2 = diacetyltartaric acid ester of diglycerides; 3 = acetic and diacetyltartaric acid esters of monoglycerides; 4 = monoglycerides; 5 = diacetyltartaric acid esters; 6 = acetyltartaric acid esters; 7 = tartaric acid esters; 8 = di(diacetyltartaric acid) esters; 9 = acetyl- and diacetyltartaric acid esters of monoglycerides.

saturated* emulsifiers is shown Fig. 4a, b and the compounds identified are listed in Table III. The different products were identified by off-line mass spectrometry of their trimethylsilyl derivatives** and direct admission into the ionizing chamber. The most typical fragmentations were at $m/e = M' - \text{CH}_3$, $M' - \text{RCOO}$ and $M' - \text{RCOOCH}_2$ where R = fatty acid radical or $(\text{CH}_3)_3\text{SiOCOCH}(\text{OR}_1)\text{CH}(\text{OR}_2)$ with $\text{R}_1, \text{R}_2 = \text{Si}(\text{CH}_3)_3, \text{CH}_3\text{CO}$. Ions $[\text{RCOOC}_3\text{H}_6\text{O}]^+$ were observed in every spectrum. These kinds of fragmentations are well-known with silanized derivatives of diglycerides¹⁸, we also observed them for trimethylsilyl derivatives of the different tartaric acid esters of monoglycerides.

TABLE III

IDENTIFIED COMPOUNDS IN SAMPLES OF ACETYLATED TARTARIC ACID ESTERS OF MONO- AND DIGLYCERIDES (E 472 e, f)

<i>Identified compound</i>	<i>Formula number in Appendix</i>
Acetyl- and diacetyltartaric acid esters of monoglycerides	6
Di(diacetyltartaric acid) esters of monoglycerides	7
Tartaric acid esters of monoglycerides	8
Acetyltartaric acid esters of monoglycerides	9
Diacetyltartaric acid esters of monoglycerides	10
Acetic and diacetyltartaric acid esters of monoglycerides	11
Diacetyltartaric acid esters of diglycerides	12

Separation of medium polar and apolar compounds was achieved with a saturated emulsifier (containing stearic acid as more than 80% of the total fatty acid content). Chromatographic separations are shown Fig. 5, and the results are in Table IV. The different products were identified by their mass spectra¹⁸ as determined by gas chromatography-mass spectrometry.

TABLE IV

MEDIUM POLAR AND APOLAR COMPOUNDS IDENTIFIED IN A SAMPLE OF DIACETYLTARTARIC ACID ESTERS OF MONO- AND DIGLYCERIDES (E 472, e)

<i>Mobile phase composition (% v/v)</i>	<i>Identified compound</i>	<i>Formula number in Appendix</i>
Hexane 2-Propanol (0.8% water)	Acetic acid	—
	Acetylated 1,3-monoglycerides	13
96.3 3.7	Acetylated 1,2-monoglycerides	14
99.1 0.9	1,3-Diglycerides	15
	Fatty acids	2
99.75 0.25	Triglycerides	16

* With nearly the same fatty acid composition, as determined after saponification, methylation and GC analysis. Stearic and palmitic acids are the major fatty acids.

