Analytical Techniques in Food Emulsifiers

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ABSTRACT

After reviewing some problems arising in the analysis of food emulsifiers, we describe an analytical technique to detect most of them. The analytical method is based on the following procedure: (a) extraction of total lipids from food with a mixture of chloroform-methanol; (b) removal of nonpolar lipids from the lipidic extract by column chromatography with an elution media based on petroleum ether-ethyl ether mixtures; (c) thin layer chromatographic identification of the polar lipids extracted from foods. The validity of the method has been practically checked on some emulsifier-based foods, i.e., chocolates, toppings, ice creams, and whippable creams.

INTRODUCTION

Emulsifiers are widely used in foods to improve the foods' physico-chemical characteristics; their use is strictly regulated, and more and more analytical means are needed to evaluate them.

Various problems arise in the detection and chatacterization of food emulsifiers, including: (a) The components of some of the emulsifiers concerned are widely distributed in nature, e.g., monoglycerides esterified with tartaric or citric acid. (b) Some emulsifiers are naturally present in oils and fats, generally in low amounts according to origin and nature of the oil, for example, the oil in food. (c) The nature of the components in consideration – most food emulsifiers are esters of fatty acids, possibly with higher alcohols. Among these monoglycerides and their derivatives are found the most important class of additives (1,2). (d) Most emulsifiers are neutral compounds with various polarities expressed in HLB values. These values give a scale from monostearate at the bottom to polyoxyethylene sorbitan esters on the top. (e) The interactions of emulsifiers with food constituents must be considered. Very often emulsifiers are applied not only for their surface tension lowering effect but also for other more specific properties in foods, e.g., interactions of monoglycerides with protein and starch. These interactions lead to appreciated properties of the food in which the emulsifier is used and which are not primarily based on the tensio-activity of the emulsifier in question (3).

A peculiarity of most commercial emulsifiers is that they are not always single compounds but can be a mixutre of several well-defined substances in quantities which vary according to their origin and manufacture (4,5). Some of these problems have also been reviewed by Bauer (6).

Accordingly, it is wise not to look for an analytical method specific to one compound but for methods which are sensitive to characteristics common to all emulsifier compounds of a certain type. Several analytical methods have been approved by governmental bodies, but these may be used only for commercial brands and blends of emulsifiers, that is for pure compounds and mixtures thereof as well as for identification and determination of a restricted number of emulsifiers in food where their application is requested or permitted (6,7). The same is true for many publications treating analytical problems of emulsifiers (8-10).

Analyses of food emulsifiers are carried out in three steps, each giving rise to specific problems: (a) extraction from the foodstuff; (b) removal of excess triglycerides from the lipidic extract; (c) identification and determination of emulsifiers in enriched lipid extract.

Isolation and extraction of polar lipids, as completely and with as few artefacts as possible, is a problem very often encountered in biological research (11) and food analysis (12); most efforts having been directed in the field of phospholipids (11). This is the reason many publications are concerned with isolation of polar lipids and phospholipids especially from biological materials, plant materials, animal tissues, and cereals.

Essentially, two methods are in use: extraction based on Folch's method (13), and, for cereals, extraction with water-saturated butanol (14) (both frequently adapted to the special purpose of the work).

Mattey (15) successfully applied Folch's extraction procedure, slightly modified to extract fat-soluble emulgators. Hubbard (12) checked different extraction modes for lipids with regard to a more complete recovery of total lipids and sterols. He found that an extraction procedure similar to the one used by Mattey gave best results.

From the lipidic extract, nonpolar triglycerides can also be removed easily by column or thin layer chromatography (16,17). Silica gel is preferrred to alumina in column chromatography; the latter, being more active, may influence the structure of absorbed molecules. Among suitable elution systems, mixtures of benzene with ethyl ether have been selected (18). An alternative, avoiding the use of benzene, proposed by Ravin et al. (19), consists of mixtures of isooctane with isopropyl ether followed by more polar eluants based on isopropyl ether.

A further elution system has been used successfully based on petroleum ether-ethyl ether mixtures with increasing concentration of ethyl ether. The final elutions were done with methanol (20).

Generally, when elution systems with increasing polarity were applied, lipids were eluted in the following order with increasing polarity of the eluant: hydrocarbons; fatty acid esters of cholesterol; triglycerides; cholesterol, higher alcohols; diglycerides; monoglycerides; and phospholipids.

