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Combined chromatography and mass spectrometry for the molecular characterization of food emulsifiers

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ABSTRACT

Food emulsifiers are widely used to stabilise water-fat emulsions such as mayonnaise and dressings. They are prepared by oligomerisation of a poly-alcohol (as e.g. glycerol or citric acid) followed by a reaction with fatty acids. In order to gain insight in the chemical composition of different emulsifiers, a range of chromatographic methods including gas chromatography, size exclusion chromatography, normal phase- and reversed phase liquid chromatography either or not in combination with mass spectrometry was deployed. The different methods turned out to be highly complementary. By combining the information from different methods the polar head group and the fatty acid part of the emulsifier can be characterised in detail. Mass spectrometry is indispensable for establishing the number of polar molecules in the head group as well as for establishing the correct combinations of fatty acids in one molecule. Ten commercial emulsifiers were described at the level of number and type of polar groups and fatty acids present.

1. Introduction

Many widely used food products such as margarines, mayonnaises and dressings are emulsions of water and oil. To stabilize such systems emulsifiers are used. These can either be natural compounds, like egg yolk or other systems rich in e.g. phospholipids, or can be dedicated, synthesized food emulsifiers. Irrespective of whether natural or synthesized, all emulsifiers consist of a polar head-group and a non-polar part, usually one or more fatty acid chains. Synthesized emulsifiers are generally prepared in a two-step reaction in which first the poly-alcohol, i.e. a molecule with multiple hydroxyl groups (e.g. glycerol) or a combined alcohol/acid (e.g. lactic acid), is oligomerized. In the second step fatty acids are grafted onto this polar head. The resulting mixtures are extremely complex and consist of multiple polyalcohol molecules linked together with different numbers of fatty acid grafts. Since many of the important properties of food emulsions, such as stability and mouth feel, are directly related to the chemical composition of the emulsifiers, insight in the composition of the complex emulsifier mixtures is crucial for the development of better food products.

The characterization of food emulsifier has received a great deal of attention in literature. A wide variety of techniques was used, including gas chromatography [1–4], thin layer chromatography [5,6], and various forms of liquid chromatography either or not in combination with mass spectrometry [7–9]. In most cases single methods were used. For such single methods it is, however, extremely difficult to characterize both the hydrophobic and the hydrophilic part of the molecule. For this reason combinations of methods have more recently also been applied. De Meulenaer et al., for example used off-line LC–GC to isolate and characterize fatty acid esters of polyglycerols [10]. Comprehensive chromatography (e.g. GC × GC, LC × LC or LC × GC) has been shown to be a valuable new tool for the analysis of (non-food) surfactants [11], but this technology has so far not been applied to food emulsifiers. This despite the fact that food emulsifiers are equally complex as surfactants for laundry applications and e.g. oil production.

Before starting complex studies of hyphenated methods and comprehensive chromatography analysis of food emulsifiers, we wanted to explore the possibilities and limitations of joint deployment of multiple chromatographic methods and common hyphenated chromatography-mass spectrometry couplings (GC–MS and LC–MS). In our strategy for food-emulsifier characterisation we combine multiple chromatographic methods to obtain knowledge about the functional groups and the molecular weight of the polymer and hence about the degree of polymerization and the composition of the molecules. Different analytical methods are presented and used to characterise 10 commercially available emulsifiers. Special attention is devoted to the class of mono- and

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Table 1
Overview of the emulsifiers studied.

Emulsifier type	Sample code
Diacetyl tartaric acid ester (commercially also known as DATEM)	E2, E3
Citric acid ester (commercially also known as CITREM)	E4, E5
Lactic acid ester (commercially also known as LACTEM)	E6, E7, E8
Polyglycerol ester	E9, E10, E11

diacylglycerol esters of fatty acids esterified with lactic, citric or tartaric acids.

2. Materials and methods

2.1. Chemicals and reagents

All reagents and solvents used in the experiment were analytical grade or better. Milli-Q water (18.2 MΩ, Millipore, Bedford, MA, USA) was used for preparation of solutions and dilutions. Dichloromethane, *n*-hexane, tetrahydrofurane (THF), methyl *tert*-butyl ether (MTBE), 2-propanol and anhydrous sodium sulfate (Na_2SO_4·0aq) were supplied by Merck (Darmstadt, Germany). Trimethylsulfonium hydroxide (TMSH, 0.2 M in Methanol) for trans-esterification of fatty acid-containing species into fatty acid methyl esters was obtained from Machery–Nagel GmbH (Düren, Germany). UPLC grade acetonitrile, formic acid and ammonium formate were supplied by Biosolve (Biosolve BV, Valkenswaard, the Netherlands). The derivatisation reagent for GC-FID analyses was bis(trimethylsilyl)trifluoroacetamide (BSTFA, Thermo Scientific, Waltham, MA, USA).

2.2. Emulsifier samples

A selection of 10 emulsifiers was obtained from various suppliers. An overview of the emulsifiers studied is presented in Table 1.