** Obtained with N,O-bis(trimethylsilyl)acetamide (BSA) in dichloromethane.

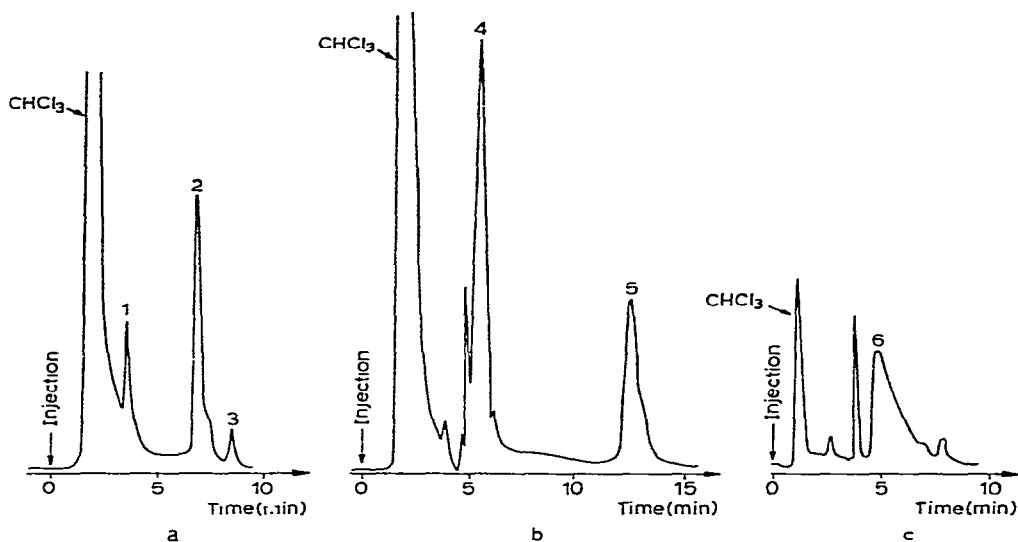


Fig. 5. Separation polar and apolar compounds in a sample of diacetyltartaric acid esters of mono- and diglycerides. Column: 15×0.47 cm I.D. Stationary phase: Partisil 5, $5 \mu\text{m}$. Flow-rate 1.3 ml/min. UV detection at 204 nm. Mobile phase: hexane-2-propanol (0.8% water) (96.3:3.7) (A), (99.1:0.9) (B) and (99.75:0.25) (C). Peaks: 1 = acetic acid, 2 = acetylated 1,3-monoglycerides; 3 = acetylated 1,2-monoglycerides; 4 = fatty acids; 5 = 1,3-diglycerides; 6 = triglycerides.

As expected from the starting materials, these emulsifiers are very complex mixtures which show only quantitative differences in liquid chromatographic analysis. These differences may be due to the processing conditions (temperature, reaction time) and the ratio and source of the starting materials.

Aging of an emulsifier sample was also studied. A sample which was previously a powder became a pasty block having a strong odour of acetic acid on storage at

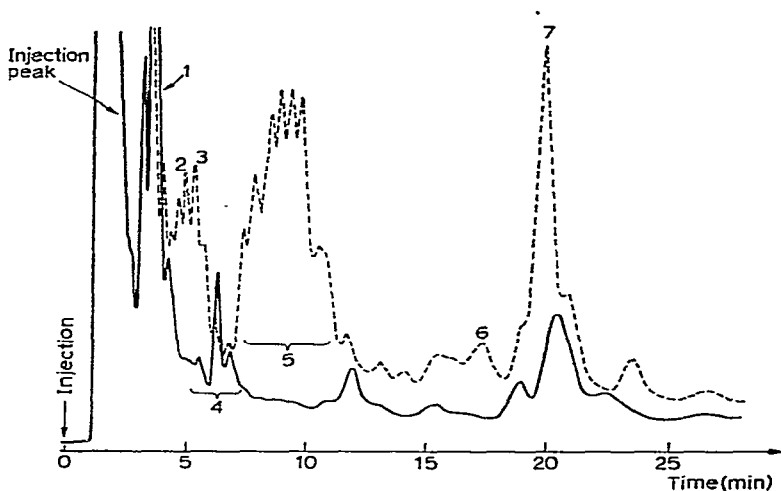
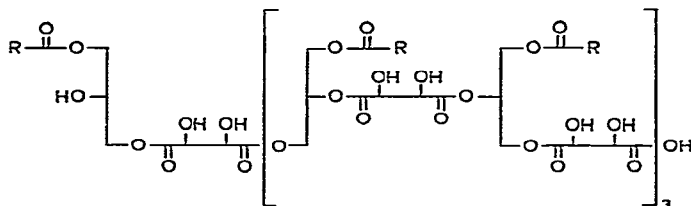


Fig. 6. Aging of an emulsifier sample. Operating conditions and peak numbers as in Fig. 4. —, Old sample (*ca.* 6 years old); ---, new sample, manufactured from hydrogenated fish oil (shown by its fatty acid analysis).

