Molecular assembly, interfacial rheology and foaming properties of oligofructose fatty acid esters

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Thesis

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Contents

List of abbreviations

LMW	Low molecular weight
CMC	Critical micelle concentration
CAC	Critical aggregation concentration
HLB	Hydrophilic lipophilic balance
DMSO	Dimethylsulfoxide
Bu^tOH	Tert-butanol
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time of flight mass spectrometry
NMR	Nuclear magnetic resonance
C8	Caprylic acid
C12	Lauric acid
C16	Palmitic acid
C18	Stearic acid
DP	Degree of polymerization
DHB	2,5-dihydroxybenzoic acid
D_2O	Deuterium oxide
$CDCl_3$	Deuterated chloroform
ADT	Automated drop tensiometer
RP-SPE	Reverse phase solid phase extraction
ELSD	Evaporative Light Scattering Detector
pC_{20}	Surfactant efficiency (the negative logarithm of the bulk concentration that is necessary to obtain a reduction in surface tension of 20 mN/m)
π_{CAC}	Surfactant effectiveness (the reduction in surface tension at the CAC)
Γ_{max}	Maximum surface excess concentration of surfactant at the air/water interface
C16:1	Palmitoleic acid
π_{CMC}	Surface pressure at the CMC
ME	Mono-esters
DE	Di-esters
LA	Lauric acid
OF	Oligofructose
CP	Crude product

OLAE	Oligofructose lauric acid esters
WPI	Whey protein isolate
BAM	Brewster angle microscopy
AFM	Atomic force microscopy
OFAE	Oligofructose fatty acid esters
C4	Butyric acid
C10	Capric acid
FT-IR	Fourier transform infrared spectroscopy
HPLC	High-performance liquid chromatography
TEM	Transmission electron microscopy
SDS	Sodium dodecyl sulfate

Chapter 1

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General introduction

In this thesis we address the properties of oligofructose fatty acid esters, which are intended to be used as food-grade surfactants, at three different length scales: the molecular, interfacial and macroscopic length scale. At the molecular scale, the synthesis of oligofructose fatty acid esters by esterification of oligofructose with fatty acids is discussed. At a larger length scale, the molecular assembly of the esters and rheological properties and microstructure of interfaces stabilized by the esters are discussed. At the largest length scale, foaming properties are discussed. In this chapter we will introduce relevant aspects of all lengths scales, starting at the macroscopic length scale, followed by the interfacial length scale and finally the molecular length scale.

General introduction

Food products are often complex systems that consist of multiple phases. A wide range of textures can be obtained by using different structural elements. One particular example are air bubbles stabilized by surfactants in mousses [1]. The properties of the interfaces in this system often play an important role in the macroscopic properties and are highly dependent on the type of surfactant that is used to stabilize the foam films [2]. In this thesis, we describe interfacial and amphiphilic functionalities of a series of oligofructose fatty acid esters; surfactants with some unusual properties compared to commonly used surfactants.

Foams

Many food products or parts of food products consist of foams [1], such as the head of beer, whipped cream, mousses, cappuccino foam, and bread [3]. Foams are dispersions of gas bubbles in a continuous phase [4, 5], which can be either liquid or solid. Since the presence of a large interfacial area increases the free energy of the system, the system will always try to minimize the interfacial area [4]. This results in foam instability.

Instability mechanisms

There are three main instability mechanisms in foams: drainage, coalescence and disproportionation [3, 5]. Drainage is the flowing of liquid downwards through nodes and channels between bubbles, and is driven by gravity and capillary action [3, 6]. Drainage can be reduced by increasing the viscosity of the continuous phase [4], which often leads to significant improvements in foam stability [7]. However, increasing the viscosity of the continuous phase affects the texture of the overall product, and is not always desired.

Drainage is also affected by bubble size and size distribution, where smaller bubbles and a narrower distribution lead to slower drainage [6].

Coalescence is the merging of two bubbles by rupture of the film between them, and causes a coarsening in the bubble size distribution [8]. Coalescence can be reduced when the interface is covered by a sterically [9-11] or electrostatically [10] stabilizing layer.

Disproportionation is the flow of gas from smaller to larger bubbles as a result of differences in Laplace pressure, and again leads to a coarser bubble size distribution [3, 5, 12]. Of all instability mechanisms, disproportionation is hardest to prevent. An approach that is used, for instance in beer, is to use a gas that has a low solubility in the continuous phase [6, 12]. Some authors claim that the presence of an elastic layer at the interface may reduce disproportionation [4, 5, 13]. Finally, it is important to realize that the three instability mechanisms are interdependent: when a foam drains the bubbles can approach closer which will increase the chances of coalescence and disproportionation [5]. Also, coarsening accelerates drainage [5, 6].

Interfaces

An interface is defined as the boundary between two immiscible phases. In the case of emulsions the two phases are usually water and oil, while in the case of foams the two phases are usually water and air. Foams contain a large interfacial area. Therefore, the properties of the air/water interfaces may contribute significantly to the properties of the foam [9, 11, 14]. However, it is important to realize that the interfacial properties are not necessarily directly related to macroscopic foaming properties, due to the complexity of the mechanisms responsible for foam destabilization [4, 7, 11, 15, 16]. Two important interfacial properties are the surface tension and the surface rheological properties.

Surface tension

Surface tension is the result of an imbalance in attractive forces between the solvent molecules that are present at the interface. It is a measure for the free energy of the surface, and defined as the work per unit area required to extend a surface under isothermal conditions. The surface tension is important for foaming because a lower surface tension facilitates the enlargement of the interfacial area, which is important during foam formation [11]. Also, a lower surface tension reduces the free energy of the system, thereby improving the stability of the foam. Furthermore, a lower surface tension reduces disproportionation, because the Laplace pressure is dependent on surface tension. Finally, a lower surface tension results in smaller bubbles, which reduces drainage [6].

Rheological properties

The rheological properties of interfaces are important because they affect instability mechanisms, such as disproportionation and coalescence. During surface rheological measurements, the response of an interface to a deformation is studied [16]. When this response is purely elastic, all energy applied to the interface during deformation is stored in the interface, and recovered when the interface returns to its original shape. When the response is purely viscous, all energy applied during deformation is dissipated, and the interface remains deformed after the deforming force is removed. Mostly, interfaces stabilized by food-grade surfactants have a response that is not completely viscous and not completely elastic. The degree of elasticity or viscosity can be determined from the phase difference between the applied deformation and the response of the interface [17].

Two different types of deformation are often used during interfacial rheology: shear and dilatation [11, 15, 16]. During shear rheology, the size of the interface remains the same while the shape changes [11]. In contrast, during dilatational rheology the size of the interface changes while the shape remains the same [11, 18]. Both types of deformation can provide useful information. Dilatational rheology is sensitive towards relaxation mechanisms under compression/expansion and the kinetics of molecules when moving to/from interfaces [15]. Shear rheology is more sensitive towards the interfacial composition and structure and the nature of the interactions between the molecules [11]. Shear deformations are often linked to drainage [11, 19], while dilatational deformations are linked to foam formation [9], coalescence, and disproportionation [13]. Both types of deformation are therefore important when considering the link between surface rheological properties and foaming properties.

The surface modulus may be studied during different types of experiments. First, time sweeps may be performed to study the development of the surface dilatational modulus as a function of the degree of surface coverage. Second, the interface may be characterized using a frequency sweep, where the ratio of the characteristic time scale that is related to the transport of molecules from bulk to interface and the characteristic time scale related to the speed of interfacial deformation is studied. Also, in more complex systems characteristic times determined by in-plane interactions are important. Third, amplitude sweeps, which are not commonly used during surface dilatational rheology, may provide important information about the presence and nature of interfacial microstructure. Finally, temperature sweeps may provide additional information about phase transitions at the interface, which can help to understand the nature of the interfacial microstructure.

Surfactants

Surfactants, a blend of the words surface active agent, are used to improve the stability of foams. For a surfactant to be an effective foam stabilizer, a few factors are important. First, the surface tension and surface dilatational modulus of the interface that is stabilized by the surfactant are important, as already explained before. These depend on the morphology of the surfactants [2] and the interactions between them. Additionally, since foam formation is a process that takes place at very short timescales, it is important that the surfactant adsorbs at the interface quickly [10]. The speed of adsorption is influenced by the molecular size and state of aggregation, which is dependent, amongst others, on the hydrophobicity of the molecule.

Surfactants used in food industry

To improve the stability of products that consist of foam, the food industry uses two major types of food-grade surfactants: proteins and low molecular weight (LMW) surfactants [11]. These two types have different characteristics. Proteins adsorb at interfaces where they lower the surface tension. This adsorption is generally followed by structural rearrangements, which may be seen as a type of interfacial denaturation. This denaturation results in a practically irreversible adsorption, and often in the formation of a two-dimensional network. In general, this network provides the interface with a high modulus and steric stabilization [10, 11].

In contrast, LMW surfactants are smaller molecules that form more compact interfacial layers with lower surface tensions [11]. However, they generally do not form interfacial layers with a high modulus [11, 20, 21]. Instead, they stabilize the interface through the Gibbs-Marangoni mechanism which relies on rapid diffusion of molecules along the interface when surface tension gradients occur as a result of deformations [10, 21]. During diffusion, the LMW surfactants drag along some of the continuous phase. This slows down drainage of liquid from the films, which will increase foam stability [21].

LMW surfactants generally lower the surface tension more when their bulk concentration is increased. However, at a certain critical concentration, they start to form aggregates in the bulk [22]. From this point onwards, the surface tension is not reduced much further. When the aggregates are micellar, the critical concentration is referred to as critical micelle concentration (CMC). When the aggregates are not micellar, but rather vesicles or lamellar phases, the critical concentration is referred to as critical aggregation concentration (CAC). The concentration at which aggregates start to form and the type of aggregates are dependent on surfactant morphology.

When proteins and LMW surfactants are mixed, often destabilization is obtained, because the stabilization mechanisms of the two molecules are mutually exclusive [10, 21, 23].

Sugar esters

Sugar esters are food-grade LMW surfactants [24, 25] and have been the subject of investigation for many years due to their functional properties as surfactants and the fact that they are non-toxic, biodegradable and produced from renewable resources [26, 27]. They can be synthesized either chemically or enzymatically [26, 27]. Major drawbacks of the chemical reaction procedure are high energy consumption, the use of toxic solvents and the low specificity which results in the formation of many by-products [24, 28-30]. Therefore, enzymatic reaction procedures have gained a lot of interest [31, 32]. They can take place under milder reaction conditions, with lower temperatures [26, 29] and less toxic solvents, and most importantly, the reaction is generally highly specific, resulting in well-defined reaction products [24, 29, 31]. Sugar esters with sucrose as the head group are commercially available and used in food products.

Reports in literature in the last ten years demonstrate that sugar esters can be synthesized with a very wide range of variation in the molecular structure. Variations that are studied include changes to the length of the fatty acid chain, degree of saturation of the fatty acid chain, degree of esterification, type of sugar, and degree of polymerization of the sugar. The length of the fatty acid chain that is esterified to the sugar group generally varies between 4 and 18 carbon atoms [33-40], although reports on esters of C12 and C14 are most frequent [41-47]. Oleic acid is the most commonly studied unsaturated fatty acid [48-50]. Mono-esters are most commonly studied, although in some studies also di-esters or higher esters are studied [34, 38, 41, 47, 48]. The sugar part is usually a small mono- or disaccharide, sugar acid or sugar alcohol such as xylitol [38, 48-50], fructose [41, 44-46, 49], sucrose [41, 42, 45, 49], glucuronic acid [33, 39], glucose [33, 35, 37, 39, 40, 42, 49], maltose [42] or sorbitol [49], although there are some reports on non-reducing oligosaccharides such as cyclodextrin [36], raffinose, melezitose, kestose and stachyose [43], and larger molecules such as inulin [34, 51] and starch [47].

Sugar esters are considered as typical LMW surfactants. They tend to decrease the surface tension of interfaces and form micelles in the bulk after a critical concentration that depends on the chemical fine structure of the molecules. The value for the surface tension of air/water interfaces that is obtained at concentrations higher than the CMC tends to be in the range of 24-44 mN/m [52-56]. Information about the rheological properties of interfaces

stabilized by sucrose esters is more scarce, probably because they generally form interfaces with low moduli. Garofalakis et al. [23] report values for the dilatational modulus of air/water interfaces stabilized by sucrose esters between 7 and 45 mN/m, while Razafindralambo et al. [39] report values between 4 and 45 mN/m for glucose octanoate and octyl glucuronate that decrease with increasing bulk concentration. Furthermore, sucrose esters can be used to stabilize foams, as reported by Husband et al. [57] and Garafalakis et al. [23]. The foaming properties depend on the chemical fine structure of the surfactant. Changes to the length of the fatty acid chain and the degree of esterification have a major impact on the foaming properties.

Oligofructose fatty acid esters

A molecule that can provide a low surface tension, like a LMW surfactant, but at the same time also provide the interface with a high modulus, like a protein, could be an excellent foam stabilizer. In an attempt to create a molecule with those properties and inspired by amphiphilic block oligomers that have interesting self-assembly properties [58], we have focused on the synthesis and functional properties of oligofructose fatty acid esters. They are structurally similar to the commercially available sucrose esters, with the exception of the hydrophilic group, which is larger in the case of oligofructose esters. Oligofructose is derived from inulin, a linear polydisperse carbohydrate, which mainly consists of β-(2, 1) fructose units (F_n), sometimes terminated by a glucose unit (GF_m, figure 1), and that is extracted from chicory roots [59-62]. To synthesize oligofructose fatty acid esters, we have esterified fatty acids to oligofructose with a degree of polymerization between 2 and 8 (average 4.4). We have chosen an enzymatic reaction procedure because of the benefits compared to chemical procedures, as already explained.

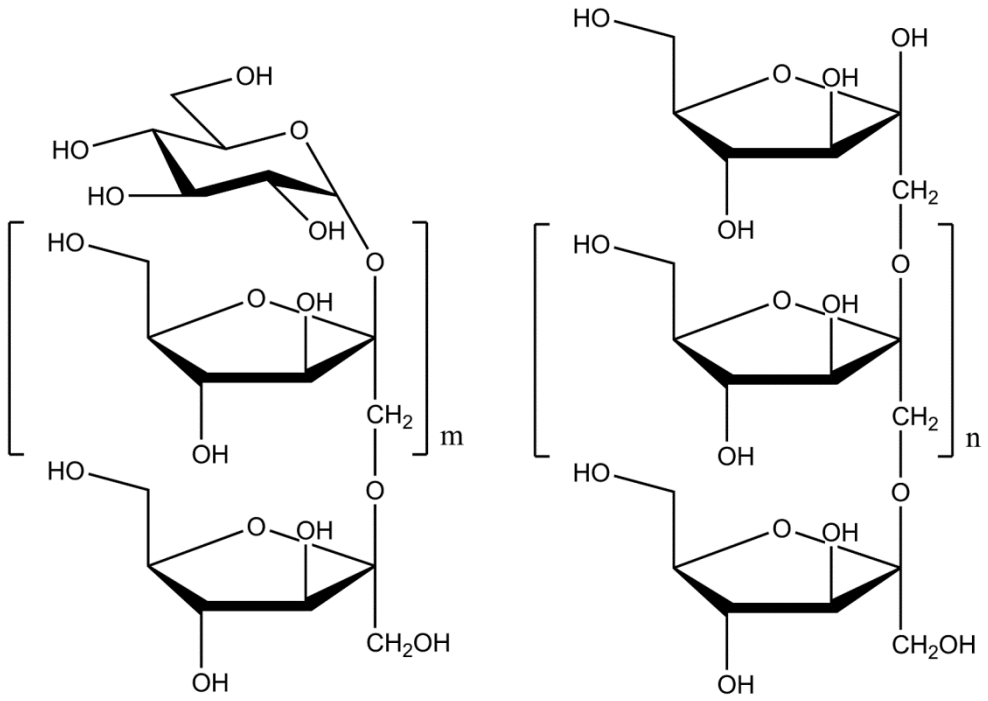

Figure 1: Structure of oligofructose. Left: GF_m. Right: F_n.

Influence of variations in molecular structure on functional properties

As already indicated, many different variations in molecular structure may be studied. Of these, the length of the fatty acid chain, degree of esterification and degree of polymerization of the hydrophilic part of the molecule will influence the balance between hydrophobicity and hydrophilicity in the molecule, which will have consequences for the functional properties. Surfactants are often characterized by their HLB (hydrophilic lipophilic balance) value. The HLB value is a numerical expression of the balance between hydrophobicity and hydrophilicity in the molecule. By changing the number and size of hydrophilic and hydrophobic groups in the molecule, the HLB value can be changed dramatically [41, 57]. The HLB value has a significant impact on the functional properties of the molecule [11, 41]. Therefore, it is of interest to study the properties of surfactants with a range of HLB values. To that end, we made changes to the length of the fatty acid and the number of fatty acids that is esterified to one oligofructose molecule. Additionally we have changed the degree of saturation of the fatty acid chain. Finally, we have studied the

influence of the size of the hydrophilic head group by comparing oligofructose esters to sucrose esters.

Aim and outline of thesis

The aim of this thesis was:

(1) to synthesize and structurally characterize oligofructose fatty acid esters with differences in degree of esterification, length of the fatty acid chains, and degree of saturation of the fatty acid chains,
(2) to study the basic functional properties of the esters, such as micelle formation and the area that a molecule occupies at the interface, and the rheological properties of air/water interfaces stabilized by the esters, and to link these properties to molecular structure,
(3) from a more practical point of view, to determine the properties of the esters when mixed with either di-esters or fatty acids, or proteins that are present in food products and finally, to determine the foaming capabilities of the esters.

This outline is schematically presented in figure 2. Table 1 presents an overview of the surfactants that were used, their abbreviations, structural characteristics and the chapters in which they were used.

Table 1: Overview of the surfactants that were used in this thesis.

	Surfactant abbrevation	Hydrophilic group	Fatty acid chain length	Degree of saturation	Ratio mono-ester	Ratio di-ester	Average molecular weight			Average HLB-value[‡]			Chapter					
							DP2	DP4.4[†]	DP8	DP2	DP4.4[†]	DP8	2	3	4	5	6	7
Mono-esters	OF-C4m	Oligofructose	4	0			412	801	1384	16.6	18.2	19.0						X
	OF-C8m	Oligofructose	8	0			468	857	1440	14.6	17.1	18.2	X	X	X			X
	OF-C10m	Oligofructose	10	0			496	885	1468	13.8	16.5	17.9						X
	OF-C12m	Oligofructose	12	0			524	913	1496	13.0	16.0	17.6	X	X	X	X		X
	OF-C16m	Oligofructose	16	0			580	969	1552	11.8	15.1	16.9	X	X	X		X	X
	OF-C18m	Oligofructose	18	0			609	997	1581	11.2	14.7	16.6	X	X	X			X
	OF-C16:1m	Oligofructose	16	1			578	967	1550	11.8	15.1	17.0		X	X			X
Di-esters	OF-C8d	Oligofructose	8	0			594	983	1566	11.5	14.9	16.8		X	X			
	OF-C12d	Oligofructose	12	0			707	1095	1679	9.7	13.3	15.7		X	X	X		X
Sucrose esters	S-C12m	Sucrose	12	0	1	0	524			13.0				X	X			X
	S-C16	Sucrose	16	0	0.8	0.2	628			10.9				X	X			X
	S-C18	Sucrose	18	0	0.75	0.25	662			10.4				X	X			X

[†] The oligofructose that was used in this thesis is a mixture of oligomers with a degree of polymerization varying between 2 and 8, with an average of 4.4

[‡] HLB: Hydrophilic lipophilic balance

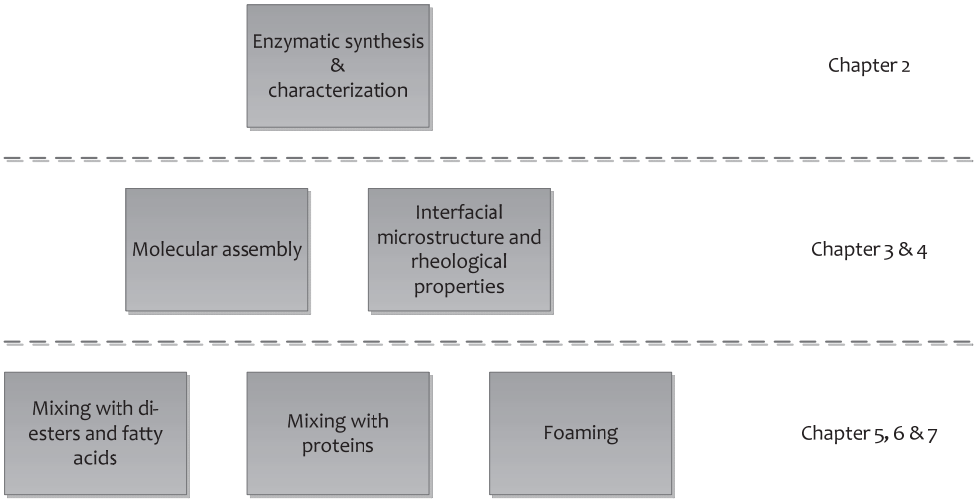

Figure 2: Schematic overview of the outline of the thesis.

In *chapter 2* we describe the synthesis and structural characterization of a series of oligofructose fatty acid esters with different fatty acid chain lengths. The oligofructose that was used was a mixture of oligomers with different degrees of polymerization, ranging from 2 to 8, with an average of 4.4 (table 1). The synthesis was performed in a mixture of DMSO and Bu^tOH, using lipase as a catalyst. The synthesis yielded a mixture of unreacted oligofructose, mono-esters, di-esters and unreacted fatty acids. Due to the specificity of the reaction, mono-esters were the main reaction products. Because of expected differences in functionality between the different fractions, the mixture needed to be fractionated. This was performed using reverse phase solid phase extraction. After purification, MALDI-TOF MS and NMR were used to characterize the products and to establish their purity.

In *chapter 3* we describe the basic functional properties of six different purified oligofructose esters; four mono-esters of fatty acid chain lengths C8, C12, C16 and C18 and two di-esters of fatty acid chain lengths C8 and C12. First, CAC curves are presented. These curves were used to establish the efficiency and effectiveness of the surfactants. Also, we estimated the maximum surface excess and the area per molecule. Then, we performed dynamic light scattering experiments to establish the size of aggregates at concentrations higher than the CAC. Finally, we performed ellipsometry experiments to establish the amount of material that adsorbed at the interface.

In *chapter 4* we describe the rheological properties of the same set of esters that was studied in chapter 3. Over a range of surface pressures, we have established the surface

dilatational modulus during time sweeps. Furthermore, we have performed frequency sweeps and amplitude sweeps. The results of the amplitude sweeps are presented in the form of Lissajous plots to maximize the amount of information that could be gathered from these experiments. Finally, we have performed temperature sweeps to establish whether the modulus was temperature-dependent. By combining the results of time sweeps, frequency sweeps, amplitude sweeps and temperature sweeps more detailed information about the presence and type of interfacial structure was gathered.

Many industrially used sucrose esters are crude products, often consisting of a mix of different degrees of esterification. In these products often traces of fatty acids are left behind. Both changes in the degree of esterification and the presence of free fatty acids have a possible influence on functionality. Before purification, the crude product of oligofructose esters also consisted of different fractions. Since mono-esters were the main fraction present in the crude product, next to di-esters, unreacted fatty acids and unreacted oligofructose, one could easily assume that these mono-esters were also responsible for the functional properties of the crude product. To test this assumption, in *chapter 5* we discuss the functional properties of mono-esters with a fatty acid chain length of C12 mixed with the different components present in the crude reaction product: di-esters, free fatty acids and unreacted oligofructose.

Results in chapters 2, 3 and 4 showed that a mono-ester with fatty acid chain length 16 was a particularly interesting ester, because of the low surface tension, combined with a high surface dilatational modulus. To study the possible implementation of this ester in foods, it is important to understand the behavior of the ester in the presence of a protein. Proteins are present in many food products, are surface active and may compete for the interface. Therefore, in *chapter 6* we have established the functional properties of this ester in the presence of whey protein isolate, a commonly used food protein.

In *chapter 7* we discuss the influence of variations in molecular structure on the foaming properties of oligofructose esters. We discuss the relation between the surface tension and surface dilatational modulus at long times (after reaching equilibrium), the surface tension at a short time scale and the foamability and foam stability.

In *chapter 8* we present a general discussion where the results from the different chapters are integrated and where we provide an outlook for future research, such as improvements to the synthesis of oligofructose fatty acid esters, further functional characterization and possible additional applications.

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Chapter 2

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Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification

Abstract

This chapter describes the synthesis of a series of oligofructose monoesters with fatty acids of different chain length (C8, C12, C16 and C18) to obtain food-grade surfactants with a range of amphiphilicity. Reactions were performed in a mixture of DMSO/Bu^{t}OH (10/90 v/v) at 60 °C and catalyzed by immobilized *Candida antarctica* lipase B. MALDI-TOF-MS analysis showed that the crude reaction products were mixtures of unmodified oligofructose and mostly mono-esters. The conversion into mono-esters increased with the length of the fatty acid chain, reflecting the specificity of the lipase towards more lipophilic substrates. Reverse phase solid phase extraction was used to fractionate the products, which lead to sufficient purity (>93%) of the fatty acid esters for functionality testing. It was shown that derivatives of longer (C16 & C18) fatty acids were more efficient in lowering the surface tension and gave a much higher dilatational modulus than the derivatives of the shorter (C8 & C12) fatty acids.

1. Introduction

Sugar esters are food-grade non-ionic surfactants [1, 2] and have many attractive properties: they are produced from renewable and largely available feedstock [3], and are biodegradable, non-toxic, odorless and tasteless [4]. They are synthesized by esterification of a sugar with a fatty acid [2, 4]. The synthesis of fatty acid esters of mono- and disaccharides has been thoroughly investigated [5-7] and lauric, palmitic and stearic esters of sucrose are manufactured and applied industrially. To our best knowledge oligosaccharides have been rarely used [8-12]. Therefore, this study will focus on oligofructose, which is derived from inulin, a readily available, water soluble, prebiotic fiber extracted from chicory roots [13]. It is a linear polydisperse carbohydrate, which mainly consists of β-(2, 1) fructose units and usually is terminated by an α-(2, 1) glucopyranose unit. By partial hydrolysis oligofructose is obtained [14].

By esterification of oligofructose with a fatty acid an amphiphilic molecule is obtained that can be used in food products. Esterification of sugars with fatty acids can be performed chemically or enzymatically [3, 4]. Chemical synthesis usually takes place in the presence of an alkaline catalyst at high temperatures, accompanied by discoloration and degradation of the carbohydrate moiety [2, 15]. One important limitation of the chemical esterification is the low regioselectivity, which, due to the multiple hydroxyl groups of the substrate, leads to the formation of a complex mixture of sugar esters, with different degrees of esterification at different locations on the monomer unit (i.e. C-2, C-3, C-6) and on the oligosaccharide chain [1, 16]. This has an impact on the functional properties of the product. Alternatively, the esterification can be performed enzymatically [15, 17]. Enzymatic catalysis has the advantage of the high regio-, stereo- and enantioselectivity, which leads to mono-esters as predominant reaction products [1, 17]. In addition, enzymatic reactions can be performed at moderate temperatures, usually below 80°C [4, 18], preventing discoloration and degradation of carbohydrate substrates and products.

One particular challenge in the design of the synthesis procedure is the choice of solvent. The difference in polarity between sugar and fatty acid limits the choice of organic solvents [1, 15]. Relatively polar media, that promote solubilisation of the sugar, reduce the stability of the lipase and the solubilisation of the fatty acid [19]. Nevertheless, previous research by our groups has shown that oligofructose can be esterified with lauric acid in a mixture of dimethylsulfoxide (DMSO) and tert-butanol (Bu^tOH) with reasonable yields, using a lipase as catalyst [19, 20].

Two major types of currently used food-grade surfactants are macromolecules (mostly proteins) and low molecular weight (LMW) surfactants. Generally, LMW surfactants

can substantially lower the surface tension but do not provide a high modulus. Proteins do provide a high modulus but cannot lower the surface tension to the same extent as LMW surfactants [21, 22]. The oligofructose lauric acid esters, that resulted from the synthesis, had the ability to lower the surface tension of an air/water interface considerably and gave a high dilatational modulus [20]. Both factors are important in surfactant performance and resulted in high foam stability. By varying the size of the hydrophobic groups, products with a wide range of amphiphilicity can be produced. This will have an impact on the functional properties of the molecule [21].

Therefore, in this chapter we report the synthesis of a range of oligofructose esters with fatty acids of different chain length (caprylic (C8), lauric (C12), palmitic (C16), and stearic (C18) acid). The synthesis was carried out in a mixture of DMSO and Bu^tOH, with lipase as a catalyst in the presence of molecular sieves. The products were purified by reverse phase solid phase extraction. The surface tension and surface dilatational modulus were determined as a first step in the functional characterization of the reaction products.

2. Experimental

2.1 Materials

Oligofructose (Orafti P95) was obtained from Beneo-Orafti (Tienen, Belgium) and had a DP that ranged between 2 and 8. It was used without further modification. Immobilized lipase from *Candida antarctica* (Novozym 435) was received from Novozymes (Bagsvard, Denmark). The enzyme activity, determined in a synthetic reaction between butyric acid and Bu^tOH, was 0.0679 U (expressed as µmol butyl butyrate formed per milligram of enzyme per minute). Dimethylsulfoxide (>99%) (DMSO), butyl butyrate, and 2,5-dihydroxybenzoic acid (>99.5%) (DHB) were obtained from Fluka (Buchs, Switzerland). Deuterium oxide (D_2O), deuterated chloroform ($CDCl_3$) and caprylic acid were obtained from Acros (Geel, Belgium). Lauric acid, palmitic acid, stearic acid, acetone, toluene, n-hexadecane and n-hexane were obtained from Merck (Darmstadt, Germany). Molecular sieves (4 Å, 8-12 mesh), 1-butanol, butyric acid and Bu^tOH were obtained from Sigma (Zwijndrecht, The Netherlands). Methanol and acetonitrile were obtained from Biosolve (Valkenswaard, the Netherlands). D_6-DMSO was obtained from Cambridge Isotope Laboratories (Andover, USA). Water was purified using a Purelab Ultra system (Elga Labwater, Ede, the Netherlands).

2.2 Methods

2.2.1 Lipase activity

Esterification activity: The esterification activity was determined for the reaction between butyric acid and butanol, in 5 mL of a mixture of toluene and Bu^tOH (70/30 v/v) containing 0.73 mmol butyric acid, 1.54 mmol butanol, 0.034 mmol n-hexadecane as internal standard and 0.5 g of molecular sieves [23]. The reaction was started by the addition of 5 mg of lipase and the reaction mixture was incubated for 30 minutes at 40 °C while stirring at 200 rpm. The reaction was terminated by separating the enzyme and molecular sieves by filtration through a Whatman Spartan 13/0.45RC filter. The amount of butyl butyrate formed was determined using gas chromatography (Focus GC with AS3000 autosampler, Interscience, Breda, Netherlands). The system was calibrated using butyl butyrate. The gas chromatograph was equipped with a Flame Ionization Detector and a Restek Rxi-5ms column with dimensions of 30 m * 0.25 mm * 0.25 µm. The carrier gas was helium and the make-up gas was nitrogen. Conditions: injection volume was 1 µL; oven temperature was 50 °C for 2 minutes and was then raised to 300 °C at a rate of 20 °C/minute and stayed at 300 °C for 2 minutes; software was Chrom-Card v2.4.1.

Hydrolytic activity: The hydrolytic activity was determined using a pH-stat device (Metrohm) with triolein as substrate at 40 °C and pH 7.5, according to the method of Hoppe and Theimer [24]. Liberated fatty acids were titrated automatically with 0.1 M NaOH to maintain a constant pH. One unit (U) of lipase activity was defined as the amount of lipase that liberates 1 µmol fatty acids per minute.

Enzyme stability: 50 mg of Novozym 435 was suspended in 5 mL of a mixture of DMSO/Bu^{t}OH (10/90, v/v) and kept at 60 °C with magnetic stirring at 200 rpm. After 1, 2, 3, 4, 5, 6, 16, 24, 40, 48 and 70 hours the enzyme was removed from the solvent by filtration and analyzed to determine the residual activity.

2.2.2 Synthesis of caprylic acid, lauric acid, palmitic acid and stearic acid esters of oligofructose by esterification

Oligofructose was dried in a vacuum oven (Gallenkamp, Loughborough, UK) at 50 °C for 48-72 hours until the water content was reduced to less than 1%. The water content of the oligofructose was determined with the Mettler Toledo DL-39 Karl Fischer Coulometer linked to a Mettler Toledo Stromboli sample oven and sample changer (Columbus, USA). The determination was performed at a temperature of 125 °C. Oligofructose (final concentration 1% (w/v)) was dissolved in dimethylsulfoxide (DMSO). Caprylic acid, lauric acid, vinyl laurate, palmitic acid or stearic acid (molar ratio fatty acid:oligofructose 3:1, calculated based on the average molecular weight of the oligofructose) was dissolved in warm (~50 °C) Bu^{t}OH. The solutions were slowly added together until all material was dissolved while stirring at 200 rpm at a temperature of 60 °C. The final composition was DMSO/Bu^{t}OH (10/90 v/v). Molecular sieves (3% (w/v)) and lipase (68 U/g oligofructose, 1% (w/v), ranging from 50% (C18) to 67% (C8) weight/weight of total substrate) were added to start the reaction. The reaction mixture was incubated for 69 hours at 60 °C while stirring at 200 rpm using overhead mechanical stirring. Samples were taken after 0.5, 1, 2, 3, 19, 27, 43 and 69 hours. After 19 and 43 hours another portion of lipase was added (2x 68 U/g oligofructose). After 69 hours the reaction was stopped by decanting to separate the molecular sieves and the lipase from the solvent. The solvents were evaporated using rotary vacuum evaporation. The product was washed two times with 50 mL of acetone and one time with 50 mL of n-hexane.

2.2.3 Purification

For reverse phase solid phase extraction Vac 35cc C18 10 g cartridges (Waters, Milford, USA) with a loading volume of 16 mL were used. The columns were activated by

washing them with 45 mL of methanol followed by 45 mL of water. 16 mL of a suspension of esters in water (14 mg/mL) was loaded onto the column. Water/methanol mixtures were used to elute the components of interest, starting with 100% water and ending with 100% methanol, using 10% increments. The last step (100% methanol) was performed twice. 45 mL was used for each step. The fractions were eluted with the help of a vacuum pump. After purification, the fractions were analyzed using MALDI-TOF MS, after which similar fractions were added together. Finally, the solvent was evaporated using rotary vacuum evaporation.

2.2.4 MALDI-TOF MS

Mass spectrometry of the products was performed according to a previously described protocol [19] on an Ultraflex MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany), using DHB as a matrix. From the spectra the relative abundance of the different components was calculated. To that end, the intensities of all identifiable peaks of both oligofructose and ester products (sodium and potassium adducts) were added and set to 100%. The relative contribution of each component was determined from the sum of the sodium and potassium adducts. A similar procedure has been used before for the same type of molecules [19].

2.2.5 Nuclear magnetic resonance (NMR) analysis

For ^{1}H NMR analysis caprylic acid mono-esters and lauric acid mono-esters were dissolved in D_2O, palmitic acid mono-esters were dissolved in D_6-DMSO, and stearic acid mono-esters were dissolved in $CDCl_3$. 100 mg of sample was dissolved in 1 mL of solvent. ^{1}H NMR spectra were recorded on a Bruker Avance III 400 spectrometer operating at 400.17MHz.

C8 mono-ester (D_2O, δ): 0.8 (t, 3H, CH_3), 1.3 (m, 8H, $(CH_2)_4$), 1.6 (m, 2H, CH_2CH_2CO), 2.4 (t, 2H, CH_2CO), 3.3-5.4 (m, CH, CH_2 and OH in oligofructose). C12 mono-ester (D_2O, δ): 0.9 (t, 3H, CH_3), 1.3 (m, 16H, $(CH_2)_8$), 1.6 (m, 2H, CH_2CH_2CO), 2.4 (t, 2H, CH_2CO), 3.5-5.4 (m, CH, CH_2 and OH in oligofructose). C16 mono-ester (DMSO-d_6, δ): 0.8 (t, 3H, CH_3), 1.2 (m, 24H, $(CH_2)_{12}$), 1.5 (m, 2H, CH_2CH_2CO), 2.5 (t, 2H, -CH_2-CO), 3.2-5.2 (m, CH, CH_2 and OH in oligofructose). C18 mono-ester (CD_3Cl, δ): 0.9 (t, 3H, CH_3), 1.3 (m, 28H, $(CH_2)_{14}$), 1.6 (m, 2H, CH_2CH_2CO), 2.3 (t, 2H, CH_2CO), 3.6-5.4 (m, CH, CH_2 and OH in oligofructose).

For the 2D NMR analysis 40 mg of lauric acid mono-esters was dissolved in 0.5 mL D_6-DMSO. NMR spectra were recorded at a probe temperature of 297 K on a Bruker Avance-III-500 spectrometer located at the Wageningen NMR Centre. The ^{1}H signal at 2.5 ppm and ^{13}C signal at 39.52 ppm of D_6-DMSO were used as chemical shift reference. 1D and 2D HMBC

and HMQC spectra were acquired using standard pulse sequences delivered by Bruker. For the [^{1}H,^{13}C]-HMBC 800 spectra of 32 scans were recorded, resulting in a measuring time of 12.5 h. For the [^{1}H,^{13}C]-HMQC 512 spectra of 16 scans were recorded, resulting in a measuring time of 4 h.

2.2.6 Determination of surface tension and surface dilatational modulus

Purified mono-ester fractions of caprylic, lauric, palmitic and stearic acid were dissolved in water at a concentration of 0.01% (w/v). The surface tension and surface dilatational modulus were monitored as a function of time using an automated drop tensiometer (ADT, ITCONCEPT, Longessaigne, France). Cycles of sinusoidal deformations were applied to determine the surface dilatational modulus. The frequency was 0.1 Hz and five cycles of oscillations were alternated with five blank cycles. The drop area was 15 mm^2 for caprylic and lauric acid derivatives while it was 10 mm^2 for palmitic and stearic acid derivatives. The deformation amplitude $\delta A/A$ was 0.05. The temperature was 25 °C. Measurements were performed at least in duplicate. If reproducibility was insufficient, measurements were performed in triplicate. Reported values for surface tension and surface dilatational modulus are equilibrium values.

3. Results and discussion

3.1 Establishment of reaction conditions

3.1.1 Esterification vs. transesterification

Elaborating on the work of Sagis et al. [20], a fatty acid was linked to oligofructose by an ester bond, to create an amphiphilic molecule that can be used as a food-grade surfactant. Because of the increased specificity and the milder reaction conditions, enzymatic procedures were preferred over chemical procedures. Therefore, lipase-catalyzed esterification and transesterification reactions were used to obtain the oligofructose fatty acid ester. Both reactions are favored at low water content to prevent the hydrolysis of the newly formed esters. During esterification, water is formed as the second product aside the ester. Therefore, molecular sieves may be added to this system to control the water content. Since the oligofructose and all solvents are dried before the reaction, the addition of molecular sieves should not be necessary during the transesterification reaction. They could, however, remove some of the water that remained after drying or absorb water that enters the system through the atmosphere. Therefore, three different reaction procedures were tested; transesterification of oligofructose with vinyl laurate (both in the absence and in the presence of molecular sieves) as well as an esterification reaction of oligofructose with lauric acid (in the presence of molecular sieves).

Table 1: Formation of oligofructose lauric acid mono-esters using three different enzymatic procedures; esterification in the presence of molecular sieves, transesterification in the presence of molecular sieves, and transesterification in the absence of molecular sieves. An additional portion of enzyme was added after 16 and 39 hours.

Reaction procedure	Molecular sieves present?	Relative abundance of mono-ester (%)			
		0h	16h	39h	63h
Esterification	Yes	0	27	24	36
Transesterification	Yes	0	29	38	37
Transesterification	No	0	25	35	28

Table 1 shows the relative abundance of mono-esters formed in time, as estimated based on the intensity of the peaks in the MALDI-TOF mass spectrum. The relative abundance of mono-esters (defined as one fatty acid esterified with one oligofructose molecule) at the end of the reaction time was about 30-40%, for all procedures (table 1). These results are comparable to previously reported data on the transesterification reaction between oligofructose and vinyl laurate [19], despite the difference in DMSO concentration

between the two studies (20% DMSO compared to 10% DMSO in this research). Although a decrease in the DMSO level reduces the solubility of the oligofructose, it will increase the enzyme activity. Apparently the combination of the two effects leads to a similar yield.

There were no major differences between the three reaction procedures with regard to the amount of product formation. Generally, transesterification reactions with for instance vinyl esters of fatty acids as acyl donors are used for enzymatic ester synthesis, since they lead to higher conversions [19]. However, in this study esterification was considered as well for a few reasons. During transesterification with vinyl esters of fatty acids, acetaldehyde is formed which can inactivate the enzyme. Furthermore, vinyl esters of fatty acids are not commercially available (at least not for all fatty acid chain lengths) while free fatty acids are commercially available. Finally, in a food-grade reaction procedure it is more desirable to use the more natural fatty acids instead of vinyl esters. Therefore, it was decided that further reactions would be esterification reactions instead of transesterification reactions.

3.1.2 Enzyme activity

To investigate whether the catalytic activity and stability of the immobilized lipase is affected under the conditions of the reaction, the enzyme was incubated at 60 °C in DMSO/Bu^{t}OH (10/90 v/v) for 72 h, with magnetic stirring. At different time intervals the residual enzyme activity was determined in (a) aqueous conditions, to compensate for reversible inactivation due to dehydration, if any, and (b) in an almost dry solvent mixture. The enzyme activity as a function of time is available in supplementary data 1. The activity of the rehydrated enzyme (i.e. hydrolytic activity, in emulsion) decreased to about 50% of the initial activity at incubation times longer than 24 h in DMSO/Bu^{t}OH (10/90 v/v). Since the enzyme was rehydrated in a medium with very high water activity, reversible inactivation due to dehydration is not an issue, and the irreversible inactivation observed can be assigned to effects of temperature and abrasion due to mechanical shear. When the residual esterification activity was measured in anhydrous organic medium without rehydration, the enzyme retained only 17% of the initial activity after 24 h incubation, with a half-life time of about 13 h. This additional inactivation can be ascribed to the reversible dehydration by the polar organic solvents, which can strip the essential water molecules from the enzyme [25]. The inactivation of immobilized lipase B from *Candida antarctica* by dehydration in polar organic solvents and by mechanical stirring has been reported earlier and many studies address solutions to diminishing these effects [19, 26-28]. Ter Haar and collaborators [19] have shown the inactivating effect of DMSO on Novozym 435 in both the

esterification and trans-esterification reaction and succeeded to minimize the loss of activity by minimizing the concentration of DMSO in a binary mixture with *tert*-butanol as co-solvent to 20% (v/v). Other approaches were to replace the polar reaction medium with a hydrophobic solvent, like hexane or toluene [28] or with a two-phase solvent system consisting of a polar solvent, i.e. DMSO, dimethylformamide, or pyridine, and a hydrophobic solvent, like hexane and diisopropyl ether [26]. Generally, reducing the speed of agitation was used to prevent the mechanical degradation and the leakage of the enzyme adsorbed on the solid support due to shear.

In this study, to compensate for the loss of activity of the biocatalyst and to enhance the product yield, in further batch experiments the addition of enzyme after 19 hours and 43 hours was incorporated in the synthesis protocol. This approach was suitable for the production of preparative amounts of oligofructose mono-esters at lab scale, but is less appropriate for industrial application due to the impact of the enzyme costs on the process economics. This limitation could be overcome by developing a continuous process for the enzymatic esterification of oligofructose using a fixed-bed reactor with immobilized enzyme and a rehydration step between the cycles. The preliminary results are very promising and the study, which is currently in progress, will be published later in a dedicated article.

3.2 Synthesis of caprylic acid, lauric acid, palmitic acid and stearic acid esters of oligofructose by esterification

To produce oligofructose fatty acid esters with differences in hydrophobicity, the length of the fatty acid was varied. Caprylic, lauric, palmitic, and stearic acid were esterified with oligofructose to obtain amphiphilic molecules.

