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Project Abstract
for Food Standards News
(approximately 150 words)

Agency funded research has further developed methods for the determination of stearoyl lactylates, (E481 and E482), sorbitan ester emulsifiers (E491 to E495) and mono- and di-acetyl tartaric acid esters of mono- and di-glycerides of fatty acids (E472 (DATEM)) in foods. These methods involve;

- **The analysis of methylated stearoyl lactylate derivatives by gas chromatography,**
- **Hydrolysis of DATEM to tartaric acid and then analysis by ion-chromatography,**
- **Hydrolysis and acetylation of sorbitan esters to sorbitan and then analysis by gas chromatography.**

In addition a partially validated method for the determination of polyphosphate has been developed, based on hydrolysis of polyphosphates to the orthophosphate anion and then ion chromatography. By determining the orthophosphate ion content before and after hydrolysis polyphosphate content can be derived.

These methods have been shown to work in a number of food matrices and have been internally validated. Once fully validated these methods would be used to enforce legislation and monitor usage and intakes of these additives.

A similar Agency funded project on the development of a method for the determination of ammonium phosphatide (E442) has recently been completed and is available from the library.

Any additional information or instructions

RESEARCH PROJECT - FINAL REPORT FORM

FOOD STANDARDS AGENCY

Section 1 : Project Details

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Development of Methods for the Determination of Emulsifiers and Polyphosphates in Foods

Executive Summary

Robust methods are required for the determination of emulsifiers permitted under the Miscellaneous Food Additives Regulations 1995 (as amended) [1]. Methods will assist enforcement of these Regulations and facilitate the study of dietary intakes.

Three emulsifier groups were selected for study: the stearoyl lactylates (E-481 and E-482), mono- and di-acetyl tartaric acid esters of mono- and di-glycerides of fatty acids (E-472e), and sorbitan fatty acid esters (E-491 to E-495). These emulsifiers are widely permitted for use in foods including staple items such as bread. An additional aspect to the project involved the development of a method to determine polyphosphates (E-452) in foods – another permitted additive for which reliable methodology is sought.

Characterisation of each of the selected emulsifiers using potential analytical strategies formed the initial objective. This process was intended to identify an appropriate analytical approach and possible sources of interference from natural food components. The second stage then aimed to develop the selected approach for the analysis of food matrices.

Throughout this project two alternative approaches have been considered for the analytical determination of the additive, either a direct analysis of intact chemical constituents or an in-direct approach where the additive is first hydrolysed and the hydrolysis products are used as the analytical indices. A model bread system was considered to be an analytically challenging matrix and was used for the development of methods for extraction of these additives.

A method has been developed for polyphosphate determination based on the conversion of polyphosphate to a single analyte, the orthophosphate anion. By determining orthophosphate content before and after hydrolysis of polyphosphates, polyphosphate content, expressed as a phosphate equivalent is derived. This in turn

can be converted to a phosphorus pentoxide basis, the basis used in the Regulations. The procedure has been validated for the determination of polyphosphates in a variety of foods including ham and processed cheese. The analysis was shown to be specific for polyphosphates and potential interferences from organic phosphate species were removed. Acceptable recovery and repeatability were achieved and a limited set of retail samples was tested.

The method developed for stearoyl lactylate determination in foods is based upon the analysis of the methylated derivatives by gas chromatography. Commercial emulsifiers have also been characterised by in-direct analysis of lactic acid following hydrolysis. The latter approach offered the attraction of a single measure for stearoyl lactylates but was shown not to be suitable as an index of stearoyl lactylates in foods. The direct analysis provided a specific measure of stearoyl lactylates for a limited set of retail samples and demonstrated good recovery and repeatability.

The method developed for DATEM determination in foods is based upon hydrolysis to tartaric acid and analysis of the latter by ion-chromatography. Commercial emulsifiers have also been examined by a direct approach using normal-phase HPLC. The latter approach produced broad peak envelopes, which varied between different DATEM sources and offered no suitable index of DATEM in foods. The final developed method provided a specific measure of DATEM when used to test a limited set of retail samples. Rates of recovery, however, were low and meant that the procedure was only semi-quantitative. Further work is suggested to improve recovery and allow quantitative determination.

The method developed for sorbitan ester determination in foods is based upon sorbitan analysis by gas chromatography after hydrolysis and acetylation. A combination of sorbitan peaks was proposed as the measurement index. Commercial emulsifiers have also been analysed by a direct analysis of sorbitan esters following silylation and gas chromatography. The latter approach produced highly complex chromatograms that were unsuitable for measurement of sorbitan esters in foods. The final developed method provided a specific measure of sorbitan esters for the majority of a set of retail sample types tested and demonstrated good recovery and repeatability.

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1. Introduction

Background

Robust methods are required for the determination of emulsifiers permitted under the Miscellaneous Food Additives Regulations 1995 (as amended) [1]. Methods will assist enforcement of these Regulations and facilitate the study of dietary intakes. Validated methods for analysis of some emulsifiers are currently unavailable. Moreover, defining which compounds should be measured to satisfy the legislation is by no means straightforward.

Commercial emulsifiers used in food are derived from edible fats, chemically modified to give both hydrophobic and hydrophilic character. The products are complex mixtures of compounds and therefore the actual analytical moieties to be determined must be established. Characterisation of each of the selected emulsifiers using potential analytical strategies formed the initial objective. This process was intended to identify an appropriate analytical approach and possible sources of interference from natural food components. The second stage then aimed to develop the selected approach for the analysis of food matrices.

Three emulsifier groups were selected for study: the stearoyl lactylates (E-481 and E-482), mono- and di-acetyl tartaric acid esters of mono- and di-glycerides of fatty acids (E-472e), and sorbitan fatty acid esters (E-491 to E-495). These emulsifiers are widely permitted for use in foods including staple items such as bread.

Stearoyl lactylates are permitted for use in a range of foods including cereal-based foods, cakes, desserts, confectionery and other products such as hot beverage powders. DATEM emulsifiers are permitted for general use in foods. Sorbitan ester emulsifiers are permitted for use in a range of foods including cakes, desserts, ices, confectionery, toppings and coatings and other less-consumed products such as beverage whiteners and emulsified sauces. However, it should be noted that despite the extensive list of permitted uses, the subsequent search of retail outlets for test samples revealed relatively few of these food products with these emulsifiers declared in the list of ingredients.

Model bread systems (with these emulsifiers added) were considered to be an analytically challenging matrix for the extraction and determination of these additives. This bread, with the inclusion of added fat, sugar and milk powder and the further complexity derived from the processing steps of fermentation and baking, was chosen as a suitable matrix for the development of extraction methods. It was anticipated that methodologies applicable to this bread would be sufficiently robust as to be applicable to a wide range of foods.

An additional aspect to the project involved the development of a method to determine polyphosphates (E-452) in foods – another permitted additive for which reliable methodology is sought.

Throughout this project two alternative approaches have been considered for the analytical determination of the additive, either a direct analysis of intact chemical constituents or an in-direct approach where the additive is first hydrolysed and the hydrolysis products are used as the analytical indices.

1.1 Determination of Polyphosphates

Polyphosphates are produced by the high temperature condensation reaction of orthophosphates (typically sodium orthophosphate) to initially form chains of two or three phosphate units and by further heating to yield longer phosphate chains of from four to thirty or more units (Fig.1). The di-, tri- and polyphosphates are permitted additives listed in the Regulations [1] as E-450, E-451 and E452, respectively.

Polyphosphates are used in a range of food products including meat and fish, processed cheese and coffee whiteners. In meat and fish their function is to solublise protein to enable binding and water retention. In processed cheese they act as emulsifiers breaking the calcium bridges between cheese proteins, converting these insoluble complexes into separate soluble protein molecules.

Analytical Approaches

Published methods for the reliable quantification of polyphosphates in food are currently not available. Indirect methods measure total and organic phosphorus but then do not differentiate polyphosphates from naturally present orthophosphate. Polyphosphates have been analysed qualitatively by TLC [2]. Ion-chromatography allows the separation of individual polyphosphate polymers with detection using conductivity or post-column reaction [3,4,5]. However, quantitative determination of the many polymeric forms is not practical. This is because of the need to resolve all forms from matrix interferences and the fact that while each form requires separate calibration, pure standards for each of these are unavailable. The Miscellaneous Food Additives Regulations 1995 (as amended) [1] express limits for polyphosphates as phosphorus pentoxide (P_2O_5). Thus the requirement is for a method that gives a single result for polyphosphate rather than separately quantifying individual polymers.

The proposed approach addresses these issues by converting polyphosphate to a single analyte, the orthophosphate anion. By determining orthophosphate content before and after hydrolysis of polyphosphates, polyphosphate content is expressed as a phosphate equivalent. This in turn can be converted to a phosphorus pentoxide basis as follows: one mole of phosphate (FW 95.0) is equivalent to half a mole of

phosphorus pentoxide (FW 141.9). Thus unit mass of phosphate can be converted to unit mass phosphorus pentoxide by multiplying by (0.5x141.9)/95.0 (i.e a factor of 0.747).

Using ion chromatography with suppressed conductivity detection, phosphate is typically well resolved from other anions and potential interferences. The analysis then requires only a measurement of phosphate before and after hydrolysis of polyphosphates to orthophosphate. Polyphosphates are known to hydrolyse relatively easily and indeed the project aimed to ensure that hydrolysis during extraction was minimised. Another requirement of this approach is that organic phosphates (e.g RNA components and phytic acid) are removed prior to the hydrolysis step. Otherwise phosphates may be formed from this source following hydrolysis rather than solely from polyphosphates.

Developing procedures for the extraction of polyphosphates from foods formed an important aspect of the project. The selection of food matrices for study was based firstly on the Directive [1] and secondly on the actual usage, i.e. by examining ingredient declarations of retail samples.

1.2 Determination of Stearoyl Lactylates

Stearoyl lactylates are the condensation products from the reaction of fatty acids and lactic acid (a hydroxy acid), neutralized to the sodium or calcium salts, E-481 and E-482, respectively (Figs.2&3). Commercial emulsifier formulations are mainly a mixture of the palmitoyl (C16) and stearoyl (C18) lactylates, present as lactylate oligomers from mono- up to possibly penta- or hexa-lactylates. Stearoyl lactylates is a collective term, covering all fatty acid variants.

Stearoyl lactylates have found particular application in the baking industry, giving increased loaf volume and improved crumb structure. In addition, they are used in other wheat flour products and may be used in cereal snacks, confectionery and desserts, for example.

Analytical Approaches

The potential routes to the determination of stearoyl lactylates in foods were considered. Firstly, the direct determination of the stearoyl lactylates moieties and secondly, indirect determination as lactic acid following hydrolysis. The latter approach offered the attraction of a single measure for stearoyl lactylates irrespective of the actual moieties present. These approaches are described below.

Although methods for the quantitative determination of stearoyl lactylates in bread and related products have been published [6,7], these are from 1981 and 1986 respectively. Since then no preferred, robust and reliable methodology has emerged. The analytical approach reported here has taken the published methods as a starting point, with rigorous development to improve and optimise the analytical procedures. Common to the determination of most commercial emulsifiers, analytical difficulties are posed by:

i) variable composition
ii) variable 'active agent' content
iii) 'active agent' is not a single compound
iv) analytical reference standards are not available

The initial priority is an analytical method to characterise commercial emulsifier formulations and identify and quantify the active agents. From this information, the most appropriate index/indices for the measurement of the emulsifier in foods should be identifiable. These analyses also provide detailed compositional data of the emulsifier as a food ingredient.

Both published methods for stearoyl lactylate determination are based upon the analysis of the methylated derivatives by gas chromatography. The approach adopted here has taken this as the fundamental means of determination and optimised both quantification and information gained. The following issues have been examined:

i) yield of methylated products (reaction conditions)
ii) maximizing compositional data from capillary GC analysis
iii) basis of quantification, identity and means of use of internal standard
iv) sample treatment and extraction of stearoyl lactylates from foods

The model bread system described earlier was used to develop extraction methodology. White breads were prepared incorporating a stearoyl lactylate emulsifier at 0.2% addition on flour weight, according to the recipe given in Appendix.

1.3 Determination of DATEM Emulsifiers

The properties of mono-glycerides, emulsifiers in their own right, can be radically altered by reacting them with tartaric acid. DATEM (E-472e) is the shortened description for diacetyl-tartaric acid esters of monoglycerides. Tartaric acid is a di-hydroxy, di-carboxylic acid. Acetic acid (or acetic anhydride) is used to acetylate the two hydroxy groups. Esterification of one of the acid groups with the terminal hydroxy group of a mono-glyceride yields DATEM (Fig.4). In common with other fat-based emulsifiers, the fatty acids present are typically stearic (C18:0) and palmitic (C16:0), though liquid DATEM preparations incorporating oleic acid (C18:1) are also available.

Reaction of monoglycerides, tartaric acid and acetic acid can lead to a complex mixture of products. For example acetylation may occur on the monoglyceride hydroxyl group (at the 2-position) and may be absent on one or both of the tartaric acid hydroxyl groups. Additionally, esterified diglycerides as well as monoglycerides may be present. Sudraud et al [8] identified at least 12 species in commercial DATEM.

DATEM are commonly used in bread improvers to increase loaf volume and improve crumb structure. Their ability to complex with flour protein, apparently strengthening the gluten produced, gives improved dough gas retention properties.

Analytical Approach

As was the case with the stearoyl lactylates, two potential routes to the determination of DATEM were considered. Firstly, the direct analysis of intact DATEM and secondly, indirect determination of tartaric acid following hydrolysis.

A number of studies employing a hydrolysis approach to the analysis of DATEM are cited in the literature. In these, tartaric acid is typically determined after hydrolysis and silylation by gas chromatography [9,10], though colorimetry has also been used [11].

An indirect approach involving the hydrolysis of DATEM to tartaric acid was developed at RHM Technology some years ago. This approach has been shown to permit the detection of DATEM addition to bread but has only been employed as a qualitative tool. This method therefore requires development to provide a quantitative and robust method.

Sudraud et al [11] used normal phase HPLC and UV detection to analyse DATEM emulsifiers directly. But these workers were concerned with identifying the chemical forms present rather than providing a quantitative method for measuring DATEM in foods. The problems with the direct approach are the same as already listed for stearoyl lactylates:

i) variable composition
ii) variable 'active agent' content
iii) 'active agent' is not a single compound
iv) analytical reference standards are not available

The proposed approach for the direct analysis of DATEM was to evaluate HPLC conditions used in-house for phospholipid analysis. This involves separation by normal-phase chromatography and detection with a light-scattering detector.

Both direct and indirect approaches were employed to characterise commercial formulations. From this evaluation, the most appropriate methodology was identified for the measurement of emulsifier in foods.

The extraction methodology was developed using the white bread model system described earlier. White breads were prepared incorporating DATEM emulsifier at 0.2% addition on flour weight, according to the recipe given in Appendix.

1.4 Determination of Sorbitan Esters

Sorbitan esters are the products from the two-step dehydration and esterification reaction of sorbitol and fatty acids. Given the possibility that commercial sorbitol may contain other polyols and, more importantly, yield a range of anhydrides (Fig.5), there is the potential for an extremely complex mixture of products. This is further compounded in commercial emulsifier usage by the approval for use of five sorbitan ester products (E491 – E495), namely, monostearate, tristearate, monolaurate (C12), monooleate and monopalmitate.

Sorbitan esters have application in the maintenance of oil in water / water in oil emulsions and may be used in products such as confectionery, cakes and desserts.

Analytical Approaches

In common with the approach followed for both stearoyl lactylates and DATEM emulsifiers, both direct and indirect routes were studied. Direct analysis of intact sorbitan esters was compared with analysis of hydrolysates. In this case though, hydrolysis yields not just a single index compound (c.f lactic and tartaric acids), but instead a mixture of sorbitol and its anhydrides (Fig.5).

As far as the authors are aware, there is only one publication [12] that refers to the analysis of sorbitan ester emulsifiers. This reports the determination of hydrolysis products by GC but has only been tested against a coconut oil matrix. Thus, there is no preferred and robust methodology for the determination of sorbitan esters in foods. The analytical approaches pursued in this work cover two phases:

i) methodology to characterise commercial emulsifiers as added to foods

ii) methodology based upon hydrolysis pre-treatment [12] and further developed to provide more compositional data on formulations. This leading to the identification and measurement of appropriate index compound(s) for the quantitative determination of sorbitan esters in foods.

The method development reported here has investigated the issues of:

i) determination of sorbitan mono-, di- and tri- esters
ii) determination of residual non-esterified sorbitans
iii) hydrolysis conditions to yield total sorbitans
iv) sample treatment and extraction of sorbitan esters from foods
v) treatment of food extracts and their derivatisation to include quantification and index of sorbitan level

White breads were prepared incorporating sorbitan ester emulsifier at 0.2% addition on flour weight, according to the recipe given in the Appendix. This model system was used to develop methods for emulsifier extraction.

2. Methods and Samples

2.1 Determination of Polyphosphates

The approach adopted for this analysis requires a measurement of orthophosphate ion before and after hydrolysis of polyphosphates to phosphate. Ion chromatography with suppressed conductivity detection was chosen for the determination of phosphate. Methods developed for the characterisation of commercial polyphosphates and the determination of polyphosphates in food matrices are described below.

2.1.1 Commercial Polyphosphate Samples

Commercial polyphosphate samples were supplied by Fiske and Rhodia (Table 3).