The resulting fractions from column chromatography were identified through analytical methods such as colorimetry, thin layer chromatography, etc.

The above-mentioned methods were of such value that they immediately became classical. Relatively few papers report the removal of triglycerides from the lipidic extract, and few investigations have been done in the field of global analyses of food emulsifiers. Braun (21) tried a general analytical scheme to cover all food additives and emulsifiers, whereas Mattey (15) limited his investigations to emulsifiers used in confectionery.

Quite recently, new analytical techniques based on liquid-liquid or gas chromatography (22-24) have been described.

EXPERIMENTAL PROCEDURES

Based on the previously cited works, we have verified the different analytical methods proposed for the three steps in the analysis of emulsifiers in foods. We have also introduced some modifications which seemed necessary to improve efficiency and recovery. For the extraction step, we adopted the method of Mattey (15); for working up of the lipid extracts, we used column chromatography based on the work of Hirsch and Ahrens (20).

The identification of the emulsifiers extracted was based on the work done in "Subcommission 23 – Emulsifiers" of the Swiss manual for food analysis (25).

Lipid Extraction

Apparatus: Standard centrifuge up to 7000 rpm; high speed homogenizer.

Sample size: 10 g finely powdered, grated, or sliced.

Method: To a weighed sample in a centrifuge tube of ca. 350 ml, the following reagents are added in order: 50 ml of chloroform, 100 ml of methanol, and 0.5 ml 1 m $MgCl_2$ $6H_2O$.

The mixture is then homogenized with a high speed homogenizer for 2 min. Chloroform (50 ml) is added and the mixture again homogenized for 2 min, followed by centrifuging for 10 min at 2000 rpm. The upper layer is decanted carefully with a pipette. (If the separation after centrifuging is incomplete, the decanted layer must be filtered.)

The residue is homogenized again for 2 min with 50 ml of chloroform and then centrifuged for 10 min, at 2000 rpm. As before, the liquid phase is decanted and the two extracts are collected in a separting funnel.

After addition of 90 ± 2 ml of water, the mixture is shaken vigorously. An emulsion may form but can easily be destroyed by the addition of ~100 mg NaCl.

The lower layer is separated and the upper layer further extracted with 50 ml of the solvent used by Folch (chloroform-methanol, 2:1, v/v).

The upper layer is discarded, and the lower layer, together with that obtained in the previous step, evaporated to dryness. After heating under vacuum at 60 C for ½ hr and cooling to room temperature, the residue is weighed.

Column Chromatography of the Lipidic Extract

Apparatus: chromatographic column, 2 cm diameter, 40 cm length, equipped with a fritted disc and a stopcock.

Reagents: Silica gel Merck No. 7729, petroleum ether, ethyl ether, chloroform, methanol.

Column preparation: 30 g of silica gel is immersed in 100 ml petroleum ether. After stirring the slurry with a glass rod to expel air bubbles, the whole quantity is added ot the column. All of the silica gel is washed into the column with additional aliquots of petroleum ether, which is then drained off so that the top of the silica gel remaines more or less covered with liquid.

Elution: 1 g of the sample in 10 ml toluene is added to the column, and then toluene solution is run into the column until the level reaches 1 cm above the adsorbent. Elution speed is adjusted to 1.5-2 ml/min. Then, elution with 7% ethyl ether in petroleum ether is begun by carefully adding 10 ml of the eluant. As soon as the level falls to 1 cm above the silica gel, 10 ml of the eluant are again added. These operations are repeated after each change of eluant.

The following fractions with volume and eluant, respectively, are collected: Fraction 1, 300 ml, 7% ethyl ether in petroleum ether (v/v); Fraction 2, 150 ml chloroform-methanol (2:1, v/v); and Fraction 3, 100 ml, methanol.

It is wise to collect 50 ml of the effluent each time and, after evaporating, immediately weigh the fraction eluted. As soon as the fraction size falls below 10 mg, the eluant is changed.

Thin Layer Chromatography of the Fractions Collected

The effect of the separation can be monitored by thin layer chromatography. The analytical procedure consists of developing thin layer chromatograms with two migration solvents followed by alkaline cleavage of the emulsifiers and separate detection of the alcohol and acidic units. For organic acids, a third migration solvent is proposed.

Apparatus: chromatographic equipment.

