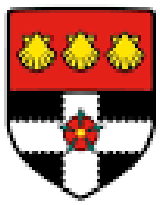

University of Reading

SYNTHESIS OF SURFACE - ACTIVE MALTODEXTRIN LAURATES BY ENZYMATIC TRANSESTERIFICATION

A Thesis Submitted for the Degree of Doctor of Philosophy

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ABSTRACT

Maltodextrins are polysaccharides that are widely used in the food industry due to their non-toxicity, low-price and functionality. Most polysaccharides are strongly hydrophilic and hence they are not suitable surface-active agents for emulsion systems. The modification of a polysaccharide's hydrophilic nature through the introduction of an ester group results in the synthesis of an amphiphilic polysaccharide. This thesis explores the use of enzymatic transesterification reaction, which involves incubating maltodextrins of different dextrose equivalent (DE), namely DE 4-7, DE 13-17 and DE 16.5-19.5 with a vinyl laurate in a mixture of DSMO and *tert*-Butyl alcohol (10:90) as solvent, and using an immobilised lipase B from *Candida antarctica* (Novozym® 435) to catalyse the reactions. The highest degree of substitution (DS) was 0.43 and was observed with maltodextrin DE16.5, indicating that the DS is influenced by steric hindrances affecting the reactivity of hydroxyl groups. However, the maltodextrin DE16.5 laurate was obtained with the lowest conversion yield (6.6 mg/g of initial substrates) indicating that from a production perspective this would be a less economically viable process. All maltodextrin laurates showed to be surface-active at a concentration of 10, 20 and 40 % (*w/v*). The maltodextrin laurates were tested for their emulsion formation ability and emulsion stability, oil-in-water (O/W) emulsion food systems. The maltodextrin DE4 laurate showed good stabilising and emulsifying properties and was more effective than the rest in reducing the emulsion creaming rate, most likely due to its higher viscosity. In addition to their emulsification properties, it was hypothesised that maltodextrin laurates can act like low molecular weight surfactants with detergency properties. The stability and compatibility of the three maltodextrin laurates in detergent formulations was tested targeting the removal of lipophilic substances (rapeseed oil) from cotton cloth. All maltodextrin laurates were shown to possess the emulsion-stabilising capacity for vegetable oil, whereas the high emulsification index

with rapeseed oil (54-66%) reflected good stability of the formed emulsion. High oil removal percentage (56-83%, *w/w*) was obtained under conditions of 0.1M Trizma buffer pH9 at 37 °C in all samples, whereas MDE4 laurate performed the best (83%, *w/w*) at a concentration of 1.0% (*w/v*) in the detergent formulation. Overall, the results of this study indicated that maltodextrin laurates have considerable potential in being used as emulsion stabilisers in foods and as surfactants in detergent formulations.

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AUTHORS' DECLARATIONS

'Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged'

Nurhayati Binti Yusof

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Chapter 1 - Introduction

1.1 Introduction

Carbohydrate fatty acid esters (CFAE) can be used as surfactants and are gaining increased attention because they can be produced from renewable, inexpensive and readily accessible feedstocks and are also biodegradable, harmless to the environment and non-toxic (Castillo et al., 2003; Tokiwa et al., 2000). With the advances in chemical synthesis and industrial biotechnology, numerous methods have been developed to the synthesise CFAEs using various carbohydrates and fat and oil derivatives such as fatty acids and fatty acid esters. Currently, commercial products broadly within the CFAE category include sucrose esters, sorbitan esters and alkyl polyglycosides (Hill & Rhode, 1999). These are commercially produced by the chemical esterification of sugar or polyol esters with a fatty acid in organic solvents (e.g. pyridine, chloroform, dimethyl formamide) using alkaline catalysts at high pressures and temperatures above 100 °C. The use of high temperatures renders the process a high-energy process. The additional disadvantage of chemical synthesis is that it is not selective and therefore produces a mixture of monoester, di- and tri-ester isomers, which reduces the purity and functionality of the end-product, and as a result often requires the inclusion of a multistep separation process (Gumel et al., 2011).

Enzymatic synthesis offers an alternative route for the production of CFAE and offers an environmentally friendly alternative process (Chang & Shaw, 2009). Enzymatic synthesis is a low-energy process as the reaction normally takes place at a temperature range between 40 and 60 °C (Gumel et al., 2011), thereby avoiding the degradation of the carbohydrate substrates and the end products (van den Broek & Boeriu, 2013). In addition, enzymatic catalysis offers the significant advantage of producing mono-esters as

the main reaction products because of the high regio-, stereo- and enantio-selectivity of the enzyme (Gumel et al., 2011).

The biosynthesis of surfactants through enzymatic synthesis can produce a wide range of surface-active compounds, depending on the carbohydrate and acyl donor used, generally called biosurfactants, with the term also including microbially produced surfactants, such as rhamnolipids and surfactin. Biosurfactants are able to lower the surface and interfacial tension using the same mechanisms as synthetic surfactants and as a result confer a range of functional properties including emulsification, detergency, solubilisation, lubrication, foaming, wetting, phase dispersion and viscosity reduction (Campos et al., 2013; De et al., 2015; Singh et al., 2007). Low-molecular-weight biosurfactants in particular are able to reduce the surface tension at air/water interfaces and the interfacial tension at oil/water interfaces, whereas the high-molecular-weight biopolymers or exopolysaccharides, often called bioemulsifiers, are more effective in stabilising oil-in-water emulsions (Banat et al., 2010; Uzoigwe et al., 2015).

Extensive research has been conducted in the past few years on the enzymatic esterification primarily of simple sugars such as sucrose (Neta et al., 2012; Ye et al., 2016), fructose (Neta et al., 2012; Ye et al., 2016) and glucose (Ren & Lamsal, 2017). However, the interest for the synthesis of polysaccharide (e.g. starch, maltodextrin) esters is increasing as polysaccharides are hydrophilic and have intrinsically a variety of functional properties, depending on their molecular mass and molecular structures; they also have more hydroxyl groups than simple sugars, which are accessible to enzymatic reaction. This gives the opportunity to design and produce a range of CFAEs with tailor-made functional properties. In support of the above, the introduction of an ester group to a polysaccharides has been shown to modify their original hydrophilic nature yielding amphiphilic polysaccharides (Horchani et al., 2010; Udomrati & Gohtani, 2014). Amphiphilic polysaccharides such as

acetylated starches with a relatively low degree of substitution (DS) are widely used in the food industry because of their unique physicochemical characteristics, such as low gelatinisation temperature, high solubility, and good cooking and storage stability (Wang & Wang, 2002). Moreover, it has been proposed recently that maltodextrin fatty acid esters, which is the focus of this thesis, can be used as food additives for emulsifying and stabilising oil-in-water (O/W) emulsions (Udomrati et al., 2016).

Maltodextrins have been used for about 35 years as a food additive and have a generally recognised as safe (GRAS) status (FDA, 21CFR 184). Maltodextrins are hydrolysis products of starch and are characterised by their dextrose equivalent (DE) value, which is usually less than 20. The DE is the main parameter that influences the rheological and functional properties of maltodextrins. Some of their important functional properties include bulking, structuring, emulsifying and stabilising properties, while they can also be used as carriers of bioactive compounds (Nurhadi et al., 2016; Pycia et al., 2016), and also to bind flavours and fat (Sadeghi et al., 2008). Recently, research has been conducted to modify maltodextrin through direct esterification with decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16) (Udomrati & Gohtani, 2014). The disadvantage of direct esterification is that it produces water as the by-product of the reaction which decreases the ester yield. The synthesis of maltodextrin esters through transesterification, using maltodextrins of different DE and various fatty acid esters, is another approach that can be used, although this to our knowledge has not been investigated previously. Transesterification using fatty acid vinyl esters would produce as by-product vinyl alcohol, since the vinyl alcohol formed in the reaction tautomerises to volatile acetaldehyde at normal temperatures, which is easily removed from the reaction mixture and could result in an increase the yield of esters. Amongst the carboxylic acids used as acyl donors, lauric acid (C-12) is a good candidate, as it is a saturated medium chain fatty acid that can form more

stable complexes with polysaccharides compared to unsaturated fatty acid (Arijaje & Wang, 2017). Vinyl laurate also soluble in organic solvent. Taking into account the above, the synthesis of maltodextrin laurate, would be a novel concept and such research would generate new knowledge in this dynamic field. The biggest problem in enzymatically synthesising polysaccharides esters, such as maltodexrin esters, is selecting the most appropriate solvent to dissolve polysaccharides. To dissolve maltodextrin, hydrophilic organic solvents, such as dimethyl sulfoxide (DMSO) are used, however, DMSO has generally a strong inactivating effect on lipase (Plou et al., 2002). This findings open up opportunities for using the commercial immobilised lipase *Candida antarctica* lipase B (Novozym® 435) that renders the process more cost-effective, which can be recycled several times. The use of mixtures with organic solvents (e.g. *tert*-Butyl alcohol, DMSO, etc) could potentially provide a more favourable environment for the enzyme and should be investigated.

Maltodextrin laurate has been reported as emulsifier and stabiliser of O/W food emulsion (Udomrati & Gohtani, 2014) however maltodextrin laurate not stable for a long period time due to their surface activity. Production of maltodextrin laurate with the low conversion yield, thus from a production perspective, this would be a less economically viable process. Because of the fact that biosurfactant formulated at low concentration in laundry detergent, thus exploring these maltodextrin laurate will provide new information for food and laundry detergent industries.

1.2 Research hypothesis and objectives

The DE of polysaccharides is inversely proportional to the average molecular weight (M_n) and the degree of polymerisation (DP), which are both commonly used to describe the size and structural characteristics of carbohydrate polymers (Sun et al., 2010). It has been shown that the physicochemical properties of maltodextrins including solubility, freezing point, and viscosity are considerably influenced by the DE value (Dokic-Baucal et al., 2004). Thus, it is important to investigate the effect of the maltodextrin DE on the conversion yield, and on the degree of substitution (DS) of the produced maltodextrin esters and their physicochemical properties.

The overall aim of this research was to develop an enzymatic transesterification process for the synthesis of novel maltodextrin laurates, used as novel biosurfactants, and characterise their physicochemical properties, including surface activity, rheological properties and emulsification capacity in model systems. Moreover, understanding the role of maltodextrin laurate in oil-in-water emulsions and its influence on emulsion stability as well as its effect on the interfacial rheology of air/water interfaces was a key part of this study. Finally, investigating the potential application of maltodextrin laurate in detergent applications, and more specifically as biosurfactants for the removal of rapeseed oil from cotton cloth was also critical. To achieve the above aims, the following objectives were set:

1. Investigate the optimum conditions of maltodextrin with three different Dextrose Equivalent (DEs) as the acyl donor in transesterification reaction with vinyl laurate catalysed by immobilised enzyme, and assess key physicochemical properties of the produced biosurfactants **(Chapter 3).**
2. Study the stabilising mechanism of maltodextrin laurate of different DE on the interfacial shear rheology at air/water interfaces and the stability of oil-in-water emulsions **(Chapter 4).**
3. Evaluate the emulsion forming and capacity of maltodextrin laurates on hydrophobic surfaces such as vegetable oils and assess the potential application of maltodextrin laurates in detergents, more specifically to remove oil from cotton cloth **(Chapter 5).**

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Chapter 2 - Literature Review

2.1 Introduction to surfactants and biosurfactants

Surfactants are amphiphilic molecules that contain hydrophilic (polar) and hydrophobic (nonpolar) moieties present within the same molecule. These amphiphilic molecules partition at the interface between liquid, gas, and solid phases such as oil–water or air-water interfaces, which have a different degree of polarity and hydrogen bridges (Campos et al., 2013). The nonpolar portion is often a hydrocarbon chain, whereas the polar portion may be non-ionic, ionic (cationic or anionic) or amphoteric as shown in Figure 2-1. A non-ionic surfactant has no charge groups in its polar portion and is generally used as an emulsifier, primarily in cosmetic products; examples include polyoxyethylene sorbitan esters (Polysorbate) and sorbitan esters (Span®). An ionic surfactant carries a net charge in the polar head group, which can be either negative (anionic surfactant) or positive (cationic surfactant). Anionic surfactants are commonly applied in various household products, such as in detergents; an example is linear alkylbenzene sulfonates (Calsoft®). Cationic surfactants, such as cetylpyridinium chloride (CPC) is commonly applied in mouthwashes and toothpaste (Asadoorian & Williams, 2008) and benzethonium chloride (BZT) which is also shown to exert antimicrobial activities, is commonly used as a disinfectant or a preservative in eye and nasal drops (Enomoto et al., 2007). A surfactant that contains a head with two oppositely charged groups is termed amphoteric (zwitterionic) surfactant. Amphoteric surfactants are often sensitive to pH and behave either as anionic surfactant at alkaline pH or cationic surfactant at acidic pH. Generally, they are used as foam stabilisers and thickening agents in shampoo and skin cleanser formulations and also as softeners for textiles (Lukic et al., 2016).

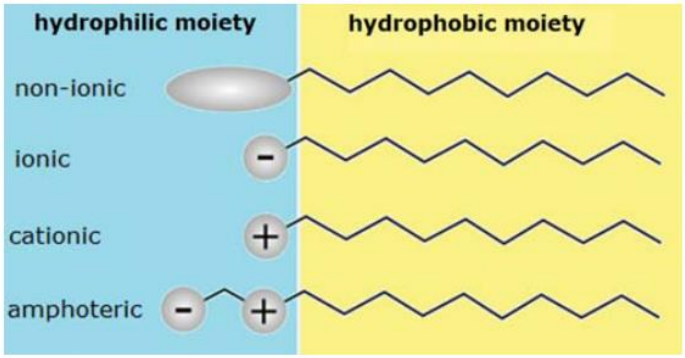


Figure 2-1: Surfactant molecular structures and classification into non-ionic, ionic, cationic and amphoteric surfactants according to the composition of their hydrophilic moieties. Adapted from Campos et al. (2013).

A molecule with surfactant properties is able to form spherical micelles or to aggregate and form cylindrical micelles and bilayers (Figure 2-2). At low concentrations in an aqueous solution, a surfactant exists as a monomers (free or unassociated surfactant molecule). When the concentration of the surfactant is above the critical micelle concentration (CMC), the surfactant monomers aggregate to form spherical micelles, cylindrical micelles, and bilayers (De et al., 2015). The CMC indicates the point at which monolayer adsorption is complete and the surface active properties are at an optimum (Farn, 2008). Surfactant monomers can self-assemble to form a closed aggregate (spherical micelle) in which the hydrophobic tail groups are shielded from water while the hydrophilic head groups are oriented towards water. This will depend on the area occupied by the hydrophilic and hydrophobic groups of surfactants; spherical micelles are commonly formed by single chain surfactants with large head group areas such as anionic surfactants (Remita et al., 2017). Additionally, changes in the properties of a solution (e.g. pH, temperature, concentration, or electrolyte strength) could potentially affect the effective size of hydrophilic head groups of a surfactant, which could consequently affect the size of the aggregate and its shape, i.e. from a spherical to a cylindrical form. Cylindrical micelles are

formed by single chain surfactants with small head group areas, such as non-ionic surfactants and ionic surfactants, particularly in solutions with high salt concentration (Farn, 2008). Bilayers are formed by double chain anionic surfactants in solutions of high salt concentration (Farn, 2008).

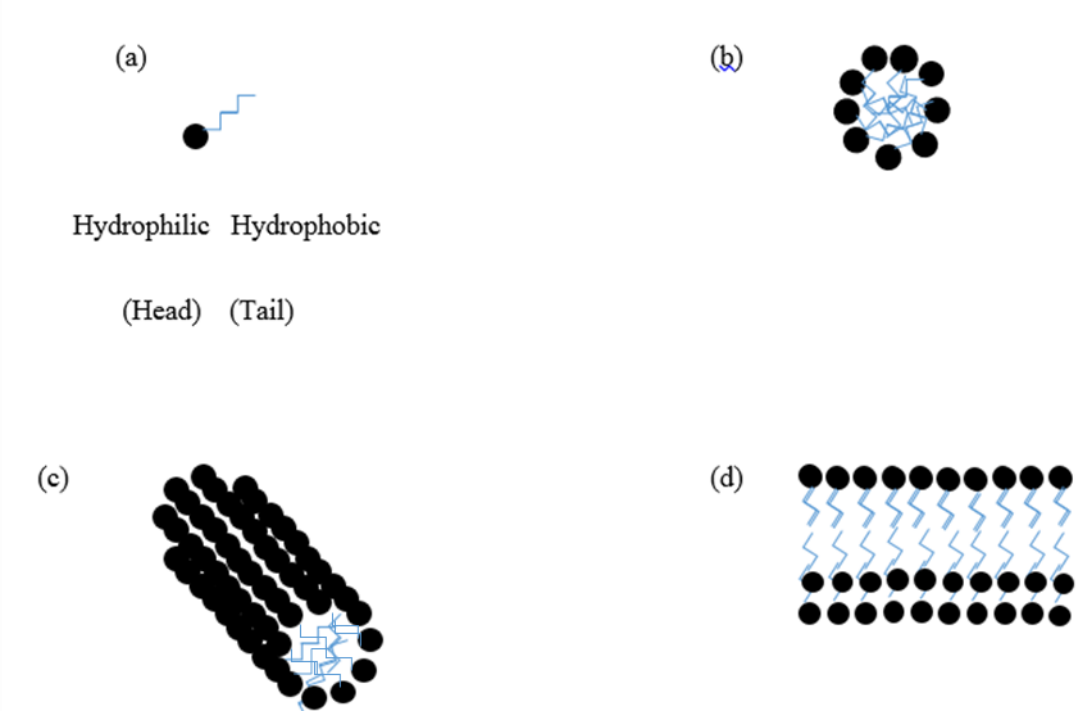


Figure 2-2: Structures of surfactants (a) surfactant monomer, indicated by a circle (representing the hydrophilic head) attached to a hydrocarbon tail; (b) spherical micelle, (c) cylindrical micelle; (d) bilayer. Adapted from Farn (2008) and Fiechter (1992).

Surfactants are characterised by their capacity to reduce the surface and interfacial tension of a liquid and form microemulsions, in which oil can solubilise in water or water can solubilise in oil (Campos et al., 2013). Such properties confer surfactants with a wide range of activities including emulsification, detergency, foaming capacity, lubrication, moisture retention, solubilisation and phase dispersion (Desai & Banat, 1997). The types of industrial applications of surfactants depend primarily on their structure (anionic, cationic, amphoteric, and non-ionic) and are summarised in Table 2-1.

Table 2-1: Types of surfactants used in industrial sectors. Adapted from Lukic et al. (2016), Alwadani and Fatehi (2018) and Sarney and Vulfson (1995).

Surfactants	**Groups**	**Major Uses**
Anionic	Carboxylates	Cleansing agents used as soap bar (fabric hand wash)
	Sulfate	Cleansing agent for cosmetics, shampoos and skin cleanser
	Sulfonates	Detergent for household products
	Phosphates	Household cleaning and industrial textile manufacturing
Cationic	Alkylamines	Cosmetic industry (hair conditioner) and antistatic agents
	Alkylimidazolines	Bactericidal agents or emulsifiers
	Quarternary ammonium salts	Fabric softeners or hair conditioners
Non-ionic	Fatty alcohols ethoxylates	Emulsifiers, wetting agents and solubilisers. Household, industrial and personal care products
	Alkyl carbohydrate esters (sugar or sucrose esters)	Food-grade ingredients used as food additives (emulsifiers and stabilisers)
	Amine oxides	Solubilisers and detergents
Amphoteric	Alkyl betaines	Cosmetics (hair conditioning agent, and skin-conditioning agent e.g. humectant)
	Alkyl dimethylamine	Foam stabiliser and thickening agents (dishwashing)

The global surfactant market in 2015 was 15 million tons with a value of $30 billion (Burn, 2016) and is expected to reach a value of $40 billion by 2021, representing a steady growth of 3–4 % per year (Acmite Market Intelligence, 2016). Most commercially available surfactants are chemically synthesised using platform chemicals as feedstocks, produced by the petrochemical industry, such as ethylene, benzene and kerosene (Campos et al., 2013; Rust & Wildes, 2008). These petrochemical-derived chemical feedstocks are processed through various chemical reactions, such as sulphation and ethoxylation to synthesise sulphonated, sulphated and ethoxylated surfactants (Knepper & Berna, 2003). In the last few years, driven by environmental concerns and with the aim to reduce fossil resources, the

chemical industry is exploiting the utilisation of renewable materials as feedstocks for chemical synthesis. To this end, the term "renewable surfactants" includes surfactants synthesised from renewable raw materials (bio-based surfactants) and surfactants synthesised by living cells or through the use of enzymes (biosurfactants) (De et al., 2015; Khan & Rathod, 2015; Le Guenic et al., 2019). Bio-based surfactants can be produced from chemicals that have been derived from sugar fermentation, e.g. alcohols (e.g. ethanol, butanol) and organic acids (e.g. acetate, lactate) and oleochemicals, such as fatty acids, methyl esters, glycerol and fatty amines, derived from plants and animals via various chemical reactions (e.g. hydrolysis, transesterification, hydrogenation) (Burk, 2010). On the other hand, there are two approaches that can be applied for the biosynthesis of biosurfactants, i.e. whole-cell biotransformation (microbial synthesis) and enzymatic synthesis to produce a range of surfactant molecules, such as sugar fatty acid esters, amino acid based surfactants and carbohydrate alkyl ester derivatives (Allen & Tao, 1999; Desai & Banat, 1997; Le Guenic et al., 2019; Sarney & Vulfson, 1995). The rapid advances in the field of biotechnology over the two decades has led to considerable interest in the development of biological methods for manufacturing biosurfactants on an industrial scale. Biosurfactants have attracted considerable interest over chemically synthesised surfactants as they are less toxic and have higher biodegradability, and the production process is more environmentally friendly (Campos et al., 2013; De et al., 2015).

Biosurfactants can be divided into low-molecular-weight biosurfactants and high-molecular-weight biosurfactants/ bioemulsifiers (De et al., 2015; Uzoigwe et al., 2015). Low-molecular-weight biosurfactants, ranging from 500 to 1500 Da, are efficient in lowering the surface and interfacial tension of a liquid to form micelles and microemulsions between two different phases (De et al., 2015). High-molecular-weight biosurfactants/ bioemulsifiers with molecular weights greater than 10,000 Da (Franzetti et al., 2010), are

more effective at stabilising oil-in-water emulsions (Banat et al., 2010; Campos et al., 2013; Rosenberg & Ron, 1999). Table 2-2 shows the major classes of biosurfactants and the microorganisms involved for their production.

Table 2-2: Microbially produced biosurfactants. Adapted from De et al. (2015), Nitschke and Costa (2007) and Uzoigwe et al. (2015).

Surfactant class	**Examples**	**Microorganisms**	**Reference**
Low-molecular-weight			
Glycolipids (<1000Da)	Rhamnolipids	*Pseudomonas aeruginosa*	Perfumo et al. (2006)
	Trehalolipids	*Rhodococcus sp.*	White et al. (2013)
	Sophorolipids	*Candida bombicola,*	Ashby et al. (2005)
Lipopeptides (1000-1500Da)	Surfactin	*Bacillus subtilis*	Ongena et al. (2007)
	Viscosin	*Pseudomonas fluorescens*	Alsohim et al. (2014)
	Lichenysin	*Bacillus licheniformis*	Coronel-León et al. (2016)
Phospholipids (1300-2000Da)	Fatty acids/neutral lipids	*Klebsiella pneumoniae*	Nwaguma et al. (2016)
High-molecular-weight			
Polymeric surfactants (~1000kDa)	Emulsan	*Acinetobacter calcoaceticus*	Su et al. (2009)
	Alasan	*Acinetobacter radioresistens*	Navon-Venezia et al. (1995)
	Liposan	*Candida lipolytica*	Cirigliano and Carman (1985)
Particulate biosurfactants (~20kDa)	Membrane vesicles	*Pseudoalteromonas antarctica*	Nevot et al. (2006)
	Whole microbial cells	*Vibrio sp.*	Hu et al. (2015)

2.2 Production of carbohydrate fatty acid esters (CFAEs)

Amphiphilic CFAE are a type of non-ionic surfactants which can be synthesised through a chemical or enzymatic process (Chang & Shaw, 2009; Neta et al., 2015; van Kempen et al., 2013b). The synthesis involves linking the hydrophobic and hydrophilic moieties; the hydrophobic moiety can be either a long-chain fatty acid or a fatty acid derivative (hydroxy fatty acid, or α-alkyl β-hydroxy fatty acid), and the hydrophilic moiety can be a carbohydrate (mono-, di-, oligo-, or poly-saccharide) (van den Broek & Boeriu, 2013). CFAEs function as low-molecular-weight surfactants by maintaining most of the carbohydrate properties such as emulsifying, gelling, and film-forming properties and are also characterised by partial water solubility (van den Broek & Boeriu, 2013).

At present, CFAEs are commercially manufactured by chemical esterification of fatty acid methyl ester with acid chlorides in organic solvents (e.g. dimethyl formamide) using alkaline catalysts, which leave residual traces in the final products. Until now, the only sugar esters available in the market are sucrose esters synthesised by a chemical process (Soultani et al., 2003). Sucrose esters have been synthesised at 90 °C by base-catalysed (K_2CO_3) transesterification of fatty acid methyl esters in solvent dimethyl formamide (Hill & Rhode, 1999). The issue with the manufacturing process is that it leads to a complex product mixture consisting of mono-, di-, tri-, tetra-, and penta-esters. Often these are very hydrophobic as a result of the multiple substitutions of the OH groups, which limits their application potential (Hill & Rhode, 1999; van den Broek & Boeriu, 2013).

Enzymatic catalysis has been investigated for the synthesis of CFAE due to the fact that it is a more selective and efficient process (Inprakhon et al., 2001b). The most common enzyme used for synthesis is lipase; the synthesis is carried out in organic solvents with a low water activity to initiate the reaction towards synthesis instead of ester hydrolysis. The

most commonly used lipases originate from *Candida antarctica, Candida rugosa, Candida cylindracea, Rhizomucor miehei, Bacillus subtilis* and *Bacillus licheniformis*, while porcine pancreatic lipase is also used. The structure, properties and activities of lipases have been previously reviewed (Contesini et al., 2010; Guncheva & Zhiryakova, 2011; Rodrigues & Fernandez-Lafuente, 2010; Sharma et al., 2001). The advantage of enzymatic over chemical catalysis is that it normally produces mono-esters as the main reaction products because of the high regio- stereo- and enantio-selectivity of the lipase (Gumel et al., 2011). This is demonstrated in Figure 2-3 which depicts the synthesis of sucrose hexadecanoate (palmitate) esters through chemical and enzymatic synthesis. The higher specificity and regioselectivity that is offered by the enzymatic synthesis method can lead to products with tailored structures and functionality.

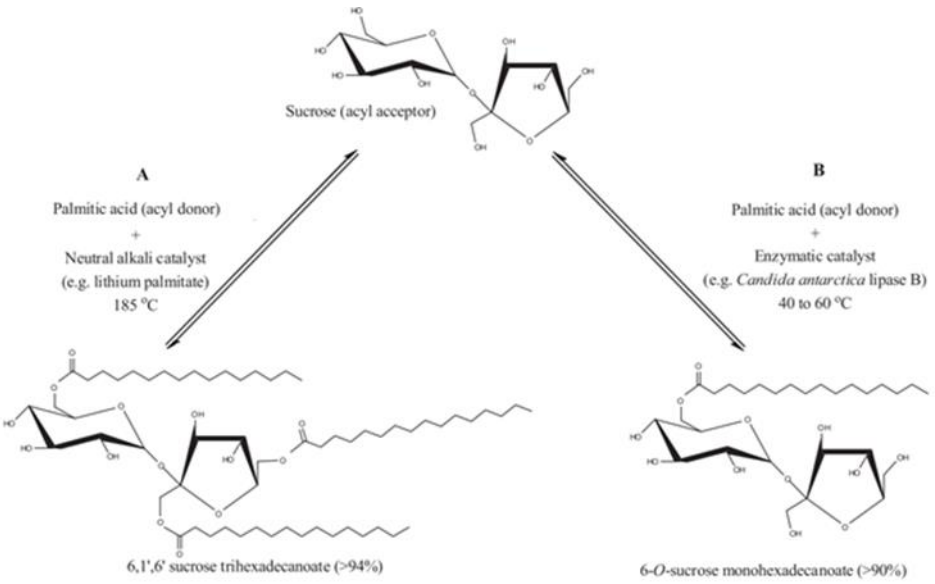


Figure 2-3: Comparison of (A) chemical and (B) enzymatic synthesis of sucrose hexadecanoate (palmitate) esters. Adapted from Gumel et al. (2011).

There are generally two types of reactions for the synthesis of the CFAE, i.e esterification and transesterification. Figure 2-4 depicts these reactions, using as an example

the synthesis reaction of glucose with either lauric acid or vinyl laurate. The esterification is an equilibrium reaction, which is thermodynamically controlled by using free carboxylic acid as the acyl donor. The by-product of such reaction is water, which is formed during ester synthesis. Therefore, in order to shift the equilibrium and increase the ester yield, continuous removal of the water produced is required (van den Broek & Boeriu, 2013). Numerous methods have been reported for the removal of water formed during esterification reactions, such as evaporation under reduced pressure (Izák et al., 2005), azeotropic distillation (Yan et al., 2002), and the use of molecular sieves (Chamouleau et al., 2001) or silica gel (Sonwalkar et al., 2003). In the case of the transesterification reaction, fatty acid esters (e.g. methyl, ethyl, and vinyl) esters are generally used as acyl donors; alcohols are formed as by-products. Vinyl esters are preferred acyl donors since the vinyl alcohol formed in the reaction tautomerises to volatile acetaldehyde at normal temperatures, which can be easily removed from the reaction mixture (van den Broek & Boeriu, 2013).

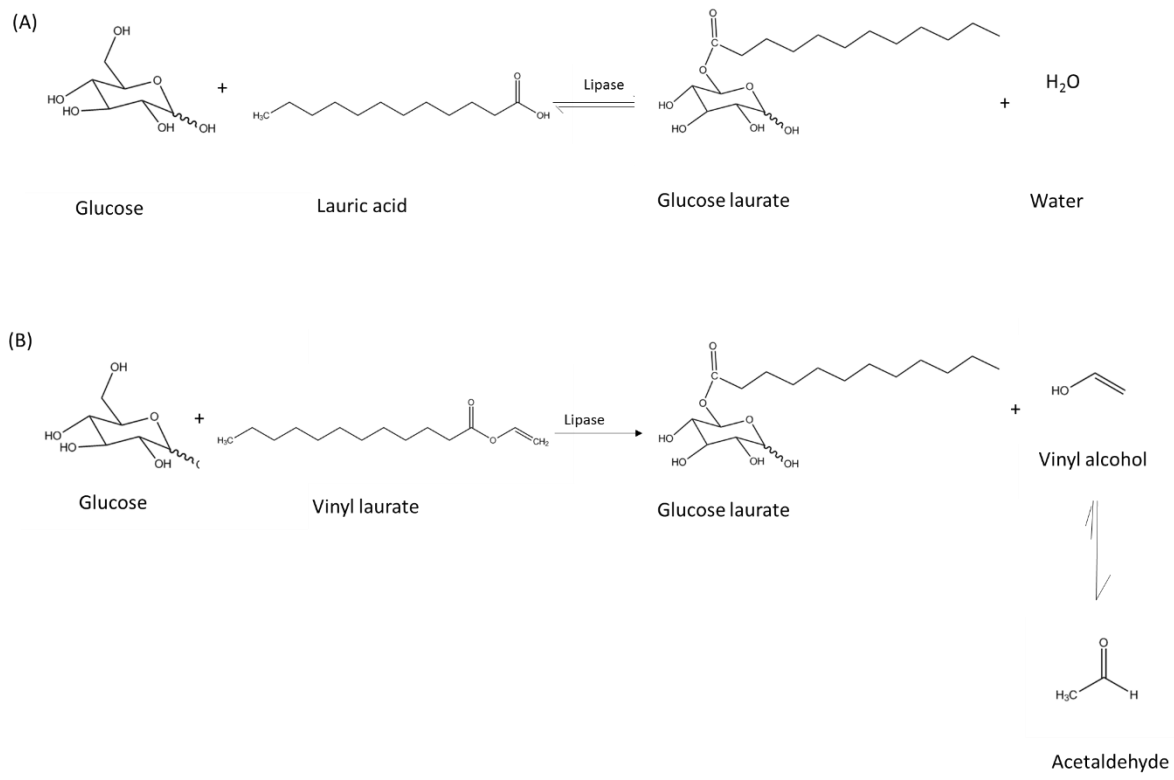


Figure 2-4: Example of (A) esterification (B) transesterification reaction of glucose with lauric acid/vinyl laurate catalysed using lipase enzyme.

