Characterisation of the stability for suberin-in-water emulsions

Thesis for M.Sc. in Chemical and Process Engineering

by

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Abstract

Title of the thesis:	Characterisation of the stability for suberin-in-water emulsions
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This thesis focuses on investigating the stability of suberin-in-water emulsions using commercial surfactants, i.e., Spans and Tweens, and hemicellulose derivatives as surfactants. Suberin, a natural biopolymer found in the cell walls of plants, has gained attention as a potential bio-friendly alternative in various applications. The objectives of this study were to determine the hydrophilic-lipophilic balance (HLB) of suberin, analyse the stability of the emulsions, and assess the suitability of hemicellulose-grafted-fatty acids (HC-FA) as surfactants for these emulsions. Experimental methods involved determining the HLB of suberin, formulating emulsions using hemicellulose derivatives as surfactants, and evaluating the stability of the emulsions. Emulsions stabilized with Spans and Tweens exhibited strong colloidal stability, while emulsions stabilized with galactoglucomannan-grafted-fatty acids (GGM-FA), a natural surfactant, displayed its potential by producing stable emulsions. The average zeta potential of around -25 mV also showed GGM-FAs potential for creating stable emulsions, despite microscopic observations confirming the presence of particle clusters. Overall, the findings contribute to understanding the stability of suberin emulsions and the potential application of hemicellulose derivatives as surfactants, providing valuable insights for the packaging and coating industries.

Keywords: Suberin, emulsion stability, hemicellulose derivatives, surfactants, hydrophilic-lipophilic balance (HLB), biopolymer, particle size

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Thomas Öhman

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Abbreviations

C	Carbon atom
CDI	1'1-carbonyldi-imidazole
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
ELS	Electrophoretic light scattering
FA	Fatty acid
HC-FA	Hemicellulose-grafted-fatty acids
Glc	Glucose
GGM	Galactoglucomannan
GGM-FA	Galactoglucomannan-grafted-fatty acids
Gal	Galactose
HLB	Hydrophilic-lipophilic balance
Man	Mannose
NMR	Nuclear magnetic resonance
O/W	Oil-in-water
PHWE	Pressurized hot water extraction
SMLS	Static multiple light scattering
TSI	Turbiscan stability index
W/O	Water-in-oil
% w/w	Weight per weight

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Introduction

The utilisation of fossil fuels as raw materials in various industries poses significant challenges due to environmental concerns and finite resources. In the pursuit of sustainable alternatives, the exploration of bio-based materials has gained attention. Suberin, a complex biopolymer found in the cell walls of certain plants, holds promise as a renewable resource in the development of bio-based binders and coatings.

Suberin, with its unique structure and composition, offers tremendous potential as a renewable resource for binder and coating applications. Its abundance in nature and remarkable properties, including hydrophobicity and film-forming capabilities, make it an attractive alternative to fossil fuel-based materials (Graça, 2015). In the packaging and coating industries, exploring the characteristics of suberin-in-water emulsions and evaluating hemicellulose-grafted-fatty acids (HC-FA) as surfactants is of interest. By utilising renewable materials like suberin, we can move towards decreasing reliance on fossil fuels and promoting more environmentally friendly solutions.

Emulsions, which are dispersed systems consisting of two immiscible phases, play an important role in various industrial applications. Gaining insights into the composition and functionality of emulsions is crucial for optimising their stability and performance. The hydrophilic-lipophilic balance (HLB), which measures the relative hydrophilicity and lipophilicity of surfactants, serves as a vital tool in selecting appropriate emulsifiers (ICI Americas, 1984).

HC-FA, particularly complexes of galactoglucomannans and fatty acids (GGM-FA), offer potential as bio-based surfactants for suberin emulsions. The determination of their suitability as surfactants aims to enhance the stability and functionality of suberin-based formulations. The use of hemicellulose derivatives as surfactants

provides a possible solution for sustainable and renewable materials in the packaging and coatings industry.

This thesis focuses on exploring the characteristics of suberin-in-water emulsions and evaluating hemicellulose-grafted-fatty acids as potential surfactants. Its main emphasis lies in characterising the stability of these emulsions. For this, the HLB system will be utilised to identify appropriate surfactants for stabilising the emulsions. The evaluation of stability will cover various analytical techniques, including measurements of stability, analysis of zeta potential, and characterisation of particle size. These analyses will provide valuable insights into the stability of the emulsions, providing a comprehensive understanding of their performance.

The goals of the thesis were the following:

- ✓ Determine the hydrophilic-lipophilic balance (HLB) of suberin through empirical testing.
- ✓ Evaluate the suitability of galactoglucomannan-grafted-fatty acids (GGM-FA) as surfactants for stabilising suberin-in-water emulsions.
- ✓ Verify the stability of suberin emulsions using different analysis methods.

1 Literature review

1.1 Suberin

During the early stages of land colonisation by plants, hundreds of millions of years ago, they acquired the ability to synthesise biopolymers, including suberin. This provided numerous benefits to the plants, as it enabled them to be protected against desiccation (Correia et al., 2020). Suberin is a lipophilic macromolecule found in the cell walls of plants. It serves as a protective barrier for the plant and its surroundings by preventing water loss and promoting the healing of wounded tissues. It also separates different tissues from each other within the plant (Franke & Schreiber, 2007). Suberin-containing cells form the periderm, a part of the outer bark in plants. Suberin is covalently linked to lignin, and in some plants, massive amounts of suberin can be found in the periderm, making it readily available (Graça, 2015). Some of the most suberised substances are potatoes and the outer bark of cork oak, which contains more than 50% w/w suberin (Franke & Schreiber, 2007). In the forestry industry, substantial quantities of bark are produced from various tree species, but unfortunately, a significant portion of it is currently being burned for energy recovery purposes (Gandini et al., 2006).

However, eco-friendliness and availability are not the only reasons for the industrial interest in suberin. Suberin is unique chemically, and the reactiveness in both ends of its monomer chains is of interest in many applications. Another advantage is brought on by the length of the carbon chains, leading to flexibility of the chain as well as hydrophobicity (Graça, 2015). This chapter focuses on the composition and structure of suberin.

1.1.1 Structure and composition

The suberised cell walls have three main layers: the primary layer, the secondary layer, and the tertiary layer (Graça, 2015). The primary and tertiary layers are thin

and made up of cellulose and lignin. The secondary layer, in turn, contains suberin. Suberin is a complex mixture of α, ω-bifunctional fatty acids and glycerol. The term "α, ω-bifunctional" refers to the fact that the monomers that make up suberin have two linking positions, one at both ends of the carbon chain. The monomers are connected similarly to polyesters. The majority of the suberin mass, about 80-90%, comes from long chains of aliphatic acids. Glycerol makes up 5-20% of the suberin mass, while the remaining mass is composed of lesser amounts of phenolic acids such as ferulic acid. The structures of the main components in suberin are illustrated in Figure 1.

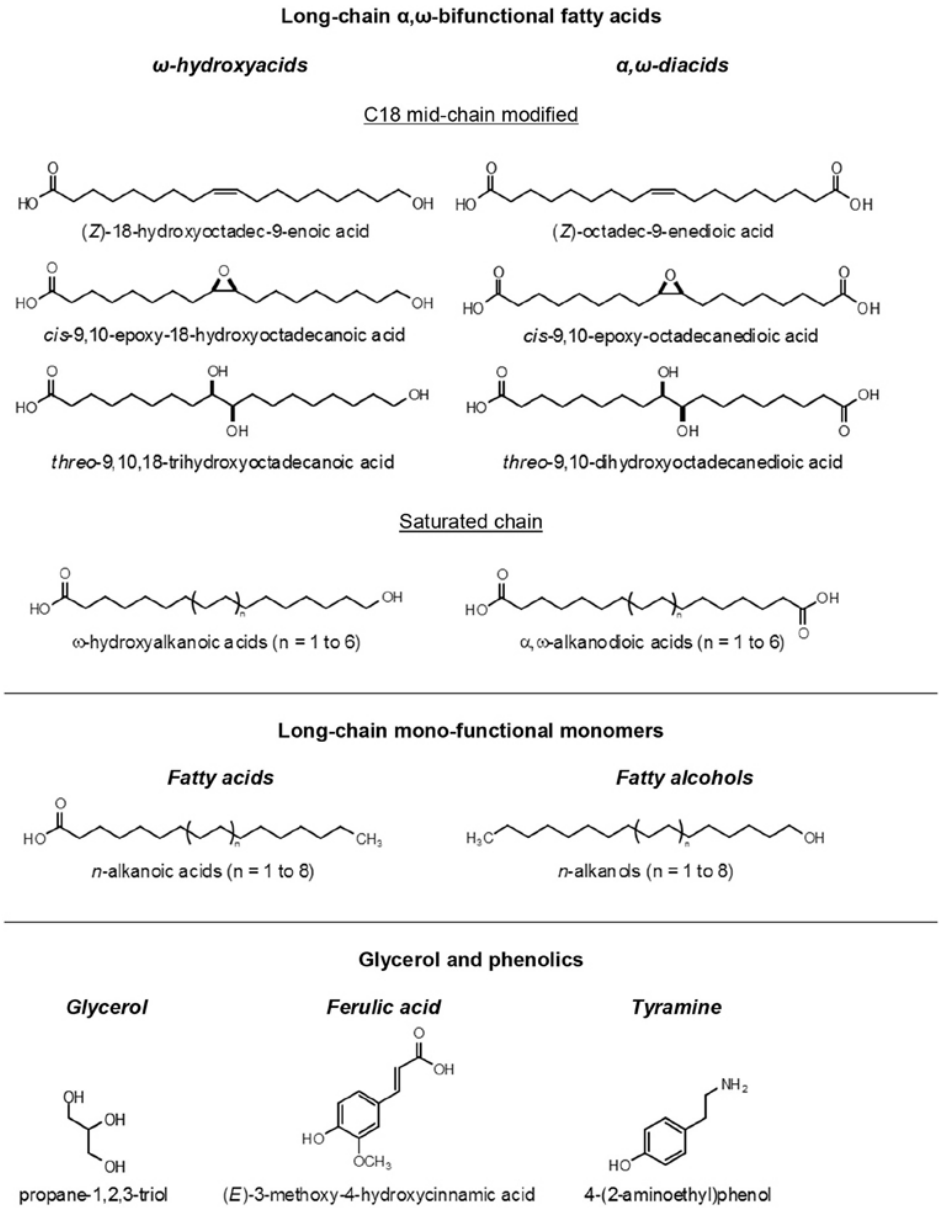

Figure 1. Chemical structures of the main components in suberin (Graça, 2015).

Fatty acids are organic compounds found in animals, plants, and microorganisms, and they form an important part of the human diet. The fatty acids in suberin can be divided into two main categories: α, ω-diacids, which have carboxylic acids at both ends of the chain, and ω-hydroxy acids, which have a carboxylic acid at the α-end and a hydroxyl group at the ω-end. While the monomers can link at other points along the chain, these end-group linkages are the minimum requirement for the monomers to be a part of the polymer chain. Both groups typically have chain lengths of 16 to 26 carbon atoms, with even numbers being most common. The fatty acids also contain smaller amounts of alkanoic acids and alkanols (less than 10%) with chain lengths of up to 30 carbon atoms. Within the α,ω-diacids and ω-hydroxyacids classes of suberin monomers, there are two types of fatty acid chains within the two main groups: one is the unsaturated chain with 18 carbon atoms and a mid-chain modification, and the other is the saturated chain without any modifications, which has either 16, or 20-28 carbon atoms. The 18-atom carbon chain has a significant role in the macromolecular structure of the polymer, as it dominates the composition in terms of quantity. Although all suberin contains these monomers, the pattern changes depending on the raw material used (Graça, 2015). Substantial differences in the amounts of the different monomers have been reported, with e.g., α, ω-diacids varying from 6% to 45% and ω-hydroxy acids varying from 11% to 62% (Gandini et al., 2006).

1.2 Hemicelluloses

Hemicelluloses are natural polysaccharides and are the second most abundant renewable component, following cellulose, in lignocellulosic biomass (Rao et al., 2023). Lignocellulosic biomass, which constitutes the primary fraction in various plant-based materials, such as wood, perennial energy crops, cereal straws, and bagasse, is composed of three main components: cellulose, hemicelluloses, and lignin (Wyman, 1994). The proportions of these components vary depending on the plant source. Together, these components form a matrix that builds the cell wall structure

in plants, pictured in Figure 2. Additionally, small amounts of pectins, extractives, and inorganic components can be found in lignocellulosic biomass (Brandt et al., 2013). The abundance of lignocellulosic biomass makes it an incredibly valuable and highly renewable natural resource worldwide (Qian, 2014). It is considered the most cost-effective and sustainable raw material available.