Reagents: thin layer plates precoated with silica gel 60 F-254 as, for example, Merck 5715.

Developing solvents	Parts per volume	Abbreviations used
petroleum ether ethyl ether acetic acid	60 40 1	(L1)
chloroform methanol water	65 25 4	(L2)
ethyl ether (water saturated) formic acid 80%	7 1	(L3)

Spray reagents	Abbreviations used
2,7′ Dichlorofluoresceine nonspecific reagent	ex Merck No. 9677 R1
Anisidine/potassium periodate (26), specific for vicinal hydroxyl groups as in α-monoglycerides, white spots on violet background	R2
Modified Dragendorff reagent (15), specific for polyoxyethylene compounds quickly appearing as orange red spots on a yellowish background	R3
Naphtoresorcine (27), specific for sugar and sugar esters of fatty acids, violet spots after heating ¼ hr at 100 C on a white background	R4
Reagent according to Dittmer and Lester (15), specific for organic phosphorous compounds, blue spots on a white background after developing	R5
Silver-nitrate reagents (28), specific for tartaric acid	R6
Bromcresol green according to Stahl, No. 31 sensitive to acid compounds.	R7

Method: For facility of execution, a 20 x 20 thin layer plate is divided into two parts, one developed with L1, the other with L2. After spraying with R1, the presence or absence of some emulsifiers can be detected using the Rf-values listed in Table I. With the spray reagent specific for the different numbers of a class (R2 for monoglycerides and polyols, R3 for "Tweens," R4 for sugars and sugar esters of fatty acids, and R5 for phosphorus-containing organic compounds such as lecithins), most emulsifiers may be characterized.

It is advisable to compare supposed emulsifiers with standards as often as possible, always taking into consideration that commercial products may vary from one supplier to another.

After this, the hydrolysis of the polar lipids extracted from foods gives more information. Polar lipid extracts (0.2 g) are heated with 1.2 ml 1 m alcoholic KOH and 1.2 ml H_2O under reflux for 1 hr. After cooling, ~1 ml Dowex 50 W (acid form washed with distilled water) are added. On shaking vigorously, a white precipitate is formed, and the pH of the aqueous solution falls to ca. 4-5. Then, 3 ml of petroleum ether are added and the mixture again shaken. On standing, the petroleum ether separates and is carefully removed. It contains fatty acids and all unsaponifiable components which are soluble in petroleum ether. The aqueous phase contains polyols, organic acids, and the water-soluble components of the unsaponifiable emulsifiers. The two layers are chromatographed with developing solvent L2 and the spray reagents R2, R6, and R7, charging the starting spots with 5 μl of petroleum ether phase, 7 μl of aqueous phase for detection of polyols; and 15 μl of aqueous phase for detection of organic acids. Hydrolyzed products are identified as shown in Table II.

TABLE I

Identification of Emulsifiers by Thin Layer Chromatography

	Rf values		Identification reaction
	L1	L2	
Triglycerides	0.8	0.9	One spot only with R1, nonspecific reaction
Fatty acids	0.6	0.7	With the spray reagents proposed
Diglycerides	0.2-0.4	0.9	
Glycerol	0.0	0.3	With R2 specific
Monoglycerides	0.0-0.15	0.7	With R2 specific, one or two spots
Monoglycerides acetylated	0.3-0.6		With R1, no specific reaction with the proposed spray reagents Dist. acetylated monoglycerides, one spot at 0.6 with L1.
Monoglycerides esterified with critric or diacetyltartaric or lactic acids	b	b	With R1, no specific reaction
Fatty acid esters of propylene glycol	0.5	0.8-0.9	With R1, no specific reaction
Stearoyl lactylates	0.5	0.7	With R1, no specific reaction, characteristic group of spots centered at the Rf-values indicated
Fatty acid esters of polyglycerols	0.0-0.7	0.4-0.9	Large chain of diffuse spots within the range *of Rf-values indicated*
Polyricinoleic acid ester of polyglycerols	0.0-0.7	0.4-0.9	Idem.
Spans	b	0.5	With R1, no specific reaction
Tweens	0.0	0.7	With R3, specific several spots
Fatty acid esters of sugar alcohols	0.0	b	With R4, specific
Lecithins	0.0	b	With R5, specific
Emulgator YN	0.0	b	With R5, specific
Polyols after hydrolises			
Glycerol	0.0	0.3	With R2, specific
Ethylene glycol	0.0	0.5	With R2, specific
Propylene glycol	0.05	0.6	With R2, specific
Sugar alcohols	0.0	0.0-0.1	With R2, specific and R4 too
Sorbitols	0.0	0.1-0.3	With R2, specific double spots With Rf (L2), ∿0.15 and 0.25
Polyoxyethylene			
Sorbitol		0.9	With R3, specific
Polyglycerols	0.0	0-0.3	With R2, specific spot at 0.3 With a pronounced tail edged at 0.0
Organic acids after hydrolysis	L1	L2	
Citric acid[d]	0	0.2-0.3	*Specific with R6*
Tartaric acid[d]	0	0.2-0.3	Specific with R6 and R7
Lactic acid[d]	0.2	0.6	Specific with R6