2.2.1 Synthesis of carbohydrate fatty acid esters (CFAEs) by transesterification

Extensive literature exists on the enzymatic synthesis of CFAEs involving mono- and di-saccharides as the carbohydrate moiety, however a limited number of reports investigate the use of oligo- and poly-saccharides (Adachi & Kobayashi, 2005; Chang & Shaw, 2009). In the case of polysaccharides, synthesis of CFAE is difficult to be achieved by direct esterification with carboxylic acids (e.g. lauric acid, palmitic acid, decanoic acid) due to the fact that these carboxylic acids have low solubility in organic solvents (e.g. *tert*-Butyl alcohol) whereas fatty acid esters, used in transesterification reactions, are soluble in most of organic solvents (Otera, 1993). Moreover, the alcohol by-products produced in the case of transesterification are easier to remove compared to the water produced in the case

of esterification, which increases the yield of the desired ester (Adachi & Kobayashi, 2005; van den Broek & Boeriu, 2013). Table 2-3 presents an overview of the different types of carbohydrates that have been esterified with fatty acids esters using enzymes.

Table 2-3: CFAE synthesis by enzymatic transesterification using fatty acids esters as acyl donor and a variety of carbohydrates as acyl acceptors.

Substrates		**Enzymes**	**Solvents**	**Yield**	**Reference**
Acyl acceptor	**Acyl Donor**				
Starch	Vinyl sterate	*Candida lanuginose* lipase	Toluene	0.8[a]	Chakraborty et al. (2005)
Maltose	Vinyl laurate	Lipase from *Humicola lanuginose* immobilised on Celite	2-Methyl-2-butanol	38%[b]	Ferrer et al. (2000)
Maltose	Vinyl laurate	Lipase from *Humicola lanuginose* immobilised on Celite	*tert*-Amyl-alcohol/ 5% DMSO	72% [b]	Ferrer et al. (2000)
Maltose	Vinyl myristate	Lipase from *Humicola lanuginose* immobilised on Celite	*tert*-Amyl-alcohol/20% DMSO	77% [b]	Ferrer et al. (2000)
Maltose	Vinyl palmitate	Lipase from *Humicola lanuginose* immobilised on Celite	*tert*-Amyl-alcohol/ 20% DMSO	82% [b]	Ferrer et al. (2000)
Maltose	Vinyl sterate	Lipase from *Humicola lanuginose* immobilised on Celite	*tert*-Amyl-alcohol/ 20% DMSO	65% [b]	Ferrer et al. (2000)
Dextran T-40	Vinyl acetate	pH imprinted-lipase AY from *Candida rugosa* lipase	DMSO	60.5%[c]	Kaewprapan et al. (2011)
Dextran T-40	Vinyl propionate	pH imprinted-lipase AY from *Candida rugosa* lipase	DMSO	58.6%[c]	Kaewprapan et al. (2011)
Dextran T-40	Vinyl decanoate	pH imprinted-lipase AY from *Candida rugosa* lipase	DMSO	48.4%[c]	Kaewprapan et al. (2011)
Dextran T-40	Vinyl laurate	pH imprinted-lipase AY from *Candida rugosa* lipase	DMSO	43.2%[c]	Kaewprapan et al. (2011)
Dextran T-40	Vinyl decanoate	pH-imprinted lipase nanogel from *Candida rugosa* lipase	DMSO	23%[c]	Ge et al. (2009)
Fructooligosaccharides	Vinyl laurate	*Candida antarctica* lipase (Novozym® 435)	*tert*-Butyl-alcohol/DMSO (80:20 *v/v*)	45%[d]	ter Haar et al. (2010)
Raffinose	Vinyl laurate	*Candida antarctica* lipase (Novozym® 435)	*tert*-Butyl-alcohol/pyridine (55:45 *v/v*)	48%[e]	Pérez-Victoria and Morales (2006)

Raffinose	Vinyl laurate	*T. lanuginosus* lipase on granulated silica (Lipozyme TL IM)	*tert*-Butyl alcohol/pyridine (55:45 *v/v*)	79%[e]	Pérez-Victoria and Morales (2006)
Melezitose	Vinyl laurate	*Candida antarctica* lipase (Novozym® 435)	*tert*-Butyl alcohol/pyridine (55:45 *v/v*)	38%[e]	Pérez-Victoria and Morales (2006)
Melezitose	Vinyl laurate	*Thermomyces lanuginosus* lipase on granulated silica (Lipozyme TL IM)	*tert*-Butyl-alcohol/pyridine (55:45 *v/v*)	54%[e]	Pérez-Victoria and Morales (2006)
Kestose	Vinyl laurate	*Candida antarctica* lipase (Novozym® 435)	*tert*-Butyl-alcohol/pyridine (55:45 *v/v*)	54%[e]	Pérez-Victoria and Morales (2006)
Kestose	Vinyl laurate	*Themomyces lanuginosus* lipase on granulated silica (Lipozyme TL IM)	*tert*-Butyl-alcohol/pyridine (55:45 *v/v)*	57%[e]	Pérez-Victoria and Morales (2006)
Stachyose	Vinyl laurate	*Candida antarctica* lipase (Novozym® 435)	*tert*-Butyl-alcohol/pyridine (50:50 *v/v*)	26%[e]	Pérez-Victoria and Morales (2006)
Stachyose	Vinyl laurate	*Thermomyces lanuginosus* lipase on granulated silica (Lipozyme TL IM)	*tert*-Butyl-alcohol/pyridine (50:50 *v/v*)	68%[e]	Pérez-Victoria and Morales (2006)
Stachyose	Vinyl laurate	methyl-b-cyclodextrin (MβCD) subtilin Carlsberg	Pyridine	76%[e]	Pérez-Victoria and Morales (2006)
Sucrose	Vinyl laurate	Thermolysin (*Bacillus thermoproteolyticus*)	DMSO	44%[f]	Pedersen et al. (2002a)

Yield as determine with the different types of analysis in the enzymatic transesterification reaction

[a] Reported as Degree of Substitution by ^{1}H NMR
[b] Conversion of monoester determined by HPLC
[c] Conversion (Degree of Substitution) by ^{1}H NMR
[d] Relative amount of Degree of Substitution Oligomers (monomer) (DSO1 for DP3) as determined by MALDI TOF
[e] Percentage of each regioisomer of saccharide monoester as found by HPLC/MS
[f] Yield by TLC analysis

2.2.2 Factors influencing the enzymatic synthesis of carbohydrate fatty acid esters (CFAEs)

The application of lipases, esterases, proteases and peptidases is commonly reported for the synthesis of CFAEs. These enzymes are stereo- and regioselective catalysts that usually operate under mild reaction conditions. Moreover, these enzymes are generally robust, easy to handle and are commercially available for industrial applications (Table 2-4). The immobilised form of the lipase obtained from *Candida antarctica* type B, also known as CALB, has often been used for enzymatic reactions because immobilisation improves the stability of the enzyme in organic solvents. Another advantage of immobilisation is its contribution to the reduction of the enzyme costs, as the enzyme can be easily separated from the reaction mixture and can be used multiple times for ester synthesis (Plou et al., 2002). Any type of immobilisation method has the potential to better stabilise the enzymes compared to their native form. For example, pH-imprinted lipase from *C. rugosa* used for the transesterification of dextran with vinyl decanoate in DMSO led to a conversion yield of 49% with a 16-fold increase compared to free lipase (Kaewprapan et al., 2007).

Table 2-4: Commercial enzymes used for the enzymatic synthesis of CFAEs. Adapted from van den Broek and Boeriu (2013).

Enzymes	**Microorganisms**	**Company**	**Type**
Novozyme 435	*Candida antarctica*	Novozymes	Immobilised
Novozyme SP525	*Candida antarctica*	Novozymes	Free enzyme
Subtilin	*Bacillus subtilis*	Sigma Aldrich	Free enzyme
Protease (Neutral)	Not known	Amano Enzyme Co.	Free enzyme
Protease (Proleather FG-F)	*Bacillus subtilis*	Amano Enzyme Co.	Free enzyme
Subtilin Carlsberg	*Bacillus licheniformis*	Novozymes; Sigma Aldrich	Free enzyme
Lipozyme IM 60	*Rhizomucor miehei*	Novozymes	Immobilised
Lipase A12	*Aspergillus niger*	Amano Enzyme Co.	Free enzyme
Lipase AY	*Candida rugosa*	Amano Enzyme Co.	Free enzyme
Lipase	*Candida rugosa*	Sigma Aldrich	Free enzyme
Lipolase TL IM	*Thermomyces lanuginosus (Humicola lanuginose)*	Novozymes	Immobilised
Lipolase 100L	*Aspergillus oryzae*	Novozymes	Free enzyme
Lipozyme TM 20	*Mucor miehei*	Sigma Aldrich	Free enzyme
Thermolysin	*Bacillus thermoproteolyticus*	Sigma Aldrich	Free enzyme

One of the key issues for enzymatically synthesising CFAEs is the selection of an appropriate solvent to dissolve the carbohydrate. Thus, such synthesis reactions are often carried out in non-aqueous media using organic solvents, supercritical carbon dioxide or ionic liquids (Karmee, 2008; Sheldon, 2001). Key parameters of consideration include the toxicity of the solvent (particularly of organic solvents) to the enzyme and the solubility of the carbohydrate in the solvent (Danieli et al., 1997; MacManus & Vulfson, 1997). Carbohydrates are soluble in hydrophilic organic solvents such as dimethyl sulfoxide (DMSO), pyridine, and dimethylformamide (DMF). On the other hand, some solvents exhibit inactivating effect on enzymes and render the control of regioesterification difficult (Plou et al., 2002). In order to reduce the effects of solvents, the use of mixtures of two or more solvents has been previously investigated (Adachi & Kobayashi, 2005; Neta et al.,

2015). For example, it has been shown that mixtures of pyridine, *tert*-Butyl alcohol (*t*-BuOH), 2-Methyl-2-Butanol (2M2B) and DMF provide a better environment for lipase and proteases (Davis & Boyer, 2001; Degn & Zimmermann, 2001).

Another approach for CFAE synthesis is the use of ionic liquids (ILs) as the reaction medium. ILs have unique properties including negligible vapour pressure, low flammability, low toxicity and ability to dissolve a wide range of organic compounds, such as proteins (Potdar et al., 2015). ILs typically consist of anions of chloride (Cl^-), dicyanamide (DCA^-), formate ($HCOO^-$) and acetate (OAc^-). Only few reports are available describing the enzymatic synthesis of CFAEs in ILs due to the relatively low solubility of carbohydrates in these ILs (Liu et al., 2005; Park & Kazlauskas, 2001). A drawback of ILs is that in order to dissolve the carbohydrates a high molar concentration of Cl^-, DCA^-, $HCOO^-$ and OAc^- anions are needed, which might lead to enzyme denaturation (Chang & Shaw, 2009; Lee et al., 2006; Shi et al., 2011).

At present, research in this area is focused on the use of supercritical carbon dioxide (SC CO_2) for enzymatic catalysis (dos Santos et al., 2016; Laudani et al., 2007). However, the disadvantage of the SC CO_2 is that it could affect the enzyme activity due to the capability of CO_2 to strip essential water molecules from the microenvironment of enzymes (Shi et al., 2011). Additionally, carbon dioxide (CO_2) could potentially interact with the enzyme structure. Moreover, there is a suggestion that CO_2 can react with free primary amino groups of lysine residues from the enzyme surface to form ammonium carbamate (salt derived from the reaction of ammonia and carbon dioxide), which leads to a decrease in the pH of the aqueous layer around the enzyme, and reduces the enzyme activity (Beckman, 2004; Knez, 2018; Shi et al., 2011). However, there is no experimental evidence in the literature supporting this hypothesis.

2.3 Applications of CFAEs

CFAEs with various carbohydrate backbones and aliphatic residues possess high surface activity and some of them high emulsifying capacity as well (Hill, 2010). Renewable surfactants (bio-based surfactants and biosurfactants) derived from low value renewable resources are gaining increasing attention due to the advantages with regards to performance and environmental compatibility. Sucrose esters, produced currently through chemical synthesis, is an example of a commercial CFAE product that has been approved as a food additive (e.g. emulsifier) in many countries (Hill, 2010). In addition, CFAEs are suitable for personal care products and cosmetic applications (Akbari et al., 2018; Khan & Rathod, 2015; Lukic et al., 2016). However, the properties and potential applications of CFAEs using sugars other than sucrose, and which have been produced enzymatically by esterification or transesterification reactions, have not been extensively studied, although some works are listed (Table 2-5).

Table 2-5: Potential applications of enzymatically synthesised CFAEs

CFAE	**Substrate**		**Potential applications**	**Reference**
	Acyl acceptor	**Acyl Donor**		
Acylated inulin oligosaccharides	Raftiline LS	Vinyl laurate	Foam stability	Sagis et al. (2008)
Dextran-based hydrogel	Dextran	Divinyl adipate	Biomedical application (tissue engineering and controlled drug delivery)	Ferreira et al. (2005)
6 -O-palmitoylmaltotriose	Maltotriose	Vinyl palmitate	Antitumor agent	Ferrer, Perez, et al. (2005)
6-O-laurylsucrose	Sucrose	Vinyl laurate	Antimicrobial effects (*Bacillus* sp., *E. coli* and *L. plantarum*)	Ferrer, Soliveri, et al. (2005)
6-O-laurylmaltose	Maltose	Vinyl laurate	Antimicrobial effects (*Bacillus sp*., *E. coli* and *L. plantarum*)	Ferrer, Soliveri, et al. (2005)
Cellulose ester	Avicell cellulose	Vinyl propionate, vinyl laurate and vinyl stearate	Thermoplastic properties (internal plasticiser)	Gremos et al. (2011)
Modified Xyloglucan Oligosaccharides	Xyloglucan oligosaccharides (XGOs)	Vinyl stearate	Biocomposites (water repellent cellulosic material for packaging)	Gustavsson et al. (2005)
Ester of hydroxypropyl cellulose	Hydroxypropyl cellulose	Lauric acid	Biodegradable plastic	Sereti et al. (2001)
Maltodextrin palmitate	Maltodextrin	Palmitic acid	Emulsifying and stabilising agent in oil-in-water (O/W) emulsions	Udomrati et al. (2016)
Xylo-oligosaccharide palmitate	Xylo-oligosaccharide	Palmitic acid	Stabilising agent in oil-in-water (O/W) emulsions	Udomrati et al. (2016)

2.4 Oil-in-water (O/W) emulsions

An oil-in-water (O/W) emulsion consists of an oil dispersion phase, an aqueous phase, a surfactant and/or a stabiliser (Udomrati & Gohtani, 2015). A system that consists of small oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion (McClements & Gumus, 2016). O/W emulsions are important components of many commercial products, including food emulsions, for example, mayonnaise, ice cream and milk; personal care and cosmetics including lotions and moisturisers; agrochemicals, for example emulsion concentrates (EWs) and crop oil sprays (Tadros, 2013). On the other hand, a system that consists of water droplets dispersed and encapsulated within the oil phase is called a water-in-oil (W/O) emulsion, for example, foods such as butter and margarine; personal care and cosmetics including sunscreen and most makeup; and nutraceuticals including cod liver oil (Ghosh & Rousseau, 2011).

Homogenisation is the process commonly used to convert two separate immiscible liquids into an emulsion, or for reducing the size of the droplets in a pre-existing emulsion. In order to disperse two immiscible liquids, an emulsifier is needed. Emulsifiers are surface-active molecules that adsorb onto the surface of freshly formed droplets during homogenisation, forming a protective layer that prevents the droplets from coming close enough to aggregate (McClements, 2015). Most emulsifiers are amphiphilic molecules, that is, they have a polar and nonpolar region on the same molecule, and have a short term stabilising effect through their interfacial action (Dickinson, 2003). Therefore, to form an emulsion that is kinetically stable (metastable) and achieve long term stability the presence of stabilisers is required. Stabilisers are components that can be used to enhance the kinetic stability of an emulsion, and include for example biopolymers such as proteins or polysaccharides, or small molecular weight surfactants, although the latter are not as effective in conferring long-term stability (Dickinson, 2003). The stability offered by

biopolymers such as polysaccharides is via viscosity modification or gelation in the aqueous continuous phase (Dickinson, 2003).

The most effective emulsifiers are non-ionic low-molecular-weight surfactants that can be used to form and stabilise O/W or W/O emulsions. Moreover, they can stabilise emulsions to prevent flocculation and coalescence. To this end, a study was conducted by Udomrati et al. (2016) investigating the effect of concentration (10–50% w/w) and types of esterified oligosaccharides on the formation and stability of O/W emulsion. Three types of esterified oligosaccharides (EO) were used, maltodextrin palmitate with dextrose equivalent of 16 and 9 (DE16 P, DE9 P), as well as xylo-oligosaccharide palmitate (Xylo P). The creaming index of the EOs decreased with increasing the concentration of EO, whereas DE9 P inhibited coalescence and creaming more efficiently than DE16 P and Xylo P, as a result of the higher viscosity of the continuous phase in the former.

2.4.1 Factors affecting emulsion stability

Emulsions are thermodynamically unstable; a stable emulsion is one with no noticeable change in the size distribution of the droplets, or their state of aggregation, or their spatial arrangement within the sample over time (Dickinson, 2003). The dominant mechanisms of instability are gravity creaming, Ostwald ripening, flocculation and droplet coalescence, as shown in Figure 2-5. Homogenising one immiscible fluid with another (such as oil and water) will create a metastable emulsion (highly susceptible to physical destabilisation). Creaming and sedimentation result from external forces, usually gravitational or centrifugal forces. When such forces exceed the thermal motion of the droplets (Brownian motion), a concentration gradient builds up in the system with the larger droplets moving faster to the top, referred to as creaming (if their density is lower than that

of the medium) or to the bottom referred to sedimentation (if their density is larger than that of the medium) of the container (Tadros, 2013). Thus, droplets in an O/W emulsion tend to cream, whereas those in a W/O emulsion tend to sediment (McClements, 2015). Flocculation involves the aggregation of two or more droplets into a clump (larger units), with each individual droplet retaining its original dimension (without any change in droplet size) (Dickinson, 2010). Hence, flocculation occurs when there is not sufficient repulsion (steric and electrostatic) to keep the droplets apart to distances where van der Waals attraction is weak (Tadros, 2013). Additionally, coalescence is the process of thinning and disruption of the liquid film between the droplets, whereby two or more droplets merge together to form a single larger droplet (Tadros, 2013; Tcholakova et al., 2006). This process tends to occur when the attractive forces acting between the droplets are greater than the repulsive forces (similar to flocculation), thus rupturing the interfacial layers around the oil droplets when the droplets come into contact (McClements & Jafari, 2018).

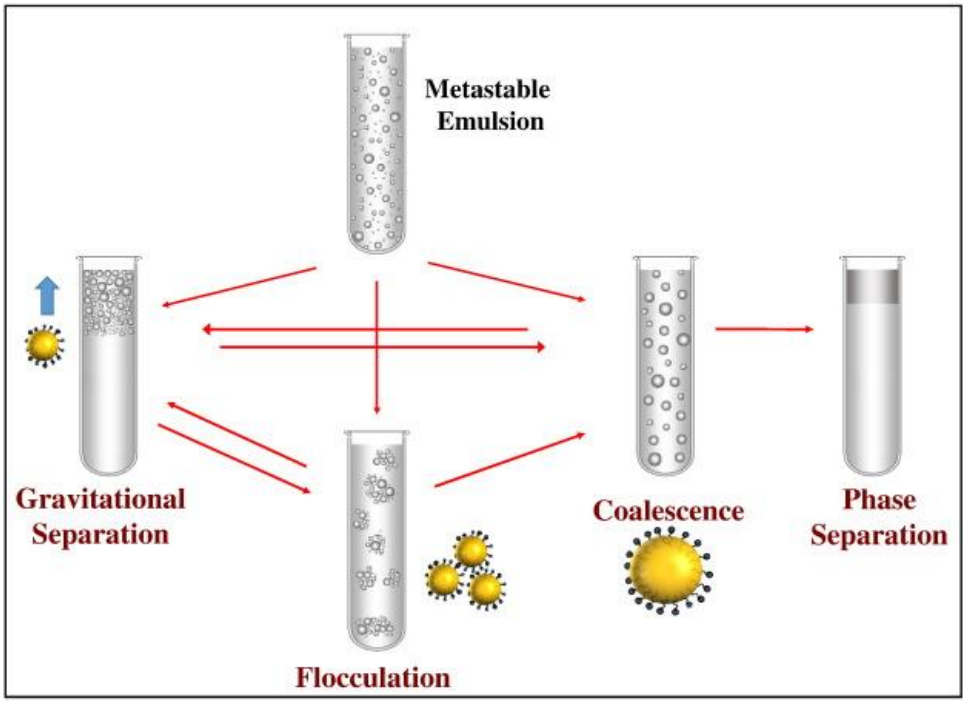
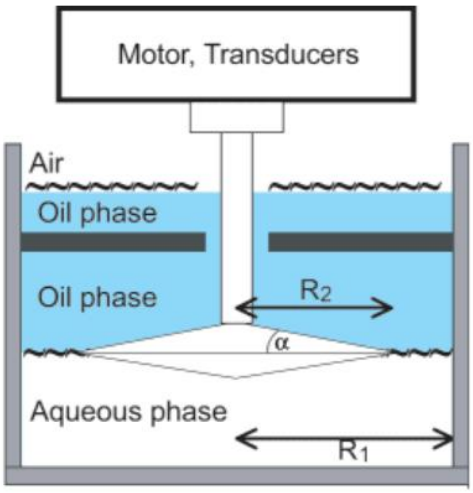


Figure 2-5: Mechanisms of emulsion instability. Adapted from McClements and Jafari (2018).

2.5 Interfacial rheology

Interfacial rheology is the study of deformation and flow of adsorption layers at O/W or W/O interfaces and is the most powerful tool for observing these phenomena at the interphase (Erni et al., 2007). Interfacial rheology studies investigate the response of mobile interfaces to shear (changes in shape at constant area) and/or dilatational deformation (changes in surface area with the constant shape) (Pelipenko et al., 2012). Interfacial rheology is divided into shear rheology and dilatational rheology; shear rheology is related to the long-term stability of dispersions while dilatational rheology provides information regarding short-term stability (Pelipenko et al., 2012). A number of experimental methods have been developed to measure the shear rheology of interfaces including the biconical bob geometry (Erni et al., 2003), and the Du Noüy ring geometry (Baldursdottir et al., 2010). The experimental set ups of these two methods are depicted in Figure 2-6.

(A)



(B)

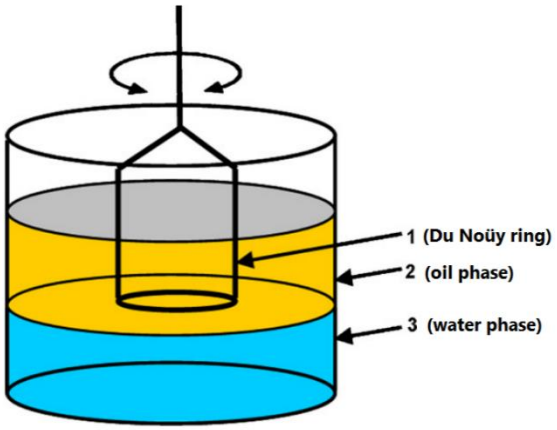


Figure 2-6: Geometry setup for interfacial shear rheology (A) rotating biconical disk (B) Du Nouy ring. Adapted from Erni et al. (2007) and Baldursdottir et al. (2010).

An emulsion undergoes different types of deformations when subjected to mechanical agitation as a result of stress (Walstra, 2003). The stress that causes different regions of the interfaces to move past each other, without altering the overall surface area is known as interfacial shear deformation. In contrast, the stress that may cause the surface area to expand or contract is known as interfacial dilatational deformation (McClements, 2015). Most interfaces have partly solid-like and partly fluid-like characteristics and therefore

exhibit viscoelastic behaviour. There are several rheological parameters used to characterise the liquid interfacial layers including the steady interfacial shear viscosity (η) and the dilatational viscosity (κ) (both in units of N m^{-1} s); these are measured in a steady interfacial shear flow or a dilatational flow. In the modulus notation, the interfacial shear moduli G′ (elastic) and G″ (viscous) and dilatational moduli E′ (elastic) and E″ (viscous) are measured, as a function of the strain (γ) and of the angular frequency of the oscillations (ω). In previous research, oligofructose fatty acid esters produced using through lipase catalysis using caprylic acid (C8), lauric acid (C12), palmitic acid (C16) and stearic acid (C18) showed a low surface tension and a high dilatational modulus, and were reported as being excellent foam stabilisers (van Kempen et al., 2014b).

Besides small molecular weight surfactants, proteins can decrease the interfacial tension upon adsorption and also form a viscoelastic (multi) layer at the interface to protect the oil droplet and to prevent flocculation (Dickinson, 2003; Fischer & Erni, 2007). Erni et al. (2007) studied the interfacial rheology of adsorption layers at the oil/water interface of *Acacia senegal* gums (hybrid polyelectrolyte containing both protein and polysaccharide subunits) and compared with adsorbed layers at the oil/water interface of hydrophobically modified starch. Both the shear and the dilatational rheological responses of the interfaces were studied and the hydrophobically modified starch showed slightly weaker viscoelasticity than *Acacia senegal* gum in the dilatational experiments. However, interfacial shear rheology indicated that the interfaces covered with the plant *Acacia* gum flow like a rigid and solid-like material with a large storage moduli and a linear viscoelastic regime. In contrast, the films formed by hydrophobically modified starch were predominantly viscous, and the shear moduli were only weakly dependent on the deformation (Erni et al., 2007).

The rheology of the interface depends on several factors, which influence the strength of the interactions between the molecules adsorbed at the interfaces, for example, surfactant

concentration, temperature, pH, and ionic strength (McClements, 2015). Generally, surfactants (e.g. small molecular weight surfactants and casein) have an interfacial viscosity or elastic moduli of several orders of magnitude less than biopolymer membranes (e.g. globular proteins and some polysaccharides) as shown in Figure 2-7. This is because biopolymers tend to undergo intermingling or cross-linking at the interface with each other through various covalent or physical forces (McClements, 2015). As a result, the interfacial rheology of globular proteins tends to increase over time due to conformational changes that lead to interfacial aggregation. The viscoelasticity of an emulsion of soy β-conglycinin was reported previously to increase significantly through the addition of soluble soy polysaccharides and gum Arabic, demonstrating the influential role of such additives in a food system (Li et al., 2018).

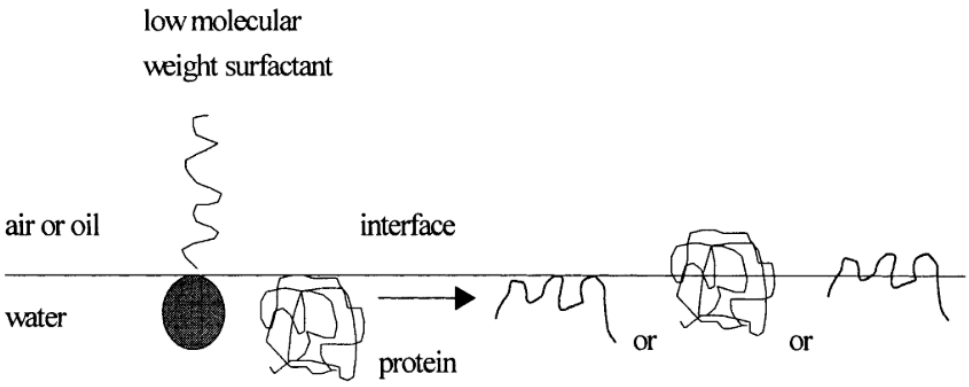


Figure 2-7: Conformation of low molecular weight surfactant and a macromolecular surfactant at a fluid-fluid interface. The two drawings on the right apply to oil-water interfaces. Adapted from Bos and van Vliet (2001).

2.6 Maltodextrins

Maltodextrins are products of partially depolymerised starch consisting of D-glucose units connected with variable chain lengths. The glucose units are linked linearly with α (1-4)-glycosidic bonds and α (1-6)-glycosidic bonds for branching (Udomrati et al., 2011). The depolymerisation of starch into maltodextrins can be achieved by acid hydrolysis, enzymatically, or by a combination of both procedures (Reineccius, 1991). The end-product of these chemical or enzymatic reactions usually consists of a mixture of simple sugars, such as D-glucose and maltose, as well as oligosaccharides and polysaccharides, such as maltotriose and maltotetraose and mixtures of higher molecular weight malto-oligosaccharides. Maltodextrins differ in their average molecular size and are classified based on their dextrose equivalent (DE). The DE is a measure of the amount of reducing sugars present in the structure of the carbohydrate, expressed as a percentage on a dry basis. i.e. glucose (dextrose) has a DE of a 100 on a dry-weight basis, so maltodextrin with a DE of 10 would have 10% of the reducing power of dextrose. This means that the higher the DE value, the higher the level of hydrolysis and as a result, the lower is the molecular weight of the maltodextrin components. Maltodextrins commonly have a DE value of less than 20 (Storz & Steffens, 2004). In addition, maltodextrins with different DE value can vary in their physicochemical properties, including solubility, freezing temperature and viscosity (Dokic-Baucal et al., 2004). On the other hand, maltodextrins with the same DE value may also have different properties depending on the hydrolysis procedure, the source of starch (e.g. maize, potato, rice), and the amylose to amylopectin ratio (Dokic-Baucal et al., 2004). By altering the hydrolysis conditions, different proportions of high and low molecular weight saccharides of similar DE can be obtained. The presence of high molecular weight components affects the properties of hydrolysed maltodextrins such as the solution stability

and solubility, whereas the dominance of low molecular weight components affects the fermentability, viscosity, crystallinity and sweetness (Cheetham & Sirimanne, 1981).

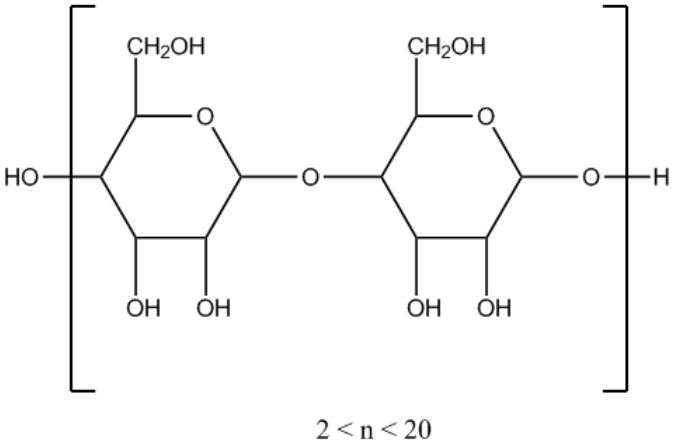


Figure 2-8: Chemical structure of maltodextrins

2.6.1 Applications of maltodextrins

Maltodextrins are soluble in water, therefore they are widely used in the food industry. They find applications as food ingredients because of their non-toxic nature, their generally recognised as safe (GRAS) status and low price. They are used as texture modifiers, gelling agents, fat replacers (Dokic-Baucal et al., 2004) and as encapsulation matrices (Che Man et al., 1999). In the food and confectionery industry maltodextrins are used as a fat replacer, e.g. in ice cream and mayonnaise, and for the prevention of crystallisation in syrups (Storz & Steffens, 2004).

Maltodextrins are widely used in food emulsions as stabilisers (Dokic-Baucal et al., 2004; Hogan et al., 2001) however emulsions containing maltodextrins as stabilisers require an additional emulsifying agent (e.g. lecithin, casein, glycerol, propylene glycol) for the

encapsulation of lipids. A few studies have been conducted studying the influence of maltodextrin as a stabiliser on the properties of O/W emulsions (Dokic-Baucal et al., 2004; Klinkesorn et al., 2004; Udomrati et al., 2011; Wangsakan et al., 2003). The effect of tapioca maltodextrin on the stability of O/W emulsion prepared with Tween 80 as emulsifier has been studied by Udomrati et al. (2011). Tapioca maltodextrins of DE values of 9 (DE9), 12 (DE12), and 16 (DE16) were used in that study and creaming was not observed at maltodextrin concentrations more than 35 % *w/w* for DE9 and 40 % *w/w* for DE12. However, DE16 showed creaming at all concentrations above the critical flocculation concentration (CFC). Maltodextrin with a lower DE inhibited creaming more effectively than maltodextrin with a higher DE because of the higher viscosity.

Besides considering their low cost, their effectiveness as a matrix component contributes to the fact that maltodextrins are used in spray drying as an encapsulating agent for vitamins, minerals and colourants (Jafari et al., 2008; Nunes et al., 2015; Sadeghi et al., 2008). Negrão-Murakami et al. (2017) reported the effect of different DE maltodextrins, i.e. maltodextrin DE10.2, DE15.2 and DE18.5 as wall materials on the physicochemical properties, antioxidant activity, and storage stability of spray-dried concentrated mate. The concentrated mate was obtained by nanofiltration from the native plant, *Ilex paraguariensis* St. Hilaire (yerba mate) and is a rich source of phenolic compounds. The results showed that the lowest DE maltodextrin, DE10.2, produced more stable microcapsules for stabilising the phenolic compounds and the antioxidant activity of the dried concentrated mate. Maltodextrins are also used to increase the processing and storage stability of powdered materials in order to reduce lumping, caking, stickiness and improve flowability (Descamps et al., 2013; Fitzpatrick et al., 2007).