2.3. LC–MS analysis

NPLC-MS analyses were performed on an Agilent 1200 binary gradient LC system and an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The NPLC separations were carried on a Pursuit XRs DIOL 3 μm 250 × 3.0 mm column (Agilent Technologies) with gradient solvents A (hexane) and B (2-propanol). Gradient: 0 min 95% A; 10 min 95% A; 30 min 50% A, flow 0.5 mL/min. The mass spectrometer was equipped with an atmospheric pressure photo ionization (APPI) source (Agilent Technologies) operated in the positive ionization mode at capillary voltage 3 kV, source temperature 150 °C, vaporizer temperature 375 °C, nitrogen sheat gas flow 2.5 L/min, nitrogen sheat gas temperature 275 °C, nebulizer pressure 10 psi, fragmentor voltage 100 V.

The RPLC separations were carried on an Inertsil ODS-3, 5 μm 150 × 2.1 mm column (GL Sciences, Tokyo, Japan) with gradient solvents A (2 mM ammoniumformate and 50 mM formic acid in milli-Q water) and B (2 mM ammoniumformate and 50 mM formic acid in 95% acetonitrile and 5% milli-Q water). Gradient: 0 min 50% A; 5 min 50% A; 20 min 2% A, flow 0.25 mL/min. The mass spectrometer was equipped with an ESI ionization source. Positive ionization, capillary voltage 4 kV, source temperature 300 °C, nitrogen gas flow 6 L/min, nebulizer pressure 15 psi, fragmentor voltage 135 V.

2.4. SEC-RI analysis

The SEC system consisted of an LC-10ADvp isocratic LC pump (Shimadzu Corporation, Kyoto, Japan) and a refractive index detector (Separations, H.I. Ambacht, the Netherlands). It was equipped with a Marathon autosampler with a 10 μL sample loop and a column oven (both from Spark Holland, Emmen, the Netherlands). Two PLgel GPC-columns of 7.5 mm × 300 mm i.d., 5 μm particle size, pore sizes 500 Å and 100 Å, respectively (Polymer Laboratories, Church Stretton, UK) connected in series were used. The mobile phase was THF at a flow rate of 0.8 mL/min and a temperature of 33 °C.

2.5. GC analysis

High-temperature GC (HTGC) with flame ionization detection (FID) and on-column injection was applied to study the intact emulsifier molecules. The experiments were done on a Trace GC8000 (Interscience, Breda, the Netherlands) equipped with a cold on-column injector. Analytical column CP-Sil 5CB capillary column (Agilent Technologies), 10 m × 0.32 mm, film thickness 0.12 μm. Carrier gas hydrogen 4.0 mL/min, oven temperature 80 °C (2 min hold) to 360 °C (5 min hold) 10 °C/min. Samples were silylated for 30 min at 70 °C using a mixture of BSTFA/pyridine (1:4, v/v) prior to GC-FID analysis.

GC-FID experiments to determine the fatty acid composition were performed on a 6890 N GC equipped with a split injector (Agilent Technologies). Analytical column: CPWAX-52CB capillary column (Agilent Technologies), 10 m × 0.15 mm, film thickness 0.2 μm. Carrier gas helium 250 kPa, oven temperature 120 °C (0 min hold) to 255 °C (6 min hold) 15 °C/min.

2.6. Sample treatment

Samples were melted in an oven at 80 °C. For NPLC-MS about 10 mg of each sample was dissolved in 2 mL hexane/2-propanol (95/5 v/v) followed by an additional 20 times dilution using the hexane/2-propanol mixture. For RPLC-MS 10 mg of each sample was dissolved in 1 mL of dichloromethane followed by an additional 40 fold dilution in acetonitrile. For SEC, approximately 100 mg of melted sample was dissolved in 2.5 mL THF. A small amount of Na_2SO_4·0aq was added prior to filtration of the sample solution over a Millex-SR 0.5 μm filter (Millipore, Bedford, MA, USA). For GC-FID, samples were either dissolved in MTBE and derivatized using TMSH (fatty acid chain analysis) according to the protocol described by El-Hamdy and Chistie [12] or were derivatized for 30 min at 70 °C using a mixture of BSTFA/pyridine in a ratio of 1:4 (v/v) (direct analysis).

3. Results and discussion

The emulsifier samples were first analysed using high-temperature GC (HTGC) with on-column injection after silylation. Fig. 1 shows a few representative HTGC chromatograms. From the chromatograms it is clear that the emulsifiers are complex mixtures. Multiple peaks are seen for all samples and, additionally, although all base-lines are flat towards the end of the run, for most samples a significant fraction of the material does not elute. For the typical molecular structures encountered here, the current HTGC set-up can elute molecules containing up to approximately 80 carbon atoms. Since several of the emulsifiers seemed to contain larger species exceeding this limit, we applied size exclusion chromatography (SEC) to estimate the average molecular weight of the oligomers and calculate the number of acid and alcohol units present.

Fig. 2 shows SEC chromatograms of the selected representative samples. Because no standards of known molecular weights are available for the emulsifiers studied, we used a polymerized triglyceride standard to estimate the approximate molecular weights of the emulsifiers. From the SEC results it is clear that most of the