The oligofructose that was used as a substrate is a mixture of oligomers with a degree of polymerization (DP) between 2 and 8, and an average DP of 4.4 (figure 1A). The esterification with fatty acids yielded a mixture of unmodified oligofructose and predominantly mono-esters (illustrated for caprylic acid esters in figure 1B).

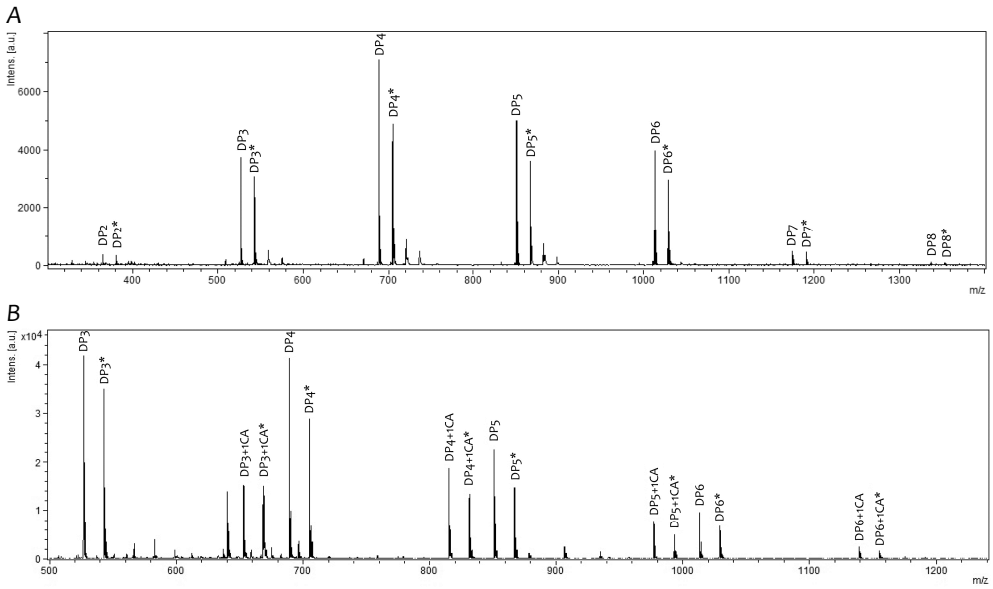


Figure 1: A) MALDI-TOF mass spectrum of unmodified oligofructose. B) MALDI-TOF mass spectrum of oligofructose caprylic acid esters after reaction (t=69 hours), before purification. Both sodium (Δm/z = 23) and potassium (Δm/z = 39) adducts appear. Potassium adducts are indicated by an asterisk. Unmodified oligofructose is indicated in the figure with DP3, DP4, DP5, DP6, DP7, and DP8. Mono-esters of oligofructose and caprylic acid (Δm/z =162, compared to unmodified oligofructose) are indicated by the addition +1CA.

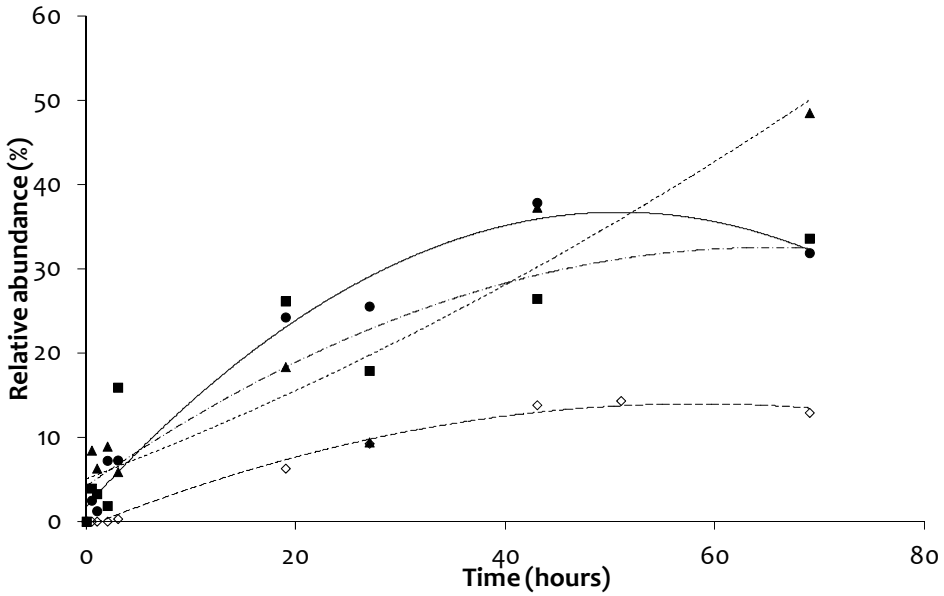


Figure 2: Formation of mono-esters during enzymatic esterification of oligofructose with different fatty acids as determined with MALDI-TOF MS; caprylic acid (--◇--), lauric acid (--■--), palmitic acid (···▲···) and stearic acid (—●—).

We studied the formation of products as a function of time and the relative abundance of mono-esters was estimated based on the intensity of the peaks in the mass spectrum (figure 2).

After 69 hours, the relative abundance of unreacted oligofructose was 87% for caprylic acid, 64% for lauric acid, 48% for palmitic acid and 67% for stearic acid. The reaction product consisted of mono- and di-esters, although mono-esters were the predominant reaction products. The amount of di-esters was below the detection limit for caprylic acid esters, 2.1% for lauric acid esters, 3.8% for palmitic acid esters, and 1.2% for stearic acid esters (data not shown). The specificity of lipase is responsible for the fact that mono-esters were the predominant reaction product [29]. A significant increase of the oligofructose conversion with the increase of the chain length of the fatty acid was observed, with the highest conversion for the C16 acid. This is consistent with earlier reports of *Candida antarctica* lipase specificity towards more lipophilic substrates [18, 30].

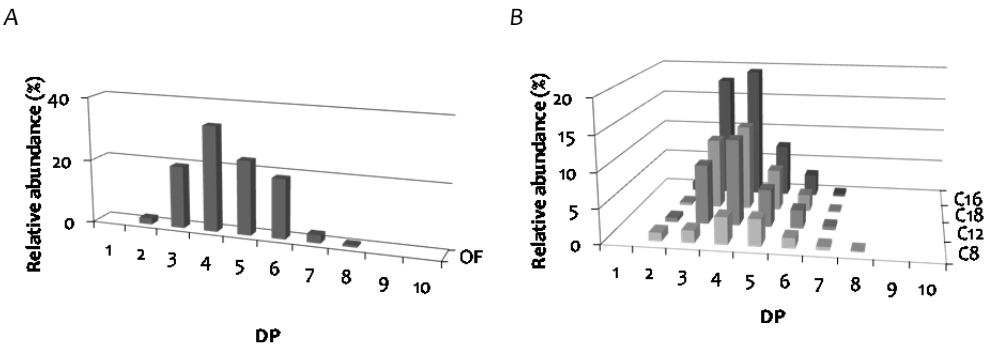


Figure 3: Relative abundance of products before and after reaction, separated into different degrees of polymerization. A) Unmodified oligofructoe (OF). B) Mono-esters of caprylic (C8), lauric (C12), palmitic (C16) and stearic (C18) acids.

The results in figure 3 clearly show that both the length of the alkyl chain of the fatty acid and the degree of polymerization (DP) of the individual oligomers in the oligofructose substrate are relevant factors for the type of products formed (figure 3B). For all oligomers, increased fatty acid chain length lead to an increase in mono-ester formation. Furthermore, when comparing the distribution of the different oligomers in the unmodified oligofructose (figure 3A) to that in the mono-esters (figure 3B), the oligomers with DP 3 and DP 4 seem to be better substrates for the enzyme than the oligomers with a higher DP, although for smaller fatty acids DP5 also seems to be a good substrate. Finally, di-esters were only formed for oligomers with a lower DP (3 and 4, results not shown). These effects could be related to the anatomy of the catalytic cleft of the lipase. It is an elliptical, steep funnel with dimension of 9.5 x 4.5 Å [31]. When larger molecules attempt to access the catalytic cleft, they may be hindered due to steric effects. This could explain why mono-esters with a higher DP of the oligofructose moiety are formed for shorter fatty acids and why di-esters are only formed for oligofructose moieties with a lower DP [32, 33].

3.3 Isolation and purification of mono- and di-esters of oligofructose

After removing molecular sieves and lipase, evaporation of the solvents and washing with acetone and hexane, the products were fractionated using reverse phase solid phase extraction (RP-SPE). RP-SPE is an effective purification method that: 1) would allow us to separate large amounts of materials in a relatively short time, 2) gives a sufficient purity for functionality experiments, and 3) requires only minor modifications when switching between products derived from fatty acids with different chain lengths.

Unmodified oligofructose was eluted from the column during the first two steps: 100% water and 10% methanol (figure 4). Mono-esters of caprylic acid eluted with 50% and 60% methanol, mono-esters of lauric acid with 70% and 80% methanol, mono-esters of palmitic acid with 90% methanol and mono-esters of stearic acid with 90% and 100% methanol. Di- and tri-esters of caprylic esters eluted with 80% and 90% methanol, di- and tri-esters of lauric acid with 90% methanol, di-esters of palmitic acid with 100% methanol, and di-esters of stearic acid only during the second washing step with 100% methanol. The difference in hydrophobicity between the different fractions is reflected in the polarity of the solvent that was needed for elution.

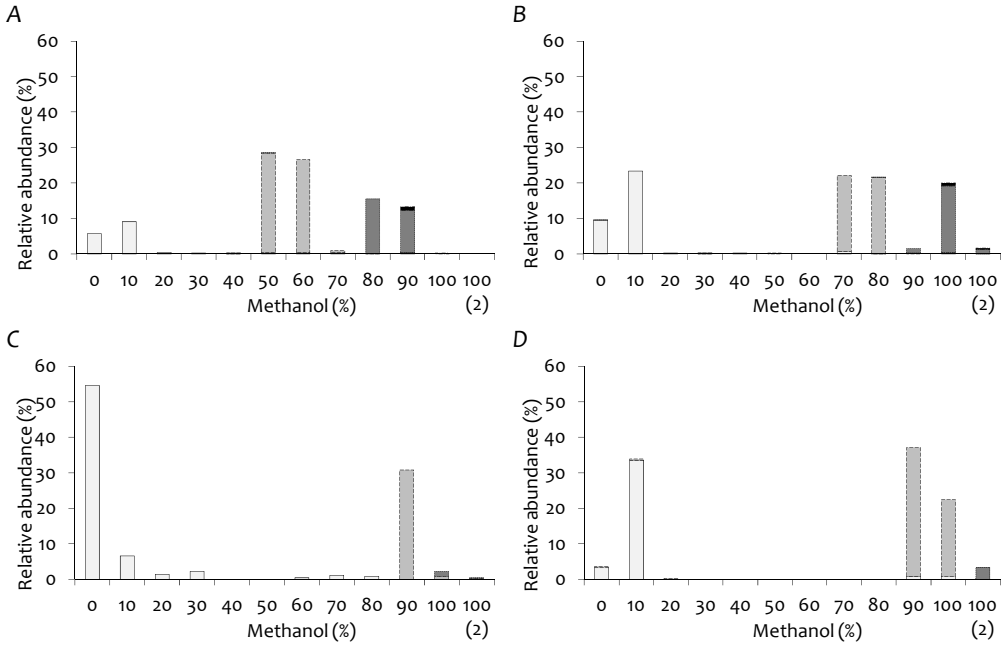


Figure 4: Composition of fractions during reverse phase separation of oligofructose fatty acid esters, as determined with MALDI-TOF MS. A) Caprylic acid. B) Lauric acid. C) Palmitic acid. D) Stearic acid. White: Unmodified oligofructose. Light grey: Mono-esters. Dark grey: Di-esters. Black: Tri-esters.

Purifications have also been performed using flash chromatography, which fractionates the material using the same column material in a larger column and a linear

gradient of water and methanol (details about methods are available in supporting information 2). Similar results were obtained and are therefore not shown.

3.4 Characterization of purified fractions

Fractionation of the products of each reaction by SPE allowed the separation of two purified fractions, one enriched in monoester (entries 1-4 in table 2) and one containing di- and tri-esters (not shown). Di-esters of palmitic acid could not be isolated in sufficient quantities.

Table 2: Relative abundance of oligofructose fatty acid ester fractions after purification with reverse phase separation. nd: not detected, below noise level.

Entry	Fraction	Composition (%)			
		Unmodified oligofructose	Mono-ester	Di-ester	Tri-ester
1	Caprylic acid mono-esters	0.4	99.6	nd	nd
2	Lauric acid mono-esters	2.7	97.3	nd	nd
3	Palmitic acid mono-esters	6.1	93.9	nd	nd
4	Stearic acid mono-esters	6.1	93.9	nd	nd

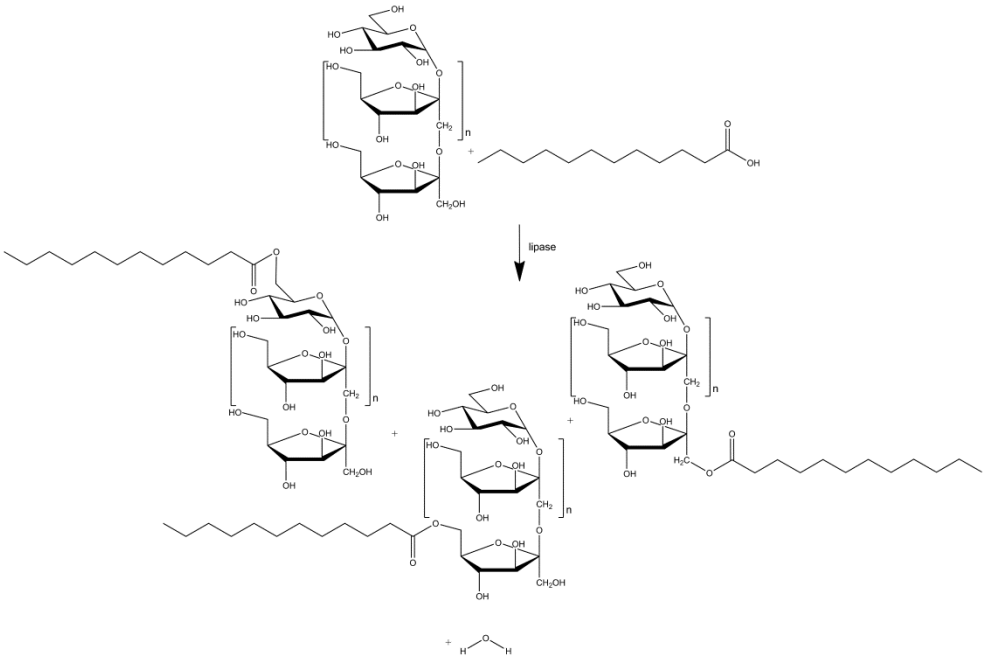
MALDI-TOF-MS analysis showed that the products obtained have sufficient purity for functional testing (table 2). The mono-ester fractions (especially caprylic and lauric acid derivatives) are highly pure. The mono-esters were mixtures of DP2 to DP8 oligomers, with predominantly DP3, DP4 and DP5. Surprisingly, low amounts of di- and tri-esters could be isolated, although they were not identified in all crude reaction mixtures using MALDI-TOF-MS analysis, probably because their concentration was below the detection limit.

^{1}H NMR spectra of the monoesters of caprylic acid, lauric acid, palmitic acid and stearic acid with oligofructose confirmed the presence of the fatty acid ester group, as illustrated for the oligofructose C12-monoester, (δ ppm, D_2O): 0.8-0.9 (br t, 3H, CH_3); δ1.1-1.3 (m, 16H, $(CH_2)_8$); δ1.4-1.6 (m, 2H, CH_2CH_2CO); δ2.2-2.4 (br t, 2H, CH_2CO); δ3.3-5.5 (multiple coupling, CH, CH_2 and OH of the oligofructose unit).

2D NMR analysis was performed for lauric acid mono-esters to further confirm the structure of the molecule. In the HMBC spectrum 3-bond couplings between the C=O of the fatty acid and protons of the oligofructose could be identified, indicating the positions where fatty acids are attached to the oligofructose chain. This attachment appeared to be

heterogeneous, since multiple overlapping signals for sugar protons coupled with C=O were found. In the HMQC spectrum the single-bond couplings between these proton signals and their adjacent carbon atoms could be identified. From the chemical shifts below 67 ppm of these carbon atoms, it is clear that the fatty acids are mainly attached at fructosyl C-1 and/or C-6 positions of fructose and glucose, showing the preference of the enzyme for primary hydroxyl groups. All NMR spectra are available in supplementary data 3.

Since except the C-1 hydroxyl group of the terminal fructosyl residue all other C-1 hydroxyls are involved in the glycosidic links, the position of the esterified fatty acid is expected to be restricted to the C-6 position and the terminal fructosyl C-1. Although 2D NMR analysis could prove the location of the esterified fatty acid on a single monosaccharide unit, it could not conclusively identify the position of the fatty acid on the oligofructose chain. However, the location of the fatty acids is most probably at the primary OH groups of either terminal glucose and fructose moieties of the oligosaccharides due to geometrical constraints in the catalytic cleft of the lipase. Therefore the reaction can be summarized according to the reaction scheme as presented in scheme 1.



Scheme 1: Enzymatic synthesis of oligofructose monoesters with long chain fatty acids, in this case lauric acid.

3.5 Functionality

As a first step in the functional characterization, the surface tension and surface dilatational modulus of air/water interfaces stabilized by purified mono-esters were determined (table 3). There is a clear dependence of these properties on the length of the fatty acid that is esterified with the oligofructose. In general, when the fatty acid chain length increases the surface tension decreases. The efficiency of a surfactant is inversely proportional to the amount of surfactant that is necessary to achieve a certain surface tension reduction. Therefore, derivatives with a longer fatty acid chain are more efficient in reducing the surface tension. Since the critical micelle concentrations of the derivatives are not yet known, no statements about the effectiveness (the maximum surface tension reduction) can be made at this point.

Table 3: Surface tension and surface dilatational modulus of air/water interfaces stabilized by purified mono-esters (bulk concentration 0.01% (w/v) in water). Nd = too low to be accurately determined.

Length of fatty acid chain	Surface tension (mN/m)	Surface dilatational modulus (mN/m)
8	54.5 ± 7.0	nd
12	54.1 ± 1.6	13.1 ± 3.2
16	37.1 ± 3.5	145.3 ± 103.1
18	28.1 ± 8.5	45.7 ± 32.7

The palmitic and stearic acid derivatives have a significantly higher dilatational modulus compared to the shorter chain derivatives. The reproducibility of the measurements, as reflected in the standard deviations of the modulus, was poor. The reason for the poor reproducibility is that the measurements are outside of the linear regime, where the stress is no longer proportional to the strain. This means that the structure at the interface, which is responsible for the high modulus, is affected by the deformation that is imposed. Unfortunately, with the currently available equipment, it is not possible to take measurements within the linear regime. This topic will be further explored in chapter 4. However, despite the large variation in the reported values, a strong dependence of the modulus on the length of the fatty acid chain is found. Air/water interfaces stabilized by proteins generally have a surface tension of 47-57 mN/m and a surface dilatational modulus ranging between 20-80 mN/m, while interfaces stabilized by LMW surfactants generally have a surface tension of 22-42 mN/m and a negligible surface

dilatational modulus [21]. For example, an air/water interface stabilized by sucrose monolaurate had a surface tension of 38 mN/m and a surface dilatational modulus of 1 mN/m [20]. This shows that, because of the low surface tension combined with a high dilatational modulus, especially oligofructose esters of palmitic and stearic acid perform well compared to commonly used food surfactants. A high dilatational modulus is often linked to high foam stability [20]. Commercially available sucrose esters of longer fatty acids are also capable of lowering the surface tension considerably and create an interface with a high dilatational modulus. However, they are often produced by chemical methods and therefore usually mixtures of esters with a wide range of degrees of substitution and even different fatty acid chain lengths. In contrast, as a result of the enzymatic synthesis, the oligofructose fatty acid esters that were created in this study are mono-esters with a single fatty acid chain length. Furthermore, because of the larger hydrophilic head, the solubility in water is increased.

These results imply that especially the oligofructose fatty acid esters with longer fatty acid chains could be efficient surfactants for use in foods.

4. Conclusions

Enzymatic esterification of oligofructose with fatty acids of different chain lengths yielded a mixture of unmodified oligofructose, mostly mono-esters and small amounts of di- and tri-esters. The fractionation using reverse phase solid phase extraction lead to a sufficient degree of purification (>93%) for most products. By combining MALDI-TOF MS, ^{1}H NMR and 2D NMR techniques, the products were identified as monosubstituted oligofructose fatty acid esters. These results show that, using relatively simple methods with only minor modifications when changing between the different fatty acids, it is possible to synthesize esters of oligofructose and a range of fatty acids. Initial functional experiments showed that the derivatives of longer (palmitic and stearic) fatty acids had a low surface tension and a high surface dilatational modulus, which makes them promising novel food-grade surfactants.

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Supplementary information

1. Enzyme activity

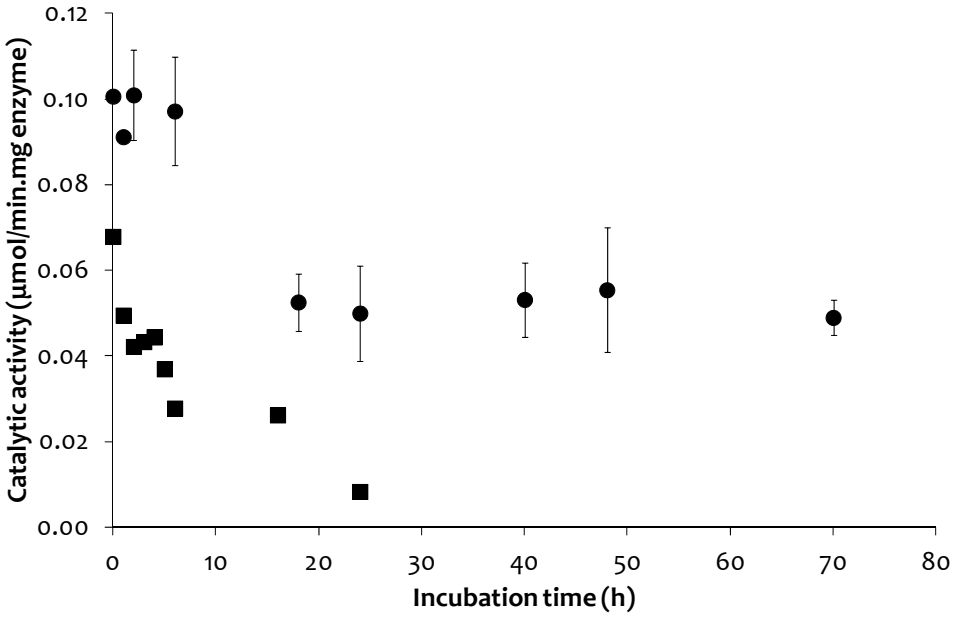

Figure 1: Catalytic activity of immobilized C. antarctica lipase B.Synthetic reaction between butyric acid and butanol, determined at 60 °C in a mixture of 10% DMSO and 90% Bu^{t}OH, expressed as µmol *butyl butyrate formed per milligram of enzyme per minute (■). Hydrolytic reaction of triolein, determined at 40 °C in water at pH 8, expressed as* µmol *of oleic acid produced per milligram of enzyme per minute (●).*

2. Methods Flash Chromatography

The reaction products were fractionated by flash chromatography using the Reveleris® Flash Forward System (Grace Davison Discovery Science). The threshold value for the ELSD (Evaporative Light Scattering Detector) of the system was set to 5 mV and the system only collected peaks exceeding the threshold. Reveleris C18 RP 80 g cartridges with a loading volume of 88 mL were used. The columns were activated by washing them with 264 mL of methanol followed by 264 mL of water. 30 mL of a 15 mg/mL suspension of esters in water were loaded onto the column. A gradient of deionized purified water and methanol was used to elute the products from the column. The gradient was different for the four products and is summarized in table 1. The gradient was linearly changed between the different steps. The flow rate of the solvents was 60 mL/min.

Table 1: Gradients used for flash chromatography

C8		C12		C16		C18	
Time (min)	Methanol (%)	Time (min)	Methanol (%)	Time (min)	Methanol (%)	Time (min)	Methanol (%)
0	0	0	0	0	0	0	0
0.5	0	2	0	1	0	2	0
5	20	8	20	9	20	8	20
6	50	9	72	10	90	15	100
14	60	19	85	18	100	25	100
15	65	21	100	21	100		

3. NMR spectra

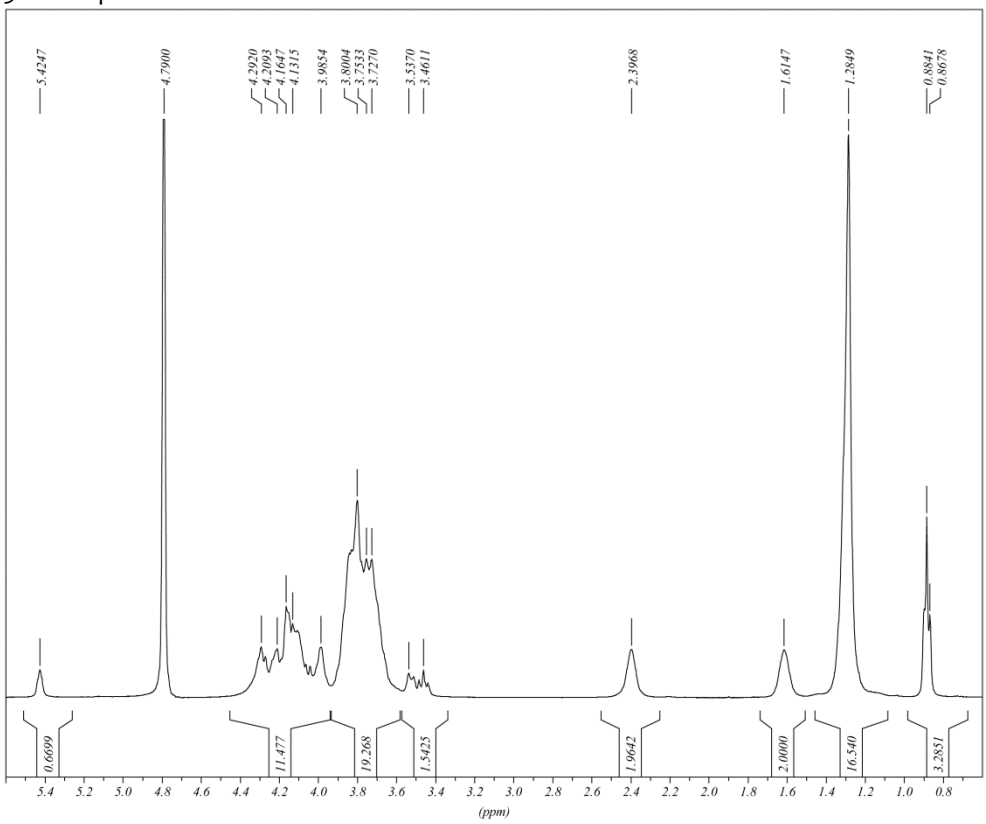

Figure 1: ^{1}H NMR spectrum of oligofructose lauric acid mono-esters.

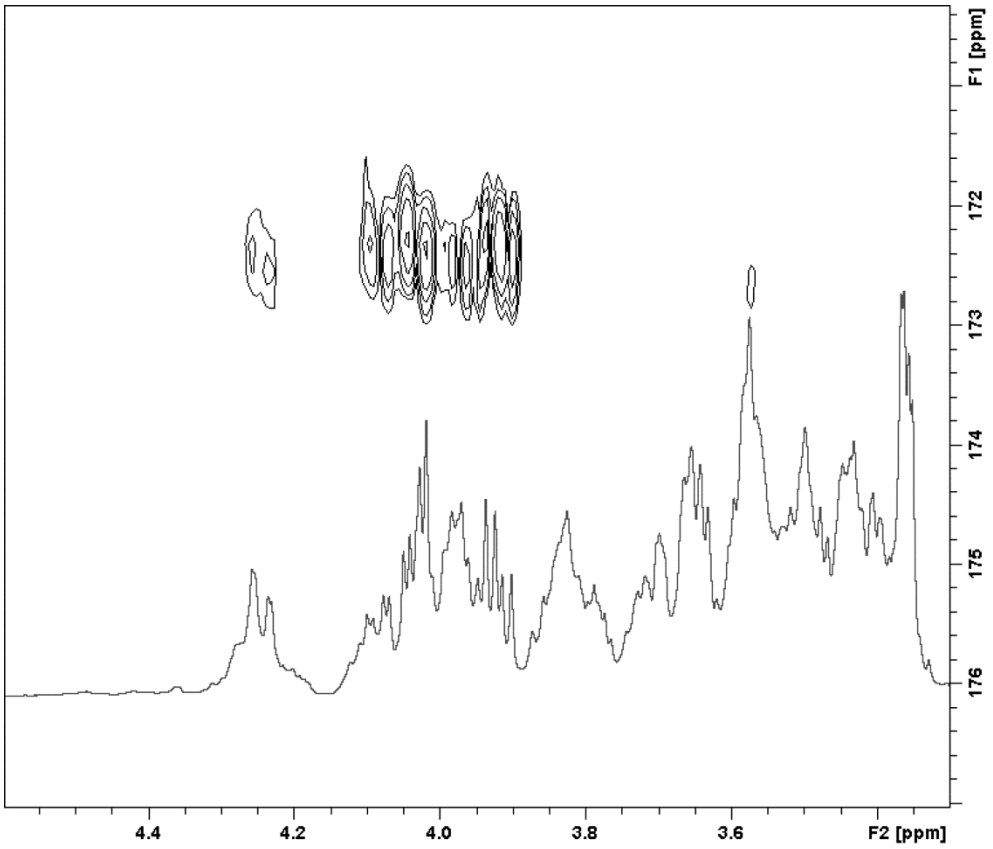

Figure 2: HMBC spectrum of oligofructose lauric acid mono-esters.

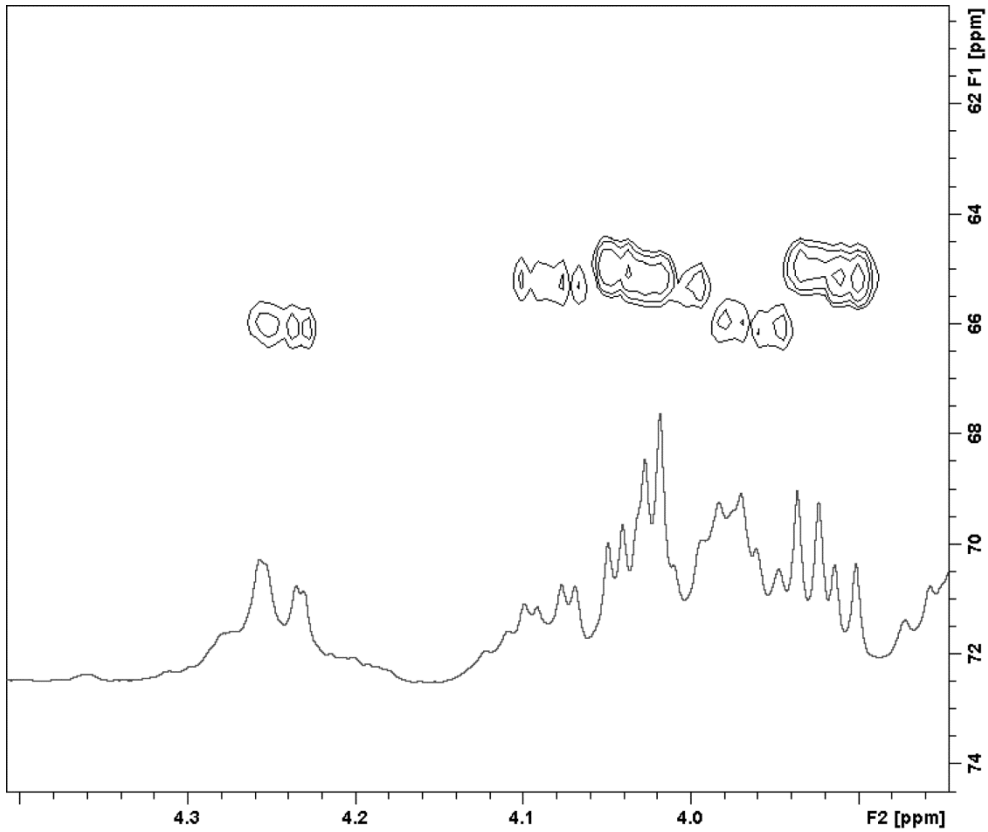

Figure 3: HMQC spectrum of oligofructose lauric acid mono-esters.

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Chapter 3

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Effect of variations in the fatty acid chain on functional properties of oligofructose fatty acid esters

Abstract

Oligofructose fatty acid esters are surfactants that considerably lower the surface tension of an air/water interface, provide the interface with a high dilatational modulus and lead to a high foam stability. In this chapter, we investigate the effect of the molecular structure of oligofructose fatty acid esters on their functional properties. We varied the length and degree of saturation of the fatty acid chain, and the number of fatty acids esterified to the oligofructose part. A tensiometer was used to establish CAC curves, light scattering to determine the size of micellar aggregates and an ellipsometer to determine the amount of material that adsorbed at the interface. Esters with a more hydrophobic character had a lower CAC and had a higher efficiency. Oligofructose mono- and di-esters with fatty acid chain lengths between C8 and C16 formed spherical micelles, while esters with a fatty acid chain length of C18 formed larger aggregates. The effectiveness was similar for all esters. Using the Gibbs adsorption model, we did not find major differences in the area per molecule for the different esters. Ellipsometry experiments also did not indicate major differences in the area per molecule for the oligofructose mono-esters. The area per molecule of oligofructose esters was larger than that of sucrose esters and independent of the degree of saturation of the fatty acid chain. We conclude that the amount of interfacial area occupied by one molecule is determined by the oligofructose part.

1. Introduction

Two major types of food-grade surfactants are proteins and low molecular weight (LMW) surfactants [1, 2]. Proteins are macromolecules with both hydrophilic and hydrophobic patches. Upon adsorption to the interface, they lower the surface tension and can unfold to create a network with high viscoelasticity [1, 3]. This network provides the interface with a high dilatational modulus. In contrast, LMW surfactants are relatively small and have a clearly defined hydrophobic and hydrophilic part. Upon adsorption to the interface, LMW surfactants can lower the surface tension much more than proteins. This is caused by a more compact interfacial structure of the LMW surfactants [3]. However, they do not typically create interfaces with a high dilatational modulus [3-5]. Instead, LMW surfactants stabilize interfaces by the Gibbs-Marangoni mechanism. This mechanism relies on rapid surface diffusion of surfactants that will reduce surface concentration gradients that can develop after deformation of the interface [4, 6]. Both surface tension and surface rheological properties are important with respect to foaming properties. A molecule that lowers the surface tension considerably and at the same time provides a high dilatational modulus could be an excellent foam stabilizer.

Previous research on oligofructose fatty acid esters showed that they have this ability to lower the surface tension of an air/water interface considerably, like a LMW surfactant, and to provide a high dilatational modulus, like a protein [7], [chapter 2]. Both factors are important in surfactant performance and resulted in high foam stability. The oligofructose that was used was a mixture of oligomers with a degree of polymerization varying between 2 and 8 (average 4.4). Consequently, the resulting esters are also mixtures. By varying the size and number of hydrophobic groups, products with a wide range of amphiphilicity can be produced. This will have an impact on the functional properties of the molecule [3].

The goal of this study was to determine the influence of variations in chemical fine structure on the functional properties of oligofructose fatty acid esters. To obtain a range of molecules with different functionality, the length of the saturated fatty acid chain (C8, C12, C16, C18) as well as the number of fatty acids per oligofructose molecule was varied (mono-esters for all four fatty acid chain lengths and also di-esters for C8 and C12). To investigate the influence of the degree of saturation on the functional properties, we also studied an ester of oligofructose and palmitoleic acid, a mono-unsaturated fatty acid with a chain length of 16. Additionally, to study the influence of the size of the hydrophilic group, sucrose esters were included. We have determined the surface tension of air/water interfaces stabilized by the different esters as a function of the bulk concentration to

establish the CAC. From the CAC curves, we have derived the efficiency, effectiveness, the maximum surface excess concentration and the cross-sectional area per molecule. We have performed light scattering experiments to determine the size of the aggregates that were present above the CAC. Finally, we have studied the amount of material that adsorbed to the interface using ellipsometry.

2. Materials and methods

2.1 Materials

Oligofructose fatty acid esters were prepared according to a previously described protocol [chapter 2], by esterification of fatty acids with oligofructose with a DP ranging from 2 to 8, using lipase as a catalyst. Five different fatty acids were used (caprylic acid, lauric acid, palmitic acid, palmitoleic acid and stearic acid). Palmitoleic acid esters were prepared according to the protocol described for palmitic acid esters. The crude products contained mostly monosubstituted esters, but also small amounts of disubstituted esters, unmodified oligofructose and unreacted fatty acids. Using reverse phase solid phase extraction, the products were fractionated. MALDI-TOF MS and NMR confirmed the purity (>90% for mono-esters, >80% for di-esters) of the purified fractions [chapter 2].

Sucrose monolaurate (≥97%) was obtained from Sigma Aldrich (Steinheim, Germany). Sucrose esters with fatty acid chain lengths C16 and C18 (RYOTO esters S1670 and P1670) were obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Both S1670 and P1670 are crude products. According to the manufacturer, S1670 consist of 75% mono-esters and 25% di-, tri-, and poly-esters. The fatty acids consist for 70% of stearic acid. P1670 consists of 80% mono-esters and 20% di-, tri-, and poly-esters. The fatty acids consist for 80% of palmitic acid. These products were used without further purification.

2.2 Methods

2.2.1 Sample preparation

The esters were dissolved in purified deionized water using a magnetic stirrer. For some of the samples (with fatty acid chain lengths of 16 and 18 carbon atoms) it was necessary to heat (maximally 70 °C) to dissolve the esters. Samples were cooled down to room temperature before starting measurements.

2.2.2 Determination of surface tension

The surface tension of the air/water interface stabilized by the esters was determined with an automated drop tensiometer (ITCONCEPT, Longessaigne, France) and with a profile analysis tensiometer (Sinterface, Berlin, Germany). All experiments were performed at 25 °C and repeated until sufficient reproducibility was obtained. Reported values represent the average of 2-10 measurements.

2.2.3 Light scattering

The size of the aggregates that were present in the solutions at concentrations higher than the CAC was determined using a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, United Kingdom). The solutions were clear, however to remove dust particles and small amounts of undissolved material that could disturb the measurements, solutions were filtered prior to the measurements using Millipore-Millex GP Hydrophilic PES filters with a pore size of 0.22 μm (Merck Millipore, Billerica, United States). Measurements were performed at 25 °C with three measurements per sample. Reported size distributions represent the average of these three measurements. Measurements were also performed at concentrations below the CAC to verify the absence of the aggregates.

2.2.4 Ellipsometry

The amount of material that adsorbed at the interface was determined using null ellipsometry. We used a Multiskop instrument (Optrel Gbr, Berlin, Germany) with the angle of incidence set at 55°. The light source was a He–Ne laser with a wavelength of 632.8 nm. From the obtained values of the ellipsometrical angles Ψ and Δ, the amount of adsorbed material was calculated using a three-layer model with a dn/dc of 0.15, which is common for small surfactants [8-10]. The variation in the dn/dc value for different materials is small (generally between 0.15 and 0.18) [10] and the actual value of dn/dc does not affect the ratio between the head group sizes that were obtained. Experiments were performed at room temperature at bulk concentrations higher than the CAC. At these concentrations, no significant changes in Ψ and Δ were found in the first ten minutes after the start of measurement. This indicates that the interfaces were already saturated. Measurements were performed in duplicate.

3. Results & discussion

In this study, the effect of variations in chemical fine structure on functional properties of oligofructose fatty acid esters was investigated. The esters were prepared by esterifying fatty acids to oligofructose. In all cases, the oligofructose was a mixture of oligomers with a degree of polymerization varying between 2 and 8 (average 4.4). Consequently, the resulting esters are also mixtures [chapter 2]. The first variation in the structure of the molecule was the length of the fatty acid chain. Four different fatty acid chain lengths were used: C8, C12, C16 and C18. The second variation was the degree of substitution. For all fatty acid chain lengths, the main reaction products, mono-esters (one fatty acid per oligofructose molecule), were studied. For the two shortest fatty acid chain lengths (C8 and C12), also di-esters (two fatty acids per oligofructose molecule) were studied. Di-esters of the longer fatty acid chain lengths were not studied due to their limited presence in the crude reaction product and due to their very high hydrophobicity which limited their solubility in water. This very low solubility in water made them unsuitable for the type of experiments that we are interested in. By changing the chemical fine structure of the molecule, the balance between hydrophobicity and hydrophilicity, expressed as HLB value, is changed. The HLB value was calculated according to the method of Griffin [11]. The structural parameters for the esters that were studied are summarized in table 1.

Table 1: Structural parameters of the oligofructose fatty acid esters used in this study. The oligofructose part was a mixture with different degrees of polymerization ranging between 2 and 8 with an average of 4.4.

	Fatty acid chain length	Number of fatty acid chains	Molecular weight			HLB value		
			DP2	DP4.4	DP8	DP2	DP4.4	DP8
OF-C8m	8	1	468	857	1440	14.6	17.1	18.2
OF-C8d	8	2	594	983	1566	11.5	14.9	16.8
OF-C12m	12	1	524	913	1496	13.0	16.0	17.6
OF-C12d	12	2	707	1095	1679	9.7	13.3	15.7
OF-C16m	16	1	580	969	1552	11.8	15.1	16.9
OF-C18m	18	1	609	997	1581	11.2	14.7	16.6

3.1 Surface tension

The first step in the functional characterization of the molecules was the determination of the surface tension of an air/water interface as a function of the bulk concentration of surfactant. Figure 1 shows that for all six esters except OF-C18m (figure 1F)

a curve with a decrease in surface tension with increasing concentration was obtained, up to a certain concentration after which the surface tension remained essentially constant. For OF-C18m it was not possible to establish this dependency. At concentrations higher than this concentration, aggregates are formed in the bulk. At this point, we do not want to make any assumptions about the size, shape and nature of aggregates. Therefore, we adopt the term critical aggregation concentration (CAC) to describe the concentration at which no more significant changes in surface tension occur [12]. The value of the CAC was determined graphically from the intercept of a linear fit in the flat part of the curve (>CAC) and a linear fit of the part of the curve lower than CAC. These fits are included in figure 1. In some cases, there is a large variability in the values for the surface tension. In chapter 4, we discuss surface rheological experiments that suggest that the oligofructose esters may form a two-dimensional glass phase after adsorption at the interface. As a result, in some cases the interface may be in an arrested state which inhibits further adsorption, and this may explain the large variation that is sometimes seen in the values for the surface tension.

The CAC depended on the molecular structure of the surfactants (table 2). When the fatty acid chain length was increased, we found a clear decrease in CAC. This is commonly observed for sugar esters [12-15]. As a result of the high variability in surface tension data, there is a certain amount of uncertainty in the CAC values. However, the differences in the CAC values *between* the esters are much larger than these errors. To evaluate the effect of the degree of substitution, the CAC of mono-esters was compared to that of di-esters. Di-esters had a much lower CAC compared to mono-esters with the same fatty acid chain length. This is also a known phenomenon for sugar esters, such as sucrose esters and fructose esters [16, 17]. Similar to mono-esters, a longer fatty acid chain length reduced the CAC of di-esters. To evaluate the effect of the size of the hydrophilic group, the value of the CAC of OF-C12m was compared to literature values of sucrose monolaurate (S-C12m). This molecule is structurally similar to OF-C12m, with the exception of a smaller hydrophilic group in the case of S-C12m. Garofalakis et al. [12] have found a CAC of 0.21 mM for S-C12m, while Husband et al. [17] found a CAC of 0.35 mM. The value that was found for OF-C12m (3.21 mM) was much larger than the literature values for S-C12m and may be attributed to the larger size of the oligofructose part compared to a sucrose group, which leads to a change in hydrophobicity.