2.6.2 Maltodextrin fatty acid esters

Maltodextrins are strongly hydrophilic and are not surface-active in emulsions, thus are not suitable for O/W emulsion systems (Udomrati & Gohtani, 2014, 2015). However, they can be modified by the attachment of hydrophobic groups through esterification/ transesterification reactions with a fatty acid or fatty acid esters. The presence of the ester group into the maltodextrin can modify the hydrophilic properties of maltodextrin and confer amphiphilic properties to the molecule. Therefore, amphiphilic maltodextrins produced through a lipase catalysed reaction could potentially function as polymeric non-ionic surfactants without altering the emulsifying, gelling and film-forming properties of the polysaccharide (van den Broek & Boeriu, 2013). This opens up a lot of opportunities for producing biosurfactants from renewable feedstocks, such as carbohydrates derived from starch. Thus, the product, maltodextrin fatty acid ester can be applied in a whole range of products. For example, the maltodextrin fatty acid ester can be used as biodegradable emulsifiers, detergents, and for the surface modification of preformed polysaccharide-based materials.

The conceptual synthesis reactions for the synthesis of maltodextrin laurate, the molecule targeted in this PhD work, through either direct esterification or transesterification with lauric acid or vinyl laurate are illustrated in Figure 2-9. A direct esterification reaction was successfully employed to synthesise amphiphilic maltodextrin with DE 16 with three fatty acids, decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16) (Udomrati & Gohtani, 2014). Interestingly, esterified maltodextrin DE16 was found to exhibit some surface, interfacial and emulsification activity and the emulsifying activity increased with increasing the chain length of the fatty acid. Besides this study, to our knowledge, there are no studies investigating the production of maltodextrin esters particularly by transesterification and their physicochemical properties. Overall, there is a lack of

knowledge on the effect of maltodextrins with different DE on the properties of the produced esters, which this PhD work aims to address.

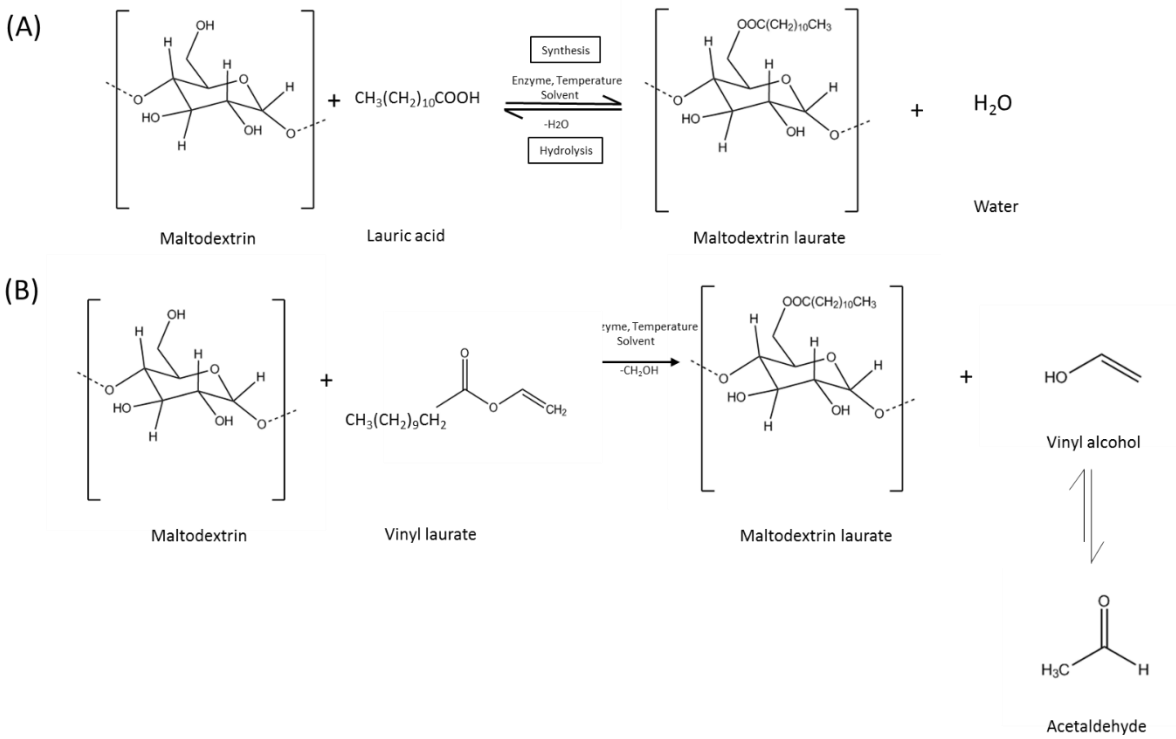


Figure 2-9: Schematic diagram of the process for converting maltodextrin and lauric acid/vinyl laurate to maltodextrin laurate through (a) esterification and (b) transesterification reactions using lipase enzyme.

Following a similar concept, dextran, a bacterial polysaccharide consisting of α-1,6 linked D-glucopyranoside residues with a small percentage of α-1,3 linked side chains, has been used for the synthesis dextran fatty esters (Kaewprapan et al., 2011). These have been synthesised by the transesterification of dextran and vinyl fatty esters (e.g. vinyl acetate, vinyl propionate, vinyl laurate, vinyl decanoate, vinyl acrylate, vinyl crotonate and vinyl pivalate) in dimethyl sulfoxide at 50°C using lipase AY from *Candida rugose.* That study reported that the highest conversion yield (96% conversion) was obtained for the dextran

vinyl decanoate ester; the ester was water-insoluble and formed nanoparticles of 150 nm diameter in water, and thus has potential applications as a drug carrier system.

2.6.3 Challenges of CFAE production

Over the past decade, research has demonstrated the high potential of enzymes, particularly lipases, for the synthesis of a wide range of CFAEs, consisting of various carbohydrate backbones and aliphatic residues. Although maltodextrins are important functional ingredients and are currently used widely as food additives, they have received significantly less attention than other polysaccharides/oligosaccharides for the development of CFAEs. Moreover, there are no commercial processes yet in place for the enzymatic production of CFAEs. Both these areas constitute significant areas where future research should focus on. More specifically, further research is needed on the optimisation of enzymatic synthesis of CFAEs, focusing on decreasing the reaction times, improving the physicochemical and functional characterisation of CFAEs and developing applications for the food as well as the detergent, cosmetics and personal care sectors. Moreover, standardised methods are needed for the determination of the degree of substitution and other process parameters. Currently, it is difficult to compare results between studies due to the different methods used to calculate the degree of substitution and the conversion yield. Such knowledge around carbohydrate esterification is important in order to develop environmentally friendly processes for the synthesis of bio-based surfactants that can find applications in various industrial sectors and decrease our dependence on petrochemical.

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Chapter 3 - Enzyme-catalysed transesterification of maltodextrins and evaluation of their surface-active properties

Abstract

The enzymatic synthesis of maltodextrin with vinyl laurate catalysed by an immobilised lipase from *Candida antarctica* (Novozym® 435) was investigated. To this end, maltodextrins with different dextrose equivalents (DEs), namely, DE4-7 (MDE4), DE13-17 (MDE13) and DE16.5-19.5 (MDE16.5), were used as substrates. The degree of substitution ranged from 0.25 to 0.43 (in the case of MDE16.5), when the molar ratio of vinyl laurate to maltodextrin was equal to 4:1, using 0.3% (*w*/*v*) of the enzyme at 60 °C for 36 hours. The yields of the obtained esters were 6.6, 11.2 and 18.4 mg per g of initial substrate for MDE16.5, MDE13 and MDE4, respectively. The produced esters were fractionated by reverse phase solid phase extraction, and ^{1}H NMR and FTIR confirmed the presence of a lauryl group attached to maltodextrins. The surface tension and rheological properties of the three maltodextrin laurates were studied in three concentrations, 10, 20 and 40% *(w/v)*. In all cases, the esters showed reduced surface tension (48.5–23.4 mN/m), compared to that of the control (Tween 20, 37.6–31.0 mN/m) when the ester concentrations increased from 10 to 40% *(w/v)*. Maltodextrin laurates had lower viscosity compared to the unmodified maltodextrin, and showed Newtonian behaviour in an aqueous system with the exception of the MDE4 laurate at the concentration of 40% (*w*/*v*), which showed a shear thinning flow behaviour.

Keywords: maltodextrin esters; transesterification; rheology; surface tension; lipase

3.1 Introduction

Carbohydrate fatty acid esters (CFAEs) are non-ionic surfactants synthesised through chemical or enzymatic reactions of a carbohydrate moiety as the hydrophilic group and one or more fatty acids as lipophilic component(s) (Chang & Shaw, 2009). Surfactants are characterised by their capacity to reduce the surface and interfacial tension of a liquid and form oil-in-water (O/W) emulsions or water-in-oil (W/O) emulsions. Several sugar-based surfactants called sorbitan esters (also known as Spans, Tween) and sucrose esters have been used in commercial applications (Le Guenic et al., 2019; Lukic et al., 2016). For example, sucrose ester is commercially obtained through chemical transesterification with fatty acid methyl ester under base catalysed environment (K_2CO_3) and carried out in solvents (e.g. dimethyl formamide at 90 °C) (Hill & Rhode, 1999). The issue with regards to the chemical esterification of sucrose esters is that it is a non-selective process and produces complex mixtures of mono-, di- and tri-esters. Additionally, primary and secondary hydroxyl groups are substituted, and ester groups are randomly distributed in the carbohydrate monomer and polymer backbone. Thus, the chemical esterification process is suitable only for the production of carbohydrate esters with a high degree of substitution (DS) (van den Broek & Boeriu, 2013).

The enzymatic synthesis of CFAEs, mainly through the use of lipases, offers many advantages over the chemical route (Inprakhon et al., 2001a). Enzymatic reactions may be performed under mild temperatures (usually at 40–60 °C) (Gumel et al., 2011), thereby preventing the discolouration and degradation of carbohydrate substrates and products (van den Broek & Boeriu, 2013). The synthesis reaction can be achieved through direct esterification reactions with a fatty acid or transesterification with fatty acid esters. Transesterification is more preferable than direct esterification since the reactivity of the fatty acid ester group is higher than that of a carboxylic acid residue. The alcohol by-products

of the reaction tautomerise to volatile acetaldehyde at normal temperatures and are easily removed from the reaction mixture (van den Broek & Boeriu, 2013).

Furthermore, the enzymatic synthesis of CFAE in an organic solvent is based on the ability of lipases to catalyse the reverse hydrolysis leading to the formation of ester bonds in a medium with low water activity. Under these conditions, the thermodynamic equilibrium of the reaction is shifted towards a synthesis reaction instead of hydrolysis (Neta et al., 2011). However, in synthesis reactions using oligo- and polysaccharides, a major problem is the low solubility of the carbohydrate in organic solvents. The difference in polarity between polysaccharide and fatty acid limits the choice of organic solvents (Borges & Balaban, 2007; Plou et al., 2002). Polar media, such as dimethylsulfoxide (DMSO), 1-methyl-2-pyrrolidone (NMP) and dimethylformamide (DMF), which promote the solubilisation of polysaccharides, reduce the stability of the lipase and the solubilisation of fatty acids (ter Haar et al., 2010). Numerous studies have been recently reported, investigating the lipase-catalysed transesterification of carbohydrates with different degree of polymerisations (DPs), including *Agave tequilana* fructans with DP lower than 6 (Casas-Godoy et al., 2016), oligofructose (Orafti P95) with DP of 2–8 (van Kempen et al., 2013b), and Raftiline LS with DP of 10 (Sagis et al., 2008).

Carbohydrates in the form of polysaccharides are produced naturally in plants and generally include starch, cellulose and hemicellulose. The hydrolysis of starch through acid hydrolysis, enzymatic reaction or their combination mainly produces maltodextrin, whichcontains linear amylose and branched amylopectin consisting of α-(1-4) and α-(1-6)-D-glucose oligomers and/or polymers (Udomrati et al., 2011). Therefore, maltodextrins differ in terms of their average molecular size and are classified according to their dextrose equivalents (DEs). In the food industry, maltodextrins are widely used because of their solubility in water. They are often applied as food ingredients because of their non-toxic

nature (deriving from corn, potato, tapioca or rice starch), their Generally Recognised as Safe (GRAS) status and low price. They are commonly used as texture modifiers, gelling agents, fat replacers (Dokic-Baucal et al., 2004) and encapsulation matrices (Che Man et al., 1999).

Currently, the esterification of polysaccharides attracts greater research interest than that of sugars (glucose, sucrose and fructose), due to the number of the reactive hydroxyl groups of polysaccharides. The condensation of a reactive group of a saccharide with a fatty acid produces surface-active compounds (i.e. an hydrophilic saccharide moiety combined with hydrophobic fatty acid moiety) (Allen & Tao, 2002). Oligofructose lauric acid esters have been previously reported to lower the surface tension of the air/water interface and provide a high dilatational modulus (van Kempen et al., 2013b). The enzymatic esterification of starch and maltodextrin has been previously studied (Jandura et al., 2000; Udomrati & Gohtani, 2014). However, the effect of different sizes of acyl acceptor (maltodextrin) on the enzymatic transesterification of polysaccharides has not been addressed, especially for the production of surface-active properties that can modify the rheological characteristics of maltodextrin, which are important properties that define surfactant performance.

This study aims to investigate the performance of maltodextrins with three different DEs in transesterification reactions with vinyl laurate, catalysed by immobilised lipase. The reaction conditions were investigated in detail with respect to substrate ratio, enzyme concentration and time. Then, the ester products were purified and their physicochemical properties were characterised and compared to those of unmodified maltodextrins.

3.2 Materials and methods

3.2.1 Materials

Maltodextrins with three different DE ranges were obtained from Sigma-Aldrich (UK), including maltodextrin DE 4–7 (MDE4), DE 13–17 (MDE13) and DE 16.5–19.5 (MDE16.5). DE is inversely proportional to the average molecular weight (M_n) and the degree of polymerisation (DP), which are both commonly used to describe the size distribution of the polysaccharide chain in the carbohydrate polymer (Sun et al., 2010).

DMSO (>99%), *tert*-Butyl alcohol (≥99.5%), acetone (>99%), methanol (≥97%) and molecular sieves (4 Å, 8–12 mesh) were purchased from Fisher Scientific. Vinyl laurate, ≥98% (GC) and hexane (≥97%) were purchased from Sigma-Aldrich (UK).

3.2.2 Enzyme

A recombinant lipase from *Candida antarctica*, expressed in *Aspergillus niger*, which was immobilised on an acrylic resin (Novozym® 435) was purchased from Sigma–Aldrich. According to the manufacturer's specifications, the specific activity was 10000 Propyl Laurate Unit (PLU)/g.

3.2.3 Enzymatic synthesis of maltodextrin laurate

Vinyl laurate was used as the acyl donor, while maltodextrins were used as the acyl acceptor for the transesterification reactions as illustrated in Figure 3-1. Approximately 1 g of dried maltodextrin was dissolved in 100 mL of DMSO to a final concentration of 1% (*w*/*v*). Vinyl laurate was dissolved in warm (~50 °C) *t*-BuOH by using vinyl laurate and

maltodextrin in molar ratios of 1: 1, 2: 1, 3: 1, 4:1 and 5:1 (mole of vinyl laurate/mole of anhydro glucose unit). Maltodextrin in DMSO (1% *w*/*v*) was slowly mixed with 90 mL of vinyl laurate in *t*-BuOH until all material was dissolved, while the final co-solvent composition was DMSO/*t*-BuOH (10/90 *v*/*v*). Molecular sieves 3% (*w*/*v*) and lipase (100 mg), (10 PLU/g of lipase activity) were added to initiate the reaction and the mixture was incubated for 24 h at 60 °C in a shaking incubator (Incu-Shake MAXI, SciQuip, UK).

The optimum vinyl laurate and maltodextrin in molar ratios of 1: 1, 2: 1, 3: 1, 4:1 and 5:1 (mole of vinyl laurate/mole of anhydro glucose unit) was obtained and used in subsequent experiment, with the enzyme concentration (0- 0.5 % (*w/v*) and incubation time 0 - 72 h was investigated. The reaction was stopped via filtration for the separation of the molecular sieves and the immobilised lipase from the solvent. The *t*-BuOH solvent was evaporated through rotary vacuum evaporation (Rotavapor, Buchi, Switzerland). The product was washed twice with 50 mL of acetone and once with 50 mL of hexane for the removal of any residual vinyl laurate. Then, the produced esters were precipitated with ethanol. The ethanol supernatant was decanted, and two additional ethanol extractions were performed prior to drying of the precipitate in an air oven (Memmert INB 200 incubator, Memmert GmBH, Netherlands) at 50 °C.

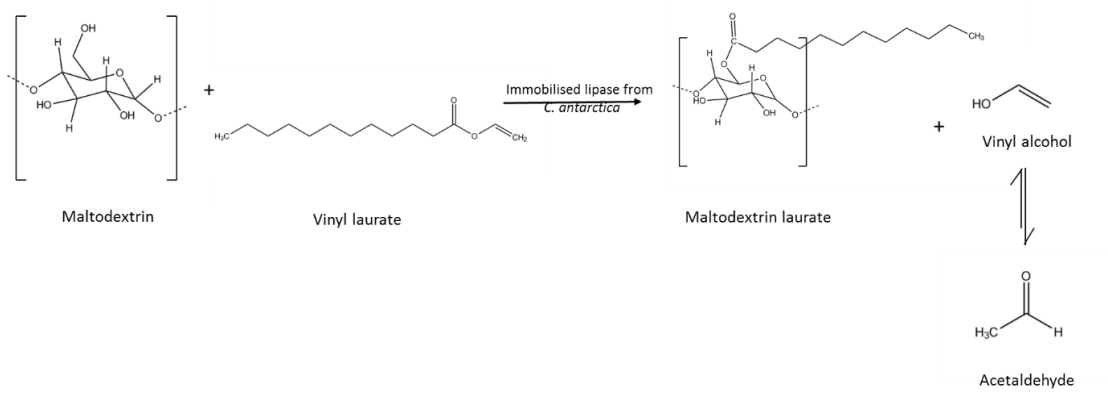


Figure 3-1: Enzymatic transesterification of maltodextrin with vinyl laurate catalysed by immobilised lipase from *C. antarctica*.

3.2.3.1 Determination of degree of substitution (DS) through proton NMR (^{1}H NMR)

Each glucose unit within a maltodextrin polymer chain contains three hydroxyl groups that can be substituted with lauric acid. Hence, the average DS can range from 0–3. The DS for a starch or amylose derivative is defined as the moles of substituents of hydroxyl groups per D-glucopyranosyl structural unit of the polymer (Kapusniak & Siemion, 2007). For the determination of the DS, approximately 10 mg of MDE4, MDE13 and MDE16.5 samples were dissolved in 0.7 mL of DMSO-$_{d6}$ at 50 °C. The ^{1}H NMR spectra were recorded with a Bruker Advance III 400 MHz spectrometer according to the method described by Udomrati and Gohtani (2014). The data were analysed by using TopSpin (Bruker, Billerica, MA). The ^{1}H NMR spectra of the maltodextrin laurate showed the three protons of the terminal methyl group of the acyl chain at approximately 0.81 ppm. The peaks between 4.58 and 5.50 ppm corresponded to the signals from the four protons of the glycoside structure. The three protons in the CH_3 terminal of the acyl chain were observed as a triplet at 0.81 ppm. The DS was obtained from the ratio of the proton peak are at 0.81 ppm to that of the proton peak between 4.58 and 5.50 ppm, calculated as follow:

$$DS = \frac{\text{I Signal}/3}{\Sigma IAGU/4} \quad ,$$

Where 3 is the number of protons from the signal of the methyl proton, and I_{AGU} is the integral for the 4 protons of the anhydroglucose unit (AGU) between 4.58 and 5.50 ppm.

3.2.3.2 Product yield of the maltodextrin laurate

The yield of the produced maltodextrin laurate was calculated according to Namazi et al. (2011).

$$Product\ Yield\ (\frac{mg}{g}) = \frac{\text{mass of maltodextrin laurate (mg)}}{\text{mass of initial maltodextrin (g)} + \text{mass of reacting vinyl laurate (g)}}$$

3.2.3.3 Solubility of maltodextrin laurates

Maltodextrin laurate powder (30-50 mg) was suspended in 5 mL of water at different temperatures (5, 25 and 50 °C), stirred for 30 min and centrifuged at 4000 rpm for 15 min (Heraeus Multifuge X3R, Thermo Fisher, USA). The precipitate was collected, oven-dried at 90 °C and then weighed. The solubility (%, *w/w*) was calculated as follows:

$$Solubility\ (\%) = \frac{\text{weight of soluble maltodextrin laurate (g)}}{\text{weight of initial dry sample (g)}} \times 100$$

Where the weight of soluble maltodextrin (g) was calculated by using the weight of the initial dry sample (g) and subtracting the weight of the precipitated maltodextrin laurate (g).

3.2.4 Purification of maltodextrin laurate

Strata® C18-U cartridges (Phenomenex) with a loading volume of 6 mL were used following the method van Kempen et al. (2013b) with some modifications. The columns were conditioned and equilibrated by washing them twice with 6 mL of methanol followed

by washing them three times with 6 mL of water. The maltodextrin laurate samples were diluted with water (1:10) and then loaded onto the column. The desired maltodextrin laurate was eluted with 6 mL of water/methanol mixtures, starting with 100% water and ending with 100% methanol, with 10% increments. The last step (100% methanol) was performed twice using a vacuum pump. Finally, the solvent of the fraction containing water/methanol mixture was evaporated through rotary vacuum evaporation. After purification, the fractions were analysed through ESI–MS as described in the next section. All samples were kept at −20 ºC overnight and freeze-dried for 3 days in a laboratory freeze dryer (VirTis Sentry 2.0, SP Scientific Inc., PA).

3.2.5 ESI-MS analysis of product fractions

Electrospray ionisation (ESI) analysis was performed with a Thermo Scientific LTQ Orbitrap XL mass spectrometer. Samples (100 µL) were diluted in 900 µL of HPLC water to a final concentration of 0.1 µL/mL and were injected directly to the spectrometer. The peaks of maltodextrin laurate were found in the positive ion mode [ESI+] by scanning from m/z 200 to m/z 4000. The capillary potential was 4.0 kV, the dry gas temperature was 274 °C and the drying gas flow was 5 µL/min. The data was analysed through Qual Browser of X-Calibur software (Thermo Scientific, USA).

The calculated mass error for each peak that within 5 ppm was chosen in this characterisation. The mass error of the theoretical value was calculated as below:

$$Mass\ error\ (ppm) = \frac{\text{Observed } (\frac{m}{z}) - \text{theoretical } (\frac{m}{z})}{\text{theoretical } (\frac{m}{z})} \times 1000000$$

3.2.6 Fourier transform infrared spectroscopy (FTIR)

For the characterisation of maltodextrin and maltodextrin laurate, FTIR was performed. One gram of dried sample was uniformly spread on the crystal surface area, covered by a flat probe tip and the spectrum recorded using a Perkin Elmer Spectrum 100 ATR- FTIR over a frequency range of 4000 cm^{-1} to 500 cm^{-1} at a resolution of 4 cm^{-1}. Sixteen scans were taken for each sample. Duplicate spectra for each sample were collected and analysed using Spectrum software (Spectrum 100, Perkin Elmer Inc., USA).

3.2.7 Determination of surface-active properties of the produced esters (air-water surface tension $\Upsilon_{a/w}$)

The surface tension was monitored using an automated Pendant Drop equipped with a needle, a camera and a light source (Drop Shape Analyzer DSA30, Krùss GmbH, Hamburg, Germany). Maltodextrin laurate was dissolved in water at concentration of 10, 20 and 40% (*w*/*v*). 20 µL of a maltodextrin laurate solution were dropped onto the surface by using a micro-syringe. The surface tension was measured from a drop suspended from a needle, which starts to detach when its weight (volume) reaches the magnitude balancing the surface tension of the liquid. The weight (volume) is dependent on the characteristics of the liquid and the surface tension was measured using the Young Laplace equation. Measurements were performed in triplicate, and the mean value was calculated.

3.2.8 Viscosity measurement of unmodified maltodextrin and maltodextrin laurate

Shear stress (τ) and viscosity of maltodextrin laurate solutions at 10%, 20% and 40% (*w*/*v*) were measured using a bob-and-cup system. The samples were measured using a CC25

measuring system (Modular Compact Rheometer MCR 102, Anton Paar, Graz, Austria). Samples were placed in the cylindrical cup of the viscometer and allowed to equilibrate at a set-point 25 °C for 1 min prior to analysis. The shear stress of the sample was measured from a shear rate of 5–100 s^{-1}. The shear rate was measured point-by-point with consecutive 20 s steps of constant shear rate. The viscosity was recorded for each point to obtain the flow curves. The flow curves were fitted to the *Herschel–Bulkley* model as follows:

$$\tau = \tau 0 + k \cdot \gamma^{n}$$

where τ is the shear stress (Pa), $\tau 0$ is the yield stress (Pa), k is the consistency coefficient (Pa sn), γ is the shear rate (s^{-1}) and n is the flow index; n < 1 corresponds to shear thinning behaviour, n > 1 corresponds to shear thickening behaviour and n =1 corresponds to Newtonian behaviour.

3.2.9 Statistical analysis

Statistical analysis was conducted using the Minitab® 18 statistical analysis software. One-way analysis of variance (ANOVA) with a Tukey's multiple comparison test was used to determine significant difference between treatments at a confidence level of 95% ($p < 0.05$). Results are presented as mean ± standard deviation.

3.3 Results and discussion

3.3.1 Effect of substrate ratio, enzyme concentration and reaction time on the synthesis of maltodextrin laurates

All three maltodextrin samples were prepared by using the same enzyme concentration (0.1% *w*/*v*) for 24 h, and the effect of the molar ratio of vinyl laurate to maltodextrin is shown in Figure 3-2 (a). Maltodextrin MDE16.5 showed the highest DS (0.20) at a substrate molar ratio of 4:1, followed by MDE13 (0.15 at molar substrate ratio of 3:1) and MDE4 (0.13 with substrate molar ratio of 2:1). The highest DS for the MDE16.5 could be explained by the highest solubility of this sample in DMSO/*t*-BuOH solvent. The solubility of the carbohydrate is the main factor that governs the level of substitution by lauric acid. Woudenberg-van Oosterom et al. (1996) observed that the more soluble the substrate, the higher the rate of the reaction is. After the reaction reached the optimum molar ratio of maltodextrin and vinyl laurate, the formation of the covalent linkage between the sugar repeat units in polysaccharides creates additional steric hindrance which might limit the formation of more substituted units (Cramer et al., 2007; Kaewprapan et al., 2012; Pedersen et al., 2002b; van Kempen et al., 2013b). In contrast, Udomrati and Gohtani (2014) achieved a low DS range from 0.002-0.084 during the esterification of tapioca maltodextrin (DE=16) with fatty acid (decanoic acid, lauric acid, palmitic acid) under optimised conditions of 60 °C, 4 h of reaction and 1:0.5 of maltodextrin/fatty acid molar ratio.

Figure 3-2 (b) shows that the DS was controlled by changing the enzyme concentration. MDE16.5 had the highest DS (0.35) ($p<0.05$) with 0.3% (*w*/*v*) (30 PLU/g) of lipase concentration. It is likely that the increased solubility of the short-chain maltodextrin in the solvent, increased the accessibility to the catalytic cleft of the lipase sites and increased the level of substitution in the produced ester. However, the DS significantly decreased when

the enzyme concentration reached 0.5% (*w*/*v*) due to the increase in the amount of vinyl alcohol generated as a by-product. Moreover, it is likely that the enzymatic synthesis of the ester caused the tautomerisation of vinyl alcohol into acetaldehyde, which could react with the free amino groups of the lysine residues of the lipase, which inactivated the enzyme (Franken et al., 2011; van Kempen et al., 2013b).

According to Figure 3-2 (c), the highest DS was observed at 36 h of reaction time for all maltodextrin samples; after this time point, the DS reached a plateau. The optimal vinyl laurate/maltodextrin ratios leading to the highest DS for MDE16.5, MDE4 and MDE13 were 4:1, 2:1 and 3:1, respectively with 0.3 % (*w/v*) of lipase for 36 h at 60 °C.

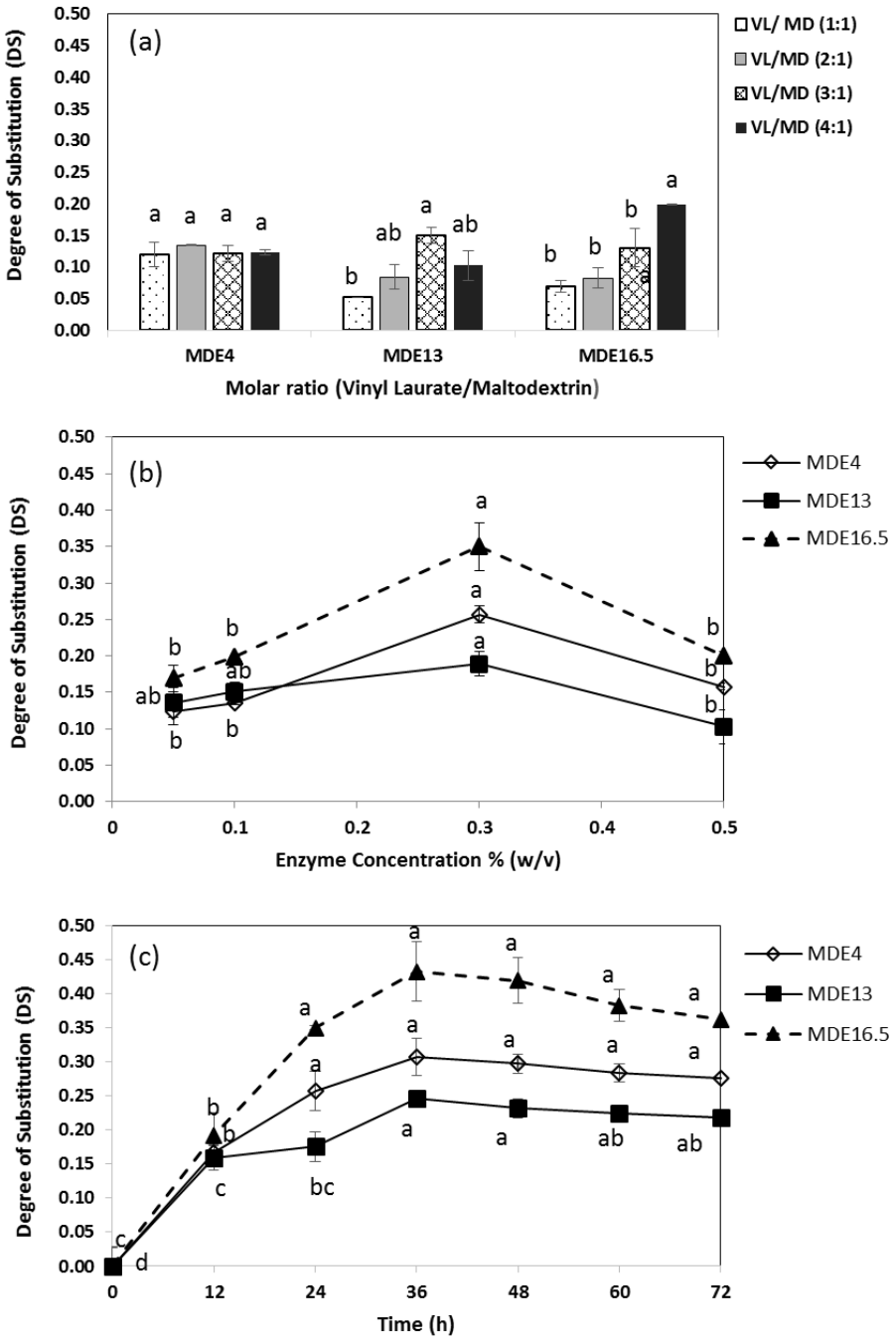


Figure 3-2: Effect of (a) molar ratio of vinyl laurate/maltodextrin (b) lipase concentration and (c) time, on the DS of maltodextrin laurate. Conditions: 60 °C, 200 rpm, with three types of maltodextrins used as substrate (MDE4, MDE13 MDE16.5). Error bars indicate standard deviation. Different letters (a, b, c, d) indicate that the DS values significantly differ ($p<0.05$) with the same maltodextrin laurate sample.

Table 3-1 shows the difference in the yield of the produced esters. The MDE4 laurate had the highest yield of 18.4 mg of maltodextrin laurate/g of initial substrate, whereas the MDE16.5 laurate had the lowest yield of 6.6 mg of maltodextrin laurate/g of initial substrate. This finding can be attributed to the solubility of the three maltodextrins in the co-solvent (DMSO and *t*-BuOH). The DMSO was added at the concentration of 10% in a binary mixture with *t*-BuOH to promote increased solubility of the polysaccharide (Degn & Zimmermann, 2001). MDE16.5 laurate was the most soluble because of the dominance of the low molecular weight components of amylose and MDE4 was the least soluble most likely due to the prevalence of high molecular weight components of amylose and amylopectin. Hence in this study, the yield was calculated from the ester obtained from the insoluble maltodextrin laurate and also the soluble maltodextrin laurate that recovered by precipitating the ester three times with ethanol. Starch esterified with fatty acid chlorides (lauroyl) produced a yield of 0.75-0.85 g/g (Namazi et al., 2011). Arijaje and Wang (2017) reported that total recovery including both soluble and insoluble starch for all unacetylated and acetylated starch-linoleic acid complexes was between 0.90 and 0.95 g/g.