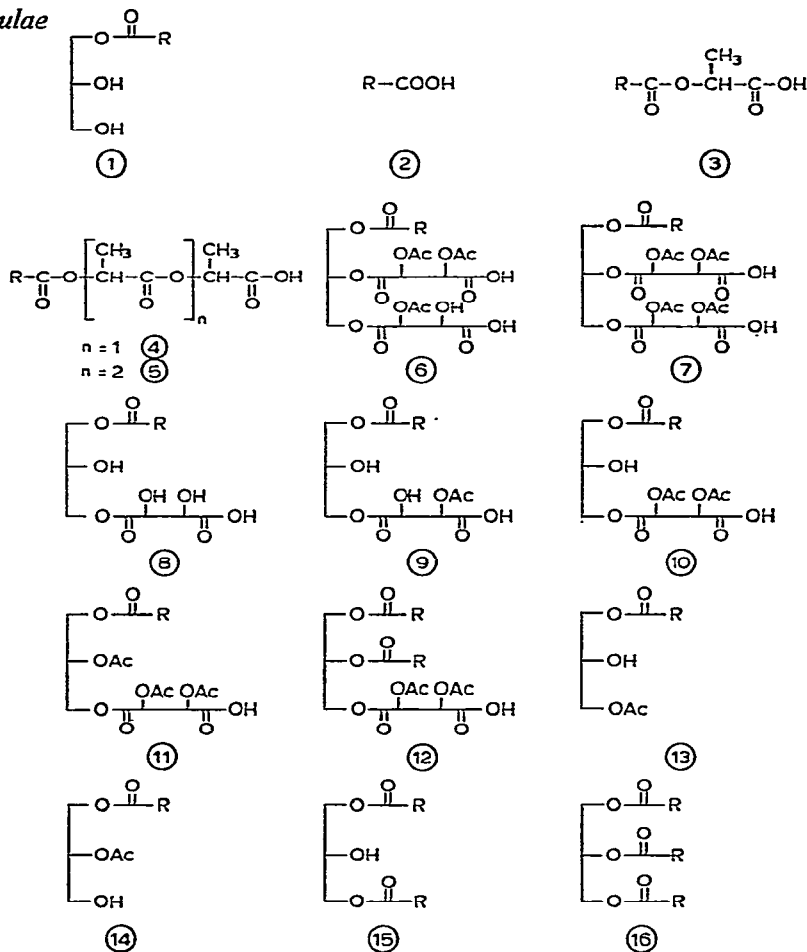
room temperature for several years. The emulsifier lost most of its acetic acid (Fig. 6) and higher-molecular-weight materials were formed (MW about 2500 Daltons). Such compounds may result from polycondensation of six to seven glycerol units linked by tartaric acid bridges with, for instance, the following structure:



Compounds with molecular weights higher than 1300 (after silylation) were also isolated by HPLC but not identified.

APPENDIX

Formulae



Only one isomer is shown. The actual structures and the number of isomers in each peak are not known. (a) $R = C_{13}H_{27}$; (b) $R = C_{15}H_{31}$; (c) $R = C_{17}H_{35}$; (d) $R = C_{19}H_{39}$.

CONCLUSION

Analysis of sodium or calcium stearoyllactylate (four samples from two manufacturers) shows that the major components are 2-stearoyl and 2-palmitoyllactic acid and their salts (or 2-stearoyloxypropionic acid and 2-palmitoyloxypropionic acid and their salts).

Acetylated tartaric acid esters of mono- and diglycerides are complex mixtures of which at least twelve different components, not including isomeric forms and homologues, were identified and isolated. On aging they lose acetic acid and form polymeric materials.

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