Table 2: Surface properties of oligofructose fatty acid esters. nd: not determined.

Surfactant	CAC (mM)		Efficiency (pC_{20})	Effectiveness (π_{CAC})	Maximum surface excess * 10^{-6} (mol/m^2)	Molecular area (Å^2)	
OF-C8m	28.0	± 7.5%	2.8	39.4	2.72	61	± 15
OF-C8d	0.209	± 7.5%	4.8	40.1	3.02	55	± 10
OF-C12m	3.21	± 20%	3.8	34.1	1.83	91	± 25
OF-C12d	0.0195	± 5%	5.5	37.0	3.63	46	± 15
OF-C16m	0.225	± 20%	5.0	35.3	1.96	85	± 15
OF-C18m	nd		nd	nd	nd	nd	

The CAC can be related to the average hydrophobicity of the total molecule. The more hydrophobic a surfactant is, the more likely it is to form micelles at lower concentrations. In figure 2A we have plotted the logarithm of the CAC value against the fatty acid chain length. Both for mono-esters (triangles) and for di-esters (squares) a strong dependence of the CAC on the fatty acid chain length was found. This was reported before by Zhang et al. [14] for surfactants based on maltose and fatty acids, and by Becerra et al. [18] for sucrose fatty acid esters. For the oligofructose mono-esters we have determined that the increase of the chain length with 1 carbon atom means that the CAC will fall by a factor 3.5 (*f*). This means that the hydrophobic energy increment per CH_2 group (calculated as $RT \ln f$) is 3.1 kJ mol^{-1}. These values are comparable to results obtained for a series of non-ionic alkyl polyoxyethylene monoethers [19] and for glucose and lactose-based surfactants [20].

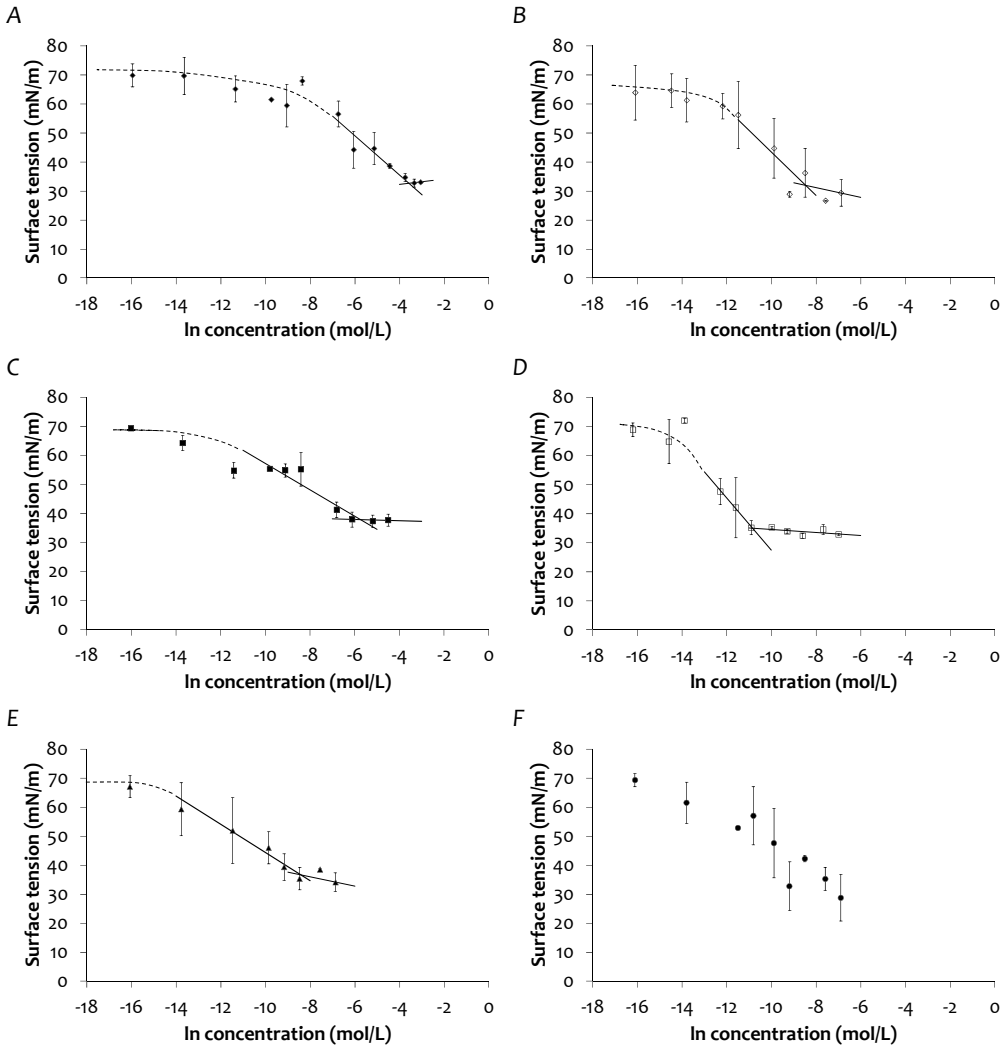

Figure 1: Equilibrium surface tension of air/water interfaces as a function of bulk concentration for OF-C8m (A), OF-C8d (B), OF-C12m (C), OF-C12d (D), OF-C16m (E) and OF-C18m (F). For OF-C12d and OF-C18m the highest concentration that was reported represents the solubility limit. For OF-C18m it was not possible to determine a CAC.

Two parameters that are often used when evaluating surfactant performance are the efficiency and the effectiveness [14]. The efficiency of a surfactant describes the concentration of the surfactant that is necessary to obtain a certain surface tension

reduction from the value of a clean interface, often 20 mN/m. It is defined by the value of the negative logarithm of the bulk concentration that is necessary to obtain this reduction and designated pC_{20}. The effectiveness of a surfactant is described as the reduction in surface tension at the CAC, (π_{CAC}) and may differ significantly among different surfactants. The values for both parameters can be found in table 2.

The efficiency of the esters was dependent on the fatty acid chain length (figure 2B). Both for mono-esters and di-esters an increased efficiency was found when the fatty acid chain length was increased. Since the efficiency of a surfactant determines the amount of material that is necessary for surface tension reduction, it has practical relevance. It is correlated to the amount of surfactant that is necessary in a product formulation and hence has a major impact on the costs. Therefore, especially the more hydrophobic components are interesting in terms of applicability in food products. However, these esters will also have lower solubility which may be a disadvantage for some applications.

The effectiveness was only weakly dependent on the molecular structure (figure 2C). In most cases π_{CAC} was similar, although it seems to slightly decrease with fatty acid chain length. Some authors have found no dependency of the size of the hydrophobic domain on the effectiveness [14]. In contrast, other authors did find a decrease in effectiveness when the fatty acid chain length was increased [12, 15, 16]. These studies were mostly done on sugar esters with a smaller hydrophilic group (lactose, lactitol, xylose, galactose, sucrose, lactose and fructose) than the oligofructose that was used in this study. The π_{CAC} depends, amongst others, on how compact the interfacial layer is, which in turn is dependent on the amount of space that one surfactant molecule occupies. Since all surfactants share the same oligofructose part and because of the similarity in effectiveness, the maximum packing could be determined by the size of the oligofructose part.

From the CAC curves in figure 1, the maximum surface excess concentration of surfactant at the air/water interface (Γ_{max}) and the area occupied by each molecule were determined. We have assumed that the Gibbs adsorption equation could be applied:

$$\Gamma_{max} = \frac{1}{RT}\frac{d\gamma}{d\ lnC}$$

where R is the gas constant, T the temperature, γ the surface tension and C the concentration. Γ_{max} was calculated directly from the slope of a γ-ln$C$ plot, where a linear fit was applied to the part of the curve below CAC (figure 1). The points at the beginning of the curve (at the lowest concentrations, dashed lines), where there was no significant deviation

from the value of a clean interface, were excluded. Subsequently, the area occupied by each surfactant, *A*, was calculated according to

$$A = \frac{1}{\Gamma_{max} N}$$

where *N* is Avogrado's constant.

The linear fits that were used as a basis for the calculations are shown in figure 1. Due to the errors in the surface tension values, the quality of the fitting results in relatively large errors for the values of the maximum surface excess concentration and the cross-sectional area per molecule. The results of the calculations are also shown in table 2. The values for the area per molecule vary between 46 and 91 $Å^2$, and do not seem to depend on the fatty acid that was used for esterification. Despite the large amount of uncertainty, we can conclude that the fatty acid chains are not closely packed, since the cross-sectional area for a closely packed linear aliphatic chain is about 20 $Å^2$ [14].

In table 3 we have summarized values for the cross-sectional area of various sugar-based surfactants taken from literature. These have mostly rather small hydrophilic groups. In general, the cross-sectional areas found in literature are smaller than the ones for the oligofructose esters in our study. The fact that the esters in this study give higher cross-sectional areas shows that the larger oligofructose part resulted in the occupation of more space on the interface and that the oligofructose part is not fully extended perpendicular to the interface but rather adopts a more tilted orientation.

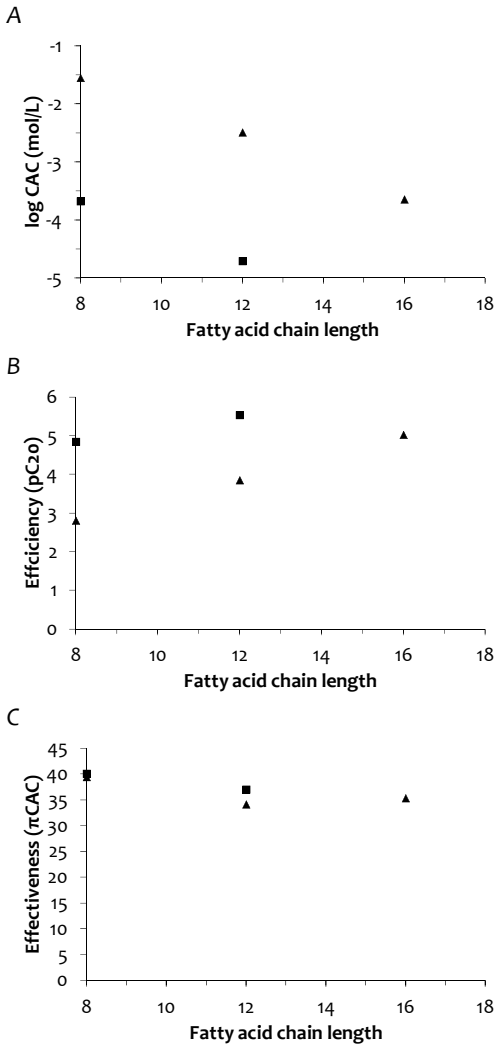

Figure 2: Log CAC (A), efficiency (B) and effectiveness (C) as a function of fatty acid chain length. Mono-esters are indicated with ▲, di-esters with ■.

*Table 3: Comparison of cross-sectional area of oligofructose esters to literature values of the cross-sectional area of sugar-based fatty acid esters. *Most values were between 50 and 65.*

Hydrophilic part	Hydrophobic part	Degree of esterification	Molecular area (Å^2)	Reference
Oligofructose	Caprylic acid	1	61	This study
Oligofructose	Caprylic acid	2	55	This study
Oligofructose	Lauric acid	1	91	This study
Oligofructose	Lauric acid	2	46	This study
Oligofructose	Palmitic acid	1	85	This study
Glucuronic acid	Caprylic acid	1	43	[21]
Fructose and sucrose	Capric acid, myristic acid, palmitic acid, stearic acid	1 or mix 1/2/higher	5-20	[16]
Xylose, galactose, sucrose and lactose	Lauric acid, myristic acid, palmitic acid	1	29-68	[12]
Sucrose	Lauric acid, palmitic acid, stearic acid	Mix 1/2/higher	10-75 (50-65*)	[22]
Lactose and lactitol	Caprylic acid, lauric acid, palmitic acid	1	32-43	[15]
Glucose, sucrose, raffinose and stachyose	Lauric acid	1	36-72	[23]

3.2 Size of aggregates

To determine the presence and size of the aggregates that are formed at bulk concentrations higher than the CAC, light scattering measurements were performed. Depending on the molecular structure, aggregates could be spherical micelles or more complex structures such as vesicles. The size of spherical micelles can be estimated. First, the length of the hydrophobic tail may be calculated using l_c (Å) = 1.5 + 1.265 * N_c, where l_c is the length of the hydrophobic tail and N_c the number of carbon atoms in the fatty acid chain [24]. We have estimated the length of the oligofructose part based on previous work by Lerk et al. [25], who used a length of 11 Å for the disaccharide sucrose. We have assumed that the groups become proportionally bigger with increasing degree of polymerization. This leads to an estimation of micellar size. Since the oligofructose that was used in this study was a mixture of oligomers with different degrees of polymerization, we have made the calculations for the smallest oligomer (DP2), the largest oligomer (DP8), and a theoretical average oligomer with DP 4.4 (table 4). The combination of these three estimations will give an idea of the size range of aggregates that should be found in the case of spherical micelles.

Table 4: Estimation of the size of spherical micelles formed by oligofructose fatty acid esters with fatty acid chain length varying from 8 to 18.

Length of fatty acid chain	Diameter micelle DP2 (nm)	Diameter micelle DP4.4 (nm)	Diameter micelle DP8 (nm)
8	4.3	6.9	10.9
12	5.3	7.9	11.9
16	6.3	8.9	12.9
18	6.8	9.4	13.4

The size distribution that was found using light scattering showed that both oligofructose mono-esters and oligofructose di-esters with fatty acid chain lengths between 8 and 16 carbon atoms formed aggregates that are within the size range of simple micelles (figure 3A and B). One exception were oligofructose mono-esters with a fatty acid chain length of 8, where particles with a very small size were found. Since the measurement was performed at a bulk concentration that was only slightly above CAC, the concentration of micelles may have been too low to detect. For mono-esters with a fatty acid chain length of 18 (figure 3C), larger aggregates with a high degree of polydispersity were found. The multimodal character of the curve is a result of taking the average of different

measurements and does not have a physical meaning. This shows that these esters do not form spherical micelles. This could be attributed to their high hydrophobicity.

To investigate the influence of the size of the hydrophilic part, we have also included sucrose esters (figure 3D). We have studied a purified sucrose mono-ester of lauric acid (S-C12m), and two crude samples of sucrose esters with fatty acid chain length 16 (S-C16) and 18 (S-C18). The micellar size found in this study for S-C12m (4.85 nm) was similar to the results reported by Lerk et al. [25] who found particles with a diameter of 5.84 nm. Both values correspond to spherical micelles. Also for S-C16 and S-C18, the experimental values correspond to the theoretical value for spherical micelles. Small deviations may be due to the fact that these two products are crude samples that contain different fatty acid chain lengths and different degrees of substitution, which affect the dimensions of the micelle.

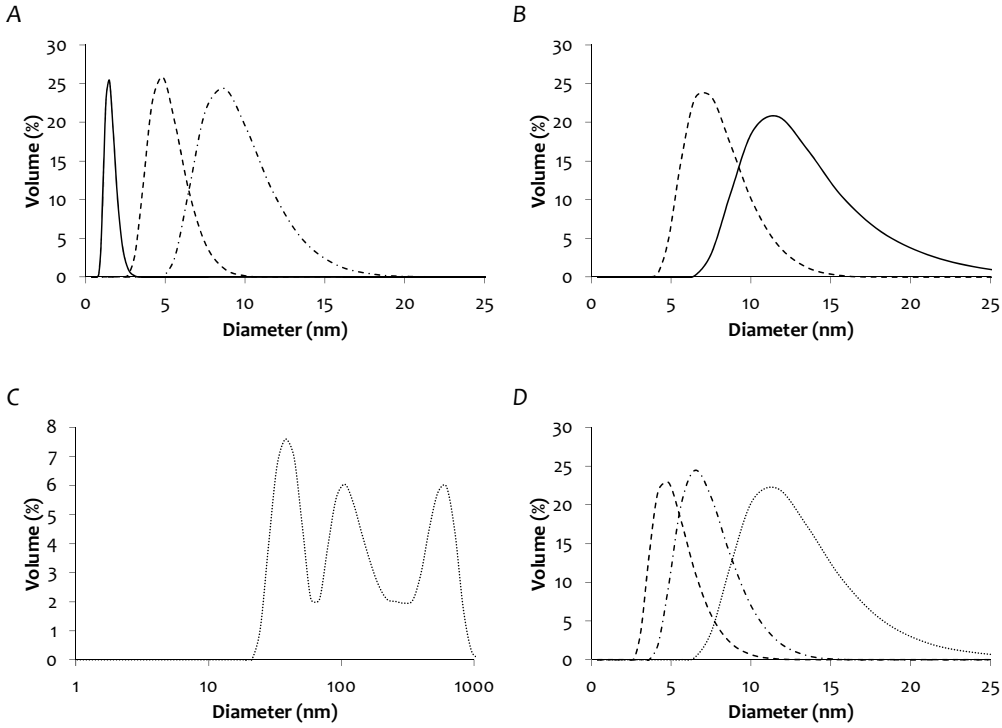

Figure 3: Size distribution of particles found in solutions of sugar esters in water at concentrations higher than the CAC. A) Oligofructose mono-esters of C8 (—), C12 (- -) and C16 (- ·). B) Oligofructose di-esters of C8 (—) and C12 (- -). C) Oligofructose mono-esters of C18(·····). D) Sucrose esters of C12 (- -), C16 (- ·) and C18 (·····).

3.3 Amount of material adsorbed at interface

To determine the amount of material that adsorbed at the interface, ellipsometry experiments were performed. From the adsorbed amount we have calculated the molecular area. Oligofructose mono-esters with different fatty acid chain lengths all had a similar area per molecule (between 76 and 94 Å^2, table 5). To verify whether the area occupied by a single molecule was determined by the size of the hydrophilic group, we have also analyzed the value for sucrose esters. Sucrose esters showed a lower area per molecule (between 56 and 58 Å^2) than oligofructose esters. The values for sucrose esters are similar to literature values for sucrose esters that are between 47 and 57 Å^2 [12, 23]. The more dense packing for sucrose esters compared to oligofructose esters may indeed be attributed to the size of the hydrophilic group. It shows that an ester with oligofructose as the hydrophilic part occupies significantly more space than an ester with sucrose as the hydrophilic part. If the oligofructose part is indeed dominant, an increase in the degree of saturation of the fatty acid tail should not make a difference to the amount of area occupied by the molecule. Therefore, the amount of material that adsorbed for an oligofructose mono-ester with a mono-unsaturated fatty acid chain with a length of 16 (OF-C16:1m) was also determined. There were no major differences in the area per molecule between the saturated (86 Å^2) and unsaturated ester (81 Å^2). Since unsaturated fatty acids contain a double bond in the molecule and are therefore not straight, the fatty acid tail should occupy a larger interfacial area than a saturated fatty acid tail. Apparently, the interfacial area of the oligofructose part is still larger than the interfacial area of the unsaturated fatty acid. When comparing di-esters to mono-esters, we find a less dense packing at the interface. An oligofructose fatty acid di-ester with chain length 12, for instance occupies an area of 113 Å^2, while an oligofructose mono-esters occupies 94 Å^2. However, the difference between oligofructose mono-esters and di-esters is less pronounced than the difference between sucrose esters and oligofructose mono-esters. A sucrose ester with chain length C12, for example, occupies an area of 58 Å^2. Two fatty acid tails apparently occupy slightly more space than the oligofructose part. Although the values for the molecular area that are found using ellipsometry seem to be slightly larger than the ones obtained from the CAC curves, these results support the conclusion already drawn from surface tension experiments that the interfacial area occupied by a molecule is mainly determined by the size of the oligofructose part.

Table 5: Amount of material adsorbed at the interface and molecular area per molecule for oligofructose and sucrose fatty acid esters with variations in molecular structure, calculated from ellipsometrical angles Ψ and Δ.

Sample	Type	Adsorbed amount * 10^{-6} (mol/m^2)			Molecular area (Å^2)		
OF-C8d	Oligofructose di-ester	1.22	±	0.05	137	±	5.7
OF-C12d	Oligofructose di-ester	1.47	±	0.11	113	±	8.4
OF-C12m	Oligofructose mono-ester	1.77	±	0.04	94	±	2.0
OF-C16m	Oligofructose mono-ester	1.93	±	0.07	86	±	3.3
OF-C16:1m	Oligofructose mono-ester	2.05	±	0.07	81	±	2.6
OF-C18m	Oligofructose mono-ester	2.23	±	0.35	76	±	12.1
S-C12m	Sucrose ester	2.86	±	0.00	58	±	0.0
S-C16	Sucrose ester	2.90	±	0.02	58	±	0.4
S-C18	Sucrose ester	2.99	±	0.06	56	±	1.2

4. Conclusions

In this chapter, the effect of variations in chemical fine structure on functional properties of oligofructose fatty acid esters was examined. The critical aggregation concentration of the esters was dependent on the balance between hydrophilicity and hydrophobicity of the molecule: more hydrophobic components had a lower CAC. Light scattering measurements showed that, with the exception of oligofructose mono-esters with a fatty acid chain length of 18 carbon atoms, all surfactants formed spherical micelles beyond the CAC. Therefore, the term critical micelle concentration (CMC) is justified for these components. The efficiency of the surfactants increased with increasing hydrophobicity, making especially the derivatives of longer fatty acids interesting as potential food-grade surfactants. The effectiveness of the surfactants was independent of the molecular structure. The amount of material adsorbed at the interface and area occupied by one molecule were determined by fitting the CAC curves with the Gibbs adsorption model and measured directly by ellipsometry. In both cases, we found a similar value for the molecular area per molecule for all oligofructose esters. We found no difference between esters with a saturated and an unsaturated chain. Furthermore, we found a higher area per molecule for oligofructose esters compared to sucrose esters. Therefore, we conclude that the amount of area occupied by a molecule is dominated by the oligofructose part. As we will demonstrate in chapter 4, this has significant consequences for the rheological behavior of interfaces stabilized by oligofructose esters.

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Chapter 4

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Non-linear surface dilatational rheology as a tool for understanding microstructures of air/water interfaces stabilized by oligofructose fatty acid esters

Abstract

In this chapter, the rheological response of air/water interfaces, stabilized by various oligofructose fatty acid esters, to oscillatory dilatational deformations was studied and compared to the response of interfaces stabilized by sucrose esters. We have followed a traditional approach to surface rheology, where the development of the modulus as a function of time is studied as well as the frequency dependence of the modulus. We also adopted a different approach where we investigate in detail the amplitude dependence of the modulus. Finally, we studied the temperature dependence. We show that for an accurate characterization of the dilatational rheology of fluid-fluid interfaces with a complex microstructure, a protocol should be used that involves not only variations of surface pressure, frequency, and temperature, but that also establishes amplitude dependence. We show that Lissajous plots of surface pressure versus deformation can be a useful tool to help interpret surface dilatational behavior in terms of interfacial microstructure. The rheological response of interfaces stabilized by oligofructose esters differed significantly from the response of those stabilized by sucrose esters. Sucrose esters behaved like typical low molecular weight surfactants, and gave interfaces with relatively low moduli, a frequency scaling of the dilatational modulus with an exponent close to 0.5, and displayed no asymmetries in Lissajous plots. In contrast, the oligofructose esters gave, dependent on the fatty acid tail, relatively high moduli, almost independent of frequency. Significant asymmetries were observed in the Lissajous plots, with strain hardening during compression and strain softening during extension. Our results suggest that the unusual rheological properties of interfaces stabilized by oligofructose esters may be the result of the formation of a two-dimensional soft glass phase by the oligofructose part of the ester.

1. Introduction

Many food products or parts of food products consist of foams [1], such as the head of beer, whipped cream, mousses, cappuccino foam, and bread [2]. To improve the stability of foams, surface active molecules are added to foam formulations. These molecules adsorb at the air/water interface where they significantly alter the interfacial properties. Although the relation between interfacial properties and macroscopic properties such as foam stabilizing capabilities is still an active area of research, most researchers agree that the study of interfacial rheology is relevant with respect to foaming properties [3-5].

For low molecular weight (LMW) surfactants, the dilatational rheological response of the interface at low surface concentrations will be mostly influenced by transport of monomers from the bulk to the interface [5]. Above the critical micelle concentration, the presence of micelles in the subphase complicates matters, since now, in addition to the transport of monomers to and from the interface, one has to take into account the transition from micelle to monomer [6]. With increasing surface concentration of the surfactant, it becomes more likely that a high modulus is caused by structure formation at the interface. As already demonstrated by Rodríguez Patino et al. [7, 8], some surfactants can exhibit rich phase behavior with moduli that are strongly dependent on the degree of surface coverage and the microstructure of the surface layer.

The dilatational rheological response of an interface will depend on the ratio of the characteristic time scale that is related to the transport of molecules from bulk to interface and the characteristic time scale related to the rate of interfacial deformation. Therefore, frequency sweeps are often used to characterize the rheological response of interfacial layers in more detail. The slope in a double logarithmic plot of modulus versus frequency can provide useful information about the interfacial kinetics. Two specific cases in the response are a slope of 0 and a slope of 0.5. A slope of 0 implies a completely elastic interface and is often found at very high frequencies, where the deformation is so fast that diffusion from the bulk to the interface is negligible. In contrast, a slope of 0.5 corresponds to processes that are completely diffusion controlled, as explained in the Lucassen van den Tempel model [9], a value which is often found at very low frequencies [10].

While frequency sweeps are often performed during the study of interfacial dilatational rheology of surfactant stabilized interfaces, amplitude sweeps are much less common. Most authors do not investigate any possible dependence of rheological properties on the magnitude of the deformation, and simply assume that they are measuring within the linear viscoelastic regime, without providing the experimental data to

support their assumption [11]. These amplitude sweeps are considered standard practice in bulk rheology to establish whether experiments are performed in the linear viscoelastic regime. When the dilatational modulus is a result of structure formation at the interface, the interfacial microstructure may be affected by the applied deformation, and we would expect a dependence of the rheological properties on amplitude. To address this issue, in this chapter we performed a detailed analysis of the amplitude dependence of the surface dilatational modulus.

Oligofructose fatty acid esters have been described recently as molecules that provide air/water interfaces with relatively high surface dilatational moduli, and that have excellent foam stabilizing capabilities [12], [chapter 2]. They are surface active components that can be synthesized with a wide range of amphiphilicity and thus functionality. This wide range is accomplished by changing the fatty acid chain length and the degree of esterification. The oligofructose that was used was a mixture of oligomers with a degree of polymerization varying between 2 and 8 (average 4.4). Consequently, the resulting esters are also mixtures. In chapter 2, we have reported the equilibrium surface tension and surface dilatational modulus of air/water interfaces stabilized by oligofructose fatty acid mono-esters with different fatty acid chain lengths. We have shown that interfaces stabilized by oligofructose esters containing longer fatty acid chains had a significantly higher dilatational modulus than those stabilized by esters containing shorter fatty acid chains. Furthermore, we observed that these high dilatational moduli were accompanied by extremely high standard deviations. We attributed these extremely high standard deviations to the fact that measurements may have been taken outside of the linear viscoelastic regime.

The purpose of this chapter was to study the dilatational rheological properties of air/water interfaces stabilized by oligofructose fatty acid esters with variations in the fatty acid chain, as a function of the surface pressure, frequency, deformation amplitude, and temperature. To study the influence of the size of the hydrophilic group, also sucrose esters were studied. First, we adopted a traditional approach to surface rheology. In section 3.1 we focus on the development of the modulus during time sweeps. We have also studied the surface rheological properties of the interfaces after reaching equilibrium. We performed frequency sweeps, which are discussed in section 3.2. Next, we adopted a different approach, where we studied in detail the amplitude dependence of the modulus, which is discussed in section 3.3. Finally, for some of the samples we have performed temperature sweeps, which are discussed in section 3.4.

2. Materials and methods

2.1 Materials

Palmitoleic acid was obtained from Sigma Aldrich (Steinheim, Germany). Oligofructose fatty acid esters were prepared according to a previously described protocol [chapter 2], by esterification of fatty acids with oligofructose with a degree of polymerization ranging from 2 to 8, using lipase as a catalyst. Four different fatty acid chain lengths were used (C8, C12, C16 and C18). The crude products contained mostly mono-esters, but also small amounts of di-esters. The products were fractionated to separate the fractions with different degrees of esterification. Using MALDI-TOF MS and NMR we established the purity (>90% for mono-esters, >80% for di-esters) of the purified fractions [chapter 2]. Additionally, an ester with palmitoleic acid (C16:1) as the hydrophobic group was produced using the procedure as previously described for palmitic acid esters [chapter 2]. Using MALDI-TOF MS [chapter 2] a purity of 100% was established.

Sucrose monolaurate (≥97%) was obtained from Sigma Aldrich (Steinheim, Germany). Sucrose esters with fatty acid chain lengths C16 and C18 (RYOTO esters S1670 and P1670) were obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Both S1670 and P1670 are crude products. According to the manufacturer, S1670 consist of 75% mono-esters and 25% di-, tri-, and poly-esters. The fatty acids consist for 70% of stearic acid. P1670 consists of 80% mono-esters and 20% di-, tri-, and poly-esters. The fatty acids consist for 80% of palmitic acid. These products were used without further purification.

2.2 Methods

2.2.1 Sample preparation

Samples were prepared by dissolving the surfactant in Millipore water (Millipore corporation, Billerica, Massachusetts, USA). For esters with longer fatty acid chains (C16 and C18) mild heating (max. 70 °C) was applied to improve the dispersing of the esters in the liquid.

2.2.2 Determination of surface tension and surface dilatational modulus

The surface tension and surface dilatational modulus of the air/water interface were determined with a profile analysis tensiometer (Sinterface, Berlin, Germany). During time sweeps, sinusoidal oscillations with an amplitude of 5% and a frequency of 0.1 Hz were applied. The experiments consisted of 25 cycles of oscillations that were alternated with 1 cycle of rest. These experiments were continued until equilibrium was reached. The time sweeps were followed by a frequency sweep where the frequency was increased stepwise

from 0.005 Hz to 1 Hz at an amplitude of 5%. The slope of a double logarithmic plot of surface dilatational modulus versus frequency was determined using linear regression. The quality of the linear regression fit was generally very high. At the highest frequencies, the fit used to establish the first-harmonic Fourier moduli was not always of sufficient quality, which reduced the accuracy of the values for the moduli. Therefore, these values were excluded from the regression fit. The experimental series was finished with an amplitude sweep, where the amplitude was increased stepwise from 1.5% to 30% at a frequency of 0.1 Hz. All experiments were performed at 25 °C.

For the temperature sweeps an automated drop tensiometer was used (ITCONCEPT, Longessaigne, France). The reported temperature is the temperature of the cuvette holder and may slightly differ from the actual sample temperature. Similar to the experiments performed with the profile analysis tensiometer, an amplitude of 5% and a frequency 0.1 Hz were used.

2.2.3 Analysis of amplitude sweeps

In a paper by Ewoldt et al. [13], these authors have demonstrated the limitations of using first-harmonic Fourier moduli, which are the common output of rheometers. The profile analysis tensiometer that was used in our study also reports first-harmonic Fourier moduli, and particularly for large deformations these dilatational moduli will have limited physical meaning.

For oscillatory shear experiments on bulk fluids Ewoldt et al. [13] have shown how the raw signal of a rheometer, expressed as a Lissajous plot of stress versus strain, may be analyzed to reveal the presence of intracycle strain thinning and thickening. They have defined a minimum strain shear modulus (G_M), a large strain shear modulus (G_L) and a strain-stiffening ratio S to quantify the degree of non-linearity:

$$S \equiv \frac{G_L - G_M}{G_L} \qquad (1)$$

Here S=0 may be interpreted as a linear elastic response, S>0 indicates intracycle strain stiffening, and S<0 corresponds to intracycle strain softening.

In this chapter, we have extended this approach to analyze surface dilatational oscillatory experiments. We have used the output of the profile analysis tensiometer to construct Lissajous plots, of the surface pressure ($\Pi=\gamma-\gamma_o$) versus deformation ($\delta A/A_o$). Here $\delta A=A-A_o$, γ and A are the surface tension and area of the deformed interface, and γ_o and A_o

are the surface tension and area of the non-deformed interface. An example of these plots is shown in figure 1.

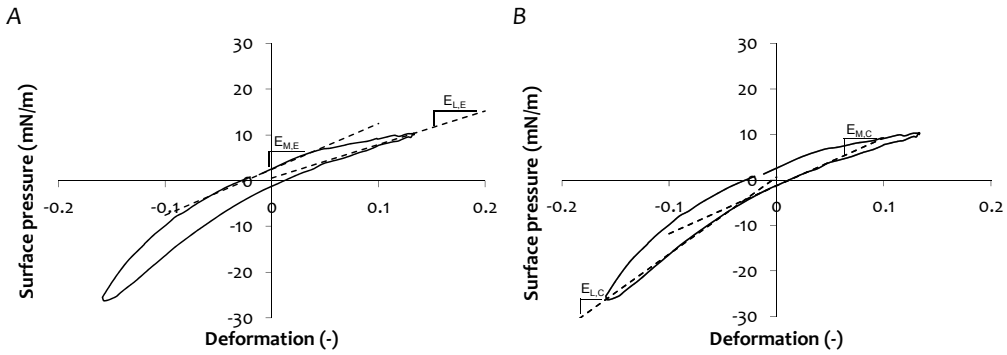


Figure 1: Determination of the minimum strain modulus and the large strain modulus in extension (A) and compression (B).

In most cases, we found a different response in compression than in extension. Therefore, we defined the following factors in the analysis of the curves in this chapter: $E_{L,E}$, defined as the large strain modulus in extension, $E_{M,E}$, defined as the minimum strain modulus in extension, $E_{L,C}$, defined as the large strain modulus in compression, and $E_{M,C}$, defined as the minimum strain modulus in compression. In figure 1 the method for determining these factors is demonstrated. Furthermore, we have defined two strain-stiffening ratios (S), S_{ext} and S_{com}:

$$S_{ext} \equiv \frac{E_{L,E}-E_{M,E}}{E_{L,E}} \quad (2)$$

$$S_{com} \equiv \frac{E_{L,C}-E_{M,C}}{E_{L,C}} \quad (3)$$

With these factors, it is possible to quantify the degree of non-linearity and distinguish between the response in compression and extension. For both factors, S=0 may be interpreted as a linear elastic response, S>0 indicates intracycle strain stiffening, and S<0 corresponds to intracycle strain softening.

3. Results & discussion

In this study, the dilatational rheological properties of air/water interfaces stabilized by oligofructose fatty acid esters were investigated. Due to the expected impact on functionality, we have studied the following variations in molecular structure: length of the fatty acid chain, degree of esterification, degree of saturation of the fatty acid chain and size of the hydrophilic group. To study the influence of the length of the fatty acid chain, oligofructose mono-esters containing saturated fatty acids with chain lengths of 8, 12, 16 and 18 carbon atoms, designated as OF-C8m, OF-C12m, OF-C16m and OF-C18m, were studied. To study the influence of the degree of esterification, in addition to mono-esters, also di-esters with fatty acid chain lengths 8 and 12, designated OF-C8d and OF-C12d, were investigated. To study the influence of the degree of saturation of the fatty acid chain, in addition to OF-C16m, an oligofructose ester with the mono-unsaturated fatty acid cis-9-hexadecenoic acid as the hydrophobic group, designated OF-C16:1m, was studied. To study the influence of the size of the hydrophilic group, sucrose esters were also included. These were a mono-ester with fatty acid chain length C12, designated as S-C12m, and two crude samples composed of a mixture of mono-esters and di-esters with fatty acid chain length C16 and C18, designated as S-C16 and S-C18. First, we follow a traditional approach where we treat the development of the surface dilatational modulus as a function of the surface pressure (section 3.1) during time sweeps and the frequency dependence of the dilatational modulus of the interfaces after reaching equilibrium (section 3.2). Next, we adopt a different approach and present a detailed study of the dependence of the moduli on the amplitude (section 3.3). Also, we focus on the temperature dependence (section 3.4) of the moduli.

3.1 Traditional approach to surface rheology: development of the modulus during time sweeps

To establish the dependence of the surface dilatational modulus on surface pressure, a range of time sweeps was performed using samples with a wide range of bulk concentrations of the ester, to cover a wide range of surface pressures. In figure 2 the results of these experiments are shown for the oligofructose (saturated) mono-esters and di-esters. In chapter 3 we have measured surface tension as a function of bulk concentration of these esters, and reported their critical micelle concentration (CMC). The surface pressure at these CMC values (π_{CMC}) differed slightly as a function of the molecular structure of the ester, and is indicated in figure 2.

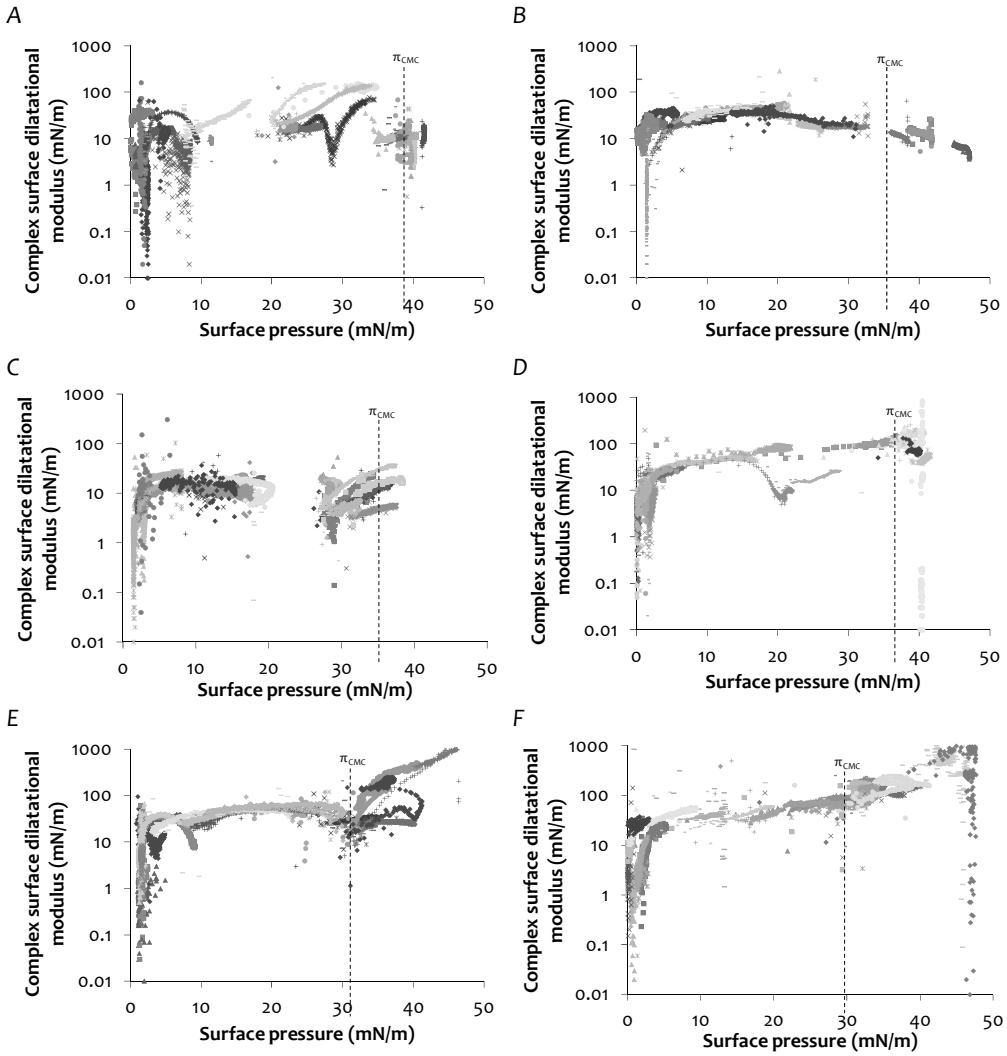


Figure 2: Surface dilatational modulus as a function of surface pressure obtained during time sweeps of air/water interfaces stabilized by oligofructose fatty acid esters: OF-C8m (A), OF-C8d (B), OF-C12m (C), OF-C12d (D), OF-C16m (E) and OF-C18m (F). Different symbols represent individual measurements on samples with varying bulk concentrations. The surface pressure at the CMC is indicated with a dashed line.

For all samples, a sharp increase in the surface dilatational modulus is observed at very low surface pressures (0-3 mN/m). In some cases there is a high degree of variability in

the values for the surface dilatational modulus at similar surface pressures. It seems, however, that after the initial increase in the surface dilatational modulus, a (semi)plateau value is reached over a wide range of surface pressures. The plateau value of the surface dilatational modulus is dependent on the molecular structure: with increasing fatty acid chain length or degree of esterification, the plateau value increases. For example, the plateau value obtained for OF-C12m (figure 2C) was around 20 mN/m, while for OF-C18m (figure 2F) it was around 60 mN/m. At surface pressures lower than π_{CMC}, there are no micelles present in the bulk. This means that during compression and extension, only single molecules will exchange between bulk and interface. Since an increasing fatty acid chain length or degree of esterification will increase the hydrophobicity of the molecule, the affinity of these molecules for the interface will also increase. This means that exchange between bulk and interface is less likely to occur, leading to a higher value for the dilatational modulus.

With increasing surface pressure, the degree of surface coverage is increased. This means that interactions between the molecules become more and more important. When the degree of surface coverage is sufficiently high, and there are sufficiently strong interactions between the molecules, it is possible that interfacial mesophases are formed. We hypothesize that this could be a glass phase formed by the oligofructose part of the esters or a crystalline phase formed by the fatty acid chains. The dilatational modulus is then no longer exclusively determined by exchange of the esters with the subphase, but also by in-plane interactions (deviatoric stresses and/or bending stresses).

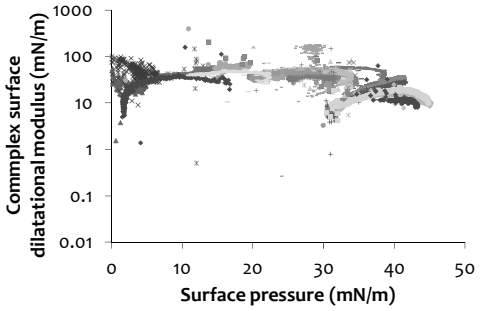


Figure 3: Surface dilatational modulus as a function of surface pressure obtained during time sweeps of air/water interfaces stabilized by OF-C16:1m.

The influence of the degree of saturation of the fatty acid chain on the rheological response was investigated by studying the surface dilatational modulus as a function of the

surface pressure for OF-C16:1m (figure 3). The shape of the curve is similar to the curves in figure 2. Also here a plateau value for the surface dilatational modulus was obtained. The plateau value of the surface dilatational modulus obtained for OF-C16:1m was comparable to the one obtained for OF-C16m.

The curves in figures 2 and 3 also present some curious features that are more difficult to explain. As already mentioned, in some cases there is a high degree of variation in the surface dilatational modulus at similar surface pressures. This degree of variation is quite pronounced in the intermediate surface pressure regime of OF-C8m (figure 2A) and the high surface pressure regime of OF-C16m (figure 2E). A possible explanation for the variations may be that the deformation amplitude used in these experiments was above the maximum linear strain, and therefore the measurements were not in the linear response regime. We will say more about this issue in section 3.3.

Furthermore, in some cases the surface dilatational modulus starts to deviate from the plateau value at surface pressures higher than π_{CMC}. For example, in the case of OF-C8d (figure 2B) the modulus decreases at $\pi > \pi_{CMC}$, while in the case of OF-C18m (figure 2F) the modulus increases at $\pi > \pi_{CMC}$. Possibly, the surface coverage may have reached a level were mesophase formation is occurring.

In addition to the oligofructose esters, the surface dilatational modulus of sucrose esters with chain lengths C12, C16 and C18 was studied. For all three sucrose esters, the modulus was determined at a fixed bulk concentration of 0.2% (w/v), higher than the CMC. For an interface stabilized by S-C12m, we have found a low surface dilatational modulus of 3.4 mN/m. For sucrose esters with chain lengths C16 and C18 we found higher dilatational moduli of 15.0 mN/m and 29.4 mN/m, respectively. The higher modulus for the more hydrophobic sucrose esters may be explained using a similar reasoning as for the oligofructose esters: with increasing hydrophobicity the affinity for the interface increases, and the exchange with the subphase is slower.

Summarizing, the dependence of the surface dilatational modulus on surface pressure of interfaces stabilized by oligofructose esters is quite difficult to interpret due to the high amount of variability in the data.

Table 1: Surface dilatational modulus and according slope of a double logarithmic plot of surface dilatational modulus versus frequency determined for air/water interfaces stabilized by sucrose esters with chain lengths 12, 16 and 18 determined at a bulk concentration of 0.2% (w/v).

Ester	Surface dilatational modulus (mN/m)			Slope		
S-C12m	3.4	±	1.0	0.26	±	0.042
S-C16	15.0	±	1.3	0.53	±	0.019
S-C18	29.4	±	4.0	0.44	±	0.014

3.2 Traditional approach to surface rheology: dependence of the modulus on frequency

To gain more information on the rheological properties of the interfaces stabilized by different esters, frequency sweeps were performed after reaching equilibrium. The slope of a double logarithmic plot of surface dilatational modulus as a function of frequency was determined. These slopes are plotted as a function of the surface pressure in figure 4.

At surface pressures lower than π_{CMC}, for all esters the slope of the curve is low. A slope that approaches zero is an indication of a purely elastic interface. It shows that the migration of the esters from the bulk to the interface and vice versa upon compression and extension is very slow and that at this time scale no dissipative processes could be measured. This slow migration is the result of the relatively low concentration of surfactant in the subphase.

There is a quite a bit of variation in the slope, especially at surface pressures higher than π_{CMC}. At similar surface pressures, different values in the slope are found for the different esters. Also in this case, the high degree of variability complicates the interpretation of the data. For OF-C16m (figure 4E), for example, at a surface pressure of 35 mN/m the slope varies between 0.12 and 0.3. In some cases, even negative values for the slopes were found. We will elaborate on these negative values in the next section. Despite these variations, for all mono-esters (figures 4A, 4C, 4E and 4F) the slopes remain relatively low at high surface pressures, and the rheological response appears not to be diffusion-controlled. This could be indicative for the presence of interfacial mesophases, such as a soft glass phase formed by the oligofructose part, or a crystalline phase formed by the fatty acid chains. Such interfacial structures would be quite brittle, meaning that they are very sensitive to structure deformations, and could fracture or yield when the amplitude of deformation is too high. This could also explain the high variability in the slopes. Only in the case of OF-C8d (figure 4B) and OF-C12d (figure 4D) the slopes approach 0.5 at high surface pressures. These esters seem to behave like typical low molecular weight (LMW)

surfactants, where the surface processes are diffusion-controlled, and adequately described by the Lucassen van den Tempel model [9].

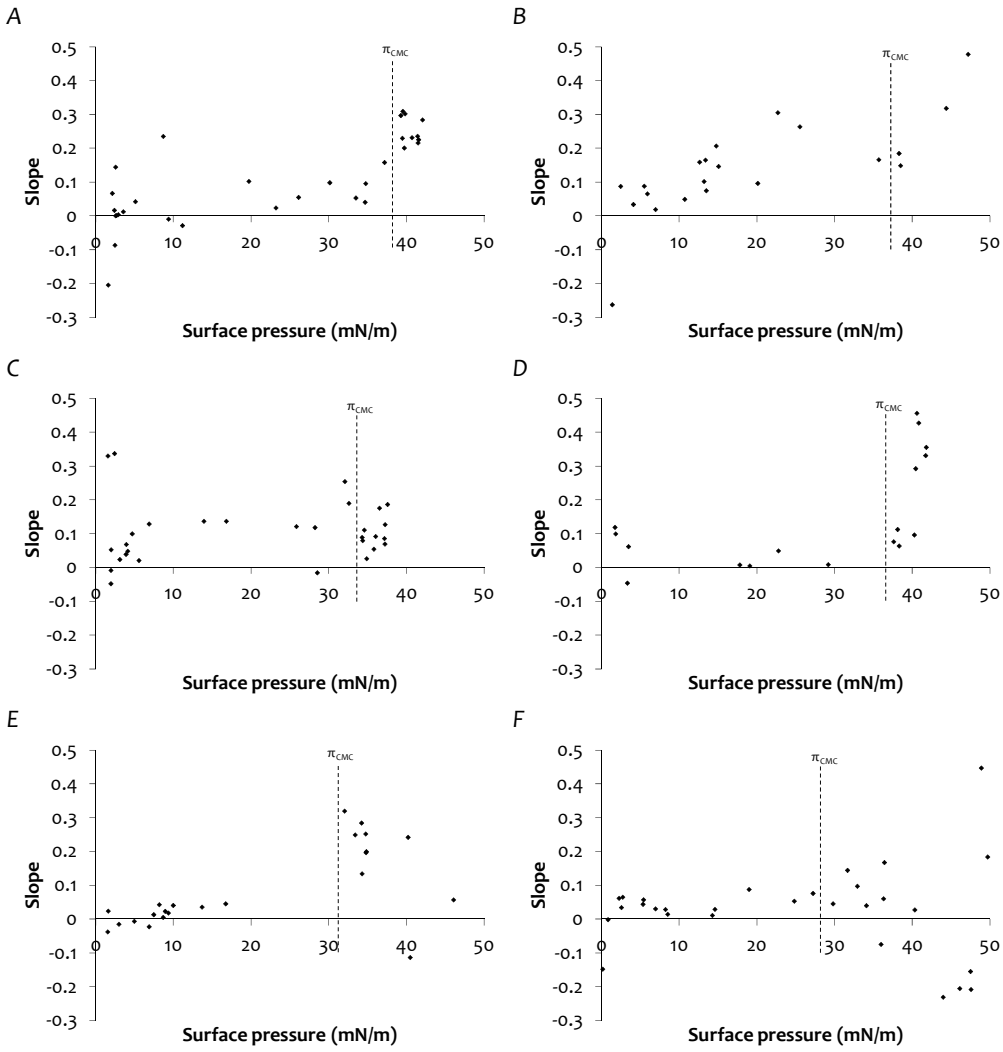


Figure 4: Slope of a double logarithmic plot of surface dilatational modulus as a function of frequency obtained for air/water interfaces stabilized by oligofructose fatty acid esters. OF-C8m (A), OF-C8d (B), OF-C12m (C), OF-C12d (D), OF-C16m (E) and OF-C18m (F). The surface pressure at the CMC is indicated with a dashed line.

In the previous chapter [chapter 3], we have reported the interfacial area per molecule occupied by the oligofructose esters. We found that both mono-esters (67-94 Å^2) and di-esters (113-137 Å^2) occupy a significantly larger area than a single fatty acid chain (approximately 20Å^2). The larger surface area per molecule is the result of the size of the oligofructose part of the molecule. Only when fatty acid chains are able to approach each other closely, will interactions between them lead to an interface with a high dilatational modulus and a low frequency dependence. Therefore, it is unlikely that the rheological properties that were found are the result of interactions between the fatty acid chains, for example crystallization. It is more likely that interactions between the oligofructose part are responsible for the rheological response, for example by the formation of a soft interfacial glass phase. The higher degree of polymerization of the oligofructose part compared to the sucrose part causes a shift in the glass transition, which may explain why the glass phase is formed for the oligofructose ester but not for the sucrose ester. Because di-esters occupy a slightly larger area than mono-esters, we hypothesize that two fatty acid tails might occupy slightly more interfacial area than the oligofructose part (figure 5). This could explain why, for di-esters, a slope of 0.5 is approached at high surface pressures: the oligofructose part cannot approach close enough to form a soft glass phase due to the presence of the two fatty acid chains.

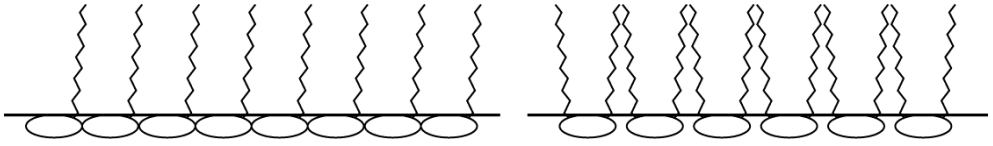


Figure 5:Schematic representation of proposed interfacial structure of oligofructose fatty acid esters. The oligofructose part is a mixture of oligomers with different degrees of polymerization between 2 and 8. Left: mono-esters, where the oligofructose part can approach closely and form a two-dimensional glass phase. Right: di-esters, where the incorporation of the second fatty acid chain prevents close approach of the oligofructose part and glass phase formation.

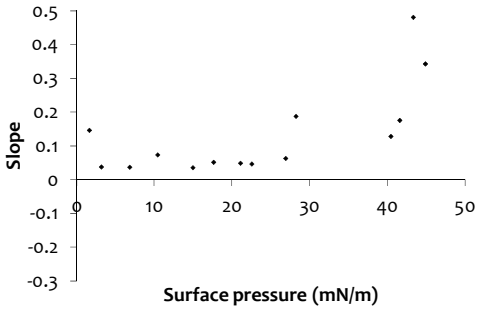


Figure 6: Slope of a double logarithmic plot of surface dilatational modulus as a function of frequency obtained for air/water interfaces stabilized by OF-C16:1m.

The frequency dependence of OF-C16:1m (figure 6) was comparable to the frequency dependence of OF-C16m (figure 4E), with low slopes at low surface pressures and a high degree of variability at higher surface pressures caused by the high brittleness of the interfacial structure.

If the oligofructose part indeed forms a soft interfacial glass phase, the rheological properties of air/water interfaces stabilized by esters with a smaller hydrophilic group should be different. Therefore, we have studied the frequency dependence of the modulus of an air/water interface stabilized by sucrose esters. For S-C12m we found a slope of 0.26 (table 1). For the other sucrose esters, we found slopes close to 0.5 (0.53 for S-C16 and 0.44 for S-C18), close to the frequency dependence predicted by the Lucassen van den Tempel model [9]. This would indicate that the surface processes are diffusion-controlled and that the higher modulus that is found for S-C16 and S-C18 compared to S-C12m is a result of slower exchange of molecules between bulk and interface. The slower exchange is caused by an increased hydrophobicity, which is caused by a longer fatty acid chain length and the presence of di-esters. This shows that the rheological response of sucrose esters is typical for LMW surfactants.

3.3 Detailed study of the amplitude dependence of the modulus

In sections 3.1 and 3.2, a traditional approach to surface rheology was followed. Some conclusions could be drawn based on the results that were obtained. However, the high degree of variability in the data that is presented in figures 2, 3, 4 and 6 complicates the interpretation of the data. We hypothesized that these variations are caused by the brittleness of the structures that are present at the interface. Therefore, in this section we will focus on the amplitude dependence of the modulus.

First-harmonic Fourier moduli

While amplitude sweeps are considered common practice in bulk rheology, in interfacial rheology they are far less commonly used. However, it is to be expected that any structure that is present on an interface (for example a two-dimensional network formed by a protein, or in our case a soft glass phase formed by oligofructose part of the ester) will be affected when the deformation amplitude becomes too high. Especially for structures that are expected to be brittle, it is essential to know if the deformation amplitude that is applied is within the linear viscoelastic regime.

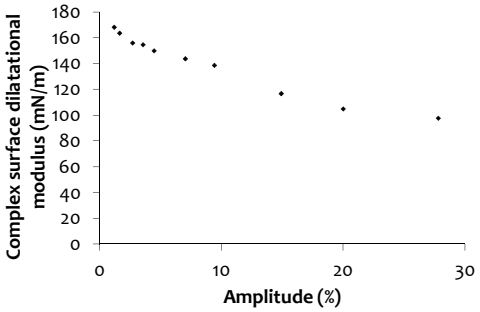


Figure 7: Surface dilatational modulus as a function of amplitude for an air/water interface stabilized by OF-C18m at a bulk concentration of 0.05%.

To find the dependence of the modulus on the deformation amplitude, an amplitude sweep was performed where, after obtaining equilibrium, the amplitude was increased from the lowest amplitude that could be properly executed by the equipment (1.5%) to a very high value (30%). Figure 7 shows an example of a typical curve of surface dilatational modulus versus amplitude obtained for air/water interfaces stabilized by OF-C18m at surface pressures higher than π_{CMC}. The results show that the surface dilatational modulus is strongly dependent on the applied amplitude. It continuously decreases as the amplitude increases. This may show that the interfacial structure is affected by the imposed deformation. Furthermore, it is obvious from these data that even the smallest amplitude that could be applied fell outside of the linear viscoelastic regime. The fact that the measurements were performed outside of the linear regime may explain the negative values for the scaling exponent in the frequency dependence of the modulus (section 3.2); during the frequency sweeps the structure was affected by the applied deformation. These results clearly indicate the importance of understanding the impact of the magnitude of

deformations on the interfacial structure and are indicative of the presence of a brittle structure at the interface.

Lissajous plots

There are certain disadvantages to using the first-harmonic Fourier moduli that are the output of the equipment. As pointed out by Ewoldt et al. [13], by using these first-harmonic Fourier moduli any nonlinearities that could be present in the raw signal are disregarded. Therefore, in this chapter the results of the amplitude sweeps are presented as Lissajous plots (figure 8). The shape of the plots strongly depends on the surface pressure. Figure 8 shows the Lissajous plots as a function of surface pressure for all oligofructose (saturated) mono-esters and di-esters. We have depicted the most common response over a certain range of surface pressures. In some cases, however, there were minor deviations.

For all esters, at extremely low surface pressures of 0-3 mN/m, the curves show only noise, which is caused by a too low surface concentration of esters.

In the range of surface pressures between 3 and π_{CMC} the response is, for all esters, predominantly elastic. These findings are consistent with the findings in figure 4, where a low frequency dependence of the modulus was found, indicating that the migration of the esters from the interface to the bulk and back again into the interface upon compression and extension was slow. Furthermore, in extension, the slope of the curves is decreasing with increasing amplitude, which points to strain softening. In contrast, in compression, the slope of the curves is increasing with increasing amplitudes, which points to strain hardening. The degree of non-linearity becomes more pronounced with increasing deformation amplitude which shows that, even at low equilibrium surface concentrations, the surface may still become concentrated at high compressions.

At surface pressures higher than π_{CMC}, the degree of variability in the shape of the plots slightly increased. For OF-C8d and OF-C12d with increasing surface pressure the shape of the curves changes from mostly elastic with strain hardening in compression and strain softening in extension, to more viscous without asymmetries. This is consistent with the frequency sweeps, where scaling exponents close to 0.5 were found at high surface pressures. For the oligofructose mono-esters, in most cases a response with strain hardening during compression and strain softening during extension was found. Combined with the low frequency dependence, this is consistent with the formation of a soft glass phase by the oligofructose part.

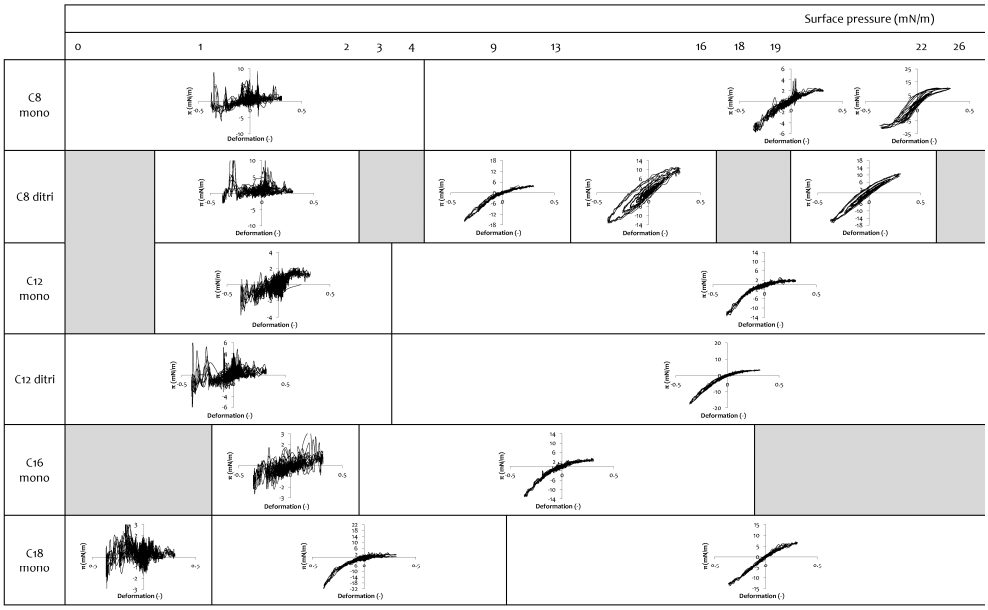


Figure 8: Typical Lissajous plots at different surface pressure ranges obtained during amplitude $\pi < \pi_{CMC}$ to $\pi > \pi_{CMC}$ is clearly marked with a triple bold line.

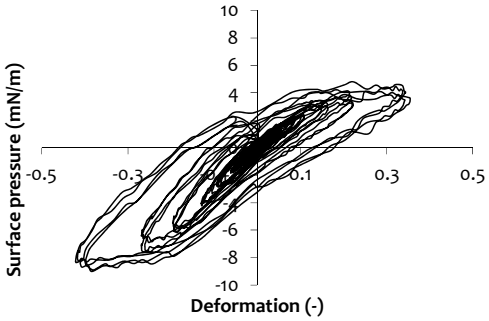
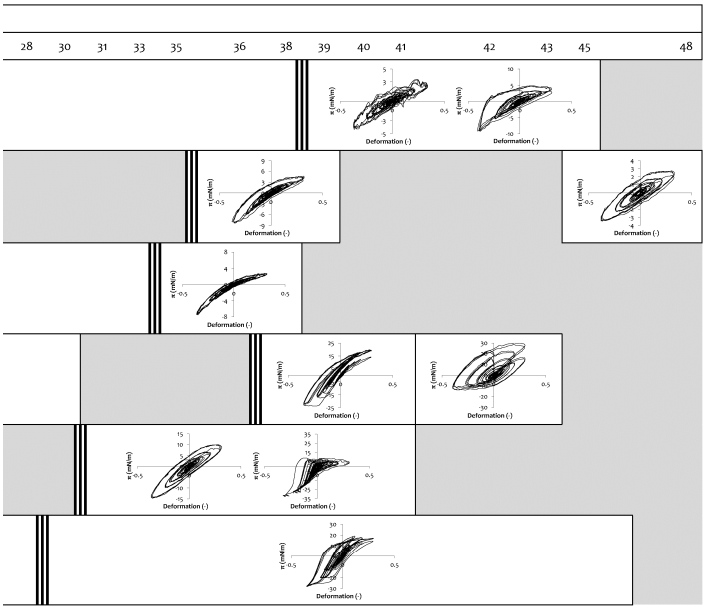


Figure 9: Lissajous plot obtained during amplitude sweep of an air/water interface stabilized by OF-C16:1m.

The shape of the Lissajous plot of OF-C16:1m (figure 9, obtained at high surface pressure) was similar to the one obtained for OF-C16m (figure 8) with strain hardening during compression and strain softening during extension. With increasing amplitude the response became more viscous. The shape of the curves of surface dilatational modulus



sweeps of air/water interfaces stabilized by oligofructose fatty acid esters. The transition from

versus surface pressure (figures 2E and 3) and of frequency dependence versus surface pressure (figures 4E and 6) were also similar. This shows that there were no major differences in the rheological response when the degree of saturation of the fatty acid chain was varied. This supports the earlier hypothesis that it is unlikely that interactions between the fatty acid chains are responsible for the rheological response. Instead, it supports the hypothesis that interactions between the oligofructose part are responsible.

The Lissajous plots that were obtained with sucrose esters (figure 10) were quite different than the ones obtained with oligofructose mono-esters. For all sucrose esters, the response was fairly viscous without any asymmetries. Combined with the exponent in the frequency dependence close to 0.5 it can be concluded that there is no mesophase formation for sucrose esters and that they behave like typical LMW surfactants.

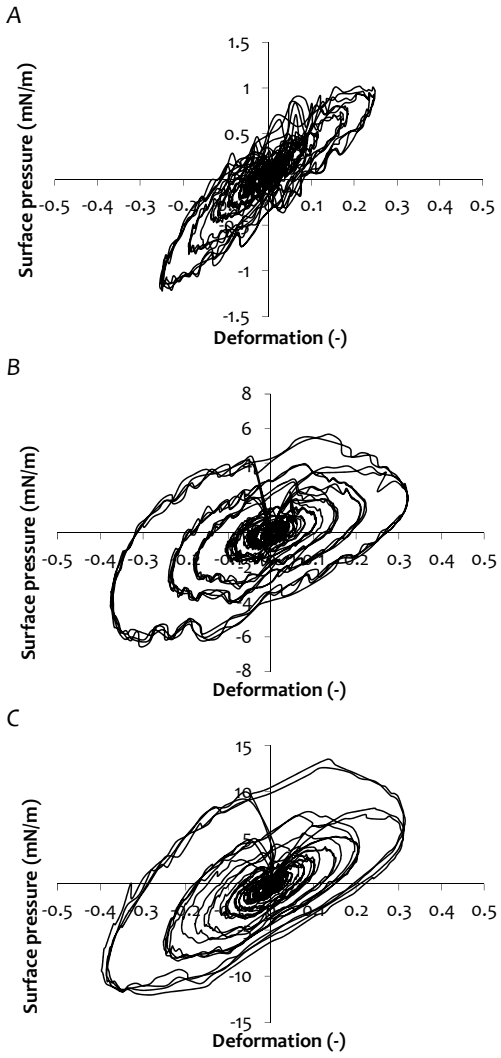


Figure 10: Lissajous plots obtained during amplitude sweep of an air/water interface stabilized by sucrose esters. S-C12m (A), S-C16 (B) and S-C18 (C).

Next, some of the Lissajous plots in figure 8 were selected for further analysis. While for some esters, for example OF-C12m, the shape of the curve does not seem to depend on the deformation amplitude, for other esters, for example OF-C18m, clear deviations in the shape of the curves are observed when the deformation amplitude increases. Therefore, the dependence of the shape of the Lissajous plot on the deformation amplitude was examined for OF-C12m and OF-C18m in more detail.

For OF-C12m, at amplitudes up to 7.5% the Lissajous plots are characterized by a mostly elastic response, without pronounced asymmetries (figures 11A-F). At higher amplitudes the response becomes increasingly more asymmetric with strain hardening during compression and strain softening during extension (figures 11G-J).

Compared to OF-C12m (figure 11), for OF-C18m the shape of the Lissajous plots changes more when the deformation amplitude is increased (figure 12). These data represent the same data set as presented in figure 7. At amplitudes up to 7.5% (figures 12A-F), the response is mostly elastic, without non-linearities. At 10 and 15% amplitude (figures 12G and H), the response is still mostly elastic, however here non-linearities appear, with strain hardening during compression and strain softening during extension. For 20 and 30% amplitude (figures 12I and J), the response becomes more viscous and is strain softening both in extension and compression.

To quantify the degree of non-linearity, we have determined S_{ext} and S_{com} for both components. For details of this procedure, the reader is referred to the materials and methods section. A value close to 0 corresponds to a linear elastic response, a positive value for the S factor refers to intracycle strain hardening, whereas a negative S factor refers to intracycle strain softening.

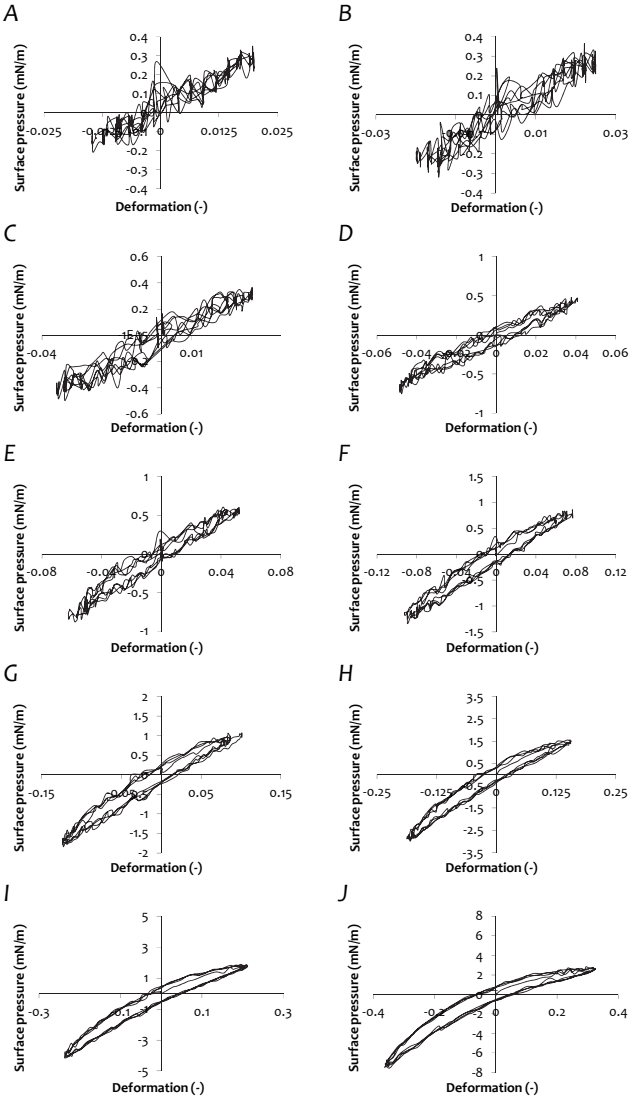


Figure 11: Lissajous plots as a function of deformation amplitude for OF-C12m at a bulk concentration of 0.2%. Applied amplitude was 1.5% (A), 2% (B), 3% (C), 4% (D), 5% (E), 7.5% (F), 10% (G), 15% (H), 20% (I) and 30% (J).

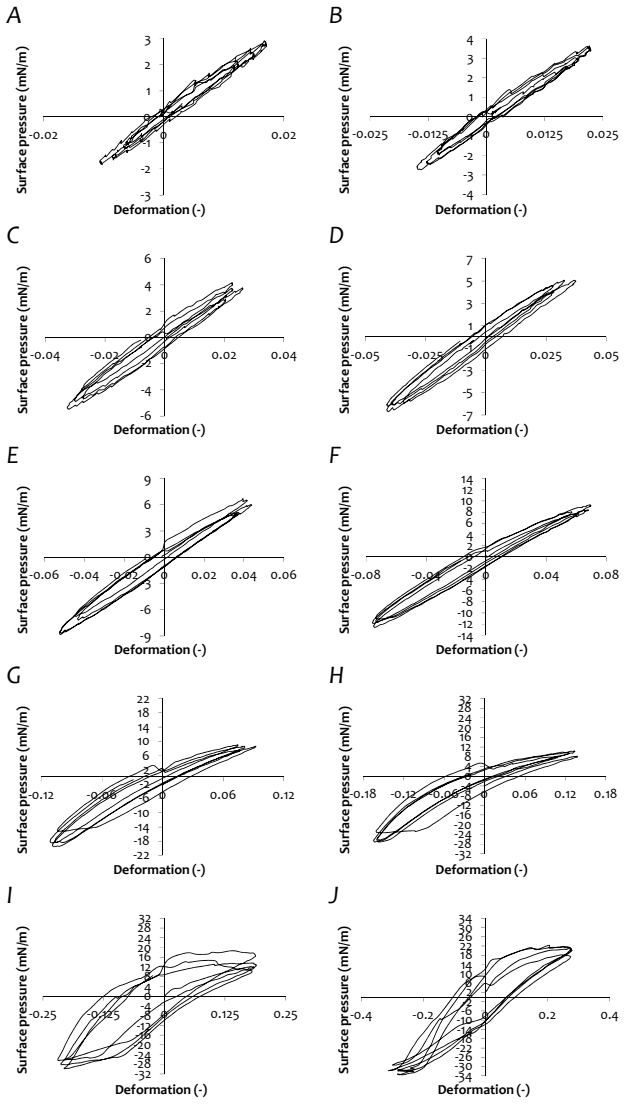


Figure 12: Lissajous plots as a function of deformation amplitude for OF-C18m at a bulk concentration of 0.05%. Applied amplitude was 1.5% (A), 2% (B), 3% (C), 4% (D), 5% (E), 7.5% (F), 10% (G), 15% (H), 20% (I) and 30% (J).

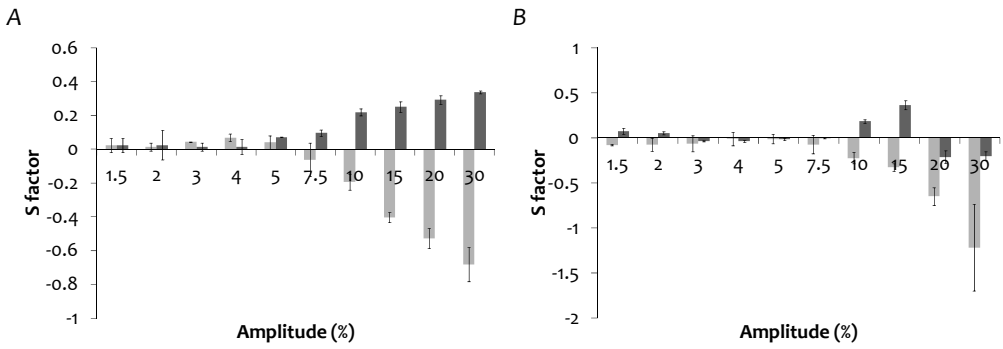


Figure 13: S factor during extension (light grey) and during compression (dark grey), determined for amplitude sweeps of air/water interfaces stabilized OF-C12m at a bulk concentration at 0.2% (A) and by OF-C18m at a bulk concentration at 0.05% (B).

Figure 13A confirms the absence of non-linearities at amplitudes up to 4% for OF-C12m. Furthermore it shows that with increasing deformation amplitude the degree of non-linearity increases, both in compression and extension. For OF-C18m, figure 13B confirms the absence of any non-linearities, with S factors close to 0, in the rheological response up to an amplitude of 7.5%. Furthermore, it shows that the degree of strain softening upon extension becomes increasingly larger when the amplitude is increased from 10% to 30%. Finally, it does confirm that up to 15% amplitude, the response is strain hardening upon compression and above 20% amplitude, the response is strain softening upon compression. The strain softening in compression for OF-C18m at amplitudes above 20% is different from the response of OF-C12m where over the whole range of amplitudes strain hardening in compression is obtained. When examining figures 12I and J in detail it is obvious that the response in compression is initially strain hardening, and changes to strain softening with increasing degree of compression. This may be attributed to the occurrence of a rearrangement in the interfacial structure. A possible scenario is that with increasing deformation amplitude, cracks begin to form in the soft glass phase that eventually lead to the presence of individual islands of glass phase. These islands could start to slide on top of each other at very high deformations leading to strain softening. Furthermore, it is possible that the interfacial structure starts to buckle, and even expels part of the interfacial structure into the bulk in the form of a micelle. This scenario would also lead to strain softening. All these hypotheses need to be confirmed using appropriate techniques, such as Brewster Angle Microscopy, particle tracking observed by microscopy [14], or grazing incidence X-ray diffraction [15].

3.4 Dependence of the surface dilatational modulus on temperature

The results that have been described in section 3.3 are in line with the hypothesis of the formation of a glass phase by the oligofructose part. Since the formation of a glass phase is a temperature-dependent process, the development of the modulus should be dependent on temperature as well. Therefore, temperature sweeps have been performed (figure 14) for OF-C16m and for OF-C18m.

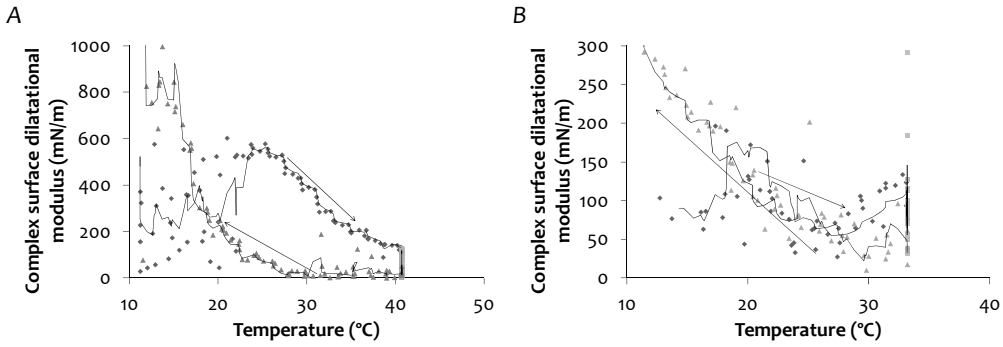


Figure 14: Dilatational modulus of air/water interfaces stabilized by OF-C16m (A) and OF-C18m (B) as a function of temperature determined at a bulk concentration of 0.02%. The arrows indicate whether data points correspond to heating or cooling of the interface.

The experiment was started with the establishment of an equilibrium at 10 °C. After equilibrium was established, the temperature was slowly increased to 42 °C in the case of OF-C16m (figure 14A) and 33 °C in the case of OF-C18m (figure 14B), and then slowly decreased again. During the warming up of the interface there is no clear dependence of the modulus on temperature; the initial modulus is also not extremely high. Since it is now clear that the interfacial structure depends on the magnitude of the imposed deformation it is likely that the interfacial structure development was hindered by the deformation. Upon cooling a strong increase in surface dilatational modulus is visible as the temperature decreases. This is indicative for an interfacial structural transition, which is in support of the hypothesis that the eventually high dilatational modulus is a result of glass formation by the oligofructose part. Since non-ionic surfactants generally become more soluble in the bulk when temperature is decreased and thus get a lower affinity for the interface, it is unlikely that the increase in dilatational modulus with decreasing temperature may be attributed to changes to the solubility of the ester.

4. Conclusions

In this chapter, we show how a standard surface rheological approach where the surface dilatational modulus is determined as a function of surface pressure and frequency does not always provide sufficient information about the interfacial structure. We show that it is especially important to understand the dependence of the surface dilatational modulus on deformation amplitude and temperature. We demonstrate that the use of Lissajous plots in interfacial rheology can be a powerful tool to increase the understanding of the link between surface rheological response and interfacial structure. Therefore, we propose that for a full rheological characterization of an interface the dependence of the surface dilatational modulus on surface pressure, frequency, amplitude and temperature should be determined and that Lissajous plots should be used to increase the understanding of interfacial microstructure. We have shown that interfaces stabilized by oligofructose esters have much different rheological properties than those stabilized by sucrose esters. While sucrose esters behave like typical LMW surfactants with low moduli, a scaling exponent in the frequency dependence close to 0.5, and fairly viscous Lissajous plots without asymmetries, oligofructose esters behave much differently. They form interfaces with relatively high moduli, low frequency dependence of the modulus and fairly elastic Lissajous plots with strain hardening during compression and strain softening during extension. We argue that the unusual rheological properties of interfaces stabilized by oligofructose fatty acid esters are most likely governed by the formation of a soft glass phase by the oligofructose part. This hypothesis needs to be further investigated using appropriate structural characterization techniques.

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Chapter 5

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The effect of di-esters and lauric acid on rheological properties of air/water interfaces stabilized by oligofructose lauric acid mono-esters

Abstract

In this chapter, the rheological properties of interfaces stabilized by oligofructose fatty acid esters are studied. First, the properties of interfaces stabilized by mono-esters (ME), di-esters (DE), lauric acid (LA), oligofructose (OF), and mixtures of ME with DE, LA or OF were studied. Finally, the properties of interfaces stabilized by the crude product (CP) containing ME, DE, LA and OF were studied. The dependency of the dilatational modulus on frequency and deformation amplitude pointed to the possible formation of a soft glass phase for ME, and a viscous interface for DE. When ME and DE were mixed at a ratio of 0.8/0.2, the experimental results suggest that the interfacial structure consists of islands of a glass phase formed by ME, dispersed in a 2D viscous phase of DE. CP stabilized interfaces, where the ratio ME/DE was higher, lead to a different rheological response. The ratio ME/DE plays an important role in the surface properties of the CP. This may have significant consequences for applications in macroscopic systems such as foams.

1. Introduction

Sugar esters have been the subject of investigation for many years. They are food-grade non-ionic surfactants [1, 2] and widely used within the food industry [3] because they have attractive properties. They are produced from renewable and largely available feedstock [4], biodegradable, non-toxic, odorless and tasteless [5]. They can be used for many different applications since they can be produced with a wide range of hydrophobicity. This wide range is accomplished by the use of fatty acids of different chain length, by varying the degree of substitution of the sugar group, and by changing the degree of polymerization of the carbohydrate.

Industrially available sugar esters are often produced by chemical synthesis methods which result in a mix of esters with different degrees of substitution. Also, there are usually traces of fatty acids left behind [3, 6]. When studying the surface properties of these crude samples, it is important to consider the contribution of the different individual components.

By increasing the number of fatty acid chains that is esterified to a sugar group, the surface activity is increased. However, the increased hydrophobicity also leads to reduced solubility. Lower solubility will lead to a slower adsorption at interfaces [7]. Therefore, a higher surface activity is expected for di-esters compared to mono-esters, as long as the hydrophobicity does not become too high. The hydrophobicity of the molecule is determined by the balance between the size of the hydrophobic and hydrophilic groups.

The contribution of the free fatty acids strongly depends on whether they are able to migrate to the interface, which in turn depends on the amount of monomers that is available in the bulk. The amount of monomers is strongly influenced by the fatty acid chain length and the degree of saturation, both of which influence the melting point. Below the melting point, the fatty acids will be present in the bulk in the form of insoluble precipitates that will not migrate to the interface. Surfactant micelles can aid in the transportation of fatty acids from bulk to interface below their melting point. Golemanov et al. [8] have shown that, by using the anionic surfactant sodium lauryl-dioxyethylene sulfate and the zwitterionic surfactant cocoamidopropyl betaine, in combination with medium chain fatty acids, it is possible to create interfaces with a high dilatational modulus. They hypothesized that mixed micelles of both types of surfactants and fatty acids were formed that transported fatty acids to the interface where they formed a condensed layer, leading to a high modulus. If a similar mechanism would occur when fatty acids are present in a crude product containing oligofructose fatty acid esters, the fatty acids in the crude product could contribute to the surface properties.

Husband et al [3] have studied the surface tension and foaming properties of sucrose lauric acid esters. They studied crude reaction products, purified mono-esters, purified di-esters and mixtures of mono-esters and di-esters. They found a higher surface tension and poorer foaming properties of di-esters compared to mono-esters. The crude product had improved foaming properties compared to either pure component. Addition of di-esters to mono-esters improved the foaming properties to that of the crude product. These results demonstrate the importance of understanding not only the contribution of each individual component present in a crude reaction product to the functional properties, but also the contribution of mixtures of these components. To the best of our knowledge similar studies, where the effects of different components present in crude products on the functional properties are investigated, are rare.

While synthesis and functional properties of fatty acid esters of mono- and di-saccharides are frequently described [9-12], reports on oligosaccharide esters are usually limited to the synthesis [13-17]. Because of their ability to create interfaces with both a low surface tension as well as a high dilatational modulus, previous research by our group focused on synthesis and functional properties of oligofructose lauric acid esters (OLAE). A study on functional properties showed that crude OLAE could lower the surface tension of an air/water interface considerably and provide a high dilatational modulus. These interfacial properties also translated into high foam stability [18]. The OLAE are produced by enzymatic esterification of oligofructose to fatty acids. The oligofructose that is used is a mixture of different degrees of polymerization (between 2 and 8). As a result, the oligofructose esters that are obtained are also a mixture of different components. Because of the specific enzymatic reaction procedure, the reaction yields mostly (67%) mono-esters (one fatty acid coupled to one oligofructose molecule). However, also small amounts of unesterified oligofructose, di-esters (two fatty acids coupled to one oligofructose molecule) and unreacted fatty acids are present [chapter 2]. After synthesis, the different fractions are separated and purified mono-esters and di-esters are obtained.

We expect major differences in the functionality of the different pure components (ME, DE, LA, OF). Furthermore, mixtures of these components can have completely different functionality compared to the pure components. Hence, the composition will affect performance of the OLAE in actual applications (such as foam or emulsion stabilization). Therefore, the purpose of this study was to investigate the contribution of the different components present in the crude product, and combinations of these components, to the properties of air/water interfaces stabilized by OLAE. First, the properties of the purified mono-ester were investigated (section 3.1). Then, the properties

of the other components present in the crude product and a mix of 80% mono-ester with 20% of this component were studied (di-ester in section 3.2, lauric acid in section 3.3 and oligofructose in section 3.4). Finally, the properties of the crude product were investigated (section 3.5). In section 3.6 a hypothesis on the interfacial microstructure is proposed to explain the rheological behavior of the different systems.

2. Materials and methods

2.1 Materials

Oligofructose with a degree of polymerization (DP) between 2 and 8 (average 4.4) was a kind gift of Beneo-Orafti (Tienen, Belgium). In some cases the oligofructose chain was terminated by a glucose unit. Lauric acid was obtained from Sigma –Aldrich (Steinheim, Germany).

2.2 Methods

2.2.1 Synthesis

Oligofructose lauric acid esters were prepared according to a previously described protocol [chapter 2]. The crude product was washed once with n-hexane to remove lauric acid. The composition of the product was estimated using MALDI-TOF MS. The composition of the detectable part of the crude product was approximately 67% mono-esters, 10% di-esters and 23% unmodified oligofructose. Although the presence of lauric acid could not be verified with MALDI-TOF MS, it is likely that small amounts of lauric acid are present in the crude product. After this, the product was fractionated to separate the different fractions. MALDI-TOF MS and NMR confirmed the high purity (97%) of the purified mono-esters [chapter 2]. For di-esters a purity of 90% was obtained. The main impurities were mono-esters, but also small amounts of unmodified oligofructose and tri-esters were present.

2.2.2 Sample preparation

Samples were prepared by dissolving the surfactant in purified deionized water. All concentrations are expressed as % w/v in the bulk. The total concentration was 0.2% in all cases, except for pure di-esters, where, due to low solubility, a concentration of 0.1% was chosen. This concentration is above the critical micelle concentration (determined as approximately 0.003%).

2.2.3 Determination of surface tension and surface dilatational modulus

The surface tension and surface dilatational modulus of the air/water interface were determined with a profile analysis tensiometer (Sinterface, Berlin, Germany). During time sweeps, sinusoidal oscillations with an amplitude of 5% and a frequency of 0.1 Hz were applied. The experiments consisted of 25 cycles of oscillations that were alternated with 1 cycle of rest. About 1 second after drop creation the equipment took the first measurement. This value was taken as the initial surface tension used in figure 3. The time sweeps were followed by a frequency sweep where the frequency was increased stepwise from 0.005 Hz

to 1 Hz, at an amplitude of 5%. The slope of a double logarithmic plot of surface dilatational modulus versus frequency was determined using a linear regression fit. Reported values represent the average slope of 2-7 measurements with the corresponding standard deviation. After the frequency sweep, an amplitude sweep was performed where the amplitude was increased stepwise from 1.5% to 30%, at a frequency of 0.1 Hz. All experiments were performed at 25 °C. Reported values represent the average of 2-7 measurements.

3. Results & discussion

In this study, the properties of air/water interfaces stabilized by oligofructose fatty acid esters are studied. The oligofructose that was used for the synthesis of these esters was a mixture of oligomers with different degrees of polymerization. Therefore, the resulting esters are also mixtures. The functional properties of several components present in the crude reaction product (mono-esters, di-esters, lauric acid and oligofructose) and mixtures of these components are discussed.

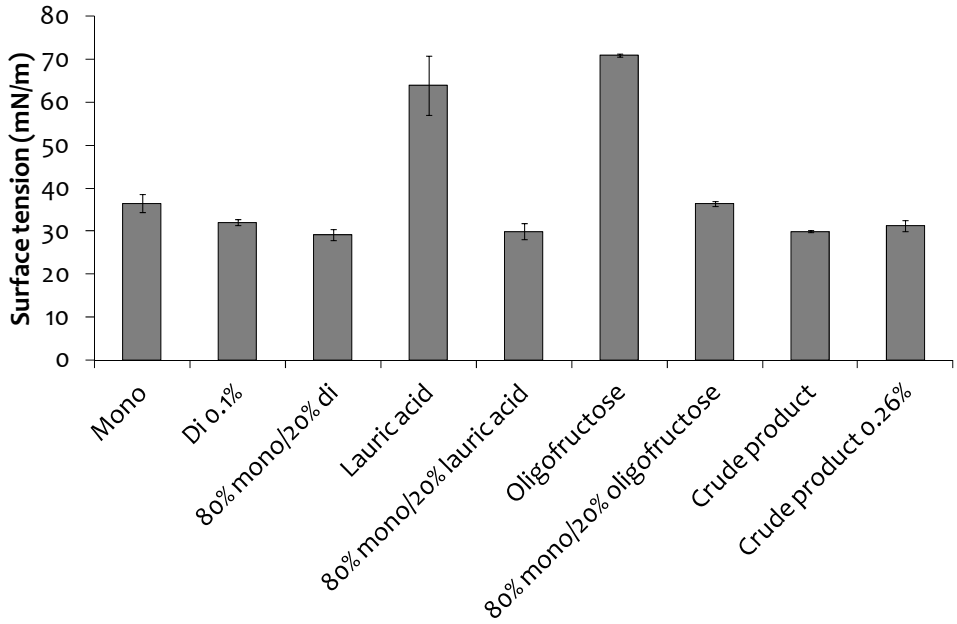

Figure 1: Equilibrium surface tension of air/water interfaces (determined at a bulk concentration of 0.2%, except when stated otherwise) stabilized by purified oligofructose lauric acid mono-esters, di-esters (0.1%), lauric acid, oligofructose, mixes of mono-ester with di-esters, lauric acid and oligofructose, and the crude reaction product (at 0.2 and 0.26%).

3.1 Mono-ester

At a surfactant concentration in the bulk of 0.2% (w/v), the equilibrium surface tension of an air/water interface stabilized by the purified mono-ester was 36.5 ± 2.1 mN/m (Figure 1), while the equilibrium surface dilatational modulus was 14.7 ± 2.8 mN/m (Figure 2). The surface tension after 1 second was 46.0 ± 1.6 mN/m (Figure 3). The fairly fast decrease in surface tension points to a molecule that can migrate to the interface rather rapidly. To gain

a deeper understanding of rheological characteristics of the interface, a frequency sweep was performed starting immediately after the time sweep. The slope of a double logarithmic plot of modulus versus frequency was 0.12 ± 0.04 (Figure 4). This value is much lower than the value of 0.5 predicted by the Lucassen and van den Tempel model [19] for low molecular weight surfactants, and points to the presence of a mostly elastic layer. This is further confirmed by the low value of the loss tangent (0.031 ± 0.008). After the frequency sweep, an amplitude sweep was performed. The results are presented as Lissajous plots of surface pressure versus deformation, in a procedure similar to Ewoldt et al. [20] (Figure 5A). Here surface pressure was taken as a measure of stress. The figure confirms the elastic nature of the interfacial layer. Furthermore, the curve is asymmetrical. In the compression part of the cycle, the curve becomes more steep with increasing deformation. Hence, the interface is strain hardening upon compression. In the extension part of the curve, the curve becomes less steep with increasing deformation. So the interface is strain softening upon expansion.

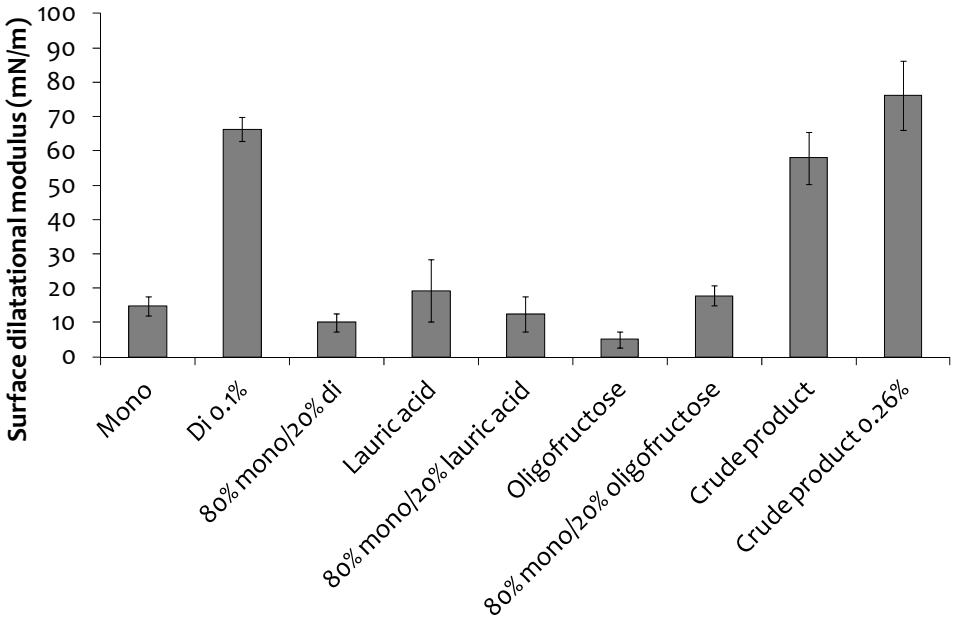

Figure 2: Equilibrium surface dilatational modulus of air/water interfaces (determined at a bulk concentration of 0.2%, except when stated otherwise) stabilized by purified oligofructose lauric acid mono-esters, di-esters (0.1%), lauric acid, oligofructose, mixes of mono-ester with di-esters, lauric acid and oligofructose, and the crude reaction product (at 0.2 and 0.26%).

The low exponent in the frequency dependence of the dilatational modulus combined with the strain hardening observed in compression and strain softening observed in extension are indications that the ME are forming a soft glass phase after adsorption to the interface [21].

The critical micelle concentration (CMC) of pure mono-esters is around 0.23% (chapter 3). Since the total concentration of surface active material was 0.2% for the samples in this study, the concentration of mono-esters was slightly below the CMC. In sections 3.2, 3.3 and 3.4, the mono-esters were mixed with other components present in the crude reaction product at a ratio of mono-ester/other component of 0.8/0.2.

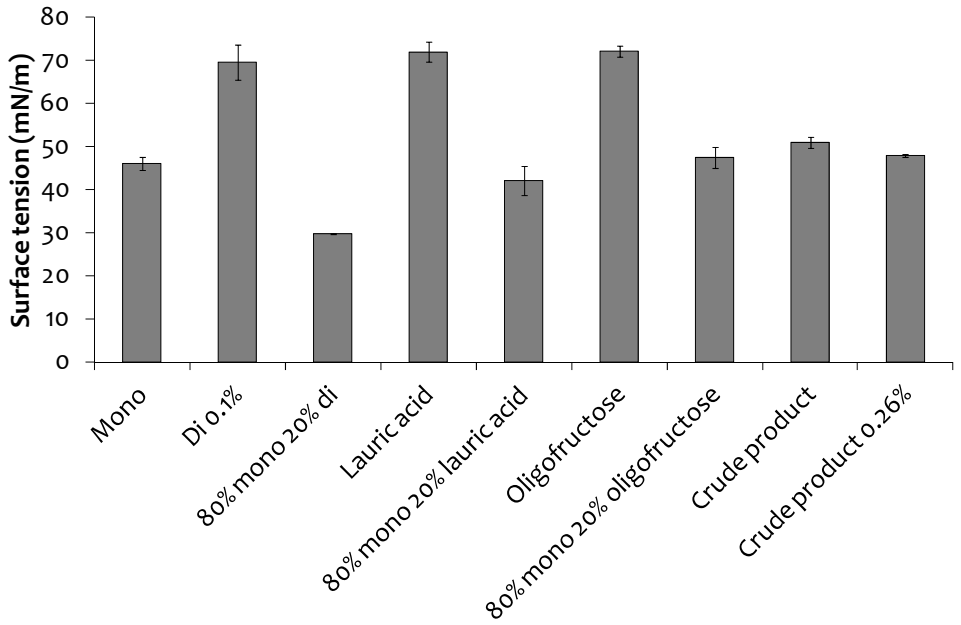

Figure 3: Surface tension 1 second after creation of air/water interfaces (determined at a bulk concentration of 0.2%, except when stated otherwise) stabilized by purified oligofructose lauric acid mono-esters, di-esters (0.1%), lauric acid, oligofructose, mixes of mono-ester with di-esters, lauric acid and oligofructose, and the crude reaction product (at 0.2 and 0.26%).

3.2 Di-ester

3.2.1 Pure di-ester

The equilibrium surface tension of an air/water interface stabilized by the pure di-esters (32.0 ± 0.7 mN/m) was lower than the surface tension of an interface stabilized by

pure mono-esters (Figure 1). The equilibrium surface dilatational modulus of an air/water interface stabilized by di-esters was 66.4 ± 3.6 mN/m and much higher than the one obtained with mono-esters (Figure 2). Closer examination of the surface tension after 1 second shows a high surface tension of 69.7 ± 4.1 mN/m (Figure 3). Apparently, the migration of the di-ester towards the interface is much slower than that of the mono-ester. This is caused by the higher hydrophobicity of the di-ester. The analysis of the frequency sweeps reveals a slope of 0.52 ± 0.02 (Figure 4). A slope of 0.5 is consistent with surface processes that are diffusion-controlled, according to the Lucassen van den Tempel model [19]. The Lissajous plot of surface pressure versus deformation (Figure 5B) reveals that the interfaces stabilized by DE are more viscous than those stabilized by ME. The curves also show asymmetries, but far less pronounced than for ME stabilized interfaces. From the slope of 0.5 in the frequency sweep and the lack of asymmetry observed in the Lissajous plots we conclude that DE stabilized interfaces do not show mesophase formation and behave similar to interfaces stabilized by simple surfactants, where the dilatational modulus depends on the rate of exchange with the bulk phase. The high modulus is therefore caused by the slow adsorption of DE from the bulk to the interface.

3.2.2 Mix of mono-ester and di-ester

Both mono-esters and di-esters adsorb at the interface. However, since the speed of adsorption and the degree of mesophase formation are different for both species, it is important to study the properties of mixed interfaces of both species. Therefore, the surface properties of an air/water interface formed from a mix of mono-esters and di-esters were studied. The surface tension of an air/water interface stabilized by a mixture of 80% mono-esters and 20% di-esters was 29.2 ± 1.2 mN/m. This value is slightly lower than the surface tension value obtained with pure di-esters (Figure 1). Similar to these results, when studying sucrose esters, Husband et al. [3] have found that the surface tension of a mix of mono- and di-esters was similar to that of the component that lead to a lower surface tension, in their case the mono-ester. Next, the surface dilatational modulus was determined. When oligofructose mono-esters and di-esters were mixed, a surface dilatational modulus of 10.0 ± 2.5 mN was obtained (Figure 2), which is similar to that obtained with the pure mono-ester. The surface tension value after 1 second was 29.8 ± 0.0 mN/m (Figure 3), which is lower than the value obtained with either pure component. Apparently, the presence of the mono-ester greatly accelerates the adsorption of the di-ester. This acceleration could be the result of the formation of mixed micelles. The analysis of the frequency sweeps reveals a slope of 0.28 ± 0.02 (Figure 4), which points neither to

diffusion-controlled processes nor to the presence of an elastic layer. The Lissajous plot (Figure 5C) reveals a more viscous interface than a pure mono-ester stabilized interface. However, it is still more elastic than a pure di-ester stabilized interface. Furthermore, there is also an asymmetry in the curve, with slight strain hardening during compression and slight strain softening during expansion. When taking into consideration the surface tension, which was equal to the one obtained with pure di-ester, combined with the data from the frequency and amplitude sweeps, which point to an intermediate between mono-ester and di-ester in terms of interfacial structure, the interfacial structure could consist of islands of a glass phase formed by the mono-ester, surrounded by di-esters. This hypothesis still has to be confirmed by structural analysis.

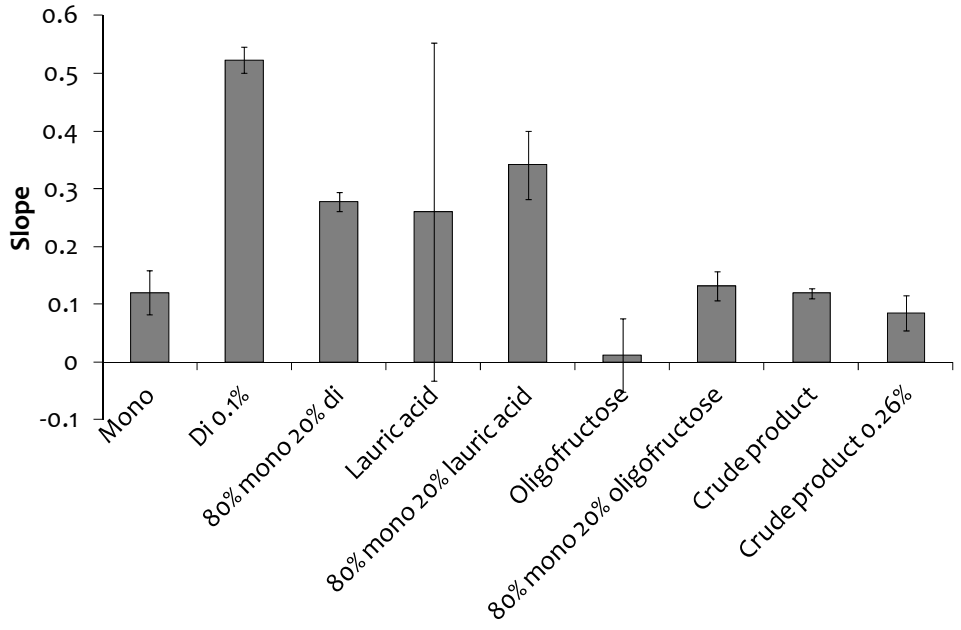

Figure 4: Slope of a double logarithmic plot of surface dilatational modulus versus frequency of air/water interfaces (determined at a bulk concentration of 0.2%, except when stated otherwise) stabilized by purified oligofructose lauric acid mono-esters, di-esters (0.1%), lauric acid, oligofructose, mixes of mono-ester with di-esters, lauric acid and oligofructose, and the crude reaction product (at 0.2 and 0.26%).

3.3 Lauric acid

3.3.1 Pure lauric acid

As a result of the synthesis methods, many crude samples of sugar esters contain traces of fatty acids. However, their possible influence on the functional properties is usually neglected [3, 6]. The surface properties of air/water interfaces stabilized by fatty acids strongly depend on the surface concentration and the temperature. The surface concentration of fatty acids depends on their ability to migrate to the interface, which is strongly dependent on the amount of monomers in the bulk phase. Below their melting point, the fatty acids will be present in the form of insoluble precipitates that will not migrate to the interface. At temperatures above the melting point, the insoluble precipitates will disappear and the individual fatty acids will migrate to the interface where they significantly lower the surface tension. Chumpitaz et al. [22], for example, have found a surface tension of 27 mN/m at 60 °C at an air/water interface stabilized by lauric acid. However, a very high dilatational modulus will only be found at temperatures below the melting point when the surface concentration is sufficiently high. In this study, we have determined the surface tension and surface dilatational modulus of an air/water interface at 25 °C to determine if sufficient monomers are present at this temperature to have a significant impact on the functional properties. The equilibrium surface tension obtained for an interface stabilized by pure lauric acid in this study was 63.9 ± 7.0 mN/m (Figure 1). The dilatational modulus was 19.2 ± 9.0 mN/m (Figure 2). These values point to a molecule that did not dissolve well in the bulk phase and therefore did not adsorb at the interface in significant quantities. At equilibrium, the surface concentration of lauric acid was still very low. The surface tension after 1 second, which was 72.0 ± 2.3 mN/m (Figure 3), confirms the poor solubility in the bulk phase. The analysis of the frequency sweeps reveals a slope of 0.26 ± 0.29 (Figure 4), which does not provide useful information due to the large error, which is caused by the extreme scattering in the data for the modulus. The data from the amplitude sweep (Figure 5D) show that the small amount of material that did adsorb at the interface formed a purely elastic layer, which is expected for insoluble fatty acids.

3.3.2 Mix of mono-ester and lauric acid

Surfactant micelles can facilitate the transportation of fatty acids from bulk to interface at temperatures below their melting point. Golemanov et al. [8] have shown that, by using the anionic surfactant sodium lauryl-dioxyethylene sulfate and the zwitterionic surfactant cocoamidopropyl betaine in combination with fatty acids, it is possible to create interfaces with a high dilatational modulus. To test whether mono-esters can have a similar effect in

increasing the amount of lauric acid that is transported from bulk to interface, thus enabling lauric acid to contribute to the interfacial properties, we have determined the surface tension and surface dilatational modulus of an air/water interface stabilized by a mixture of 80% mono-esters with 20% lauric acid. The surface tension of an air/water interface stabilized by a mixture of mono-esters and lauric acid was 29.9 ± 1.9 mN/m (Figure 1). This value for the surface tension is lower than the surface tension of a pure mono-ester stabilized interface. Apparently, the presence of mono-esters caused an increase in the amount of lauric acid that was present at the interface. This could be the result of the formation of mixed micelles. The surface dilatational modulus of an air/water interface was similar for mono-esters, lauric acid and for a mix of lauric acid and mono-esters (Figure 2). Apparently, although there was an increase in solubility, the surface concentration of the lauric acid at equilibrium was not high enough to cause an increase in the modulus. Golemanov et al. [8] have found a sharp increase in modulus when the surface tension of their system was around 25 mN/m. The fact that the surface tension in our system was higher supports the conclusion that the surface concentration of lauric acid is too low to achieve a high surface dilatational modulus. The surface tension after 1 second (42.0 ± 3.4 mN/m, figure 3) was similar to the surface tension of a pure mono-ester stabilized interface, which points to an interface that was initially dominated by mono-esters. With time, more and more lauric acid was delivered to the interface, leading to the formation of a more compact interfacial layer, which resulted in a lower surface tension. The fact that this decrease took a much longer time compared to the case of a mixed mono-ester/di-ester interface may be attributed to a lower degree of solubilisation by the mono-esters. Analysis of the frequency sweeps shows a slope of 0.34 ± 0.06 (Figure 4). Like in the case of mixed mono-ester/di-ester interfaces, the value of the slope points neither to diffusion-controlled processes nor to the presence of an elastic layer. The analysis of the Lissajous plot (Figure 5E) reveals an interfacial layer with intermediate viscoelasticity, comparable to figure 5C. There is asymmetry with slight strain hardening upon compression and slight strain softening upon expansion. Similar to the case of a mixed mono-ester/di-ester interface, this could be the result of the formation of a mixed layer, consisting of patches of a glass phase formed by mono-ester surrounded by lauric acid molecules.

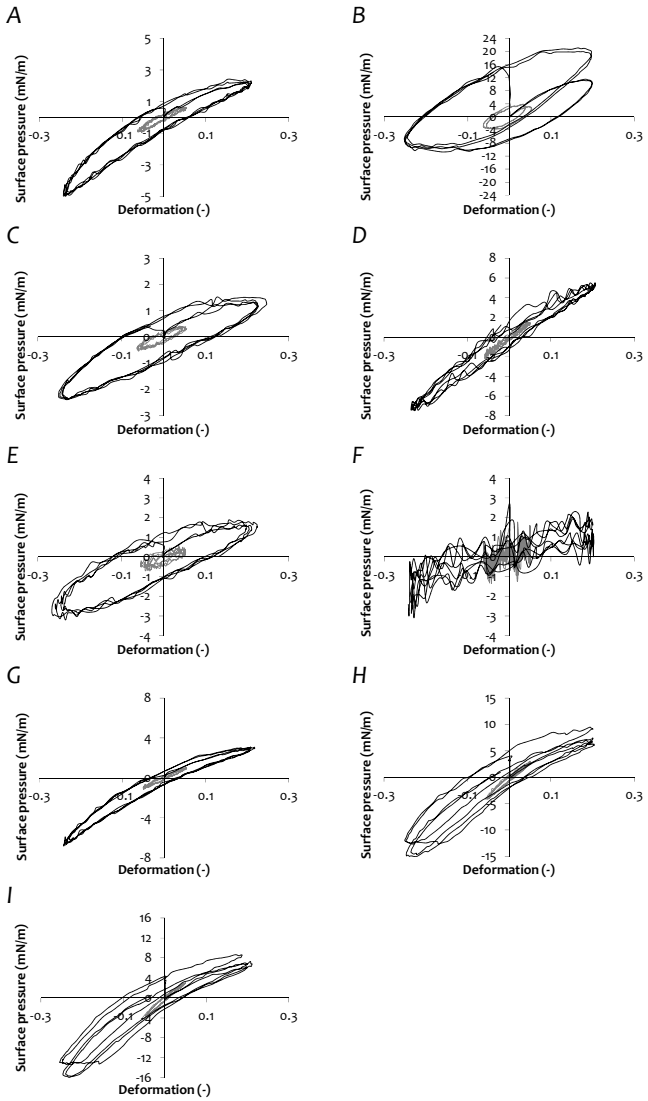

Figure 5: Lissajous plots of surface pressure versus deformation, obtained during amplitude sweeps (bulk concentration of 0.2%, except when stated otherwise) for mono-esters (A), di-esters (0.1%)(B), mix of mono-esters and di-esters (C), lauric acid (D), mix of mono-esters and lauric acid (E), oligofructose (F), mix of mono-esters and oligofructose (G), crude reaction product (H), crude reaction product (0.26%)(I). Figures show single measurements that were representative for all measurements. During amplitude sweeps, the amplitudes were varied from 1.5% to 30%. For sake of simplicity, only 5% and 20% amplitude are plotted.

3.4 Oligofructose

3.4.1 Pure oligofructose

The oligofructose that was used is a mixture of oligomers with different degrees of polymerization, with only hydrophilic parts. As a consequence, the oligofructose is not expected to be surface-active. The lack of surface activity is reflected in the high equilibrium surface tension (70.7 ± 0.2 mN/m) (Figure 1), the surface tension after 1 second (72.5 ± 1.6 mN/m) (Figure 3) and the low dilatational modulus (5.9 ± 1.6 mN/m) (Figure 2). The analysis of the frequency sweeps (Figure 4) and amplitude sweeps (Figure 5F) do not present any useful information due to the lack of surface activity.

3.4.2 Mix of mono-esters and oligofructose

Since oligofructose is not surface-active, it is not likely that it will affect the surface properties of mono-esters. However, for the sake of completeness and to verify the absence of any unforeseen effects, we have determined the properties of a mix of mono-esters and oligofructose. The surface tension of an air/water interface stabilized by a mixture of 80% mono-esters and 20% oligofructose was 36.4 ± 0.7 mN/m (Figure 1), while the surface dilatational modulus that was obtained (Figure 2) was 17.8 ± 2.9 mN/m. Both values are similar to pure mono-esters. The surface tension after 1 second was 47.4 ± 2.5 mN/m (Figure 3), which is similar to the surface tension obtained with pure mono-ester after 1 second. The analysis of the frequency sweeps shows a slope of 0.13 ± 0.02 (Figure 4), which is the same as the slope of the curve of pure mono-ester. Finally, the Lissajous plots (Figure 5G) are also similar. Since oligofructose is not a surface active molecule, its only contribution to the functional properties was the diluting of the mono-esters, which did not have a significant effect on the functional properties because the concentration was still close to the CMC.

3.5 Crude product

The composition of the crude product was estimated to be around 67% mono-esters, 10% di-esters and 23% unmodified oligofructose. The amount of lauric acid was not determined but is not likely to exceed a few percent [3, 6]. Since in section 3.3.2 it was established that even at a ratio of mono-ester/lauric acid of 0.8/0.2 the effect of lauric acid on the functionality was very small, the role of lauric acid in the crude product is expected to be limited. The ratio mono-ester/di-ester in this mixture is 0.87/0.13. This means that the relative amount of mono-ester in the product is higher than in section 3.2. Furthermore, the amount of surface active material in the sample is only 78%. The di-ester has a low CMC of

approximately 0.0031% (to be published), so the concentration of di-ester is still above the CMC. However, the CMC of the mono-ester is around 0.23% (to be published). With only 67% mono-ester in the sample at a concentration of 0.2%, the concentration of mono-ester is below the CMC. The surface tension of an air/water interface stabilized by the crude product was 29.9 ± 0.2 mN/m (Figure 1) and similar to the surface tension of an air/water interface stabilized by the di-ester alone, and to the mixes of mono-ester with di-ester or lauric acid. This value is lower than the surface tension that was obtained in a previous study on oligofructose lauric acid esters (39 mN/m) [18]. Besides the fact that in the previous study the concentration was slightly lower (0.1% compared to 0.2% in this study), it is important to consider that the degree of polymerization of the oligofructose groups and the ratio mono-ester/di-ester in both products were not the same. An air/water interface stabilized by the crude product showed a rather high surface dilatational modulus of 57.9 ± 7.5 mN/m (Figure 2), which is comparable to the modulus of an interface stabilized by pure di-esters. The surface tension after 1 second was 50.9 ± 1.3, which is similar to the surface tension measured for pure mono-ester. Analysis of the frequency sweep shows a slope of 0.12 ± 0.01, which is low and similar to the mono-ester. For most amplitudes, the Lissajous plot (figure 5H) shows elastic behavior with strain hardening in compression and strain softening in expansion, similar to the pure mono-ester. However, at higher amplitudes the response becomes more viscous, especially during compression. Concluding, results from the frequency and amplitude sweeps show behavior similar to a system stabilized by pure mono-ester. However, based on the similarity between the surface tension of interfaces stabilized by pure di-ester and the crude product, and the fact that the modulus is much higher than for a pure mono-ester interface, the interfacial structure cannot be similar to that of the mono-ester. Furthermore, when the amplitude is increased during the amplitude sweep, the modulus decreases while tan delta increases. This points to the formation of a firm elastic structure at the interface, that is sensitive to changes in deformation and is broken down when amplitudes become too high.

Surface properties were also determined for the crude product at a higher bulk concentration of 0.26%. At this concentration, the amount of surface active material is 0.2% and the same as in the previous sections. Therefore, the system can be compared to sections 3.2 and 3.3 more easily. There were no major differences between the properties of the crude product at a bulk concentration at 0.2% and 0.26%, as shown in figures 1-4 and 5I. Therefore, the difference in surface properties between the crude product and the individual components or mixes cannot be attributed to the lower concentration of surface active components in the crude product.

3.6 Hypothesis on interfacial microstructure

To summarize the findings until now, an interface stabilized by pure mono-esters showed a low frequency dependency of the modulus, combined with strain hardening in compression and strain softening in expansion. Also, the modulus was low. We concluded that a soft glass phase must have been present. In contrast, an interface stabilized by pure di-esters showed a high frequency dependence corresponding to the Lucassen van den Tempel model [19] without asymmetries. This is typical simple surfactant behavior. The modulus was high and reflected the slow speed of adsorption. For the crude product with a ratio of mono-ester/di-ester of 0.87/0.13, we found an elastic interface with strain hardening in expansion and strain softening in compression and a high modulus. For the mix of mono-esters and di-esters with a ratio of mono-ester/di-ester of 0.8/0.2, we found intermediate frequency dependence and slight strain hardening in expansion and strain softening in compression. This was combined with a low modulus.

There are two hypotheses to explain the differences between the rheological characteristics of the crude product (ratio mono-ester/di-ester 0.87/0.13) and the mix of mono-ester with di-ester (ratio mono-ester/di-ester 0.8/0.2). First, the structure at the CP stabilized interface might be composed of a mixture of mono-ester, di-ester and lauric acid. However, it seems unlikely that this explains the observed difference because the amount of lauric acid in the crude product is rather low. Second, the ratio between mono-ester and di-ester might be crucial for the development of the structure at the interface. If the mono-ester indeed forms a soft glass, it should be quite brittle. This means that the structure can easily be disrupted when the deformation amplitude becomes too high. Indeed, we never observe a linear response regime for the purified ME interfaces. This may explain the relatively low value for the modulus of a pure ME stabilized interface. When the surface fraction of DE increases, the systems goes from a soft glass to a dispersion of glassy ME patches in a viscous DE phase. Initially, when the fraction of DE is small, the ME patches still dominate the interface and create an interface that is less brittle, but still elastic with a high modulus. With increasing DE fraction, the amount of glassy ME patches reduces which leads to a loss of connectivity. Eventually, this leads to a loss of the elastic character and of the high modulus. The proposed mechanism is summarized in figure 6. Although a lot of information about the interfacial structure can be gathered from rheological data, it is very important to confirm the hypotheses that were proposed in this chapter using appropriate methods, such as Brewster Angle Microscopy, particle tracking observed by microscopy [23], or grazing incidence X-ray diffraction [24].

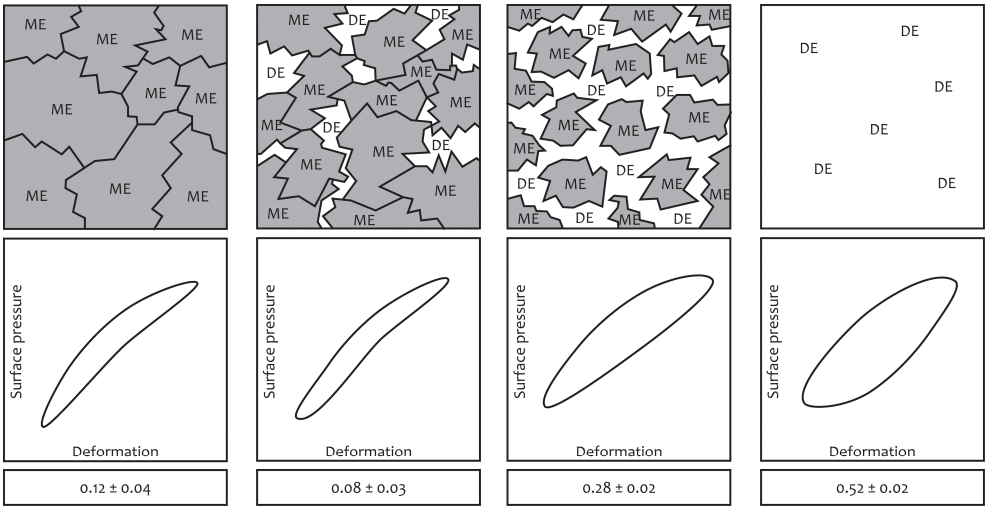

Figure 6: Top row: proposed interfacial structure of OLAE. Middle row: schematic Lissajous plots. Bottom row: Slope of a double logarithmic plot of surface dilatational modulus versus frequency. From left to right: a soft glass formed by pure mono-esters, a mixed interface formed at ratio of mono-ester/di-ester of 0.87/0.13, a mixed interface formed at ratio of mono-ester/di-ester of 0.8/0.2, a pure di-ester stabilized interface.

4. Conclusions

Although they are the main components in the crude product, the surface properties of the product are not solely governed by mono-esters. Di-esters and free lauric acid, which are often present in crude samples, do contribute to the functional properties. To apply this type of materials as a foam or emulsion stabilizer, the effect of other components present in the crude product must always carefully be considered. Small variations in composition may lead to significant changes in functionality.

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Chapter 6

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Interfacial properties of air/water interfaces stabilized by oligofructose palmitic acid esters in the presence of whey protein isolate

Abstract

To study the applicability of oligofructose palmitic acid esters (OF-C16m) as novel surfactants in food systems, the functional properties of OF-C16m were studied in the presence of whey protein isolate (WPI). Surface tension measurements, surface dilatational rheology, foam stability tests and Brewster Angle Microscopy were used to study the competitive adsorption of WPI and OF-C16m and the displacement of WPI by OF-C16m.

Pure WPI stabilized interfaces had a moderate surface tension (48 mN/m) and a dilatational modulus of 90 mN/m, while pure OF-C16m stabilized interfaces had a low surface tension (30 mN/m) and a dilatational modulus of 50 mN/m. The stabilization mechanisms of WPI (elastic network formation) and of OF-C16m (surface solidification) are very different, and the combined adsorption of these two components led to a structure with a much lower dilatational modulus. At the lowest WPI concentrations (0.5% and 1%), the equilibrium surface tension was similar to a pure OF-C16m stabilized interface, pointing to a low WPI surface concentration. However, apparently still sufficient WPI had adsorbed either at or just below the interface, to prevent the OF-C16m from solidifying. Despite the low moduli, the foam stability for the mixed systems was high. The interfaces were probably stabilized by the Gibbs-Marangoni mechanism. In contrast, at the highest WPI concentration (2%), the equilibrium surface concentration of WPI was sufficiently high to decrease the interfacial mobility of OF-C16m, which decreased the Gibbs-Marangoni effect and resulted in decreased foam stability.

Finally, OF-C16m could also displace a fully developed WPI network from the interface.

1. Introduction

Many food products consist of foams, which are thermodynamically unstable systems. Foam stability can be improved by the addition of surfactants, which lower the surface tension. A lower surface tension facilitates the enlargement of the air/water interfacial area, resulting in a higher foamability [1]. Proteins and low molecular weight (LMW) surfactants are two major types of food-grade surfactants. Proteins are macromolecules containing both hydrophilic and hydrophobic patches. Upon adsorption to the interface, they lower the surface tension and tend to form a viscoelastic network [1]. This network provides the interface with a high dilatational modulus. Interfaces with a high dilatational modulus show decreased disproportionation, which increases foam stability [2]. In contrast, LMW surfactants are relatively small and have a clearly defined hydrophobic and hydrophilic part. Upon adsorption to the interface, LMW surfactants can lower the surface tension much more than proteins. This is caused by a more compact interfacial structure of the LMW surfactants [1]. The lower surface tension that is obtained with LMW surfactants will improve foam stability. However, they do not typically form interfaces with high viscoelasticity [1, 3, 4]. Instead, LMW surfactants stabilize the interface by the Gibbs-Marangoni mechanism. This mechanism relies on rapid surface diffusion of LMW surfactants that will reduce surface concentration gradients that may develop as a result of deformations of the interface. During diffusion, LMW surfactants drag along some of the continuous phase. This slows down drainage of liquid from the films, which will increase foam stability [3].

A surfactant that can considerably lower the surface tension while also providing a high dilatational modulus would be an excellent foam stabilizer. To create a molecule with those properties, we focused on synthesis and functional properties of oligofructose lauric acid esters (OF-C12) [5]. The previous study on functional properties of these esters showed that OF-C12 can lower the surface tension of an air/water interface considerably and provide a high dilatational modulus. These interfacial properties also translated into high foam stability. The functional properties were also compared to a typical LMW surfactant, sucrose laurate. This molecule showed a much lower foamability and foam stability and also a much lower surface dilatational modulus. This showed that the OF-C12 did not behave like a typical LMW surfactant. The study on OF-C12 was extended to oligofructose esters of fatty acids with different chain lengths. Here it was found that oligofructose esters of palmitic acid (OF-C16m) were particularly promising new food-grade surfactants [chapter 2]. Even more than OF-C12, they provided a low surface tension and a high dilatational modulus to air/water interfaces. However, the previous studies were performed in model systems consisting of

only air, water and the oligofructose fatty acid ester. Before this ester can be implemented as a novel food-grade surfactant, its behavior must be studied in an environment that more closely resembles a food system. Most food systems are multi-component systems and this can affect the functionality of the esters. Although food systems contain many ingredients that possibly influence the functionality, the most important food ingredients to consider in respect to interfacial properties are proteins, as they are surface active and may compete for the interface.

The interactions between proteins and LMW surfactants have been of great interest to many researchers so far [2, 6-8]. Both proteins and LMW surfactants are, on their own, able to stabilize foams. However, they use completely different stabilization mechanisms. Proteins form a viscoelastic network at the interface while LMW surfactants stabilize the interface through the Gibbs-Marangoni mechanism [4, 6, 9]. These mechanisms are antagonistic: the LMW surfactant dilutes the protein and prevents network formation, while the protein network interferes with rapid surface diffusion of the LMW surfactant. Generally, this leads to a decrease in stability. The process is known as competitive destabilization [3].

Mixed interfaces can be studied in two different ways; the competitive adsorption of both species from the bulk or the displacement of an interfacial protein network by LMW surfactants.

The competitive adsorption of proteins and LMW surfactants strongly depends on concentration. During the initial phase of adsorption, the rate of adsorption is proportional to the concentration in solution [6]. Therefore, if the protein is present in higher concentration, first an interface is formed which contains predominantly proteins. However, since the LMW surfactant can lower the surface tension more than the protein, eventually the LMW surfactant will displace the protein. Research showed that at sufficiently high LMW surfactant concentrations, the protein contributed little to the surface properties [3, 10, 11].

When the starting point is a protein-stabilized interface, a viscoelastic network is present. As LMW surfactants can lower the surface tension more than proteins, they will compete for the surface area [4, 12, 13]. Proteins tend to form a viscoelastic network with variations in thickness and density. When a LMW surfactant approaches the surface, it will adsorb in the region with the lowest thickness and/or density or at a defect in the protein film [14, 15]. These initial adsorption locations act as nucleation sites for surfactant adsorption, leading to the growth of these nucleation sites into small domains. Subsequent expansion of the surfactant domains compresses the protein network, which initially

increases in density without increasing in thickness. The increased density of the protein film will discourage adsorption of LMW surfactants in locations other than the nucleation site. Eventually, when sufficient LMW surfactants have adsorbed, the protein is displaced from the surface [15, 16]. This mechanism is referred to as orogenic displacement [9, 17, 18].

Since OF-C16m is not a typical LMW surfactant, due to its high surface dilatational modulus and high foam stability, it is unknown whether the previously described mechanisms for mixed interfaces are also valid here. Therefore, the objective of this study was to investigate the functional properties of oligofructose palmitic acid esters (OF-C16m) in the presence of whey protein isolate (WPI), a commonly used food protein. Several techniques such as surface tension measurements, surface dilatational rheology, determinations of foam stability and Brewster angle microscopy were combined to gain insight into the functional properties of mixed systems. Two types of experiments were performed: competitive adsorption of WPI and OF-C16m and the disruption of a WPI protein network by OF-C16m.

2. Materials and methods

2.1 Materials

BiPRO Whey Protein Isolate (WPI) was obtained from Davisco Food (Le Sueur, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Merck (Darmstadt, Germany). Oligofructose palmitic acid mono-esters were prepared according to a previously described protocol [chapter 2], by esterification of palmitic acid with oligofructose with a degree of polymerization ranging from 2 to 8, using lipase as a catalyst.

2.2 Methods

2.2.1 Sample preparation

Samples were prepared by dissolving WPI and/or OF-C16m in a 10 mM phosphate buffer at room temperature. The WPI concentration was 0.5%, 1% or 2%, the OF-C16m concentration was 0.05%, 0.1% or 0.2%. For the mixed systems, the OF-C16m concentration was always 0.05%. All concentrations are expressed as % (w/v in the bulk).

2.2.2 Automated drop tensiometer

The surface tension and surface dilatational modulus of the air/water interface were monitored using an automated drop tensiometer (ADT, Teclis Tracker; ITCONCEPT, Longessaigne, France). Sinusoidal deformations with an amplitude of 5% and a period of 10 seconds were applied to an air bubble with an area of 10 mm^2. Five periods of oscillations were alternated with 5 periods of rest. The experiment was carried out at 22 °C ± 1 °C. The measurements were taken at least in duplicate, and if reproducibility was poor, the measurement was repeated a third time.

2.2.3 Foam stability

The foam stability was determined by pouring 15 mL of the sample into a glass foaming tube with a glass grid (pore size 40-100 µm) at the bottom and an inner diameter of 2 cm (L.G.S. B.V., Ubbena, the Netherlands). The mixture was aerated with nitrogen gas from the bottom of the tube. The flow rate was set to 10 mL/s with the help of a flow control device. Aeration was continued until the foam height reached 30 cm. After that, changes in foam and liquid height as well as foam structure were observed by eye as a function of time. Experiments were performed in duplicate and at room temperature. The error margin in the foam height was generally within 5%.

2.2.4 Brewster angle microscopy

A Brewster Angle Microscope (Multiskop, Optrel GBR, Sinzing, Germany) equipped with a Langmuir trough (MicroTroughXL, Kibron Inc, Espoo, Finland) was used to visualize the interface. The trough was filled with 100 mL of a 1% (w/v) WPI solution, and the interface was left to stabilize for one hour. Then, 1 mL OF-C16m (bulk concentration 0.2% (w/v)) was injected below the surface. The final bulk concentration of OF-C16m was 0.002% (w/v). The surface tension was continuously monitored using the Du Nouy-Padday method. Pictures were taken at 1 second intervals using a Brewster angle microscope set at 50.8° and the polaroid filter set at 240°, with a 10x magnification.

3. Results & discussion

3.1 Protein and surfactant competitive adsorption

In the first part of the study, the competitive adsorption of whey protein isolate (WPI) and mono-substituted oligofructose palmitic acid esters (OF-C16m) was studied. The surface tension, surface rheology and foaming capabilities of interfaces stabilized by pure WPI and pure OF-C16m as well as WPI/OF-C16m mixtures were examined.

3.1.1. Surface tension

The dynamic surface tension of WPI was independent of concentration (Figure 1A), showing that the surface was already saturated at the lowest bulk concentration. After formation of the air bubble, the surface tension quickly dropped, indicating that the proteins instantly adsorbed to the surface. After this, the surface tension slowly decreased further. This decrease may be explained by partial denaturation of the proteins at the interface [19]. The equilibrium surface tension between 45 and 48 mN/m is similar to values found in literature [4, 12, 17, 20-22].

The surface tension of the different OF-C16m stabilized interfaces was stable over time (figure 1B). The equilibrium was reached relatively fast, and did not significantly change after that. The surface tension of OF-C16m stabilized interfaces was lower than those stabilized by WPI, even though the concentrations of OF-C16m were a factor 10 lower than the concentrations of WPI. The lower surface tension shows that OF-C16m was able to form a more compact interfacial layer, which is typical for low molecular weight surfactants [1, 3]. Garofalakis et al. [23] have studied the surface tension of palmitic acid esters with different mono-, di- and oligosaccharides as the hydrophilic head group and found similar values (between 34 and 43 mN/m) for the surface tension.

When WPI (0.5% or 1%) was mixed with OF-C16m (0.05%), the surface tension was very close to the surface tension of an interface stabilized by pure OF-C16m (figure 1C). The similarity in surface tension between mixed systems and pure OF-C16m systems leads to the conclusion that the interface is predominantly covered by the OF-C16m. Even though the WPI is present at much higher concentrations in the bulk, the higher surface activity of the OF-C16m causes the preferential adsorption of OF-C16m at the interface. The preferential adsorption of LMW surfactants over proteins has been observed before [10, 16, 24, 25]. The surface concentration of WPI was sufficiently low as to have no influence on the surface tension. When 2% WPI was mixed with 0.05% OF-C16m, the surface tension (36 mN/m) deviated from the surface tension of the pure OF-C16m (30 mN/m). This difference suggests

that the surface concentration of WPI was high enough to have an influence on the surface tension, an effect that has been described before [26].

3.1.2 Surface dilatational modulus

The dynamic surface dilatational modulus of WPI showed two distinct regions. First, a sudden increase in modulus was observed followed by a period in which the modulus slowly increased even further (figure 2A). In the first region, the WPI started to adsorb at the interface and build a protein network, which was also reflected by the decrease in surface tension. In the second region, the surface tension change was less dramatic, indicating that no additional WPI was adsorbed at the interface. The increase in the modulus in the second region can be attributed to molecular rearrangements [3, 17]. Globular proteins generally form strong viscoelastic networks due to the formation of a dense layer with strong intermolecular interactions (hydrogen bonds, electrostatic and hydrophobic interactions) [17]. The values for the dilatational modulus that were found here are similar to those found by others [8, 27, 28].

The surface dilatational loss tangent, tan δ, was continuously lower than 1 (figure 2B), indicating that the elastic behavior was predominant over the viscous behavior, which was expected [21, 27, 29]. As time progressed, tan δ slowly decreased. This shows that the protein network became more and more elastic, which may be explained by the increase of crosslinks between the proteins that result from the previously mentioned molecular rearrangements. This slow decrease in tan δ was not observed for the sample with the lowest bulk concentration (0.5%). Because of the lower bulk concentration a less elastic network was formed. Rodriguez Patino et al. [27] found a loss tangent for this system of around 0.4, which is similar to our results.

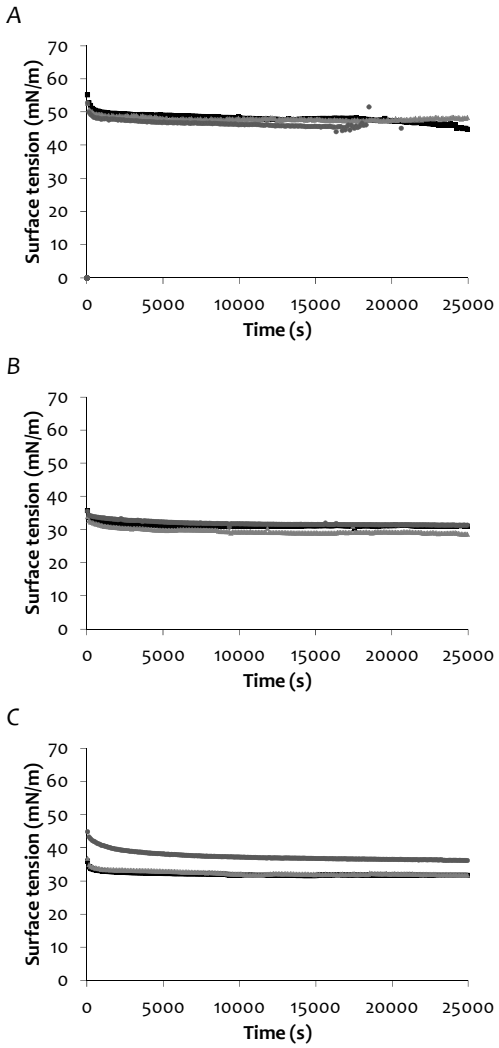

Figure 1: Dynamic surface tension of air/water interfaces stabilized by; (A) pure WPI at three different concentrations (■ 0.5%, ▲ 1% and ● 2%), (B) OF-C16m at three different concentrations (■ 0.05%, ▲ 0.1% and ● 0.2%), (C) WPI/OF-C16m mixtures. OF-C16m concentration was fixed at 0.05%, WPI concentration was ■ 0.5%, ▲ 1% or ● 2%.

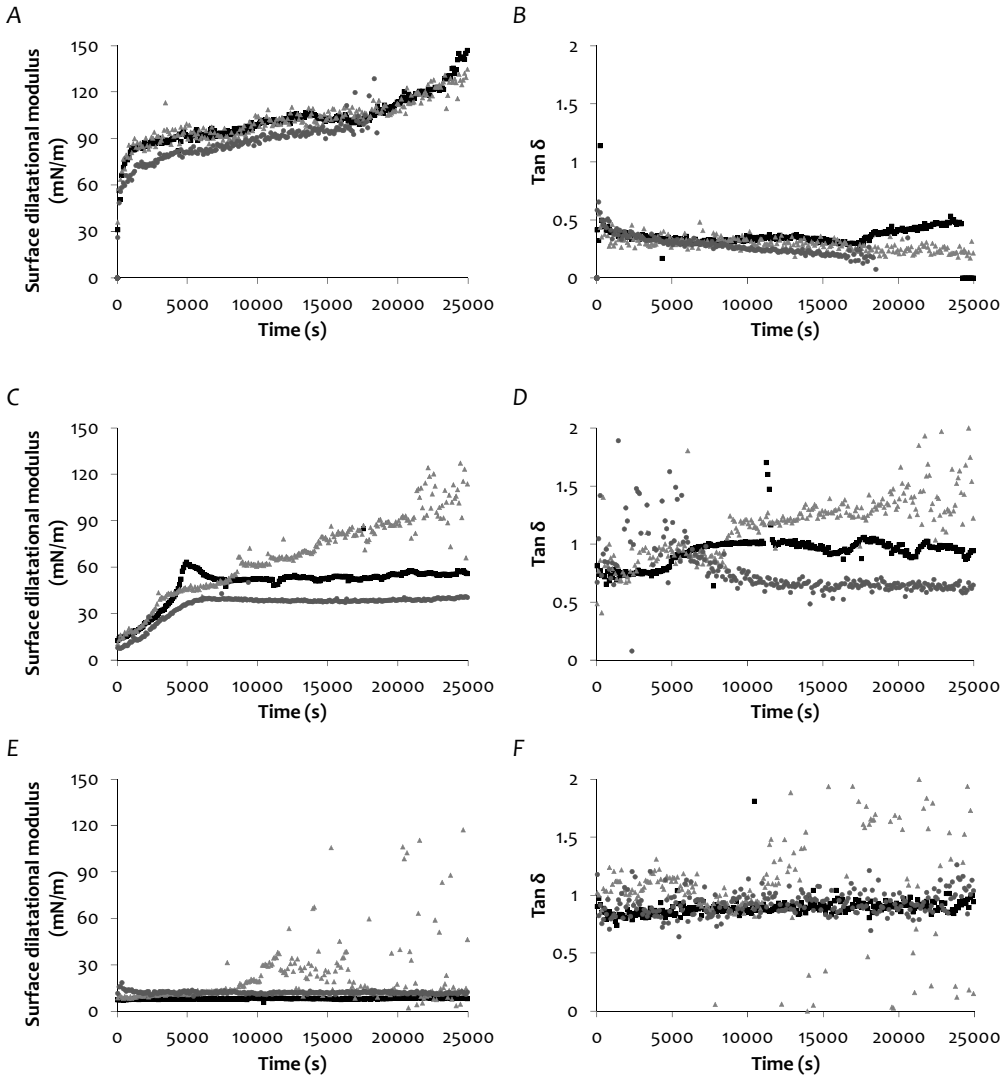

Figure 2: Dynamic surface dilatational modulus of air/water interfaces stabilized by; (A) pure WPI at three different concentrations (■ 0.5%, ▲ 1% and ● 2%), (C) pure OF-C16m at three different concentrations (■ 0.05%, ▲ 0.1% and ● 0.2%), (E) WPI/OF-C16m mixtures. OF-C16m concentration was fixed at 0.05%, WPI concentration was ■ 0.5%, ▲ 1% and ● 2%. Tan δ of; (B) pure WPI at three different concentrations (■ 0.5%, ▲ 1% and ● 2%), (D) pure OF-C16m at three different concentrations (■ 0.05%, ▲ 0.1% and ● 0.2%), (F) WPI/OF-C16m mixtures. OF-C16m concentration was fixed at 0.05%, WPI concentration was ■ 0.5%, ▲ 1% and ● 2%.

The surface dilatational modulus of OF-C16m (figure 2C) showed a slow but steady increase first, after which the modulus either decreased, remained at the same level, or slowly increased further. It is not likely that the increase in modulus is the result of elastic network formation like in the WPI. Like for a WPI stabilized interface, the modulus of an OF-C16m stabilized interface kept increasing while the surface tension already reached equilibrium. This indicates that the development of the modulus is not a result of adsorption of additional molecules to the interface, but rather of molecular rearrangements that lead to a higher connectivity parallel to the interface. This points to the development of the high dilatational modulus as a result of the formation of a soft two-dimensional glass phase at the interface.

If there is indeed a glass phase at the interface, it should be very brittle. Therefore, it is extremely important in a system stabilized by OF-C16m to make sure that any deformation is carried out within the linear regime. Deformations that are too large will result in structural rearrangements, that will affect the equilibrium value of the modulus (figure 2C) [30]. Previous studies (unpublished) on oligofructose stearic acid esters have already shown that amplitudes higher than 0.5% are outside the linear regime. Since in the current study an amplitude of 5% was applied and because of the structural similarity between the molecules in these studies and ours, we expect that also in this study the experiment is performed outside of the linear viscoelastic regime. This explains the discontinuities in the dynamic surface dilatational modulus and the lack of reproducibility (not shown). It also explains the relatively high value for tan δ (figure 2D).

It is important to consider that once a foam has been formed only small and slow deformations are applied to the interface. Therefore, it may be expected that in a foam system a full soft glass phase will develop. This structure will have a high value for the dilatational modulus.

The dynamic dilatational modulus of interfaces stabilized by WPI/OF-C16m mixtures is different from that of the two individual components (figure 2E). Although by means of different mechanisms, both WPI and OF-C16m provide a high surface dilatational modulus, while in the mixtures the value of modulus is continuously low. The surface tension values for the mixtures (figure 1C) indicate that the interface should be predominantly covered by OF-C16m. However, apparently there are sufficient protein molecules adsorbed at or just below the interface to prevent the soft glass formation of OF-C16m. This leads to the conclusion that if both OF-C16m and WPI are present, neither mechanism prevails since neither the WPI network nor the OF-C16m soft glass phase could completely cover the interface. The storage and loss modulus were equal (figure 2F), indicating that neither the

viscous or elastic behavior was predominant. This supports our hypothesis of protein molecules acting as structure blockers of the soft glass phase.

3.1.3 Foam stability

The foam stability of systems containing pure WPI, pure OF-C16m and WPI/OF-C16m mixtures was determined in glass foam tubes. After foam creation, three factors were observed over time: foam height, liquid height and foam structure.

All foams stabilized by pure WPI were immediately coarse. Over time, the foams quickly coarsened even more. To evaluate the influence of surface tension on the foamability and thus the initial foam structure, the first data points that could be acquired for surface tension (at 0.2 seconds) are listed in table 1. For all WPI concentrations, the initial surface tension was high. This high value may explain the coarse structure. Coarsening progressed until the bubbles became so large that the system could not be characterized as a foam anymore. Between 120 and 150 minutes, the foams collapsed. Although the foams did retain their height quite well for the first 100 minutes (figure 3A), the coarsening of the foam led to a significant reduction in foam quality.

Table 1: Surface tension at 0.2 seconds of air/water interfaces stabilized by pure WPI at three different concentrations; 0.5%, 1% and 2%, pure OF-C16m at three different concentrations; 0.05%, 0.1% and 0.2%, WPI/OF-C16m mixtures. OF-C16m concentration was fixed at 0.05%, WPI concentration was 0.5%, 1% or 2%.

Pure systems	Surface tension (mN/m)
0.5% WPI	60.7
1% WPI	59.1
2% WPI	58.5
0.05% OF-C16m	50.6
0.1% OF-C16m	41.3
0.2% OF-C16m	40.9

Mixed systems	Surface tension (mN/m)
0.05% OF-C16m + 0.5% WPI	44.2
0.05% OF-C16m + 1% WPI	47.2
0.05% OF-C16m + 2% WPI	51.6

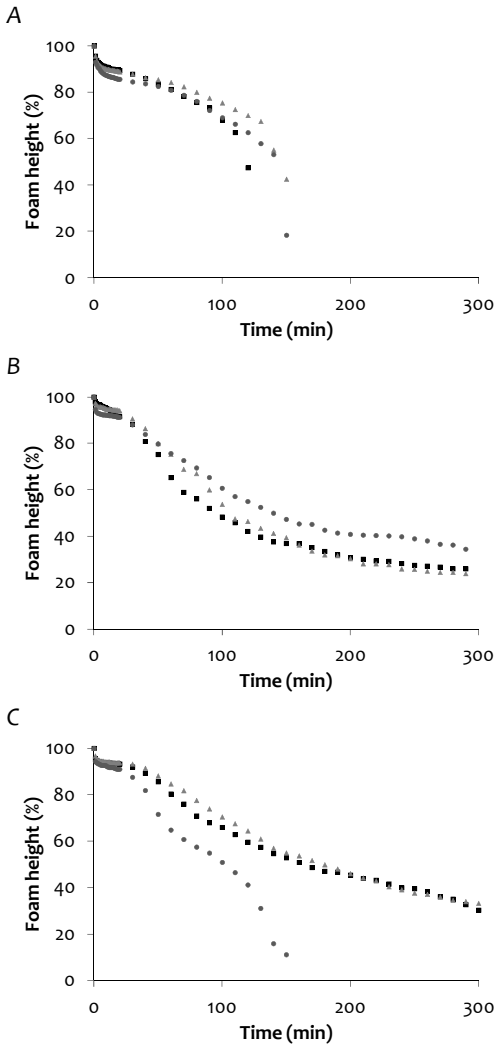

Figure 3: Foam stability of; (A) pure WPI at three different concentrations; ■ 0.5%, ▲1% and ● 2%, (B) pure OF-C16m at three different concentrations; ■ 0.05%, ▲ 0.1% and ● 0.2%, (C) WPI/OF-C16m mixtures. OF-C16m concentration was fixed at 0.05%, WPI concentration was ■ 0.5%, ▲1% or ● 2%.

The foam height of pure OF-C16m systems (figure 3B) was slightly lower than that of pure WPI. However, there was a clear difference in foam structure. All OF-C16m stabilized foams showed a uniform bubble size distribution with small bubbles. The initial surface

tension of these systems was much lower than the pure WPI systems (table 1). This lower surface tension can explain the finer bubble size distribution. During the 300 minutes in which the foam was monitored, hardly any changes in bubble size could be observed. After 150 minutes, when the WPI foams already collapsed, the OF-C16m foams were still stable. Even after 24 hours, there was still a small amount of foam present. The lack of change in bubble size distribution shows that OF-C16m can adequately prevent disproportionation, which is not common for LMW surfactants. The ability to prevent disproportionation is often claimed to be related to a high dilatational modulus [31]. However, recent research by Erni et al. [32] shows that a high dilatational modulus does not necessarily prevent disproportionation.

The foam stability of the mixtures of WPI and OF-C16m was different for 0.5% and 1% compared to 2% WPI (figure 3C). Foams stabilized with 0.05% OF-C16m and 0.5% or 1% WPI had a very fine initial foam structure with very little coarsening. The foam height of these two products was slightly higher than that of the pure OF-C16m foams. The very fine foam structure is likely the result of the low initial surface tension that was obtained for these mixtures (table 1). The initial surface tension of the mixed systems was lower than the initial surface tension of the pure systems. The lower surface tension points to the formation of a mixed interface. With higher protein concentrations in the bulk, the initial surface tension is increased. This increase in surface tension points to an increase in ratio between protein and surfactant at the interface. Over time, the surface tension values of the mixtures with 0.5% WPI and 1% WPI return to values similar as the pure OF-C16m (figure 1C). This indicates that WPI is displaced from the interface by OF-C16m and that the surface concentration of WPI is low. The presence of a small amount of WPI molecules at the interface could prevent the formation of the glass phase of the OF-C16m and thus lead to a low dilatational modulus. However, despite the low surface dilatational modulus, there was little coarsening. Apparently, the influence of the dilatational modulus on disproportionation is much lower than expected. Therefore, it is likely that the air bubbles in the foam are stabilized by the Gibbs-Marangoni mechanism. This is a tentative conclusion, since there are other factors that could have had an influence on foam stability, for example the width of the initial bubble size distribution. If these mixtures had a much narrower bubble size distribution than the pure component interfaces, disproportionation would also be slower. Since reliable data on the size distribution cannot be obtained from the simple foaming experiment we used here, we could not assess the effect of this parameter.

Foams stabilized with 0.05% OF-C16m and 2% WPI were immediately very coarse. Over time, the foams quickly coarsened, even more than the pure WPI foam. Also, the foam

height was lower than either of the pure solutions. The dilatational modulus of the 2% WPI mixture was similar to that of the 0.5% and 1% WPI mixtures. As initially a mixed interface is formed, over time, more and more WPI is displaced from the interface. The equilibrium surface tension of the 2% WPI mixture was higher than the 0.5% or 1% WPI mixture, or the pure OF-C16m. This indicates that in the mixture with 2% WPI, there are still sufficient protein molecules present at the interface to decrease the mobility of the OF-C16m and hence also decrease the stabilizing Gibbs-Marangoni effect [18, 25]. This could explain the large difference in foam stability between the 0.5% and 1% WPI mixtures as compared to the 2% WPI mixture.

Apparently the concentration of WPI has a significant effect on the stability of mixed samples, which was also found by other authors [6, 17, 33]. Also, it was shown before that a high protein/surfactant ratio results in an unstable foam, while a stable foam can be produced when the ratio is such that the surfactant can stabilize the entire surface [17, 18, 34].

3.2 Protein displacement by surfactant

Results from section 3.1 indicate that during the competitive adsorption of WPI and OF-C16m, OF-C16m will dominate the interface. However, when a WPI network with a high dilatational modulus has already been formed at the interface, it may be more difficult for the OF-C16m to access the interface [3, 35].

Therefore, in the second part of the study the displacement of WPI by OF-C16m was studied. The surface tension, surface dilatational modulus and visual appearance of an air/water interface stabilized by WPI were studied, while adding OF-C16m to the subphase.

3.2.1 Surface tension and surface dilatational modulus

To study the displacement of WPI from the interface by OF-C16m, the surface tension of pure WPI was monitored (figure 4A). After 15000 seconds, OF-C16m was introduced into the subphase after which an immediate drop in surface tension could be observed. This indicates that OF-C16m is capable of displacing WPI from the interface. It has been observed by other authors that most LMW surfactants, with a lower surface tension than proteins, can displace proteins from interfaces [1, 16, 36]. Closer examination of the surface tension drop upon the introduction of OF-C16m reveals that the surface tension is more quickly reduced in the 1% WPI system compared to 2% WPI system. Also, the equilibrium surface tension value is slightly higher for 2% WPI than for 1% WPI. This higher

surface tension in a system with 2% WPI was also obtained during the competitive adsorption (figure 1C).

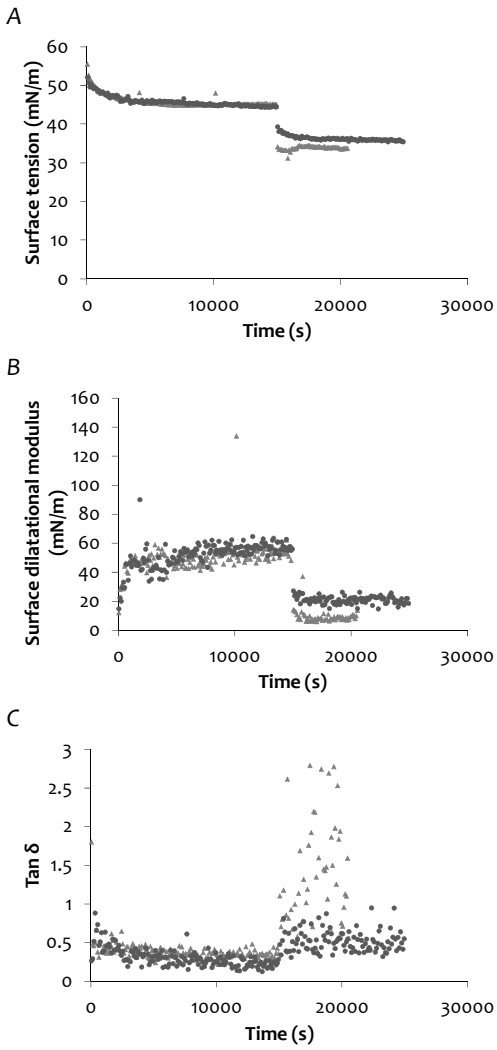

Figure 4: Surface tension (A), surface dilatational modulus (B) and tan δ (C) of WPI at two different concentrations (▲1% and ● 2%) followed by the introduction of 0.05% OF-C16m at 15000s.

The data on surface dilatational modulus support the findings from the surface tension measurements (figure 4B). When only WPI was present in the system, the modulus slowly built up until it reached equilibrium. After the introduction of OF-C16m at 15000 seconds, there was a quick decrease in the modulus. It reached a value that was similar to the value that was obtained during competitive adsorption of the two components (figure 2E). Similar results were obtained by Rouimi et al. [17] when studying the competitive adsorption of milk proteins and sucrose esters. The quick decrease in modulus coincided with an increase in tan δ (figure 4C). This indicates that the protein network that was present at the interface was disrupted when OF-C16m was introduced to the subphase. After this, neither a protein network nor a soft glass phase stabilized the interface. The values for tan δ (figure 4C) show very similar values compared to the systems in which both components adsorbed competitively (figure 2F). The modulus value for the 2% WPI system (figure 4B) is slightly higher than for the system where both components adsorbed competitively (figure 2E). This higher value for modulus leads to the conclusion that once a strong protein network has formed, it becomes more difficult for OF-C16m to completely displace it.

3.2.2 Brewster angle microscopy

The interface was studied using Brewster angle microscopy (BAM), to visualize structural changes that occur when OF-C16m is introduced to a WPI stabilized interface. During these microscopic observations, also the surface tension was monitored.

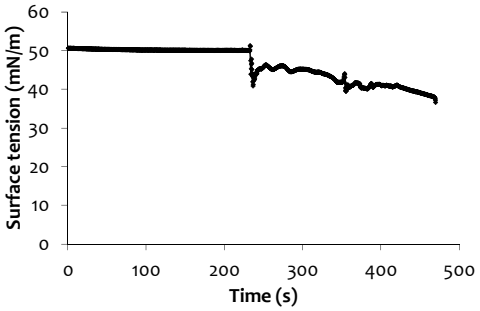

Figure 5: Surface tension an air/water interface stabilized by 1% WPI followed by the introduction of 0.002% OF-C16m at 233s. Experiment was performed simultaneously with the Brewster angle microscope experiment (figure 6).

The WPI solution was left to equilibrate for 60 minutes, after which the surface tension measurement was started (figure 5). At 233 seconds after the start of the surface tension measurement, OF-C16m was introduced in the system. Although the concentrations of WPI (1%) and OF-C16m (0.002%) differed even more than in the previous experiment (figure 4), surface tension still quickly decreased. This decrease shows that even a small amount of OF-C16m can displace WPI from the interface. However, the decrease in surface tension is slower and the surface tension does not decrease as much as with a higher surfactant concentration. These results demonstrate that it is more difficult to displace a protein network at lower surfactant concentrations.

Figures 6A-D represent BAM images of the WPI stabilized interface before OF-C16m was added. The images were taken at 1 second intervals. Reflections from the laser and the bottom of the trough are clearly visible. These images do not show many interfacial features, with the exception of dust particles. They are indicated with a circle. There is hardly any difference between images 6A-D, indicating that the interface was immobile. A protein network is expected to be immobile. Images 6E-H show the interface after adding OF-C16m. Immediately after OF-C16m was added, the interface started to become mobile. This mobility is reflected in the change of the position of the dust particles between images 6E-H. The results are in agreement with the rheological results that showed a quick decrease in modulus upon the addition of OF-C16m to a WPI-stabilized interface. A similar result was found by Garofalakis et al. [8] when studying the displacement of β-lactoglobulin by sucrose esters. In their work, dust particles were also immobilized when a pure protein network was present at the interface, while mobility also increased after surfactant was added.

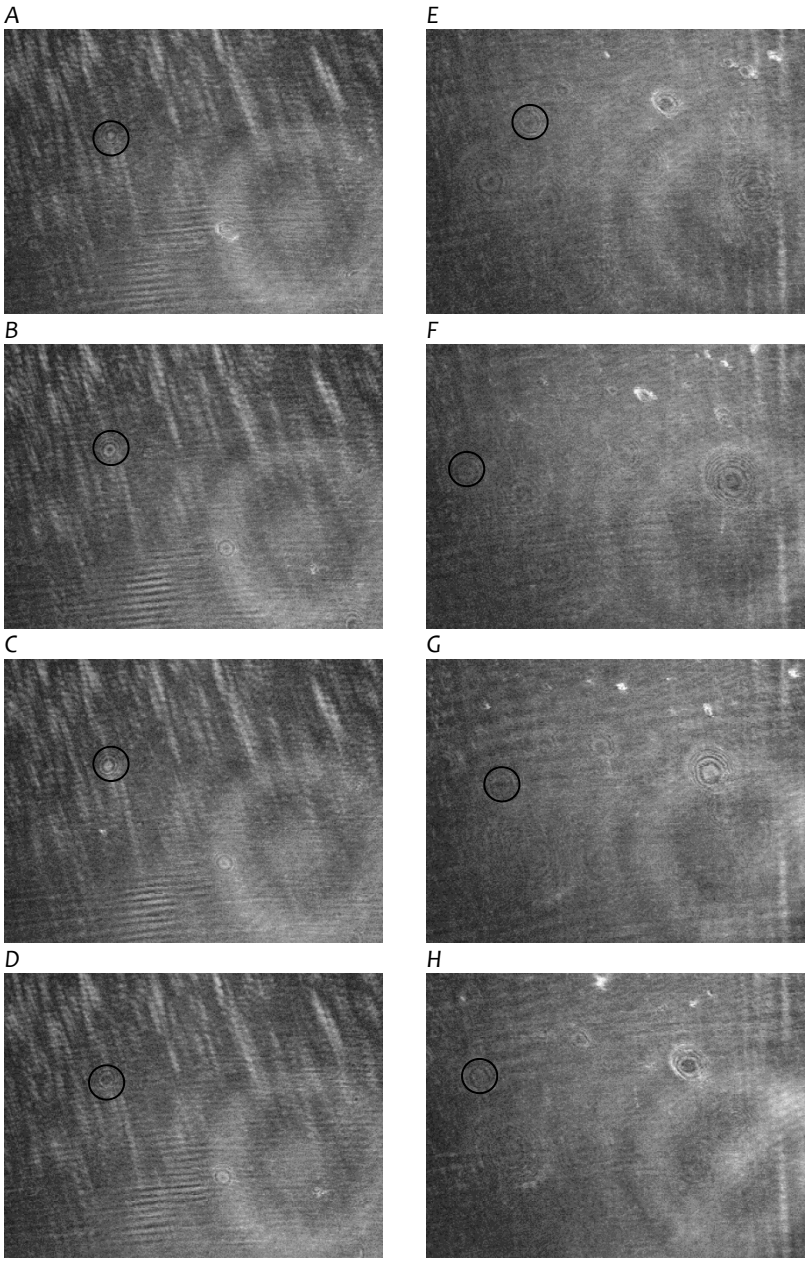

Figure 6: Brewster angle microscopy pictures of an interface stabilized by pure WPI (A-D), and of the interface after the introduction of OF-C16m (E-H). A-D and E-H were taken successively with 1 second intervals. Dust particles are indicated with a circle.

In these images (Figures 6E-H), no evidence of the mechanism of displacement can be found. In the case of orogenic displacement, the displacement of proteins by LMW surfactants leads to the formation of slowly growing domains of LMW surfactants. These domains eventually completely displace the protein. These surfactant domains have been visualized before by AFM [37, 38] and BAM [39, 40]. Depending on both protein and surfactant type and surface concentration, the size of the surfactant domains in protein films varies from several tens of nanometers to several tens of micrometers. More specifically, surfactant domains of a few micrometers in size were found when β-lactoglobulin was displaced by a non-ionic surfactant (Tween 20/Tween 60) [15, 39]. Since the magnification of the Brewster Angle Microscope that was used in this study was only 10 times, surfactant domains may have been present but simply were not large enough to be seen. Furthermore, it is possible that, under the current experimental conditions, the relative reflectivity of the OF-C16m is similar to that of the protein. Any domain would then be invisible for the Brewster angle microscope in this case. Although observations of the interface using Brewster Angle Microscopy could not give a decisive answer about the mechanism of displacement, they do support the main result of this study: the sudden increase in interfacial mobility upon the addition of surfactant. This has been found before for other systems [39, 41].

4. Conclusions

In this study, the foam stability and interfacial properties of systems containing OF-C16m were studied in the presence of WPI. The studies were performed in two ways: competitive adsorption of both species from the bulk and displacement of a WPI stabilized interface by OF-C16m.

During the competitive adsorption of WPI and OF-C16m, the initial surface tension values were lower than the surface tension values in either pure system, which points to the formation of an initially mixed interface. The ratio between the initial surface concentration of WPI and the surface concentration of OF-C16m was dependent on the bulk concentration of WPI, as evidenced by the surface tension values. When both OF-C16m and WPI were present, neither stabilization mechanism (elastic network formation for WPI and surface solidification for OF-C16m) prevailed. This resulted in a low modulus. Over time, as the surface tension of the system got closer to that of a pure OF-C16m stabilized system, more and more WPI was displaced by OF-C16m. Because at the lowest WPI concentrations the equilibrium surface tension was similar to that of a pure OF-C16m stabilized system, the equilibrium surface concentration of WPI was low. But apparently there was still sufficient protein adsorbed at or just below the interface to prevent the OF-C16m from forming a soft glass phase. The foam stability for these systems was high. Probably the interfaces were stabilized by the Gibbs-Marangoni mechanism. In contrast, at the highest WPI concentration, it is likely that the equilibrium surface concentration of WPI was sufficiently high to decrease the interfacial mobility of OF-C16m. The lack of mobility could have affected the stabilizing Gibbs-Marangoni mechanism, which lead to a decrease in foam stability. Results from the second type of experiment showed that OF-C16m was also capable of displacing a fully developed WPI network from the interface.

The aim of the current study was to study the functional properties of OF-C16m in a protein containing system. When the WPI concentration was not too high, the interface was dominated by OF-C16m, also when a WPI network had already been formed at the interface. Also, under these conditions, the foam stability was high. In the current study, a very low OF-C16m concentration was used. We expect that with higher OF-C16m concentrations the interfaces will be dominated even more by OF-C16m, also at higher protein concentrations. Although proteins are not the only food ingredients that possibly influence the functionality of the esters, the results indicate that OF-C16m could be successfully used to stabilize a protein containing food product.

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Chapter 7

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Effect of variations in the fatty acid chain of oligofructose fatty acid esters on their foaming functionality

Abstract

In this chapter the influence of variations in the fatty acid chain of oligofructose fatty acid esters (OFAE) on foamability and foam stability is described. First, oligofructose (OF) mono-esters containing saturated fatty acid chains ranging between C4 and C18 were studied. Additionally, a mono-ester containing a C16 mono-unsaturated fatty acid chain and a C12 di-ester were studied. Finally, to investigate the influence of the size of the hydrophilic group, commercially available sucrose esters were studied. The surface tension and surface rheological properties of air/water interfaces stabilized by the esters were determined, as well as the foaming properties of the esters at a bulk concentration of 0.2% (w/v). OF mono-esters with intermediate fatty acid chain lengths (C10-C16) were able to migrate quickly to the interface producing foams with small bubbles (0.4 mm), a relatively narrow bubble size distribution, and a high stability. For oligofructose mono-esters containing fatty acids C4 and C8, the bulk concentration of 0.2% (w/v) was below the CMC, resulting in insufficient surface coverage, and low foamability and foam stability. The OF C18 mono-ester and the OF C12 di-ester were slow to migrate to the interface resulting in low foamability. Despite similar surface tension values, the foam half-life time of OFAE was higher than of the corresponding sucrose esters. OFAE had higher surface dilatational moduli compared to sucrose esters. Based on the frequency dependency of the modulus and analysis of Lissajous plots, we propose that OFAE may be forming a soft glass at the interface.

1. Introduction

Foams are dispersions of gas in a continuous phase [1, 2] and are thermodynamically unstable [3]. Such a dispersed system will always try to minimize its surface area [2], resulting in foam destabilization. Foam destabilization occurs through drainage and coarsening, and the latter may be subdivided into coalescence and disproportionation [1, 4, 5]. Foam drainage is the flow of liquid through channels and nodes between bubbles, and is driven by gravity and capillarity [4, 5]. Drainage can be slowed down significantly when the viscosity of the continuous phase is increased [2]. This often leads to satisfactory foam stability [6]. However, increasing the viscosity of the continuous phase changes the texture of the foam which is not always desired. The first form of coarsening, coalescence, is the merging of two bubbles into one, and is accompanied by the rupture of the film between the two bubbles. Rupture can, for example, be prevented by using film-stabilizing surfactants such as proteins [4]. The second form of coarsening, disproportionation, occurs when gas flows from small bubbles with a high Laplace pressure to large bubbles with a low Laplace pressure [1]. The exchange of gas will only have a significant effect on stability when the films separating the bubbles are thin [4]. Of all instability mechanisms, disproportionation is most difficult to prevent. An interface with high elasticity could provide an energy barrier that slows down bubble shrinkage [1, 2]. It is important to realize that the instability mechanisms affect each other: drainage is followed by a close approach of neighboring bubbles which promotes coalescence and leads to foam collapse, loss of gas and loss of structure [1]. Furthermore, coarsening can accelerate drainage [1, 4].

The stability of foams can be greatly improved by the addition of surface active molecules. Surface active molecules used in foods to stabilize interfaces and foams are customarily divided in two classes: low molecular weight (LMW) surfactants, that form a compact layer with low surface tension, and proteins, that typically form a visco-elastic irreversibly adsorbed layer [3, 6]. LMW surfactants are more effective in lowering surface tension than proteins. However, foams stabilized by LMW surfactants are generally less stable against coalescence than foams stabilized by proteins, because LMW surfactants lack the ability to form a highly visco-elastic layer that provides steric stabilization [7]. Therefore, proteins are the most widespread foaming agents used in foods [1, 2].

Foam formation has a typical characteristic time scale of sub-milliseconds [3]. Therefore, the ability of a surfactant to adsorb rapidly is critical for foam formation. Surfactants adsorb most rapidly when they are in their monomeric form. However, at higher concentrations surfactants tend to form aggregates in the bulk. The concentration at which

aggregates are formed and the morphology of these aggregates greatly differs, depending on surfactant structure. The concentration at which aggregates are formed is referred to as CMC, if the aggregates are micellar, or critical aggregation concentration (CAC), if the aggregates have some other morphology such as vesicles or lamellar phases. These latter types of aggregates are generally formed if the monomeric solubility is very low [6]. In systems with a low CMC/CAC, the amount of monomers in the bulk will be low and the majority of molecules will be present in the form of aggregates. This will lead to slower adsorption at the interface. The speed of surfactant adsorption also depends on molecular weight, due to its influence on diffusion speed [8].

The correlation between surface rheological parameters and the formation and stability of foams has been the subject of investigation for many years. The conditions that are used during rheological measurements differ significantly from the conditions during foam formation in terms of time scales, stresses, strain and rate of strain [7]. Conditions during foam formation are often turbulent and non-equilibrium. Therefore, the correlation between foam formation and surface rheology may be very weak or even non-existent [2, 6, 7, 9, 10]. The link between surface rheological parameters and foam stability under quiescent conditions may be stronger [7].

In previous chapters, we have reported the properties of air/water interfaces stabilized by oligofructose fatty acid esters. They are surface active components that can be synthesized with a wide range of amphiphilicity and thus functionality. This wide range is accomplished by changing the fatty acid chain length and the degree of esterification. The oligofructose that was used was a mixture of oligomers with a degree of polymerization varying between 2 and 8 (average 4.4). Consequently, the resulting esters are also mixtures. We have studied the influence of the length of the fatty acid chain on functional properties and found that the equilibrium surface tension and surface dilatational modulus of air/water interfaces stabilized by oligofructose fatty acid esters are highly dependent on the surfactant structure, giving a low surface tension and a high dilatational modulus for the esters consisting of C16 and C18 fatty acids. This combination of low surface tension and high modulus makes them promising novel food-grade surfactants.

The aim of this study was to investigate how variations in the fatty acid chain, and thus interfacial properties, influence the foam stabilizing capabilities of oligofructose fatty acid esters. We have examined the effect of the degree of esterification, the fatty acid chain length, the degree of saturation in the fatty acid chain, and the size of the hydrophilic group. Hereto we have determined the surface tension one second after creation of the interface. We have also determined the surface tension and surface dilatational modulus at

equilibrium. Furthermore, we have determined the frequency and amplitude dependency of the modulus. Finally, we have established the foamability and stability of foams produced from aqueous solutions of the esters.

2. Materials and methods

2.1 Materials

Palmitoleic acid, vinyl butyrate and vinyl decanoate were obtained from Sigma Aldrich (Steinheim, Germany). Oligofructose fatty acid esters were prepared according to a previously described protocol [chapter 2], by esterification of caprylic acid (C8), lauric acid (C12), palmitic acid (C16) and stearic acid (C18) with oligofructose with a degree of polymerization ranging from 2 to 8, using lipase as a catalyst. The crude products contained mostly mono-esters, but also small amounts of di-esters. The products were fractionated to separate the fractions with different degrees of esterification. MALDI-TOF MS and NMR confirmed the purity (>90%) of the purified fractions [chapter 2]. Additionally, an ester with palmitoleic acid (C16:1) as the hydrophobic group was produced using the procedure as previously described for palmitic acid esters [chapter 2]. Furthermore, esters of butyric acid (C4) and capric acid (C10) were prepared by transesterification of vinyl esters of the fatty acids to the oligofructose. The reaction conditions were identical, except in this case a mixture of 20/80 v/v DMSO/Bu^{t}OH was used, instead of the previously described 10/90 v/v [chapter 2]. The C4 and C10 esters were fractionated using reverse phase solid phase extraction, as previously described [chapter 2], only with minor changes to the water/methanol gradient that was used. Using MALDI-TOF MS [chapter 2] we found a purity of 100% for esters containing the fatty acids C16:1, C4 and C10.

Sucrose monolaurate (≥97%) was obtained from Sigma Aldrich (Steinheim, Germany). Sucrose esters with fatty acid chain lengths C16 and C18 (RYOTO esters S1670 and P1670) were obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Both S1670 and P1670 are crude products. According to the manufacturer, S1670 consist of 75% mono-esters and 25% di-, tri-, and poly-esters. The fatty acids consist for 70% of stearic acid. P1670 consists of 80% mono-esters and 20% di-, tri-, and poly-esters. The fatty acids consist for 80% of palmitic acid. These products were used without further purification.

2.2 Methods

2.2.1 Sample preparation

Samples were prepared by dissolving the surfactant in Millipore water (Millipore corporation, Billerica, Massachusetts, USA) at a bulk concentration of 0.2% (w/v). For esters with longer fatty acid chains (C16 and C18) mild heating (max. 70 °C) was applied to improve the dispersing of the esters in the liquid.

2.2.2 Determination of surface tension and surface dilatational modulus

The surface tension and surface dilatational modulus of the air/water interface were determined with a profile analysis tensiometer (Sinterface, Berlin, Germany). About one second after drop creation, the equipment took the first measurement. This value was taken as the surface tension one second after interface creation. During time sweeps, sinusoidal oscillations with an amplitude of 5% and a frequency of 0.1 Hz were applied. The experiments consisted of 25 cycles of oscillations that were alternated with 1 cycle of rest. The time sweeps were followed by a frequency sweep where the frequency was increased stepwise from 0.005 Hz to 1 Hz, at an amplitude of 5%. The slope of a double logarithmic plot of surface dilatational modulus versus frequency was determined using linear regression. After the frequency sweeps, amplitude sweeps were performed where the amplitude was increased from 1.5% to 30% at a frequency of 0.1 Hz. The raw data from these amplitude sweeps were used to construct Lissajous plots. All experiments were performed at 25 °C. Reported values represent an average of 2 measurements for most measurements. Only when reproducibility was insufficient (for S-C12m, OF-C8m and OF-C12m), additional measurements were performed (up to 8).

2.2.3 Foam stability

The foam stability was determined by pouring 15 mL of the sample into a glass foaming tube with a glass grid (pore size 40-100 µm) at the bottom and an inner diameter of 2 cm (L.G.S. B.V., Ubbena, the Netherlands). Aeration was performed with nitrogen gas from the bottom of the tube. Aeration was continued until the foam height reached 30 cm. Pictures were taken in the middle of the foam column using a commercially available camera. Changes in foam and liquid height as well as foam structure were observed as a function of time. The foam half-life time is specified as the time at which the foam height was 15 cm. All experiments have been performed in duplicate.

3. Results & discussion

In this study, the surface tension and surface dilatational modulus of air/water interfaces stabilized by oligofructose fatty acid esters with variations in the fatty acid chain were examined at a bulk concentration of 0.2% (w/v). Furthermore, the foaming properties of these esters have been determined. We have used oligofructose fatty acid mono-esters containing fatty acids ranging between C4 and C18. These are designated as OF-C4m, OF-C8m, OF-C10m, OF-C12m, OF-C16m, and OF-C18m. To study the influence of the degree of saturation of the fatty acid chain, a mono-ester with the mono-unsaturated palmitoleic acid as the hydrophobic group, designated as OF-C16:1m, was tested. To investigate the influence of the degree of esterification a di-ester with fatty acid chain length C12, designated as OF-C12d, was tested. To study the influence of the size of the hydrophilic group, sucrose esters were also included. These were a mono-ester with fatty acid chain length C12, designated as S-C12m, and two crude samples composed of a mixture of mono-esters and di-esters with fatty acid chain length C16 and C18, designated as S-C16 and S-C18.

3.1 Surface properties

3.1.1 Surface tension

The equilibrium surface tension of air/water interfaces stabilized by sugar esters (both sucrose esters and oligofructose esters) varied for esters with different fatty acid chain lengths, but did not vary when the size of the hydrophilic group, the degree of esterification, and the degree of saturation of the fatty acid chain were changed. For esters with fatty acid chain lengths of C10 and longer, the equilibrium surface tension was close to 30 mN/m (Figure 1A). For the esters with shorter fatty acid chain lengths (OF-C4m and OF-C8m), the equilibrium surface tension increased as the fatty acid chain length decreased. This deviation of OF-C4m and OF-C8m may be attributed to a lower surface concentration. The critical micelle concentration (CMC) of the esters is dependent on the fatty acid chain length: esters with a longer fatty acid chain are expected to have a lower CMC due to their increased hydrophobicity. The CMC of OF-C12m was estimated to be around 0.29%, while the CMC of OF-C8m was estimated to be around 2.4% [chapter 3]. Since the bulk concentration of the esters during this study was 0.2%, OF-C4m and OF-C8m are significantly below the CMC. This means that the interface was not fully saturated, which explains their much higher surface tension.

For a surfactant to be a good foam stabilizer, it is essential that it migrates to the interface quickly. The equilibrium surface tension is not relevant during foam formation, because the time scales are so short that equilibrium will not be reached [11]. The relevant

time scale for foam formation is shorter than the time scale that can be recorded using the profile analysis tensiometer. However, the first value that is recorded by the equipment (around 1 second after creation of the interface) gives qualitative information about the speed of adsorption.

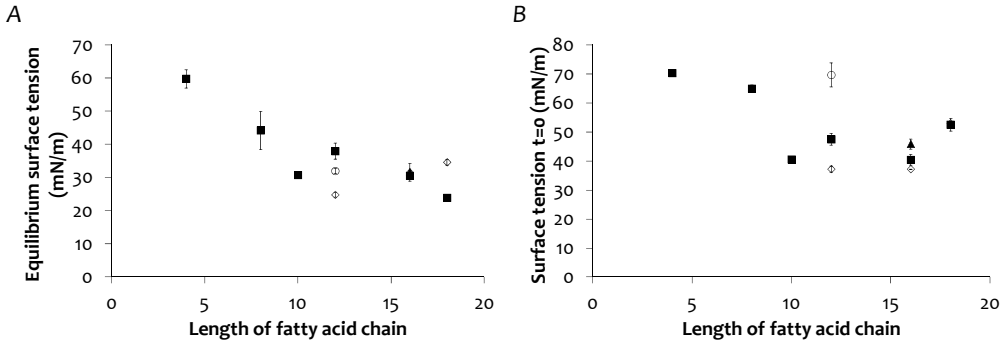

Figure 1: Equilibrium surface tension (A), and surface tension about 1 second after the creation of the interface (B) for air/water interfaces stabilized by sugar fatty acid esters with different fatty acid chain lengths. Oligofructose mono-esters (■), mono-unsaturated oligofructose mono-esters (▲), oligofructose di-esters (○) and sucrose esters (◊).

Only sucrose esters and oligofructose mono-esters with intermediate fatty acid chain length (C10, C12 and C16) were able to reach a low surface tension at a short time scale (figure 1B). Since the concentration of esters with the shortest fatty acid chain lengths (OF-C4m and OF-C8m) was significantly below the CMC, the interfaces were not saturated, resulting in a high surface tension. The adsorption speed is determined by the molecular size due to its influence on the diffusion rate [8]. Therefore, with increasing fatty acid chain length the adsorption speed should decrease. More importantly, molecules with higher hydrophobicity have a lower CMC [12-15]. Therefore, with increased fatty acid chain length or degree of esterification, a smaller amount of molecules will be present in the form of single molecules which results in a slower transport to the interface [16]. Therefore, at short time scales a higher surface tension is expected for mono-esters with a longer fatty acid chain or di-esters. This is indeed observed for the C18 mono-ester and the C12 di-ester. No significant differences are observed between esters with chain lengths C10, C12 and C16 (both for oligofructose esters and sucrose esters). It is likely that these differences are only visible at the sub-second time scale which could be not be measured using our equipment.

3.1.2 Surface rheology

Next, a rheological characterization of the air/water interfaces stabilized by the sugar fatty acid esters was performed.

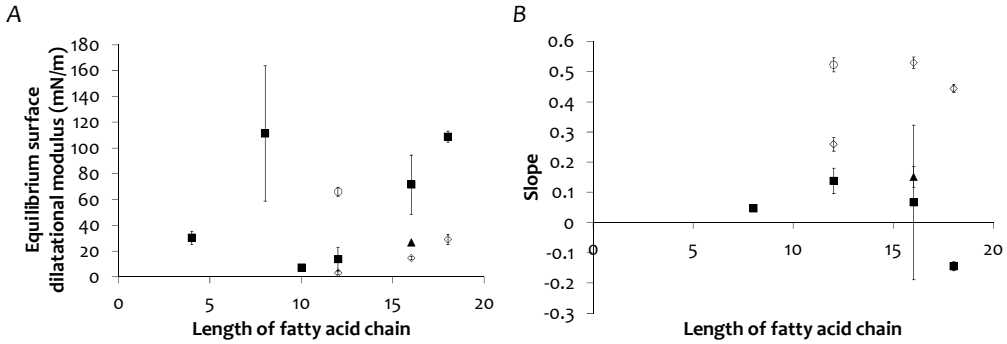

Figure 2: Surface dilatational modulus (A) and slope of a double logarithmic plot of surface dilatational modulus versus frequency (B) for air/water interfaces stabilized by sugar fatty acid esters with different fatty acid chain lengths. Oligofructose mono-esters (■), mono-unsaturated oligofructose mono-esters (▲),oligofructose di-esters (○) and sucrose esters (◊).

First, the surface dilatational modulus was determined during time sweeps (figure 2A). For all sucrose esters, a relatively low surface dilatational modulus was found. For the oligofructose esters, there were significant differences in the modulus. A high dilatational modulus was found for some of the esters (for example OF-C8m, OF-C12d and OF-C18m) while a low dilatational modulus was found for others (for example OF-C12m). To be able to explain the origin of these differences in terms of interfacial microstructure, additional rheological characterizations were performed in the form of frequency sweeps and amplitude sweeps. For the frequency sweeps, the slope of a double logarithmic plot of surface dilatational modulus versus frequency was determined. The value of this slope is presented in figure 2B. Both S-C18 and S-C16 had a slope close to 0.5, which is consistent with surface processes that are diffusion-controlled, according to the Lucassen van den Tempel model [17]. This would point to an interface without any mesophase formation, typical for LMW surfactants. For S-C12m an intermediate slope of around 0.25 was found. It is likely that also for S-C12m the surface processes are diffusion controlled. For OF-C12d a high slope close to 0.5 was obtained, similar to the sucrose esters. In contrast, for the oligofructose mono-esters low slopes close to 0.1 were found. One exception was the

oligofructose mono-ester with chain length C18 where a negative slope was found. See below for further elaboration on this negative value.

The raw data from the amplitude sweeps were used to construct Lissajous plots. There is an important difference in the rheological response of oligofructose esters and sucrose esters. In figure 3 this difference is demonstrated for esters with chain length of C12. These plots are representative for the esters with other chain lengths. An interface stabilized by S-C12m had a response that barely could be detected and that did not differ in compression or extension (figure 3A). This is typical behavior for small surfactants, where the dilatational response is governed by the speed of adsorption of the molecules. Despite the frequency scaling exponent close to 0.25 for S-C12m, we conclude that also this molecule behaves like a typical LMW surfactant, similar to S-C16 and S-C18. In contrast, an interface stabilized by OF-C12m had a much different rheological profile (figure 3B). There is a clear difference between the response in compression and the response in extension. During extension, the curve becomes less steep with increasing deformation which shows that the interface is strain softening. During compression, the curve becomes more steep with increasing deformation which shows that the interface is strain hardening.

The low slope in the frequency dependency combined with the characteristic shape of the Lissajous plot points to the possible formation of a soft glass phase by the oligofructose groups [chapter 4], [18]. This interfacial glass structure is expected to be quite brittle, making it sensitive to structure degradation when deformations are too high. This could explain the fact that at the same strain for some esters a high modulus was found while for others a low modulus was found. Furthermore, it could explain the negative value of the slope for OF-C18m; during the frequency sweep the structure was affected due to the fact that the measurement was performed in the non-linear regime.

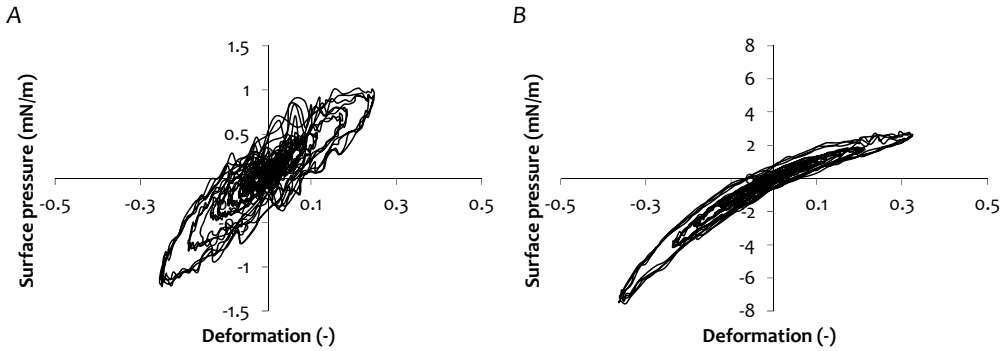

Figure 3: Lissajous plots obtained during amplitude sweeps of air/water interfaces stabilized by sucrose esters (A) and oligofructose mono-esters (B) with fatty acid chain length C12.

3.2 Foaming properties

3.2.1 Foamability

The foaming properties of the esters were tested in glass foam tubes with a glass grid at the bottom. The foams were aerated from below until a foam height of 30 cm was reached. After that, the foam and liquid height as well as the foam structure were monitored as a function of time. With all esters except OF-C4m and OF-C12d, it was possible to reach the designated foam height of 30 cm (figure 4). For OF-C4m, during aeration approximately 1 cm of foam was formed. However, when aeration was discontinued, the foam collapsed immediately. For OF-C12d, it was not possible at all to form a foam. While the foams stabilized by esters with a fatty acid chain length of C8, C10 and C12 did not drain significantly during foam creation, leading to a reported liquid level of around 0 cm, the foam stabilized by esters with a chain length of C16 or C18 did drain during foam creation, leading to liquid levels higher than 0 cm. For all esters, practically all liquid drained from the foam during the first five minutes after foam creation. Since the foaming solution was an aqueous solution of ester at low bulk concentration, the viscosity of the liquid phase was very low. This is one of the reasons for the quick drainage.

The foam structure immediately after foam creation is shown in figure 5. While the foams stabilized by esters with fatty acid chain lengths C8 to C12 had mostly spherical bubbles, the foams stabilized by esters with fatty acid chain lengths C16 and C18 were more polyhedral. This was most pronounced for the sucrose esters. The bubble shape was related to the amount of liquid that drained during foam formation (figure 4). Bubbles that were more polyhedral were present in foams where more liquid drained, while spherical bubbles

were observed when only little liquid drained. However, these spherical bubbles quickly became more polyhedral as a result of drainage.

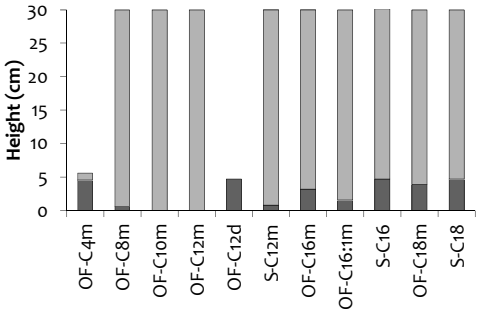

Figure 4: Foam (light grey) and liquid (dark grey) level of foams stabilized by oligofructose and sucrose fatty acid esters, immediately after creation. All foams were created in glass foam tubes with a glass grid at the bottom by aeration from below. The starting level of the liquid was 4.7 cm in all cases. Aeration was discontinued when the foam height reached 30 cm.

When looking at bubble size, for the sucrose esters there was a dramatic increase in the bubble size as the length of the fatty acid increased. While S-C12m formed small bubbles (around 0.4 mm) with a relatively uniform bubble size distribution, S-C16 (around 1.3 mm) and S-C18 (around 3.5 mm) formed much coarser foams with a higher degree of polydispersity. This was most pronounced in the case of S-C18. For the oligofructose esters, the bubble size also strongly depended on the length of the fatty acid chain. For OF-C8m, a rather coarse and very polydisperse bubble size distribution (0.1 - 2.6 mm) was found. Already during foam formation, the foam started to coarsen. For OF-C10m and OF-C12m, a quite uniform bubble size distribution with small bubbles (around 0.4 mm) was found. For OF-C16m and OF-C16:1m, we found a slightly more polydisperse bubble size distribution with slightly larger bubbles (around 0.5 mm) than OF-C12m and OF-C10m, although still smaller than the bubbles in the foam stabilized by S-C16 (1.3 mm). Finally, OF-C18m had the largest bubbles of all oligofructose esters (around 1.6 mm), although bubbles were still smaller than for S-C18 (3.5 mm). In addition to the bubble shape, also the bubble size of the foams can be related to the amount of drainage: foams with a large and polydisperse bubble size distribution, like S-C18 already drained significantly during foam formation while foams with a small and monodisperse bubble size distribution, like OF-C12m, did not drain significantly during foam formation. This observation is consistent with reports in literature [4, 19].

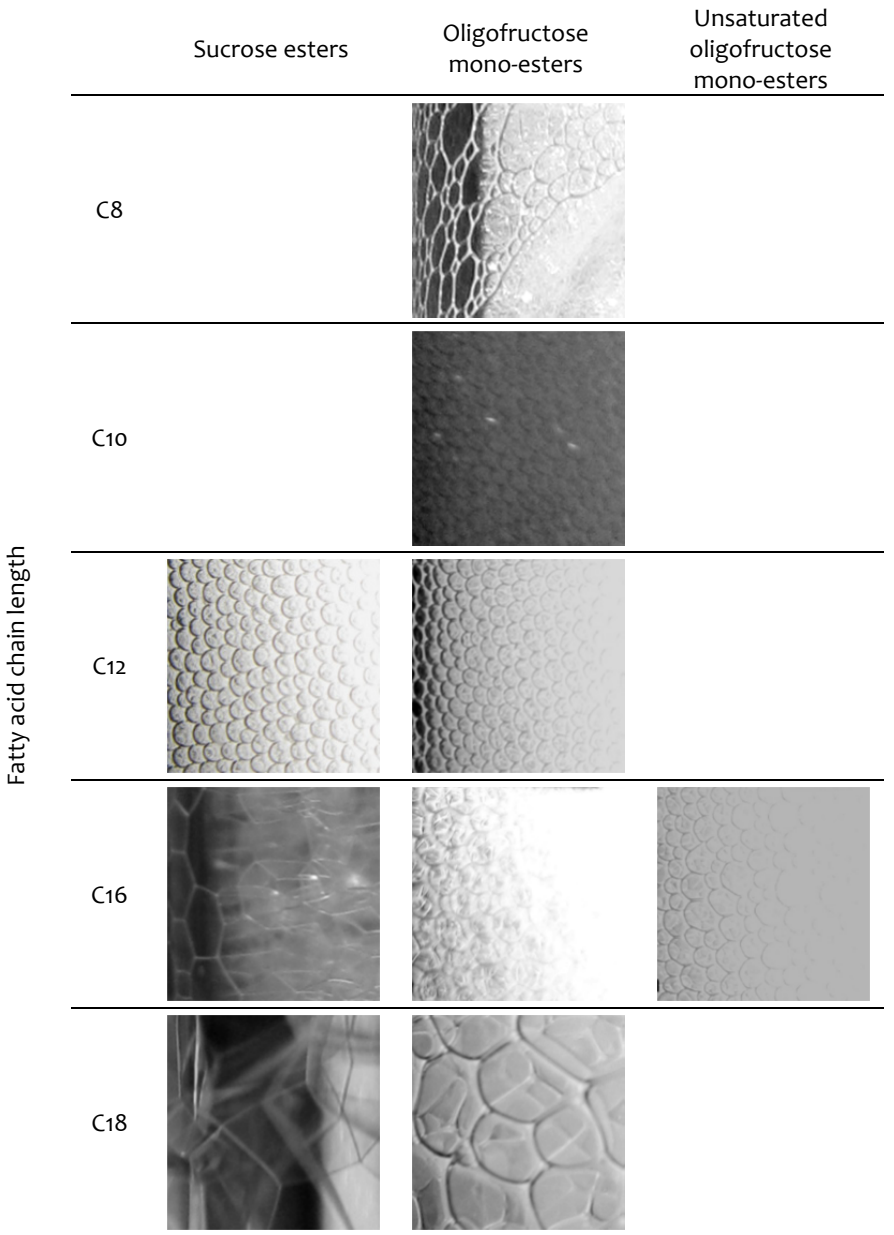

Figure 5: Structure of foams stabilized by sugar esters immediately after creation. Width of each picture corresponds to an actual size of 5 mm.

3.2.2 Foam stability

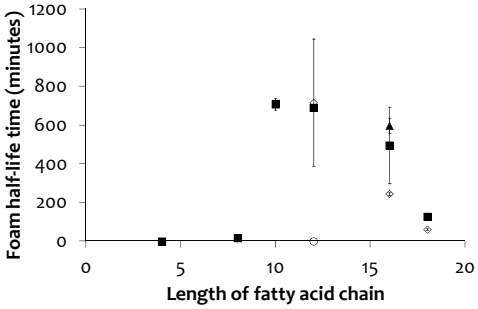

Figure 6: Foam half-life time of foams produced with sugar fatty acid esters with different fatty acid chain lengths. Oligofructose mono-esters (■), mono-unsaturated oligofructose mono-esters (▲), oligofructose di-esters (○) and sucrose esters (◊).

After foam creation, the foam and liquid height were monitored as a function of time. Figure 6 shows the half-life time values of the foams stabilized by the different esters. As already indicated, it was not possible to create a foam with OF-C12d and the foam stabilized by OF-C4m collapsed immediately after discontinuation of aeration. Therefore in both cases a half-time of 0 minutes was found. OF-C8m had a very short half-life time of 17 minutes. Coarsening progressed very quickly in this foam and resulted in fast foam collapse. OF-C10m, S-C12m, OF-C12m, OF-C16m and OF-C16:1m all had a very high half time between 500 and 700 minutes. S-C16 had a significantly shorter half-life time (245 minutes) compared to the corresponding oligofructose esters with the same fatty acid chain length. Both OF-C18m (127 minutes) and S-C18 (61 minutes) had a short half-life time. In all of the foams, coarsening was observed during the experimental time frame. Concluding, esters with intermediate chain lengths have the best foaming properties. This was found before for sucrose esters [20] and fructose esters [21].

3.3 Relation between foaming properties and interfacial properties

The foam structure immediately after creation (figure 5) may be related to the initial surface tension (figure 1B). Esters with fatty acid chain lengths between C10 and C16 had a low initial surface tension (<50 mN/m) which indicated that they were able to quickly migrate to the interface. This resulted in a foam with small bubbles. The esters with chain lengths of C4, C8 and C18 had a high initial surface tension (>50 mN/m) indicating that they were not able to migrate quickly to the interface, either as a result of insufficient surface

activity or of too high hydrophobicity, and resulted in a foam characterized by large bubbles and/or high polydispersity.

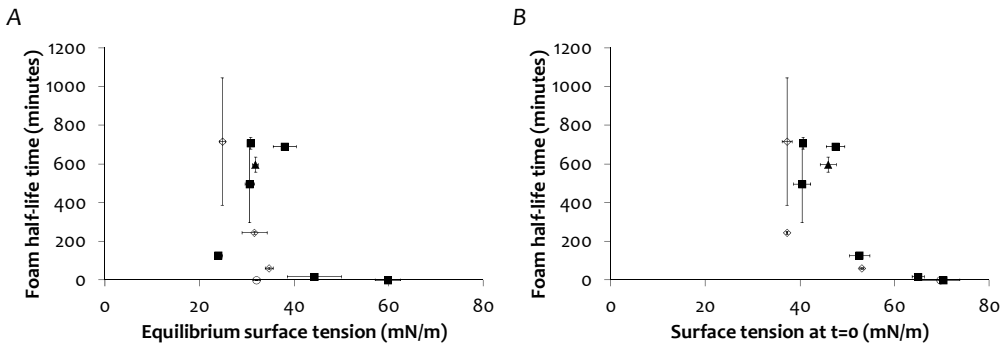

Figure 7: Foam half-life time as a function of surface tension at equilibrium (A) and surface tension about 1 second after the creation of the interface (B) of foams stabilized by sugar fatty acid esters with different fatty acid chain lengths. Oligofructose mono-esters (■), mono-unsaturated oligofructose mono-esters (▲), oligofructose di-esters (○) and sucrose esters (◊).

In figure 7A the foam half-life time is plotted as a function of the surface tension at equilibrium. All foams with a long half-life time had a low surface tension at equilibrium (< 40 mN/m). However, the opposite statement that a low equilibrium surface tension leads to a long half-life time is not true. OF-C18m, for example, had a low equilibrium surface tension and a low half-life time. Therefore, equilibrium surface tension is not an accurate predictor of foam stability. The correlation between surface tension at short time scale and the foam half-life time (figure 7B), however, was much stronger. Foams that were stabilized by surfactants with a low surface tension at short time scale (<50 mN/m), had a higher stability. These surfactants were able to quickly migrate to the interface, resulting in foams that were characterized by a uniform bubble size distribution with small bubbles, which led to higher foam stability. In contrast, foams stabilized by esters with a high initial surface tension (>50 mN/m) had a much lower stability. When the initial surface tension was close to 70 mN/m, which was the case for OF-C4m and OF-C12d, a half-life time of 0 was obtained. The results in this section clearly demonstrate the link between speed of surfactant migration from bulk to interface, initial foam structure and foam stability.

Although there is a correlation between initial surface tension and foam stability, there seems to be a difference between sucrose esters and oligofructose esters. At similar initial surface tensions, the foam stability of oligofructose esters is higher than the foam

stability of sucrose esters. The rheological profile of the interface has important consequences for drainage and coarsening, and therefore also on foam stability. The rheological response of sucrose esters was much different from the rheological response of oligofructose esters. Sucrose esters formed viscous interfaces with a relatively low modulus, high frequency dependency and did not show non-linearities, typical for low molecular weight surfactants. In contrast, oligofructose esters formed interfaces with a relatively high modulus, low frequency dependency and were characterized by strain hardening during compression and strain softening during extension. It is hypothesized that this rheological response could be attributed to the presence of an interfacial glass phase formed by the oligofructose part of the esters, which will provide mechanical stability to the foam film. It is also possible that, due to the larger hydrophilic part, the presence of oligofructose esters at the interface results in increased hydrodynamic drag, which leads to higher foam stability [22].

4. Conclusions

In this chapter, the effect of variations in the fatty acid chain of oligofructose fatty acid esters on the stability of foams produced from aqueous solutions of these esters was studied. Oligofructose mono-esters with intermediate fatty acid chain lengths (C10-C16) led to the highest foam stability. These esters were able to migrate to the interface quickly, and formed foams with small bubbles and a relatively narrow bubble size distribution, resulting in high foam stability. Oligofructose mono-esters with short fatty acid chain lengths (C4 and C8) were not able to fully cover the interface due to their very high CMC values. This lead to low foam stability. Oligofructose mono-esters with the longest fatty acid chain length (C18) and oligofructose C12 di-esters migrated to the interface slowly due to their high hydrophobicity, leading to low foamability. Despite the similarity in surface tension between oligofructose esters and sucrose esters with the same fatty acid chain length, oligofructose esters had higher foam stability. While oligofructose esters formed interfaces with a relatively high modulus, low frequency dependency and were characterized by strain hardening during compression and strain softening during extension, the rheological response of sucrose esters was much different. They formed interfaces with a relatively low modulus, high frequency dependency and did not show non-linearities. This difference in rheological response is suggested to be attributed to the presence of an interfacial glass phase formed by the oligofructose part of the esters, which provides mechanical stability to the foam films. Concluding, changes to the size of the hydrophilic group have a significant impact on foam functionality of sugar esters. The higher foam stability of oligofructose esters compared to sucrose esters may be attributed to major differences in interfacial microstructure.

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Chapter 8

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General discussion

1. Introduction

The food industry produces food products with a wide range of textures. These textures are obtained using a wide variety of functional ingredients and structural elements, for example, three-dimensional networks formed by gluten proteins in bread [1], partially coalesced fat structures formed by milk fats in ice creams [2], and air bubbles stabilized by surfactants in mousses [3]. In this thesis, we have focused on the latter type of product, aerated food products.

The production of aerated products with sufficient stability throughout their shelf life is complicated. Air bubbles can be introduced into a food product by for instance whipping, shaking, or gas injection [3]. After the introduction of air bubbles, they need to be stabilized, so that at least during the shelf life of the product no significant changes in product texture occur. The stability of the bubble depends on different factors. First, it is important that during the formation of the air bubble, surfactants adsorb quickly at its interface [4]. Next, the functional properties of the continuous phase are important. A continuous phase with a high viscosity leads to slower drainage, which is one of the instability mechanisms in foams [5-8]. Furthermore, the functional properties of the interfaces between air and water influence disproportionation and coalescence, two other instability mechanisms in foams [5, 9-14]. The interfacial properties are highly dependent on the type of surfactant that is used to stabilize the foam films [15].

Two types of surfactants that are mostly used in the food industry are proteins and low molecular weight (LMW) surfactants [13]. Proteins stabilize the interface by lowering the surface tension and by formation of a viscoelastic layer upon adsorption to the interface [4, 8, 13]. LMW surfactants generally lower the surface tension more than proteins and stabilize the interface through the Gibbs-Marangoni mechanism [4, 13, 16-18], which relies on rapid surface diffusion of surfactants, that will reduce surface concentration gradients that can develop after deformation of the interface [17, 19].

A surfactant that can lower the surface tension significantly, like a LMW surfactant, and at the same time provide the interface with a high dilatational modulus, like a protein, would be an excellent foam stabilizer. In this thesis we describe the properties of a series of molecules that obey these criteria: oligofructose fatty acid esters. The molecular structure of the esters is expected to have a major impact on the functional properties [13, 20]. Therefore, the following variations in molecular structure were investigated: degree of esterification (mono-ester versus di-ester), length of the fatty acid chain, degree of saturation of the fatty acid chain, and the size of the hydrophilic part. After synthesis, purification and structural characterization (*chapter 2*), the basic functional properties, such

as micelle formation and the area per molecule were studied (*chapter 3*), as well as rheological properties of air/water interfaces stabilized by the esters (*chapter 4*). Next, the functional properties of the mono-esters were determined when mixed with components present in the crude reaction product (*chapter 5*) or proteins (*chapter 6*). Finally, the potential of the esters as foaming ingredients was studied (*chapter 7*). The outline of this thesis is graphically presented in figure 1. The results represent different length scales: molecular, interfacial and macroscopic, that are all interdependent. In this final chapter we will elaborate on details of the results to appreciate the interdependencies between the different chapters and focus on new insights that were gained in this thesis.

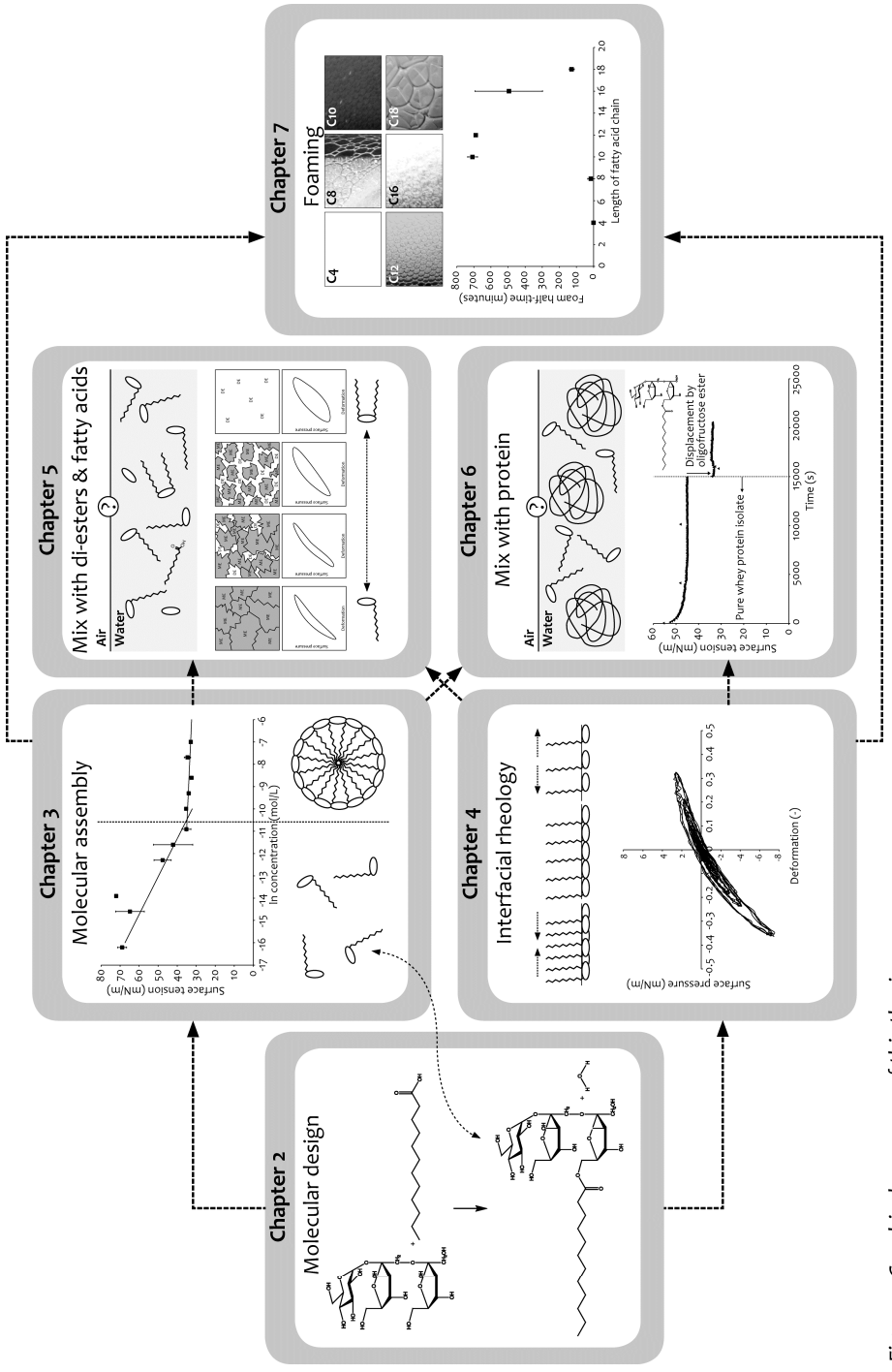

Figure 1: Graphical summary of this thesis

2. Synthesis and structural characterization

In *chapter 2* the synthesis of oligofructose esters with different fatty acid chain length is discussed. Many different approaches may be considered to synthesize oligofructose fatty acid esters. Traditionally, sugar esters are manufactured using a chemical reaction procedure, which has certain disadvantages, such as the use of alkaline catalysts at high temperatures [21, 22]. Furthermore, an important limitation of the chemical reaction procedure is the low selectivity, which leads to a mixture of esters with different degrees of esterification [23-26]. To overcome these disadvantages, an enzymatic reaction procedure was used. During this procedure, lower temperatures are employed, and due to the high specificity of the reaction, a more well-defined reaction product is obtained [23, 25, 27, 28]. Lipase B from *Candida antarctica* was selected due to the fact that this is a commonly used enzyme in similar reaction procedures [24, 29-33], and due to the fact that it was already successfully used to synthesize oligofructose esters [34, 35]. At a later stage in the research, a range of enzymes was screened for their transesterification activity in a model reaction between 1-octanol and vinyl laurate in hexane. There were both immobilized and free enzymes, and the enzymes were from different origins (yeasts, bacteria and plants). As expected [36], immobilized enzymes displayed significantly higher activities than free enzymes. Of all enzymes tested, the immobilized lipase B from *Candida antarctica* had the highest activity. This confirmed that the earlier choice to employ this enzyme for the synthesis reactions was the right one.

Due to the difference in polarity between the oligofructose and fatty acids, it is important to carefully consider what (combination of) solvent(s) will be used. Previous research [34, 35] had already shown that reasonable yields could be obtained when using a combination of DMSO and Bu^tOH. Therefore, this solvent combination was used for synthesis of oligofructose esters.

As a first step in the establishment of the synthesis reaction conditions, three different reaction procedures were considered: 1) esterification in the presence of molecular sieves, 2) transesterification in the absence of molecular sieves, 3) transesterification in the presence of molecular sieves. Transesterification using vinyl esters of fatty acids is often used for these types of reactions because it leads to higher conversions [34]. However, based on the fact that these vinyl esters are not readily available for every fatty acid chain length, and the fact that fatty acids are more natural and thus more desirable in a food-grade product, esterification was considered as well. During the esterification reaction water molecules are released that could hydrolyze the formed esters. Therefore, molecular sieves are employed to control the water content. Prior to the

reaction, all solvents and substrates are dried. Since during transesterification reactions no water molecules are released, molecular sieves should not be necessary. However, molecular sieves could absorb some of the water that remained in the solvents and substrates and therefore lead to a higher yield [29]. No major differences in yield were found between the three different reaction procedures. These results are similar to recently reported results obtained during the esterification of mannose myristic acid esters in DMSO/Bu^{t}OH 0.1/0.9 [37]. In that study no major differences in yield between esterification with molecular sieves and transesterification without molecular sieves were found. Because a similar yield was obtained for all reaction procedures, esterification reactions were selected for further syntheses of oligofructose esters.

Next, the inactivation of the enzyme was studied under the selected reaction conditions. The enzyme activity significantly decreased as a result of temperature, mechanical abrasion and stripping of the essential layer of water molecules. These results are similar to results recently reported by Nott et al. [37], who found a significant decrease in the yield of mannose myristic acid esters when the DMSO fraction was increased. They attributed this to the disruption of the hydration shell of the enzyme. In our study, after 24 hours, only 17% of the original activity was retained. Since the total incubation time was 68 hours, after 19 and 43 hours an additional batch of enzyme was added to compensate for enzyme inactivation.

For all fatty acid chain lengths, as a result of the highly specific reaction procedure [38], mono-esters were the main reaction products. The conversion of oligofructose into esters increased with fatty acid chain length, and is consistent with earlier reports of enzyme specificity towards more lipophilic substrates [25, 39].

The oligofructose was a mixture with different degrees of polymerization (DP). Not all oligomer sizes are necessarily good substrates for the enzyme. Therefore, it was established whether there were differences in the DP distribution before and after synthesis. We found that for all fatty acid chain lengths, DP 3 and 4 were slightly better substrates for the enzyme than DP 5, 6, 7 and 8. For the shorter fatty acids DP5 was also a slightly better substrate than the longer oligomers. Furthermore, di-esters were only formed for the shortest fatty acids. These differences may be related to the lack of space in the catalytic cleft of the enzyme, which limits the access of oligomers with a higher DP and mono-esters [40-42].

The reaction yielded a mixture of mono-esters, di-esters, unreacted oligofructose and unreacted fatty acids. Because of expected differences in functionality between the different fractions, which are discussed in *chapter 5*, it was desired to fractionate the crude

reaction product. Reverse phase solid phase extraction was used to fractionate the mixture into the different individual components. A gradient of methanol and water was used and the composition of the solvent that eluted the esters from the column could be correlated to the hydrophobicity of the esters.

The purified fractions were characterized using MALDI-TOF MS and (2D) NMR. Using MALDI-TOF MS, a purity of >90% was obtained for mono-esters and a purity of >80% for di-esters. For the mono-ester fraction the main impurity was unreacted oligofructose, for di-esters the main impurities were mono-esters, but also small amounts of unmodified oligofructose and tri-esters were present. Using (2D) NMR techniques, it was possible to show that the location of the esterification was restricted to the C-1 and C-6 positions of the monosaccharides. Unfortunately, the analysis did not conclusively show on which position on the oligofructose chain the esterification was located. However, due to geometric constraints in the catalytic cleft of the enzyme, it is likely that the esterification locations were at either end of the oligofructose chain.

3. Effects of molecular structure on molecular assembly and interfacial properties

As already indicated, it is expected that differences in the molecular structure of the ester, such as the length of the fatty acid or the degree of esterification, have a major impact on the functionality of the esters. Therefore, the influence of molecular structure on some basic functional properties of these esters was studied. These include micelle formation, the amount of space that a molecule occupies at the air/water interface and rheological properties of interfaces stabilized by those esters.

Micelle formation

Due to their amphiphilicity, surfactants that are present in the bulk will migrate to the interface where they lower the surface tension. With increasing bulk concentration of the surfactant, more molecules will migrate to the interface, where they further lower the surface tension. At a certain bulk concentration, the surfactants start to form aggregates. After this critical concentration is reached, the surface tension will not decrease much further. It is referred to as critical micelle concentration (CMC) and is an important specification of a surfactant [43]. The CMC is expected to depend on the molecular structure of the ester. Therefore, in *chapter 3* it was determined how the surface tension of air/water interfaces stabilized by oligofructose esters with different molecular structure depended on the bulk concentration. For all esters, a typical CMC curve was obtained. The CMC decreased with increasing fatty acid chain length (both for mono-esters and di-esters).

Furthermore, di-esters had a lower CMC than the corresponding mono-esters. Similar effects were found by other authors when studying sugar esters [20, 44-50]. Additionally, light scattering experiments were performed to establish the nature of the aggregates that were formed at concentrations higher than the CMC. For all esters except the mono-ester with a fatty acid chain length of 18 carbon atoms, aggregates in the size range of simple micelles were found. For the mono-esters with a fatty acid chain length of 18 carbon atoms, the size distribution was characterized by large and polydisperse aggregates. The nature of these aggregates is unclear. They could be caused by the presence of insoluble aggregates, as a result of the high hydrophobicity of the ester. Furthermore, it is possible that the long fatty acid chain affects the critical packing parameter of the esters and leads to micelles with a different morphology, such as worm-like micelles [51] or rods [47].

From the CMC curves that are described in *chapter 3*, it is possible to determine the effectiveness and efficiency of the esters. These terms are often used to characterize surfactants [45]. The efficiency of a surfactant gives information about the amount of material that is necessary to obtain a certain surface tension reduction, often 20 mN/m. The efficiency has practical relevance because it determines the amount of material that is necessary in a product formulation. The efficiency of the esters increased with increasing hydrophobicity. Therefore, especially esters of longer fatty acid chains seem to be interesting as potential food-grade functional additives [50]. The effectiveness is defined as the surface pressure that is obtained at the CMC and is not necessarily the same among a series of surfactants. In the case of oligofructose esters, a similar effectiveness was found for all esters, which may imply that the amount of space occupied by a molecule is determined by the oligofructose head group.

Surface area per molecule

The amount of space occupied by a single molecule after adsorption at the air/water interface gives information about the interactions between the molecules. Therefore, it is important for the rheological behavior of interfaces. In *chapter 3* two different methods were used to determine the amount of space occupied by a single molecule at the interface. First, the CMC curves were fitted with the Gibbs equation for non-ionic surfactants, as often performed for sugar based esters [20, 47, 50, 52, 53]. Furthermore, this parameter was determined using ellipsometry. As far as we are aware, this method was not applied before for sugar esters, possibly because the accuracy decreases as interfacial layers get thinner [54]. With both methods, a similar area per molecule was found for the oligofructose esters with different fatty acid chain lengths.

Indications were found that di-esters occupy slightly more area than mono-esters, which means that two fatty acid tails could occupy more area than the oligofructose part of the molecule. The consequences for the rheological response and microstructure of interfaces stabilized by these esters are discussed in *chapter 4 and 5*. To study the influence of the size of hydrophilic group, oligofructose esters were compared to sucrose esters. It was found that oligofructose esters occupy a larger molecular area than the corresponding sucrose esters with the same fatty acid chain length. All (sucrose and oligofructose) esters occupied significantly more area than a single fatty acid chain. This shows that the oligofructose part determines the amount of space occupied by one molecule and that the fatty acid chains are relatively far apart. Therefore, it is likely that the rheological response of interfaces stabilized by oligofructose fatty acid esters is largely determined by interactions between the oligofructose part of the molecule, and is less affected by the fatty acid tail-tail interactions.

Rheological properties

An important reason to study the rheological properties of air/water interfaces is their link to foaming properties [55-57]. The shear and dilatational properties of interfaces have been linked to instability mechanisms of foams, such as disproportionation and coalescence [58]. In this thesis, the focus was on dilatational deformation.

Traditional approach

First, the development of the surface dilatational modulus was studied as a function of surface pressure during time sweeps. A sharp increase in surface dilatational modulus at very low surface pressures was followed by a plateau value that was sustained over a broad range of surface pressures. The plateau value of the modulus increased with increasing hydrophobicity. In some cases the value of the modulus started to deviate from the plateau value after the surface pressure became higher than π_{CMC}. These results are in contrast to Garofalakis et al. [59] who have studied the dilatational rheology of insoluble esters composed of mono- or di-saccharides and stearic acid, and did not find this typical plateau value. Instead they found an increase in modulus with increasing surface pressure followed by a decrease. They attributed their results to the relaxation phenomena related with collapse (desorption and multilayer formation). The dilatational modulus can either be the result of the speed of surfactant migration from bulk to interface, or the formation of a mesophase at the interface. At very low surface pressures, when the surface concentration of the ester is also low, interactions between the molecules are not expected to play a

significant role. Therefore, the increased value for the modulus for more hydrophobic molecules may be attributed to a higher affinity for the interface. At higher surface pressures and thus higher surface concentrations, mesophase formation becomes more likely. This mesophase could either be a soft interfacial glass phase formed by the oligofructose part of the molecule or a crystalline phase formed by the fatty acid chains. Based on the amount of area occupied by the oligofructose ester that was established in *chapter 3*, a soft interfacial glass phase formed by the oligofructose part of the molecule is most likely. This hypothesis is confirmed by the results in the next section. An interface stabilized by an oligofructose ester with a mono-unsaturated chain followed the same trend as the one with a saturated fatty acid chain. This supports the hypothesis that interactions between fatty acid chains do not play a significant role in the rheological response.

To further characterize the rheological response of the interfaces, frequency sweeps were performed after reaching equilibrium. For all oligofructose esters (mono-esters with saturated and unsaturated fatty acid chains and di-esters) a low scaling exponent in the frequency dependency was found at surface pressures lower than π_{CMC}, as a result of slow transport from bulk to interface caused by the low concentration of molecules in the bulk. When the surface pressure was higher than π_{CMC} the degree of variability in the scaling exponents increased. However, for the oligofructose mono-esters they remained relatively low. This could again be indicative for the presence of a soft interfacial glass phase formed by the oligofructose part of the molecule [60]. For di-esters in some cases a scaling exponent in the frequency dependency close to 0.5 was obtained. This indicates that processes were diffusion-controlled and adequately described by the Lucassen van den Tempel model [61], which is typical for LMW surfactants. For sucrose esters, scaling exponents that approached 0.5 were obtained which indicates typical LMW surfactant behavior without mesophase formation. The difference in rheological response between oligofructose esters and sucrose esters and the fact that they have the same fatty acid chain makes it unlikely that interactions between the fatty acids play a major role in the surface rheological response.

Novel approach

The conclusions about interfacial microstructure had to be based on curves with a high degree of variability. It was hypothesized that this high degree of variability was a result of having measured outside of the linear viscoelastic regime. Therefore, attention was paid to the dependency of the modulus on deformation amplitude. First, an illustrative result in the form of the first-harmonic Fourier moduli was reported. The surface dilatational

modulus of oligofructose mono-esters with fatty acid chain length 18 strongly depended on the applied deformation amplitude. With increasing deformation amplitude, the surface dilatational modulus became lower. This could be interpreted as significant structural changes in the interfacial microstructure. In fact, no linear viscoelastic regime could be identified within the range of applied deformation amplitudes between 1.5% and 30%. This could point to the presence of a brittle layer, and could support the hypothesis of the formation of a soft interfacial glass phase by the oligofructose part of the molecule as introduced in *chapter 3*. Similar results were obtained by Humblet-Hua et al. [62], when studying oil/water interfaces stabilized by complexes of high methoxyl-pectin and proteins (ovalbumin and lysozyme) and by protein (ovalbumin and lysozyme) fibrils. The dependence of the modulus on the amplitude deformation is not specific for oligofructose esters and we stress the importance of including strain sweeps during rheological characterizations of interfaces.

Using the first-harmonic Fourier moduli, non-linearities in the raw signal are disregarded, as already pointed out by Ewoldt et al. [63]. To acquire information about non-linearities, the results of the amplitude sweeps were presented in the form of Lissajous plots, where surface pressure was taken as a measure of stress. We show that over the whole range of surface pressures, oligofructose mono-esters had a predominantly elastic response. Furthermore, the Lissajous plots showed asymmetries where during compression the slope of curve increased with increasing strain, and during expansion the slope of the curve decreased with increasing strain. This points to strain hardening during compression and strain softening during extension. Also these results support the hypothesis that the oligofructose part of the molecule forms a soft interfacial glass phase. For oligofructose di-esters at high surface pressures, the response became more viscous and non-linearities disappeared. Combined with the scaling exponent in the frequency dependence close to 0.5, we conclude that the presence of the two fatty acid chains interfered with the formation of the soft interfacial glass phase by the oligofructose part of the molecule. For sucrose esters a rather viscous response without asymmetries was found, typical for low molecular weight surfactants. These results demonstrate that Lissajous plots can be very useful in understanding the link between surface rheological properties and interfacial microstructure.

The fact that the surface dilatational modulus was dependent on temperature was indicative of a structural transition upon cooling of the interface. This is another argument that supports the hypothesis of a soft interfacial glass phase formed by the oligofructose part of the molecule.

To summarize, interfaces stabilized by oligofructose mono-esters had relatively high dilatational moduli, low scaling exponents in the frequency dependency, and elastic and highly asymmetrical Lissajous plots with strain hardening during compression and strain softening during expansion. These properties were the same for esters with saturated or unsaturated fatty acid tails. Furthermore, the moduli were dependent on temperature. In contrast, sucrose esters had relatively low moduli, scaling exponents in the frequency dependency close to 0.5, and fairly viscous Lissajous plots without asymmetries. Based on these facts, we conclude with a fair amount of certainty that the oligofructose part of the ester forms a soft interfacial glass phase. In this thesis we have shown that especially for interfaces with complex microstructures, it is essential to study the dependence of the modulus on deformation amplitude. Therefore, we propose that the study of the rheological properties of interfaces during oscillatory dilatational deformations should always include strain sweeps. Furthermore, we have shown that Lissajous plots of surface pressure versus deformation can be powerful tools in understanding the link between surface rheological properties and interfacial microstructure.

4. Mixing with other components

After having established the basic functional properties of purified mono-esters and di-esters, it is important to consider what will happen when the esters are mixed in the bulk with other components. First, one should realize that the crude reaction product that is obtained is a mixture of esters with different degrees of esterification, and of unreacted oligofructose and fatty acids, as described in *chapter 2*. Since purification is a time-consuming and expensive process, one may wonder, from a practical point of view, what are the differences in functionality between the crude and purified products and what is the contribution of each component that is present in the crude product to the functional properties. Second, when thinking about the application in food products, it is important to consider that many food products contain components that are surface active, mostly proteins. Hence, one must carefully consider what the interfacial structure of such a system will look like. Therefore, in *chapter 5 and 6* mixed systems are discussed.

<u>Mix of mono-ester with di-ester and fatty acid</u>

As described in *chapter 2*, oligofructose fatty acid esters are produced by esterification of fatty acids with oligofructose. After synthesis, the reaction mixture is composed of unreacted oligofructose, unreacted fatty acids, mono-esters and di-esters, which are expected to have completely different functional properties.

Due to the low solubility of fatty acids in the bulk at room temperature, properties of interfacial layers stabilized by fatty acids are often studied after spreading of the fatty acid on the interface [64]. In *chapter 5* we have taken a different approach, because we wanted to know to what extent lauric acid could migrate from the bulk to the interface at room temperature, despite its low solubility. Pure lauric acid was present in the form of (visible) aggregates and hardly migrated to the interface, as evidenced by a high surface tension and a low dilatational modulus. The small amount of material that did migrate to the interface formed a purely elastic layer. The presence of mono-esters caused a small increase in the surface concentration of fatty acids, possibly by the formation of mixed micelles, as hypothesized by Golemanov et al. [65] for their system containing both surfactants and fatty acids. The mixed interface of oligofructose mono-ester and fatty acid was characterized by an intermediate frequency dependency (scaling exponent of 0.34) and a slightly asymmetric Lissajous plot. This could be caused by the presence of islands of glass phase formed by mono-ester surrounded by lauric acid.

Oligofructose was not surface active and the mixing of oligofructose with mono-ester only diluted the system, which did not lead to significant changes in surface properties because the concentration of mono-esters was, even after mixing, still close to the CMC.

So the impact of lauric acid and oligofructose on the properties of mixed interfaces was limited. The two main surface active components, mono-esters and di-esters, had a more pronounced influence on interfacial properties. In *chapter 3* we have established that both mono-esters and di-esters migrated to the interface. However, it is possible that, as a result of increased hydrophobicity, di-esters are present in the form of aggregates that will only migrate to the interface slowly. In *chapter 5*, we have assessed the surface tension of air/water interfaces stabilized by both mono-esters and di-esters. Additionally, in *chapter 5* we have focused on differences in the speed of surfactant adsorption that were not addressed in *chapter 3*. Since in *chapter 4* major differences were found in the rheological response of mono-esters and di-esters, mixing of mono-esters and di-esters at different ratios may have significant consequences for the interfacial microstructure.

Pure OF mono-esters could lower the surface tension more quickly than pure OF di-esters. However, at equilibrium, di-esters lead to a lower surface tension. This means that the increase in hydrophobicity as a result of the second fatty acid chain does slow down the migration to the interface, but the reduction in solubility is insufficient to render the di-esters surface-inactive. This is in contrast to the behavior reported for smaller hydrophilic groups. Ferrer et al. [50] describe how the incorporation of a second acyl chain into a sucrose ester led to a significant loss in solubility that made it impossible to collect reliable

CMC data. Husband et al. [48] describe how the surface tension obtained with sucrose di-ester was much higher than the surface tension obtained with sucrose mono-ester. Di-esters also had lower foamability. They attributed these differences to a less compact structure and to an increase in the amount of micelles that was present in the bulk leading to slower adsorption kinetics. In the case of oligofructose esters these effects are much less pronounced as a result of the increased hydrophilicity due to the larger hydrophilic group. Oligofructose mono-esters were studied at a concentration around the critical micelle concentration as established in *chapter 3*, which means that the surface concentration of the mono-esters was sufficiently high for the formation of the soft interfacial glass phase, as described in *chapter 4*. The surface rheological characterization confirmed this. A low modulus was combined with a low exponent in the frequency dependency, and a Lissajous plot that was mostly elastic with strain hardening during compression and strain softening during expansion. Di-esters led to a high modulus, combined with a high frequency dependency of the modulus, and a fairly viscous and symmetric Lissajous plot, similar to the results obtained in *chapter 4*.

When mono-esters and di-esters were mixed at a ratio of 0.8/0.2, the initial surface tension was lower than the surface tension obtained with either pure product. This shows that the presence of mono-esters increased the speed of migration of di-esters from bulk to interface, possibly through the formation of mixed micelles. The equilibrium surface tension was similar to that of di-ester, indicating that there was a considerable amount of di-ester on the interface. We have found a low surface dilatational modulus, combined with intermediate frequency dependency and asymmetry in the Lissajous plots. The asymmetry was far less pronounced than in the case of pure mono-ester but strain hardening during compression and strain softening during expansion were still observed. This might be caused by a structure composed of islands of mono-ester glass phase surrounded by a viscous phase formed by di-ester. In the crude product, where the ratio between mono-esters and di-esters was higher compared to the previous case, there were a few differences. Here the surface tension was also similar to the di-ester but the modulus was high. The scaling exponent in the slope was low, and the Lissajous plot asymmetric, similar to the mono-ester. The hypothesis was formulated that here the interfacial structure is also composed of islands of glass phase present in a viscous phase formed by di-esters. However, in contrast to the previous case, here the surface fraction of di-esters was low, which means that the glassy patches formed by mono-ester dominate the interface.

The results from *chapter 5* show that there may be significant differences in the interfaces stabilized by crude products and purified products, which may have consequences for the application of the esters.

Mix of mono-ester with whey protein isolate

The functional properties of mixes of surfactants and proteins at interfaces have been the subject of investigation for many years [58, 66-68]. One of the reasons for studying these mixes is that protein formulations often contain traces of small lipid-like surfactants that are considered as contaminants [15]. Since proteins generally lead to higher foam stability than small surfactants, proteins are the preferred substance for interface stabilization [6, 10]. However, small surfactants generally lower the surface tension more than protein and will therefore always try to displace the proteins, leading to a dramatic decrease in stability when the interface is mixed [17]. When virtually all protein is displaced the stability increases again [17, 69, 70].

Since the oligofructose fatty acid esters are intended as food ingredients, it should be considered what happens when the ester is added to a protein-containing formulation. In contrast to other studies, in *chapter 6* the ester is the preferred molecule at the interface instead of the protein. Because of the low surface tension combined with a high dilatational modulus as established in *chapter 3* and *chapter 4*, an oligofructose mono-ester with a chain length of 16 carbon atoms was used. Whey protein isolate was selected as a protein source, because it is a widely used protein in the food industry and because it is a globular protein, known to form a viscoelastic layer with a high modulus [14] that might be difficult to displace. As expected, whey protein isolate formed an interfacial layer with intermediate surface tension and a high dilatational modulus, which may be attributed to the formation of a viscoelastic network. The pure oligofructose fatty acid ester formed a layer with a lower surface tension than the one obtained with WPI at all three of the concentrations that were studied. The similarity in surface tension may be explained by the fact that all three concentrations were higher than the CMC, which was established in *chapter 3*. Furthermore, the interfaces had a high dilatational modulus, attributed to the presence of a soft interfacial glass phase formed by the oligofructose part of the molecule, as established in *chapter 4*. The development of the modulus was highly irregular, with sometimes sudden decreases following a steady increase. These observations were attributed to the high brittleness of the interfacial layer, which means that the amplitude of deformation was too high, leading to structural rearrangements, and leading to a lower surface dilatational modulus after reaching equilibrium [71], as detailed in *chapter 4*. Mixes of protein and

oligofructose fatty acid ester were studied at three different concentrations of the protein. Only at the highest bulk concentration of the protein did the surface tension obtained with the mix deviate from the surface tension obtained with pure oligofructose ester, indicating that there was a significant amount of protein at the interface. At all protein concentrations, the modulus of the mixed system was low. This shows that at the lowest protein bulk concentrations, the surface concentration of protein was low, but still high enough to prevent the formation of the soft interfacial glass phase by the oligofructose ester. We conclude that the stabilization mechanisms of proteins and oligofructose esters are mutually exclusive, which may have consequences for the foaming properties of their mixtures.

The higher foam stability of the pure oligofructose ester compared to the protein was attributed to differences in speed of surfactant adsorption and thus surface tension reduction, which were not discussed in *chapter 3*. Despite the low modulus that was obtained at the lowest protein concentrations, in these cases still a high foam stability was obtained. This may be attributed to the occurrence of the Gibbs-Marangoni mechanism. At the highest protein concentration, the surface concentration of protein was sufficiently high to interfere with the Gibbs-Marangoni mechanism, which resulted in a dramatic decrease in the foam stability [72, 73].

Because a fully developed WPI network may be more difficult to access by the esters [74, 75], the next issue that was addressed was whether the oligofructose ester would be capable of displacing WPI. Immediately upon addition of the ester to the subphase of a maturated WPI stabilized interface, the surface tension and surface dilatational modulus dropped dramatically, indicating that the oligofructose ester could displace part of the WPI. Similar results were reported when studying mixed interfaces of milk proteins and sucrose esters [14]. At the highest whey protein isolate concentration, a more connected network was formed that was more difficult to disrupt. In an attempt to gain understanding about the displacement mechanism, a similar experiment was performed while observing the interface using Brewster Angle Microscopy. The interface was immobile when stabilized by pure WPI. The mobility of the interface quickly increased after the oligofructose ester was injected below the surface, which supports the rheological results. Unfortunately, there were no other features visible that could increase the understanding of the displacement mechanism, possibly due to the small difference in relative refractivity or due to the small magnification of the camera.

Results from *chapter 6* showed that oligofructose esters have the potential to be used as a food additive, even in the presence of proteins.

5. Foaming properties

In *chapter 7* the thesis deals with the application of the oligofructose fatty acid esters as foam stabilizers. Depending on the surfactant structure, major differences in the functional properties were found. All esters were studied at the same bulk concentration. Oligofructose fatty acid esters containing short fatty acids of 4 and 8 carbon atoms have a relatively hydrophilic character and a relatively high affinity for the aqueous phase. As described in *chapter 3*, they only start to form micelles in the bulk at concentrations much higher than the concentration that was studied in *chapter 7*. Due to this, the interface could not be fully saturated when using these molecules. This led to incomplete surface coverage and high surface tensions, both at short time scales and after reaching equilibrium. In contrast, oligofructose mono-esters (both saturated and unsaturated chains) and di-esters containing fatty acids with chain lengths of 10 carbon atoms or longer all had a similar low surface tension at equilibrium, which means that they were able to completely saturate the interface. The fact that they could reach a low surface tension may be attributed to their higher affinity for the interface, which is a result of increased hydrophobicity as described in *chapter 3*. The similarity in surface tension may be attributed to the fact that the amount of area occupied by one molecule is determined by the size of the oligofructose part of the molecule as established in *chapter 3*. However, there were differences in the surface tension at short time scales that have not been addressed in *chapter 3*. For the esters with the highest hydrophobicity, which were the mono-esters with the longest fatty acid chains and the di-esters, the surface tensions at short time scales were higher. These observations for the surface tension values had serious consequences for the foamability of the aqueous solutions of the esters. Only esters with intermediate hydrophobicity were able to generate foams with small bubbles and a monodisperse bubble size distribution, which resulted in high foam stability. The conclusion that was drawn in *chapter 3*, that esters of longer fatty acid chains are the most promising food-grade additives, is therefore not valid for this specific application as foam stabilizer. For this application, it is more important that a surfactant is able to migrate to the interface quickly. It was shown that the surface tension at short time scale is the most accurate predictor of foam stability. However, at similar surface tensions at short time scale the foam stability of oligofructose esters was higher than the foam stability of sucrose esters. This is likely caused by the differences in rheological response of the interfacial layers as described in *chapter 4*. Interfaces stabilized by sucrose esters had relatively low moduli, scaling exponents in the frequency dependency close to 0.5, and Lissajous plots that were fairly viscous and free from asymmetries. These results support the idea that interfaces were stabilized by the Gibbs-Marangoni mechanism.

However, with increasing fatty acid chain length, the speed of surfactant adsorption also reduces, which will decrease the efficiency of this mechanism. The oligofructose esters, whose interfaces were characterized by relatively high moduli, low scaling exponents in the frequency dependency, and asymmetric Lissajous plots with strain hardening during compression and strain softening during expansion, will lead to interfaces with high mechanical stability and resistance to compression.

Results from *chapter 7* show that oligofructose esters may be used as high-performance foaming ingredients.

6. Perspectives for future research

In this thesis, we have described the synthesis and functional properties of oligofructose esters with variations in molecular structure. We have shown that these esters form interfacial layers with low surface tensions and high surface dilatational moduli. The interfacial layers are characterized by brittle structures, due to the presence of a soft interfacial glass phase. The esters could also be used to stabilize foams. This makes oligofructose esters interesting molecules, both from a scientific and from a practical perspective. In this last section we will identify further research lines that can be derived from this thesis.

Synthesis

The methods described in *chapter 2* to synthesize the oligofructose fatty acid esters were based on previous works by ter Haar et al. [34] and Sagis et al. [35] on oligofructose lauric acid esters. In this thesis we have successfully used these methods to synthesize esters with different fatty acid chain lengths. Since the main focus of this thesis was on functional properties of the esters, the methods for the synthesis were not fully optimized. In the following section possible improvements to the synthesis methods will be discussed that could be used to further increase the efficiency.

One of the complications that one faces when producing these types of derivatives in liquid solvents is the difference in polarity between the oligofructose part of the molecule and the fatty acid. This limits the choice of organic solvents [23, 76]. Relatively polar solvents that lead to high solubility of the oligofructose part of the molecule decrease the solubility of the fatty acid and decrease the stability of the lipase [34]. As a compromise, in this research a mixture of DMSO and Bu^tOH was used. The ratio of DMSO/Bu^tOH was set at 0.2/0.8 for most reactions. Although the research by ter Haar et al. [34] discussed the effect of a change in this ratio, it was not thoroughly investigated in this thesis. Nott et al. [37]

have recently showed that during lipase-catalyzed esterification the yield of mannose myristic acid esters was highest when a ratio DMSO/Bu^{t}OH of 0.1/0.9 was used. Hence, the ratio between DMSO and Bu^{t}OH could be addressed in future research. Also, it is possible that with (a combination of) different solvents, for example acetone, diethyl ether, hexane or 2-methyl-2-butanol [29, 77], a higher yield could be obtained.

It was established that the enzyme is inactivated rather quickly under the experimental conditions that were employed. In this thesis, we have overcome this problem by adding additional batches of enzyme during the incubation time. However, from an economic point of view, this is not an optimal solution to the problem. Many different approaches may be considered to reduce enzyme inactivation. Changes to the solvent will also affect the activity of the enzyme. It was proposed, for example, to replace the solvent by a hydrophobic solvent such as hexane or toluene [78]. To reduce inactivation due to mechanical shear the speed of agitation may be reduced. Another approach, which was tested during preliminary experiments, is the development of a continuous process for the enzymatic esterification of the oligofructose ester using a fixed-bed reactor with immobilized enzyme and a rehydration step between the cycles. Using this set-up, it was possible to synthesize oligofructose esters using both an esterification reaction and a transesterification reaction. The yield of the transesterification was much higher than the yield of the esterification reaction. These results justify further investigations.

Šabeder et al. [33] have shown that the yield of an esterification reaction between fructose and palmitic acid depended on the concentration of molecular sieves. At concentrations higher than 10% they observed significant decreases in the yield, attributed to excessive removing of essential water. Therefore we recommend to further investigate the effect of the concentration of molecular sieves.

The consequence of the wide range of the fatty acid chain length that was esterified to the oligofructose part of the molecule is that there are major differences in the hydrophobicity of the molecules. This difference in hydrophobicity will have major consequences for the partitioning of the esters between the solvents that are used during synthesis, the washing steps, and the purification. Therefore, to obtain the highest yield it is recommended to optimize all procedures separately for all esters.

During the purification steps a small yield was obtained. The cause was not further investigated. Possible causes are insufficient attachment of the esters to the column material, losses that occurred during the evaporation of solvent, or hydrolysis of the esters on the column. Whatever the cause, further investigations are necessary to prevent such excessive losses in the future.

The combination of FT-IR, MALDI-TOF MS and (2D) NMR provided adequate information about the molecular structure of the esters. However, certain points for improvement were identified. The fact that the oligofructose was a mixture with different degrees of polymerization complicated the interpretation of the 2D NMR spectra. As a result, we were not able to conclude on which oligomers the esterifications with fatty acids take place. Although it is likely, based on the geometry of the catalytic cleft of the lipase, that the esterifications are at either end of the oligomer chain, this could not be proven. The location of the esterification is likely to influence the functional properties of the esters, more specifically the arrangement of the molecule at the interface. The importance of the position of the esterification is demonstrated by Ferrer et al. [50] for di-esters who found a much lower surface tension for the 6,6'-di-ester compared to the 6,1'-di-ester. Razafindralambo et al. [81] have demonstrated how the location of the esterification can significantly change both surface tension and surface rheological properties. Therefore, we recommend to further investigate this. One approach that would facilitate the interpretation of the 2D NMR spectra would be the fractionation of the oligofructose on the basis of DP. Furthermore, while a method was used to draw (semi-) quantitative conclusions based on MALDI-TOF MS spectra, it is recommended to develop an HPLC method to give more accurate information on sample composition.

Most importantly, since major differences in functionality were observed between the oligofructose esters and sucrose esters as described in *chapters 3, 4 and 7*, the size of the hydrophilic moiety apparently plays an important role in the functional properties of interfaces stabilized by those esters. Therefore, we recommend that further research should focus on ways to separate the oligomers with different degrees of polymerization from each other, either before or after synthesis. To obtain oligofructose with a more defined DP, one can focus on improving the selectivity of the partial enzymatic hydrolysis of inulin, using an endo-inulinase, or by making use of oligofructose being synthesized from sucrose using fructosyl-transferase [79, 80].

Functionality

In the next paragraphs we will identify some aspects of the functionality of oligofructose esters that deserve further investigations. Additionally, some applications that were not investigated (in much detail) are mentioned.

In *chapter 3*, light scattering was used to investigate the size of the aggregates that were formed at concentrations larger than the CMC. For almost all surfactants we have found aggregates in the size range of simple micelles. For OF-C18m, however, larger

aggregates were found. The molecular microstructure of these aggregates could not be identified based on light scattering. Therefore, future research should focus on ways to visualize the aggregates that are present in the solutions. Preliminary experiments with transmission electron microscopy (TEM) did not lead to usable results. It is recommended to use a different method, for example cryo-TEM.

For the ellipsometry experiments described in *chapter 3*, we have used a dn/dc factor of 0.15 based on literature values for low molecular weight surfactants [82-84]. The variation in the dn/dc value for different materials is generally between 0.15 and 0.18 [84]. The actual value of dn/dc does not affect the ratio between the head group sizes that were obtained but it does influence the actual value of the head group area. Therefore, to be able to compare obtained results better to literature values, it is recommended to determine dn/dc experimentally.

In *chapter 4* we have focused on dilatational rheology. Dilatational rheology is sensitive towards relaxation mechanisms under compression and expansion, and the kinetics of molecules when moving to/from interfaces [85]. Dilatational deformations are often linked to foam formation [12], coalescence and disproportionation [58]. However, shear deformations are also relevant for foaming properties. They provide information about the interfacial composition and structure and the nature of the interactions between molecules [13]. Shear deformations are often linked to drainage [13, 86]. Therefore, we recommend to further explore the rheological responses of the interfaces during shear deformations. Exploratory shear rheology experiments on a rheometer equipped with a double wall ring geometry gave highly irreproducible results. It is recommended to reproduce these experiments on a rheometer with increased sensitivity, for example a magnetic rod interfacial stress rheometer [87].

All hypotheses in *chapter 4, 5, 6 and 7* about interfacial microstructure that were formulated based on rheological information need to be confirmed using appropriate optical techniques such as Brewster Angle Microscopy, particle tracking observed by microscopy [88], or grazing incidence X-ray diffraction [89].

Husband et al. [48] have studied the interfacial and foaming properties of sucrose esters. They studied a crude sample, a pure mono-ester and a pure di-ester and found a higher foamability and foam stability of the crude sample compared to either component. Furthermore, they found that the addition of di-ester to mono-ester could improve the foaming properties to the level of the crude sample. In *chapter 5* we have shown that the interfacial properties of mixed interfaces of mono-esters and di-esters are strongly dependent on the ratio between mono-ester and di-ester. We expect that this difference in

interfacial structure will have significant consequences for the foaming properties. In this thesis this aspect was not yet addressed. Therefore, we recommend to further investigate the foaming properties.

When mixing oligofructose esters with whey protein isolate in *chapter 6*, the properties of the interfaces were dominated by the oligofructose esters, as long as the protein concentration was not too high. This is a first indication that these esters can be successfully implemented in aerated food products, because proteins are the main surface active components in food products and other components such as sugars and salts have a limited influence on foaming functionality. However, this statement needs to be further supported with experiments using different types of proteins, at different concentrations of surfactant and in actual food products.

For the foam experiments that were described in *chapter 6 and 7*, we recommend to use a more standardized method for foam creation and observations. Since in these chapters the foam and liquid height and foam structure were observed by eye, there is a possible source of human error in these experiments. An example of a more standardized method is the commercially available FoamScan which employs image analysis and conductivity measurements, and has been successfully used for the study of foams created from aqueous solutions of surfactants [90].

Preliminary experiments on oil/water interfaces have shown that oligofructose fatty acid esters can significantly lower the interfacial tension of that interface. Oligofructose mono-esters with fatty acids of 8, 12 and 16 carbon atoms lowered the interfacial tension more than the reference products whey protein isolate and SDS. Furthermore, they could be used to stabilize emulsions. It is recommended to further elaborate on these results and focus on the rheological properties and microstructure of the oil/water interfaces stabilized by the esters and to investigate their potential as emulsion stabilizers.

During exploratory experiments, we have also esterified fatty acids to maltodextrin and konjac glucomannan (with a DP up to 15). These derivatives could not be synthesized enzymatically, possibly caused by the fact that due to their larger size compared to oligofructose they could not access the catalytic cleft of the enzyme. Rather, their synthesis was catalyzed by molecular sieves, as demonstrated by ter Haar et al. [34]. Because an enzymatic reaction procedure was the preferred procedure in this thesis, these esters were not selected for further investigations. However, we do believe that they have potential as novel food ingredients because we were able to show that also these derivatives stabilized foams and emulsions.

Dressaire et al. [91] have shown that a mixture of sucrose monostearate and sucrose distearate can be used to form microbubbles with extremely high stability. They attribute this stability to the formation of a self-assembled surfactant layer with nanometer-scale hexagonal patterning. The size of the domains in this patterning depended on the ratio mono-ester/di-ester. We have performed some exploratory experiments to see if oligofructose fatty acid esters could be used to form similarly stable microbubbles. This exploratory experiment involved the use of oligofructose lauric acid esters as the stabilizer and sonification as the production method. However, further investigations are necessary using a different production method, for example shearing, and using oligofructose esters with differences in molecular structure to explore the potential of the esters to stabilize microbubbles.

Sucrose esters are widely used in Japan as antibacterial agents in canned drinks [32]. Habulin et al. [92] have shown that by using sucrose monolaurate, the growth of *Bacillus cereus*, a food poisoning bacteria, could be inhibited. Ferrer et al. [32] have shown that sucrose monolaurate and maltose monolaurate could inhibit the growth of *Bacillus* sp. and *Lactobacillus plantarum*. The mechanism by which sucrose esters are thought to inhibit bacterial growth is inducing autolysis processes that result in cell death [93]. Possibly, sugar esters reorganize the cellular membrane which results in an increased permeability and losses of important metabolites [94]. We recommend to investigate if oligofructose fatty acid esters also can inhibit microbial growth, because this would make them attractive multi-functional food additives.

Discher et al. [95, 96] have shown that synthetic block co-polymers can self-assemble into highly stable vesicles. Besheer et al. [97] have shown that block-copolymers based on hydroxyethyl starch and fatty acids can form micelles and vesicles. A similar type of structure, when produced using food-grade methods and materials, has the potential to be used as an encapsulating material for the food industry, where the encapsulated material is protected from moisture, heat or other extreme conditions. Furthermore, capsules can be used to mask odors and tastes. Therefore, we recommend to establish the potential of oligofructose fatty acid esters to form vesicles to be used as delivery systems.

7. Concluding remark

In this thesis, we have shown that by using an oligosaccharide instead of a disaccharide as the hydrophilic head group in a sugar ester, dramatic changes in functionality are induced. When using an oligosaccharide, as a result of the larger hydrophilic group, interactions between the head groups are present on a much larger

length scale than in the case of a disaccharide. This leads to the formation of an interfacial soft glass. This different interfacial microstructure has pronounced consequences for the rheological response of the interface, which eventually leads to differences in macroscopic properties. Oligofructose fatty acid esters are surface-active molecules that combine the best properties of both LMW surfactants and proteins; a low surface tension and a high dilatational modulus.

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Summary

Aerated food products consist of air bubbles that are surrounded by a matrix that can be either liquid or solid. Due to the large number of air bubbles that are generally present in aerated products, these systems contain a large interfacial area. Therefore, the properties of the interfaces are considered to contribute significantly to the macroscopic properties of the system. The properties of these interfaces are largely determined by the type of surfactant that adsorbs. Two major types of surfactants that are used within the food industry are proteins and low molecular weight (LMW) surfactants. Proteins are macromolecules consisting of hydrophilic and hydrophobic patches that adsorb at the interface, where they lower the surface tension and can unfold to create a two-dimensional network that can provide a high modulus. In contrast, LMW surfactants are molecules with a well-defined hydrophilic and hydrophobic part. They can form more compact surface layers than proteins, leading to lower surface tensions. They generally do not provide the interface with a high modulus, instead they stabilize the interface through the Gibbs-Marangoni mechanism that relies on rapid diffusion of surfactants after deformations of the interface. A molecule that can lower the surface tension considerably, like a LMW surfactant, and at the same time provide a high modulus, like a protein, has the potential to be an excellent foam stabilizer. In this thesis we focus on a series of molecules that obey these criteria: oligofructose fatty acid esters. We address the influence of changes in chemical fine structure (fatty acid chain length and degree of saturation, degree of esterification and size of the hydrophilic group) on the functional properties.

These esters are synthesized by esterification of fatty acids to oligofructose, which is a mixture of oligomers with different degrees of polymerization. As we show in *chapter 2*, reasonable yields are obtained when using lipase as the catalyst in a mixture of DMSO and Bu^tOH. The conversion into mono-esters increased with increasing fatty acid chain length and is consistent with the preference of the enzyme for more hydrophobic substrates. The crude reaction product consisted of a mixture of unreacted oligofructose and fatty acids, the main reaction products mono-esters and small amounts of di-esters. The crude product was fractionated using RP-SPE. MALDI-TOF MS and (2D) NMR were used to confirm the structure and purity of the esters; >90% for mono-esters and >80% for di-esters.

Similar to typical LMW surfactants, the oligofructose esters formed spherical micelles in the bulk after a certain critical concentration. As we show in *chapter 3*, the CAC depended on the hydrophobicity of the molecules. The efficiency also increased with increasing hydrophobicity and the effectiveness was similar. The area occupied by a single molecule at the interface was determined by fitting the CAC curves with the Gibbs adsorption model and measured directly using ellipsometry. The area occupied at the

interface was larger for oligofructose mono-esters compared to sucrose esters. Furthermore, oligofructose di-esters occupied slightly more area than sucrose esters. All esters occupied significantly more area than a single fatty acid chain. This shows that the oligofructose group dominates the area occupied at the interface.

The rheological properties, as studied in *chapter 4*, were determined using a traditional approach, where the dependency of the surface dilatational modulus on surface pressure and frequency was determined, and using a novel approach, where we show how the surface dilatational modulus is dependent on deformation amplitude and temperature. Furthermore, we show how Lissajous plots of surface pressure versus deformation may be used to gain information about the correlation between surface rheological properties and interfacial microstructure. Sucrose esters behaved like typical LMW surfactants, with low surface dilatational moduli, scaling exponents in the frequency dependency close to 0.5, and fairly viscous Lissajous plots without significant asymmetries. In contrast, oligofructose mono-esters formed interfaces with high surface dilatational moduli, low scaling exponents in the frequency dependency and asymmetric Lissajous plot with strain hardening during compression and strain softening during expansion. We conclude that the oligofructose mono-esters form a two-dimensional soft glass. The oligofructose di-esters behaved like typical LMW surfactants at high surface pressures, showing that the presence of the second fatty acid chain prevent the formation of the glass by the oligofructose part.

In *chapter 5* we focus on the difference in functionality between the crude reaction product, the individual components that are present in the crude product and mixes of these products. Unreacted fatty acids migrated to the interface only in very small amount, due to the low solubility in the bulk. The addition of mono-esters slightly improved the amount of fatty acid that could migrate to the interface. Oligofructose was not surface active and its addition to the mono-ester only diluted the mono-ester which did not lead to significant changes in functional properties because the concentration of mono-ester was still close to the CMC. When mono-esters and di-esters were mixed, the rheological results showed that the ratio between mono-ester and di-ester was very important for the rheological profile. In both cases the results suggest the presence of islands of glass phase formed by the mono-esters surrounded by a viscous phase formed by the di-esters. When the surface concentration of mono-esters was high, the glassy patches dominated the interface, leading to a high modulus, low frequency dependency and Lissajous plots with a high degree of asymmetry. When the surface concentration of mono-esters decreased, the lower connectivity between the glassy patches lead to a low modulus, intermediate frequency dependency, and Lissajous plots with moderate asymmetry.

To study the potential of oligofructose esters as food grade surfactants it is important to consider that many food products contain ingredients with the potential to be surface active. Therefore, in *chapter 6* we have studied the functional properties of an oligofructose mono-ester in the presence of whey protein isolate, a commonly used food protein. Except for at the highest protein concentration, the surface was dominated by the oligofructose ester. The stabilization mechanisms of oligofructose ester and WPI were mutually exclusive, leading to interfaces with a low surface dilatational modulus. Since the foaming properties were not negatively affected, we conclude that the Gibbs-Marangoni mechanism occurred. Only at the highest protein concentration, the surface concentration of WPI was sufficiently high to interfere with this mechanism, leading to a significant decrease in foam stability. Oligofructose esters were also able to displace a fully developed WPI network.

In *chapter 7* we discuss the foaming properties of the esters. We show that only esters of intermediate hydrophobicity are able to form foams with small bubbles and a uniform bubble size distribution that lead to high foam stability. The affinity of esters with shorter fatty acid chains, up to 8 carbon atoms, for the interface was quite low as a result of the relatively hydrophilic nature of the molecules. Therefore, they were not effective foam stabilizers. The most hydrophobic components (mono-ester with a chain length of 18 carbon atoms and di-ester with a chain length of 12 carbon atoms) were too slow to migrate to the interface. Therefore, also these components were poor foam stabilizers. We show that the surface tension at short time scales is the most accurate predictor of foam stability. However, despite similar initial surface tension values, oligofructose esters lead to higher foam stability. This could be attributed to the oligofructose part that forms a two-dimensional glass phase and provides mechanical stability to the foam films.

In the general discussion that is presented in *chapter 8* we integrate the results from the different chapters. One of the factors that is persistent throughout the different chapters is the rheological profile of the interfaces. We have shown that by using amplitude sweeps and Lissajous plots, a lot more information on the interfacial microstructure can be extracted from rheological data than by using more conventional methods. In the last part of the general discussion improvements to the synthesis are discussed, as the optimization of the synthesis was not considered in this thesis. Furthermore, improvements for the functional experiments and additional applications were identified.

Samenvatting

Levensmiddelen die bestaan uit schuim zijn opgebouwd uit luchtbelletjes die omringd worden door een matrix die zowel vloeibaar als vast kan zijn. Door het grote aantal luchtbelletjes dat in dit soort producten zit bevatten deze producten een groot grensvlak. Daarom dragen de eigenschappen van dit grensvlak significant bij aan de macroscopische eigenschappen van het systeem. De eigenschappen van het grensvlak worden vooral bepaald door het type molecuul dat aan dit grensvlak adsorbeert. Twee belangrijke types oppervlakte-actieve stoffen die gebruikt worden in de levensmiddelenindustrie zijn eiwitten en oppervlakte-actieve stoffen met een laag moleculair gewicht (LMG). Eiwitten zijn macromoleculen die hydrofiele en hydrofobe stukken bevatten en adsorberen aan grensvlakken waar ze de oppervlaktespanning verlagen. Daar kunnen ze ontvouwen en een tweedimensionaal netwerk vormen dat het grensvlak een hoge modulus geeft. LMG oppervlakte-actieve stoffen, daarentegen, zijn moleculen met een goed gedefinieerd hydrofiel en hydrofoob gedeelte. Zij vormen lagen aan het grensvlak die compacter zijn dan die van eiwitten, wat tot een lagere oppervlaktespanning leidt. Over het algemeen leiden deze moleculen niet tot een grensvlak met een hoge modulus. In plaats daarvan stabiliseren zij het grensvlak door middel van het Gibbs-Marangoni mechanisme. Dit mechanisme omvat snelle diffusie van oppervlakte-actieve stoffen na vervormingen van het grensvlak. Een molecuul dat de oppervlaktespanning aanzienlijk kan verlagen, zoals een LMG oppervlakte-actieve stof, maar ook een hoge modulus geeft, zoals een eiwit, heeft het potentieel om een zeer goede schuimstabilisator te zijn. In dit proefschrift hebben we een serie moleculen bestudeerd die aan deze criteria voldoen: oligofructose vetzuur esters. Er is gekeken naar de invloed van variaties in de chemische fijnstructuur (lengte en mate van verzadiging van de vetzuurstaarten, de mate van esterificatie en de grootte van de hydrofiele groep) op de functionele eigenschappen.

Deze esters zijn gesynthetiseerd door een vetzuur aan een oligofructose te veresteren. De oligofructose was een mengsel van oligomeren met verschillende mate van polymerisatie. In *hoofdstuk 2* laten we zien dat een redelijke opbrengst wordt verkregen als lipase als katalysator wordt gebruikt in een mengsel van DMSO en Bu^tOH. De omzetting in mono-esters nam toe als de lengte van de vetzuurstaart toenam. Dit komt overeen met een voorkeur van het enzym voor meer hydrofobe substraten. Het ruwe reactieproduct bestond uit een mengsel van ongereageerde oligofructose en vetzuren, het belangrijkste reactieproduct mono-esters en kleine hoeveelheden di-esters. Het werd gefractioneerd met behulp van RP-SPE. Door middel van MALDI-TOF MS en (2D) NMR werd de structuur en zuiverheid van de esters vastgesteld; >90% voor mono-esters en >80% voor di-esters.

Net als typische LMG oppervlakte-actieve stoffen, vormen de oligofructose esters bolvormige micellen in de bulk bij concentraties hoger dan een bepaalde kritische concentratie (KAC). In *hoofdstuk 3* laten we zien dat de KAC afhangt van de hydrofobiciteit van de moleculen. The efficiëntie nam ook toe met toenemende hydrofobiciteit. De effectiviteit, daarentegen, was vergelijkbaar. De hoeveelheid oppervlak die een molecuul inneemt op het grensvlak is bepaald door het fitten van de KAC curves met het Gibbs adsorptie model. Deze parameter werd ook direct gemeten door middel van ellipsometrie. Oligofructose mono-esters namen meer plek in beslag dan sucrose esters. Verder namen oligofructose di-esters iets meer plek in beslag als oligofructose mono-esters. Alle esters namen veel meer plek in beslag dan een enkele vetzuurstaart. Dit laat zien dat het oligofructose-deel de hoeveelheid plek die een molecuul inneemt domineert.

De reologische eigenschappen, beschreven in *hoofdstuk 4*, zijn bepaald volgens een traditionele aanpak, waarbij de dilatatiemodulus van het grensvlak gemeten is als functie van oppervlaktedruk en frequentie. Verder hebben we een nieuwe aanpak gebruikt, waar we laten zien dat deze modulus afhangt van de amplitude en temperatuur. Verder laten we zien hoe Lissajous curves waar de oppervlaktedruk wordt weergegeven als een functie van de vervorming gebruikt kunnen worden om meer informatie te verkrijgen over de relatie tussen reologische eigenschappen van het grensvlak en de microstructuur van het grensvlak. Sucrose esters gedroegen zich als typische LMG oppervlakte-actieve stoffen, met lage moduli, een exponent in de frequentie-afhankelijkheid dicht bij 0.5, en Lissajous plots karakteristiek voor redelijk viskeuze grensvlakken, zonder asymmetrie. Oligofructose mono-esters, daarentegen, vormden grensvlakken met een hoge modulus, een lage frequentie-afhankelijkheid en asymmetrische Lissajous plots met een grensvlak dat harder wordt als het wordt ingedrukt en zachter wordt als het wordt uitgerekt. Deze resultaten wijzen erop dat de oligofructose mono-esters een tweedimensionale glasfase vormen. De oligofructose di-esters gedroegen zich als typische LMG oppervlakte-actieve stoffen bij hoge oppervlaktedruk, wat laat zien dat de aanwezigheid van de tweede vetzuurstaart het ontwikkelen van de glasfase verhindert.

In *hoofdstuk 5* richten we ons op de verschillen in functionaliteit tussen het ruwe reactieproduct, de individuele componenten die aanwezig zijn in dit product, en mengsels van deze componenten. Ongereageerde vetzuren konden slechts in beperkte hoeveelheden naar het grensvlak migreren door hun lage oplosbaarheid. Als mono-esters werden toegevoegd kon er iets meer vetzuur naar het grensvlak migreren. Oligofructose was niet oppervlakte-actief en verdunde alleen de mono-ester. Dit had geen belangrijke gevolgen omdat de mono-ester concentratie nog steeds dicht bij de KAC zat. Wanneer

mono-esters en di-esters gemengd werden, lieten de reologische resultaten zien dat de verhouding tussen mono-esters en di-esters belangrijk was voor het reologische profiel. In beide gevallen waren er eilandjes van een glasfase gevormd door de mono-ester, omringd door een viskeuze fase gevormd door de di-ester. Wanneer de grensvlakconcentratie van mono-esters hoog was werd het grensvlak gedomineerd door de eilandjes. Het grensvlak had een hoge modulus, lage frequentie-afhankelijkheid en asymmetrische Lissajous curves. Wanneer de grensvlakconcentratie van de mono-esters afnam, was er minder connectiviteit tussen de eilandjes, wat resulteerde in een lagere modulus, gemiddelde frequentie-afhankelijkheid, en Lissajous curves met een gematigde asymmetrie.

Voor het toepassen van de esters als functionele ingrediënten in levensmiddelen is het belangrijk om te realiseren dat veel levensmiddelen ingrediënten bevatten die mogelijk ook oppervlakte-actief zijn. Daarom hebben we in *hoofdstuk 6* gekeken naar de functionele eigenschappen van de oligofructose mono-ester in de aanwezigheid van wei-eiwit isolaat, een veel gebruikt eiwit. Behalve bij de hoogste eiwit-concentratie, werd het grensvlak gedomineerd door de oligofructose esters. De stabilisatie-mechanismen van eiwit en oligofructose ester sloten elkaar uit, wat leidde tot een grensvlak met een lage modulus. De schuimeigenschappen werden niet negatief beïnvloed. Daarom concluderen we dat de grensvlakken gestabiliseerd werden door het Gibbs-Marangoni mechanisme. Alleen bij de hoogste eiwit-concentratie was de grensvlakconcentratie van het eiwit hoog genoeg om te interfereren met dit mechanisme. Dit leidde tot een lagere schuimstabiliteit. Oligofructose esters konden ook bij een volledig ontwikkeld eiwitgrensvlak het eiwit van het grensvlak verstoten.

In *hoofdstuk 7* bestuderen we de schuimeigenschappen van de esters. We laten zien dat alleen esters met gemiddelde hydrofobiciteit schuimen kunnen vormen met kleine bellen en een uniforme distributie van bellengrootte. Dit leidde tot hoge schuimstabiliteit. De esters met korte vetzuurstaarten hadden een lage affiniteit voor het grensvlak door hun relatief hoge hydrofiliciteit. Daarom waren zij geen goede schuimstabilisatoren. De meest hydrofobe componenten (mono-ester met een ketenlengte van 18 koolstofatomen en di-esters met een ketenlengte van 12 koolstofatomen) migreerden te langzaam naar het grensvlak. Daarom waren zij ook slechte schuimstabilisatoren. De oppervlaktespanning op de korte tijdschaal was de meest betrouwbare voorspeller van schuimstabiliteit. Maar, ondanks vergelijkbare oppervlaktespanning op korte tijdschaal hadden oligofructose mono-esters een hogere schuimstabiliteit dan sucrose esters. Dit komt door het oligofructose deel dat een glasfase vormt, wat zorgt voor een betere mechanische stabilisatie van de schuimfilms.

In de algemene discussie in *hoofdstuk 8* integreren we de resultaten van de verschillende hoofstukken. Een van de concepten die in veel hoofdstukken voorkomt is het reologische profiel van de grensvlakken. We hebben laten zien dat door middel van amplitude variaties en Lissajous curves, veel meer informatie over de microstructuur van het grensvlak kan worden verkregen uit reologische gegevens vergeleken met meer traditionele methoden. In het laatste deel bespreken we verbeteringen aan de synthese, aangezien deze niet geoptimaliseerd is in deze thesis. Verder worden verbeteringen voor de functionele experimenten en meer toepassingen besproken.

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Curriculum vitae

Silvia van Kempen was born on the 30th of August 1984 in Venray. She attended the Raayland college where she received her VWO diploma in 2002. After that, she studied food technology at Wageningen University. During the BSc, she performed a minor in nutrition and health and received the degree in 2006. During the MSc, she did an internship in product development at Campina. After that, she performed a thesis at the chair group of physics and physical chemistry of foods. This thesis was about the transglumatinase-induced gelation of sodium caseinate. She received the MSc degree in 2008. In the same year, she started working as a PhD researcher in the same chair group. The results of this research are presented in this thesis.

Publications

Van Kempen, S.E.H.J., C.G. Boeriu, H.A. Schols, P. De Waard, E. Van der Linden, and L.M.C. Sagis, Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification. Food Chemistry, 2013. 138(2-3): p. 1884-1891.

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Van Kempen, S.E.H.J., H.A. Schols, E. Van der Linden, and L.M.C. Sagis, Molecular assembly, interfacial rheology and foaming properties of oligofructose fatty acid esters. Submitted., 2013.

Overview of completed training activities

Discipline specific activities
Food hydrocolloids, Wageningen, The Netherlands, 2009
NWO meeting Liquids and Interfaces, Lunteren, The Netherlands, 2009
Han-Sur-Lesse Winterschool, Han-sur-Lesse, Belgium, 2009
Dutch Polymer Institute Cluster Review meeting, Deventer, The Netherlands, 2009
International symposium on food rheology and structure 2009, Zurich, Switzerland, 2009
Rheology course, Leuven, Belgium, 2009
Delivery of functionality 2009, Wageningen, The Netherlands, 2009
Food colloids 2010, Granada, Spain, 2010
Dutch Polymer Institute Cluster Review meeting, Arnhem, The Netherlands, 2010
Dutch Polymer Institute Cluster Review meeting, Eindhoven, The Netherlands, 2011
Conference of the European Colloid and Interface Society 2011 (including training course Surfactant-polymer interactions), Berlin, Germany, 2011
International symposium on Food rheology and structure 2012, Zurich, Switzerland, 2012 (presentation)
Surfactants in solution 2012, Edmonton, Canada, 2012 (presentation)
The XVIth International Congress on Rheology, Lisbon, Portugal, 2012 (presentation)

General courses
PhD week, Bilthoven, The Netherlands, 2009
Organising and supervising MSc thesis work, Wageningen, The Netherlands, 2009
PhD competence assessment, Wageningen, The Netherlands, 2009
Philosophy and ethics of food science and technology, Wageningen, The Netherlands, 2010
Techniques for writing and presenting a scientific paper, Wageningen, The Netherlands, 2010
Teaching methodology and skills for PhD students, Wageningen, The Netherlands, 2011
Scientific writing, Wageningen, The Netherlands, 2012
Career perspectives, Wageningen, The Netherlands, 2012

Optionals
PhD excursion, Japan, 2010
Organisation of PhD excursion Japan, 2010
Preparation PhD research proposal, 2008
Science meetings Food physics, 2008-2012

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