Table 3-1: Yield of the maltodextrin laurates

Sample	**Yield (mg of maltodextrin laurate/g of initial substrate)**
MDE4 laurate	18.4 ± 0.11
MDE13 laurate	11.2 ± 0.01
MDE16.5 laurate	6.6 ± 0.08

3.3.2 Solubility of maltodextrin laurates in aqueous solution

Table 3-2 shows the solubility of the three maltodextrin laurate in water at three different temperatures (5, 25 and 50 °C). The solubility was directly related to the DS of the ester, as the MDE 16.5 laurate had the highest DS, indicating its lowest solubility in water, whereas the MDE13 laurate had the lowest DS, indicating its highest solubility in water ($P<0.05$). This result was consistent with that of Namazi et al. (2011), reporting that the variability in the solubility properties of hydrophobically modified starch was dependent on the DS. Unmodified maltodextrin is highly soluble in water, however, after hydrophobic modifications, its solubility decreases. Maltodextrin laurates are amphiphilic macromolecules that are mainly constituted of a hydrophilic backbone and hydrophobic side chains, rendering them insoluble in water, since some of their free hydroxyl groups are substituted. This property affects the strong hydrophobic character of the fatty ester chains, by reducing the possibility of hydrogen bond formation between hydroxyl groups among maltodextrin and water (Rajan et al., 2008; Winkler et al., 2013).

Table 3-2: Solubility of the maltodextrin laurates at different temperature

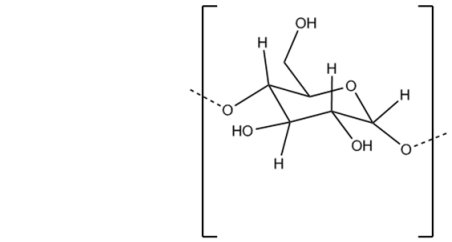
Sample	Solubility (%, *w/w*) in water		
	5 °C	25 °C	50 °C
MDE4 laurate	83.54 ± 3.91^{aB}	92.99 ± 3.25^{abA}	94.04 ± 2.14^{aA}
MDE13 laurate	90.46 ± 4.70^{aA}	96.54 ± 1.68^{aA}	96.45 ± 1.92^{aA}
MDE16.5 laurate	82.30 ± 1.00^{aA}	89.37 ± 2.27^{bA}	88.18 ± 3.97^{aA}

Different letters (a, b, A, B) represents significantly differ ($p<0.05$).
^{a,b} in the same column indicate comparison with different maltodextrin lauric acid sample of the same temperature.
$^{A, B}$ letters in the same row indicate comparison with different temperature with the same maltodextrin lauric acid sample.

3.3.3 ^{1}H NMR analysis of maltodextrin laurates

Structural characterisation of unmodified maltodextrins and maltodextrin laurate esters was performed through ^{1}H NMR analysis (Figure 3-3). The β-(1,4)-linked D-glucose units are characterised by the signals at 3.57, 3.64, 4.58, 5.10, 5.40 and 5.50 ppm which correspond to H-5, H-3, OH-6, H-1, OH-2 and OH-3, respectively (Figure 3-3 (a)). Similar proton assignments have been reported for native starch (Kapusniak & Siemion, 2007; Namazi et al., 2011).

(a)


OH
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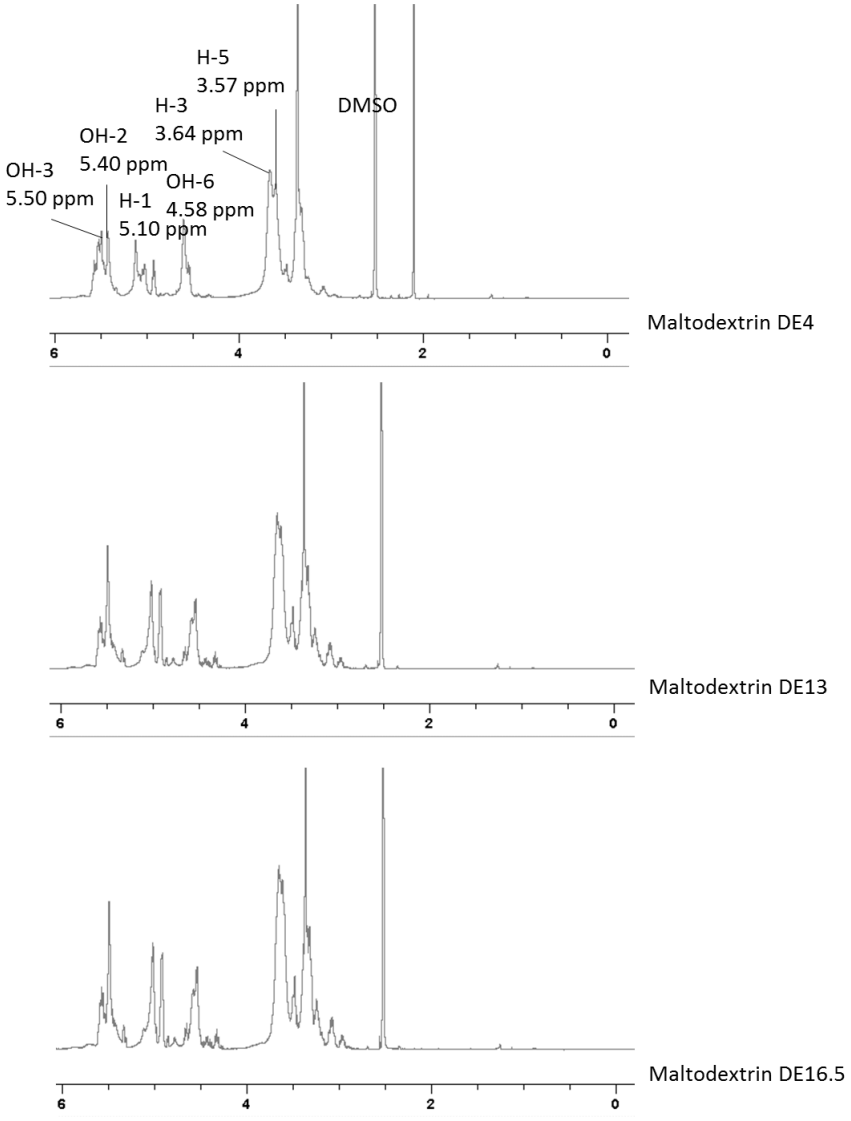
H-5
3.57 ppm
H-3
3.64 ppm
DMSO
OH-2
5.40 ppm
OH-3
5.50 ppm
H-1
5.10 ppm
OH-6
4.58 ppm
6
4
2
0
Maltodextrin DE4
Maltodextrin DE13
Maltodextrin DE16.5

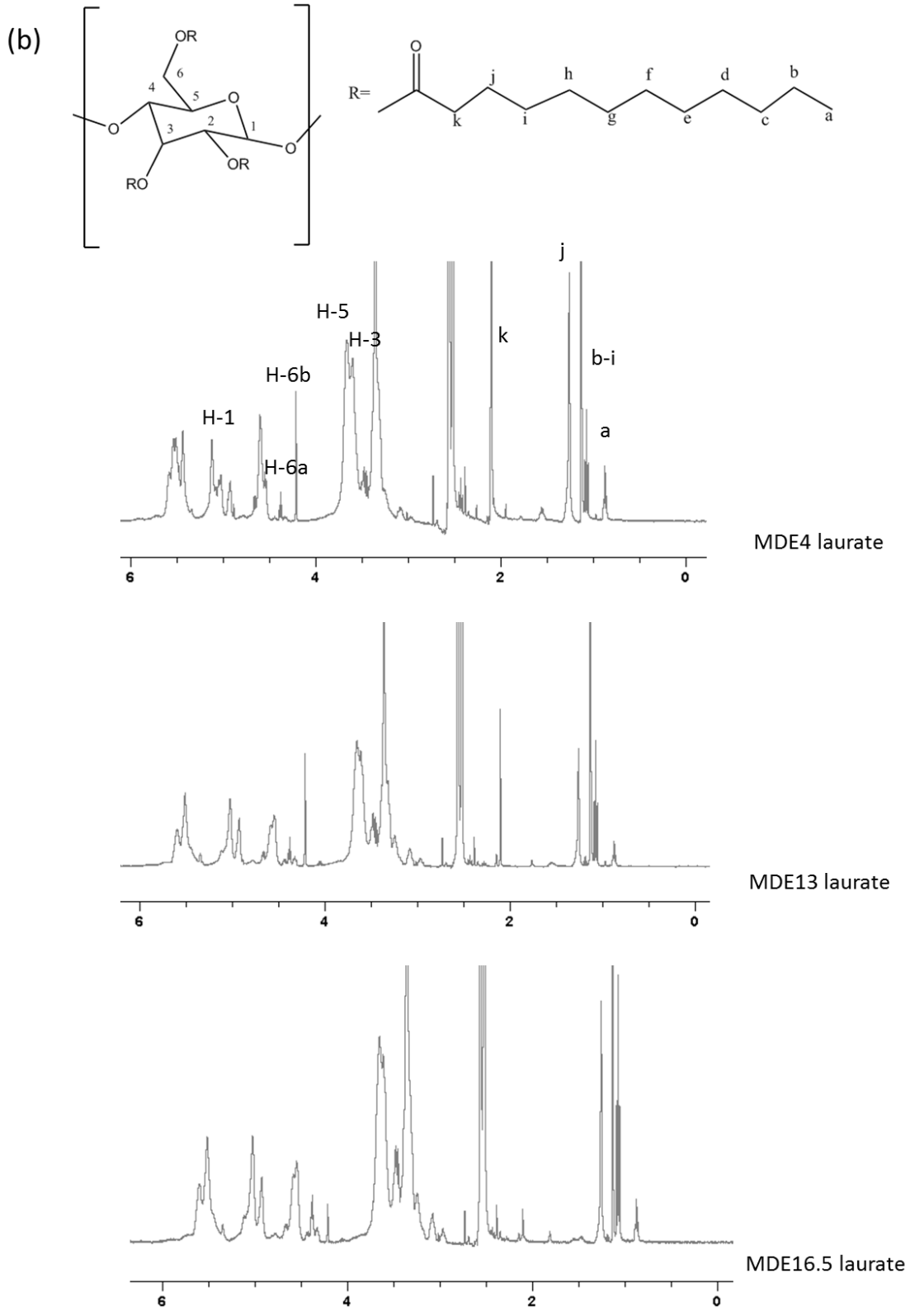

Figure 3-3: ^{1}H NMR spectra of (a) unmodified maltodextrins (b) maltodextrin laurate synthesised by immobilised lipase (Novozym® 435) at 60 °C for 36 h.

Figure 3-3 (b) shows the NMR spectra of the maltodextrin laurate without prior purification and the chemical shift for each peak is shown in Table 3-3. With the esterification process, acetyl groups were attached into the maltodextrin, and proton resonances of the anhydroglucose unit showed some changes compared to unmodified maltodextrin. The ^{1}H NMR spectra of the maltodextrin laurate showed three protons of the terminal methyl group (–CH_3–) of the acyl chain as a triplet at approximately 0.81 ppm (peak a). The methylene group (–CH_2–) (peak k), which is beside the carbonyl group as shown by the peak at 2.04 ppm and the one at 1.20 ppm (peak j), is the methylene group that is directly before it. All other methylene groups have a peak at 1.07 ppm (peaks b–i). The clear broadening of the peaks for the methylene groups that are close to the ester bond (at 2.04 and 1.20 ppm) indicates successful attachment of the lauric acid group to the maltodextrin group and hence successful esterification (Namazi et al., 2011).

Table 3-3: Chemical shifts (δ) of ^{1}H NMR of the unmodified and maltodextrin ester in DMSO-$_{d6}$

	Unmodified			Ester		
	MDE4	MDE13	MDE16.5	MDE4	MDE13	MDE16.5
Glucose						
H-1	5.00	5.01	5.01	5.00	4.97	4.97
H-2	3.46	3.46	3.46	3.46	3.42	3.41
H-3	3.65	3.63	3.63	3.65	3.6	3.6
H-4	3.34	3.3	3.35	3.3	3.3	3.3
H-5	3.58	3.6	3.6	3.58	3.55	3.55
H-6a	4.52	4.52	4.52	4.33	4.32	4.32
H-6b	4.31	4.31	4.31	4.15	4.15	4.15
Lauric acid						
CH_3 (peak a)	-	-	-	0.81	0.81	0.81
CH_2–CO (peak k)	-	-	-	2.04	2.05	2.04
CH_2–CH_2-CO (peak j)	-	-	-	1.2	1.2	1.2
12H, chain (peak b–i)	-	-	-	1.07	1.07	1.07

3.3.4 Characterisation of maltodextrin laurate by ESI-MS

Reverse phase solid phase extraction (RP–SPE) was performed to fractionate the maltodextrin laurate mixtures into various components of mono- and di-ester of lauric acid. According to van Kempen et al. (2013b), RP–SPE is an effective purification method that could generate large amounts of materials within a relatively short time and usually results in a sufficient purity for functionality experiments. A gradient of methanol and water was used, and the composition of the solvent used to eluted the esters from the column could be correlated with the hydrophobicity of the esters (van Kempen et al., 2014a). After purification, the obtained maltodextrin laurates with various degree of polymerisation (DP) values were analysed through ESI–MS. Due to the complexity of maltodextrin laurate mixture obtained, the lack of standards for these molecules and the fact that ESI-MS can provide measurements on accurate mass, this technique was chosen to characterise the substitution of lauric acid following the method of Casas-Godoy et al. (2016).

In ESI-MS, in positive ion mode, the sugar molecules usually form singly-charged ions by adding Na^+. Thus, the theoretical average *m/z* value of sugars with a degree of polymerization (DP) value of *n* in ESI-MS could be calculated as follows: $[C_{6n}H_{10n}+O_{5n+1} + Na]^+$, where n≥2. Thus, the theoretical *m/z* value of unmodified maltodextrin (DP = 2) was 365.1059 in ESI-MS as shown in Supplementary Table 3-6. The ESI–MS spectra of the unmodified MDE4 eluted with 90:10 (water/methanol) and MDE4 laurate eluted with 20:80 (water/methanol) were chosen because they show the comparison of elution with the dominant substitution of the lauric acid peak between two different polarities of the solvent. Figure 3-4 (a) shows that DP2 consists of disaccharides of the unmodified maltodextrin, the representative adduct peak $[M+Na^+]$ at *m*/*z* of 365.1067 (theoretical *m/z* 365.1059). After esterification, a major adduct peak at *m*/*z* of 547.2726 was observed corresponding to the molecular formula of DP=2 $[C_{12}H_{20}+O_{11} + Na]^+$ and one lauric acid, indicating the synthesis of $C_{24}H_{44}O_{13}Na^+$ (calculated *m*/*z* 547.2730) (Figure 3-4 (b)). Both peaks had a molecular mass displacement of approximately *m*/*z* 182.1671. Considering a molecular mass of *m*/*z* of 226.1933 for lauric acid and the fact that it loses the vinyl group ($-C_2H_3O$) and a hydrogen (H) in esterification reactions (final *m*/*z* 182.1671), the peaks correspond to the mono ester of maltodextrin laurate, respectively. The criterion for matching an observed ion with a theoretical must be that it is within 5 ppm. The mass error measured the difference between an individual measurement and the true value was accurately < 5 ppm.

One important factor that has to be considered for the esterification of polysaccharides is their DP distribution. Figure 3-4 (a) shows the profile of unmodified MDE4 with adducts peaks $[M+Na^+]$, ranging proximately from 365.1067 *m*/*z* to 2471.8033 *m*/*z*, and corresponding to a DP distribution from 2 to 15. However, some peaks (DP14-15) were not labelled due to low signal of peak detection. In Figure 3-4 (b), the possible substitution of monoesters of lauric acid (1LA) can be observed, ranging from 385.2200 *m/z*

to 2329.8556 *m/z* corresponding to a DP distribution from 1 to 13 (DP12-13 not labelled). Additionally, the substitution of diesters of lauric acid (2LA) can be observed from 729.4399 m/z to 1539.7094 m/z, corresponding to a DP distribution from 2 to 7. All the DP distributions with monoester (1LA) and diester (2LA) of lauric acid at different elution of water: methanol are shown in Supplementary Table 3-7. This result is in good agreement with van Kempen et al. (2013b), who reported that unmodified oligofructose was eluted from the column during the first two steps (100% water and 10% methanol), monoesters of lauric acid were eluted with 70% and 80% methanol and di- and tri-esters of lauric acid were eluted with 90% methanol. The difference in hydrophobicity between the different fractions is reflected in the polarity of the eluting solvent (van Kempen et al., 2013b).

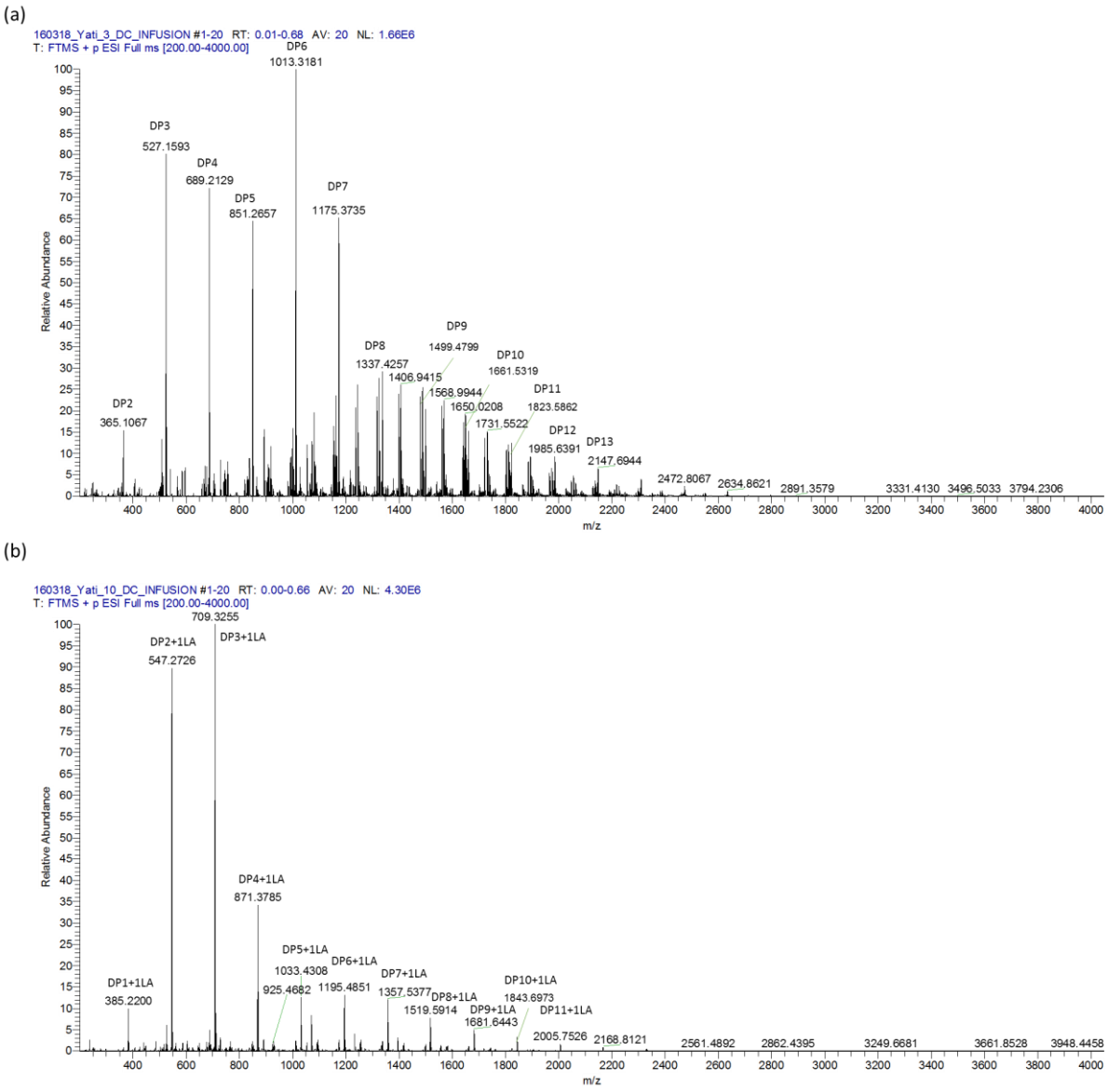

Figure 3-4: ESI –MS mass spectrum in the positive ion mode of (a) MDE4 eluted with 90:10 water/methanol elution (b) ESI–MS mass spectrum of MDE4 laurate after purification with 20:80 water/methanol elution. Sodium (Δm/z=23) adducts appear. MDE4 is indicated in the figure with DP2, DP3, DP4, DP5, DP6, DP7, DP8, DP9, DP10, DP11, DP12 and DP13. Mono-esters of MDE4 and lauric acid (Δm/z=182.1671, compared with unmodified maltodextrin) are indicated by the addition +1LA and +2LA.

The transesterification reaction of vinyl laurate with other oligosaccharides has been carried out successfully with *A. tequilana* fructan with a DP distribution from 2 to 8 and the

substitution of mono- and di-ester of lauric acid was reported from DP3 to 8 (Casas-Godoy et al., 2016). van Kempen et al. (2013b) observed that the esterification of oligofructose (inulin) with caprylic acid produced unreacted oligofructose with DP distribution from 2 to 8 and substitution of one caprylic acid from DP3 to 6. The mono-esters were the predominant reaction products due to the regio-selectivity of the immobilised *C. antarctica* lipase B for the primary hydroxyl group at the C-6 position of a sugar (Woudenberg-van Oosterom et al., 1996). In addition, di-ester of lauric acid was also observed at the C-1 hydroxyls of the terminal glucose residue. Fatty acids are mainly attached at the C-6 position and the terminal reducing end C-1, showing the preference of the enzyme for the primary hydroxyl group. Apart from the C-1 hydroxyl group of the terminal reducing end residue, all other C-1 hydroxyls are involved in the glycosidic links, thus are not available for esterification. ter Haar et al. (2010) demonstrated that immobilised *C. antarctica* lipase B only catalyses mono-ester synthesis of fructooligosaccharide-lauryl ester with DP 3–8 at both 20% and 40% (v/v) of DMSO in *t*-BuOH. Figure 3-5 (a) shows the profile of unmodified MDE13 with adducts peaks [$M+Na^+$], ranging approximately from 365.1071 *m/z* to 2633.8453 *m/z* and corresponding to a DP distribution from 2 to 16. The substitution of monoesters of lauric acid (1LA), ranging from 385.2204 *m/z* to 1843.6958 *m/z* corresponded to a DP distribution from 1 to 10 as shown in Figure 3-5 (b) (monoester of DP9-10 not labelled due to low signal of the peak). The substitution of diesters of lauric acid (2LA) was observed from 567.3864 m/z to 1701.7589 m/z, corresponding to a DP distribution from 1 to 8 (Supplementary Table 3-8).

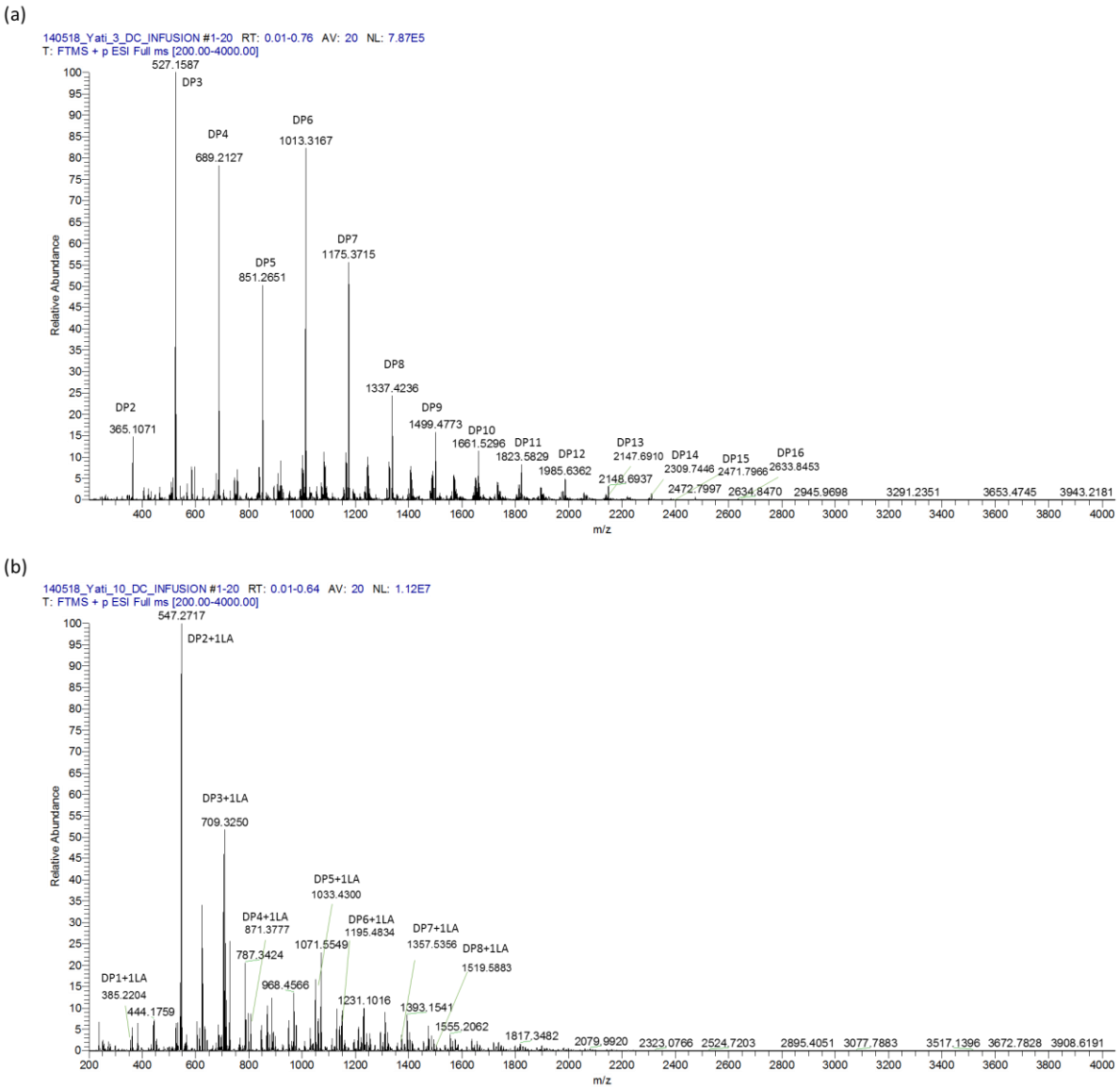

Figure 3-5: (a) ESI–MS mass spectrum in the positive ion mode of MDE13 eluted with 90:10 water/methanol elution; (b) ESI–MS mass spectrum of MDE13 laurate after purification with 20:80 water/methanol elution. Sodium (Δm/z=23) adducts appear. MDE13 is indicated in the figure with DP2, DP3, DP4, DP5, DP6, DP7, DP8, DP9, DP10, DP11, DP12, DP13, DP14, DP15 and DP16. Monoesters of MDE13 and lauric acid (Δm/z=182.1671, compared with unmodified maltodextrin) are indicated by the addition +1LA and +2LA

In addition, for MDE16.5, the ESI–MS detection ranging from 365.1065 m/z to 2795.9113 m/z corresponded to a DP distribution from the 2 to 17 of unmodified maltodextrin as shown in Figure 3-6 (a). However, some peaks (DP 2 and 17) were not labelled due to low signal of peak detection. The substitution of monoesters of lauric acid (1LA), ranging from 385.2198 *m/z* to 2491.9088 *m/z* corresponded to a DP distribution from 1 to 14 as shown Figure 3-6 (b) (monoester of DP12-14 not labelled due to low signal of peak). The substitution of di-esters of lauric acid (2LA) was observed from 567.3865 *m/z* to 1053.5445 *m/z*, corresponding to a DP distribution from 1 to 4 as shown in Supplementary Table 3-9. All the maltodextrin laurate samples showed that DP 2-11 were better substrates for the enzyme for the substitution of one lauric acid, whereas a lower DP distribution of 2 to 4 observed for diester of lauric acid. This result is consistent with other findings in which DP3-8, (oligomers with low DP), are better substrates for the lipase enzyme (Novozym® 435) than oligomers with a high DP; the di-esters of lauric acid were only formed for oligomers with DP3-DP4 (ter Haar et al., 2010; van Kempen et al., 2013b). These effects could be related to the anatomy of the catalytic cleft of the lipase, i.e. when larger molecules attempt to access the catalytic cleft, they may be hindered due to steric effects (Cramer et al., 2007; Pedersen et al., 2002b).

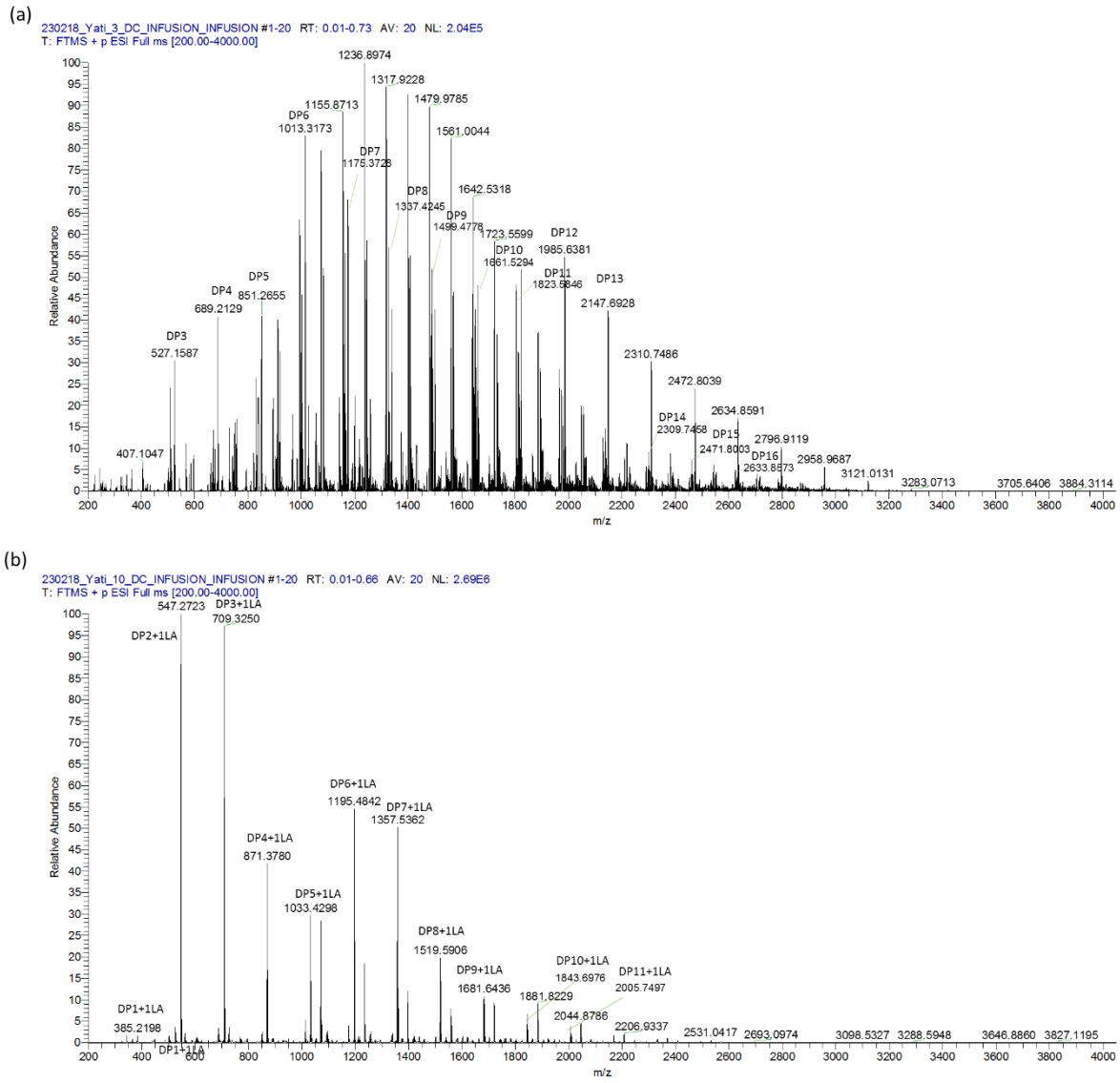

Figure 3-6: (a) ESI–MS mass spectrum in the positive ion mode of MDE16.5 eluted with 90:10 water/methanol elution; (b) ESI–MS mass spectrum of MDE16.5 laurate after purification with 20:80 water/methanol elution. Sodium (Δm/z=23) adducts appear. MDE16.5 is indicated in the figure with DP3, DP4, DP5, DP6, DP7, DP8, DP9, DP10, DP11, DP12, DP13, DP14, DP15, and DP16. Monoesters of MDE16.5 and lauric acid (Δm/z=182.1671, compared with unmodified maltodextrin) are indicated by the addition +1LA and +2LA

3.3.5 FT-IR of unmodified maltodextrin and maltodextrin laurate

The FT-IR spectra of the unmodified maltodextrins and maltodextrin laurate are shown in Figure 3-7. In the spectrum of the unmodified maltodextrin, a strong set of peaks between 992–1148 cm^{-1} costing of three peaks is the most characteristic band for a polysaccharide, and is attributed to the C–O bond stretching (Kapusniak & Siemion, 2007) and overlaps with the stretching vibrations of C–OH the side groups (Fan et al., 2014). Another characteristic peak was observed between 3000–3500 cm^{-1}, which corresponds to stretching vibrations of the hydroxyl group (Fan et al., 2014). A broad peak due to the hydrogen-bonded hydroxyl groups (O–H) appeared at 3301 cm^{-1}, which was attributed to the complex vibrational stretches associated with free, inter- and intra-molecular bound hydroxyl groups that together make up the gross structure of starches (Fang et al., 2002). The peaks at 1112 and 1049 cm^{-1} are characteristic of the anhydroglucose ring O–C stretch.