[a]Rf values may vary somewhat according to the preparation of the developing solvent, but in any event they indicate the order of the spots.
[b]The sequence of spots may vary according to the origin of the emulsifier.
[c]Diacetyltartaric acid is converted to tartaric acid during hydrolysis.
[d]Rf-values for L3 are 0.3, 0.2, and 0.6 for citric acid, tartaric acid, and lactic acid respectively.

TABLE II

Comparison of the Proposed Extraction Method with Mojonnier (%)

	Proposed method	Mojonnier	Deviation	Recovery in % of Mojonnier
Milk chocolate	29.0	30.0	-1.0	97
Milk powder I	27.2	28.2	-1.0	96
Milk powder II	25.7	26.2	-0.5	98
Ice cream powder I	17.2	17.3	-0.1	98
Ice cream powder II	16.9	17.0	-0.1	99
Whippable cream				
Spray dried powder no. 1	48.2	49.4	-1.2	98
Spray dried powder no. 2	58.2	58.6	-0.4	99
Spray dried powder no. 3	58.4	59.2	-0.8	99
Spray dried powder no. 4	56.5	56.2	+0.3	101

TABLE III

Examples of Some Food Analyses

	Composition of the lipidic extract		
	Nonpolar lipids	Polar lipids	Identification with TLC
Coating Mass I	93	8	Monoglycerides, Spans, Tweens, YN (the latter three amounts 3%)
Coating mass II	92	6	Spans lecithins
Dark chocolate	89	8	Lecithins and other polar lipids
Whippable cream			
Spray dried powder no. 1	58	42	Monoglycerides pure and lactylated, propylene glycol esters
Spray dried powder no. 2	93	14	Monoglycerides pure and lactylated, propylene glycol esters
Spray dried powder no. 3	80	20	Monoglycerides pure and lactylated
Spray dried powder no. 4	80	22	Monoglycerides pure and acetylated and lactylated

RESULTS AND DISCUSSION

The extraction method described here overcomes two of the limitations to those proposed by Mattey (15). Firstly, we have reduced sample size to 10 g. When the sample is very voluminous, much of the liquid extract is retained. We have observed that, on limiting sample size to 10 g, losses can be reduced to a negligible amount. Secondly, we have introduced an extra extraction step with 50 ml of Folch's solvent (chloroform-methanol, 2:1, v/v) of the aqueous layer resulting in the washing of the combined solvent phases after centrifuging. These two modifications enabled us to improve recoveries considerably (see Table III).

It is clear that extraction works best with dried and pulverized samples, and care must be taken that separation from the sludge is complete, otherwise the extracts must be filtered.

Removing excess triglycerides from the lipidic extract can be done quantitatively by column chromatography described previously. The system of eluants chosen based on a mixture of petroleum ether with ethyl ether, allows careful adjustment of the polarity of the eluant thus giving much better separation of polar lipids than in the methods of Quinlin and Weiser (18) and Ravin et al. (19). Column chromatography, as proposed, directly gives the separation of polar and nonpolar lipids in the lipidic extract; some results are reported in Table IV.

A gross estimation of polar lipids can be obtained by heating a 10% solution of the lipidic extract in methanol under reflux. After cooling in the refrigerator, the methanol is removed and evaporated to dryness. The recovered residue is suitable for TLC.

The polar lipid fraction is then subjected to thin layer chromatographic analysis. With the analytical schema described, it is possible to identify and characterize about 90% of emulsifiers used. The spray reagents used indicate directly the presence of a certain chemical type of an emulsifier, whereas liquid-liquid or gas liquid chromatography gives this sort of information only indirectly. On the other hand, the two methods mentioned give quantitative composition of the polar lipid fraction very quickly. Recent publications show great possibilities in this field, and it is possible that chromatographic analysis may replace to a great extent, TLC (22-24).

However, TLC can be executed very quickly without expensive apparatus. The spray reagents proposed are very specific to the class of compounds. At present, we cannot see any interference from excess foreign matters.

Unfortunately, we cannot distinguish between natural and synthetic emulsifiers, but, taking into account spot size on the plate and the expected size, along with the amount of polar lipids present, the addition of an emulsifier can be supposed. Hydrolysis of lactylated compounds gave problems which we overcame by carrying out a hydrolytic splitting in an aqueous-alcoholic medium.

In detection of lactic acid, its relatively high volatility must be taken into account. After development with the acidic migration solvent, L3, the thin layer plates must be dried carefully to remove the least traces of acid prior to spraying with R6. Therefore, we propose development of the aqueous layer after hydrolysis with development solvent L2 and spray reagents R6, whereas citric and tartaric acid, being less volatile, can best be identified with L3 and R7.

The previously described method allows easy isolation, detection, and identification of most food emulsifiers. In some cases the time required for an analysis may be reduced by omitting the column chromatography step for the detection of very polar emulsifiers, without affecting the precision of the result.

With further study of the possibilities offered by the new analytical methods, such as liquid-liquid or gas liquid chromatography, it might be possible to overcome the disadvantage of TLC. For the time being, however, TLC is a very useful analytical method not requiring complicated and expensive apparatus, and its efficiency can be greatly improved with experience.

REFERENCES

1. Petrowski, G.E., Food Rechnol. 29'52 (1975).
2. Petrowski, G.E., Ibid. 30:36 (1976).
3. Krog, N., JAOCS 54: 124 (1977).
4. Seher, A., and J. Janssen, Fette Seifen Anstrichm. 72:773 (1970).
5. Neissner, R., Ibid. 79:24 (1977).
6. Bauer, F.J., JAOCS 50:85 (1973).
7. FAO/WHO Specifications of the Idantity and Purity of Food Additives, Part V-VII/WS/E5468, Rome, 1973.
8. Nash, N.H., and L.M. Brickman, JAOCS 49:457 (1972).
9. Murphy, J.M., and H.R. Hibbert, J. Food Technol. 4:227 (1969).
10. Lehmann, G., and M. Junk, Gordian 77:88 (1977).
11. Kuksis, A., J. Chromatogr. 143:3 (1977).
12. Hubbard, W.D., A.J. Sheppard, D.R. Newkirk, A.R. Prosser, and T. Osgood, JAOCS 54:81 (1977).
13. Winter, E., Z. Lebensm. Unters. Forsch. 123:205 (1963).
14. Daftary, R.D., and Y. Pomeranz, J. Food Sci. 30:577 (1965).
15. Mattey, M.E., BFMIRA Technical Circular No. 509 (April 1972).
16. Stein, R.A., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 8, Part 3, Edited R.T. Holman, Pergamon Press, Elsmford, NY, 1966, p. 375.
17. Padly, F.B., in "Chromatographic Review," Vol. 8, Edited by M. Lederer, Elseviers Publishing, Amsterdam, 1966, p. 208.
18. Quinlin, P., and H.J. Weiser, JAOCS 35:325 (1958).
19. Ravin, L.J., R.J. Meyer, and T. Higuchi, Ibid. 34:281 (1957).
20. Hirsch, J., and E.H. Ahrens, J. Biol. Chem. 233:311 (1958).
21. Braun, G., E. Hieke, and H. Suckes, Z. Lebensm. Unters. Forsch. 146:67 (1971).
22. Aitzetmüller, K., J. Chromatogr. 113:231 (1975).
23. Dick, R., and A. Miserez, Mitt. Geb. Lebensmittelunters Hyg. 67:472 (1976).
24. Brüschweiler, H., Ibid. 68:46 (1977).
25. "Swiss Manual for Analysis of Food," Subcommission 23: Emulsifiers, Chairman Prof. H. Streuli, Rorschach.
26. Halvarson, H., and O. Qvist, JAOCS 51:162 (1974).
27. Pastuska, G., Z. Anal. Chem. 179:427 (1961).
28. Kröller, E., Fette Seifen Anstrichm. 64:602 (1962).

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