2.1.2 Determination of Orthophosphate by Ion-Chromatography

Orthophosphate was determined by ion-chromatography on an anion-exchange column with suppressed conductivity detection, using the following conditions:

Column:	Dionex Ionpac AS11 250mm x 4mm +
	Ionpac AG11 guard column 50mm x 4mm
Mobile phase:	Sodium hydroxide/methanol gradient.
	1mM to 45mM NaOH / 10% to 20% methanol
Flow rate:	2ml/min
Injection volume :	50µl
Detection:	Suppressed conductivity

Phosphate eluted at around 13 minutes.

2.1.3 Hydrolysis of polyphosphates to orthophosphate

Both alkaline and acid hydrolysis conditions were evaluated by boiling solutions of di- and tri-phosphates with sodium hydroxide solution or dilute hydrochloric acid (HCl). Orthophosphate concentrations were measured by ion-chromatography to determine the degree of hydrolysis (see above for conditions). Quantitative hydrolysis

was readily achieved using dilute HCl and optimisation of acid concentration and boiling time lead to the conditions described below for the characterisation of commercial polyphosphates and for their determination in foods.

2.1.4 Characterisation of Commercial Polyphosphates

Polyphosphates were diluted to give concentrations of around 500ppm. These solutions were mixed 1:1 (5ml of each) with dilute HCl (0.5M) and boiled for 60 minutes. After this time the solution was boiled to dryness to remove residual HCl and after making up in water (50ml, a 10-fold dilution), orthophosphate was measured by ion-chromatography.

2.1.5 Extraction and Determination of Polyphosphate in Foods

The method described below was selected after optimising both the trichloroacetic acid (TCA) concentration and holding time prior to TCA removal. TCA concentrations from 2% to 10% and holding times up to 20 hours were evaluated.

A 5g sample was homogenised using a top drive macerator with 80ml ice-cold trichloroacetic acid (10%) for one minute. The mixture was centrifuged for 10 minutes at 3500 rpm with sub-ambient cooling. The supernatant was filtered through GF/C glassfibre paper and 50-60 ml transferred to a 500ml fat flask with a 50-60ml side-tube. Water-saturated diethyl-ether (50-55ml) was added and the two phases mixed vigorously using a magnetic stirrer bar for 5 minutes. After standing to allow the phases to separate, the lower aqueous layer was tipped into the side-tube and the ether layer decanted off to waste. Two further extractions were performed, each with 50ml ether before blowing residual ether off using a nitrogen stream. The aqueous extract was then diluted 10-fold (e.g. 5ml to 50ml).

A portion of this solution was passed through a 1g C18 SPE cartridge (previously conditioned with methanol and then water). After discarding the first 2ml to elute, 4-5ml was collected for analysis of free-phosphate.

A second portion of the solution was also cleaned using a conditioned 1g C18 SPE cartridge. After discarding the first 2ml to elute, 6-7ml was collected. A 5ml aliquot of this cleaned solution was added to a 50ml plastic screw-capped centrifuge tube. 5ml of 0.5M HCl was added, the tube capped and placed in a boiling water bath for 1 hour. After this time the cap was removed and boiling continued until virtually all the water has evaporated. The last traces of water were removed by heating at 70°C in a vacuum oven for 30 minutes. The residue was re-dissolved in water (10ml) before analysis of total phosphate by ion-chromatography.

2.2 Determination of Stearoyl Lactylates

A number of different analytical approaches for the characterisation of commercial lactylates and the determination of lactylates in food matrices were tested. These are outlined below.

2.2.1 Commercial Stearoyl Lactylate Samples

Commercial stearoyl lactylate samples were supplied by Abitech, Croda, Danisco Cultar and Quest. These included both sodium and calcium lactylate formulations (Table 14).

2.2.2 Analysis of Emulsifier Formulations by Direct Determination of Lactylates (SLs) Following Methylation

Initial work gave highly variable results (yields) for methylated SLs, which was subsequently proven to be due to the labile nature of the methylated derivatives in the presence of the methylating reagent, boron trifluoride (3%) in methanol. The following procedure was therefore developed for the quantitative characterisation of the emulsifiers.

Emulsifier (50 mg) was mixed with internal standard, methyl tricosanoate [C23methyl ester] (2ml of a 1 mg/ml solution in hexane) in a McCartney bottle and the hexane removed under nitrogen. This was treated with boron trifluoride/methanol (3.0 ml), sonicated for 1-2 min and reacted for 90 min at room temperature. Hexane (2.0 ml) was added and mixed vigorously, rapidly followed by the addition of water to stop the reaction. After separation of the hexane phase, this methyl ester extract was removed for GC analysis.

2.2.3 GC Determination of Stearoyl Lactylates

The conditions for GC analysis were primarily selected for the separation of mono-, di- and tri-lactylates but were also changed to allow for the detection of any higher oligomers.

Both published methods for SLs used non-polar capillary columns, but this work has found that polar columns (as typically used for fatty acid analysis) provide a preferred separation, even though these do not elute tetra- or higher lactylates under the

conditions described below. The following conditions were used for the analysis of both formulations and food extracts:

Column: 30m x 0.22mm x 0.25 micron BPX-70
Carrier gas: hydrogen - 100 kPa
Injection: split mode – injector 275 °C
Detector: FID – 275 °C
Column temperature: 160°C (hold 1 min), ramp 4 °C /min to 250°C (hold 5 min)

The following conditions were only used for checking selected formulations for the presence of oligomers higher than tri-lactylates:

Column: 15m x 0.25mm x 0.1 micron Rtx-1
Carrier gas: hydrogen – 30 kPa
Injection: split mode – injector 300 °C
Detector: FID – 325 °C
Column temperature: 160°C (hold 1 min), ramp 4 °C /min to 300°C (hold 5 min)

2.2.4 Total Lactic Acid Method

A sample of the emulsifier (1g) was weighed into a 50ml screw-capped plastic centrifuge tube and 15ml sodium hydroxide (1M) added. The mixture was then heated in a boiling water bath for one hour. After cooling, the hydrolysate was transferred quantitatively with water to a 250ml volumetric flask and made to volume. Excess fatty acids were then removed from the aqueous solution by transferring to a 400ml beaker, adding approximately 30ml hexane and then adding 15ml HCl (1M) by pipette, whilst stirring with a magnetic stirrer bar. A portion of the aqueous layer was removed and filtered through GF/C filter paper prior to HPLC analysis.

2.2.5 Free Lactic Acid Method

A sample of the emulsifier (0.5g) was dispersed in 100ml water by stirring with a magnetic stirrer. The pH of the solution was adjusted to 6.0-6.5 with sodium hydroxide solution (0.1M) before transferring to a volumetric flask (250ml) and making to volume with water. A portion of this solution was passed through a conditioned (washed with methanol and then water) C18 SPE cartridge (500mg/3ml). After allowing 1.5ml to go to waste, 1.5 ml was collected for HPLC analysis.

2.2.6 HPLC determination of lactic acid

Lactic acid was determined by reverse-phase HPLC with UV detection using the following conditions:

Columns : 2 x Inertsil ODS-3 250mm x 4.6mm in series + C18 guard cartridge

Mobile phase: Isocratic conditions during elution of lactic acid (100% phosphate buffer pH 2.75) followed by a column wash with 70% buffer /30% acetonitrile.

Flow rate :1ml/min
UV detection : 210nm

Lactic acid eluted at around 10 minutes.

2.2.7 Extraction and Analysis of Stearoyl Lactylate Emulsifiers from Foods

Comminuted sample (10-12g) was weighed into a 250ml Duran bottle and MES/TRIS buffer (40ml) and alpha-amylase (Sigma) (0.3ml) added. The bottle was loosely capped and placed in a shaking water bath at 70°C for 1-2 hours. The mixture was then cooled and homogenized in situ with a top drive macerator. This digest was stored in a refrigerator until required.

MES/TRIS Buffer was prepared by dissolving 4.88g MES (4-morpholine ethanesulphonic acid) and 3.05g TRIS (Tris(hydroxymethyl)aminomethane) in water and making to 500ml.

The enzyme digests were extracted as follows:

Internal standard, methyl tricosanoate (C23methyl ester) (5.0ml x 0.2mg/ml chloroform) was added to the total digest in the Duran bottle followed by chloroform/methanol (50ml) and the two phases mixed rigorously with a magnetic stirrer for 15 min. Chloroform (100ml) was then added and stirring continued for a further 10 min. The phases were allowed to separate and an aliquot taken of the chloroform layer (30-50ml) and evaporated to dryness. The resulting dry residue was then methylated according to the conditions described above.

2.3 Determination of DATEM Emulsifiers

A number of different analytical approaches for the characterisation of commercial DATEM formulations and the determination of DATEM in food matrices were tested. These are outlined below.

2.3.1 Commercial DATEM Samples

Commercial DATEM samples were supplied by Abitech, Danisco Cultar and Quest (Table 21).

2.3.2 Determination of tartaric acid content of emulsifier formulations

A sample of DATEM (1g) was weighed into a 50ml screw-capped plastic centrifuge tube and water (15ml) and concentrated hydrochloric acid (5ml) were added. The mixture was then heated in a boiling water bath for one hour. After cooling, the hydrolysate was transferred quantitatively with water to a 400ml beaker. The hydrolysate was then heated on a boiling steam bath to dryness. The beaker was then placed in a vacuum oven at 70°C for an hour to remove final traces of water, hydrochloric and acetic acids.

Approximately 80ml of water was added to the beaker which was heated again on a steam bath to melt fatty acids and then placed on a magnetic stirrer to re-dissolve tartaric acid. The solution was transferred to a volumetric flask (250ml) and made to volume with water. Approximately 20ml of this solution were filtered through GF/C filter paper and then diluted 20-fold with water (5ml→100ml) in a volumetric flask. Before making to volume, 0.1M sodium hydroxide (1ml) was added to make the solution alkaline.

A C18 SPE cartridge (500mg/3ml) was conditioned by washing with methanol (3ml) and water (3ml). The alkaline extract was passed through this cartridge allowing about 1.5ml to go to waste before collecting about 3ml for HPLC analysis.

2.3.3 Determination of Tartaric Acid by Ion-Chromatography

Tartaric acid was determined by ion-chromatography on an anion-exchange column with suppressed conductivity detection, using the following conditions:

Column:	Dionex Ionpac AS11 250mm x 4mm +
	Ionpac AG11 guard column 50mm x 4mm
Mobile phase:	Sodium hydroxide/methanol gradient.
	1mM to 45mM NaOH / 10% to 20% methanol
Flow :	2ml/min
Injection volume :	50µl
Detection :	Suppressed conductivity

Tartaric acid eluted at around 10 minutes.

2.3.4 Extraction of DATEM Emulsifiers from Foods

10-12g of comminuted sample was weighed into a 250ml Duran bottle. MES/TRIS buffer (40ml) and 0.3ml alpha-amylase (Sigma) was added, the bottle capped loosely and placed in a shaking water bath at 70°C for 1 hour. The mixture was then cooled and homogenised in-situ with a top-drive macerator. This digest was stored in the refrigerator until required.

MES/TRIS Buffer was prepared by dissolving 4.88g MES (4-morpholine ethanesulphonic acid) and 3.05g TRIS (Tris(hydroxymethyl)aminomethane) in water and making to 500ml.

Chloroform/methanol 2:1 (50ml) was added to the bottle and the two phases intimately mixed with a magnetic stirrer. After 5 minutes stirring, 100ml chloroform was added and stirring continued for a further 5minutes. The contents of the bottle were then transferred to a 250ml separating funnel, the chloroform layer allowed to separate and then run off into a 500ml round-bottomed flask. The aqueous phase was then shaken with two further portions of 80ml chloroform. Each chloroform extract was evaporated on a rotary evaporator and the combined extracts finally evaporated to

dryness. The lipid residue was re-dissolved in chloroform (2 x 10ml portions) and transferred to a 400ml beaker. The chloroform was evaporated on a steam bath to leave a dry lipid extract, which was further dried in a vacuum oven at 70°C for 30 minutes.

15ml water and 5ml concentrated hydrochloric acid were added to the dried lipid extract in a 400ml beaker. The beaker was covered with a watch glass or similar and heated on a boiling steam bath for 1 hour. After an hour the cover was removed and heating continued to dryness. The beaker was then placed in a vacuum oven at 70°C for an hour to remove final traces of water, hydrochloric and acetic acids.

Sodium hydroxide (20ml of 0.1M) was added to the beaker which was heated again on a steam bath for 2-3 minutes to melt fatty acids and then placed on a magnetic stirrer to re-dissolve tartaric acid. 30ml of water was then added to the solution (giving a total volume of 50ml), which was then filtered through GF/C filter paper.

A C18 SPE cartridge (500mg/3ml) was conditioned by washing with methanol (3ml) and water (3ml). The alkaline extract was passed through this cartridge allowing about 1.5ml to go to waste before collecting about 3ml for HPLC analysis.

2.3.4 Direct analysis of DATEM in commercial formulations

Chloroform solutions of DATEM emulsifier (1%) were analysed by normal phase HPLC using light-scattering detection. The HPLC conditions were as follows:

Column : 100mm x 4.6mm Hypersil Silica 3µ

Mobile Phase: Solvent A : Methanol
Solvent B : Dichloromethane (60%); Methanol (34%); Water (6%)

Gradient	Mins	A	B
	0	98	2
	7	2	98
	12.5	2	98
	14	98	2
	20	98	2

Flow Rate : 1.25 ml/min

Detection : Light-scattering (Varex ELSD)
Gas flow (nitrogen) 50 litre/min
Heater Temperature 130°C

2.4 Determination of Sorbitan Esters

A number of different analytical approaches for the characterisation of commercial sorbitan ester formulations and the determination of sorbitan esters in food matrices were tested. These are outlined below.

2.4.1 Commercial Sorbitan Ester Samples

Commercial sorbitan ester samples were supplied by Abitech, Croda, Danisco Cultar and Quest (Table 26).

2.4.2 Analysis of Emulsifier Formulations by Direct Determination of the Sorbitan Esters and Residual Reactants Following Silylation

As far as the authors are aware there is no published information on the actual composition of commercial emulsifier formulations as added to foods. Thus, the initial priority was to develop methodology to establish the basic composition of these food ingredients. Given the potentially wide polarity range of the compounds possibly present in these formulations, silylation (formation of trimethylsilyl ether [TMS] derivatives) followed by GC determination was taken as a general practical procedure for their analysis.

A detailed qualitative analysis of all formulations was initially carried out to compare the products and also identify internal standards and establish the degree of any interferences. Following this preliminary investigation, the procedure below was developed for the quantitative characterisation of sorbitan emulsifiers.

Emulsifier (20mg) is mixed with internal standard I (0.5ml x 1 mg/ml β-phenylglucopyranoside pyridine) and internal standard II (0.5ml x 2 mg/ml 1,3-dipalmitin pyridine) in a 4ml vial. The emulsifier is dissolved in the pyridine solution followed by the addition of hexamethyldisilazane (HMDS) (0.9ml) and trifluoroacetic acid (TFA) (0.1ml) and the mixture heated for 60 min at 70°C. The cooled mixture is analysed directly by GC.

2.4.3 GC Determination of Sorbitan Esters and Residual Reactants

The conditions for GC analysis were required to determine a very wide molecular weight range from C6 polyols through to sorbitan tri-esters. Comparison of various GC columns and operating conditions lead to the two-stage GC analysis provided by the conditions below.

The following conditions allowed for the determination of:

a) sorbitol dianhydrides, sorbitol monoanhydrides, sorbitol and fatty acids against the internal standard β-phenylglucopyranoside.

b) sorbitan mono- and di-esters against the internal standard 1,3-dipalmitin:

Column: 15m x 0.25mm x 0.1 micron Rtx-1
Carrier gas: hydrogen – 35 kPa
Injection: split mode – injector 300°C
Detector: FID – 325 °C
Column temperature: 100°C (hold 2min), ramp 6 °C /min to 320°C (hold 10min)

The following conditions allowed for the determination of sorbitan mono-, di- and tri-esters against internal standard 1,3-dipalmitin:

Column: 10m x 0.25mm x 0.1 micron TAP (Triglyceride analysis phase – Chrompak)
Carrier gas: hydrogen – 35 kPa
Injection: split mode – injector 320 °C
Detector: FID – 340°C
Column temperature: 180°C (hold 2 min), ramp 6 °C /min to 340°C (hold 10 min)

2.4.4 Analysis of Emulsifier Formulations Following Hydrolysis and Silylation

Direct analyses revealed that these emulsifiers were all such extremely complex mixtures that this approach could not possibly be applied to their determination in foods. Thus, these emulsifiers were further characterised by analysis of alkaline hydrolysates. The published procedure [12] was used as the basis for that described below.