Ester compounds were confirmed by the presence of the C=O bond in the molecule. The wave number for the ester (C=O) typically ranges from 1716–1751 cm^{-1} (van den Broek & Boeriu, 2013). The reaction products exhibited a characteristic ester band (1728–1730 cm^{-1}) in the FTIR spectra. The existence of two peaks of strong intensities upon esterification at 2914–2918 and 2848–2851 cm^{-1} is attributed to the C–H stretching of the CH_2 groups of the alkyl chain at 2914–2918 cm^{-1} (symmetric) and 2848–2851 cm^{-1} (asymmetric), respectively (Sagis et al., 2008). Characteristic bands between 3000 and 3500 cm^{-1} were assigned to the hydroxyl group stretching vibrations (Kapusniak & Siemion, 2007). The maximum of that peak moved from 3290-3305 cm^{-1} for unmodified maltodextrin, to over 3329-3317 cm^{-1} for the MDE esters. This change can be attributed to the decrease in the concentration of hydrogen-bonded hydroxyl groups during their conversion into ester groups (Fang et al., 2002; Kapusniak & Siemion, 2007). These results are in agreement with Kapusniak and Siemion (2007), who reported that the concentration of hydrogen-bonded hydroxyl groups

of native starch moved from the original 3299 cm^{-1} for native starch and 3383 cm^{-1} for heated plain starch to over 3400 cm^{-1} for the ester of starch.

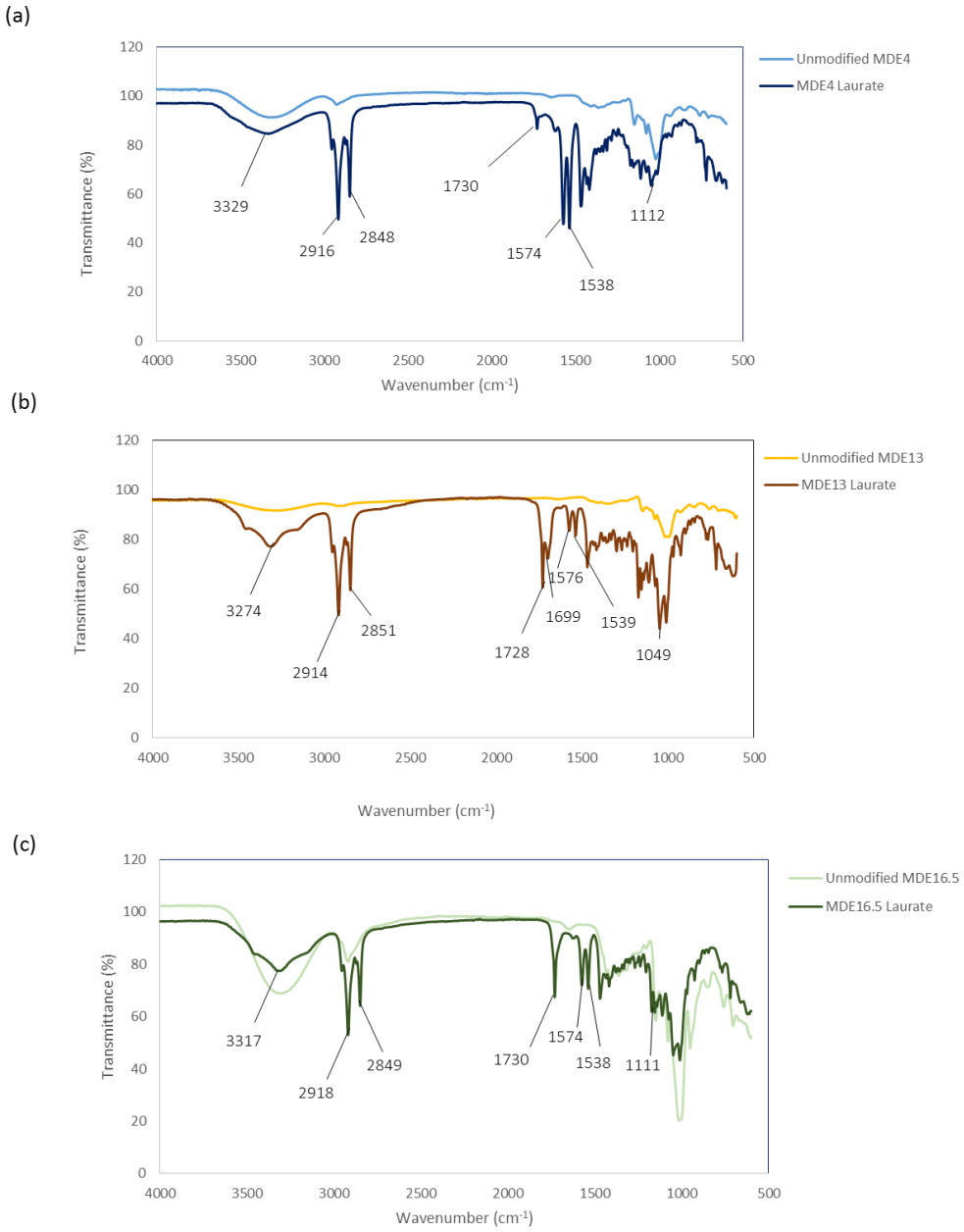

Figure 3-7: FT-IR spectra of the unmodified and maltodextrin laurate for (a) MDE4 (b) MDE13 and (c) MDE16.5 products with transesterification with vinyl laurate by lipase (Novozym® 435) at 60 °C for 36 h.

3.3.6 Determination of surface activity (air-water surface tension) of unmodified maltodextrin and maltodextrin laurate

Surface tension is an important parameter that determines the effectiveness of surfactants and measures the force of attraction between the molecules of liquids, which is considerably reduced when the surfactant concentration in an aqueous medium is increased and micelles are formed (Campos et al., 2013). The surface tension (γ) of unmodified maltodextrins and maltodextrin laurate was measured at different concentrations at 25 °C. As a comparison, a small molecule surfactant, Tween 20 was used. Tween 20 belongs to the group of sugar-based surfactants (polysorbate non-ionic surfactant with a hydrophobic dodecanoic tail) that are widely used as an emulsifier, by reducing the air-water surface tension and oil-water interfacial tension. Table 3-4 shows that the γ value of all unmodified maltodextrins at 10% (*w*/*v*) was lower compared to water (72 mN/m to 66.8–69 mN/m), which is probably caused by the slow movement to the water–air and water-oil interfaces (Garti et al., 1997).

The surface tension of maltodextrin laurate was also tested at different concentrations and it was found that it was more efficient in lowering the surface tension than unmodified maltodextrin. Unmodified MDE16.5 showed the highest surface tension 69.0 mN/m at 10% (*w/v*), and after esterification, the ester product exhibited the lowest surface tension amongst other MDE esters (23.4 mN/m) at 40% (*w/v*). In addition, all maltodextrin laurates showed a reduction of the surface tension, γ value to 23.4-27.8 mN/m when the concentration increased up to 40% (*w/v*), probably due to the saturation of hydrophobic macromolecules at the air-water surface or interface (Garti et al., 1997).

In addition, the surface tension of the maltodextrin laurate at different concentrations except for 10% of MDE4 laurate was lower than the Tween 20. According to Garti et al. (1997), at low concentrations (i.e. 10%-20%, *w*/*w*), the reduced surface tension was probably

due to the adsorption of short-chain saccharides, which can migrate better to the surface. The slight reduction from concentration of 20% to 40%, *w/v* probably corresponds to the behaviour of macromolecules at the surface. Also, the measurement was not reproducible when concentration increased because of the build-up of high viscosity in the solution. This study found that the esterification of maltodextrin leads to a decrease in the surface tension of aqueous media (e.g. air-water). A good surfactant can lower the surface tension of water from 72 to 30 mNm^{-1} (Mulligan, 2005). Therefore, these results indicate the potential application of maltodextrin laurate in the preparation of oil-in-water (O/W) emulsion.

Table 3-4: Surface tension of unmodified maltodextrin and maltodextrin laurate at a concentration of 10%, 20% and 40% (*w/v*).

Sample	Surface tension (mN/m)		
	10% (w/v)	20% (w/v)	40% (w/v)
Unmodified			
MDE4	66.8±2.3[a]	65.3±3.0[a]	60.9±1.3[a]
MDE13	68.5±0.4[a]	63.7±1.6[a]	58.9±3.7[a]
MDE16.5	69.0±1.6[a]	65.2±1.7[ab]	60.7±1.2[b]
Tween 20	37.6±0.7[a]	34.1±0.1[b]	31.0±0.0[c]
Ester			
MDE4 laurate	48.5±1.4[a]	28.9±4.1[b]	27.8±3.8[b]
MDE13 laurate	26.8 ±1.2[a]	25.9±0.0[a]	24.5± 0.7[a]
MDE16.5 laurate	27.1 ±0.5[a]	24.9±1.2[a]	23.4±1.6[a]

Data are reported as the mean from the three replicates for each sample. [a–d] Different letters within the same row are significantly different ($p<0.05$).

3.3.7 Rheological Properties of unmodified and maltodextrin laurate

The rheological properties of the unmodified maltodextrin and maltodextrin laurate were studied under three different concentrations (10%, 20% and 40% *w/v*) in aqueous solution. Figure 3-8 shows that the viscosity of the unmodified maltodextrin was higher than that of maltodextrin laurates. This was due to the fact that in maltodextrin laurates, the

formation of the ester bond hinders starch gelatinisation, thereby reducing the formation of viscous gel (Rajan et al., 2008). This finding is in agreement with Rajan et al. (2006), where modified starch showed a drastic loss in viscosity compared to raw starch. This phenomenon might be due to the hydrophobic nature attained by starch due to modification.

In a Newtonian flow behaviour, the viscosity remains constant when the shear rate increases at a constant temperature (25 °C) (i.e., the viscosity was independent of shear rate and time). MDE13 and MDE16.5 laurates showed the Newtonian flow behaviour at all concentrations (10-40% (*w/v*)). However, the MDE4 laurate showed a Newtonian flow behaviour at 10 and 20% (*w/v*), however, a shear thinning behaviour for MDE4 laurate was induced at higher concentration (40%, *w*/*v*), with the higher viscosity. This finding agrees with that of Udomrati and Gohtani (2015), where the viscosity of longer-chain molecules of MDE4 laurate is induced with increased concentration. As the concentration increases, the entanglement of longer-chain polymers produces highly ordered stiff entangled molecules, which result in high viscosity at low shear rates. Thus, with increasing the applied shear, the long-chain molecular aggregates seem to be progressively disrupted, resulting in non-Newtonian, shear thinning flow (Nor Hayati et al., 2009). At a concentration of 40% (*w/v*), MDE4 laurate shows high viscosity at a low shear rate, suggesting its suitability as a thickener (Qiao et al., 2006). However, MDE13 and MDE16.5 laurate have low viscosity at any concentration; hence no viscous gel formation. This suggest that with the combination of surface activity, this amphiphilic maltodextrin can be used as an emulsifier and polymeric surfactant. It also may be used as an ingredient in applications where viscosity and hydrophobic interactions are desired, for example in food products (e.g. mayonnaise, margarine, sauce) and cosmetics (e.g. toothpaste, shampoo, lotions).

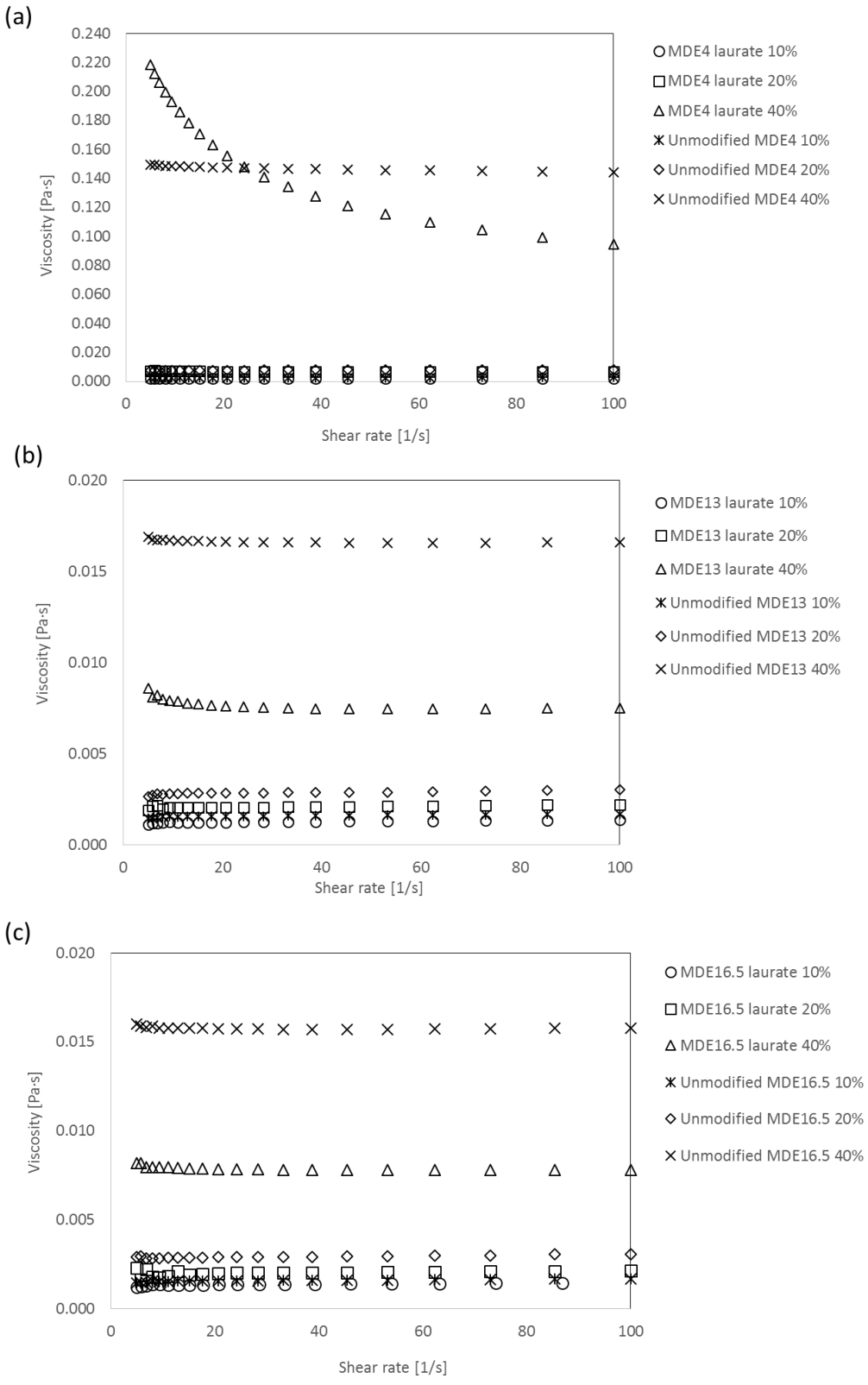

Figure 3-8: Rheological characteristics of the unmodified maltodextrin and (a) maltodextrin laurate MDE4, (b) MDE13 (c) MDE16.5, at concentration of 10%, 20% and 40% (*w/v*).

Maltodextrin flow curves were fitted to the *Herschel–Bulkley* model, which is commonly used to describe the flow properties of the emulsified system according to Liu et al. (2007). The yield stress, *τ0* (Pa), consistency coefficient, *k* (Pa s^n) and flow index, *n* values of the samples at concentrations of 10%, 20% and 40% (*w/v*) are listed in Table 3-5. A polymer possesses a liquid-like behaviour when *n* is close to 1, and as shown in Table 3-4, *n* is almost 1 at 10% unmodified maltodextrin and all three maltodextrin laurates, which indicates a Newtonian flow behaviour. However, at a concentration of 20% (*w/v*) of MDE4 laurate and 40% (*w/v*) of unmodified and MDE4 laurate, *n* is significantly less than 1 ($p<0.05$), which corresponds to shear thinning behaviour. These results are consistent with a previous report that unmodified maltodextrin solution exhibits Newtonian flow at low concentration and that an increase in the maltodextrin concentration resulted in a decrease in the *n* emulsion until the maltodextrin concentration reached 20% (*w/w*) for DE9, DE12 and 25% (*w/w*) for DE16 (Udomrati et al., 2013).

Many complex fluids, such as network forming polymers, surfactants and emulsions do not flow until the applied stress exceeds a certain critical value, known as the yield stress. The yield stress, τ0 is defined as the stress that must be applied to the sample before it starts to flow. The τ0 value of MDE13 and MDE16.5 laurate at a concentration of 10%, 20% and 40% (*w/v*) did not change because of no difference of the strength of the attractive force in the systems (Udomrati & Gohtani, 2014). However, the τ0 was higher ($p<0.05$) for MDE4 laurate at 40% because of the increase in the viscosity of the aqueous phase, indicating a non-Newtonian, shear thinning phase (Nor Hayati et al., 2009). In terms of the consistency coefficient, *k*, for MDE13 and MDE16.5, the unmodified maltodextrin showed higher consistency ($p<0.05$) than maltodextrin laurate; results showed that MDE13 and MDE16.5 laurate were less viscous than the unmodified maltodextrin. However, MDE4 laurate at concentration 40% (*w/v*) showed the highest consistency compared to unmodified MDE4 at

the same concentration. Thus, *k* is an indicator of the viscosity of an emulsion system (Nor Hayati et al., 2009).

Table 3-5: Modelling of the flow curve between 10 and 100 s^{-1} of shear stress of the unmodified and maltodextrin esters by using *Herschel–Bukley* model: $\tau = \tau 0 + k \cdot \gamma^n$

Sample	***Herschel-Bulkley* factors**								
	10% (*w/v*)			**20% (*w/v*)**			**40% (*w/v*)**		
	n	***k* (Pa s^n)**	**τ0 (Pa)**	***n***	***k* (Pa s^n)**	**τ0 (Pa)**	***n***	***k* (Pa s^n)**	**τ0 (Pa)**
Unmodified MDE4	1.057± 0.007^{a}	0.0020± 0.0001^{a}	0.0017± 0.0004^{a}	1.022± 0.008^{b}	0.0072± 0.0005^{a}	0.0023± 0.0011^{a}	0.985± 0.008^{b}	0.1553± 0.0159^{b}	0.0147± 0.0071^{b}
Unmodified MDE13	1.067± 0.012^{a}	0.0012± 0.0001^{c}	0.0008± 0.0006^{a}	1.056± 0.005^{ab}	0.0023± 0.0001^{b}	0.0016± 0.0003^{a}	1.004± 0.004^{ab}	0.0162± 0.0004^{c}	0.0027± 0.0003^{b}
Unmodified MDE16.5	1.063± 0.005^{a}	0.0013± 0.0000^{c}	0.0006± 0.0006^{a}	1.060± 0.005^{a}	0.0023± 0.0001^{b}	0.0022± 0.0007^{a}	1.007± 0.004^{ab}	0.0152± 0.0003^{c}	0.0033± 0.0012^{b}
MDE4 laurate	1.059± 0.016^{a}	0.0016± 0.0003^{b}	0.0015± 0.0011^{a}	0.987± 0.031^{c}	0.0071± 0.0022^{a}	0.0026± 0.0025^{a}	0.656± 0.008^{c}	0.4709± 0.1423^{a}	0.2625± 0.2634^{a}
MDE13 laurate	1.082± 0.016^{a}	0.0009± 0.0001^{d}	0.0009± 0.0006^{a}	1.069± 0.012^{a}	0.0016± 0.0002^{b}	0.0017± 0.0003^{a}	1.017± 0.003^{a}	0.0063± 0.0008^{c}	0.0035± 0.0031^{b}
MDE16.5 laurate	1.077± 0.009^{a}	0.0010± 0.0001^{cd}	0.0009± 0.0005^{a}	1.055± 0.015^{ab}	0.0017± 0.0003^{b}	0.0008± 0.0007^{a}	1.004± 0.022^{ab}	0.0077± 0.0011^{c}	0.0022± 0.0036^{b}

Data are reported as the mean from two independent replications (n = 2) for each sample. $^{a–d}$ Letters that differ within the same column are significantly different ($p<0.05$). τ, shear stress; $\cdot\gamma$, shear rate; τ0, yield stress; *k*, consistency coefficient; *n*, flow index.

3.4 Conclusions

The synthesis of three different DEs of maltodextrin laurate through lipase-catalysed transesterification reactions were successfully optimised in a DMSO and *t*-BuOH mixture solvent system. The DE of the maltodextrin was found to affect the DS of the produced maltodextrin laurate. The factor governing the end-product was the DE value of the maltodextrin laurate and the concentration of the maltodextrin laurate. At different concentrations, the maltodextrin laurate was found to reduce the surface tension compared to Tween 20. Unmodified and maltodextrin laurate showed shear thinning behaviour when their concentrations increased. These data suggested that different DE of maltodextrin laurate could be used to create emulsions with different physicochemical properties for various industrial applications. The produced maltodextrin laurates can potentially impart emulsifying properties with important technical characteristics including creamy mouthfeel, high yield stress for suspension ability and to a certain extent, long-term stability.

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Supplementary materials

Supplementary Table 3-6: Theoretical *m/z* of unmodified maltodextrin and maltodextrin laurate. MW stands for molecular weight. DP for the degree of polymerisation. MDE for maltodextrin. Na^+ for sodium (adduct ion used) and L for lauric acid.

DP	**MW Maltodextrin (*m/z*)**		**MW Maltodextrin ester (*m/z*)**	
	MDE	**MDE + Na^+**	**MDE-L + Na^+**	**MDE-L-L + Na^+**
1	180.0634	203.0531	385.2202	567.38725
2	342.1162	365.1059	547.2730	729.44005
3	504.1690	527.1587	709.3258	891.49285
4	666.2218	689.2115	871.3786	1053.54565
5	828.2746	851.2643	1033.4314	1215.59845
6	990.3274	1013.3171	1195.4842	1377.65125
7	1152.3802	1175.3699	1357.5370	1539.70405
8	1314.4330	1337.4227	1519.5898	1701.75685
9	1476.4858	1499.4755	1681.6426	1863.80965
10	1638.5386	1661.5283	1843.6954	2025.86245
11	1800.5914	1823.5811	2005.7482	2187.91525
12	1962.6442	1985.6339	2167.8010	2349.96805
13	2124.6970	2147.6867	2329.8538	2512.02085
14	2286.7498	2309.7395	2491.9066	2674.07365
15	2448.8026	2471.7923	2653.9594	2836.12645
16	2610.8554	2633.8451	2816.0122	2998.17925
17	2772.9082	2795.8979	2978.0650	3160.23205
18	2934.9610	2957.9507	3140.1178	3322.28485
19	3097.0138	3120.0035	3302.1706	3484.33765
20	3259.0666	3282.0563	3464.2234	3646.39045

Supplementary Table 3-7: ESI –MS mass spectrum in the positive ion mode of MDE4 laurate at different gradient of water: methanol.

Degree of Polymerisation (DP)	MW of MDE4 laurate (*m/z*)										
	Elution (Water: methanol) Gradient										
	100:0	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**	**40:30**	**30:70**	**20:80**	**10:90**	**0:100**
DP1 + 1LA						385.2197	385.2202	385.2200	385.2200	385.2205	385.2206
DP1 + 2LA										567.3873	
DP2 + 1LA						547.2724	547.2727	547.2726	547.2726	547.2730	547.2733
DP2 + 2LA								729.4401	729.4399	729.4402	
DP3 + 1LA							709.3263	709.3258	709.3255	709.3261	709.3272
DP3 + 2LA								891.4937	891.4931	891.4938	
DP4 + 1LA							871.3793	871.3792	871.3785	871.3798	871.3805
DP4 + 2LA								1053.5473	1053.5457	1053.5464	
DP5 + 1LA							1033.4309	1033.4313	1033.4308	1033.4320	
DP5 + 2LA									1215.6009	1215.6008	
DP6 + 1LA							1195.4865	1195.4855	1195.4851	1195.4870	
DP6 + 2LA									1377.6523	1377.6553	
DP7 + 1LA							1357.5391	1357.5379	1357.5377	1357.5394	
DP7 + 2LA									1539.7094	1539.7094	
DP8+1LA							1519.5896		1519.5914	1519.5933	
DP9+1LA							1681.6401		1681.6443	1681.6460	
DP10+1LA									1843.6973	1843.7011	
DP11+1LA									2005.7536		

DP12+ 1LA									2167.8043		
DP13+ 1LA									2329.8556		

MW stands for molecular weight, DP stands for the degree of polymerisation, 1LA for monosubstitution of lauric acid, 2LA for disubstitution of lauric acid

Supplementary Table 3-8: ESI –MS mass spectrum in the positive ion mode of MDE13 laurate at different gradient of water: methanol.

Degree of Polymerisation (DP)	MW of MDE13 laurate (*m/z*)										
	Elution (Water: methanol) Gradient										
	100:0	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**	**40:60**	**30:70**	**20:80**	**10:90**	**0:100**
DP1 + 1LA						385.2199	385.2206	385.2207	385.2204	385.2199	385.2207
DP1 + 2LA							567.3878	567.3875	567.3864	567.3853	
DP2 + 1LA						547.2714	547.2723	547.2723	547.2717	547.2711	547.2721
DP2 + 2LA							729.4406	729.4409	729.4390	729.4372	
DP3 + 1LA						709.3247	709.3263	709.3262	709.3250	709.3239	709.3255
DP3 + 2LA							891.4935	891.4947	891.4921	891.4904	
DP4 + 1LA						871.3770	871.3792	871.3792	871.3777	871.3763	871.3785
DP4 + 2LA							1053.5472	1053.5462	1053.5436	1053.5422	
DP5 + 1LA							1033.4312	1033.4309	1033.4300	1033.4281	1033.4308
DP6 + 2LA								1215.5983	1215.5957	1215.5957	
DP6 + 1LA							1195.4851	1195.4851	1195.4834	1195.4813	1195.4843
DP7 + 2LA									1377.6488	1377.6481	
DP7 + 1LA							1357.5365	1357.5370	1357.5356	1357.5337	1357.5368
DP7 + 2LA									1539.7007	1539.7016	
DP8 + 1LA							1519.5918		1519.5883	1519.5882	
DP8 + 2LA									1701.7589		
DP9 + 1LA									1681.6376	1681.6437	
DP10 + 1LA									1843.6958	1843.6930	
DP11 + 1LA										2005.7570	

MW stands for molecular weight, DP stands for the degree of polymerisation, 1LA for monosubstitution of lauric acid, 2LA for disubstitution of lauric acid

Supplementary Table 3-9: ESI –MS mass spectrum in the positive ion mode of MDE16.5 laurate at different gradient of water: methanol.

Degree of Polymerisation (DP)	MW of MDE16.5 laurate (*m/z*)										
	Elution (Water: methanol) Gradient										
	100:0	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**	**40:60**	**30:70**	**20:80**	**10:90**	**0:100**
DP1 + 1LA						385.2203	385.2199	385.2198	385.2198	385.2196	385.2213
DP1 + 2LA									567.3865	567.3857	
DP2 + 1LA						547.2733	547.2729	547.2725	547.2723	547.2713	547.2745
DP2 + 2LA							729.4408	729.4396	729.4396	729.4383	
DP3 + 1LA						709.3261	709.3261	709.3251	709.3250	709.3244	709.3279
DP3 + 2LA							891.4931	891.4943	891.4940	891.4916	
DP4 + 1LA						871.3797	871.3786	871.3780	871.3780	871.3771	871.3811
DP4 + 2LA								1053.5453	1053.5445	1053.5427	
DP5 + 1LA						1033.4328	1033.4306	1033.4301	1033.4298	1033.4285	1033.4338
DP5 + 2LA										1215.5967	
DP6 + 1LA						1195.4859	1195.4850	1195.4846	1195.4842	1195.4830	1195.4885
DP6 + 2LA										1377.6499	
DP7 + 1LA						1357.5382	1357.5367	1357.5361	1357.5362	1357.5349	1357.5417
DP8 + 1LA						1519.5928	1519.5908	1519.593	1519.5906	1519.5916	1519.5956
DP9 + 1LA							1681.6439		1681.6436	1681.6448	1681.6477
DP10 + 1LA							1843.7002		1843.6976	1843.7032	
DP11 + 1LA							2005.7470		2005.7497		
DP12 + 1LA									2167.8022		
DP13 + 1LA									2329.8571		
DP14 + 1LA									2491.9088		

MW stands for molecular weight, DP stands for the degree of polymerisation, 1LA for monosubstitution of lauric acid, 2LA for disubstitution of lauric acid

Chapter 4 -Effect of maltodextrin laurate on the interfacial shear rheology at the air/water interface and its application in oil-in-water (O/W) emulsions

Abstract

The ability of enzymatically produced maltodextrin laurates to act as emulsifiers and stabilisers was investigated. More specifically, the stabilising effects of emulsion droplets and foam bubbles of the three esters at the air/water interface was investigated by interfacial shear rheology, whereas their effect on the formation and stability of oil-in-water emulsions was studied by flow behaviour, droplet size distribution, microstructure and creaming stability. The results demonstrated that all three esters formed a close-packed layer at the air/water interface and exhibited higher elastic moduli than viscous moduli during shear time sweep. Emulsion formation and stability of oil in water was evaluated at 40% (*w/v*) of maltodextrin laurate with 10% (*w/v*) of rapeseed oil and compared with Tween 20 (polyoxyethylene sorbitan monolaurate). MDE 4 laurate emulsions showed the highest viscosity at low shear rates. MDE4 laurate and Tween 20 emulsions exhibited sheer thinning behaviour while MDE13 and MDE16.5 laurate exhibited Newtonian behaviour. All freshly prepared emulsions with MDE13 and MDE16.5 laurate exhibited broad and mono-modal distribution while MDE4 laurate exhibited broad and bi-modal droplet-size distributions ranging between 0.92-0.65 μm, larger than Tween 20 (0.36 μm). MDE4 laurate inhibited creaming more efficiently than Tween 20, most likely due to the immobilisation of the oil droplets in a weak gel-like network. These results indicated that enzymatically synthesised maltodextrin laurate with low DE (MDE 4) effectively stabilised O/W emulsions. Such esters could potentially have applications in food emulsions such as salad dressing and mayonnaise, as well as cleaning agents in the food industry.

Keywords: maltodextrin ester; transesterification; emulsion stability, droplet size, surface tension

4.1 Introduction

An emulsion consists of two immiscible liquids (usually oil and water), with one of the liquids being dispersed as small spherical droplets in the other and classified according to the relative spatial distribution of the oil and aqueous phases. A system that consists of water droplets dispersed in an oil phase is called a water-in-oil (W/O) emulsion, whereas when oil is present as the dispersed phase and water as the dispersion medium (continuous phase) is called an oil-in-water (O/W) emulsion. Thus, the stability of emulsions developed by the dispersed and continuous phase during emulsification depends on the composition and concentration of the emulsifier and also the external energy input (homogenisation). After the emulsifying process, the disruption on the two interfaces results in coalescence and the heterogeneous size distribution of droplets, and as a consequence droplets are separated into their individual phase. Therefore, the stabilisation against coalescence is one of the most important parameters that define emulsion quality.

In general, most emulsions are thermodynamically unstable, however, their stabilisation may be enhanced by the adsorption of amphiphilic molecules to the surface of the dispersed phase (Sagis, 2011). There are two classes of emulsifying agents commonly used in food processing: macromolecules such as proteins (e.g. milk and egg) and low molecular weight (LMW) surfactants (e.g.monoglycerides, polysorbate, sucrose ester). Proteins stabilise the emulsion interface by the formation of viscoelastic layer upon adsorption on an oil droplet surface, thus form a physical barrier to coalescence (Bos & van Vliet, 2001; Wilde et al., 2004). However, LMW surfactants can be oil or water-soluble, may lower the surface tension more than proteins and usually form a compact adsorbed layer

(Bos & van Vliet, 2001; Wilde, 2000). This LMW surfactant layer depends on the charge repulsion or the Gibbs–Marangoni mechanism or weak electrostatic repulsion to stabilise foams and emulsions (Wilde et al., 2004). For the Gibbs–Marangoni mechanism, rapid diffusion or migration of emulsifiers at the interface reduces any surface concentration gradients that may arise. The rapid movement of adsorbed emulsifiers drags the associated continuous phase (water in foams), which restores the presence of fluid between bubbles or droplets and hence prevents coalescence. Therefore, the structural characteristics of emulsifiers are important parameters that define their surface and stabilisation properties (Fruhner et al., 2000).