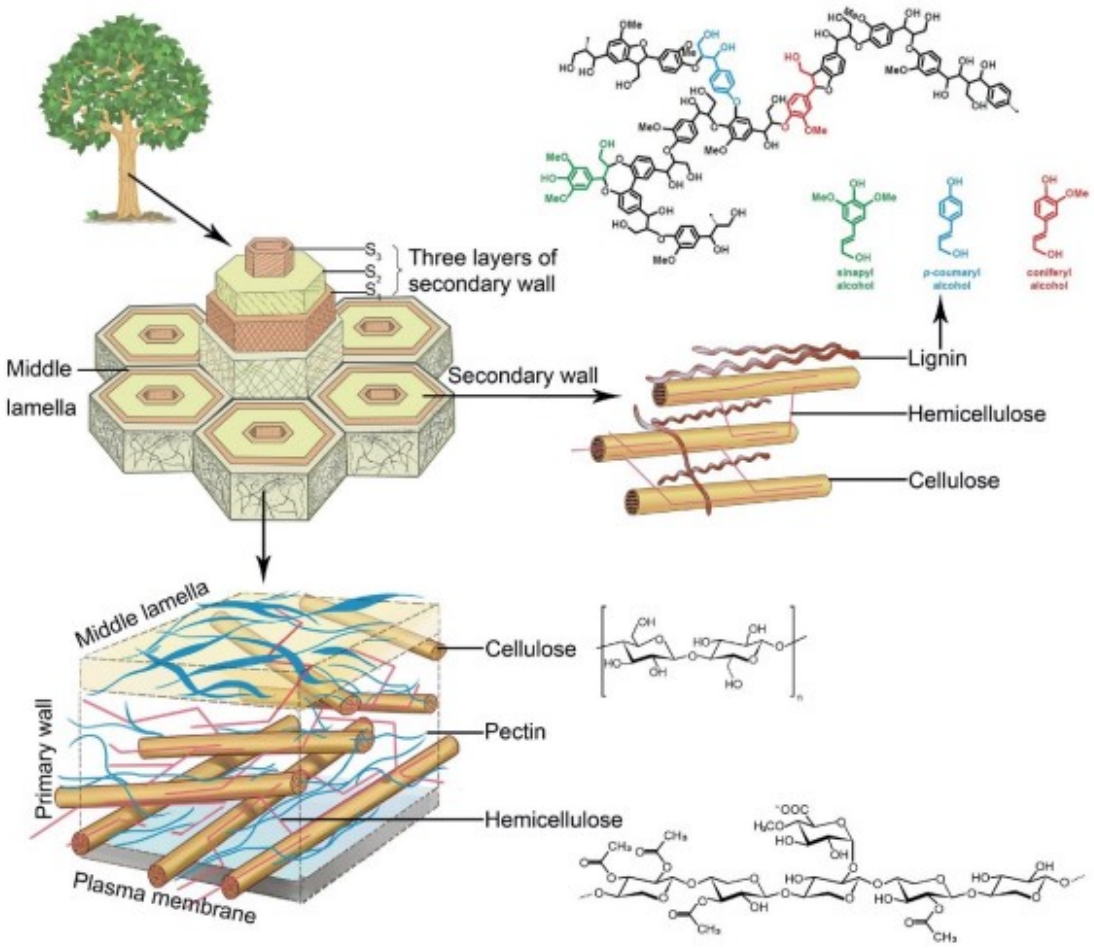

Figure 2. The components in lignocellulosic biomass (Kvikant, 2022).

With a global annual production of approximately 60 billion tons, hemicelluloses offer a nearly unlimited supply and possess exceptional physical and chemical properties (Rao et al., 2023). The versatile polysaccharides find applications in various industries due to their unique characteristics. They are extensively used in the production of materials such as emulsifiers, films, and hydrogels (Rao et al., 2023).

However, it is important to note the variety in different hemicellulose samples. Hemicelluloses, complex carbohydrates found in plant cell walls, display diverse structures and compositions that are influenced by the specific plant species and tissue types (Pauly et al., 2013). They serve important functions by interacting with cellulose through hydrogen bonds and forming covalent bonds with lignin, contributing to the strength and integrity of the cell walls.

Hemicelluloses are commonly obtained from plant cell walls using potent agents like alkali, excluding cellulose glucan chains (Pauly et al., 2013). Nevertheless, when hemicelluloses are located outside the cell wall, they can be readily extracted with water. They typically constitute about one-third of the dry weight of cell walls. To effectively utilise lignocellulosic materials in biorefineries, optimised conversion processes are essential. The materials in end applications need to be both environmentally sustainable and economically viable.

In addition to its use in materials, hemicelluloses also serve as valuable precursors in the production of various chemicals. One reason for this is that when hemicellulose forms glycosidic bonds, it acts as a condensation polymer, eliminating a water molecule from each linkage (Bajpai, 2018). This allows the hemicellulose to be hydrolysed to oligomeric and monomeric saccharides, which can be used in the production of e.g., xylitol, ethanol, and furfural (Rao et al., 2023).

Over the past few decades, research efforts have focused heavily on hemicellulose derivatives, primarily due to their potential for modification and improved compatibility with other materials (Rao et al., 2023). The ability to alter the structure of hemicelluloses through chemical modification enables the development of derivatives with desirable properties, further expanding its range of applications.

While the exploration of hemicellulose derivatives continues in many fields, their utilisation remains in the exploratory stage for numerous applications. However, ongoing research and advancements in this area hold great promise for unlocking the full potential of hemicellulose and its derivatives (Rao et al., 2023).

Hemicelluloses are usually branched heteropolysaccharides consisting of different types of sugar units, including pentoses (such as xylose and arabinose), hexoses (such as glucose, mannose, and galactose), and uronic acids (such as 4-O-methyl glucuronic acid, glucuronic acid, and galacturonic acid) (Zhou et al., 2017). The main types of hemicelluloses, such as xylans, glucomannans, or galactoglucomannans, are composed of these various sugar units. Due to the presence of multiple different sugar units and various glycosidic bonds in hemicellulose molecules, they often appear in an amorphous form, lacking a well-defined crystalline structure (Rao et al., 2023).

In hemicelluloses, most of the monosaccharides have the D configuration, except for arabinose, which has the L configuration. As mentioned, the composition of hemicelluloses vary depending on the source of the biomass. In softwood, a large part of the hemicellulose is composed of glucomannans, such as galactoglucomannans. In hardwood, xylans are the predominant hemicellulose type, a common one being glucuronoxylan, illustrated in Figure 3 (Su, 2012).

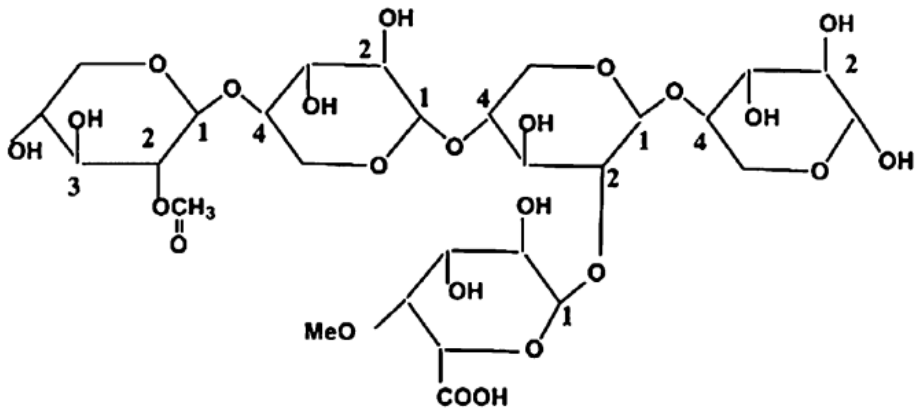

Figure 3. Chemical structure of glucuronoxylan in hardwood (Su, 2012).

1.2.1 Galactoglucomannan (GGM)

Galactoglucomannans (GGMs) make up about 15-20% of the total hemicellulose content in softwood (Willför et al., 2003). It consists of a linear backbone made of

(1->4)-linked β-D-Man*p* (mannose) and (1->4)-linked β-D-Glc*p* (glucose) units. Additionally, there are (1->6)-linked α-D-Gal*p* (galactose) units attached to the side. Some of the mannose units have O-acetyl groups at their C2 and C3 positions, making the hemicellulose partially acetylated (Willför et al., 2003). A specific segment of this structure is illustrated in Figure 4 (Dax, 2014). The ratio of the sugar units in softwood hemicellulose is usually around 3.5-4.5:1:0.5-1.1 (Man:Glc:Gal) (Willför et al., 2008).

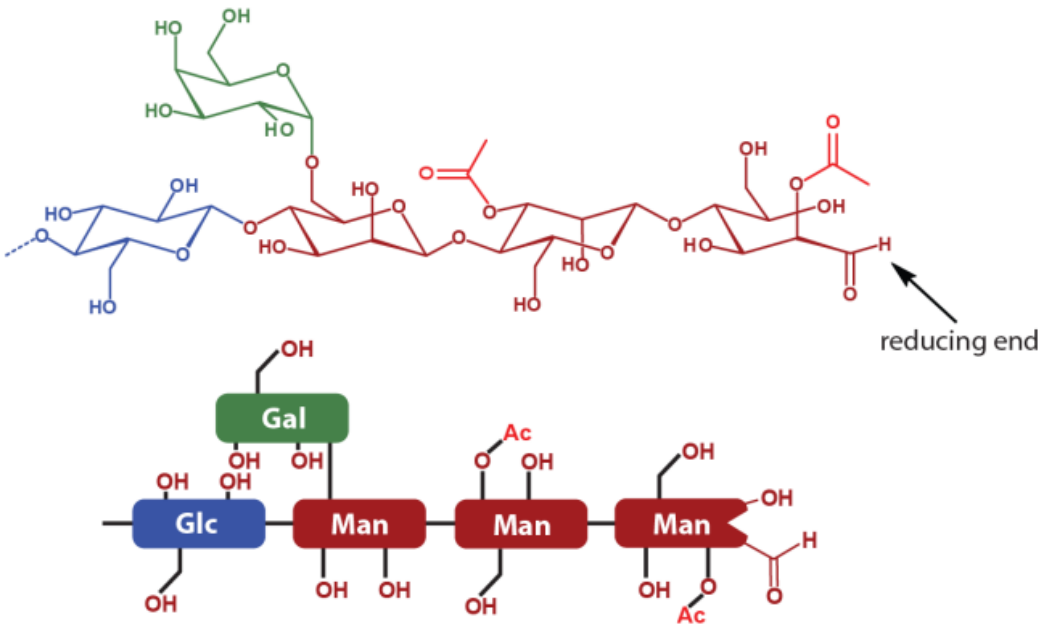

Figure 4. Chemical structure of O-acetyl galactoglucomannan with an open form of the reducing end sugar (Dax, 2014).

GGM can be obtained directly from wood using pressurized hot water extraction (PHWE) or recovered from waste streams in the paper and pulp industry (Willför et al., 2003). If isolated from the process waters of thermomechanical pulp (TMP), it is done through ultrafiltration (Lindqvist et al., 2013).

Due to the presence of acetyl groups, hemicellulose is highly soluble in water. The solubility increases as the molar mass decreases (Dax, 2014). When dissolved in water, GGM solutions turn brown, especially when increasing the concentration. GGM can also be dissolved in dimethyl sulfoxide (DMSO), which is a polar organic solvent commonly used in various synthesis processes (Dax, 2014). These solubility

characteristics of GGM make it possible to chemically modify it to introduce new functional properties. GGM esterified with fatty acids introduces hydrophobicity, which enhances its interaction with hydrophobic substances, such as suberin.

1.3 Emulsions

Emulsions are important formulations in many industries, including food, cosmetics, and pharmaceuticals, among others. Emulsions play a vital role in the creation of products with specific characteristics and properties, and as such, understanding how to create stable and suitable emulsions is crucial. This chapter aims to provide an overview of the basics of emulsions, including the different types of emulsions, the required HLB of an oil phase, the choice of emulsifiers, and the effect of mixing emulsifiers.

1.3.1 Definition

An emulsion is a mixture of two immiscible liquids, such as oil and water, stabilised by an emulsifier (Spasic & Hsu, 2005). The term "emulsion" refers to the dispersion of one liquid in another, creating a mixture that is stable and homogeneous. There are different types of emulsions, and there are many factors that affect the type of emulsion being created. The most crucial factor is the proportion of the oil and aqueous phases, but when they are nearly equal, other factors play a role in determining the type of emulsion formed. The distinction between different types of emulsions lies in which liquid is dispersed in the other. For instance, water can be dispersed in oil or oil can be dispersed in water. One of the most common types of emulsions is the oil-in-water (O/W) emulsion, in which tiny droplets of oil are dispersed in the aqueous phase. An emulsifier is used to help distribute the oil droplets evenly and prevent separation, creating a smooth and consistent mixture.

1.3.2 Stability

After the droplets have been formed, they must be kept stable during the whole lifetime of the products. The stability of emulsions can be threatened by various physicochemical processes, including gravitational separation, flocculation, coalescence, and phase separation. Gravitational separation can be affected both directly and indirectly by emulsifiers. The direct effect comes from the emulsifier's ability to rapidly adsorb onto the droplets, which affects the size of the droplets. The smaller the droplets are, the more stable they are against gravitational separation, as described by Stoke's law (Hong et al., 2018). The indirect effect comes from the increase in density when the emulsifier is adsorbed onto the surface of the lighter oil droplets. When the oil is lighter than water, and water is lighter than the emulsifier, the difference in density between the oil-phase and the water decreases, which decreases the creaming velocity (McClements & Gumus, 2016). Another thing that affects the stability is the amount of emulsifier. An increase in emulsifier concentration up to a certain point improves the stability of the emulsion and helps maintain its properties over time (Derkach, 2009).

There are four criteria which need to be fulfilled to avoid a suspension from being aggregated. The first one, is that the droplets or particles need to be covered completely by the surfactant. Any spots left uncovered will increase the possibility of flocculation due to cohesion of the dispersed phase. The second criterion is that the adsorption between the surfactant and the surface of the dispersed phase needs to be strong. The adsorption between these is driven by lowering the free energy of the phase boundary (Tadros, 2006). The adsorption process with polymeric surfactants in suspensions is complicated. It involves interaction between the polymer and the surface, between the polymer and the solvent, and between the solvent and the surface of the dispersed phase. The long polymer chains form loops and tails, with which they completely cover the particles (Tadros, 2009). The third criterion is that there needs to be strong hydration of the stabilising chain to enable effective steric stabilisation, and the final criterion is that the adsorbed layer needs to be thick enough so that flocculation does not occur (Tadros, 2009).

Flocculation refers to the process where multiple droplets come together to form a cluster while maintaining their individual sizes. Coalescence, on the other hand, is when two or more droplets merge to form a single, larger droplet. In the formation of emulsions, flocculation and coalescence can take place when attractive forces between droplets surpass the repulsive forces (McClements & Gumus, 2016). To prevent this from happening, emulsifiers are utilised as they generate strong repulsive forces through electrostatic, steric, or both mechanisms. In some cases, however, attractive forces can still be created due to hydrophobic interactions between the exposed non-polar regions of the emulsifier molecule and the droplets, or when there are high levels of non-adsorbed emulsifier leading to depletion attraction. The properties that play a role in these interactions include electrostatic interaction, steric repulsion, hydrophobic interactions, and covalent interactions (McClements & Gumus, 2016).

One way to determine colloidal stability is by measuring turbidity with e.g., the instrument Turbiscan. The Turbiscan Stability Index (TSI) is a computation directly based on the raw data obtained from the Turbiscan analysis. TSI is a tool that allows for the rapid and quantitative ranking and comparison of samples. It sums up all the variations in the sample to give a unique number that reflects the degree of destabilisation in the sample. The higher the TSI, the lower is the colloidal stability of the sample. The TSI can be divided into different zones of the sample, including the top, middle, and bottom. The TSI global is based on the overall height variation of the sample (Turbiscan, n.d.).

The number that reflects the destabilisation of the sample correlates to a letter on the TSI scale (Fig. 5), which ranges from A+ to D (Turbiscan, n.d.). TSI values below 1 typically suggest slight to no destabilization, whereas the destabilization usually become visible to the eye somewhere between TSI values from 3 to 10. The letter B (TSI values 1-3) indicates a “Visual pass”, meaning that samples within this range show some variations and correspond to the beginning of destabilization. Over 90% of destabilizations remain non visual within this range.

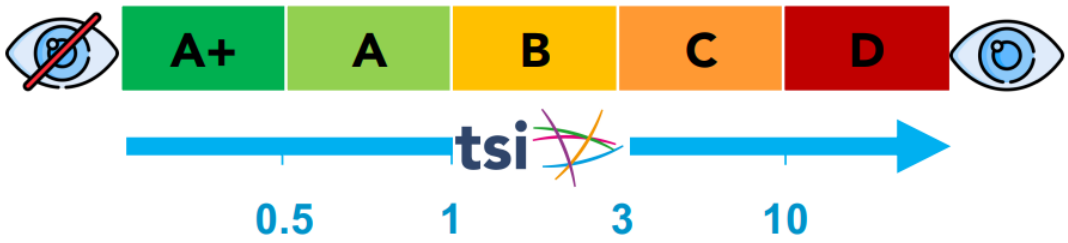

Figure 5. TSI scale indicating the destabilization of a sample (Turbiscan, n.d.)

Emulsifiers play two crucial roles in the formation of stable emulsions. The first step is to allow for the formation of small lipid droplets during the homogenisation process, and the second step is to enhance the stability of these droplets once formed. To achieve this, emulsifiers must rapidly adsorb onto the interface, lower the interfacial tension, and facilitate droplet breakup. In the second step, the emulsifiers must generate strong repulsive forces, form a resistant interfacial layer, and prevent droplets from aggregating. This high-energy approach using a homogeniser involves first dissolving the emulsifier in the aqueous phase, then combining the oil and water and mixing at high shear rates to form a coarse emulsion (McClements & Gumus, 2016). The effectiveness of natural emulsifiers in maintaining the stability of emulsions under different conditions varies greatly. This is because the characteristics of the protective layer they create around droplets, such as electrical charge, layer thickness, polarity, and reactivity, differ from one another (McClements et al., 2017). The rapidity of emulsifier adsorption is crucial, as if it is not fast enough coalescence can occur, which can negatively impact the final emulsion. The emulsifier adsorption rate must be faster than the droplet fragmentation rate, as if it is not, the droplets will not be fully covered with emulsifier (McClements & Gumus, 2016).

Polysaccharides form a thick and hydrophilic layer around oil droplets, which results in strong and long-range steric repulsion. This means that oil droplets coated with polysaccharides are less sensitive to changes in pH and ion concentration compared to droplets coated with proteins, which mainly rely on only electrostatic repulsion for stability. Steric stabilisation involves the adsorption of nonionic surfactants or polymers onto the surface of particles, resulting in a strong repulsion between the

particles and droplets in a dispersion. This repulsion prevents the particles from coming getting too close to each other and aggregating, ensuring stability (McClements et al., 2017).

1.3.3 The hydrophilic-lipophilic balance (HLB)

The HLB value of a surfactant indicates how hydrophilic or lipophilic the surfactant is, as the value describes the balance between the hydrophilic and hydrophobic parts of the surfactant molecule. The HLB values range from 0 to 20, and if a surfactant has a low HLB value (below 9.0), it is considered hydrophobic whereas a high value (above 11.0) indicates it is hydrophilic. The HLB system is a tool used to choose the most appropriate emulsifier for a particular material. With numerous options available, the goal is to find one or two emulsifiers that will effectively emulsify the material (ICI Americas, 1984).

To use the HLB system, the first step is to determine the optimal HLB value for the oil phase which is to be emulsified. Then, an emulsifier or a combination of emulsifiers with the same HLB value can be selected. However, this process is not always straightforward, as the results can vary from batch to batch due to variations in the composition of the material. For example, when determining the optimal HLB value for suberin in water emulsions, the results can differ from batch to batch because the composition of suberin may vary. Similarly, the HLB value of a hemicellulose derivative as a surfactant can also vary because two batches of hemicellulose are unlikely to be exactly the same (ICI Americas, 1984).

The selection of an appropriate emulsifier for a specific material involves more than just its HLB value. The compatibility between the structure of the emulsifier and the oil phase is also crucial. According to Table 1, the general HLB value range for oil-in-water (O/W) emulsifiers is 8-18. This suggests that when determining the optimal HLB value for suberin in water emulsions, the value should be within this range.

Table 1. General use of emulsifiers depending on their HLB value (ICI Americas, 1984).

HLB Range	Use
4-6	W/O emulsifiers
7-9	Wetting agents
8-18	O/W emulsifiers
13-15	Detergents
10-18	Solubilizers

The HLB value needed for a successful emulsion of a specific material can vary, with each material having its own unique requirement. For example, if the required HLB of a material is 8, it implies that an emulsifier with an HLB value of 8 would result in a more stable emulsion compared to any other HLB value. However, just having an emulsifier with the right HLB value is not enough, due to the chemical compatibility playing a crucial role in the stability of the emulsion. Therefore, in the example where the required HLB is 8, trying emulsifiers with HLB values of 5 or 13 would be pointless, as the optimal HLB value for that specific material would be somewhere around 8±1 (ICI Americas, 1984).

The required HLB of any oil phase in an oil-in-water emulsion can be determined through an experimental method that involves testing different combinations of emulsifiers with known HLB values. This involves creating a series of emulsions with the specific oil phase and emulsifiers and observing which HLB value results in the most stable emulsion. This HLB value is then defined as the required HLB for that particular oil phase in the oil-in-water emulsion.

To perform this experiment, matched pairs of Span and Tween emulsifiers can be used. Spans and Tweens are a family of mild non-ionic surfactants that are utilised in various industries, such as cosmetics, food, and pharmaceuticals. The Spans are a type of sorbitan ester, which is produced by dehydration of sorbitol. Span emulsifiers are lipophilic, meaning that an increasing amount of Span leads to a lower HLB value

and higher solubility in lipophilic materials. On the other hand, Tweens are water-soluble, and their solubility increases as the degree of ethoxylation increases (Hong et al., 2018). These emulsifiers are further divided into different esters such as "Span 20" and "Tween 20" (laurate esters) or "Span 80" and "Tween 80" (oleates). By mixing these emulsifiers, any desired HLB value can be achieved (ICI Americas, 1984).

Once the required HLB has been determined for a particular oil phase, the next step is to select the appropriate emulsifier or combination of emulsifiers. Simply choosing an emulsifier at random with the correct HLB may result in poor performance due to the importance of chemical compatibility between the emulsifier and the ingredients. Mixing emulsifiers should result in a more effective and stable emulsion compared to using a single emulsifier. This allows for customisation of the emulsifier to best suit the ingredients. Additionally, the use of a mix of emulsifiers eliminates the need to adjust the raw materials or compromise on the suitability of the emulsifier. Usually, one emulsifier in the mix is more hydrophilic while the other is more lipophilic. Emulsifiers with saturated carbon chains, such as laurates, tend to interact better with saturated oils, while emulsifiers with unsaturated carbon chains, such as oleates, tend to interact better with unsaturated oils (ICI Americas, 1984).

1.4 Natural emulsifiers

There is an increasing demand from consumers for commercial products to be more natural, sustainable, and environmentally friendly. This trend is also relevant in the production of oil-in-water emulsions, which are essential in various applications, including packaging. In this context, companies are striving to replace synthetic emulsifiers with natural alternatives, such as polysaccharides and their derivatives. An emulsifier is a surface-active substance that decreases the interfacial tension between two substances (Hong et al., 2018). In an emulsion, the emulsifier allows the oil and the aqueous phase to mix. This is because of emulsifiers having both a hydrophobic and a hydrophilic part in the same molecule (Kruglyakov, 2000).

Although polysaccharides require relatively high concentrations to be effective as surface-active agents, they are highly resistant to environmental changes (McClements & Gumus, 2016). However, oil-in-water emulsions are thermodynamically unstable, making emulsifiers imperative ingredients in these systems. Emulsifiers serve to stabilise the emulsion, extend its shelf-life, and provide functional attributes. Currently, the majority of surfactants used in commercial-scale emulsions are synthetic or animal-based, such as gelatine, egg, or protein. Companies are eager to embrace this shift towards eco-friendliness by creating fully natural products that can be labelled green, which is attractive.

As with many other products from natural materials, there are numerous challenges and advantages in trying to make these products commercially available in the competition against their synthetic counterparts. The advantage of natural emulsifiers is that the resulting droplets can withstand fluctuations in pH, ion concentration, or temperature (McClements et al., 2017). However, during the homogenisation process, the resulting droplets are often quite big because polysaccharides do not adsorb that quickly and do not effectively decrease the tension at the interface, which leads to the need for large amounts of emulsifier to handle the high surface loads.

One important thing to consider is the source of the emulsifier. The ideal source should be abundant, cost-effective, and homogeneous (McClements et al., 2017). The process used to extract the emulsifier from the source should be straightforward and consistent. However, many natural emulsifiers can be unpredictable, with the composition varying from batch to batch depending on the origin and extraction methods. To be a viable option for commercial use, natural emulsifiers must be priced competitively with synthetic surfactants. One advantage of natural emulsifiers is that they can often be sourced from waste streams from other processes, making them a more economical choice. Additionally, natural emulsifiers tend to be less toxic compared to synthetic emulsifiers.

1.4.1 GGM-grafted-fatty acids (GGM-FA)

One example of a natural emulsifier is GGM-FA, which is a hemicellulose derivative. Due to GGM being very water-soluble on its own, making a stable emulsion between water and hydrophobic suberin is not possible with pure GGM. Because of this, the GGM needs to be chemically modified to make it amphiphilic. By introducing amphiphilic properties, the modified GGM can better interact with both the water phase and the hydrophobic suberin phase, increasing the chances of stable emulsions. One way to do this is via esterification, where the hydroxyl groups along the carbon chain are modified. During this reaction, the hydroxyl groups react with an activated carboxylic acid (Voepel et al., 2011).

Fatty acids are hydrophobic, organic compounds found in animals, plants, and microorganisms, and they form an important part of the human diet. Fatty acids can be classified into groups based on their chemical structure, with one common categorisation being into saturated and unsaturated fatty acids. Saturated fatty acids are fully saturated with hydrogen atoms, meaning that they lack carbon-carbon double bonds. Due to the presence of the carboxyl group at the end of the chain, fatty acids have some solubility in water (Rustan & Drevon, 2005). The solubility is dependent on pH, with an increase in pH gradually leading to higher solubility (Strand, 2013).

In the context of this study, stearic acid was chosen to modify GGM in order to turn it into a surfactant. Stearic acid is a saturated fatty acid with a straight carbon chain composed of 18 carbon atoms and no double bonds (Sampath & Ntambi, 2005). Its chemical structure is illustrated in Figure 6. This structure enables stearic acid to undergo various chemical reactions, including esterification, which can form stearate esters. Stearate esters are commonly used as surfactants and lubricants (Sampath & Ntambi, 2005).

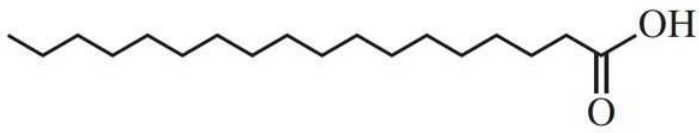

Figure 6. Chemical structure of stearic acid (ACS, 2019).

Dax (2014) synthesized GGM-FA using the following simplified procedure: Initially, stearic acid was dissolved in dimethyl sulfoxide (DMSO). Subsequently, 1,1'-carbonyldi-imidazole (CDI) was added gradually as an activation agent, chosen for its ability to provide milder reaction conditions suitable for GGM. After ensuring complete activation by monitoring the absence of carbon dioxide production, the mixture was stirred for an additional hour. To determine the yield of the activated fatty acid, a small sample was subjected to ^{1}H NMR analysis, from which the yield was calculated. Once the yield was established, the required amount of GGM was calculated based on the desired degree of substitution (DS).

The GGM was dissolved in DMSO and mixed overnight. Subsequently, the mixture was precipitated in acetone, and the resulting product was isolated through filtration. After thorough washing until the filtrate cleared, the product was dried in a vacuum oven. The process is illustrated in Figure 7, with the chemical reactions outlined in Figure 8.

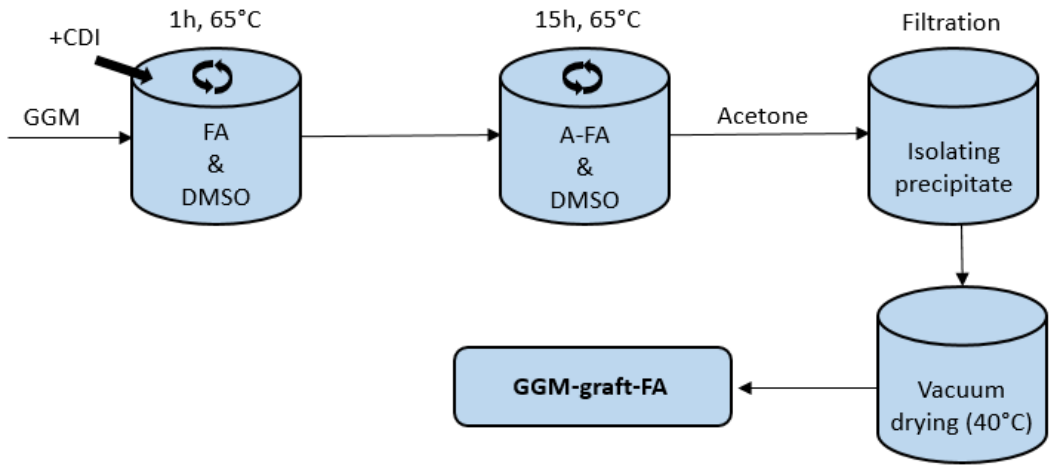

Figure 7. The main steps of the GGM-FA synthesis process.

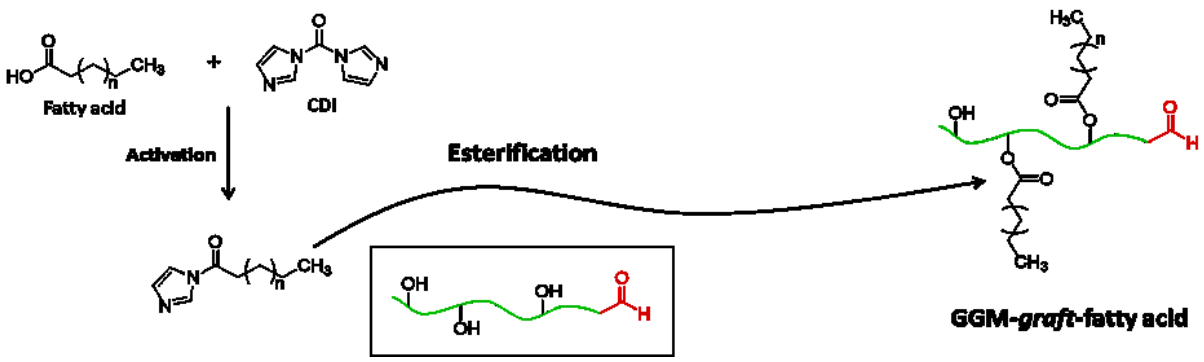

Figure 8. The chemical reactions of the GGM-FA synthesis process (Drawn by Minette Kvikant).

2 Experimental

2.1 Materials and equipment

The experiments in this study were conducted using commonly available laboratory equipment, including pipettes, spoons, magnetic stirrers, hot plates, and containers of varying sizes and shapes. To produce the emulsions, the Span and Tween emulsifiers were provided by Sigma-Aldrich, while the suberin used was obtained from VTT Finland. The solids content of the suberin was determined using a Sartorius MA 35 balance, which calculates the solids content using IR radiation drying. The solids content ranged from 50% to 60% throughout the laboratory work. The GGM-FA used was synthesized following Dax's (2014) methodology, as described in chapter 1.2.2, resulting in a DS of 0.14. The emulsions were prepared using a Kinematica Polytron PT3000 homogeniser.

2.2 Methods

2.2.1 Emulsions using Spans and Tweens

To produce the suberin-in-water emulsions, blends of Span and Tween emulsifiers with different concentrations were prepared to cover different HLB values. Two series were completed, one using Span 80 (Sorbitan oleate) and Tween 80 (Polyoxyethylene sorbitan oleate), and one using Span 80 and Tween 20 (Polyoxyethylene sorbitan laurate). The molecular formula and HLB value for each surfactant is found in Table 2.

Table 2. Molecular formulas for Span and Tween surfactants.

Surfactant	Molecular formula and synonyms	HLB
Span 80	$C_{18}H_{33}O_2$ (Sorbitan oleate)	4.3
Tween 20	$C_{26}H_{50}O_{10}$ (Polyoxyethylene sorbitan laurate)	16.7
Tween 80	$C_{64}H_{124}O_{26}$ (Polyoxyethylene sorbitan oleate)	15.0

For both series, HLB values from 6 to 9 were tested.

The percentages of each surfactant for each HLB value were calculated by:

Equation 1

$$\%Span = \frac{100(x - HLB_{Tween})}{HLB_{Span} - HLB_{Tween}}$$

$$\%Tween = 100 - \%Span$$

$$x = HLB\ value\ of\ the\ blend\ wanted$$

The amounts of each surfactant used for different HLB values in both series are seen in Table 3.

Table 3. Ratio of surfactants needed for different HLB values in both series.

Surfactant blend (%)		Calculated HLB
Span 80	**Tween 80**	
84.11	15.89	6
74.77	25.23	7
65.43	34.57	8
56.07	43.93	9
Span 80	**Tween 20**	
86.29	13.71	6
78.23	21.77	7
70.16	29.84	8
62.10	37.90	9

Within the context of the SUSBINCO project, previous work by Akhlamadi (2023) provided valuable insights and set some of the parameters for the experimental work conducted in this study. She established the HLB of suberin to be between 6 and 9 and identified that emulsions containing 20% suberin and 80% water had favourable stability. Her findings also indicated that increasing emulsifier amounts, from 0.2 g to 0.8 g, generally improved stability. This means that the stability plateau had not yet been reached at emulsions containing 0.8 g surfactant.

The 20% suberin-in-water emulsions were prepared as follows: The emulsifiers were weighed, mixed and magnetically stirred for 30 minutes until the solution was homogeneous. The solids content of suberin was determined and the desired amount of suberin (2 g) was weighed. In a small beaker, the suberin was melted using an oil bath set at 70 °C in an aluminium box with a magnetic stirrer set to 400 rpm until the suberin had melted completely. The emulsifier was dissolved into the suberin by adding the correct amounts of emulsifier and stirring for 10 minutes at 800 rpm. After the suberin and emulsifier were thoroughly mixed, 8 g of 80 °C distilled water was slowly added to the mixture. The water was added dropwise while stirring, and the stirring was continued for 5 minutes after the water had been added, at 800 rpm. Subsequently, the emulsions were prepared using a homogeniser. The homogeniser was run for five minutes at 18000 rpm. This process resulted in creamy emulsions, which were then ready for analysis. The preparation process is pictured in Figure 9.

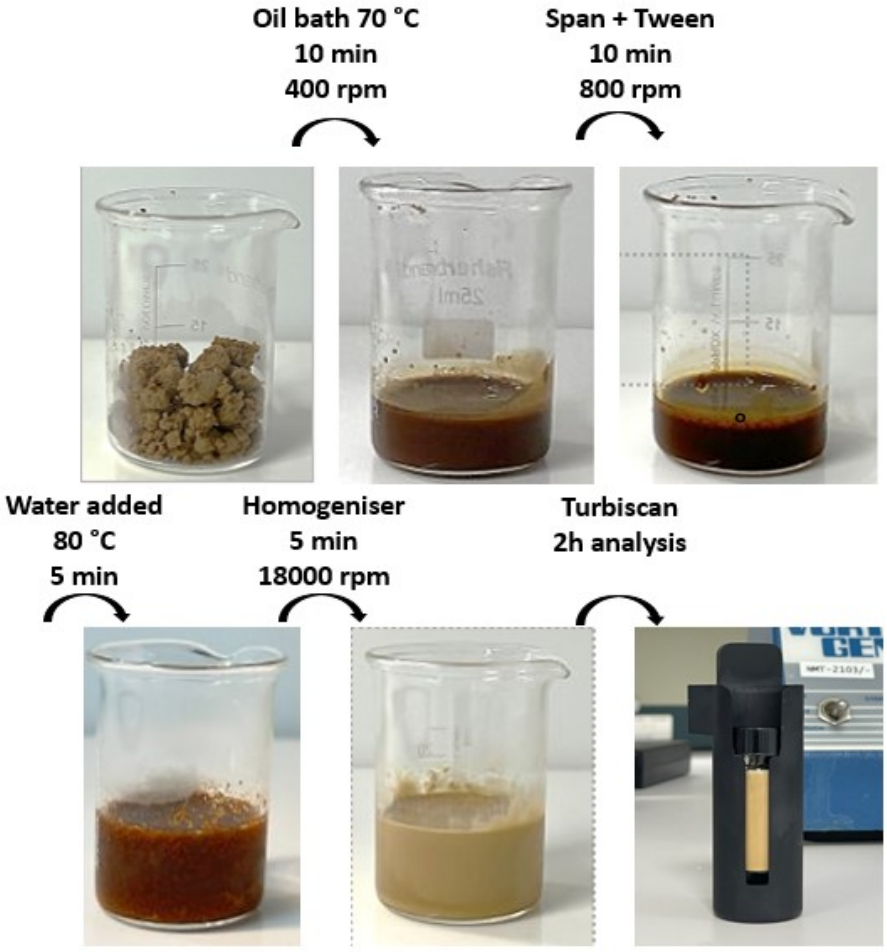

Figure 9. The preparation of suberin-in-water emulsions with Spans and Tweens.

2.2.2 Solubility of GGM-FA in water

The solubility of the GGM-FA in water was determined in order to ensure that there would not be any issues in mixing the solid surfactant with the water and the suberin. The determination of the maximum solubility of GGM-FA (C18, 0.14) in water was performed using the following procedure: The total mass of the distilled water, GGM-FA, and the beaker were weighed. The water was then prepared by heating it to 90 °C. The GGM-FA was added slowly to the hot water while continuously stirring at 700 rpm using a magnetic stirrer. This initial stirring with heat was performed for 2 hours to promote the dissolution of GGM-FA. The temperature was maintained at 90 °C throughout this process. After the 2-hour stirring period, the heating was turned off. It was then stirred overnight at 500 rpm at room temperature to further enhance dissolution and to achieve a homogeneous solution.

The weight of the mixture was measured again after the overnight stirring period. By calculating the difference in weight, the amount of water evaporated during the process was determined, and that amount of water was added back. Subsequently, the mixture was homogenised at 5000 rpm for 3 minutes using a high-speed homogeniser to ensure uniform dispersion of GGM-FA particles within the solution. The stability of the GGM-FA solution was then analysed using Turbiscan.

2.2.3 Emulsions using GGM-FA

The preparation of the emulsions using GGM-FA as emulsifier, instead of Spans and Tweens, was slightly different due to the fact that the GGM-FA is solid at room temperature, whereas Spans and Tweens are liquid.

To prepare the emulsions with GGM-FA as the stabiliser, the following procedure was followed: The solids content of suberin was measured, and a total of 2 g of suberin was placed in an oil bath heated to 70 °C. The suberin was melted by stirring at 400 rpm for 10 minutes using a magnetic stirrer.

In parallel, the already prepared mixture of water and GGM-FA was heated to 80 °C using a water bath. The heating time was kept at a minimum in order to avoid evaporation. The heated water + GGM-FA mixture was then added dropwise to the melted suberin while stirring at 800 rpm. This procedure was done slightly quicker than for the Span and Tween emulsions, as the water here contained the emulsifier. The stirring of the mixture continued for an additional 5 minutes at 800 rpm to enhance emulsion stabilisation. After that, the emulsion was subjected to further homogenisation using a high-speed homogeniser at 18000 rpm for 8 minutes. After the homogenisation step, the emulsions were analysed immediately.

2.3 Characterisation

2.3.1 Stability index

The main device used for the analysis used in this study was the Turbiscan Lab, which utilises static multiple light scattering (SMLS) to determine the stability of a dispersion. The Turbiscan Lab method assesses the physical destabilisation of samples based on measurements of particle migration, also known as creaming and sedimentation, and variation in particle size, which can lead to coalescence (Liu et al., 2014).

The Turbiscan head consists of a pulsed near-infrared light source with a wavelength of 880 nm and two synchronous detectors. One of the detectors is a transmission detector that receives the light passing through the sample at a 0° angle from the incident beam, while the other is a back-scattering detector that receives the light scattered by the sample at a 135° angle from the incident beam (Mengual et al., 1999; Abismaïl et al., 2000; Liu et al., 2014). The reading head moves vertically along the analysis cell, scanning the sample and acquiring data every 20 µm. Measurements are taken over time, and variations in the backscattering and transmission levels caused by sample instability are recorded (Turbiscan, n.d.).

After the emulsions were prepared, they were immediately analysed using the Turbiscan. The samples were placed in 4 mL vials, ensuring they were flat, neat, and without air bubbles. The Turbiscan program executed in this study involved 206 measurements, with one taken every 35 seconds, resulting in a total analysis time of 2 hours, at 25 °C. Each TSI value reported in this thesis is the result after 2 hours.

2.3.2 Zeta potential and particle size

For the zeta potential and particle size measurements, a Malvern Zetasizer was used. The zeta potential measurement provides information about the surface charge of particles or colloidal systems. With knowledge of the magnitude and sign of the zeta potential, the stability of the emulsion can be determined. The device uses electrophoretic light scattering (ELS) to determine the zeta potential (Malvern Panalytical, n.d.). This technique involves applying an electric field to particles, from which the electrophoretic mobility can be obtained (Clogston & Patri, 2011).

The other measurements conducted with this device were the particle size and particle size distribution. The device utilises dynamic light scattering (DLS) to measure the particle size (Malvern Panalytical, n.d.). Additionally, the Malvern Zetasizer provides information about the size distribution of particles within a sample. By analysing the intensity of scattered light at different angles, the instrument generates a profile showing the range and frequency of particles at various sizes. This provides information about the uniformity or variability of the samples. To prepare the emulsions for the Zetasizer, they were diluted with water to a concentration of 0.01% and subsequently stirred.

Microscopy pictures were also taken of both Span and Tween emulsions and GGM-FA emulsions. The Nikon Microphot FXA microscope was used, helping in the analysis of particle sizes and potential flocculations and decrease in stability over time within the emulsions.

3 Results and discussion

3.1 Emulsions using Spans and Tweens

A series of suberin-in-water emulsions using different amounts of Span 80 and Tween 80 with HLB values 6-9 was completed, and the results are presented in Figure 10. The emulsion formulations were prepared by testing different amounts of emulsifier, from 1.0 g to 1.6 g, in order to find the emulsion with maximum stability. The maximum stability was generally achieved at 1.2 g of emulsifier for each HLB value, with the stability not significantly increasing after this point for any series. The highest stability in this series was reached with 1.2 g of HLB 8, where a TSI value of 1.47 was observed. Emulsions with HLB 8 also exhibited the highest stability on average.

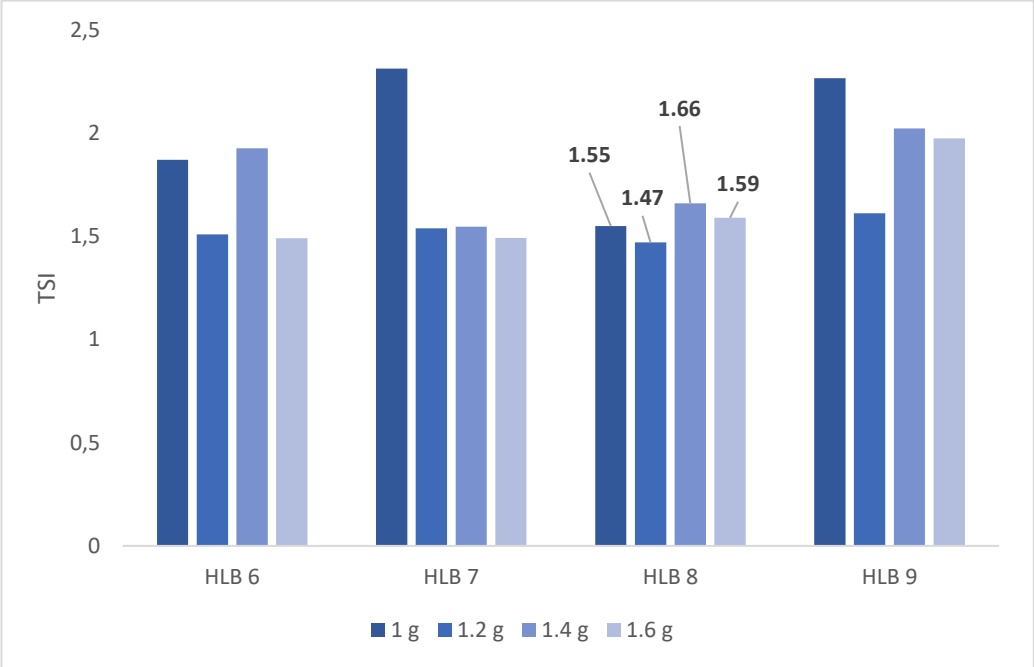

Figure 10. TSI results for suberin-in-water emulsions using different amounts of Span 80 and Tween 80 with HLB values 6-9.

This series, which was completed using Span 80 and Tween 80, continued on from Akhlamadi's (2023) work. As she had analysed amounts from 0.2 g to 0.8 g emulsifier

(2.0% to 7.4% of total weight), the experiments in this study started from 1.0 g emulsifier in order to find the stability plateau. The results obtained here align with the theoretical expectation that once the optimum amount of emulsifier is reached, which for this series was at 1.2 g, further increases in concentration will not enhance the stability (Derkach, 2009).

The presence of discrepancies in the stability outcomes of the suberin-in-water emulsions can be attributed to several factors, including experimental variability, inconsistent suberin solids content, and inconsistent sample placement in the Turbiscan vial.

The appearances of the emulsions in this series were consistent, regardless of HLB value and amount of emulsifier, with no noticeable differences after homogenisation. At times, there were visible separate phases before homogenisation, but the stability results did not correlate with this appearance. Figure 11 shows an emulsion before homogenisation, as well as different emulsions after homogenisation, with different HLB and TSI values.

Figure 11. A stable looking emulsion before homogenisation, with three examples of homogenised emulsions. From the left: TSI 1.47 (1.2 g HLB 8), TSI 2.27 (1 g HLB 9), and TSI 1.60 (1.6 g HLB 8).

In the second series of experiments, Span 80 and Tween 20 were investigated as alternative surfactants for suberin-in-water emulsions.

Interestingly, the TSI values for a couple of the HLBs increased with higher amounts of emulsifier (Fig. 12), which deviates from the expected behaviour seen in the experiment with Span 80 and Tween 80 (Fig. 11). This unexpected outcome suggests that Tween 20 may not be suitable as a surfactant for these emulsions, despite some TSI values being lower than the lowest recorded in the other series.

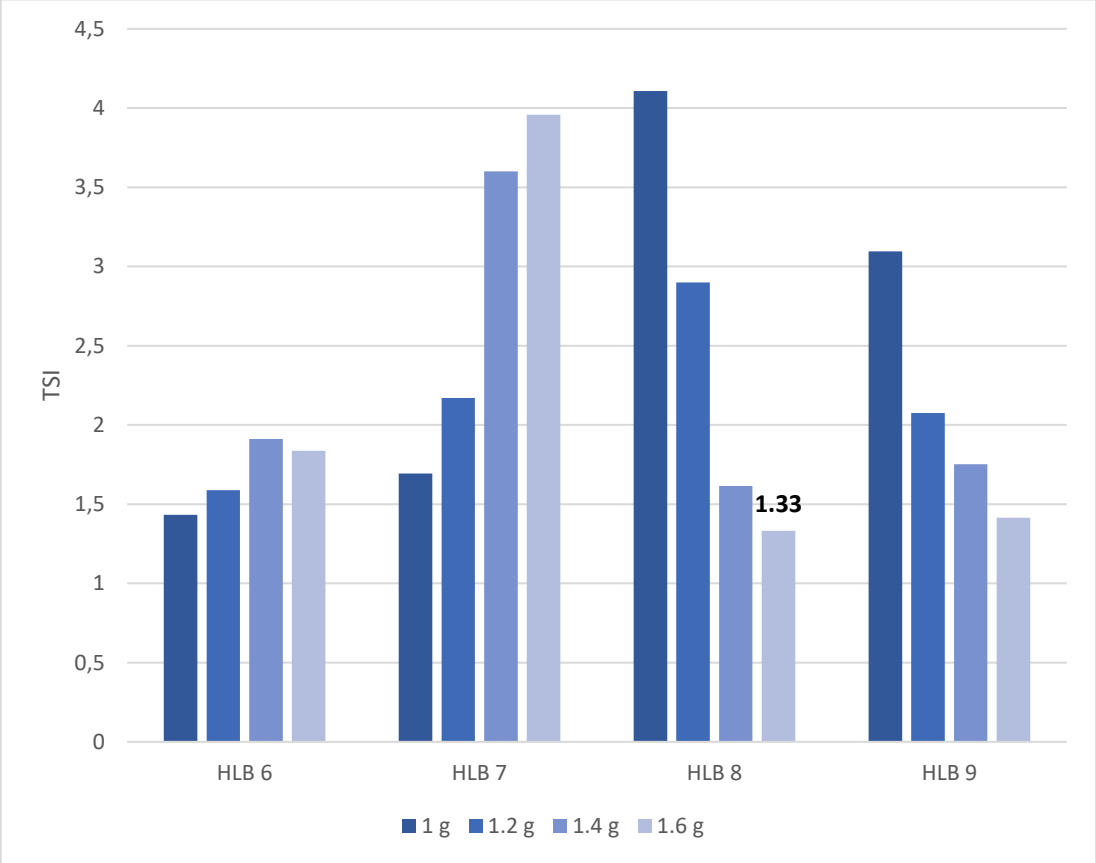

Figure 12. TSI results for suberin-in-water emulsions using different amounts of Span 80 and Tween 20 for HLB values 6-9.

A notable factor that could have affected the instability is the significant difference in HLB between Span 80 (HLB 4.3) and Tween 20 (HLB 16.7). A substantial HLB disparity between surfactants may lead to unstable emulsions (Takamura et al., 1979).

As mentioned in previous chapters, the findings obtained in the experiments with Spans and Tweens, can be explained based on the properties of Tween 20 and Tween 80. According to research, Tween 20, being a laurate, tends to interact better with saturated oils. Tween 80, an oleate, in turn demonstrates stronger interactions with unsaturated oils (ICI Americas, 1984). Additionally, studies by Graça (2015) highlight that one of the main compounds in suberin consists of unsaturated C18 chains. Therefore, it is expected that suberin would interact more favourably with oleates due to their affinity for unsaturated oils. Hence, the observed instability and inconsistencies with Tween 20 compared to that of Tween 80, align with the theory of the interactions of these surfactants with suberin.

The emulsions made with Span 80 and Tween 20 (Fig. 13) were also of impaired quality visually, compared to those made with Span 80 and Tween 80 (Fig. 11). They often contained many air bubbles, which made it difficult to add the emulsions to the turbiscan vials for analysis. Before the homogeniser, there were often significant differences in solubility, occasionally resulting in completely separate phases. The suberin particles before homogenisation were generally larger in these emulsions compared to the ones made with Span 80 and Tween 80, further indicating that the emulsions were not as stable. Surprisingly, there was no correlation between the appearance of the emulsions before the homogenisation and their TSI. This is shown in Fig. 13, where the left emulsion would be expected to have a higher TSI than the emulsion on the right. Again, after the homogeniser step all the emulsions looked the same, except for some cases where there were many air bubbles present in the sample.

Figure 13. Two emulsions before the homogenising step. From the left: TSI 1.75 (1.4 g, HLB 9), TSI 3.60 (1.4 g, HLB 7).

The emulsion with HLB 8 was found to be the most stable, as it consistently resulted in the lowest TSI results for the Span 80 and Tween 80 series, as well as reaching the lowest TSI result for the Span 80 and Tween 20 series.

To confirm that the HLB value of suberin is 8, additional experiments were conducted with Span 80 and Tween 80 using the HLB 8 value. Three new series of experiments were performed, again ranging from 1.0 g to 1.6 g of emulsifier. Overall, the TSI results became progressively worse with each series, as shown in Figure 14. The exact reason for this trend is unknown but could be attributed to factors discussed earlier. It is possible that inconsistencies in the suberin sample, which was taken from a large container, played a role. Additionally, the solids content of suberin varied significantly during the measurements.

The first series yielded an average TSI of 1.57 and a standard deviation of 0.08. The second series reached the highest stability, with a TSI of 1.25 achieved at an emulsifier amount of 1.2 g.

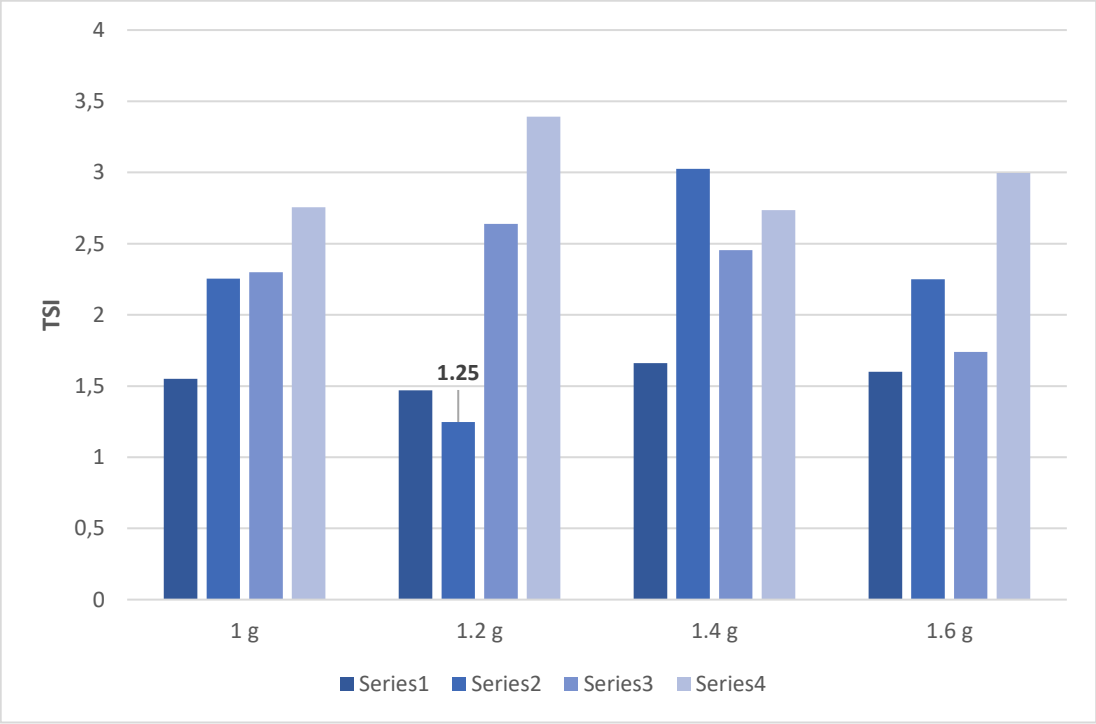

Figure 14. TSI results for parallel experiments of different amounts of Span 80 and Tween 80 with HLB 8.

Microscopy was chosen as an appropriate method to assess the size and amount of aggregates and to gain insight into the particle size and size distribution within the emulsions. Figure 15 shows two microscopy images, one captured at 4x zoom on the left and another at 10x zoom on the right. In the left image, larger flocks can be observed with approximate diameters of 20-50 µm. These flocks indicate the presence of particle agglomerates or clusters within the emulsion. The right picture, taken at 10x zoom, reveals many smaller particles.

These pictures suggest that the particle sizes in the emulsion vary a lot. The presence of flocks indicates that particles are sticking together, possibly because the surfactant does not cover each particle well or the emulsifier does not attach quickly enough to the surface of the suberin.

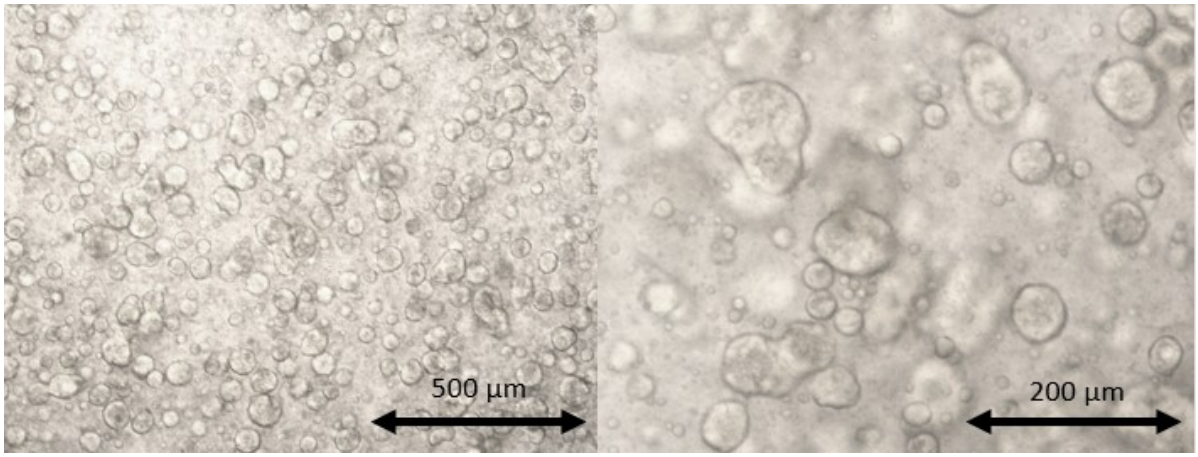

Figure 15. Microscopic images of flocks in a suberin emulsion with Spans and Tweens (1.2 g, HLB 8) as emulsifier.

3.2 Solubility of GGM-FA in water

The stability measurements of GGM-FA in water are presented in Figure 16. Concentrations ranging from 1% to 10% were tested, which resulted in higher concentrations up to 20% after water evaporation.

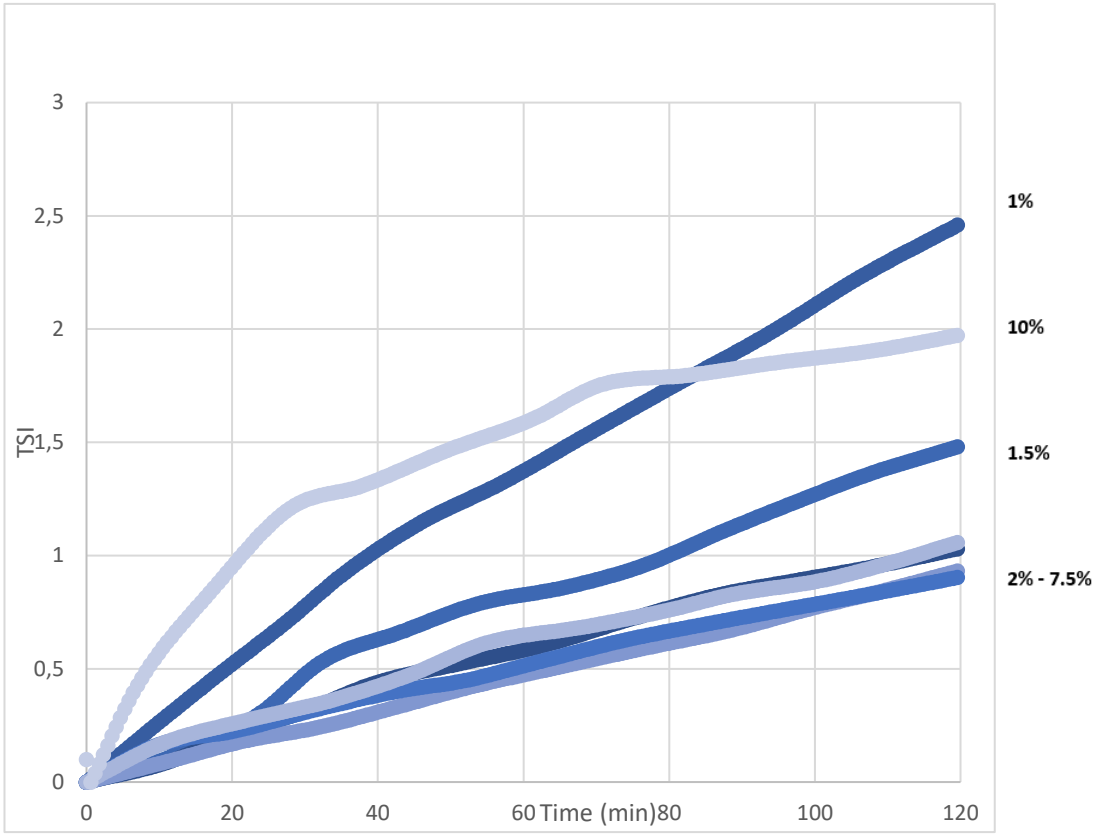

Figure 16. TSI values as a function of time for different concentrations of GGM-FA in water.

At concentrations ranging from 2% to 7.5%, the emulsions showed excellent stability, as evidenced by some of the TSI values around 0.9 (Fig. 16). TSI values actually became lower for each concentration up to 5% (6.64% after evaporation), which could possibly be due to scratches in the Turbiscan vials affecting the clearer solutions more. Overall, the results for the lower concentrations indicate that the emulsions remained stable for an extended period, making them suitable for use in suberin emulsions.

For the higher concentrations (7.5% and 10%), some issues arose. Their TSI values did not indicate instability (1.06 and 1.98, respectively), however, they were quite thick. Pre-experimenting, the poor water solubility of GGM-FA was considered as the biggest risk, but problems with the viscosity of the mixture appeared before. The high viscosity led to problems with pipetting, which would then in turn lead to difficulties when adding the mixture to the suberin emulsion. Nevertheless, visual assessments indicated that the emulsions appeared stable, suggesting that higher concentrations could potentially be utilised for some other applications.

The observed stability of the GGM-FA emulsions up to 5% concentration (6.64% after evaporation) demonstrated their potential suitability for use in the emulsions. Although sedimentation was observed after 72 hours (Fig. 17), the emulsions were easily mixed again through vigorous shaking. Images depicting the sedimentation of the samples after 72 hours are presented in Figure 17.

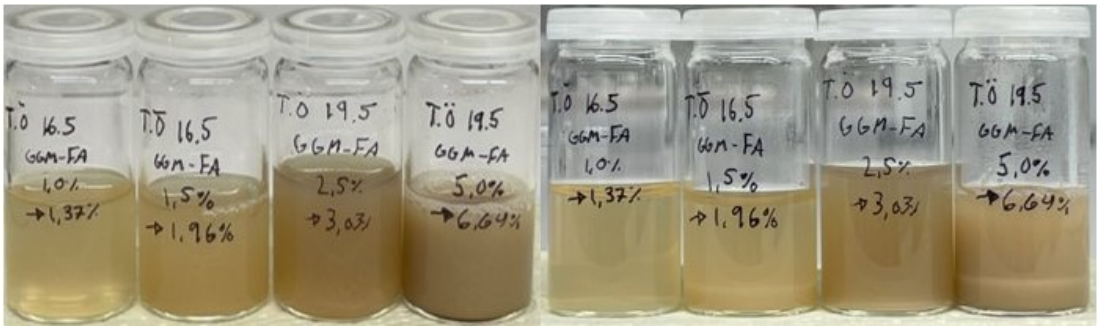

Figure 17. Sedimentation of different concentrations of GGM-FA in water over time. 0 hours (left picture), and 72 hours (right picture), with increasing concentrations.

3.3 Emulsions using GGM-FA

3.3.1 Stability

Suberin emulsions with GGM-FA as the surfactant were prepared starting from 0.2 g of GGM-FA, 2 g of suberin, and 8 g of water. The TSI results are presented in Figure 18.

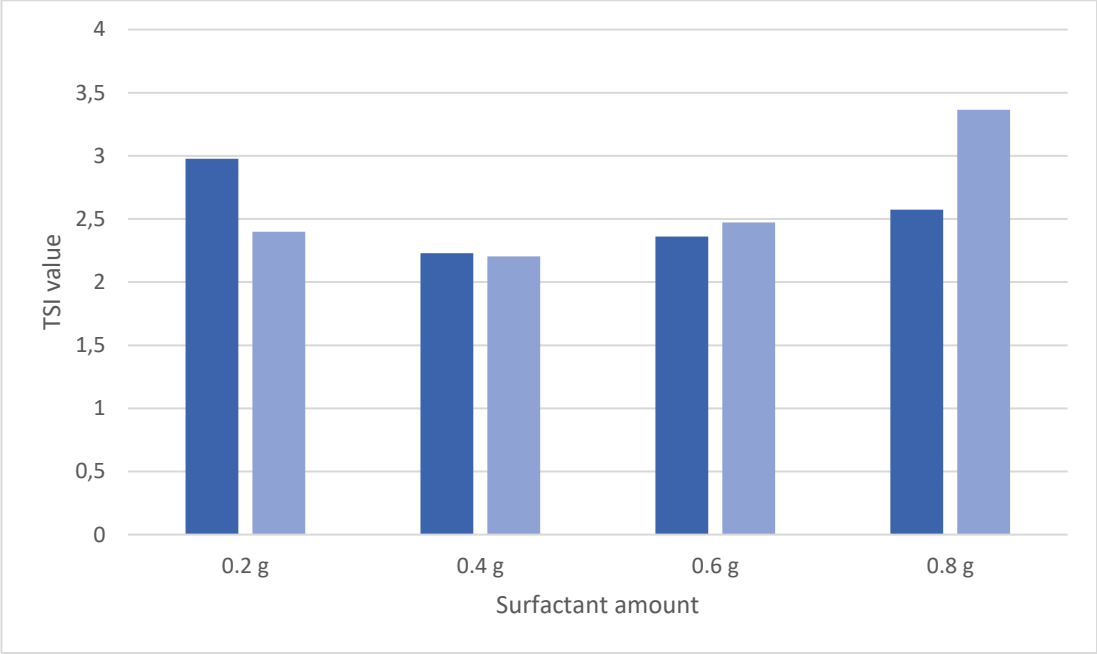

Figure 18. TSI results for suberin-in-water emulsions using different amounts of GGM-FA (C18, 0.14) as surfactant.

Emulsions with different amounts of GGM-FA were prepared from 0.2 g to a maximum of 0.8 g. At 0.6 g, the emulsions began to thicken, and at 0.8 g, they became very thick. Notably, the emulsion with 0.8 g of GGM-FA became quite solid after 2 hours in the Turbiscan. The viscosity also lead to challenges in pipetting the emulsions into the Turbiscan vial, resulting in the presence of air bubbles that could have influenced the stability analysis of the emulsions with more than 0.6 g of GGM-FA.

Two series of emulsion concentrations were completed, with the maximum measured stability observed at 0.4 g of GGM-FA. Emulsions with higher amounts of

GGM-FA still appeared very stable, however, there were issues with high viscosity. The TSI values in both series were approximately 2.2 with 0.4 g of GGM-FA, while values ranged between 2.5 and 3 for the higher and lower amounts of emulsifier. Compared to the best TSI results with Spans and Tweens, which were around 1.5, these emulsions were a bit less stable.

The appearance of the suberin emulsions with GGM-FA (Fig. 19) was very similar to those with Spans and Tweens (Fig. 11). Before homogenisation, the emulsions often exhibited two separate phases, but after homogenisation, they appeared identical to the emulsions prepared with Spans and Tweens.

Figure 19. The different stages of a suberin emulsion with GGM-FA as surfactant. From the left: the emulsion before using the homogeniser, the emulsion while being homogenised, and the emulsion after being transferred to the vial.

These findings indicate that GGM-FA can be used effectively as a surfactant for suberin emulsions, although there were challenges with high viscosity at higher GGM-FA concentrations. The similarity in appearance to the emulsions with Spans and Tweens also suggests that GGM-FA can be a viable alternative surfactant option, but merely a TSI value does not give enough information about the stability. The results from the zeta potential, particle size, and particle size distribution will further prove whether or not GGM-FA is a viable option to be used as a surfactant.

3.3.2 Zeta potential

During the zeta potential measurements, two separate experiments were conducted using varying amounts of GGM-FA as the emulsifier, specifically 0.2 g, 0.4 g, and 0.6 g. The obtained zeta potential results for the emulsions consistently averaged around -25 mV, as seen in Figure 20.

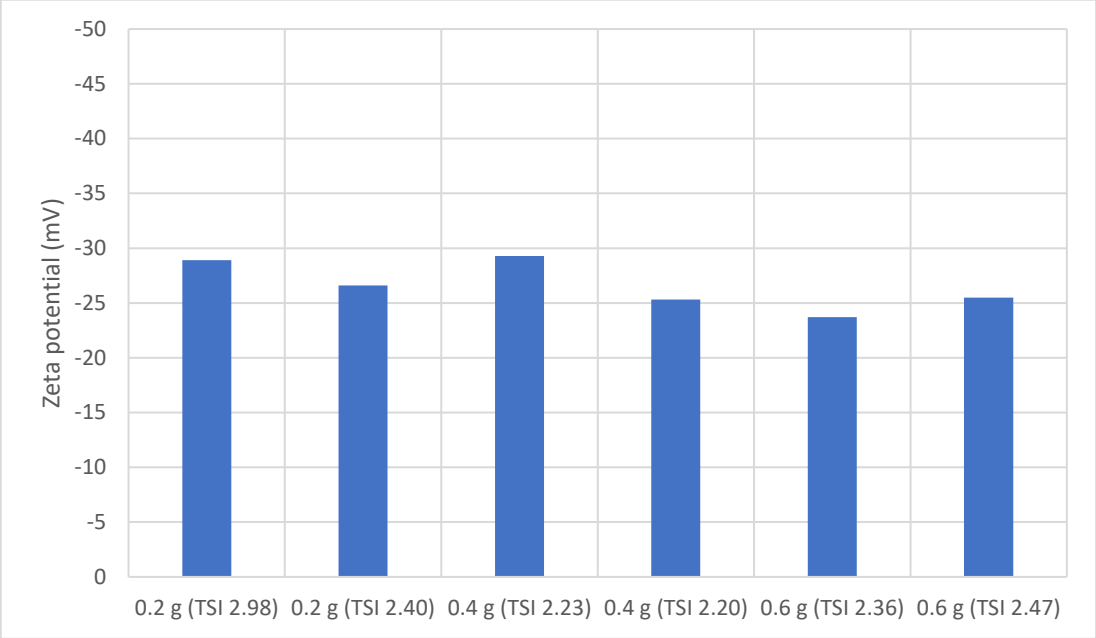

Figure 20. Zeta potential of suberin emulsions with different amounts of GGM-FA.

According to Duffy et al. (2012), emulsions are generally considered stable when their zeta potential is below -30 mV or above +30 mV. However, it's important to note that stability is not solely determined by zeta potential. Other factors, like particle interactions, size, and the nature of the substances used, also significantly impact emulsion stability. The measured zeta potential values in this study fall slightly below the stability threshold, indicating that the emulsions are relatively stable, but that there is room for improvement.

Notably, one emulsion prepared with 0.4 g of GGM-FA displayed the highest zeta potential value of -29.3 mV, while another emulsion formulated with 0.2 g of GGM-FA exhibited a zeta potential of -28.9 mV. However, the emulsions prepared with

0.6 g of GGM-FA demonstrated zeta potential values similar to the overall average, hovering around -25 mV.

These findings suggest that the presence of GGM-FA as the emulsifier contributes to a negative surface charge, which can contribute to the stability of the emulsions. However, given the slightly lower zeta potential values observed, further optimisation of the emulsion formulation or adjustment of the emulsifier concentration may be necessary to enhance their stability and achieve zeta potential values closer to the desired stability range.

The zeta potential distribution analysis of the most stable emulsion showed a single peak with a narrow width, as seen in Figure 21. This indicates that the particles in the emulsion have a similar surface charge. Having a single, narrow peak suggests that the emulsion particles have consistent electrical properties and a uniform surface charge. This uniformity in surface charge helps to prevent the particles from sticking together and enhances the long-term stability of the emulsion.

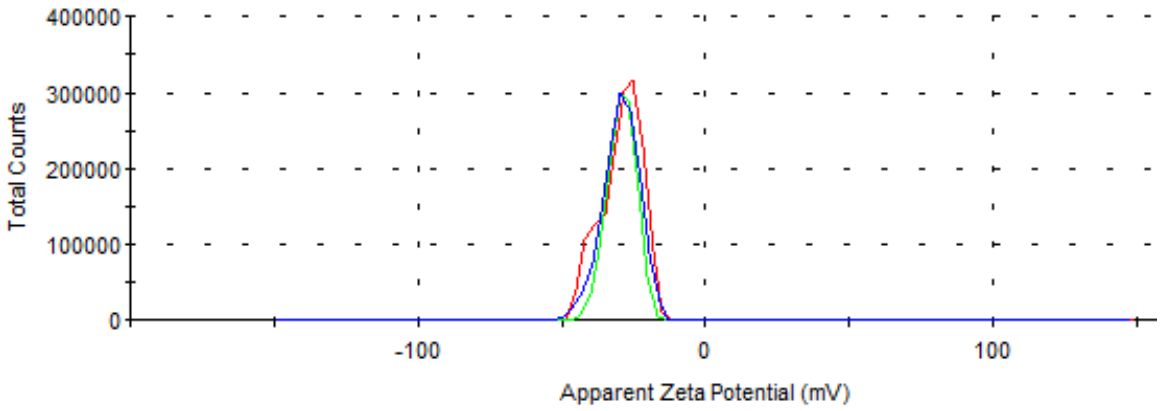

Figure 21. Zeta potential distribution of emulsion with 0. 4g GGM-FA, with the highest absolute zeta potential (-29.3) and lowest TSI (2.2) recorded.

3.3.3 Particle size

The particle size measurements conducted on the samples revealed average particle sizes ranging from 1000 to 2000 nm for most of the samples, as seen in Figure 22. One exception to this was the otherwise most stable emulsion (lowest TSI, highest zeta potential), containing 0.4 g of GGM-FA, which had a slightly higher average size of 3343 nm. This could be due to the device analysing more of the clusters in this sample or perhaps due to the other samples having clusters too big for the device to analyse.

Surprisingly, all samples only displayed peaks consistently occurring around 300-400 nm in their particle size distribution. The lack of peaks at larger diameters, despite the average particle sizes falling within the range of thousands of nanometres, raises some interesting observations. Typically, one would expect to observe peaks around the average particle size, indicating the predominant size of particles present in the emulsion. However, in this case, the presence of peaks at a much smaller size range suggests the existence of smaller particles and aggregates within the emulsion system. This in turn means that the particle size distribution is broad, as there has to be a lot of larger particles or clusters in the scale of thousands of nanometres, which for some reason do not appear as a peak in the results.

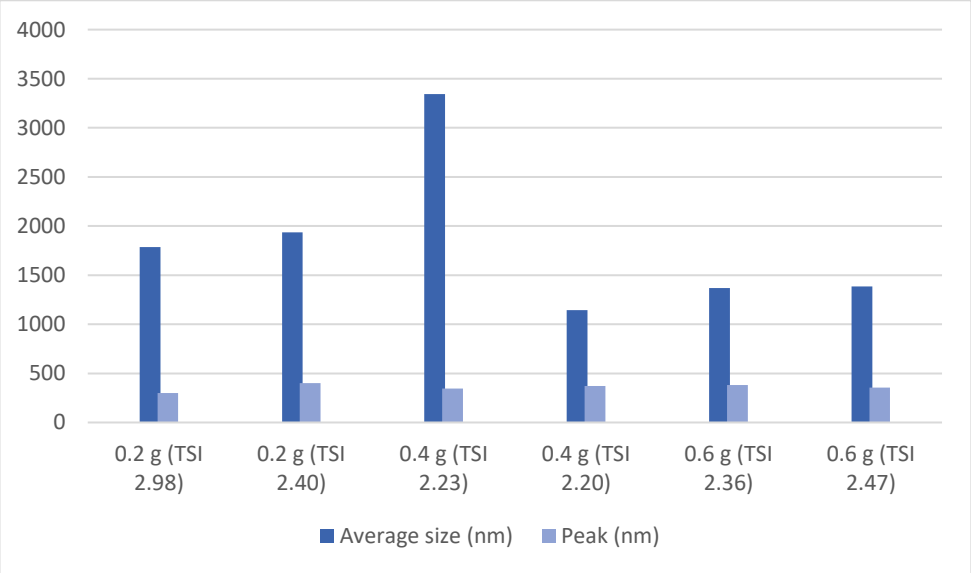

Figure 22. Average particle sizes and average peaks of the suberin emulsions with GGM-FA as surfactant.

In Figure 23, the typical result of a particle size distribution of a suberin emulsion is shown. Narrow peaks are found at very small particle sizes as well as a broader one around 400 nm.

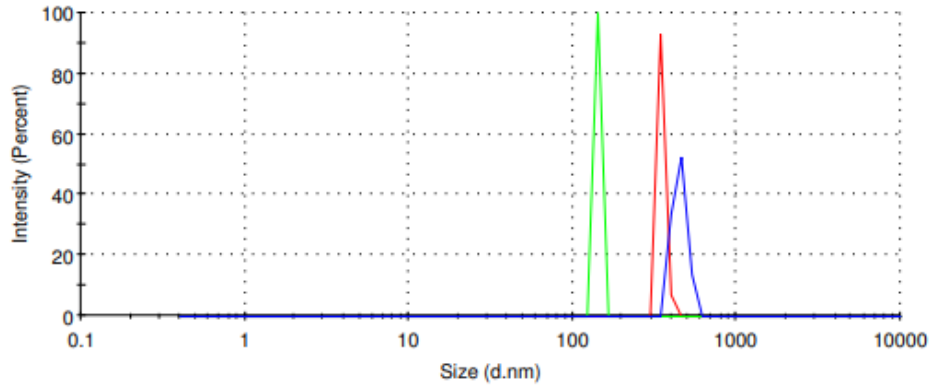

Figure 23. Example of a particle size distribution of a suberin emulsion with GGM-FA as surfactant.

Microscopic pictures were taken to visually validate the findings from the particle size measurements. One of the emulsion samples was examined under the microscope, revealing the presence of larger clusters as well as smaller particles, as seen in Figure 24. These microscopy pictures provide visual evidence that supports the particle size measurements, demonstrating the polydispersity of the emulsion including individual particles and clusters.

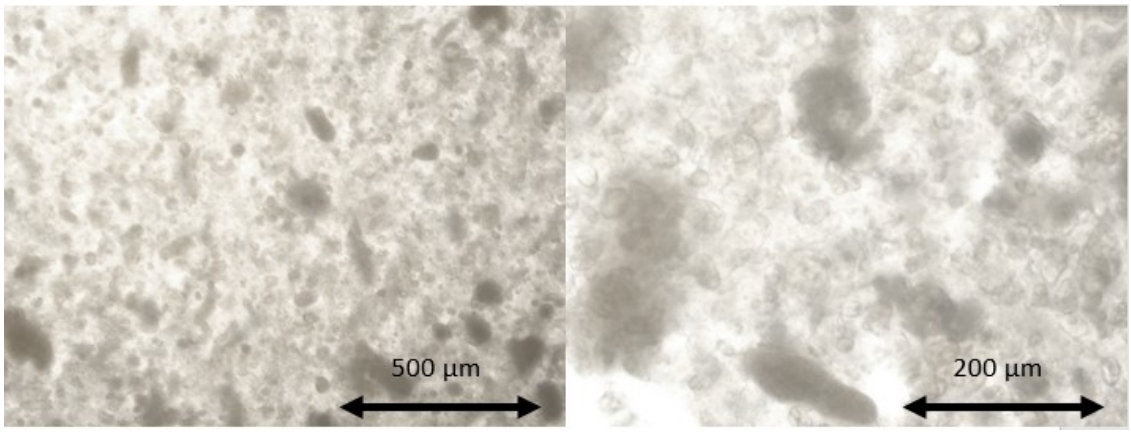

Figure 24. Microscopic images of a relatively stable suberin emulsion with GGM-FA (0.4 g) as emulsifier.

4 Conclusions

The main aim of the larger project, which this thesis is a part of, is to develop sustainable bio-based binders and coatings as environmentally friendly alternatives to fossil-based materials in various product applications. In line with this overall objective, the specific objectives of this study were to determine the HLB of suberin through empirical testing, evaluate the suitability of galactoglucomannan-grafted-fatty acids (GGM-FA) as surfactants for stabilising suberin-in-water emulsions, and to analyse the stability of these emulsions using different analytical methods.

The results of this study have provided valuable insights into the characteristics and potential applications of suberin-in-water emulsions. The HLB of suberin was determined to be around 8, enabling the selection of suitable surfactants for stable formulations. Emulsions created with Spans and Tweens initially showed good stability, achieving TSI values around 1.5. The most stable emulsions were obtained when 1.2 g of emulsifier (HLB 8), 2 g of suberin, and 8 g of water were used, despite varying results for parallel tests. Microscopic observations also revealed the presence of clusters, indicating a relatively high polydispersity in the emulsions.

Moreover, GGM-FA demonstrated surface activity and showed potential as a bio-based surfactant for stabilising suberin emulsions. TSI values around 2.2 were achieved with amounts as low as 0.4 g of GGM-FA, which is comparable to the stability levels reached of emulsions with Spans and Tweens. Additionally, average particle sizes between 1000 and 2000 nm, with peaks around 300-400 nm, were observed. This is also supported by the microscopic images, indicating that there are larger clusters as well as tiny particles in the emulsions. The zeta potential averaged around -25 mV, slightly below the threshold of -30 mV for stable emulsions.

In conclusion, this study contributes to the development of sustainable bio-based binders and coatings by exploring the characteristics and stability of suberin-in-water emulsions. The results indicate that suberin can be successfully used in stable

emulsions, and GGM-FA shows promise as a bio-based alternative to conventional surfactants. However, further research is needed to improve the consistency of results, by optimising the emulsion protocol and surfactant combination for enhanced stability.

5 Swedish summary - Svensk sammanfattning

Karaktärisering av stabiliteten hos suberin i vatten-emulsioner

Användningen av fossila bränslen som råmaterial inom olika industrier ställer oss inför betydande utmaningar med avseende på miljöpåverkan och begränsade resurser. Därmed har forskningen av hållbara alternativ blivit allt mer viktig. En lovande lösning inom detta område är utvecklingen av biobaserade material. Bland dessa material finns suberin, en komplex biopolymer som finns i cellväggarna hos vissa växter, som visar stor potential som en förnybar resurs inom förpackningsindustrin. Suberin har en unik struktur och sammansättning och erbjuder intressanta egenskaper, såsom hydrofobicitet och förmågan att bilda filmer, vilket gör det till ett attraktivt alternativ till fossila material (Graça, 2015).

Målen med denna avhandling var att undersöka stabiliteten hos suberin i vatten-emulsioner samt att utvärdera förmågan av hemicellulosa modifierat med fettsyror att fungera som surfaktant. Genom dessa undersökningar eftersträvas en djupare förståelse för suberin och hemicellulosa samt deras potential som hållbara alternativ inom bindemedels- och beläggningsindustrin.

Suberin är en viktig komponent i trä som finns i barkens cellväggar och spelar en avgörande roll för deras egenskaper. Cellväggen är uppbyggd av tre huvudsakliga lager: primär-, sekundär- och tertiärlagret. Primär- och tertiärlagren är tunna och består av cellulosa och lignin, medan sekundärlagret innehåller suberin. Till strukturen är suberin är en komplex blandning av fettsyror och glycerol. Största delen av suberinmassan, cirka 80–90 %, består av långa kedjor av alifatiska syror. Glycerol utgör 5–20 % av suberinmassan, medan resten av massan består av mindre mängder fenoliska syror som ferulinsyra (Graça, 2015).

Hemicellulosor är viktiga komponenter som finns i växters cellväggar och utgör en betydande del av den totala biomassan i trä. De är heterogena polysackarider som

består av olika monosackarider, såsom glukos, galaktos och mannos, som är förenade via olika typer av kemiska bindningar.

En specifik typ av hemicellulosa, känd som galaktoglukomannan (GGM), har visat sig vara särskilt intressant för sin potential som en förnybar resurs för flera användningsändamål. GGM består av en kombination av galaktos-, glukos- och mannosenheter. Problemet med att använda GGM i sin naturliga form är att det är vattenlösligt, vilket gör att det inte växelverkar med suberin. Genom kemisk modifiering får hemicellulosan ökad hydrofobicitet, vilket gör att den kan samverka bättre med vattnet och suberinet i emulsionerna. Denna modifiering möjliggör alltså en bättre interaktion mellan komponenterna i emulsionerna, vilket ger emulsionerna förbättrad stabilitet och funktionalitet. GGM-derivatet som undersöktes i detta projekt var GGM-fettsyror (GGM-FA), där fettsyror är bundna till GGM-strukturen. Dessa hemicellulosa-fettsyror har visat sig ha ytaktivitet och kan fungera som surfaktanter i emulsioner. Det innebär att de kan bidra till att stabilisera suberin i vatten-emulsioner genom att minska på ytspänningen mellan olje- och vattenfasen.

Emulsioner spelar en viktig roll inom många industrier. Emulsioner består av två oblandbara faser, vanligtvis en oljefas och en vattenfas. För att uppnå stabila emulsioner krävs tillsats av en surfaktant. Ett verktyg för att välja rätt surfaktant är hydrofil-lipofil-balansen (HLB), som används för att bedöma hurdan surfaktant som behövs för att stabilisera de specifika komponenterna i emulsioner.

Två välkända och kommersiellt tillgängliga surfaktantgrupper är Span och Tween. Spansurfaktanter är oljelösliga (lipofila) medan Tweensurfaktanter är vattenlösliga (hydrofila). Genom att kombinera dessa surfaktanter med olika HLB-värden, kan man styra emulsionens egenskaper och stabilitet. HLB-värdet är ett mått på den relativa hydrofiliteten och lipofiliteten hos en surfaktant. Det hjälper till att bestämma vilken typ av emulsion (olja-i-vatten eller vatten-i-olja) som kan erhållas och hur stabil den kommer att vara (ICI Americas, 1984).

För att bestämma suberinets HLB-värde gjordes emulsioner med kända HLB-värden av Span och Tween. Sedan undersöktes vilket HLB-värde av Span och Tween som ledde till den mest stabila emulsionen, vilket gav suberinets HLB-värde.

Emulsionerna tillverkades enligt följande: Surfaktanterna vägdes, blandades och rördes om i 30 minuter tills lösningen var homogen. Suberinets torrhalt mättes och den önskade mängden suberin (2 g) vägdes upp. Suberinet värmdes upp till sin smältpunkt i en liten bägare i oljebad inställt på 70 °C, i en aluminiumlåda med en magnetisk omrörare inställd på 400 rpm, tills det hade smultit. Surfaktanten lades till suberinet, och lösningen rördes om i 10 minuter på 800 rpm. Efter att suberinet och surfaktanten blandats väl tillsattes 8 g destillerat vatten (80 °C) långsamt till blandningen. Vattnet tillsattes droppvis under omrörning och omrörningen fortsatte i 5 minuter efter att vattnet hade tillsats. Därefter homogeniserades emulsionerna med hjälp av en homogenisator. Emulsionerna homogeniserades i fem minuter vid 18 000 rpm, tills emulsionen var krämig och homogen.

Först genomfördes en serie experiment med olika Span och Tween som surfaktanter för att bestämma suberinets hydrofil-lipofil-balans (HLB) och för att identifiera lämpliga surfaktanter. HLB-värden från 6 till 9 testades genom att variera förhållandet mellan Span och Tween i emulsionerna, och tre serier genomfördes för att säkerställa resultatens pålitlighet. Dessa emulsioners stabilitet analyserades med hjälp av Turbiscan Stability Index (TSI), som ger ett värde på hela provets stabilitet. Ju lägre TSI-värde, desto högre stabilitet. Utöver detta togs också mikroskopibilder för att få en uppfattning om partikelstorleken och storleksfördelningen.

Därefter undersöktes vattenlösligheten hos GGM-FA för att bedöma dess lämplighet som surfaktant för suberinemulsioner. Detta gjordes eftersom GGM-FA är i fast form vid rumstemperatur, till skillnad från Span och Tween som är i vätskeform. Detta innebar att emulsionsprocessen behövde ändras för att kunna blanda GGM-FA med suberin och vatten. Genom att analysera GGM-FA:s löslighet i vatten var det möjligt att bestämma hur mycket GGM-FA som kan användas i en emulsion.

Slutligen framställdes emulsioner med GGM-FA som surfaktant för att utvärdera deras stabilitet. Olika koncentrationer av GGM-FA användes för att optimera emulsionernas stabilitet och reologi, varefter emulsionerna analyserades med hjälp av TSI, zetapotential, partikelstorleksanalys och mikroskopi.

Emulsionerna med Span och Tween som surfaktanter gav goda resultat. En central aspekt som undersöktes var suberinets HLB-värde, vilket visade sig ligga kring 8. Detta indikerar att emulsioner med HLB-värden kring 8 bör vara mest stabila. Initialt visade emulsionerna god stabilitet och uppnådde TSI-värden kring 1,5. De mest stabila emulsionerna uppnåddes när 1,2 g surfaktant (HLB 8), 2 g suberin och 8 g vatten användes, trots att upprepningar av försöket gav varierande resultat. Mikroskopiska observationer avslöjade även förekomsten av flockar, vilket indikerar en relativt hög polydispersitet i emulsionerna.

GGM-FA visade också ytaktivitet, och resulterade i stabila emulsioner. Trots att resultaten inte uppnådde samma nivå av stabilitet som optimal blandning av Span och Tween, visade GGM-FA potential som surfaktant för att stabilisera suberinemulsioner. TSI-värden kring 2,2 uppnåddes med endast 0,4 g GGM-FA, vilket är jämförbart med stabilitetsnivåerna hos emulsionerna med Span och Tween. Partikelstorleken i emulsionerna var i genomsnitt mellan 1 000–2 000 nm, med toppar kring 300–400 nm. Detta stöds även av de mikroskopiska bilderna och indikerar att det finns både större flockar och små partiklar i emulsionerna. Zetapotentialen hade ett genomsnitt på -25 mV, vilket ligger något under tröskelvärdet på -30 mV för stabila emulsioner.

Sammanfattningsvis visar resultaten att suberin kan användas framgångsrikt i stabila emulsioner och att GGM-FA visar lovande potential som ett biobaserat alternativ till vanliga surfaktanter. Ytterligare forskning behövs dock för att förbättra resultaten genom att optimera emulsionsprocessen och surfaktantkombinationen för ökad stabilitet.

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