Emulsifier (20mg) is mixed with 1ml x 0.5M sodium hydroxide/methanol in a 4ml capped vial and heated for 3 hours at 80°C. The cooled mixture is neutralised with 0.1ml x 5M hydrochloric acid and evaporated to dryness. The residue is mixed with internal standard (1.0ml x 2mg/ml β-phenylglucopyranoside pyridine), HMDS (0.9ml) and TFA (0.1ml) and heated for 60 min at 70°C. The cooled mixture is analysed directly by GC. The following conditions allowed for the detailed resolution and determination of sorbitol dianhydrides, sorbitol monoanhydrides, sorbitol and fatty acids.:

Column: 15m x 0.25mm x 0.1 micron Rtx-1
Carrier gas: hydrogen – 35 kPa
Injection: split mode – injector 275 °C
Detector: FID – 325 °C
Column temperature: 100°C (hold 2 min), ramp 6 °C /min to 200°C, ramp 20 °C /min to 320°C (hold 5 min)

2.4.5 Analysis of Emulsifier Formulations Following Hydrolysis and Acetylation

The analysis of sorbitans (from emulsifier hydrolysates) as TMS derivatives appeared to offer possible indices for the determination of sorbitan esters in foods. However, when applied to the model bread sample, it was not possible to qualitatively identify the sorbitan profile or to obtain credible quantitative data, despite various method modifications. The acetate derivatives of the sorbitans were considered to be potentially more selective than the TMS ethers described above and the following procedure was therefore developed. This includes a modification to the hydrolysis conditions, which allowed for a more rapid and more convenient procedure (hydrolysis for 1 hour and faster evaporation of methanol cf. ethanol)

Emulsifier (20mg) is mixed with 0.5M sodium hydroxide/methanol (2ml) and internal standard, erythritol (0.25ml x 1mg/ml) in a McCartney bottle, capped and heated for 1 hour at 70 °C with occasional shaking. The hydrolysate is neutralised with 5M hydrochloric acid (0.2 ml) and evaporated to dryness at 55 °C under nitrogen. The residue is dissolved in water (0.5ml) and mixed with 1-methylimidazole (0.5ml) and acetic anhydride (5ml). After 10 min, bromophenol blue (about 0.5ml), ethyl acetate (2ml) and water (8ml) are added and mixed. This mixture is reacted with sodium hydroxide (5ml x 7.5M), cooled and a further 5ml sodium hydroxide added. When cooled, the ethyl acetate layer (yellow) is recovered for analysis by GC. The following conditions allowed for the detailed resolution of both sorbitol dianhydrides and monoanhydrides:

Column: 30m x 0.22mm x 0.25 micron BPX-70
Carrier gas: hydrogen – 100 kPa
Injection: split mode – injector 275 °C
Detector: FID – 275 °C
Column temperature: 160°C (hold 1 min), ramp 4 °C /min to 250°C (hold 10 min)

2.4.6 Extraction and Analysis of Sorbitan Ester Emulsifiers from Foods

Comminuted sample (10-12g) was rigorously mixed with a known weight (30-40g) of anhydrous sodium sulphate using a coffee grinder, or a pestle and mortar for high fat/moisture samples. The mixture was left overnight to equilibrate, briefly re-mixed and a weighed aliquot (approx 30g) packed into an extraction thimble. The mixture was extracted with chloroform:methanol (90:10) in a syphoning Soxhlet apparatus for 5 hours. The cooled extract was evaporated to reduce the volume below 100ml and then made to 100ml volume with chloroform. An aliquot (50ml) was evaporated to dryness under nitrogen at 55°C with the final evaporation step in a McCartney bottle. The residue was then treated by the procedure above for hydrolysis and acetylation but with the following additional step. After neutralisation, the hydrolysate was extracted with hexane (3x) as a clean-up step, primarily to remove fatty acids.

3. Results and Discussion

3.1 Determination of Polyphosphates

The strategy proposed for this analysis is based on the hydrolysis of polyphosphate to orthophosphate, the latter being measured by ion-chromatography.

3.1.1 Hydrolysis of Polyphosphate to Orthophosphate – Optimisation of Conditions

Establishing the conditions for the quantitative hydrolysis of polyphosphates to orthophosphate was the first objective in developing a method. Both alkaline and acid hydrolysis were evaluated.

For the initial studies of reaction conditions, sodium pyrophosphate was used as the substrate since this allowed calculation of the expected yield of orthophosphate. Alkaline hydrolysis using sodium hydroxide was shown to be ineffective. Hydrolysis using hydrochloric acid was investigated and the optimum conditions established. The choice of acid is important since the added anion is a potential interference in the separation of phosphate by ion-chromatography. Hydrochloric acid was chosen in preference to sulphuric acid because it could be readily removed by evaporation. Also chloride in comparison to sulphate elutes much earlier than phosphate. The optimum hydrolysis conditions were established as boiling a 1:1 mixture of standard substrate solution and 0.5M HCl for one hour (Table 1). Hydrolysis of tri-phosphate standards confirmed that these conditions achieved complete hydrolysis for longer chain lengths (Table 2).

3.1.2 Characterisation of Commercial Polyphosphates

The commercial polyphosphates listed in Table 3 were tested for polyphosphate content by determining phosphate before and after acid hydrolysis (Fig.6). Typical chromatograms are shown in figure 6 and the results are presented in Table 4. Polyphosphate contents range from 53 to 72% (as orthophosphate) while free phosphate contents ranged from 0 to 28%.

Whilst this analytical data provides useful compositional information, a knowledge of polyphosphate contents is not necessary for polyphosphate quantitation in foods. This is because the analytical approach measures actual polyphosphate content and not formulation content.

3.1.3 Extraction of Polyphosphate from Foods

Extraction of polyphosphates associated with meat, fish or cheese requires denaturing of the protein to release entrapped salts. Trichloroacetic acid (TCA) and perchloric acid have both been used as protein precipitants [2]. Removal of the excess reagent normally follows. TCA is readily removed by extraction into diethyl ether and perchloric acid is neutralised with an alkali such as sodium carbonate. TCA was selected because it can be removed without introducing interfering anions. The ether extraction serves a secondary purpose by removing lipids and in particular phospholipids. The latter are a potential source of organic phosphate.

3.1.4 Polyphosphate Stability in Trichloroacetic acid

The use of TCA to extract polyphosphates introduces the potential for hydrolysis. Significant losses prior to the acid hydrolysis step would undermine the basis of the analytical approach. To investigate this, the stability of commercial tri- and polyphosphate samples in 2% TCA solution was measured over a period up to 5 days (Table 5). Losses were less than 5% in the first hour and were still only 8% after 24 hours. These experiments were performed at room temperature whereas TCA extractions are typically performed under ice-cold conditions which would reduce hydrolysis further. Removal of TCA after extraction will reduce the rate of further hydrolysis. TCA therefore appears to be suitable as an extractant as long as contact times are kept to a minimum.

3.1.5 TCA Extraction Conditions

To evaluate the recovery of polyphosphates from meat, spiked samples of pork were prepared by blending macerated meat with a polyphosphate solution. Both raw and cooked pork were tested and additionaly spiked raw pork was then 'cooked' by heating the mixture in an oven. The results obtained using 2% TCA as extractant are

presented in Table 6. Recovery from raw pork was poor – around 50% and further optimisation of extraction conditions was carried out. When used as a protein precipitant much higher TCA concentrations are generally used [5]. Standing the TCA extract in the refrigerator has also been recommended [2]. The effect of TCA concentration and standing time was evaluated using spiked samples of raw pork (as showing the poorest recovery in initial experiments). Samples were macerated with ice-cold TCA and then held at 5°C before the TCA removal stage for periods from 0 to 20 hours (Table 7). The results clearly show that 10% TCA gives the best recovery and that additional standing times only result in loss of polyphosphate. An additional benefit of the higher TCA concentration was that no emulsion was formed when mixing with diethyl ether. With a 2% TCA concentration an emulsion was formed very readily and this required prolonged standing to clear. The efficiency of TCA removal was presumably reduced in this case.

After ether extraction the level of residual TCA was still sufficient to cause interference in the ion-chromatographic measurement of phosphate. The use of a C18 SPE cartridge reduced this peak considerably by removing the interference problem. An additional benefit of this TCA removal was that the possiblity of premature hydrolysis of polyphosphate was lessened; allowing measurement of the free phosphate within a reasonable time-frame. The un-hydrolysed extracts were best analysed on the same day (usually run overnight) but only small losses (<5%) were observed if the extracts were run again 24 hours later (stored refrigerated).

Recovery experiments were repeated on pork samples using 10% TCA and no standing time. The results shown in Table 8 demonstrate that acceptable recoveries around 80% were obtained.

3.1.6 Analysis of Retail Samples

Samples were collected from local supermarkets aiming to cover the foods listed in the additives directive [1] for E-450, E451 and E452, di-, tri and poly-phosphates respectively (Table 9). The samples included a range of cold meats (ham, chicken and turkey), processed cheese and processed fish. No potato products (chips, waffles etc.) could be found that contained these additives (ortho-phosphate only). Nor were any

desserts identified as containing polyphosphates. The results for whiteners and cheeses are signicantly higher than those for the cold meats (Table 10). This reflects the limits set for these foods in the additives directive and presumably usage levels. The very low concentrations in the cold meats may also be due to the higher water activity of these products and the resultant losses due to hydrolysis. Other workers have reported such losses during processing [13]. Chromatograms illustrating the separation achieved for ham and coffee whitener samples are shown in figures.7-10.

It will be noted from these chromatograms that the TCA peak is virtually absent in hydrolysates, suggesting that it is lost by evaporation.

3.1.7 Further Method Validation

Further recovery data was obtained for ham and processed cheese samples. Excellent recoveries were obtained for cheese (90 and 105%), though a single ham sample gave a poorer recovery of 45% (Table 11).

The method was further validated by testing repeatability. Replicate results for processed cheese and cold meat samples are given in Table 12. The relative standard deviation of the method is 15 to 20%. This is judged to be acceptable bearing in mind that additional errors are introduced through measuring a difference in two measurements of phosphate concentration.

Method linearity was tested using a polyphosphate sample at a range of concentrations. An acceptable R^2 value of 0.9 was obtained.

A major factor affecting method robustness was considered to be the potential for organic phosphates not being removed during the C18 cleanup stage. The SPE cartridge used for this is twice the standard 500mg capacity to increase retention and prevent breakthrough. The removal of interferences at this stage was initially demonstrated using a pork sample (Table 8) but has been further evaluated by testing polyphosphate-free products (ham and cheese). The results of 0.20 and 0.08 g/kg, respectively, are below the realistic limit of quantitation of the method (~0.5g/kg) and indicate removal is successful (Tables 9 & 10).

3.2 Determination of Stearoyl Lactylates

3.2.1 Characterisation of Commercial Emulsifiers

The optimum conditions for the methylation of stearoyl lactylates were found to contrast with those of a published method (7) (using the same methylation reagent) and gave much higher yields (Table 13). The labile nature of methylated stearoyl lactylates in the boron trifluoride/methanol reagent under either extensive time periods at room temperature or the influence of heat, has not been previously recognised. It is a critical factor in identifying optimum conditions providing quantitative determination. Constrained by these conditions, which may not give quantitative methylation of a reactive internal standard, such as a fatty acid; quantification is based upon the addition of an inert internal standard. Methyl tricosanoate (C23) was established as an ideal internal standard; initially added post-reaction but later incorporated before methylation (as preferred) with identical results. A set of commercial lactylates (Table 14) were characterised by this method. A typical analytical profile is shown in Figure 11 and the results are summarised in Tables 15 and 16.

The total stearoyl lactylate content of sodium variant samples was generally in the range 35-37%; with results for the calcium variants at approximately 30%.

Three samples with tri-lactylate levels above 2% (SSL1, SSL3, CSL1) were analysed further for the presence of higher lactylate oligomers. Tetra-lactylates were 0.6, 0.6 and 1.0% and penta-lactylates 0.1, 0.1 and 0.4% respectively. Higher oligomers are therefore not significant.

A typical simplified lactylates profile follows:

Mono 25%, di 9%, tri 2%, tetra <1% and penta <0.1%.

The mono-lactylates were selected as an index for the determination of stearoyl lactylates in foods.

The lactic acid content of the commercial stearoyl lactylates was determined with a view to using this as an alternative means of quantification. Total lactic acid contents were determined by alkaline hydrolysis followed by reverse-phase HPLC (Table 17). Results were in agreement with the manufacturer's specifications (25-35%).

Free lactic acid was also determined as a means of measuring by difference the lactic acid present as lactylates. Sodium hydroxide was used to convert free lactic acid to its salt form. Stearoyl lactylate was then removed by passing the solution through a C18 SPE cartridge. Care was needed in this analysis to avoid hydrolysis of lactylates. Results of around 5% free lactic were obtained (Table 17) making the lactic acid present as lactylates between 20 and 30%. This appears much higher than expected when compared with the concentrations of around 30% stearoyl lactylates obtained by the direct method. One explanation is that the free lactic measurement is not taking into account di-lactic and possibly tri-lactic acid present in the formulations. These will almost certainly be present since these emulsifiers are usually prepared in a single process; lactic acid condensation and fatty acid esterification typically occurring simultaneously. These were not measured by the HPLC method and standards were not available to identify and quantify them if they had been separated. Since the free acid components of the emulsifier formulations will not be present in extracts from foods, only the stearoyl lactylate entity, lactic acid can only be used as an index of emulsifer content if its proportion of this entity is certain. The use of lactic acid as an index was therefore abandoned in favour of the direct approach.

3.2.2 Extraction of Stearoyl Lactylate Emulsifiers from Foods

Methodology for the determination of stearoyl lactylates in foods has been developed using white bread prepared with the addition of emulsifier at 0.2% flour weight, as the test matrix. Recoveries are based on the assumption that crumb and dough are 60% flour.

Since stearoyl lactylates complex with amylose, treatment with α-amylase is an essential step in the analysis of these emulsifers. Initial experiments using Soxhlet extraction gave very poor recoveries from spiked bread. Several separate determinations, using the enzyme digest procedure and chloroform/methanol

extraction, gave acceptable and consistent recovery of the order of 70% (Table 18), based upon mono-lactylate levels found. (For comparison, SL added as a chloroform solution to an enzyme digest of control bread crumb gave recoveries of 89 and 95 %). A typical bread extract chromatogram is shown in Figure 12.

The results from a selection of retail food products declaring E481/E482 (Table 19) are presented (Table 20) and an example chromatogram is shown in Figure 13. The results show that only the mono-lactylates can be used as an index of SL level, since interferences may preclude the determination of di- and tri-lactylates. Total lactylates, including the latter, could be calculated by applying a correction factor of 1.41 (the mean ratio of total:monolactylates, Table 15) and assuming similar recoveries. These results from a range of food products support the conclusion that the method developed provides a degree of selectivity such that it is broadly applicable to the determination of stearoyl lactylates in foods.

3.3 Determination of DATEM Emulsifiers

3.3.1 Characterisation of DATEM Emulsifiers

The conditions used here for the hydrolysis of DATEM emulsifiers to tartaric acid were established in previous unpublished work at RHM Technology. Several other workers have followed this hydrolysis approach [8,9], though using alkaline saponification instead of acid hydrolysis. We have found that boiling DATEM with hydrochloric acid readily hydrolyses the ester linkages releasing tartaric acid, acetic acid, fatty acids and glycerol. Acetic acid and hydrochloric acid are removed by evaporation, whilst fatty acids are removed by passing an alkaline solution of the tartrate extract through a C18 SPE cartridge. Tartrate is not retained but fatty acid salts are. The removal of fatty acids was found to significantly improve peak shape, thereby increasing resolution (Figs. 14 & 15). This methodology was used to characterise the commercial DATEM samples (Tables 21 & 22). The results indicate a typical tartrate content of 20% in DATEM emulsifiers.

Free tartaric acid in the formulations was also measured by making solutions of the emulsifiers alkali before cleanup on C18 SPE cartridges. Results mainly fell in the range 2-3%, though it is not certain that the alkaline preparation conditions did not result in some hydrolysis of DATEM (Table 22). This may be the reason for the high result of 6% for one sample.

These results demonstrate that tartaric acid is a good index to use for DATEM determination.

The direct analysis of DATEM emulsifiers was investigated using normal-phase HPLC on a silica column and light-scattering detection. Separation of broad peak envelopes was observed which was to be expected given the complex mixture that make up commercial DATEM formulations. However, no consistent fingerprint profile could be seen among the set of emulsifiers and no common peaks identified (Fig.16). Because of this and the poor chromatographic separation achieved, it was

concluded that the direct approach was unsatisfactory for the study of formulations and offered no potential for measurement in foods.

3.3.2 Determination of DATEM in Foods

Methodology for the determination of DATEM in foods has been developed using white bread prepared with the addition of emulsifier at 0.2% flour weight, as the test matrix. This equated to 0.11% on a bread basis. Two procedures have been evaluated: direct soxhlet extraction with ethyl acetate and chloroform extraction after treatment with α-amylase. The latter procedure was initially attempted by mixing the liquid digest with anhydrous sodium sulphate prior to soxhlet extraction with chloroform. However, the resulting extracts were found to suffer from significant interference from co-extracted anions (particularly sulphate). An alternative liquid/liquid extraction with chloroform/methanol gave little interference and proved more robust.

Extraction is followed by a hydrolysis step where the dried lipid extracts are treated with boiling hydrochloric acid. After reducing to dryness, the residues are re-dissolved in water and made alkaline before a cleanup using C18 SPE cartridges. Tartaric acid is determined by ion-chromatography with conductivity detection.

It is expected that like stearoyl lactylates, DATEM will complex with amylose and therefore require treatment with α-amylase. Indeed initial experiments using soxhlet extraction gave very poor recoveries (10%) from spiked bread (Table 23). Determinations using the enzyme digest procedure and chloroform/methanol extraction, gave better but still low recoveries of the order of 20% (Table). This may be a result of poor extraction but might also be due to losses due to hydrolysis in bread making. The ability of DATEM to complex with proteins may offer an explanation. The use of methanol in the extraction procedure may bring about some protein denaturation but this is possibly insufficient. The addition of a pepsin enzyme alongside α-amylase treatment may be advantageous. Clearly these aspects need further study.

A small set of retail samples found to be declaring E-472e were obtained from local supermarkets (Table 24). The results obtained for these samples are presented in Table 25 and example chromatograms in Figures 17 & 18. Separation of tartaric acid from interferences was on the whole satisfactory though baseline separation was not achieved. An alternative high-capacity version of the column was evaluated for this analysis (Fig. 19). This gave greater retention of tartaric acid but did not appear to offer better resolution from interferences in sample extracts.

Recoveries were determined on selected samples: cream slices, cream buns and white bread. These samples were spiked with 0.2% DATEM emulsifier, added as a chloroform solution and then evaporated. The results were better overall than those achieved with the model bread system (Table 25). The result of 39% recovery from a spiked bread is particularly encouraging though it should be borne in mind that addition of spikes prior to analysis does not reproduce binding and hydrolysis that may occur in bread manufacture.

Whilst recoveries are disappointing, these results from a range of food products do show that this method provides a degree of selectivity such that it is broadly applicable to the semi-quantitative determination of DATEM in foods.

3.4 Determination of Sorbitan Esters

3.4.1 Characterisation of Sorbitan Ester Formulations

Direct analysis of sorbitan esters as the TMS derivatives provided a method of characterisation from residual reactants; sorbitol dianhydrides, sorbitol monoanhydrides, sorbitol and fatty acids, through mono-, di- and tri-esters. The results obtained are summarised in Table 27 and a typical profile, up to di-esters, of a mono-ester formulation is shown in Figure 20. These results show that further method development is required should complete overall quantitative be deemed necessary. However, this is probably a low priority given the subsequent findings reported below. The major source of error is considered to be the determination of the tri-esters. [Note the results for the two tri-ester products, which show the greatest deviation from 100%.] This might be reduced if GC analysis was carried out with on-column injection. Free fatty acids are relatively minor components (<10 %) and are not significant components in the identification and determination of sorbitan esters.

3.4.2 Analysis of Hydrolysates after Silylation

The characterisation data and profiles clearly indicate that with their extreme complexity it is unlikely that methodology could be developed to detect and measure their presence in foods. Thus a more selective analytical approach is required. Hydrolysis of sorbitan esters to total sorbitans yields dianhydrides, monoanhydrides and sorbitol. The latter may possibly be present in foods from other sources but this is much less likely for the anhydrides. Thus, the anhydrides may provide an index/indices for determination of sorbitan esters.

Sorbitan ester formulations were further characterised as hydrolysates;
the results are summarised in Table 28 and a typical profile is shown in Figure 21. These appear to offer scope for application to determination in foods. However, although the TMS ether derivatives provided a means of analysis for emulsifier formulations, this approach was unsatisfactory when applied to food (bread) due to gross interferences. At the qualitative level it was not possible to distinguish the

sorbitan profile, even for the presence of a known added emulsifier and quantification for the very few individual anhydrides that could be identified was poor. Various GC analysis strategies were explored but the interferences could not be resolved.

3.4.3 Analysis of Hydrolysates after Acetylation

Given the difficulties described above, removal of the interferences by clean-up techniques was considered an unfavourable approach. Since acetates are more selective than TMS derivatives (which may be formed for a wide range of polar molecules) for polyols, they offered a potential route to sorbitan determination.

Sorbitan ester formulations were therefore further characterised as acetate derivatives of the hydrolysates and the results are summarised in Table 29 and a typical profile is shown in Figure 22. Comparison of these results with those for TMS derivatives show reasonable agreement to within about 10%. However, total dianhydrides or total monoanhydrides are more likely to be subject to interferences than if individual sorbitans are the index of sorbitan content. This/these must be of similar concentration across all formulations since the actual sorbitan ester will be unknown when analysing foods.

The sorbitan acetate profiles for individual sorbitans are given in Table 30. Initial inspection would appear to show a very wide range of results. However, the food products purchased for the testing declared the use of only the mono-ester. If the results for the (nominal) tri-esters (SORB 2 &4) are excluded then the results are in a narrower range and peak IDs 3,5,6,9 and 15/16 are potential indices for sorbitan determination. SORB 1 is an outlier, in that only peak ID 3 is a possible index. The means of the results for these individual sorbitans are given below and show the range and hence the potential error in their use as indices for sorbitan determination.

Peak ID No.	Mean (6)	Mean(5)
3	2.30 +/- 0.22 (9.5%)	2.38 +/- 0.16 (6.6%)
5	-	2.02 +/- 0.43 (21.2%)
6	-	2.95 +/- 0.61 (20.7%)
9	-	3.33 +/- 0.12 (3.7%)
15+16	-	4.88 +/- 0.16 (3.3%)

These results show that if these sorbitans can be quantitatively determined in foods, they (particularly ID Nos. 3, 9 and 15+16) could provide a means of measuring sorbitan ester levels.

3.4.4 Extraction and Analysis of Sorbitan Ester Emulsifiers from Foods

Methodology for the determination of sorbitan esters in foods has been developed using the model white bread matrix prepared with the addition of emulsifier at 0.2% flour weight.

Analysis of Sorbitan Esters in Bread Crumb

Sorbitan esters do not complex strongly with amylose and therefore enzyme digestion is not required. However, their extraction demands exhaustive extraction. Several separate determinations employing the Soxhlet extraction procedure gave acceptable

recoveries, although covering a range from 80 – 126% depending upon the index compound. The recoveries are given in Table 32. A control bread sample gave an almost clean trace with minor interferences no greater than equivalent to 0.02% emulsifier. However, it is presumed that a likely explanation for recoveries in excess of 100% is mainly due to the influence of minor interferences. Figure 23 shows the analysis of a bread extract with the sorbitan profile clearly distinguishable.

Analysis of Sorbitan Esters in Food Products

The results from the five food products obtained in a search of the supermarkets for candidate samples are given in Table 34 and an example chromatogram is shown in Figure 24. These are the final results from an interpretation of the results based on sorbitan IDs 3,5,6,9 and 15+16, the details of which are given in Table 35. It can be assumed that high results are due to interferences (and can be excluded) and that lower results will be closer to the true value. Where more than one sorbitan leads to a similar result this provides further support for this value. Since we only have data on few food products it is not possible to conclude which sorbitan index is the most reliable, therefore the results have been quoted as the means of IDs 3, 5 and 9.

Interferences are variable and presumably product dependent. In contrast to the above, a ready-to-eat chocolate dessert (no emulsifier declared) gave a clean sorbitol anhydride analysis equivalent to < 0.01% emulsifier. A spiked sample gave a clearly defined and characteristic sorbitan profile with a recovery of 79-91%, based on the five indices above.

These results show that this method still requires further development, in particular, more food products need to be analysed to identify where interferences most commonly occur, followed by extension of the procedure to reduce them.

4. Conclusions

Methods have been successfully developed for the determination of stearoyl lactylate, DATEM and sorbitan ester emulsifiers in foods. In addition a partially validated procedure has been developed for the extraction and determination of polyphosphates in foods.

These methods provide a significant advance over those previously published which have not been derived and tested on such a wide selection of commercially available additives nor applied to a diverse range of food types.

Commercial samples of these additives (8-11 of each additive) have been characterised by a number of analytical procedures. These initial studies allowed selection of the most promising approach for determination of each additive in food matrices. Based upon the range in composition of the commercial food samples analysed, we can conclude that each of the final methods provides a degree of specificity for measurement of the additive. The extent to which each of the methods achieves the goals of good recovery of analyte, good repeatability and specificity across the food types of interest differs:

The polyphosphate and stearoyl lactylate methods were shown to be highly specific across a range of food types. Interfering peaks in the chromatographic analysis were not found to be a problem. Additionally these methods performed well in terms of recovery and repeatability. The polyphosphate method achieved recoveries of 77-84% from pork and 90-105% from processed cheese. Relative standard deviations of 14.5% and 19.6% were achieved for processed cheese (high level of additive) and chicken slices (low additive level), respectively. For the stearoyl lactylate method recoveries of 70-75% were achieved from white bread. Repeatability is illustrated by good duplicate results (0.096 vs. 0.096g g/kg for Pizza base; 0.346 vs. 0.326 g/kg for Danish pastry and 0.169 vs. 0.249 g/kg for white bread). It is suggested that

collaborative testing is the next step to fully validate these two methods. Surveillance studies of retail foods could then follow.

The DATEM and sorbitan ester methods generally demonstrated poorer specificity than the polyphosphate and stearoyl lactylate methods and the measurement indices (tartaric acid and sorbitans) were subject to varying degrees of interference dependant on food type. Good recoveries from 80 to 100% were obtained with the sorbitan ester method and duplicate results were generally within 3% of the mean. This method principally requires the study of a wider range of foods to identify the best and most robust sorbitan indices to use provided that only mono-esters need to be considered.

In addition to the problem with interferences, the DATEM method demonstrated recoveries no better than 40% and further work is required to improve this. It is suggested that protein-DATEM complexes may be the problem and an enzyme treatment step could give better recoveries.

5. References

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6. Appendices

Tables

TABLE 1 HYDROLYSIS OF PYROPHOSPHATE

	Final Concentration of Orthophosphate (mg/l)				
Time/Conc	0.1M	0.5M	1M	2.5M	5M
30 min	21				
60 min	22	26*	26	26	26
90 min	24	27	26	26	
120 min	25				

(Theoretical Yield = 25 mg/l)

* Optimum hydrolysis conditions established as boiling 1:1 with 0.5M HCl for one hour.

TABLE 2 HYDROLYSIS OF TRI-PHOSPHATE

	Final Concentration of Orthophosphate (mg/l) *	
Time	Sodium salt anhydrous (theoretical yield = 23 mg/l)	Sodium salt hexa-hydrate (theoretical yield = 18 mg/l)
30 min	23	18
60 min	23	18
90 min	24	18

* Hydrolysis conditions : boiling 1:1 with 0.5M HCl

TABLE 3 COMMERCIAL POLYPHOSPHATE SAMPLES OBTAINED

Supplier	Product Name	Composition
Fiske	Puron AG Ref 786	Di-phosphpate
Fiske	Puron K Ref 787	Di-phosphate
Fiske	Puron sr Ref 788	Di-phosphate
Fiske	Puron ss Ref 789	Tri-phosphate
Fiske	Calgon PT Ref 789	Polyphosphate (n=12)
Fiske	Calgon 696 Ref 790	Polyphosphate (n=25)
Fiske	Tetron Ref 791	Diphosphate
Rhodia	Calgon PT F/G Powder	Polyphosphate (n=12)
Rhodia	Tetron F/G Powder	Di-phosphate
Rhodia	Puron F	Di-phosphate

TABLE 4 CHARACTERISATION OF COMMERCIAL POLYPHOSPHATES

Product Name	n	FW	Calculated Poly-Phosphate (%)	Total phosphate (%)	Free Phosphate (%)	Poly-Phosphate as PO_4^{3-} (%)
Puron AG Ref 786	2	222	79.3	83.8	22.2	61.6
Puron K Ref 787	2	266	82.7	68.7	12.4	56.4
Puron sr Ref 788	2	222	79.3	81.3	20.8	60.5
Puron ss Ref 789	3	368	81.3	72.0	0.0	72.0
Calgon PT Ref 789	12	1293	75.1	80.6	27.5	53.1
Calgon 696 Ref 790	12	1293	75.1	83.3	14.4	68.9
Tetron Ref 791	2	266	82.7	61.3	0.0	61.3
Calgon PT F/G Powder	12	1293	75.1	83.7	12.1	71.6
Tetron F/G Powder	2	222	79.3	65.9	0.0	65.9
Puron F	2	222	79.3	77.7	23.9	53.8

TABLE 5 POLYPHOSPHATE STABILITY IN TCA

	Tri-phosphate	Polyphosphate (n=12)
	% of Total	
H2O	99.0	100.0
2% TCA T= 1 hour	98.6	96.3
2% TCA T =1 day	94.7	91.7
2% TCA T =5 days	92.0	-

TABLE 6 RECOVERY FROM PORK - RAW AND COOKED (2% TCA)

	g/kg PO_4^{3-}			
	Free Phosphate	Total Phosphate	Polyphosphate	Recovery (%)
Pork PP-free	1.19	1.14	0	-
Spike on raw pork	1.88	3.36	1.47	50.4
Spiked on raw - cooked	2.11	3.89	1.78	64.0
Spiked on cooked	1.89	4.03	2.14	74.4

TABLE 7 EFFECT OF TCA CONCENTRATION AND STANDING TIME ON RECOVERY

	% Polyphosphate Recovery from Pork (2.5 g/kg added)		
Standing Time (h)	2% TCA	5% TCA	10% TCA
0	74	91	109*
1	69	87	97
2	72	88	87
20	34	56	82

* optimum extraction conditions established as 10% TCA with no standing time.

TABLE 8 RECOVERY FROM PORK AFTER REMOVAL OF ORGANIC PHOSPHATE

Sample	Polyphosphate g/kg	Recovery (%)
Pork	<0.01	-
Pork + 1.1g/kg PP (1)	0.93	84
Pork + 1.1g/kg PP (2)	0.85	77

TABLE 9 POLYPHOSPHATE CONTENTS OF STUDY SAMPLES

Sample	Lab Reference	Phosphate Content g/kg		
		Free phosphate	Total phosphate	Polyphosphate
Coffee Whitener	1193	11.60	16.70	5.06
Processed Cheese	1194	12.88	22.28	9.40
Processed Cheese	1195	6.96	12.42	5.45
Processed Cheese	1196	7.35	19.94	12.59
Processed Cheese	1197	6.24	15.05	8.81
Processed Cheese	1198	10.98	23.99	13.00
Ham	1199	4.48	4.82	0.34
Ham	1200	4.53	5.73	1.21
Ham	1201	3.24	3.77	0.54
Gammon	1202	3.40	3.96	0.55
Gammon	1203	3.42	3.81	0.38
Gammon	1204	3.87	4.28	0.44
Bacon	1205	1.98	2.32	0.34
Turkey Slices	1206	4.71	5.10	0.39
Turkey Slices	1207	3.84	4.85	1.01
Chicken Slices	1208	3.92	5.14	1.22
Chicken Slices	1209	4.44	5.14	0.70
Processed Cheese	1482	5.80	23.10	17.30
Processed Cheese	1483	7.92	22.72	14.80
Processed Cheese	1484	5.20	1.98	14.63
Processed Cheese – No PP	1485	1.13	1.33	0.20
Processed Cheese	1486	6.58	7.60	1.02
Processed Cheese	1487	6.68	17.51	10.84
Processed Cheese	1488	11.80	15.97	4.17
Ham– No PP	1489	3.55	3.62	0.08
Fishcakes	1490	1.36	1.67	0.31
Fishcakes	1491	1.13	1.38	0.25

No PP = Polyphosphates not declared

TABLE 10 RECOVERY FROM PROCESSED CHEESE AND HAM

	Polyphosphate g/kg	Recovery (%)
Cheese 1485 + spike (1.15g/kg)	1.32	90
Cheese 1486 + spike (1.15g/kg)	2.33	105
Ham 1489+ spike (1.15g/kg)	0.59	45

TABLE 11 SAMPLE REPLICATION

	Polyphosphate g/kg	
Replicate No.	Processed Cheese Ref. 1195	Chicken Slices Ref.1208
Rep #1	4.89	0.94
Rep #2	3.64	0.60
Rep #3	4.33	0.75
Rep #4	3.66	0.91
Mean	4.13	0.80
SD	0.60	0.157
RSD(%)	14.5	19.6
Uncertainty	±1.2	±0.31

TABLE 12 LACTYLATE METHYLATION CONDITIONS

Overall Yield of Methylated Lactylates from Sodium Stearoyl Lactylate against Reaction Conditions[1]

Conditions	% Formulation[2]
30 min @70°C	2 – 6 (erratic)
5 min @70°C	23
30 min @ RT	27
45 min @ RT	32
60 min @ RT	35
75 min @ RT	36
90 min @ RT	36
120 min @ RT	35
18h @ RT	1

[1] All treated with 3ml boron trifluoride/methanol. Lactylate determined against methyl tricosanoate as internal standard assuming a response factor of 1.
[2] Sum of mono-, di- and tri-lactylates. RT = room temperature
All results at RT are means of duplicates; the latter were within 2% of the mean.

TABLE 13 COMMERCIAL STEAROYL LACTYLATES

Sodium and Calcium Stearoyl Lactylate Formulations
(Commercial Products April 2001 plus older samples obtained previously)

Code	Commercial Emulsifier
SSL1	Danisco Cultar-Grinstead P55Veg
SSL2	Quest-Admul 2012
SSL3	Abitech – SSL
SSL4	Croda- Crolactil
SSL5	Quest-Admul 2004 (1989)
SSL6	Quest-Admul (1997)
SSL7	Danisco Cultar (1997)
CSL1	Abitech CSL
CSL2	Quest CSL

TABLE 14 CHARACTERISATION OF COMMERCIAL STEAROYL LACTYLATES (1)

	Results as % formulations			
Product	Mono-Lactylate	Di-lactylate	Tri-Lactylate	Total
SSL1	25.1	8.9	2.3	36.3
SSL2	28.8	6.9	1.2	36.9
SSL3	25.0	9.1	2.4	36.5
SSL4	24.2	8.5	2.3	35.0
CSL1	19.2	7.0	2.6	28.8
CSL2	24.7	6.5	1.4	32.6

(Commercial Products April 2001)

All results are means of duplicates; the latter were within 1% of the mean.

TABLE 15 CHARACTERISATION OF COMMERCIAL STEAROYL LACTYLATES (2)

	Results as % formulations			
Product	Mono-Lactylate	Di-lactylate	Tri-Lactylate	Total
SSL5[1]	27.8	-	-	27.8
SSL6[2]	28.2	7.1	1.1	36.4
SSL7[2]	25.4	9.1	2.4	36.9

(Commercial Products 1989[1] and 1997[2])

All results are means of duplicates; the latter were within 1% of the mean

TABLE 16 CHARACTERISATION OF COMMERCIAL STEAROYL LACTYLATES (3)

	Results as % formulations	
Product	Total Lactic Acid	Free Lactic Acid
SSL1	36	5
SSL2	32 (32)[1]	9
SSL3	35 (31-34)[1]	5
SSL4	32	5
CSL1	24 (20-26)[1]	2
CSL2	24 (25)[1]	4

[1] Manufacturer's Specification

TABLE 17 RECOVERY OF STEAROYL LACTYLATES FROM SPIKED BREAD

Sample	Recovery %
SSL2 Crumb	
A	69.6
B	67.2
C	69.5
D	70.4
CSL2 Crumb	69.9
SSL2 Dough	69.9

TABLE 18 STEAROYL LACTYLATES (E481,E482) IN FOODS - PRODUCTS

Sample	Full Description
SL1	Pizza Base
SL2	Chocolate Cup Cakes
SL3	Crispbakes
SL4	Sesame Bagels
SL5	Raspberry Danish Twist
SL6	Cream Belgian Buns
SL7	White Sliced Bread 800g
SL8	White Sliced Bread 800g
SL9	White Unsliced Bread 800g

TABLE 19 STEAROYL LACTYLATES IN FOODS - RESULTS

Sample	Mono-Lactylates[1] g/kg
SL1 A	0.096
B	0.096
SL2	0.059
SL3	0.426
SL4	<0.005
SL5 A	0.346
B	0.326
SL6	0.296
SL7	0.368
SL8 A	0.169
B	0.249
SL9	<0.005
Bread Crumb Check	75% recovery

[1] Sum of palmitoyl and stearoyl lactylates.

TABLE 20 COMMERCIAL DATEM EMULSIFIERS

Code	Supplier	Material
DATEM 1	Danisco Cultar ex RHMT Test Bakery	Panodan GB Veg
DATEM 2	Danisco Cultar ex RHMT Test Bakery	Panodan 90 Veg
DATEM 3	Danisco Cultar	Panodan AB100 Veg-FS
DATEM 4	Danisco Cultar ex RHMT Test Bakery	Panodan M2010
DATEM 5	Danisco Cultar	Panodan GB Veg
DATEM 6	Danisco Cultar	Panodan A2020
DATEM 7	Quest	Admul DATEM 1914
DATEM 8	Quest	Admul DATEM 1982
DATEM 9	Quest	Admul DATEM 1943
DATEM 10	Abitech	Abitem V80H
DATEM 11	Abitech	Abitem V80GB

(obtained August 2001)

TABLE 21 CHARACTERISATION OF COMMERCIAL DATEM EMULSIFIERS

Code	Total Tartrate %	Free Tartrate %
DATEM 1	20.9	3.2
DATEM 2	21.8	1.1
DATEM 3	17.8	0.7
DATEM 4	21.6	3.4
DATEM 5	22.5	3.6
DATEM 6	14.7	2.6
DATEM 7	25.7	2.2
DATEM 8	20.3	3.1
DATEM 9	18.1	2.2
DATEM 10	16.3 (24-26)[1]	6.3
DATEM 11	12.4 (18-20)[1]	3.4

[1] Manufacturer's Specification

TABLE 22 RECOVERY OF DATEM FROM BREAD PREPARED WITH EMULSIFIER (0.1%)

Sample	Recovery %
DATEM7 Crumb	
A	10[a]
B	25[b]
C	14[b]

[a] Soxhlet extraction with ethylacetate
[b] α-amylase digest/chloroform-methanol extraction

TABLE 23 DATEM IN FOODS – PRODUCTS

Sample	Full Description
DAT1	Naan Bread
DAT 2	Reduced Fat Crackers
DAT 3	Reduced Fat Digestives
DAT 4	Raspberry Danish Twist
DAT 5	Hot Cross
DAT 6	Danish Pastry
DAT 7	Cream Slices
DAT 8	Cream Belgian Buns
DAT 9	White Sliced Bread 800g
DAT10	White Unsliced Bread 800g

TABLE 24 DATEM IN FOODS – RESULTS

Sample	Tartaric acid mg/kg	DATEM equivalent g/kg[1]
DAT1	7	0.04
DAT 2	36	0.18
	20	0.10
DAT 3	48	0.24
	37	0.19
DAT 4	27	0.14
DAT 5	88	0.44
	88	0.44
DAT 6	27	0.14
DAT 7	<1	<0.01 (10%)[2]
DAT 8	11	0.06
	11	0.06 (32%)[2]
DAT 9	114	0.57
	146	0.49
DAT10	21	0.11
	17	0.09 (39%)[2]

[1] Tartaric acid content of 20% assumed for DATEM

[2] Recovery of 0.2% DATEM Spike

TABLE 25 COMMERCIAL SORBITAN ESTER FORMULATIONS (E491-5) – PRODUCTS

Code	Supplier	Material
SORB 1 (M)	Danisco Cultar	Spans –mono stearate
SORB 2 (T)	Danisco Cultar	Spans – tristearate
SORB 3 (M)	Quest	Span 60K (mono)
SORB 4 (T)	Quest	Span 65K (tri)
SORB 5 (M)	Quest	Span 60 (mono)
SORB 6 (M)	Abitech	Span (mono oleate)
SORB 7	Croda	Crill 3
SORB 8	Croda	Crill 4

TABLE 26 CHARACTERISATION OF COMMERCIAL SORBITAN ESTERS –RESULTS (1)

	Results as % formulation				
Product	**S**	**SME**	**SDE**	**STE**	**Total**
SORB 1 (M)	3.9	30.8	40.4	26.0	101
SORB 2 (T)	0.1	4.7	30.7	39.7	75
SORB 3 (M)	5.0	34.4	40.8	27.1	107
SORB 4 (T)	0.1	5.9	30.0	39.1	75
SORB 5 (M)	4.9	34.4	39.4	23.1	102
SORB 6 (M)	4.0	30.7	32.9	29.4	97
SORB 7	4.5	32.2	36.2	23.7	97
SORB 8	2.5	28.5	36.7	23.0	91

S = sorbitan dianhydrides + sorbitan monoanhydrides + sorbitol

SME - sorbitan mono-esters
SDE - sorbitan di-esters
STE - sorbitan tri-esters
M - labelled as mono-ester
T - labelled as tri-ester

Free sorbitan components determined against β-phenyl glucopyranoside as internal standard. Sorbitan esters determined against 1,3-Dipalmitin as internal standard.

All results are means of duplicates; data for sorbitans and sorbitol were within 1% of the mean; for SME and SDE generally within 3% of the means but those for STE showed considerable variation but within 10% of the mean.

TABLE 27 CHARACTERISATION OF COMMERCIAL SORBITAN ESTERS –RESULTS (2)

Sorbitan Ester Hydrolysates – Trimethylsilyl Ether Derivatives				
	Results as % formulation			
Product	**SDA**	**SMA**	**S**	**Total**
SORB 1 (M)	2.8	14.3	3.9	20.9
SORB 2 (T)	0.9	9.1	3.2	13.2
SORB 3 (M)	3.9	19.0	1.0	23.9
SORB 4 (T)	1.4	11.0	1.8	14.2
SORB 5 (M)	3.9	18.4	1.1	23.4
SORB 6 (M)	4.5	16.8	0.7	22.0
SORB 7	3.9	18.3	2.1	24.3
SORB 8	4.0	16.5	0.8	21.3

SDA - sorbitan dianhydrides
SMA - sorbitan monoanhydrides
S - sorbitol
M - labelled as mono-ester
T - labelled as tri-ester

Sorbitan components determined against β-phenyl glucopyranoside as internal standard.

All results are means of duplicates; the latter were within 2% of the mean for SDA, SMA and S

TABLE 28 CHARACTERISATION OF COMMERCIAL SORBITAN ESTERS –RESULTS (3)

Sorbitan Ester Hydrolysates – Acetate Derivatives				
	Results as % formulation			
Product	**SDA**	**SMA**	**S**	**Total**
SORB 1 (M)	2.1	16.3	5.5	23.9
SORB 2 (T)	0.7	10.3	4.0	15.0
SORB 3 (M)	2.6	20.8	1.1	24.5
SORB 4 (T)	1.1	12.0	1.9	15.0
SORB 5 (M)	2.6	21.0	1.5	25.1
SORB 6 (M)	2.9	18.2	0.8	21.9
SORB 7	2.7	19.3	2.6	24.6
SORB 8	2.6	17.7	1.0	21.3

SDA - sorbitan dianhydrides
SMA - sorbitan monoanhydrides
S - sorbitol
M - labelled as mono-ester
T - labelled as tri-ester
Sorbitan components determined against erythritol as internal standard.

All results are means of duplicates; the latter were within 3% of the mean for SDA, SMA and S

TABLE 29 CHARACTERISATION OF SORBITAN ESTER FORMULATIONS - RESULTS (4)

Sorbitan Ester Hydrolysates – Acetate Derivatives								
	Results as % formulation							
SORB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
PEAK ID NO								
2	0.05	0.04	0.24	0.10	0.18	0.24	0.16	0.18
3	1.93	0.65	2.11	0.94	2.39	2.58	2.46	2.34
4	0.11	0.03	0.24	0	0/05	0.10	0.07	0.09
5	0.99	0.35	2.19	0.50	2.71	2.06	1.66	1.48
6	7.35	1.14	3.09	0.91	4.02	2.78	2.70	2.16
7	0.32	0.27	0.77	0.35	0.97	0.79	0.71	0.65
8	0.25	0.31	0.66	0.55	0.61	0.62	0.70	0.75
9	1.68	1.86	3.41	2.42	3.29	3.17	3.52	3.28
10	0.11	0.23	0.20	0.43	0.18	0.15	0.22	0.22
11	0.17	0.09	0.52	0.14	0.40	0.35	0.31	0.30
12	0.25	0.30	1.71	0.50	0.57	0.60	0.70	0.73
13	0.63	0.92	1.09	0.96	1.20	1.19	1.35	1.28
14	0.39	0.22	1.11	0.31	1.15	0.98	0.74	0.72
15/16	2.95	3.07	4.89	3.56	4.78	4.68	5.16	4.91
17	1.11	1.31	0.77	0.94	0.87	0.60	1.27	0.96
18	0.13	0.25	0.36.	0.38	0.23	0.23	0.23	0.23

Peak ID N os. 2,3 and 4 are dianhydrides

Peak ID Nos. 5-18 are monoanhydrides

All results are means of duplicates; the latter were within 2% of the mean.

TABLE 30 RECOVERY OF SORBITAN ESTERS FROM BREAD PREPARED WITH EMULSIFIER

SAMPLE	RECOVERY %
SORB 3 Crumb A	n/a, 84, 80, 85, n/a
SORB 3 Crumb B	96, 99, 96, 90, 113
SORB 3 Crumb C	78, 89, 90, 89, 111
SORB 6 Crumb A	126, 94, 85, 96, 109
SORB 6 Crumb B	125, 99, 97, 100, 125
SORB 6 Crumb C	113, 102, 105, 102, 124

(Recoveries are given for sorbitan peak IDs 3,5,6,9, 15+16 respectively.)

TABLE 31 SORBITAN ESTERS IN FOODS – PRODUCTS

Sample	Full Description
SOR 1	Christmas Pudding Slices
SOR 2	Chocolate Torte
SOR 3	Cherry Bakewells
SOR 4	Cream Slices
SOR 5	Bakewell Tart

TABLE 32 SORBITAN ESTERS IN FOODS – RESULTS (1)

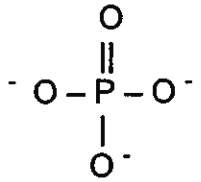
Sample	Sorbitan Esters g/kg
SOR 1	0.08
SOR 2	<0.05
SOR 3	1.11 1.02
SOR 4	0.07
SOR 5	0.72

TABLE 33 SORBITAN ESTERS IN FOODS – RESULTS (2)

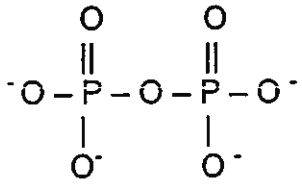
	Results as g/kg					
PRODUCT/ PEAK ID NO	3	5	6	9	15+16	MEAN (3,5,9)
SOR 1	**0.069**	**0.067**	0.441	**0.094**	0.311	0.077
SOR 2	**<0.05**	**1.608**	1.900	**<0.04**	0.769	<0.05
SOR3A	**1.038**	**1.078**	3.480	**1.227**	2.096	1.114
SOR 3B	**1.033**	**0.912**	3.672	**1.121**	1.791	1.022
SOR 4	**<0.06**	interference	Interference	**0.086**	interference	0.07
SOR 5	**0.698**	**0.644**	2.252	**0.825**	1.344	0.722

Figures

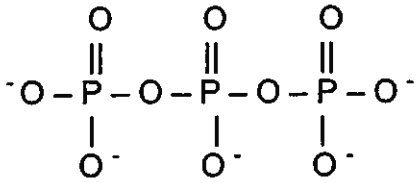
FIG.1 POLYPHOSPHATE STRUCTURES



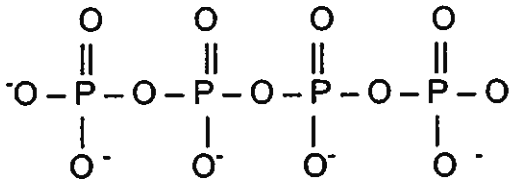
PO_4^{3-}
Orthophosphate



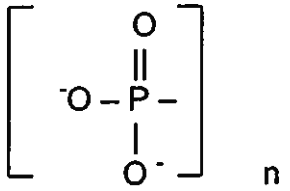
$P_2O_7^{4-}$
Di-phosphate (pyrophosphate)



$P_3O_{10}^{5-}$
Tri-Phosphate



$P_4O_{13}^{6-}$
Tetra- phosphate



$(PO_3^{2-})_n$
Polyphosphate

FIG.2 LACTIC ACID

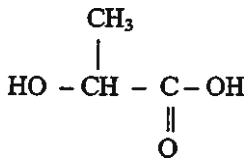
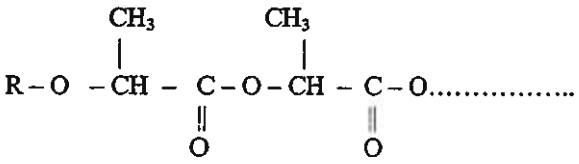
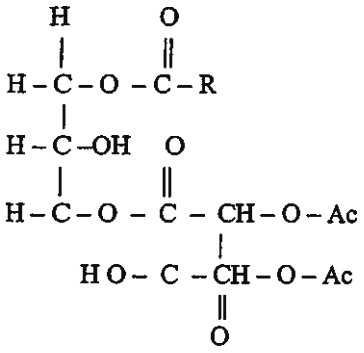


FIG.3 STEAROYL LACTYLATES



R = C16 / C18 Fatty Acid

FIG.4 DATEM EMULSIFIERS



Ac = Acetyl
R = Fatty Acid

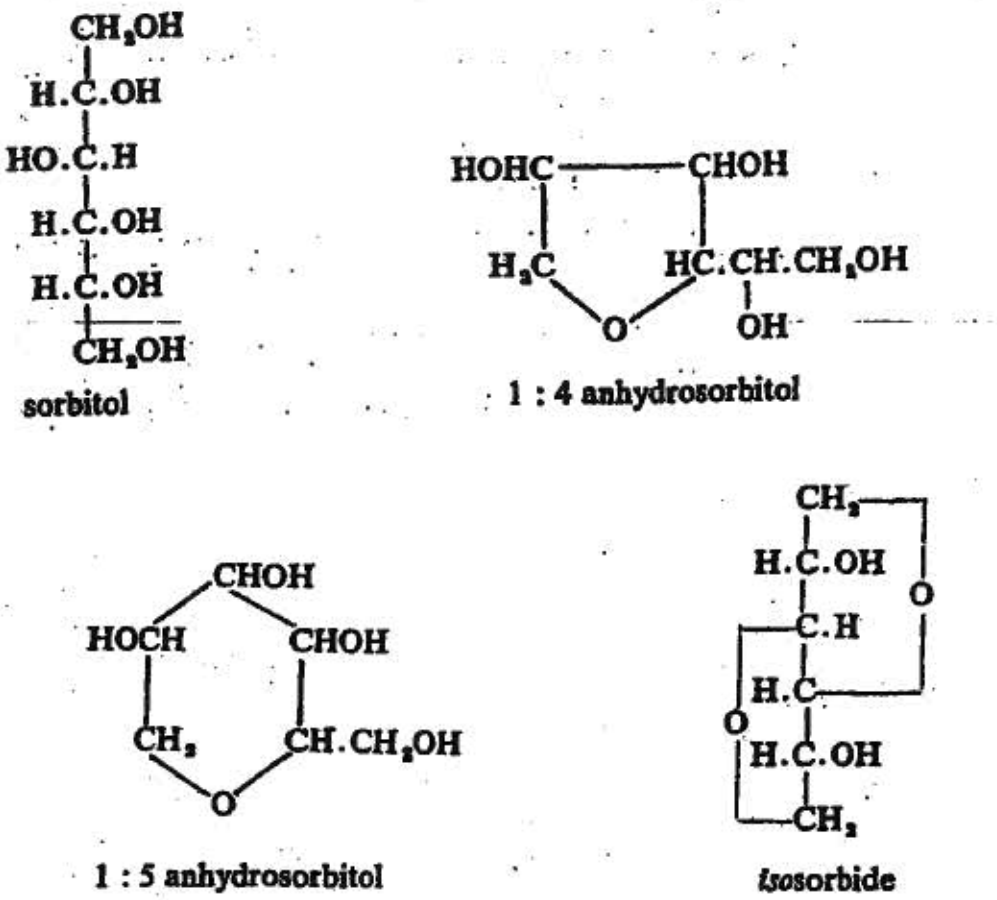


FIG. 5 SORBITANS PRESENT IN COMMERCIAL SORBITAN ESTERS

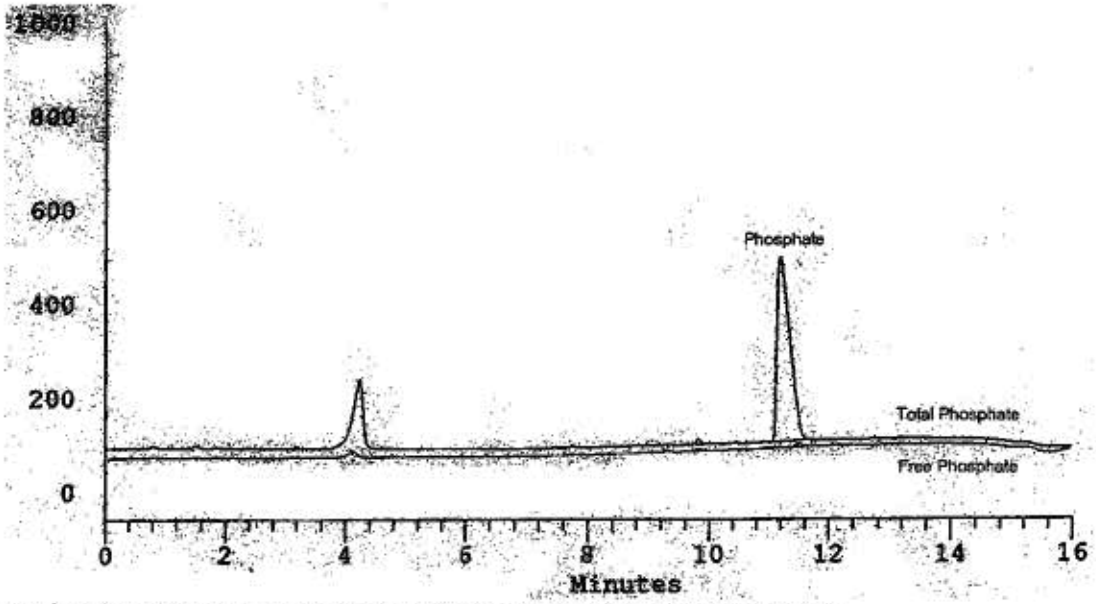


FIG.6 FREE AND TOTAL PHOSPHATE ANALYSIS OF COMMERCIAL POLYPHOSPHATE

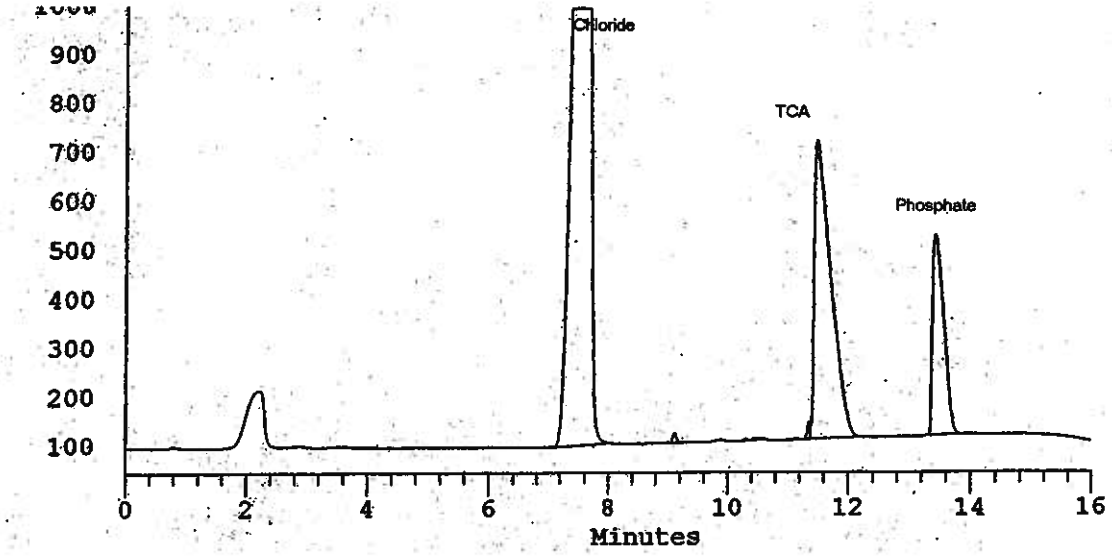


FIG.7 CHROMATOGRAPHIC SEPARATION OF FREE PHOSPHATE IN HAM EXTRACT

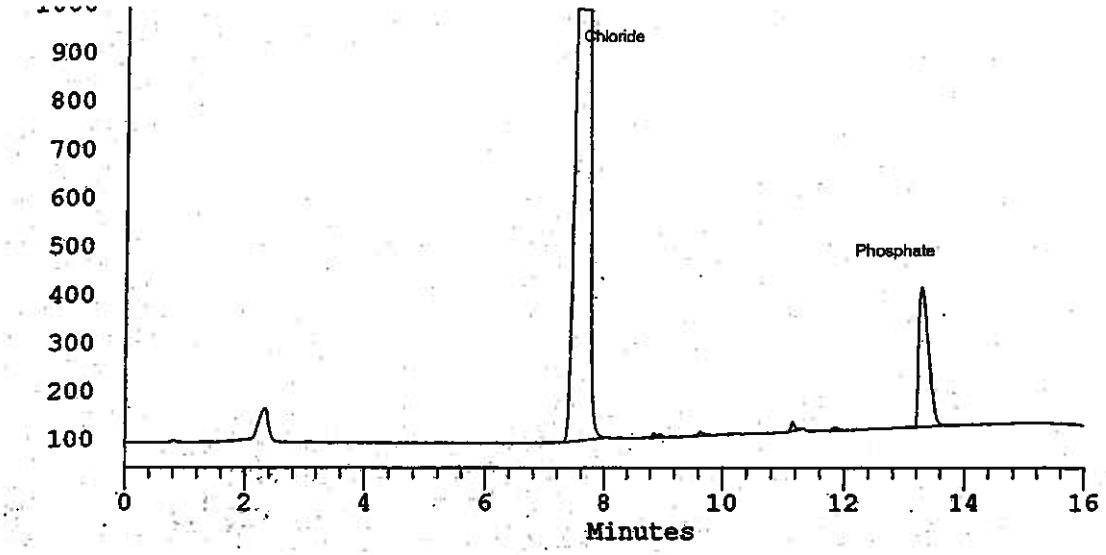


FIG.8 CHROMATOGRAPHIC SEPARATION OF TOTAL PHOSPHATE IN HAM HYDROLYSATE

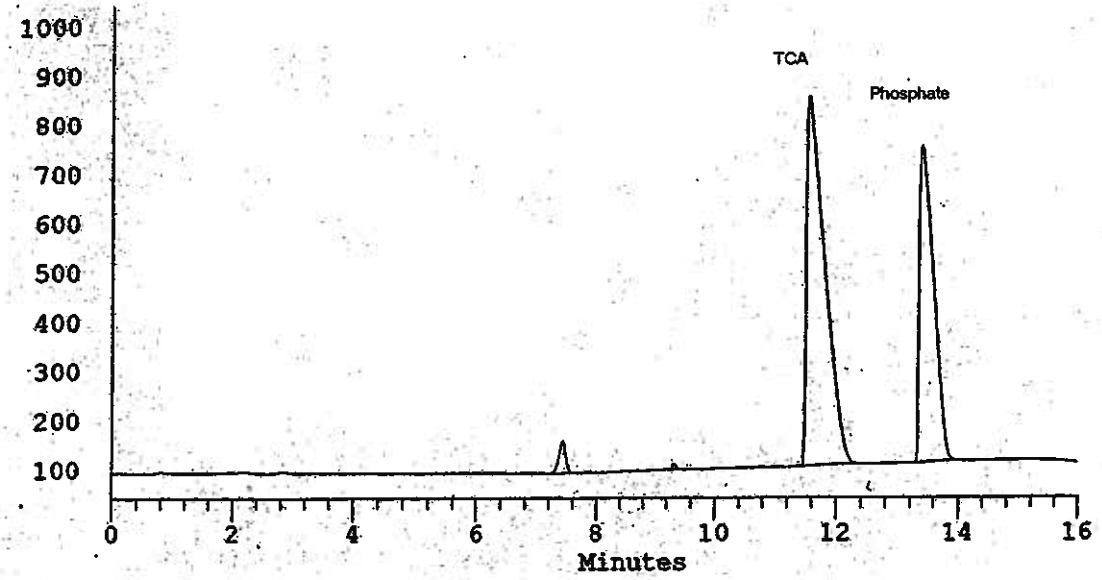


FIG.9 CHROMATOGRAPHIC SEPARATION OF FREE PHOSPHATE IN COFFEE WHITENER EXTRACT

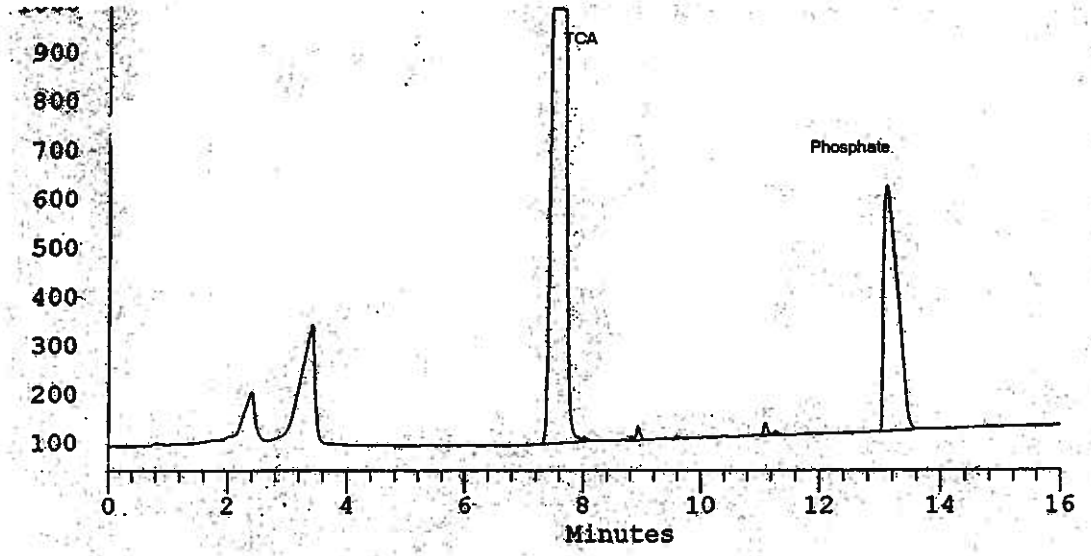


FIG.10 CHROMATOGRAPHIC SEPARATION OF TOTAL PHOSPHATE IN WHITENER HYDROLYSATE

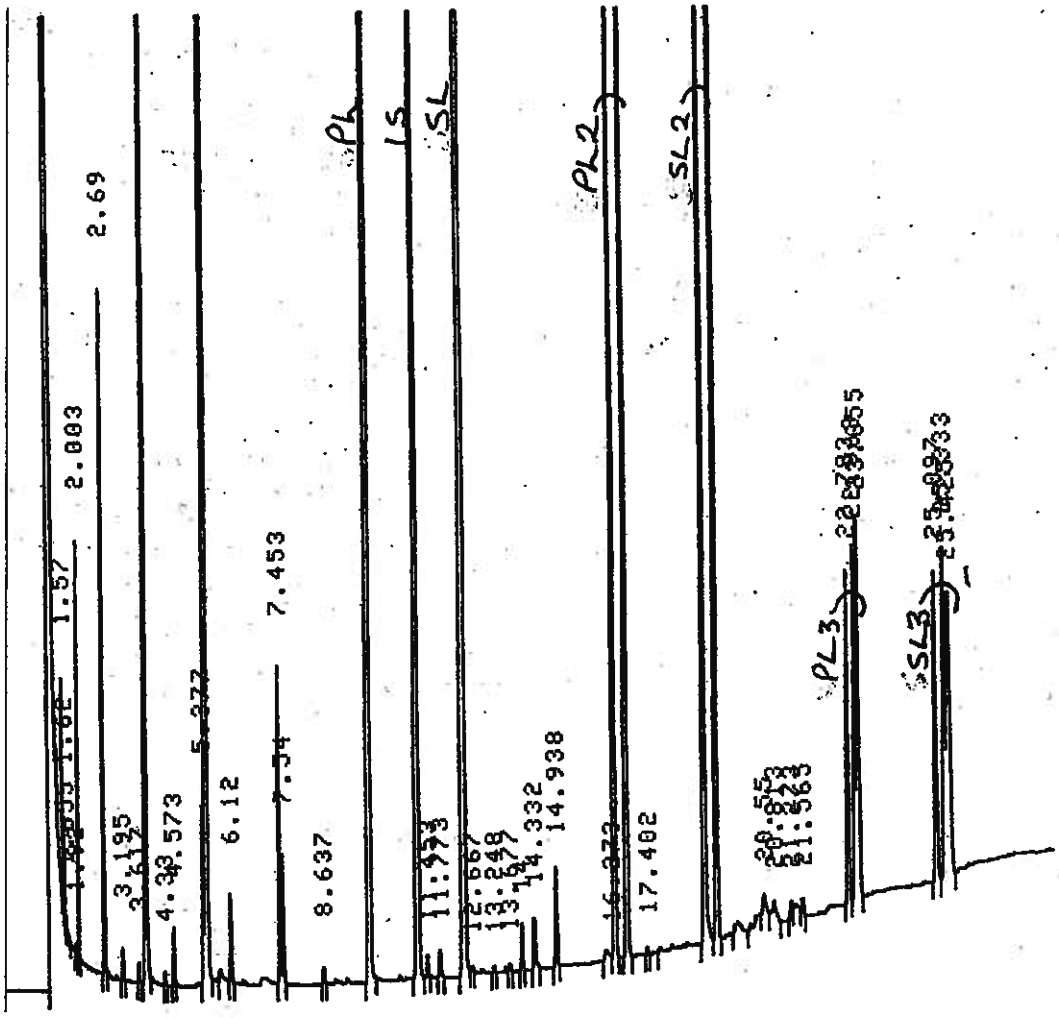


FIG. 11 CHROMATOGRAPHIC SEPARATION OF STEAROYL LACTYLATES IN COMMERCIAL EMULSIFIER
P – Palmitoyl, S – Stearoyl , L - lactylate , L2 – di- lactylate, L3 –tri- lactylate, IS- internal standard

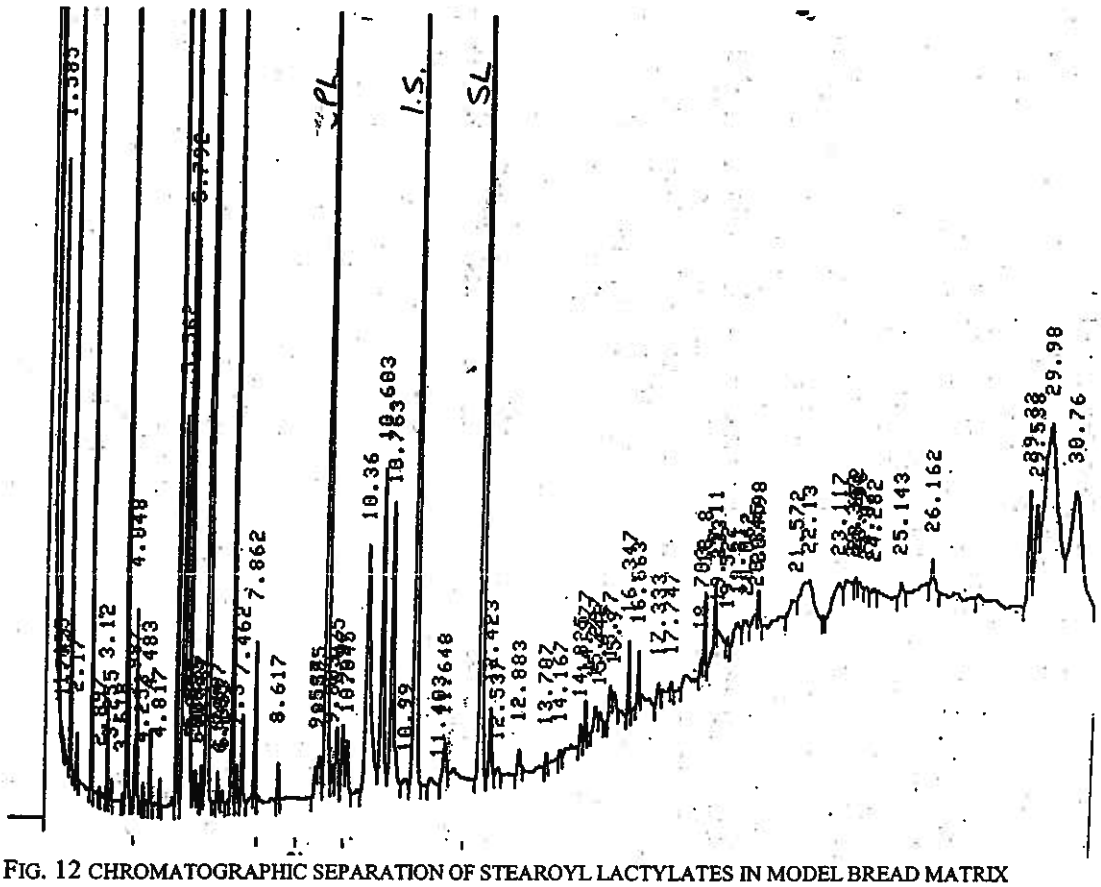


FIG. 12 CHROMATOGRAPHIC SEPARATION OF STEAROYL LACTYLATES IN MODEL BREAD MATRIX
P – Palmitoyl, S – Stearoyl , L - lactylate , IS- internal standard

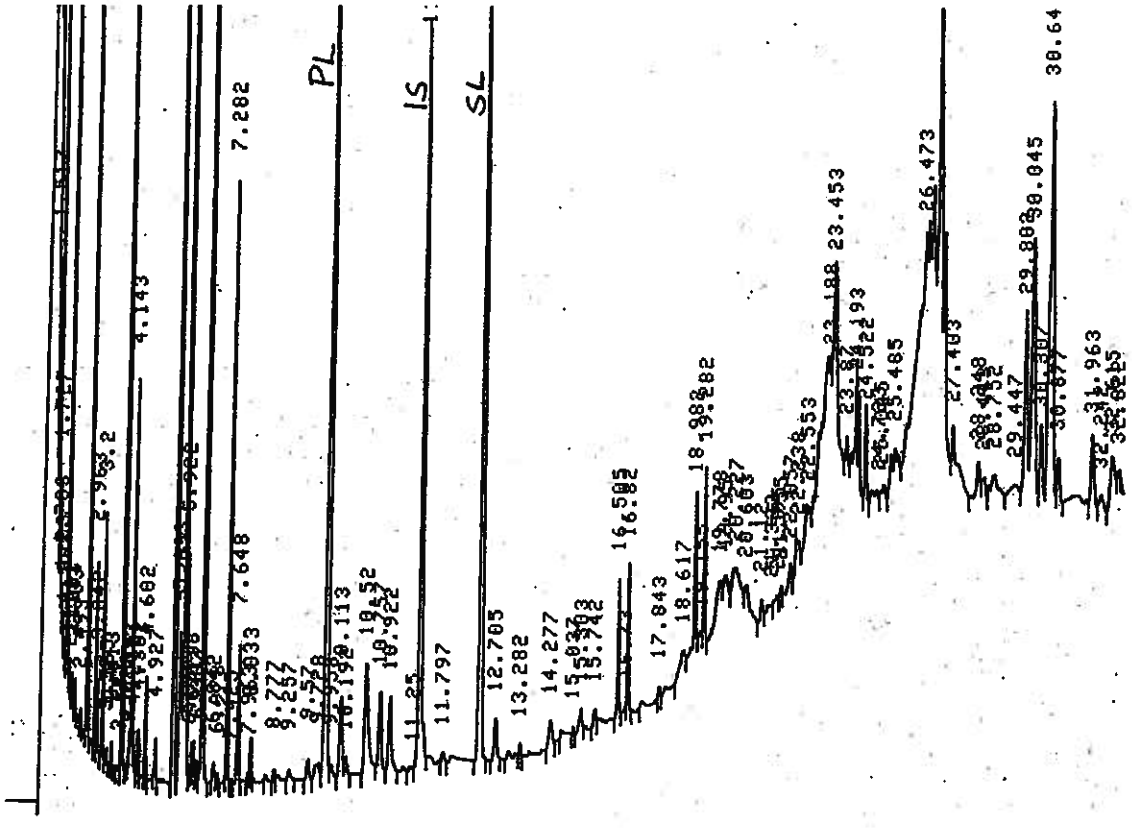


FIG. 13 ANALYSIS OF STEAROYL LACTYLATES IN CREAM BUNS
P – Palmitoyl, S – Stearoyl , L - lactylate, IS- internal standard

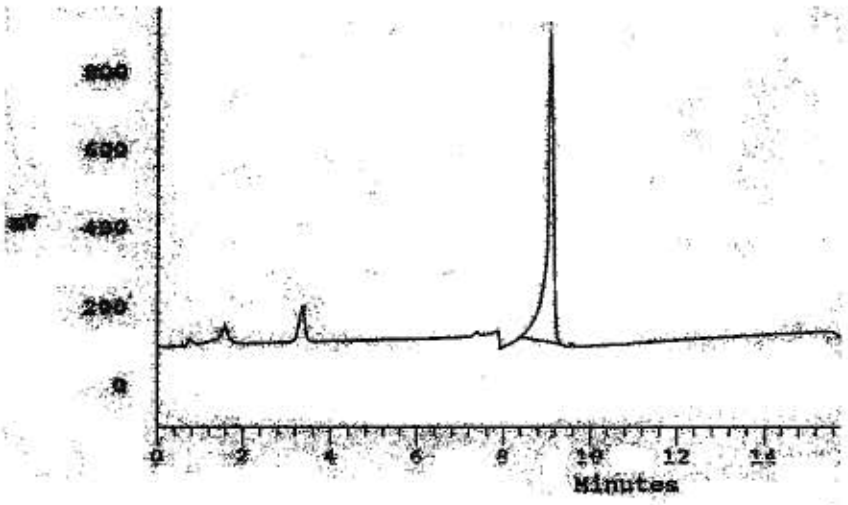


FIG.14 CHROMATOGRAPHIC SEPARATION OF TARTRATE IN DATEM HYDROLYSATE – NO CLEANUP

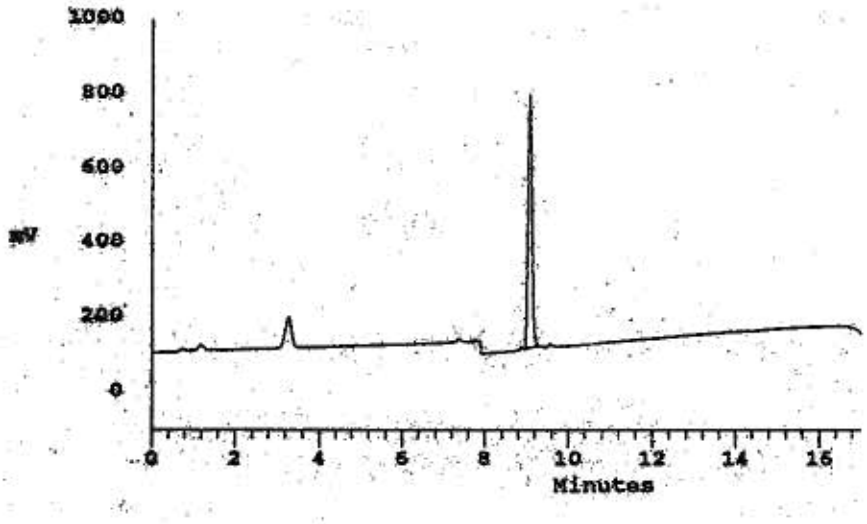


FIG.15 CHROMATOGRAPHIC SEPARATION OF TARTRATE IN DATEM HYDROLYSATE – WITH CLEANUP

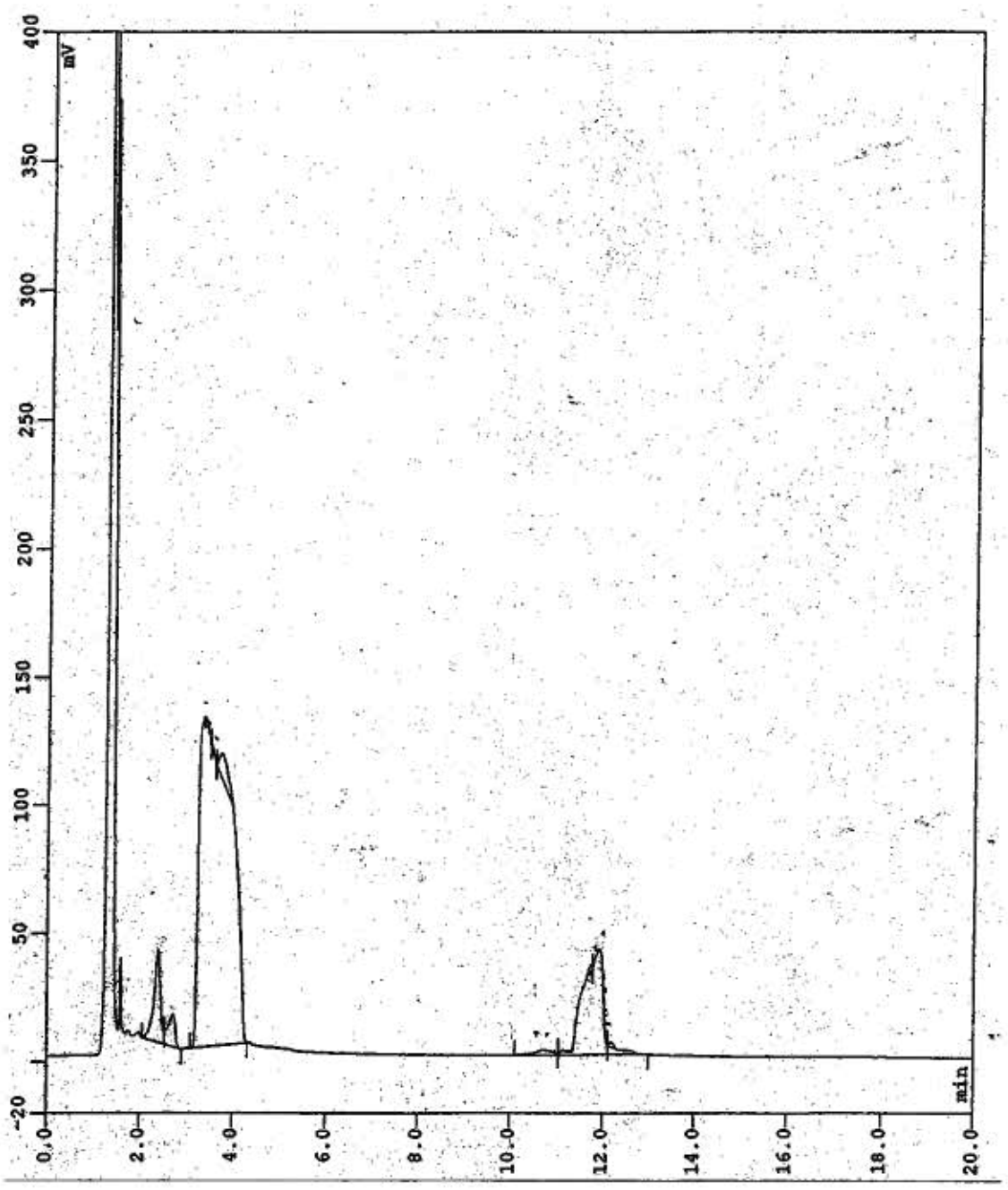


FIG.16 DATEM EMULSIFIER –DIRECT HPLC ANALYSIS

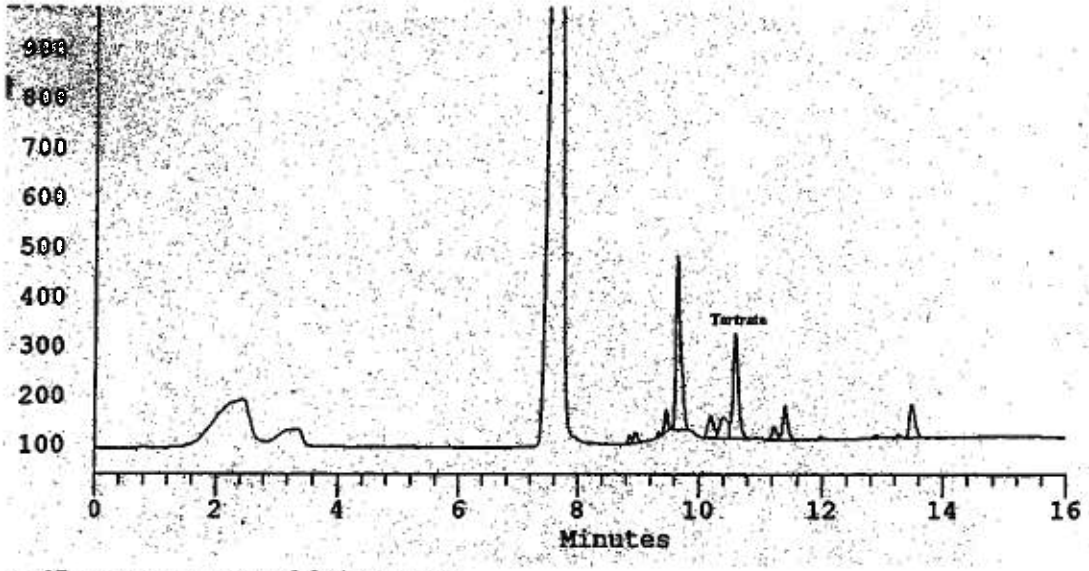


FIG.17 DIGESTIVE BISCUITS 0.2G/KG DATEM

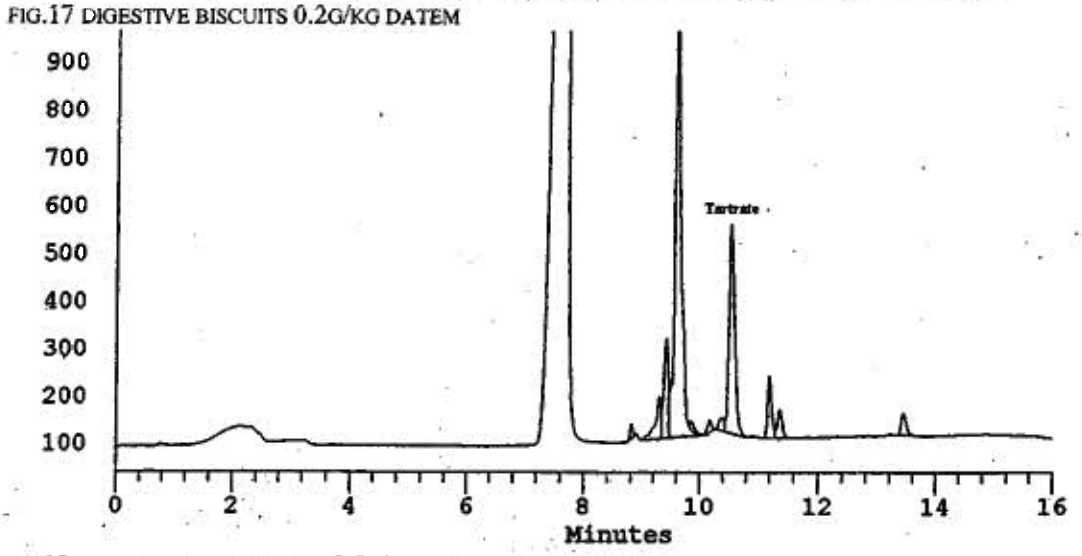


FIG.18 PREMIUM WHITE BREAD 0.5G/KG DATEM

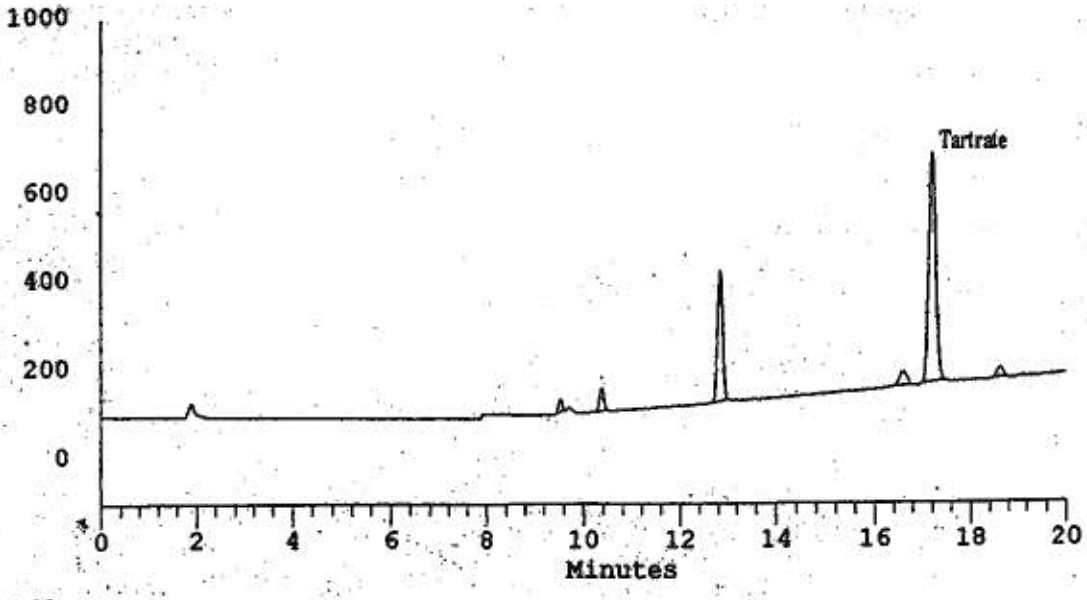


FIG.19 DATEM EMULSIFIER –ALTERNATIVE ION CHROMATOGRAPHIC CONDITIONS

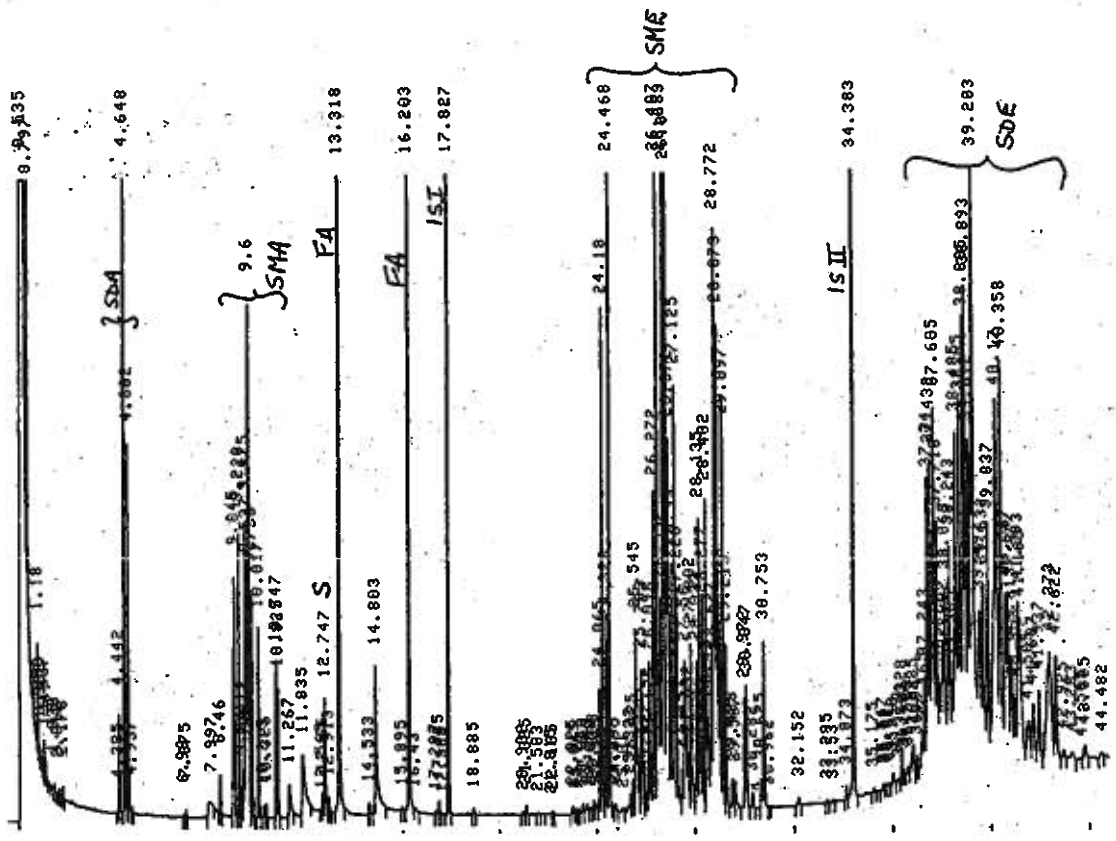


FIG. 20 DIRECT ANALYSIS OF SORBITAN ESTERS IN COMMERCIAL EMULSIFIER
S – Sorbitol, SDA – sorbitol dianhydrides, SMA – sorbitol monoanhydrides , SME – sorbitan monesters, SDE – Sorbitan diesters, FA – fatty acids, ISI- phenyl glucopyranoside, ISII – 1.3- dipalmitin

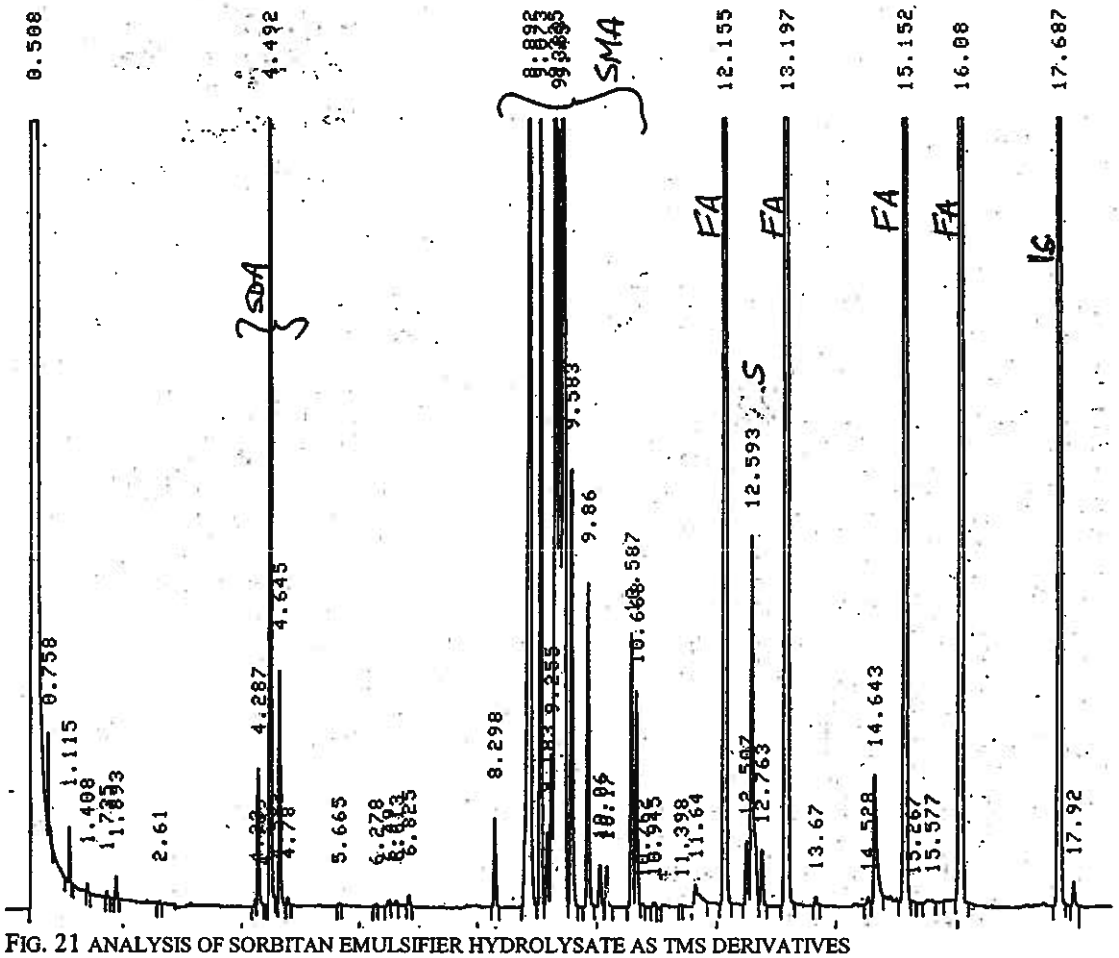


FIG. 21 ANALYSIS OF SORBITAN EMULSIFIER HYDROLYSATE AS TMS DERIVATIVES
S – Sorbitol, SDA – sorbitol dianhydrides, SMA – sorbitol monoanhydrides , SME – sorbitan monesters, SDE – Sorbitan diesters, FA – fatty acids, IS – internal standard

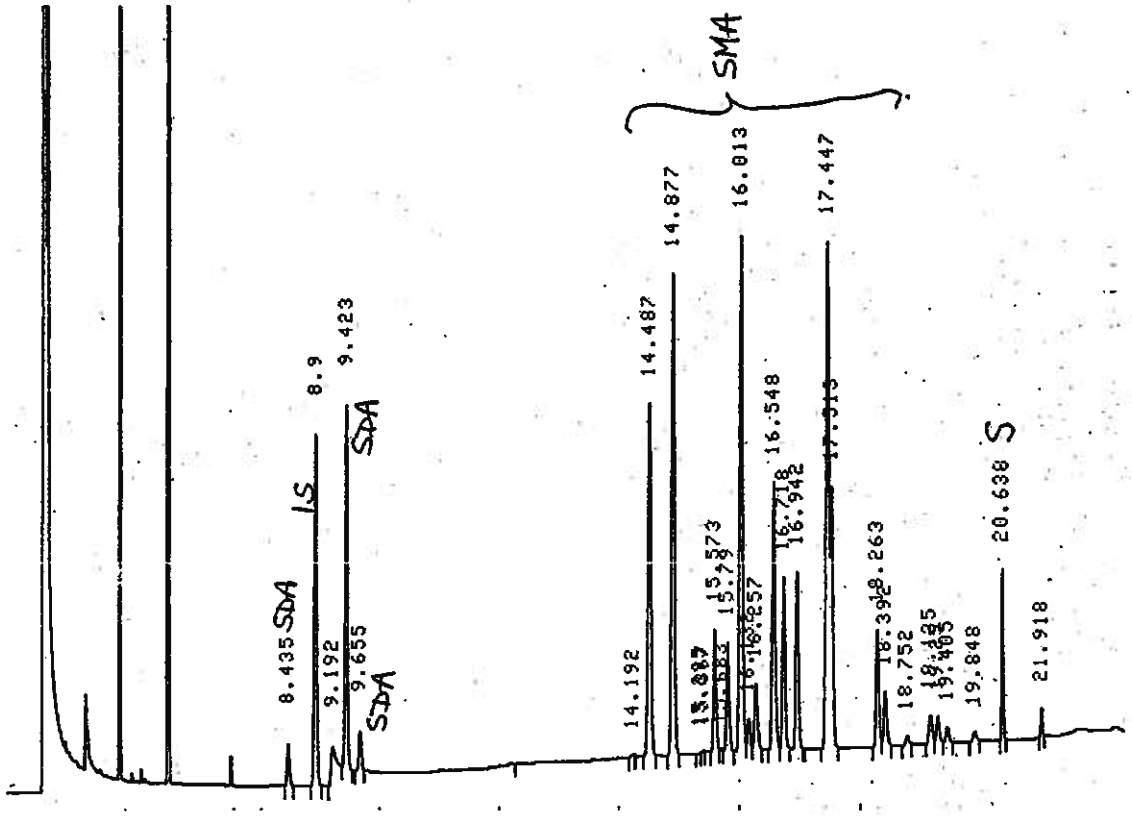


FIG. 22 ANALYSIS OF SORBITAN EMULSIFIER HYDROLYSATE AS ACETATE DERIVATIVES
S – Sorbitol, SDA – sorbitol dianhydrides, SMA – sorbitol monoanhydrides , SME – sorbitan monesters, SDE – Sorbitan diesters, FA – fatty acids, IS – internal standard

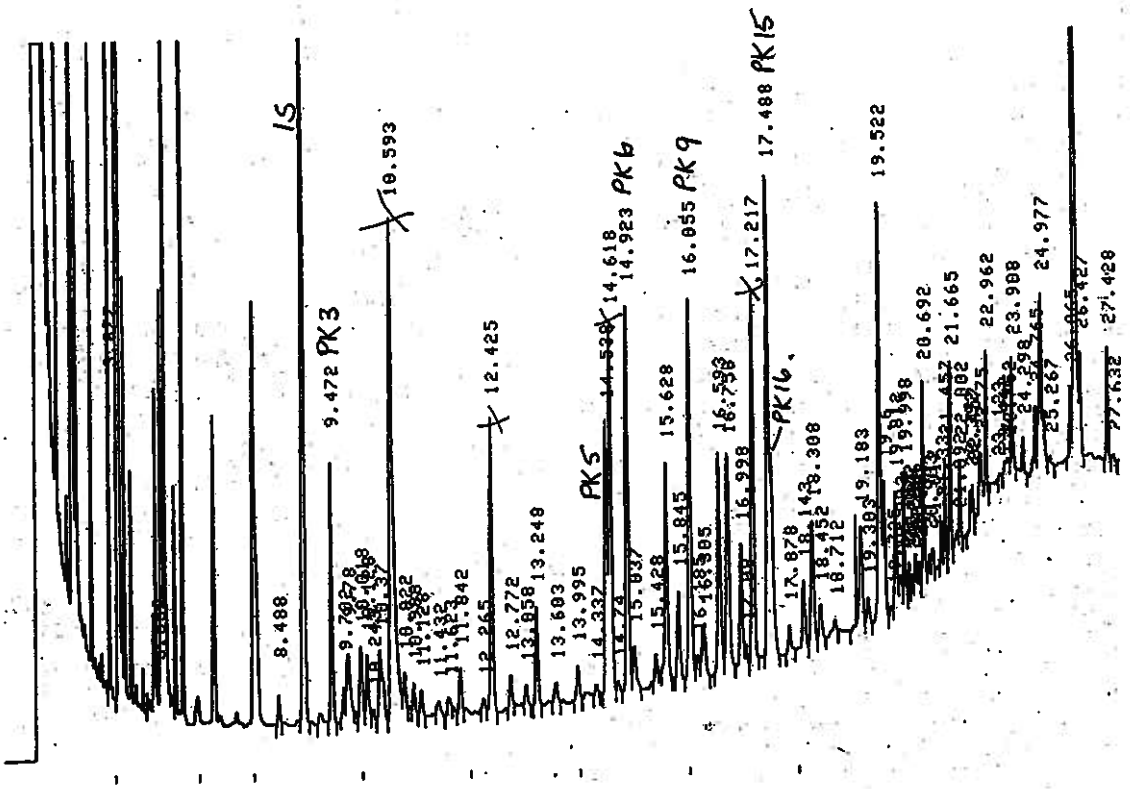


FIG. 23 ANALYSIS OF SORBITAN EMULSIFIER IN MODEL BREAD MATRIX
PK 3,5,6,9,15/16 – Sorbitan indices, IS – internal standard

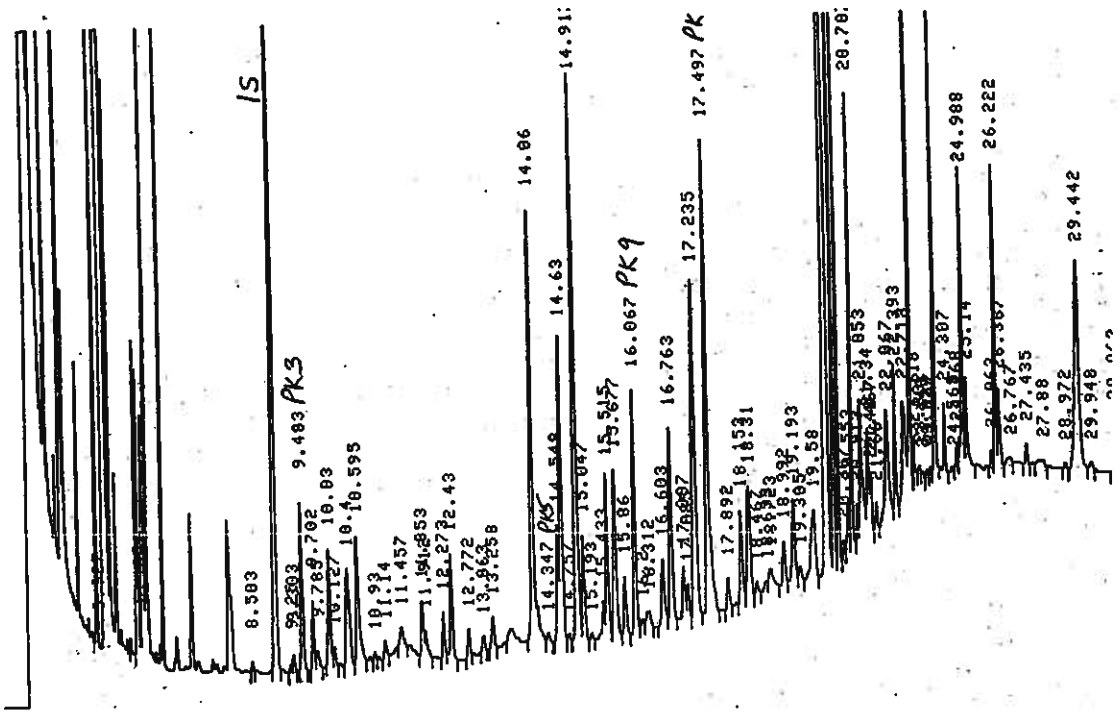


FIG. 24 ANALYSIS OF SORBITAN EMULSIFIER IN BAKEWELL TART
PK 3,5,6,9,15/16 – Sorbitan indices, IS – internal standard

Bread Sample Preparation

Samples were prepared using a Goodmans Cuisine model GHB174, home bread maker; normal programme with light setting

Recipe

275ml water
450g flour (Hovis Super strong white bread flour)
7.5g skimmed dried milk powder
9.5g salt
25g butter
3.2g easy blend dried yeast (Hovis fast action)
9g sugar

For added emulsifiers, 0.9g of powdered emulsifiers were added to 50g of flour, mixed then added to remaining 400g of flour.

For liquid emulsifiers (Datem 7), 0.9g of emulsifier were added to 275ml water and mixed well.

Loaves were allowed to cool overnight, weighed, crusts removed and remaining portion crumbed.

Emulsifiers	Wt. of emulsifier (g)	Wt. of Loaf (g)	Wt. of Loaf minus crusts
Control	-	685.67	514.38
SE3	0.917	686.12	513.70
SSL2	0.915	662.36	497.81
SE2	0.901	678.70	527.71
CSL2	0.908	680.53	505.88
Datem7	0.902	687.71	523.98

Emulsifiers	Wt. of emulsifier (g)	Wt. of Loaf (g)	Wt. of Loaf minus crusts
SE4	0.9050	676.45	459.70
SE6	0.9930	664.06	472.98
SSL2	0.958	Dough only	-
SSL2-2	0.946	685.41	461.90