Two methods are used to characterise liquid interfacial layers: the interfacial shear viscosity η and dilatational viscosity κ (both in units of N m^{-1} s), measured in steady interfacial shear flow or compression/dilatational flow. In the modulus notation, the interfacial shear moduli G′ (elastic) and G″ (viscous) and dilatational moduli E′ (elastic) and E″ (viscous) are used, as a function both of the strain γ and of the angular frequency of the oscillations (ω). The interfacial shear rheology of surfactants and macromolecules characterises the interfacial adsorption layer at gas/liquid and the liquid/liquid interface, and is relevant in a wide range of applications such as multiphase fluids processing, foam and emulsion stability, or oil recovery (Brenner, 2013; Sagis, 2011). Interfacial shear rheology is related to the intermolecular interactions and can be affected by the strength of the interfacial film (Pelipenko et al., 2012), and involves shearing deformations of an interfacial area element while retaining its area (Erni et al., 2007). Research on the formation of an interfacial layer of surfactants, amphiphilic molecules, and particles by using dilatational and shear viscosity measurements has contributed to further understanding of their adsorption kinetics and stabilisation effect (Erni et al., 2003; Fischer & Erni, 2007; Maldonado-Valderrama & Patino, 2010).

Carbohydrate fatty acid esters (CFAE) are amphiphilic polymers that have a hydrophilic (polar) and hydrophobic (nonpolar) region on the same molecule so they can act as low molecular weight surfactants (Sadtler et al., 2002). Through the attachment of an ester group into polysaccharide, the polysaccharides' original hydrophilic nature is altered into an amphiphilic polysaccharide (Udomrati & Gohtani, 2014). The latter have surface-active molecules properties that adsorb to the surface of freshly formed droplets during homogenization, forming a protective layer that prevents droplets coalescence during homogenisation (McClements, 2015).

The aim of this study was to investigate the interfacial shear rheology of maltodextrin laurate at the air/water interface in order to provide a better understanding of their possible stabilising mechanisms. To this end, different maltodextrin laurate esters were investigated for their emulsion formation and stability in oil-in-water systems.

4.2 Materials and methods

4.2.1 Materials

Maltodextrins with three different Dextrose Equivalent (DE) ranges were obtained from Sigma-Aldrich (UK) including maltodextrin DE 4-7 (MDE4), maltodextrin DE 13-17 (MDE13) and maltodextrin DE 16.5-19.5 (MDE16.5). The DE value is inversely proportional to number average molecular weight (M_n) and the degree of polymerization (DP), both of which are commonly used to describe the size distribution of the polysaccharide chain in the carbohydrate polymer (Sun et al., 2010).

Dimethylsulfoxide (>99%) (DMSO), *tert*-Butyl alcohol (≥99.5%), acetone, hexane, and molecular sieves (4 Å, 8-12 mesh) were purchased from Fisher Scientific. Vinyl laurate, ≥98% (GC) Aldrich purchased from Sigma-Aldrich (UK).

4.2.2 Interfacial rheology measurements

Maltodextrin laurates were enzymatically produced as described in **Chapter 3**. Interfacial shear rheology measurements were performed using a Modular Compact Rheometer MCR 102 (Anton Paar, Graz, Austria) and a bicone geometry (Bi-C 68-5, diameter 68.28 mm, angle 5Θ). The bicone was first positioned at the water-air interface (Figure 4-1). About 100 ml of the water was placed in a separate beaker and the bicone was lowered to make contact with the surface. After accurately positioning the bicone at the surface of the water, about 3 g of MDE laurate were dissolved in 1 ml water and 0.5 ml of hexane was carefully pipetted on top. Recording of data started about 20 minutes after the hexane was evaporated and the water/sample interface was formed.

In all interfacial shear experiments at the air/water interface, a time sweep was applied, followed by a frequency sweep and an amplitude sweep (Tamm & Drusch, 2017). The time sweep characterised the structure formation of the interfacial layer and the elastic modulus (G'), viscous modulus (G"), and interfacial complex viscosity (η) was performed for 5 h with strain amplitude (γ = 0.1%) and frequency (f = 1 Hz). Frequency sweep was carried out with a strain amplitude γ = 0.1% at f = 0.01-1 Hz, with 20 min without perturbation before each frequency step. Finally, the interface was subjected to amplitude sweeps (γ = 0.1-100%, f = 0.3 Hz) and the interfacial layer was left undisturbed for 20 min before each measurement. All interfacial shear experiments were performed at a constant temperature of 20 °C.

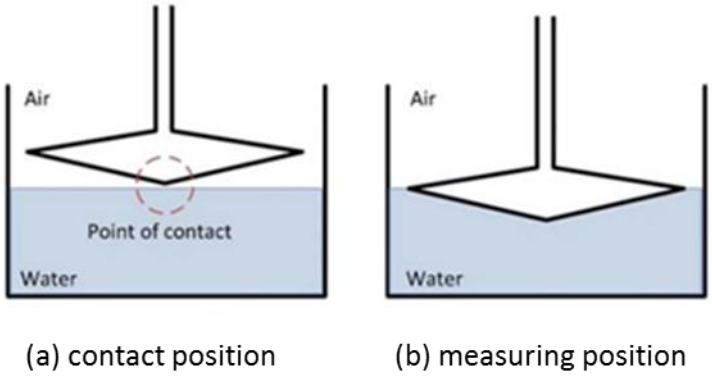


Figure 4-1: Positioning of the geometry in the interfacial shear rheology measurements. Adapted from Humblet-Hua et al. (2013)

4.2.3 Preparation of emulsions

Maltodextrin laurate was dispersed in pure water containing 0.02% (*w/v*) sodium azide, as an antimicrobial agent, in a concentration of 40% (*w/v*), and the mixture was stirred by a magnetic stirrer for 30 min. Emulsion preparation was slightly modified from the method of Udomrati et al. (2016). One millilitre (1 ml) of rapeseed oil was added to 9 ml of maltodextrin laurate suspension and then homogenised in a rotor-stator device (Ultra-Turrax red line) at 15,000 rpm for 2 minutes. The final emulsion contained 10% (*w/v*) of rapeseed oil and 40% (*w/v*) of maltodextrin laurate sample. The emulsion was stored in an incubator at 25 °C for a total of 56 days.

4.2.4 Viscosity measurement

Shear stress (τ) and viscosity of the emulsions were measured using a bob-and-cup system. The samples were measured with a CC25 measuring system (Modular Compact Rheometer MCR 102, Anton Paar, Graz, Austria). Samples were placed in the cylindrical cup of the viscometer and allowed to equilibrate at a set-point 25 °C for 1 min prior to analysis. The shear stress of the sample was measured in the range of shear rate 5-135 s^{-1}.

The shear rate was measured point by point with consecutive 20 s steps of constant shear rate. The viscosity was recorded for each point to obtain the flow curves.

$$\tau = \tau 0 + k \cdot \gamma^{n}$$

Where τ is the shear stress (Pa), $\tau 0$ is the yield stress (Pa), k is the consistency coefficient (Pa sn), γ is the shear rate (s^{-1}) and n is the flow index ($n < 1$ corresponds to shear thinning behaviour, $n > 1$ corresponds to shear thickening behaviour, and $n = 1$ corresponds to Newtonian behaviour).

4.2.5 Determination of emulsion droplet size and droplet size distribution analysis

The mean droplet size is typically expressed as the mean Sauter diameter $d_{3,2}$ which is the diameter of a spherical droplet having the same area per unit volume, S_V. The total collection of droplets in the emulsion was determined using by a laser diffraction method using a Mastersizer 2000 (Malvern Instruments Ltd., Worcenshire, UK). The droplet size distribution was estimated by the Dispersion Index (*Span*) which is calculated as follows (Nor Hayati et al., 2009).

$$Span = \frac{d[90] - d[10]}{d[50]}$$

Where, $d[10]$, $d[50]$, and $d[90]$ values are size values corresponding to the cumulative distribution at 10%, 50% and 90%, respectively. Thus, the $d[10]$ represents a size value below which 10% of the cumulative distribution is present. Emulsions were diluted in distilled water to a droplet concentration of less than about 0.05 % *w/w* (to eliminate multiple scattering effects) and gently stirred (to increase the homogeneity) prior to measurements.

Drops of emulsions were introduced into the sample presentation unit until the concentration reached the optimum one, as indicated by the instrument.

4.2.6 Microstructure observation

The droplet images of emulsions were captured by optical microscopy at room temperature. A small drop of the emulsion was placed onto the microscope slide. The slide placed on the stage of an Olympus Microscope CX41 kept at 25 °C; micrographs were obtained at 40 × magnification by a digital camera connected to the microscope.

4.2.7 Determination of creaming stability

The emulsions were poured into glass tubes and stored at 25 °C for 56 days according to a method by Udomrati and Gohtani (2015). Since the density of the liquid oil was lower than that of the aqueous phase, oil droplets tended to move upward. After storage, some emulsions were separated into a serum layer at the bottom and an opaque cream layer at the top. The height of each of the layers was determined visually using a ruler. The emulsion stability was calculated by the following equation:

$$Creaming\ index\ (\%) = \frac{\mathrm{H}s}{\mathrm{H}E} x\ 100$$

Where H_S (m) is the height of the serum layer and H_E (m) is the height of the emulsion.

4.2.8 Statistical analysis

Statistical analysis was conducted using the Minitab® 18 statistical analysis software. One-way analysis of variance (ANOVA) with a Tukey's multiple comparison tests was used to determine the significant difference between treatments, at a confidence level of 95% ($p < 0.05$). Results are presented as mean ± standard deviation.

4.3 Results and discussion

4.3.1 Interfacial shear rheological behaviour of maltodextrin laurates at the air/water interface

In order to study the adsorption of maltodextrin laurates and Tween 20 at the air/water interface, time sweeps were conducted as shown in Figure 4-2. From the start of the measurement, all maltodextrin laurates exhibited a G' value larger than that of G", showing that all components formed highly elastic layers at the air/water interface; MDE 13 laurate exhibited the highest adsorption compared to MDE4 and MDE16.5 laurate. This higher shear elastic moduli of MDE13 laurate could be attributed to the more efficient packing and adjacent intermolecular interactions of the molecules (Tamm & Drusch, 2017). However, the commercial surfactant, Tween 20 did not show any adsorption layer as the G" was higher than G', being predominantly viscous (Figure 4-2 (b)). Tween 20 is water soluble and it was found that a water-soluble surfactant was more effective at destabilising the emulsion (Cornec et al., 1996). It can be seen that the elastic moduli (G') for MDE13 laurate increased and then decreased after 30 minutes and continued to do so until the end of the test (300 min). For MDE16.5 laurate, the elastic moduli (G') was linear, however the viscous modulus (G") was enhanced during adsorption after 250 minutes. In contrast, the elastic

moduli (G') for MDE4 laurate was steady and linear during adsorption. The differences in charge, neutral sugar side chain, and molecular weight have been reported to affect the stabilisation behaviour between different polysaccharides (Roudsari et al., 2006). In addition, most likley the structure of MDE4 laurate is more branched than the other maltodextrin laurates, with maltodextrin with lower DE having large branched neutral chains, asmaltodextrin mainly consists of linear amylose and branched amylopectin chain. Thus, the branched portions of the MDE4 laurate molecules may stabilise the oil droplets more effectively by steric stabilisation (Roudsari et al., 2006). Emulsifiers (e.g. LMW surfactants) form a mobile interfacial layer and a compact adsorbed layer with a low viscoelastic surface. This layer stabilises foams and emulsions through the Gibbs–Marangoni mechanism (Pelipenko et al., 2012; Wilde et al., 2004). However, the interfacial rheology of LMW surfactants has not received as much attention as the related field of surface-active polymers primarily because of their low viscoelasticity compared to that of polymers (Erni et al., 2005). Due to this, this results will be compared with polymer-surfactant solutions that are currently used in many aqueous formulations, i.e. polymers for the control of rheology, and surfactants for the control of surface properties. In general, at the air-water interface, the surface layer is made of a surfactant monolayer, below which the polymer is adsorbed in a flat configuration (Taylor et al., 2007). The low value of surface shear moduli for the three maltodextrin laurate indicates that the surface layer is rather mobile and weakly entangled thus offering little resistance to interfacial flows (Bain et al., 2010). Monteux et al. (2006) also observed the formation of a saturated and dense layer of Poly(N-isopropylacrylamide) that forms rapidly at the air-water interface and does not rearrange over time.

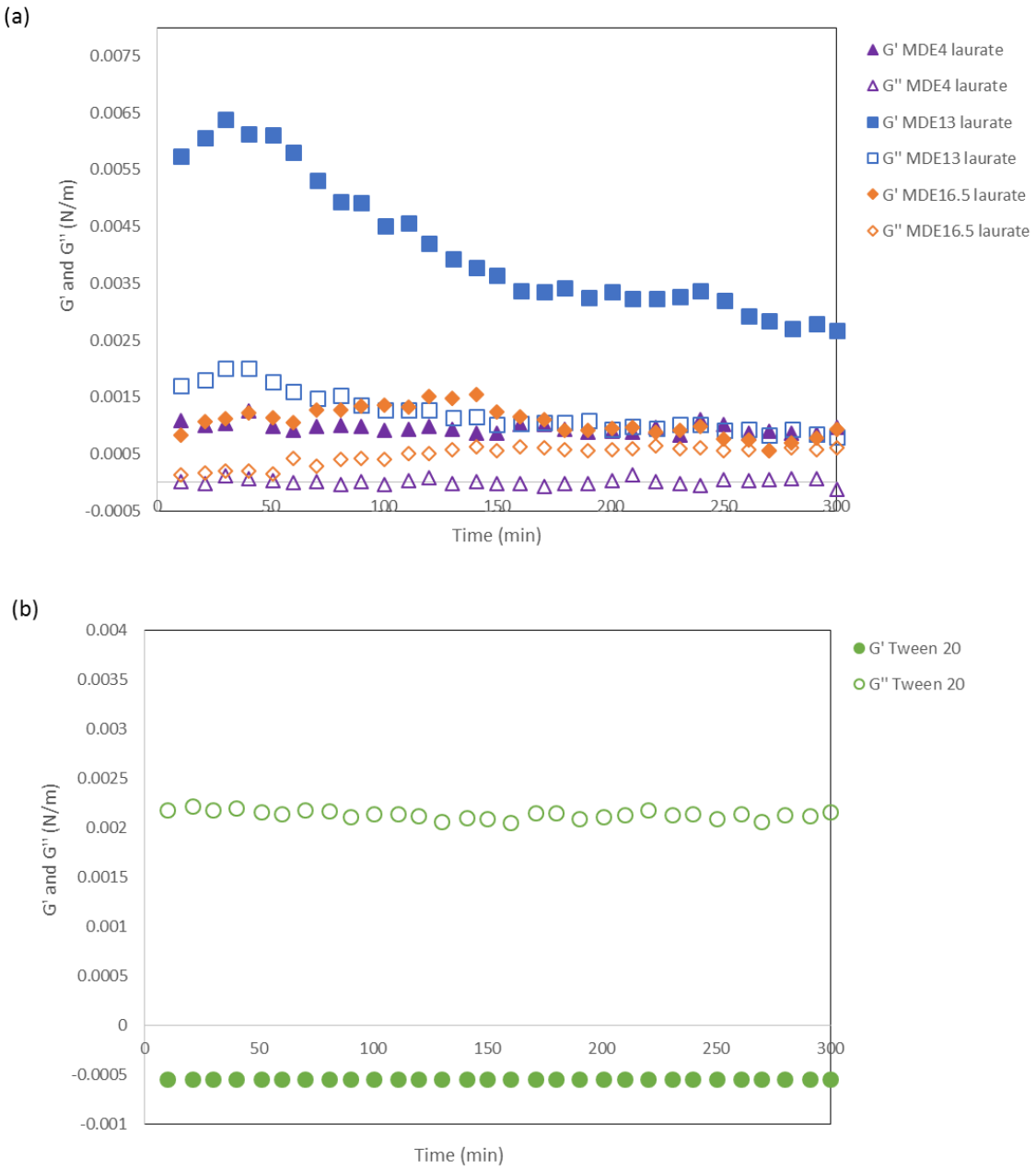


Figure 4-2: Time dependence on the interfacial shear moduli, elastic modulus (G') and viscous modulus (G'') of the adsorption layer at the air/water interface (a) maltodextrin laurate (b) Tween 20 (T=20 °C, strain amplitude, γ = 0.1%, frequency (*f*)= 1 Hz).

Loss tangent tan (δ) indicates whether elastic or viscous properties predominate in a sample (Liu et al., 2007). As shown in Figure 4-3 (a), tan δ was always less than 1, indicating that the viscous modulus was lower than the elastic modulus. During the measurement, a slow increase in the phase angle (δ) in the MDE13 laurate indicates that the viscous

behaviour was enhanced during adsorption. In contrast, the tan δ for MDE16.5 and MDE4 laurate was steady over time with no change in flow behaviour.

Figure 4-3 (b) shows that the interfacial complex viscosity (η*) was higher for MDE13 laurate, however, decreased during adsorption. The interfacial complex viscosity (η*) is the frequency-dependent viscosity function that can be determined for a viscoelastic fluid when subjecting it to oscillatory shear stress. However, η* almost linear for MDE 4 and MDE16.5 laurate adsorption, the maltodextrin laurate adsorption is achieved through a compact adsorbed layer.

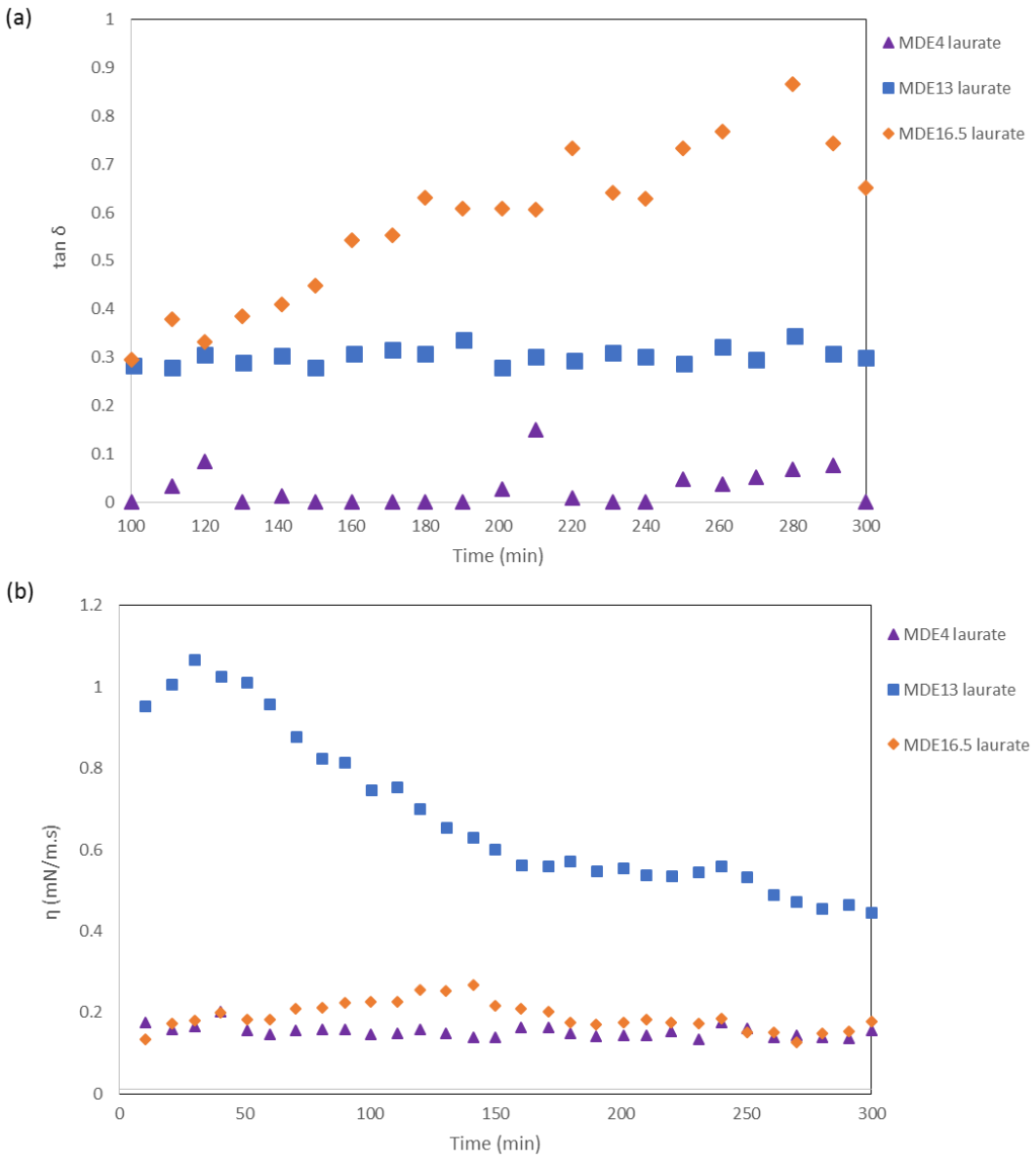


Figure 4-3: The (a) phase angle (δ) and (b) complex interfacial viscosity (η) of the adsorption layer at the air/water interface (T=20 °C, strain amplitude, γ = 0.1%, frequency (*f*)= 1 Hz). Y-axis measured is the interfacial loss factor (tan δ= G''/ G'); complex interfacial viscosity (η) measures the frequency dependent viscosity function determined for a viscoelastic fluid by subjecting to oscillating shear stress unit in mN/m.s.

To probe the structural relaxation of the film, constant strain sweep measurements were carried out and frequency sweep tests were conducted at γ = 0.1% at temperature, 20 °C with *f*= 0.01-1 Hz; the results are shown in Figure 4-4. Over the measured frequency

range, both the elastic and viscous moduli were found to be essentially dependent on frequency. The interfacial film formed by MDE13 and MDE16.5 laurate exhibited elastic behaviours with G' > G" at all frequencies. However, for MDE4 laurate, the low-frequency region where an elastic behaviour is observed (G'>G") shifts to a higher frequency, more than 0.1 Hz; the viscous moduli crossover (G">G') and purely viscous behaviour can be observed at higher frequencies.

The adsorbed layers of MDE4, MDE13, MDE16.5 laurate have a three-dimensional bonded (crosslinked) chemical network because over the frequency range (0.01-1 Hz), the G'>G" or G">G' (Mezger, 2017). MDE13 and MDE16.5 laurate with G'>G" had a solid structure and therefore a stable dispersion. However, MDE4 laurate with G">G' displays liquid behaviour and thus has no stability. A similar finding with MDE4 laurate was reported in hydrophobically modified starch that is predominantly viscous throughout the frequency sweeps (Erni et al., 2007). Torcello-Gómez et al. (2011) observed a viscous behaviour with G" >G' at low frequencies of an adsorbed layer of LMW surfactant (Span 65) with a frequency range between 0.001-1 Hz and a strain amplitude of 0.1%. However, at higher frequencies, a crossover from viscous to elastic behaviour was observed with G'>G", towards a plateau.

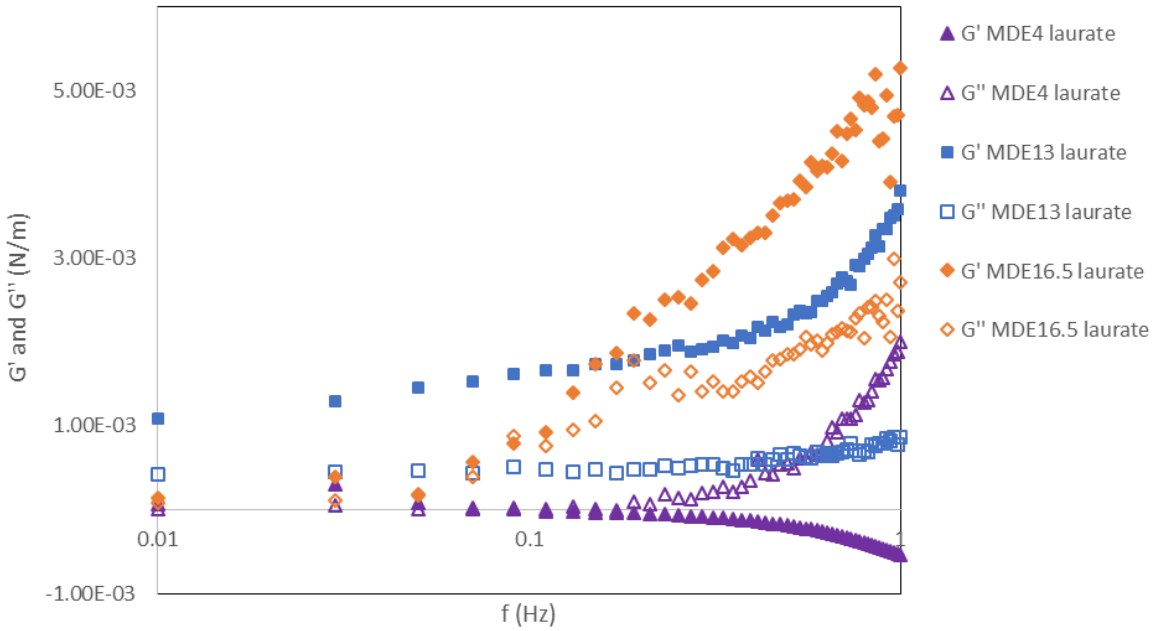


Figure 4-4: Frequency sweep on the interfacial shear moduli, elastic modulus (G') and viscous modulus (G'') of the adsorption layer at the air/water interface (T=20 °C, strain amplitude, γ = 0.1%, frequency (*f*)= 0.01-1 Hz).

Strain sweep measurements were conducted to investigate the possible fracture mechanisms of the interfacial films (the strength of the maltodextrin laurate monolayers). The shear-stress-amplitude sweeps were carried out and revealed that the strain dependence of the elastic modulus (G') and viscous modulus (G") of maltodextrin laurate stabilised at the air/water interface (Figure 4-5). This system exhibits a very weak shear rheological response, dominated completely by the fluid-like sample, (G″>G'). The linear viscoelastic fluid until 10% of the strain amplitude, and after that the breaking of the interfacial film can be seen from the fact that G" decreased abruptly. Torcello-Gómez et al. (2011) observed the linear viscoelastic regime of the interfacial films of Span 65 with the small strains. Thus, this behaviour was reported to correspond to type III, weak strain overshoot. According to Hyun et al. (2002), some interactions take place and become more pronounced under large

deformations. Due to this, weakly structured complexes are formed, partially due to hydrogen bonding resisting against deformation up to a certain strain value, where the G' increases. Then the complexes are destroyed by large deformation above the critical strain, aligning with the flow field and decreasing G".

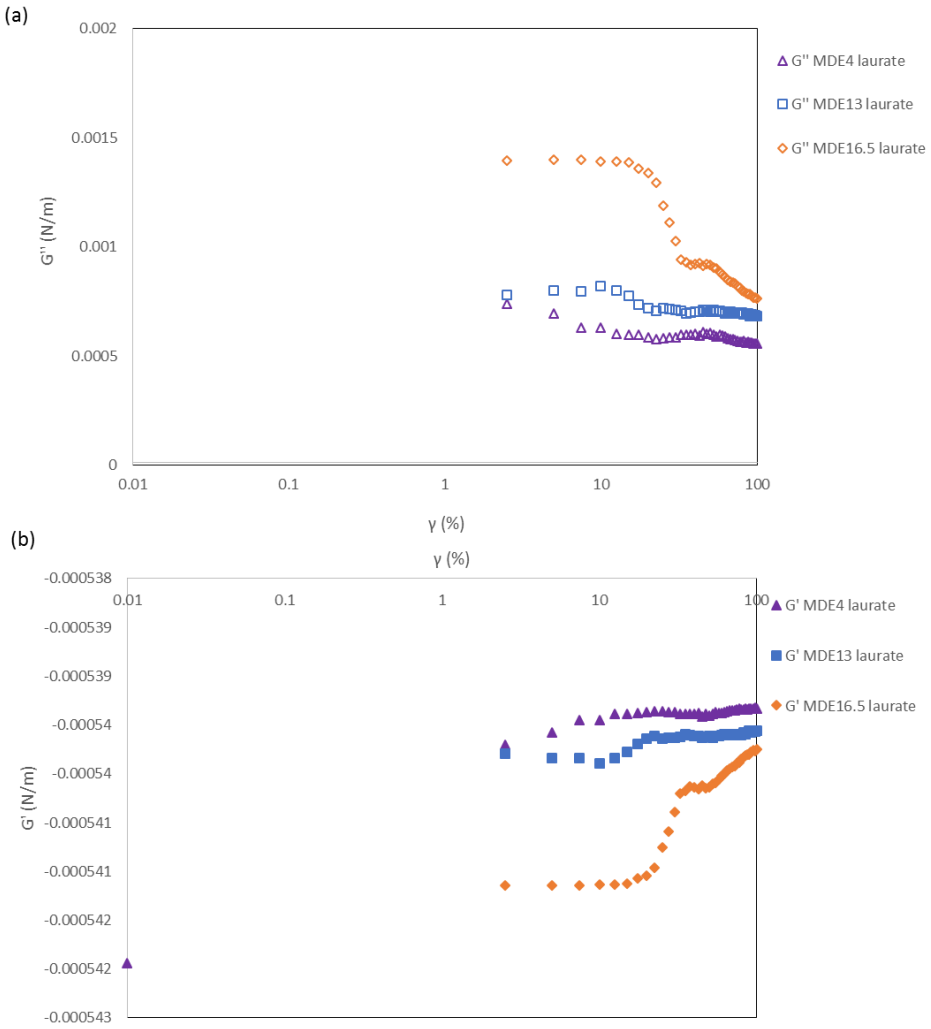


Figure 4-5: Strain dependence on the interfacial shear moduli, elastic modulus (G') and viscous modulus (G'') of the adsorption layer at the air/water interface (a) elastic modulus (b) viscous modulus (T=20 °C, strain amplitude, γ = 0.1-100%, frequency (*f*)= 0.3 Hz).

4.3.2 Flow behaviour

Rheological properties such as viscosity values in O/W emulsions characterise the close packing of the droplets as they interact with one another in the matrix, mainly in the concentration emulsion (e.g. mayonnaise). As such, the closer the droplets, the higher will be the viscosity as a consequence of the higher droplet-droplet interactions (Depree & Savage, 2001; Giacintucci et al., 2016).

The plots of shear stress versus the shear rate of maltodextrin laurate and Tween 20 are shown in Figure 4-6. The emulsions containing high DE of maltodextrin (MDE13 laurate and MDE16.5 laurate) exhibited Newtonian behaviour, whereas in the emulsions that contained low DE maltodextrin, MDE4 laurate showed a shear thinning behaviour, similar to that of the control (Tween 20) at shear rates ranging from 5 to 135 s^{-1}. The high viscosity of the MDE4 laurate emulsion was reflected by the viscosity of the native maltodextrin DE4-7 and may be attributed to its long-chain molecules, which are more efficient in increasing the resistance to flow (İbanoğlu, 2002) (see also **Section 3.3.7**). In the dispersion, maltodextrins with a high degree of polymerisation aggregate into a parallel position through hydrogen bonding and form a polymer entanglement when subjected to shear forces, thus producing highly ordered entangled stiff molecules (Dokic-Baucal et al., 2004). This finding was similar to Udomrati et al. (2016), where esterified maltodextrin, DE 9 with palmitic acid exhibited the highest viscosity compared to others esterified oligosaccharides synthesised with palmitic acid, maltodextrin DE16 and xylo-oligosaccharide.

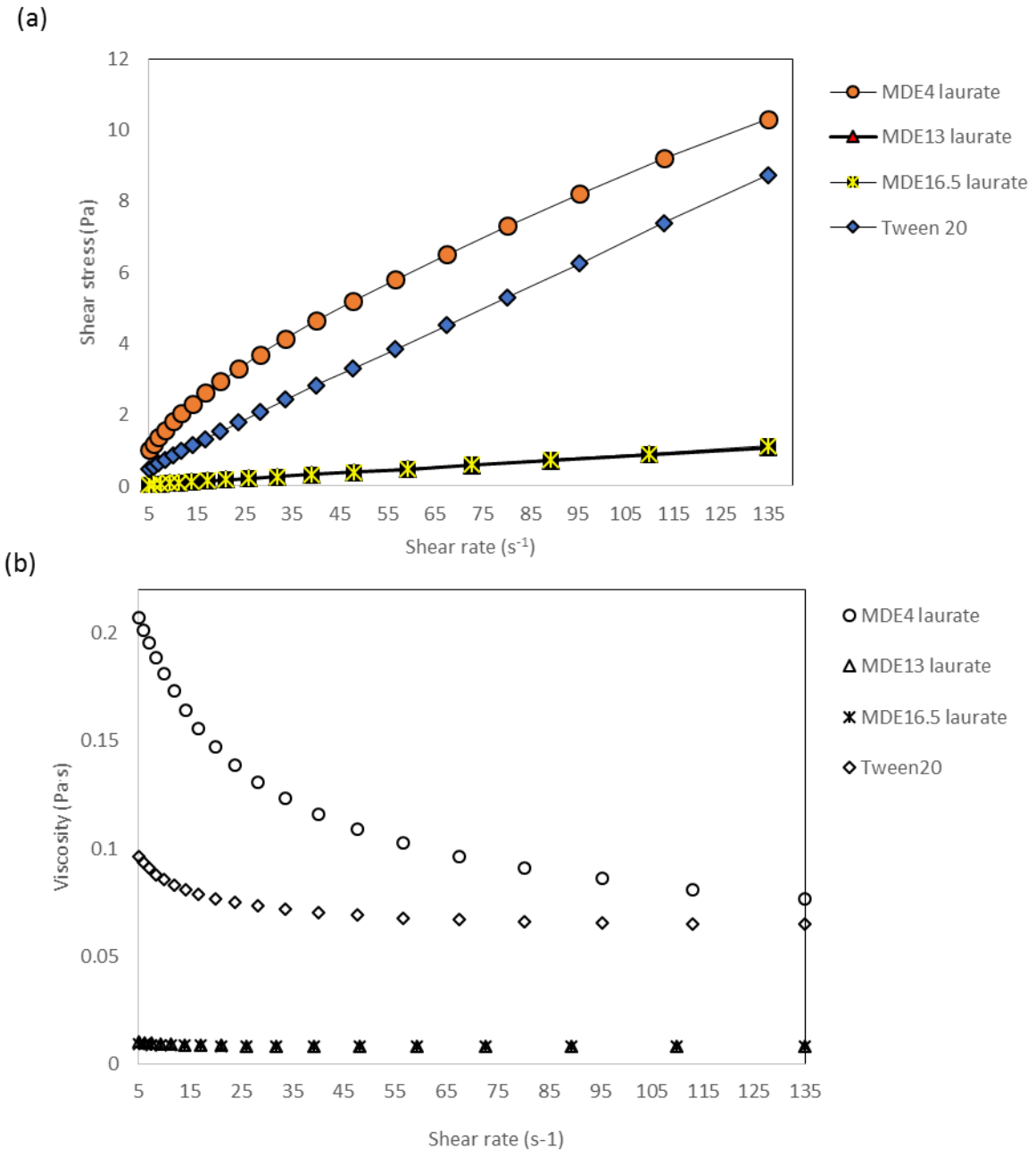


Figure 4-6: Comparison of (a) flow curves and (b) viscosity curves of O/W emulsions prepared with 10% (*w/v*) rapeseed oil and 40% (*w/v*) maltodextrin laurate

Table 4-1 summarises the flow factors of the emulsions as determined by the *Herschel–Bulkley* model. The sheer thinning behaviour can be indicated by the flow behaviour index (*n*), which decreases when sheer thinning increases. The MDE4 laurate emulsion was found to be sheer thinning with the lowest *n*=0.633 whereas the other maltodextrin laurates with different DE behaved as Newtonian liquids (n=1). It has been

reported that sheer thinning behaviour decreases with a decrease in molecular mass (Monsanto, 2001). The high *n* value for control (Tween 20) (almost 1) indicates that tween 20 behaves as a Newtonian liquid. In terms of consistency coefficient (*k*), the emulsion with MDE4 laurate showed the highest consistency. The consistency is an indicator of the emulsion's viscous nature. The high consistency in MDE4 laurate and Tween 20 could be related to a high viscous nature due to weaker attraction forces among the droplets (Nor Hayati et al., 2009). There was a noticeable decrease in yield stress (τ0) for MDE4 laurate, MDE13 laurate and MDE16.5 laurate emulsions. As mentioned before, the emulsion viscosity dropped due to the molecular mass of the maltodextrin (different DE). When the DE decreases, the attraction forces among the droplets became weaker and thus sensitive to lower shearing forces, giving lower yield stress values (Nor Hayati et al., 2009; Udomrati & Gohtani, 2014).

Table 4-1: Modelling of the flow curve between 5 and 135s^{-1} of shear stress of the fresh emulsions using the Herschel-Bulkley model: $\tau = \tau 0 + k \cdot \gamma^n$

Sample	***Herschel-Bulkley* factors**		
	n	***k* (Pa s^n)**	**τ0 (Pa)**
MDE4 laurate	0.633±0.007[c]	0.4724±0.025[a]	0.3816±0.091[a]
MDE13 laurate	1.013±0.006[a]	0.0075±0.000[c]	0.0145±0.002[c]
MDE16.5 laurate	1.022±0.005[a]	0.0074±0.000[c]	0.0096±0.001[c]
Tween 20	0.963±0.002[b]	0.0760±0.001[b]	0.1552±0.009[b]

Data are reported as the mean from two independent replications (n = 2) for each sample. [a–c] Different letters within the same column are significantly different ($p<0.05$). τ, shear stress; $\cdot \gamma$, shear rate; τ0, yield stress; *k*, consistency coefficient; *n*, flow index.

4.3.3 Emulsion droplet size and droplet-size distribution

In this study, emulsions of maltodextrin laurate at a concentration of 40% (*w/v*) and 10% (*w/v*) of rapeseed oil were prepared. The particle size of maltodextrin laurate O/W emulsions ranged between 0.65-0.92 μm, considerably larger than that of Tween 20 (0.36 μm) (Figure 4-7). In terms of the higher DE value, MDE4 16.5 laurate exhibited the smallest emulsion droplet size which was attributed to the ability of the ester to rapidly adsorb to the surface of the droplet during homogenisation (Qian & McClements, 2011). In contrast, enzymatically esterified oligosaccharides, maltodextrins and xylo-oligosaccharides have been reported to exhibit a particle size between 15 to 20 μm in emulsions of 40% (*w/w*) (Udomrati et al. (2016). Small particle size distribution offers much better stability to gravitational separation due to the Brownian motion effects that lead to gravitational forces (McClements, 2015; Tadros et al., 2004). In addition, small particle size is also likely to better support stability against droplet flocculation and coalescence because with decreasing the particle size, the range of the attractive forces acting between the droplets decreases while the range of the steric repulsion is less dependent on particle size (McClements, 2015; Tadros et al., 2004).

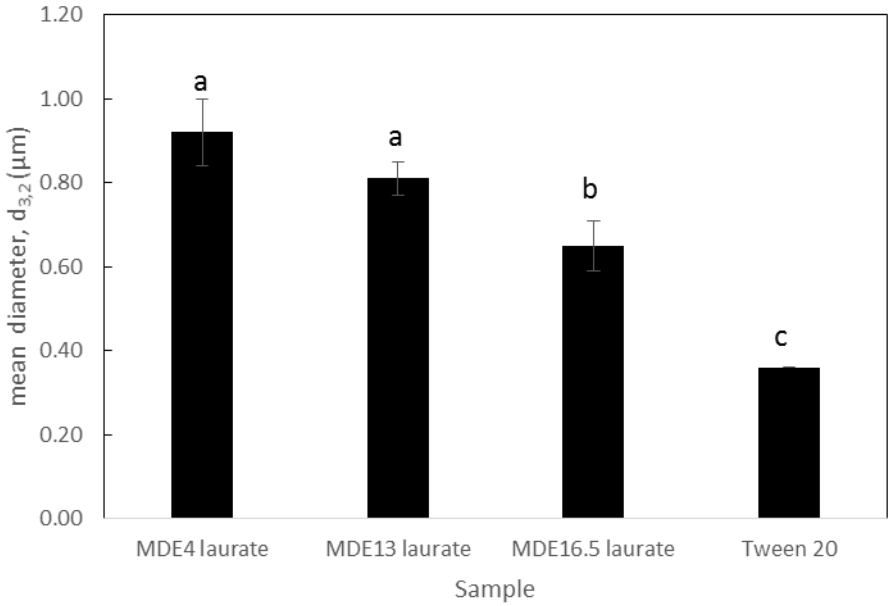


Figure 4-7: Oil droplet means diameters of maltodextrin laurate stabilised O/W emulsion. Different letters a,b,c, indicate that the droplet diameters of the emulsion are significantly different at the 95% confidence level.

According to Figure 4-8, Tween 20 emulsion exhibited a mono-modal droplet-size distribution, however, freshly prepared emulsions with MDE13 and MDE16.5 laurate exhibited broad and mono-modal distribution. On the other hand, MDE4 laurate exhibited broad and bi-modal droplet-size distributions. Similar findings have been reported by Udomrati et al. (2016), in that esterified oligosaccharides (maltodextrin DE 16 and DE 4 and xylooligosaccharides) prepared by enzymatic synthesis with palmitic acid stabilised O/W emulsion and exhibited broad and bi- or tri-modal droplet size distributions in concentrations of 10% to 50%. Nor Hayati et al. (2009) explained that a bi-modal of this oil droplet distribution can be the result of a short time of homogenisation and the type of homogeniser employed. The high-speed homogeniser was applied in this study to prepare the emulsionis only capable of generating fairly low energy inputs to disrupt and mix the oil and water phases and therefore is incapable of producing small droplet sizes comparable to Tween 20. The emulsions were homogenised for a considerably short time (2 min), resulting in only a

small fraction of the emulsion over time in the region where the most intensive disruptive forces are generated.

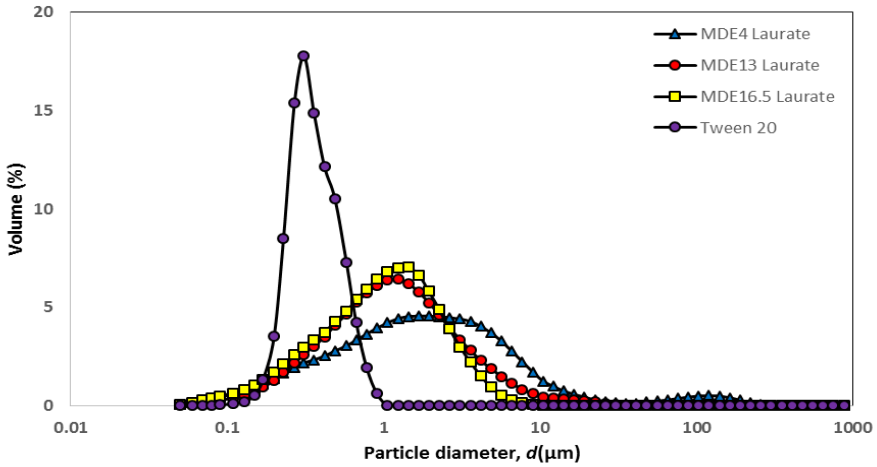


Figure 4-8: Droplet size distribution of freshly prepared emulsions. Data points are presented in an average of duplicate.

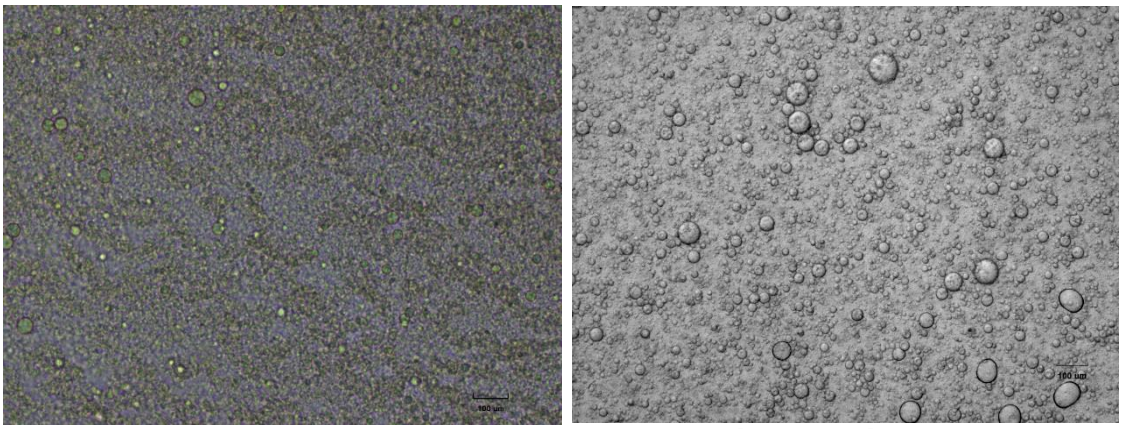
Span values give an indication of the extent of polydispersion in an emulsion. Polydispersion of the emulsion droplets is confirmed in Table 4-2. *Span* values tended to increase over the DE of the maltodextrin, and ranged from 2.49 to 3.42. A *span* value of >1 clearly indicates that the droplet size is not uniform. MDE16.5 laurate showed the smallest value of *span* among maltodextrin laurate, while MDE4 laurate showed the presence of large droplets that give rise to higher polydispersity. However, the *span* value of the Tween 20 was 1.09 thus has the lowest degree of polydispersion, indicating a higher uniformity of the droplet size. The presence of the polysaccharides with different DE resulted in the differences in the polydispersity of the emulsions; the smallest *span* value for the lowest DE could be due to an increase in the droplet size produced during homogenisation because of the ability of the poysaccharides to suppress the formation of small eddies during turbulence (Nor Hayati et al., 2009).

Table 4-2: Span of freshly prepared emulsion.

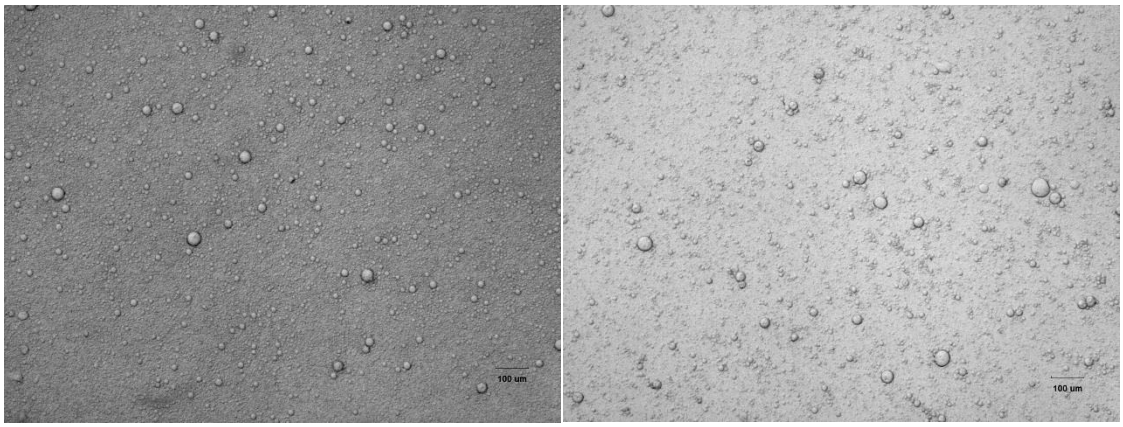
Sample	***Span***
MDE4 laurate	3.42
MDE13 laurate	3.30
MDE16.5 laurate	2.49
Tween 20	1.09

4.3.4 Droplet microstructure

Maltodextrin laurate emulsion droplets were observed under a normal-light microscope as shown in Figure 4-9. Figure 4-9 (b-d) clearly shows the presence of a polydisperse distribution of droplet size in the emulsion; the droplet size is not uniform. MDE4 laurate produced an emulsion with the biggest oil droplets, followed by MDE13 laurate and MDE16.5 laurate. MDE16.5 laurate produced an emulsion with the smallest oil droplets, indicating a product with better emulsifying ability. On the other hand, the maltodextrin laurate particles adsorbed on the oil surface and formed a stabilising layer around the oil droplets.



(a) Tween 20 (b) MDE4 laurate



(c) MDE13 laurate (d) MDE16.5 laurate

Figure 4-9: Micrograph of freshly prepared emulsions of concentration of maltodextrin laurate at 40% (*w/v*)-stabilised emulsion with 20 × magnification; (a) Tween 20, (b) MDE4 laurate, (c) MDE13 laurate and (d) MDE16.5 laurate.

4.3.5 Creaming stability

Figure 4-10 shows the percentages of creaming indices of the emulsions; these were determined by measuring the height of the serum layer formed at the bottom of the emulsions after 24 h of storage at room temperature (25 °C) for 56 days. A distinct serum layer was observed in most emulsions, the height of which was dependent on the different DE of the amphiphilic maltodextrin laurate. No creaming was observed for MDE4 laurate due to the higher viscosity of the high molecular fractions of low DE. Specifically, high molecular fractions of low DE maltodextrin can associate in a different manner as branched amylopectin added to linear amylose can form tightly packed segments, forming a network

structure within the continuous phase. The oil droplets are strongly held in this three-dimensional gel network and cannot be easily rearranged and expel serum from the emulsion structure; this delays droplet creaming (Dickinson, 2003; Dokic-Baucal et al., 2004). No creaming in emulsions with high concentrations of xanthan gum has also been reported because of the high yield stress of the continuous phase, which slowed down the movement of flocculations or dispersed emulsion droplets (Chivero et al., 2015; Hemar et al., 2001). On the contrary, the emulsion with MDE16.5 laurate creamed quickly (after the first couple of days), followed by Tween 20 and MDE13 laurate emulsions. These observations may be attributed to the fact that the MDE16.5 laurate cannot form tightly packed structures of low molecular weight fractions, thus cannot form a network structure within the continuos phase to stabilise the emulsion droplets (Udomrati et al., 2011; Udomrati et al., 2016). Small molecular surfactants, e.g. Tween 20, are not so effective in conferring long-term stability. As such, the use of polysaccharides as stabilisers to extend the long term stability of emulsions by viscosity modification or gelation in the aqueous continuous phase, is often needed. A similar finding was reported by Udomrati et al. (2016), who noted that maltodextrin DE9 palmitate had high viscosity and was more effective in reducing the creaming rate of emulsions compared to maltodextrin DE16 palmitate and xylo-oligosaccharides palmitate.

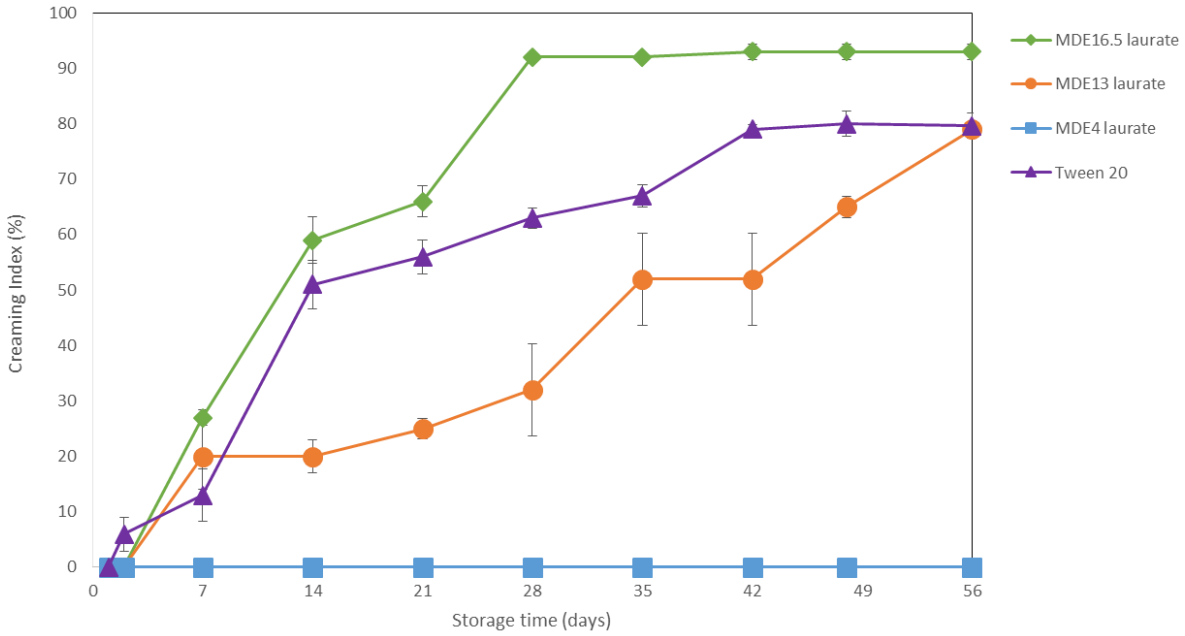


Figure 4-10: Creaming indices of stored maltodextrin laurate stabilised O/W emulsion prepared with 10% (*w/v*) of rapeseed oil and 40% (*w/v*) of maltodextrin laurate (MDE4, MDE13 and MDE16.5) and Tween 20.

After 7 days of storage at 25 °C, the emulsions of MDE13 laurate and MDE16.5 laurate showed a distinct phase separation comprising a top layer of an emulsion (or cream) phase and a bottom layer of an aqueous phase (Figure 4-11). The high syneresis could be attributed to a very weak emulsion structure with a low viscosity of the continuous phase. Thus, this led to very high droplet mobility and subsequently a high degree of syneresis. MDE4 laurate, on the other hand, showed excellent stability with no phase separation. This emulsion is likely to provide a thicker and stronger emulsion network in order to reduce the collision frequency of the droplets (Nor Hayati et al., 2009). Similar to properties of maltodextrin, MDE4 laurate have ability to form gels, they could be used in producing emulsions as texture modifiers, bulking agents and particularly in food emulsions to certain extent for substitution of fat (Dokic-Baucal et al., 2004).

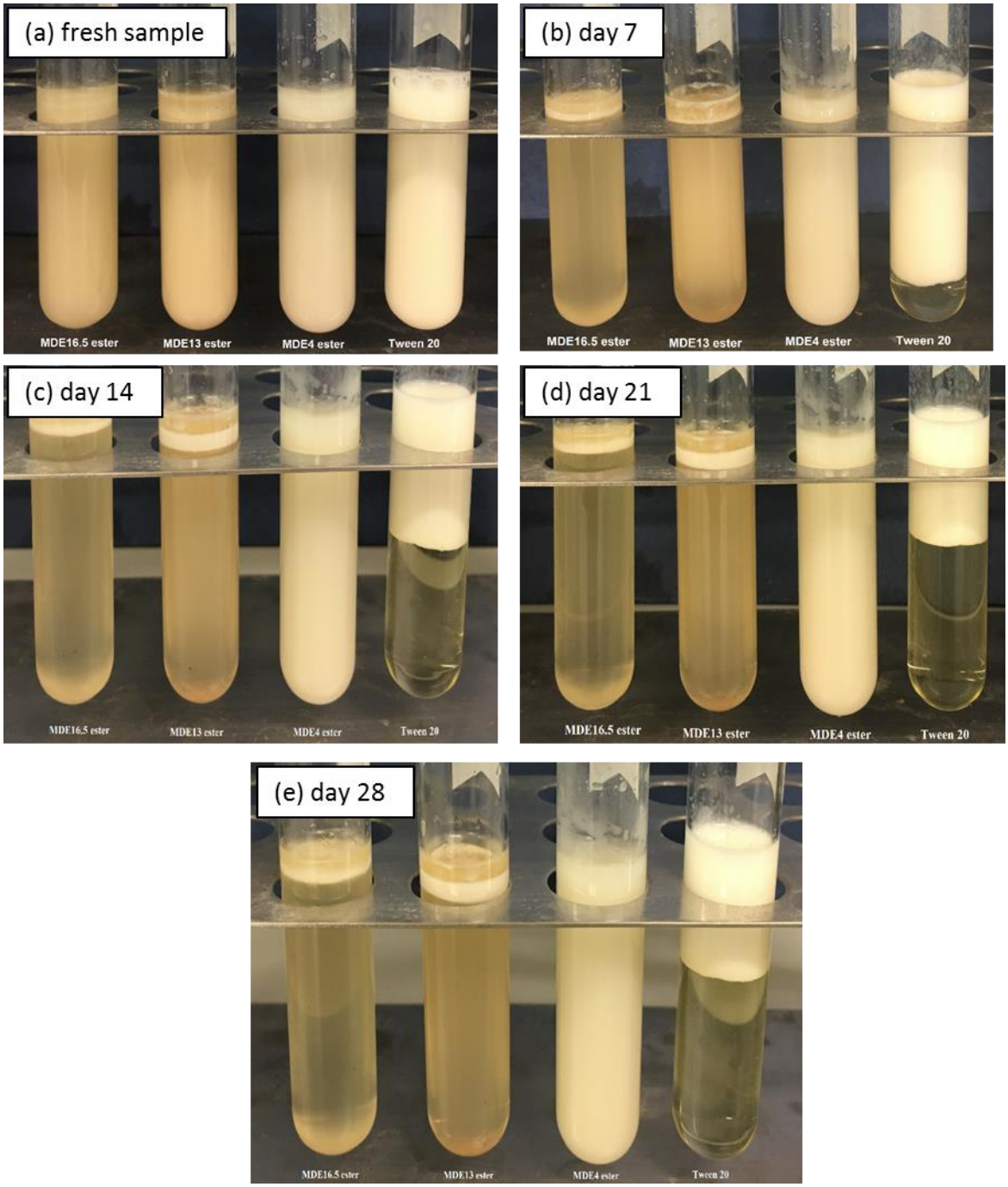


Figure 4-11: Visual appearance of phase separation after storage (25 °C, 28 days) (a) freshly prepare (b) day 7 (c) day 14 (d) day 21 and (e) day 28.

4.4 Conclusions

The interfacial shear rheology of maltodextrin laurate has been studied and all maltodextrin laurates adsorbed at the air/water interfaces, suggesting the formation of a dense, saturated layer surface. The non-linear response of the surface film depended on the molecular weight and the general structure of the molecules at the interface. Based on the frequency sweep results, MDE13 and MDE16.5 laurate could stabilise the air/water emulsion as indicated by G'>G", however MDE4 laurate shows G">G' which indicates instability at the air/water interface.

At a concentration of 40% (*w/v*) of maltodextrin laurate, MDE16.5 and MDE13 exhibited the smallest oil droplet size, larger than Tween 20; however, they were not stable for a long period of time due to their surface activity. Thus, such maltodextrin laurates could be used as a stabiliser in LMW surfactant (e.g. Tween 20, Tween 80, sucrose ester) emulsions. On the other hand, MDE4 laurate exhibited the highest viscosity due to its high content of high molecular weight fractions and stabilised the emulsion for longer. Such properties could be used in products where viscosity and hydrophobic interactions are desired (e.g. mayonnaise, sauce).

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Chapter 5 - Application of maltodextrin laurate as laundry detergent

Abstract

The aim of this study was to investigate the stability and compatibility of three maltodextrin laurate (MDE4, MDE13 and MDE16.5) in detergent formulations, targeting the removal of lipophilic substances (rapeseed oil) from cotton cloth. All maltodextrin laurates were shown to possess emulsion-stabilising capacity for vegetable oil, whereas the high emulsification index with rapeseed oil (54-66%) reflected a good stability of the formed emulsion. Tween 20 (control) and MDE4 laurate showed compatibility with non-bio commercial detergent (Persil) and enhanced oil removal (76%, *w/w*) compared to that of detergent alone (68%, *w/w*). High oil removal percentage (56-83%, *w/w*) was obtained under conditions of 0.1M Trizma buffer pH 9 at 37 °C in all samples, whereas MDE4 laurate performed the best (83%, *w/w*) at concentration of 1.0% (*w/v*) in the detergent formulation. The oil removal percentage was improved in low concentrations of saline (1%, *w/v* NaCl) for all samples. The surface tension of surfactants was reduced when the concentration of saline increased up to 7%, (*w/v*) ($p<0.05$). The results of this study indicate that maltodextrin laurate are compatible and stable when used with commercial detergent and also have potential as additives in detergent formulation targetting effective oil removal.

Keywords: *Oil, Salinity, Carbohydrate fatty acid ester, Surface tension, Solubility, Detergent*

5.1 Introduction

A laundry detergent composition generally comprises of surfactants, builders, enzymes and bleaching agents (Mukherjee, 2007). To date, most surfactants used in detergents are chemically synthesised surfactants which include soaps and alkylbenzene sulfonates (anionic), and ethoxylated fatty acids (non-ionic). However, previous research studies have reported the acute toxicity of laundry detergent components to fresh-water living organisms (e.g. fish and crustaceans) (Warne & Schifko, 1999) and their non-easily biodegradable nature (Makkar & Cameotra, 2002). The main contributors to the toxicity of detergents are surfactants and sodium silicate solutions, whereas other detergent components such as enzymes and builders also contribute slightly toward the toxicity when present at low concentrations (Warne & Schifko, 1999).

Increased environmental awareness about the hazards and risks associated with chemical surfactants has stimulated the search for eco-friendly and natural substitutes in laundry detergents. In recent years, renewable surfactants including surfactants synthesised from renewable raw materials (bio-based surfactants) and surfactants synthesised by living cells or through the use of enzymes (biosurfactants), have gained increasing attention (Allen & Tao, 2002; Le Guenic et al., 2019; Sarney & Vulfson, 1995). This is due to their low toxicity, biodegradable nature, their low anti-irritating effects and compatibility with skin (Makkar & Cameotra, 2002; Mukherjee, 2007; Mulligan, 2005; Yu et al., 2008). In addition, they are also effective in a wide range of extreme conditions including temperature, pH and salinity (Banat et al., 2000; Mukherjee, 2007; Mulligan, 2005). Recent work has been reported on biosurfactants from microbial cell surface that are compatible with detergents and can exhibit enhanced oil removing capacity from the soil and cotton cloths (Jain et al., 2012; Mukherjee, 2007; Santa Anna et al., 2007; Urum & Pekdemir, 2004).

Detergency is the process that removes unwanted substances (soils) from a solid surface upon contact with liquid (Bajpai & Tyagi, 2007; Tongcumpou et al., 2003). Soil removal is dependent on several factors, such as the nature and composition of the washing solution, type of soil, hydrodynamic conditions, water hardness, temperature, and electrolyte level, as well as the nature of the solid surface. Fabric detergency of oily soil is a complicated process which has been studied for many years. The removal of oily soils from fabric generally occurs by roll-up and snap-off (otherwise known as emulsification–solubilisation) mechanisms (Miller & Raney, 1993; Tungsubutra, 1994). Both roll-up and snap-off are assisted by reducing the interfacial tension (IFT) between oil (soil) and water. Hence, a major problem in laundries is the removal of adsorbed lipids from fabrics, usually vegetable oils; triglycerides are the main components in vegetable oils that have a polar nature due to the unique structure of a bulky glycerol group and three long hydrocarbon chains. The commercial approach for vegetable oil detergency is to use high alkalinity or enzymes to hydrolyse the triglycerides and fatty esters to free fatty acids, di and mono-acylglycerols, and glycerol, as these are easier to remove (Snabe et al., 2005; Thirunavukarasu et al., 2008). Therefore, formulating surfactant systems to achieve low IFT for highly hydrophobic oily soils, such as vegetable oils is a challenge.

Carbohydrate fatty acid esters (CFAE) are non-ionic surfactants synthesised from a carbohydrate and a fatty acid ester using an enzyme (mainly lipase) and the end product of synthesis demonstrates amphiphilic properties by containing both hydrophilic and hydrophobic moieties in the same molecule (Allen & Tao, 2002; Martínez et al., 2011; Neta et al., 2012; Sarney & Vulfson, 1995). To the best of our knowledge, no information is available in the literature on the CFAE compatibility with non-bio detergents and also on detergency improvement. Moreover, limited information is available on the effect of pH, salinity and temperature upon detergency performance. The aim of this work was to assess

the emulsion forming and capacity of maltodextrin laurates on hydrophobic surfaces such as vegetable oil. Then, maltodextrin laurates were evaluated for their compatibility with commercial non-bio laundry detergents (Persil). The effect of buffer pH, salinity, concentration and temperature on oil removal efficacy from cloths compared to a chemical surfactant (Tween 20) was also investigated.

5.2 Materials and Methods

5.2.1 Materials

Maltodextrins with three different Dextrose Equivalent (DE) ranges were obtained from Sigma-Aldrich (UK). These were maltodextrin DE 4-7 (MDE4), maltodextrin DE 13-17 (MDE13) and maltodextrin DE 16.5-19.5 (MDE16.5). The DE value is inversely proportional to number average molecular weight (M_n) and the degree of polymerisation (DP); both are commonly used to describe the size distribution of the polysaccharide chain in the carbohydrate polymer (Sun et al., 2010). Cotton cloth was purchased from a UK cloth retailer Primark (Reading, UK).

Dimethylsulfoxide (>99%) (DMSO), *tert*-Butyl alcohol (≥99.5%), petroleum ether, acetone, hexane, and molecular sieves (4 Å, 8-12 mesh) were purchased from Fisher Scientific, vinyl laurate,(purity ≥98% and the dye (Sudan III) were purchased from Sigma-Aldrich (UK), while sunflower oil, rapeseed oil and olive oil was purchased from the Co-op Food store (at University of Reading).

5.2.2 Emulsification assay

Maltodextrin laurates were enzymatically produced as described in **Chapter 3**. The protocol for the emulsification assay of maltodextrin laurate was based on the method described by Freitas et al. (2009) with some modifications, used sunflower oil, rapeseed oil and olive oil as the test substances. Briefly, the emulsification activity was measured by adding 6.0 ml of vegetable oil to 4.0 ml of 1% (*w/v*) of maltodextrin laurate solution and then homogenising the mixture using a high-speed homogeniser (Ultra Turrax T25, IKA Janke and Kunke, Germany) at 10,000 rpm for 2 min. The resulting mixture was kept at room temperature (25°C) for 24 h and the emulsification index (E_{24}) was calculated as follows:

$$E24 = \frac{he}{ht} x\ 100$$

where *he* (mm) is the height of the emulsion layer and *ht* (mm) is the overall height of the mixture.

5.2.3 Preparation of soiled rapeseed oil cotton fabric

Cotton fabric cloths (5 x 5 cm) were degreased by boiling in chloroform for 5 h according to Saraswat et al. (2017) to ensure all lipid material was removed from the cotton textile prior to soiling. The soiling was done by spotting with 0.5 ml rapeseed oil onto the cotton fabric and leaving it overnight at room temperature to dry.

5.2.4 Washing solution, procedures and rapeseed oil quantification

About 50 mL of six different compositions of washing solution were prepared (Table 5-1). Washing was done by soaking the cotton fabric soiled with rapeseed oil in different washing solutions in a shaking flask at 37°C and agitating at 120 rpm for 60 min. Then, the fabric was rinsed with 100 ml distilled water twice for 3 min and dried at 40°C overnight. Rapeseed oil was extracted from the fabric with petroleum ether for 4 h using a Soxhlet extractor. The quantification of rapeseed oil was performed gravimetrically by weighing the residual rapeseed oil after complete evaporation of petroleum ether from the extract. The percentage of rapeseed oil removed was calculated taking into account the weight of rapeseed oil stain before and after washing and was expressed by the following equation:

$$\%\ Removal = \frac{(\mathrm{w1} - \mathrm{w2})}{w1}\, x\ 100$$

where w1 stands for the weight of rapeseed oil before washing and w2 for the weight of rapeseed oil after washing. For visual examination and demonstration of detergent efficiency, cotton pieces were stained with Sudan red dyed rapeseed oil. After the same treatment, cloths were taken out and rinsed twice with distilled water, dried and visually inspected.

Table 5-1: Different washing solution and their compositions for compatibility with detergent

Components	W (Control)	W+D	W+S	W+S+D	B	B+S
Distilled water (ml)	50	40	40	40	50	40
1% (*w/v*) detergent solution (ml)	-	10	-	5	-	-
1% (*w/v*) surfactant (mL)	-	-	10	5	-	10
Total (ml)	50	50	50	50	50	50

W = Distilled water
S = Surfactant (Tween 20 and maltodextrin laurate)
D = Detergent
B = Tris Buffer (pH 7), Trizma Buffer (pH 8-9), bicarbonate buffer (pH 10)

5.2.5 Surface tension measurement.

The surface tension of a washing solution formulated with buffers of varying pH (pH 7, pH 8, pH 9, pH10 10), surfactant concentrations (0.25, 0.5, 1.0, 1.5, 2.0, 3.0 % *w/v*) and NaCl concentrations (1, 2, 3, 5, 7 % *w/v*), was measured by using an automated Pendant Drop analyser (Drop Shape Analyzer DSA30, Krüss GmbH, Hamburg, Germany). 20 µL of washing solution were dropped on the surface using a microsyringe and the surface tension was measured using the Young-Laplace equation. The surface tension was measured from a drop suspended from a needle that starts to detach when its weight (volume) reaches the magnitude balancing the surface tension of the liquid. The weight (volume) is dependent on the characteristics of the liquid. The measurements were repeated twice, and the mean value was calculated.

5.2.6 Solubility of surfactants in washing solutions with different salinity

Washing solutions containing 1 and 2% (*w/v*) of surfactant concentration were prepared in 0.1 M Trizma Buffer pH 9 with different NaCl concentrations (0, 1, 2, 3, 5, 7 % *w/v*) and incubated at 37°C for 1 h. The solution was centrifuged at 4000 rpm for 15 min. The precipitate was collected, oven-dried at 90 °C and then weighed. The solubility (%) was calculated as follows:

$$Solubility\ (\%) = \frac{\text{weight of dry soluble maltodextrin laurate (g)}}{\text{weight of dry sample (g)}} \times 100$$

Where the weight of soluble maltodextrin (g) was calculated by using the weight of the initial dry sample (g) subtracted the weight of precipitate maltodextrin laurate (g).

5.2.7 Statistical analysis

Statistical analysis was conducted using the Minitab® 18 statistical analysis software. One-way analysis of variance (ANOVA) with a Tukey's multiple comparison test was used to determine significant difference between treatments at a confidence level of 95% ($p < 0.05$). Results are presented as mean ± standard deviation.

5.3 Results and Discussion

5.3.1 Emulsification capacity of maltodextrin laurate

The emulsification index (EI) is an important parameter for evaluating the power of an emulsifier (Freitas et al., 2009; Neta et al., 2012). An emulsion is defined as stable if it is able to maintain at least 50% of the original emulsion volume 24 h after its formation (Willumsen & Karlson, 1997). The effective EI of an emulsion can be increased by increasing the esterified maltodextrin concentration, since they are known to be able to cover more of the emulsion droplet surface (Udomrati & Gohtani, 2014). In this study, the emulsifying properties of the maltodextrin laurate were initially evaluated at concentration of 1% (*w/w*) with three types of vegetable oils (olive oil, rapeseed oil and sunflower oil), with a view that this concentration could be acceptable in laundry detergent formulations. Figure 5-1 shows that all maltodextrin laurates possessed emulsion-stabilising capacity for vegetable oil, whereas the high EI with rapeseed oil (54-66%) reflected a good stability of the formed emulsions. Olive oil and sunflower oil gave less stable water/oil emulsions as their EI was lower than 50%.

Furthermore, MDE16.5 laurate was more efficient in forming an emulsion with the tested oils, since MDE16.5 laurate had the highest degree of substitution (DS) of lauric acid. DS is the average value of acetyl groups replacing the hydroxyl groups in the anhydroglucose units of maltodextrin (de María & Martinsson, 2009). Higher DS means more lauric acid groups attached along the polysaccharide backbone, and these are expected to have a stronger interaction with the oil surface (Udomrati & Gohtani, 2014).

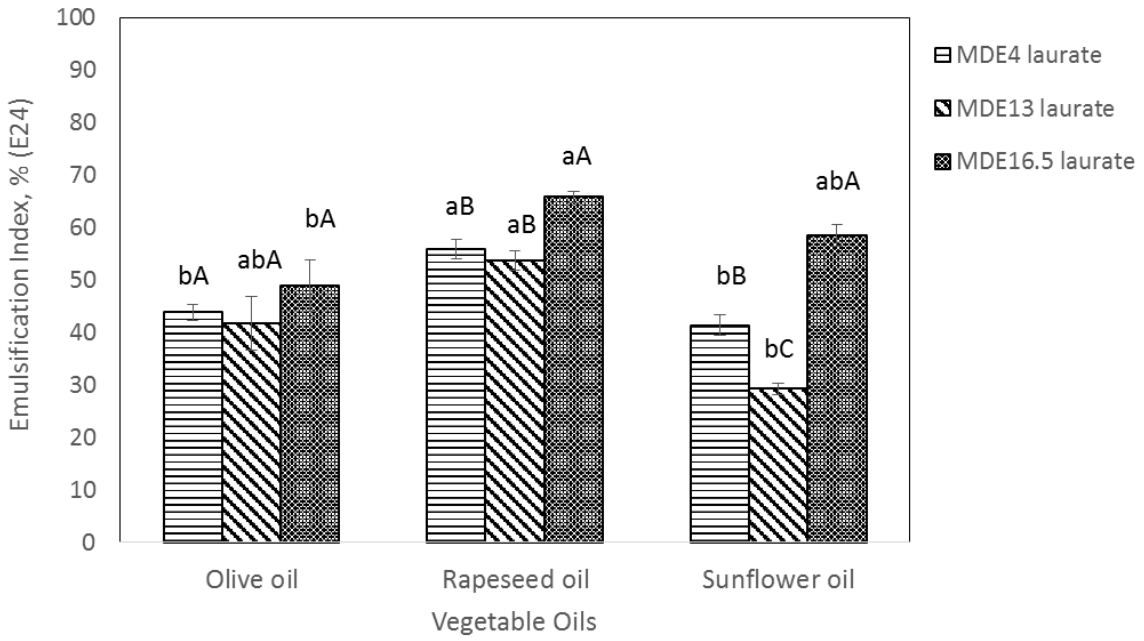

Figure 5-1: Emulsification index (E_{24}) of 1% (*w/v*) maltodextrin laurate with olive oil, rapeseed oil and sunflower oil. Each value represents the mean ± sd of three experiments. Different letters (a, b, c) indicate that the E_{24} values significantly differ ($p<0.05$) with the same sample. Different letters (A, B, C) indicate that the E_{24} values significantly differ ($p<0.05$) with the same vegetable oils sample.

5.3.2 Stability and compatibility of surfactants in water and detergent

The stability in water and compatibility of any component with the detergent is a prerequisite for its inclusion in detergent formulations. Thus, the stability of surfactants (Tween 20 and maltodextrin laurate) in water and their compatibility within a detergent formulation was tested by the ability to remove rapeseed oil from the soil cotton cloth as shown in Figure 5-2. The rapeseed oil removal percentage from cotton cloth by distilled water was about 49%, (*w/w*). MDE4 laurate at 1% (*w/v*) was observed to be sufficient even on its own, as it removed 63% (*w/w*) of rapeseed oil from cloth, compared to Tween 20 (52%, *w/w*) at the same concentration. However, previous research by Jain et al. (2012) reported that 1%, (*w/v*) of biosurfactant produced from an alkaliphilic bacterium, *Klebsiella* sp. strain RJ-03 can lead to a 80% *w/w* removal of lubricant oil. Additionally, biosurfactants

obtained after cultivation of *Candida glabrata* UCP 1002 in mixtures of glucose and cotton seed oil led to oil removal from the soil by 84%, *w/w* (de Luna et al., 2009).

When adding 1% (*w/v*) surfactant with the 1% (*w/v*) of detergent (D), the washing solution of D+Tween 20 and D+MDE4 in water improved the oil removal percentage by 24%, *w/w* and 13%, respectively, *w/w* due to the hydrophobic and hydrophilic parts of the surfactants that can adsorb to non-polar and polar materials at the same time (Bajpai & Tyagi, 2007). MDE4 laurate and Tween 20 showed compatibility and stability with commercial non-bio laundry detergents and led to a higher oil removal (76%, *w/w*) compared to washing solution with detergent alone (68%, *w/w*). This suggests that the wash performance in detergents was improved in the presence of MDE4 laurate due to due to their bulky molecular weight and complex biodegradable structure (Jain et al., 2012). Maltodextrin laurate has surface active properties as it is an amphiphilic compound that has both hydrophilic and lipophilic moieties, which are needed for forming and stabilising foams and emulsions (Maier, 2003). Similar to the lipopeptide biosurfactant molecules, maltodextrin laurate is of intermediate size in comparison with small surfactant molecules and high molecular weight proteins, diffuses and orients rapidly at water-oil interfaces, thus efficiently reducing the surface and interfacial tension (Deleu et al., 1999; Mukherjee, 2007). Jain et al. (2012) reported oil removal from the cotton cloth during washing supplemented with 0.5% biosurfactant produced from an alkaliphilic bacterium *Klebsiella* sp. strain RJ-03 with different commercial detergents (67% with Wheel, 76% with Tide and 81% with Surf excel). Our results showed that MDE4 laurate showed satisfactory stability and compatibility with commercially available detergents, along with enhanced washing performance.

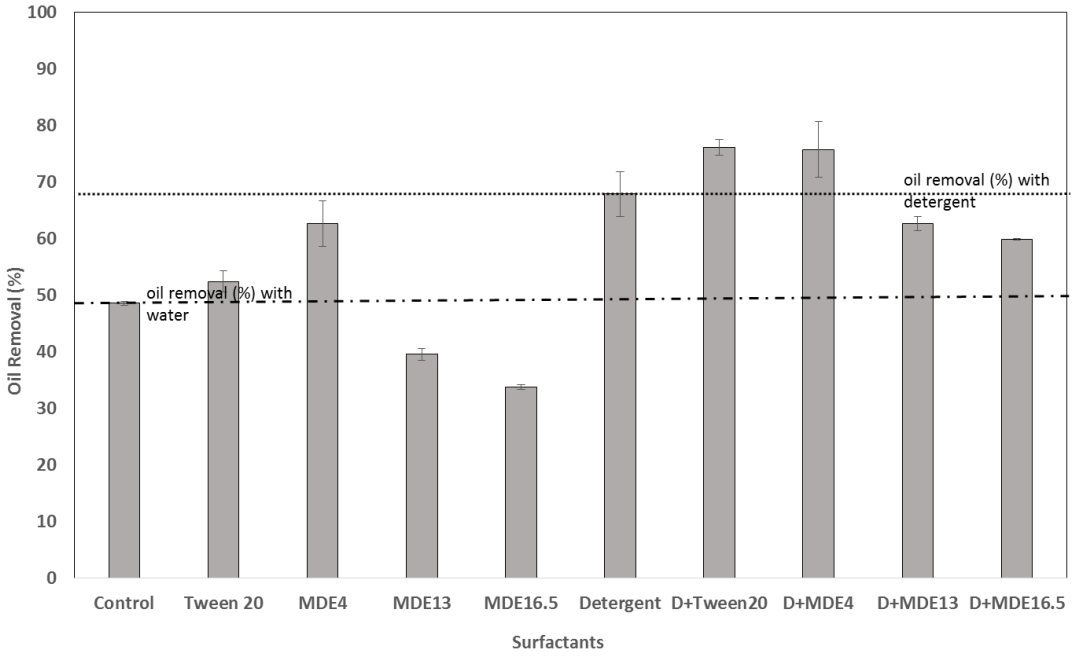

Figure 5-2: Stability of surfactants in water and compatibility test for improvement of wash performance of commercial laundry detergent. The two lines ..... represents the oil removal (%) with detergent and _._._. represents the oil removal (%) with water. Each value represents the mean ± sd of four experiments.

Table 5-2 shows the pH and surface tension of the washing solution. Tween 20 and maltodextrin laurate were more acidic compared to control (water). The detergent formulations usually have a pH between 9.0-12.0 (Haddar et al., 2009). With the addition of maltodextrin laurate, the pH of the detergent formulation was reduced from 9.80 to 9.71-9.61. The surface tension of detergent (control) was 30.42 mN/m and lower than the tested Tween 20 and maltodextrin laurate when used as additives in detergent formulation. However, all maltodextrin laurate in water exhibited reduced surface tension (from 71.15 mN/m to 66.39-69.55 mN/m).

Table 5-2: pH and surface tension for the surfactants in water and detergent

Sample	pH		SFT [mN/m]	
	water	detergent	water	detergent
Control	6.83	9.80	71.15 ± 0.78^{a}	30.42 ± 0.53^{c}
Tween 20	6.05	9.61	38.39 ± 1.96^{e}	38.84 ± 1.19^{a}
MDE4 laurate	5.52	9.71	69.55 ± 0.74^{b}	33.01 ± 1.35^{b}
MDE13 laurate	5.13	9.62	66.39 ± 1.70^{d}	33.43 ± 0.78^{b}
MDE16.5 laurate	5.01	9.62	68.55 ± 0.66^{c}	30.71 ± 1.52^{c}

5.3.3 Effect of pH of the buffer on the wash performance and surface tension

Detergent performance can be influenced by several factors such as the pH of washing solution, detergent composition as well as wash temperature (Haddar et al., 2009). Thus, any surfactant incorporated into detergent formulations must exhibit significant oil removal percentage at a certain pH. The effectiveness of cleaning can be enhanced by adjusting the level of the pH of laundry water. Many commercial and industrial laundry cleaning systems utilise high pH laundry water solutions to improve cleaning performance (U.S. Patent No. 5,972,870, 1999) . However, this practice can also result in fabric discolouration and reduction of fabric tensile strength. All samples showed significantly high oil removal at pH 9, with the MDE4 laurate being high, 83%, *w/w* ($p<0.05$) (Figure 5-3). In addition, 0.1 M Trizma Buffer pH 9 as control showed the highest oil removal (77%, *w/w*) amongst the different pH values ($p<0.05$).

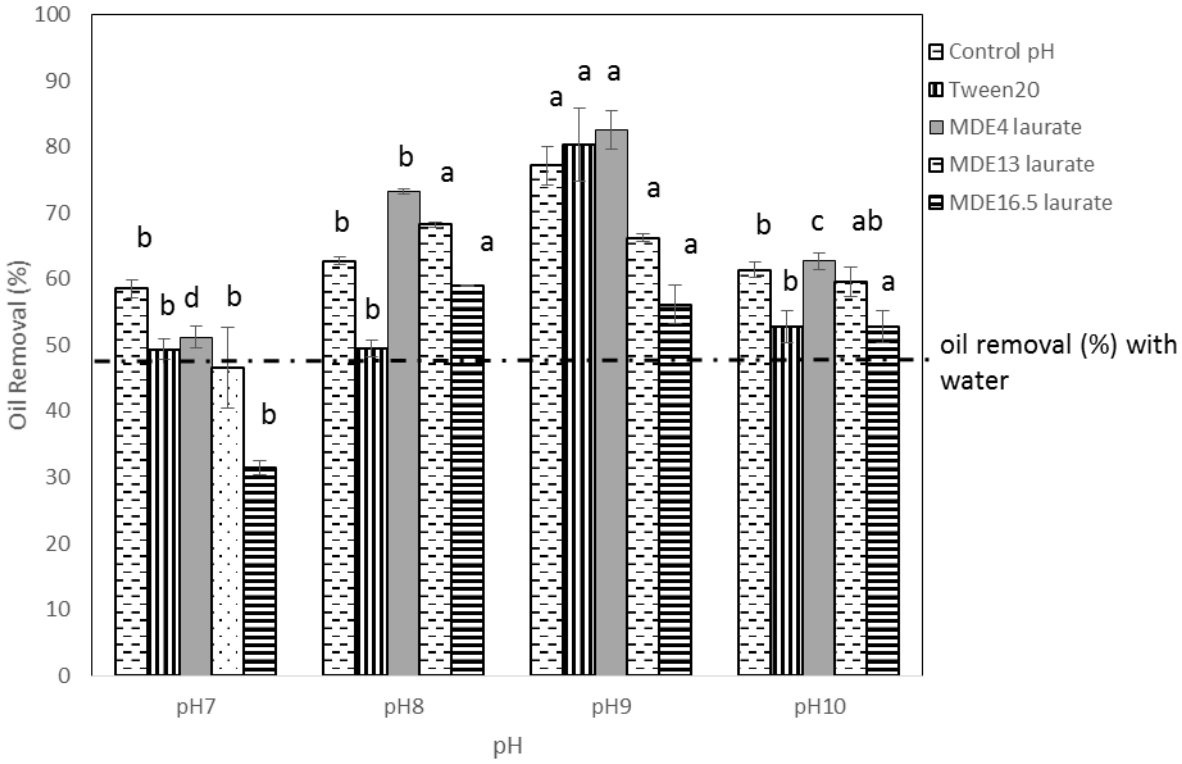

Figure 5-3: Effect of the pH of the wash solution on oil removal percentage after incubation for 1h at 37 °C. Each value represents the mean ± sd of four experiments. The line _ . _ . . represents oil removal with water. Different letters (a, b, c) indicate that the oil removal (%) values significantly differ ($p<0.05$) with the same sample.

Adding surfactants to a liquid reduces its surface tension (the affinity that the liquid's surface molecules have for each other), and therefore increase the liquid's spreading and wetting properties (U.S. Patent No. 5,972,870, 1999) . As shown in Figure 5-4, Tween 20 and maltodextrin laurate were stable in the entire pH range from 7 to 10. The 0.1 M Tris buffer pH7, Trizma buffer with pH 8-9 and bicarbonate buffer pH 10 as control, did not reduce the surface tension, however the addition of the surfactant to the buffer at the pH 7-10 led to the reduction of the surface tension ($p<0.05$) (Supplementary Table 5-3). Mukherjee (2007) reported that crude cyclic lipopeptide biosurfactants from *B.subtilis* strains were stable in pH values from 7 to 12. In addition, biosurfactants produced by the yeasts *Candida glabrata* cultivated in the glucose and cotton seed oil as substrates

demonstrated effective stability in pH 2-12 (de Luna et al., 2009). Similar to this finding, the surface tension values for the biosurfactant of *Nocardia sp*. L-417, also remained stable in all the pH values tested (from 2 to 12), indicating that the variation of the pH did not have a significant effect on the surface tension (Kim et al., 2000).

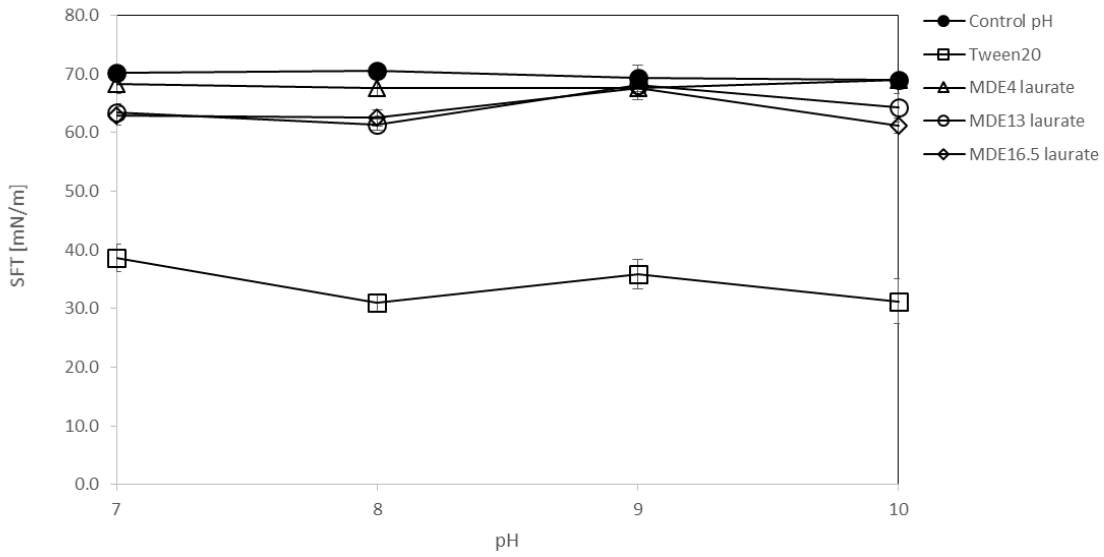

Figure 5-4: Influence of the pH on surface tension reduction property of surfactants. Each value represents the mean ± sd of two experiments.

5.3.4 Effect of surfactant concentration on the wash performance and surface tension

The effect of different concentrations of surfactants on rapeseed oil removal was investigated and the results are shown in Figure 5-5. Cotton cloth was treated with the washing solution at different concentration of surfactants (0.25-3.00%, *w/v*) at 37 °C for 1 h. As the surfactant concentration increased up to 1.0%, (*w/v*) all solutions showed improved rapeseed oil removal. The maximum oil removal percentage obtained by 1.0 %, (*w/v*) of MDE4 laurate was 83% ($p<0.05$). This was probably due to the fact that in higher surfactant

concentrations, as the surfactants get in contact with the soil/cotton and oil system they increase the contact angle and reduce the force of attraction between soil and oil. Thus, the removal of oil may be attributed to the reduction of surface and interfacial tensions by surfactant solutions. This contributes to the increase in the mobility of oil and consequently enhances their separation from the soil due to the reduction of capillary forces that are holding the soil cloth and oil together (Urum et al., 2004).

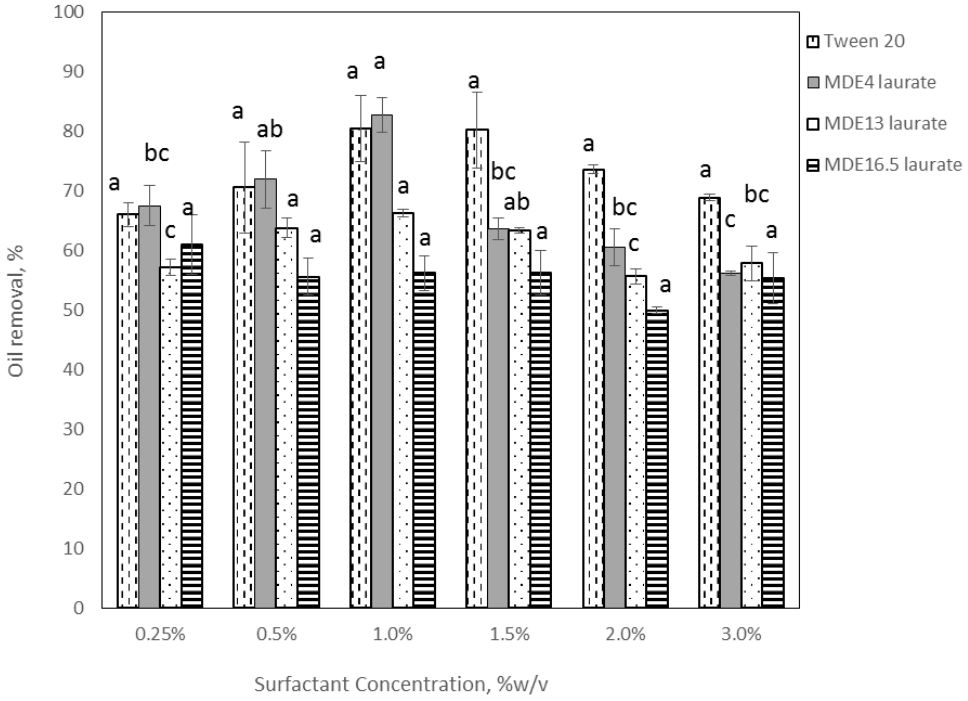

Figure 5-5: Effect of surfactants concentration on oil removal percentage after incubation for 1h at 37 °C. Each value represents the mean ± sd of four experiments. Different letters (a, b, c) indicate that the oil removal (%) values significantly differ ($p<0.05$) with the same sample.

Furthermore, for concentrations of 1.0%, (*w/v*) and above, the removal of oil was reduced (Figure 5-6). However, there was a significant reduction in the surface tension from 49.3-37.3 mN/m and 69.1-62.7 mN/m for Tween 20 and maltodextrin laurate. It is likely that

the surfactant solution could not enhance the removal of oil from the soil at concentrations greater than their critical micelle concentration (CMC) value (Abdul et al., 1990; Deshpande et al., 1999). Based on this, the suggested mechanism for oil removal is that of mobilisation, which occurred because of the reduction of interfacial tension and due to the fact that maximum crude oil removal was obtained at concentrations below the CMC. Urum and Pekdemir (2004) also reported that at greater concentrations, the low removal of oil could be attributed to the change in micelle shape and sizes. Surfactants with bulky molecular structures often lead to a change in micelle shape and size, which in turn causes micelle instability and reduction of detergency. So, oil removal may depend on the surfactant properties and the combined behaviour of surfactant/crude oil/soil systems. Thus, 1.0% (*w/v*) and 2.0% (*w/v*) of surfactant concentration was investigated further.

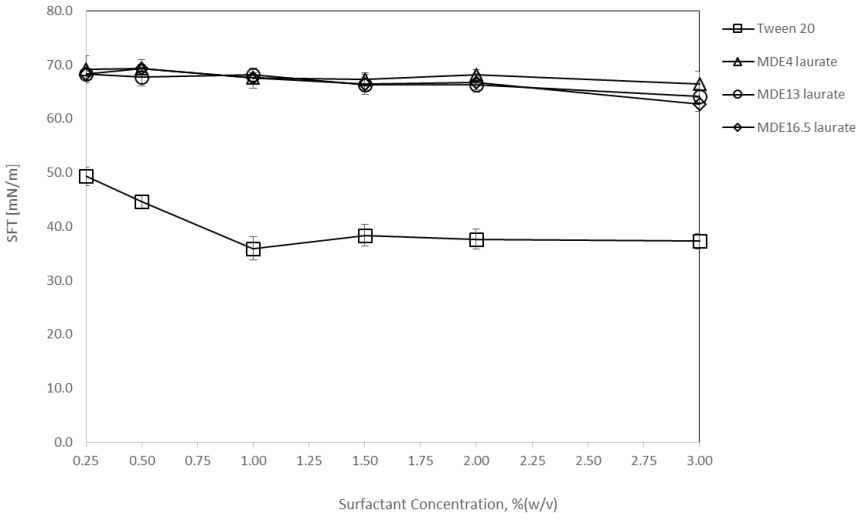

Figure 5-6: Influence of the surfactant concentration on surface tension reduction property of Tween 20 and maltodextrin laurate. Each value represents the mean ± sd of two experiments.

5.3.5 Effect of the salinity of surfactants on oil removal, solubility and surface tension

The effect of salting-out (lyotropic) of the hydrophobic group by electrolytes such as NaCl on the oil removal and surface tension was investigated. Figure 5-7 shows the oil removal as a function of salinity with concentrations of maltodextrin laurate equal to 1% and 2% (*w/v*) during treatment at 37 °C for 1 hour. At 1.0% (*w/v*) of surfactants concentration, the addition of 1% (*w/v*) NaCl improved the oil removal about 6-16% and reached the oil removal maximum of the Tween 20 (86%, *w/w*) ($p>0.05$) [Figure 5-7 (a)]. After that, increasing the salinity up to 7% (*w/v*) reduced the detergency performance for rapeseed oil removal ($p<0.05$). Then, the effect of salinity was further investigated by increasing the surfactant concentration to 2.0%, (*w/v*). The oil removal reached a maximum at 1%, (*w/v*) NaCl with the highest one being demonstrated by Tween 20 (85%, *w/w*) as shown in Figure 5-7 (b). All maltodextrin laurates (MDE4, MDE13 and MDE16.5) showed an increase of oil removal from 50-60% to 62-80% ($p<0.05$), *w/w* with the addition of 1% (*w/v*) NaCl. Additionally, increasing the NaCl up to 7% (*w/v*) reduced the detergency performance ($p<0.05$).

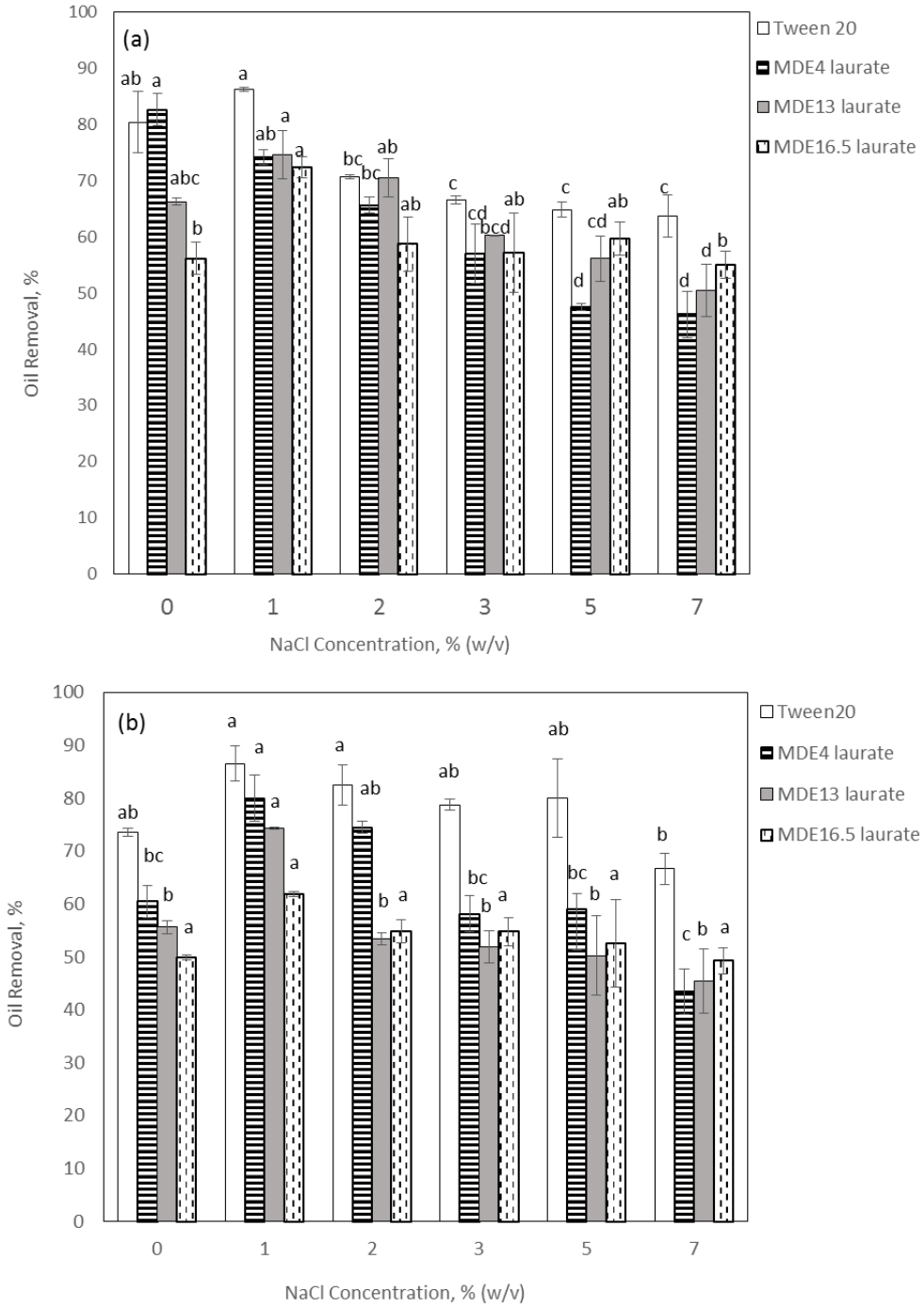

Figure 5-7: Effect of surfactants concentration and salinity on the wash performance with (a) 1% (*w/v*) surfactants and (b) 2% (*w/v*) surfactants in 0.1 M Trizma Buffer at pH 9 after incubation at 37 °C for 1 h. Each value represents the mean ± sd of four experiments. