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Review Article

Analysis of Emulsifier in Food Using Chromatographic Techniques

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ABSTRACT

Emulsifiers so far are important class of additives used in food products. Food industries and regulatory authorities have striven for the continuous development of analytical methods to determine the emulsifiers in foods. Chromatography is one of the powerful analytical techniques used in the analysis of food components due to its capability for the separation and quantitative analyses of emulsifiers. This article describes some chromatographic techniques, namely gas chromatography (GC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and planar chromatography for detection and quantification of emulsifiers in food. Sample preparation involved in the analysis of emulsifiers has been also highlighted.

Keyword: analysis, emulsifier, chromatography, food

1. Introduction

An emulsifier is a type of surfactant usually used to maintain emulsion (the mixtures of two immiscible fluids consisting of water and oil phases) in good dispersion. Chemically, the emulsifiers have hydrophobic and hydrophilic groups. They will surround oil and other immiscible substances to form a protective layer so that the oil molecules cannot "clump" together (Mc Clements, 2002). In foods, emulsifiers have been classified as group A (acceptable) or Group B (provisionally acceptable). Some items classified in group A are lecithins; polyoxyethylene, sorbitan monolaurate, monooleate, monopalmitate, mono-stearate and tristearate (Tweens 20, 80, 40, 60 and 65); carboxymethylcellulose (CMC), monoglyceride (MG) and diglyceride (DG), acetic acid esters of MG and DG, and polyglycerol esters of fatty acids; and sucrose esters of fatty acids. Some substances classified in Group B are carrageenan, lactic and citric acid esters of MG and DG of fatty acids, mono- and diacetyltartaric acid, esters of MG and DG of fatty acids, propylene glycol esters of fatty acids, sorbitan monostearate, tri-tearate, monolaurate, monooleate and monopalmitate (Spans 60, 65, 20, 80 and 40), and polyglycerol esters of dimerized fatty acids of soybean oil (Copestake, 1992). The chemical structures of some emulsifiers are shown in Figure 1.

In the quality control, the analytical importance of food emulsifiers comes from several stages, namely in production control, for comparison of emulsifiers purchased from different suppliers, and for the detection of the type of emulsifiers used in a commercial food and pharmaceutical products in order to assure that the emulsifiers comply with its specifications. Authorities may also require analyses to meet health regulations. Since most food emulsifiers are complex mixtures of several isomers and derivatives, it is quite tedious and cumbersome to analyze their composition (Garti *et al.*, 1983).

The development of analytical methods for analysis of emulsifiers in food samples is rather not easy due to three major reasons: (1) commonly, food emulsifiers have many chemical similarities which make it difficult to be separated and distinguished when they are present in mixtures. (2) None of the emulsifiers is a single compound and most are quite heterogeneous mixtures. Chromatography can overcome these 2 problems due to its capability to separate emulsifiers into each component, and (c) the nature of emulsifiers makes them difficult to remove

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from constituent proteins and carbohydrates present in finished foods. Therefore, a suitable sample preparation was needed in order to obtain the acceptable results (Baur, 1973). Analytical method for determination of emulsifier are closely related to or derived from methods commonly used for analysis of fats and oils (Hasenhuettl, 1997). In this article, chromatographic techniques for analysis of emulsifiers commonly used in food products are described.

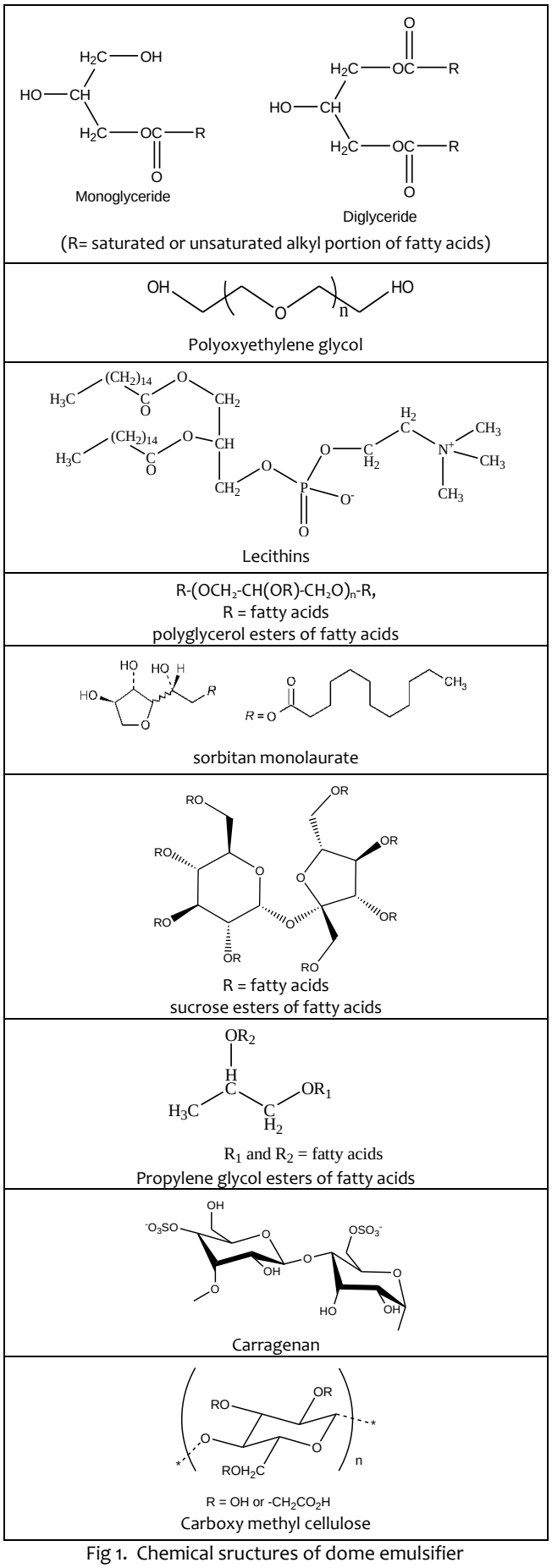

Fig 1. Chemical sructures of dome emulsifier

2. Sample preparation

Before being analyzed, the emulsifiers must be extracted from the sample matrix. In order to extract the emulsifier, analyst must consider the solubility of emulsifiers in some organic solvents, either alone or in the combination (Watkins, 1989). Compared with other lipids, emulsifiers are more polar, because emulsifiers have hydrophobic and hydrophilic groups, therefore chloroform or mixture of chloroform/methanol are sufficiently polar to extract emulsifiers from food samples (Flor and Prager, 1980).

Some solvents used for extraction of emulsifiers have been described by Baur (1973). Chloroform is used for extraction the esters of polysorbates and sugar in baked goods and cake mix. The mixture of MeOH-$CHCl_3$ is suitable for extraction of ethoxylated monoglycerides in baked and bread, whereas the combination of $CHCl_3$-EtOH is used for extraction of polysorbates in cake mix and lecithin in cocoa. Polysorbates, succinated monoglycerides in food matrix can be isolated using *n*-propanol-water. Furthermore, MeOH has been used to extract the esters of citric acid polyglycerol, sugar, and lactylic acid in margarine and pastry food samples.

Extraction may not result in complete recoveries; therefore, solid phase extraction (SPE) using specific cartridge has been introduced to minimize the use of large amounts of organic solvents (Myher and **Kuksis, 1995).** Neff et al. (1992) has used SPE using silica sorbent and MeOH as elution solvents for fractionation of mono-glyceride (MG). The procedure using aminopropyl SPE columns has also been used to separate diglyceride (DG), MG, and other classes of lipids (Pinkart et al., 1998). Burdge et al (2000) have developed a method for rapid separation of lecithin by SPE using aminopropyl silica columns.

3. Analysis of emulsifiers using chromatographic technique

As previously described, the presence of emulsifiers in food systems is in the form of complex mixture; therefore, the purpose of a chromatographic technique is to analyze the emulsifiers, either qualitatively or quantitatively and to separate the emulsifier classes in good resolution (Myher and Kuksis, 1995).

Analysis of emulsifiers in food systems can be performed by several chromatographic techniques, namely gas chromatography (GC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and planar chromatography (paper and thin layer chromatography).

3. 1. High performance liquid chromatography (HPLC)

HPLC might become the method of choice for determination of emulsifying substances. Most of these substances are UV-transparent (e.g., they contain only saturated fatty acids), and therefore do not contain a chromophoric group to be detected using spectrophotometer UV (Aitzetmuller, 1975). For this reason, emulsifiers are best detected using universal detectors available for HPLC such as evaporative light scattering detection (ELSD), refractive index (RI) and mass spectrometer (MS) (Myher and Kuksis, 1995). However, some emulsifiers have chromofores, especially those contained unsaturated functional groups, which can be detected using UV or diode-array detectors.

HPLC has been developed for the separation of mono-, di-, and triglycerides of fatty acids on a 25-cm column packed with LiChrosorb DIOL. The glycerides are eluted isocratically with *iso*-octane-isopropanol (95:5 v/v) within I0 min, and the components were detected using UV-absorption at 213 nm (Riisom and Hoffmeyer, 1978). Liu et al. (1993) has developed normal phase HPLC using ELSD for analysis MD and DG. Furthermore, Berner and Dieffenbacher (1999) used HPLC with normal phase and ELSD to analyze the common MD and DG in vegetable oils and fats for collaborative studiesd. Using a ternary gradient HPLC instrument equipped with an ELSD; column of reversed-phase RP-8 end-capped and two consecutive binary gradients consisting of ACN-water plus acetic acid (0.1%, v/v) and ACN-CH_3Cl as the mobile phase, Marcato and Cecchin (1996) simultaneously analyzed various substituted glycerides, and also the corresponding saturated fatty acids that are found as by-products in commercial glycerol monostearates.

A reliable liquid chromatography/atmospheric-pressure chemical ionisation mass spectrometry (LC–APCI-MS) method has been developed for the quantitative determination of food emulsifiers composed of MG and DG of fatty acids (E471 series) in complex food matrices by Suman et al (2009). The emulsifiers analyzed are some MGs (monolaurin, monomyristin, monopalmitin + monoolein, and monostearin), and some DGs (1,3-dimyristin, 1,2-dimyristin, 1,3-dipalmitin + 1,2-dipalmitin, and 1,3-distearin + 1,2-distearin). Limits of detection (LOD) and quantification (LOQ) reported are in the range of 0.3 to 4.0 mg/kg and li 0.9 to 12mg kg–1, respectively. Another use of HPLC to analyze emulsifier is shown in Table 1.

3.2. Supercritical fluid chromatography (SFC)

Capillary SFC on a 25% cyanopropyl stationary phase with a mobile phase of CO_2 at 100 - 150°C can be successfully used by Artz and Myers (1995) for analysis of selected emulsifiers, which included acetylated MGs, lactylated MGs, hexaglycerol distearate, triglycerol mono/dioleate and decaglycerol decaoleate. Samples of acetylated MGs were placed in a SFC cell on a glass bead bed and extracted for 15 min at 50°C at different pressure (340, 408, 544 and 680 atm) with CO_2. Giron et al. (1992) clearly demonstrated the selectivity and accuracy of the SFC method for the analysis of MG and DG standard mixtures using flame ionization detector. The results of MG and DG obtained using SFC were comparable with GC.

Polyethoxylated octylphenols has been analyzed using SFC using a column of 5 m x 50 µm SB-Biphenyl 30 with 0.25 µm film thickness and FID detector. SFC-grade CO_2 (99.99%) was used as the mobile fluid with isothermal linear pressure. The validity of the capillary SFC methods was examined and demonstrated by comparing the SFC results with corresponding data obtained from HPLC analysis (Wang and Fingas, 1993).

3.3. Gas chromatography (GC)

Analysis using gas-liquid chromatography (GLC) has become an important tool for the analysis of many emulsifiers. The development of auto-sampling technique has made GLC more reproducible, which is necessary for quantitative analysis. The new generation of gas chromatographs with computer connection is a good and almost necessary help for the calculation of emulsifier concentration. For more complicated separations of components in emulsifiers, GC capillary columns can be used with good results (Soy, 2006).

The main problem arising is their separation into groups before GC analysis (Dickes, 1979). GLC has been used for detection of volatile compounds. Unfortunately, emulsifiers are non volatile, therefore to use this technique; emulsifiers must be derivatized such as in the form of trimethylsilyl (TMS) derivate (Hasenhuettl, 1997). GLC has been used for the study of partial glycerides in crude, fractionated and refined palm oils. MG and DG were derivatized using TMS prior to GLC analysis (Goh and Timms, 1985). The parameters affecting the separation and quantification of TMS ethers of MG and DG can be investigated using GLC with QF-1 and SE-30 as stationary phases and FID. The isothermal characteristics of a range of TMS ethers of mono- and diglycerides on both stationary phases showed that log retention volume was directly proportional to C number and inversely proportional to absolute temperature (Watts and Dils, 1969).

Separation, identification and quantitative estimation of mono-, di- and tri-fatty acid esters of sorbitol and its anhydrides has been carried out by Sahasrabudhe and Chadha (1969). Lipid classes were separated by liquid partition column chromatography and TLC. The individual mono- and di-fatty acid esters and the polyols were analyzed using GLC as TMS ethers. Recoveries of known compounds in mixtures were in the range of 92% to 100%.

Kuhrt et al (1952) analyzed MG in lard and bread using GLC. GC-based method has been adopted as a standard method for the determination of MG and DG in vegetable oils and emulsifiers (Firestone, 1997). This determination requires the presence of an internal standard which has a retention time in the chromatogram such as monomargarin. See et al (1990) used octadecyl glyceryl ether (batyl alcohol) as internal standard for determination of MG and propylene glycol

Table 1. Analysis of emusifiers using HPLC

Emulsifier	Column	Mobil phase	Detector	References
Sorbitan and MGs	Lichrosorb RP-18 (25 cm x 4.6 mm, 10 µm)	88% isopropanol and 12% water.	UV 220 nm	Garti et al. (1983)
Sorbitan Tristearate	Shodex SUGAR SC (300 mm × 8 mm i.d).	water/acetonitrile (985:15, v/v). flow rate was 0.8 mL/min	refractive index	Thyssen and Andersen (1998)
Propylene glycol esters of fatty acids	Inertsil 5C8	acetonitrile and water (90:10)	UV 230 nm	Murakami et al (1997)
MG, DG, and triacylglycerol	Econosil CN, 25 cm x 4.6 mm i.d; 5 µm	2% (0.5% AA in TBE) in henane (v/v) for 6 min., linear from 6-34 min to 100% (0.5% AA in TBE) hold 10 min then return to initial conditions 44-54 min. The flow rate was 1.0 mL/min. AA = acetic acid, TBE: tert-butyl ether	Flame Ionization detector	Neff et al (1997)
MG	LiChrosorb RP-I8 (15 cm x 0.46 cm i.d 10 µm)	Acetonitrile; flow rate 0.9 mL/min	UV 204 nm	Sudraud et al. (1981)
polyethylene glycols	polymeric styrene divinylbenzene PRP-1 (150 **x** 4.1 mm i.d.,10 µm)	ammonium acetate (0.1 M, pH 6.0)-acetonitrile (79:21, v/v). The flow rate was set to 1.0 ml/min.	MS	Auriola (1993)

ester emulsifiers. As indicated by the recovery results, batyl alcohol was comparable with monomargarin.

A simple and rapid method based on SPE and GC has been developed by Fagan et al (2004) for the determination of MG and DG at low-concentration levels in milk and dairy ingredients. The LOD of MG and DG reported were in the range of 5–8 and 10–17 µg/ml, respectively. MG and DG in milk and milk products can be analyzed using GC with fused silica capillary column DB-5, 25 m x 0.247 mm I.D., film thickness 0.25 mm. The carrier gas used was hydrogen with a flow rate of 1 ml/min; injector temperature was 80 °C and detector temperature 360 °C. The temperature programme was 210 °C (held for 0.5 min), increasing to 350 °C (held for 10.5 min), at a heating rate of 10 °C/min. The recovery rates of MG were about 98% for skim milk and 99% for dessert. Spiked DGs were found with 101% in skim milk and 94% in dessert. LOQ was 0.001% for MG and 0.006% for DG (Bareth et al., 2003). GC is also used for rapid analysis of polysorbates in food using saponification of the ester, after a minimum extractive clean up on a soda lime column. Separation and determination was passed through column of 15% Carbowax 20 M/Chromosorb-T at 190°C, using detector of thermal conductivity detector and helium as carrier gas (Lundquist and Meloan, 1971).

Gas chromatography-mass spectrometry (GC/MS) has been used for the detection of four kinds of dietary emulsifiers, namely glycerin, sucrose, sorbitan and propylene glycol monoesters of fatty acids in beverages by Matsumoto et al (2003). The emulsifiers were homogenized and extracted from beverages using tetrahydrofuran-ethyl acetate (6:4 v/v). The extract was cleaned up on a silica gel column and subsequently on C_8 cartridge column, followed by acetylation. The authors claimed that established method enabled to characterize these emulsifiers. Currently GC in combination with time-of-fight (TOF)-MS was developed by our group to analyze MD and DG in several fats and oils, namely lard, butter, sun flower, corn, and palm oils (Indrasti *et al*., 2010). Using the combination of two column (DB17, length 17 m as first column and SLB-5 ms, length 0.6 m), the presence of MG and DG of studied fats and oils can be successfully quantified.

3.4. Planar chromatography

Planar chromatography refers to thin layer chromatography (TLC) and paper chromatography (PC). TLC is the dominant planar chromatographic method, especially associated with the use of kinetically optimized layers prepared from particles of a narrow size distribution (Poole and Poole, 1995). TLC and PC have been used for determination of emulsifiers. The method was simple, rapid, and reasonably reliable tools for identification the presence of emulsifiers in lipid components (Murphy and Scott, 1969).

3.4.1.Thin layer chromatography

In TLC, the MG and DG are separated from each other and then analyzed gravimetrically or densitometry. TLC can be performed using simple equipment, but the procedure is time-consuming and does not measure individual MGs and DGs. Although the technique has

been automated by conducting the separation on adsorbent-coated micro rods followed by detection and quantification by flame ionization detector, it still does not allow for the determination of individual MAG and DAG.

Due to their importance in lipid metabolism and as components of lipid membranes, phospholipids have been widely studied and analyzed. TLC has been developed for analysis of phospholipids (Erdahl, 1973). Determination of pure lecithin in different phosphatide-extracts from soybean oil, yellow of the egg, heart muscle and cerebrum has been carried out by Wagner (1961) using simple and exact procedure based on thin-layer-chromatography on Silica gel G. The separation of MG and DG was also carried out using TLC by reaction with hydroxylamine in an alkaline medium. The resulting hydroxamic acids gave colored complexes with Fe^{3+}, which were determined colorimetrically (Beldowicz and Szczepanska, 1967). Separation of common polyglycerol products into their components was carried out by dissolving higher linear polyglycerols in EtOH and separated at ambient temperature with ethyl acetate-isopropyl alcohol-acetone-methanol-H_2O (50:15:15:4:16 v/v) on plates coated with a slurry of equal weight of Kieselguhr G (Dallas and Stewart, 1967).

In addition, Murphy and Scott (1969) have used TLC for analysis of emulsifiers of polyoxyethylene containing substantial amounts of free polyethylene glycol. The emulsifier is extracted with $CHCl_3$, cleaned up on an alumina column and analyzed using TLC. The chromatogram obtained was sprayed with modified Dragendorff reagent. The authors reported that this method is at least ± 15 % accurate down to an emulsifier level of 0.01 % in fats and 0.001 % in baked foods and food mixes. The presence of MGs in fats and oils in low levels (< 0.5%) has been determined using TLC by Halvarson and Qvist (1974). The MGs are enriched with ACN and analyzed using the combination of TLC-GC. The proposed method offers good results in terms of the selectivity, yield, and reproducibility. TLC can be also used for separation of trace of MGs, DGs, and TGs as the hydroxamic derivates. MGs were eluted with boiling EtOH, DGs with benzene–ethanol (2:1 v/v) (Grynberg *et al*., 1967). Peyrou et al. (1995) have used TLC coupled with flame ionization detector to analyze MG of monoolein. Good separation of MG from other lipids was obtained using the mixture of hexane-diethyl ether-formic acid (65:35:0.04, v/v/v).

3.4.2.Paper chromatography (PC)

PC has been used by Wetterau et al (1964) for the determination of sorbitan monostearate in cake and cake mixes. The method involves the extraction of analyte from the sample, partial purification of the extract by silica gel chromatography, and analysis by PG procedures using Whatman No. 4 paper. The emulsifiers of lecithin, lysolecithin, lysophosphatidylethanolamine, and phosphatidylethanolamine were investigated using PC by Collier (1962). Paper was impregnated for 5 min with ZnO in 3.5% HCl, spotted before drying the treated paper, and developed with dibuthyl ether-propionic acid (2:1 v/v), and subsequently saturated with $ZnCl_2$. The Rf values of lecithin, lysolecithin, lysophosphatidyl-ethanolamine, and phosphatidylethanolamine are 0.20-0.25, 0.05-0.08, 0.13-0.18, and 0.32-0.40, respectively.

The identification of the glycerides of tartaric and acetyltartaric acids in margarine is described. Tartaric glycerides were extracted from sample using MeOH, precipitated with alcoholic-Pb acetate and paper-chromatographed with a propanol-ammonium hydroxide (1: 1 v/v). The spots were developed with aqueous $AgNO_3$ with Rf of tartaric glyceride are 0.15. Furthermore, acetyltartaric acids of glycerides was developed using acetic acid or calcium acetate with Rf value of 0.06 (Kroeller, 1962). Identification of mono fatty acid esters of sorbitan, polyoxyethylene, and polyoxyethylenesorbitan can be performed using PC. The esters are extracted using chloroform, saponified, and acidified, and fatty acids are subsequently removed with hexane, and the residue is neutralized to pH 7 with 0.5N NaOH. Separation was developed using BuOH saturated with water and $Pb(OAc)_4$ reagent. The Rf values of the polyhydroxy moieties released from the esters are: sorbitol (0.06), glycerol (0.3), polyoxyethylenesorbitol (0.15- 0.2), and polyoxyethylene 0.5-0.6 (Schrepfer and Egle, 1958).

PC is also used for analysis of polyoxyethylene glycol (PEG 400) monostearate and polyoxyethylene sorbitan monostearate (Tween 80) at 0.1-0.5% in bread, cakes, flour, and flour products using a modified Dragendorff reagent in paper chromatograms. The selected solvent is BuOH: glacial acetic acid: H_2O 4:1:5 (Boari, 1959).

4. Conclusion

Chromatography is method of choice for analysis of emulsifiers in food samples, qualitatively and quantitatively, due to its capability for separation and quantification of emulsifiers. After being extracted, the type of emulsifiers can be determined using certain types of chromatography. Paper and thin layer chromatographies have been widely used which attributed to their simplicity and relatively low costs. Gas liquid chromatography can only be applied to emulsifiers which are converted into volatile derivates. The drawback of GLC can be overcome by high performance liquid chromatography and supercritical fluid chromatography, in which emulsifiers do not need to be volatilized prior analysis.

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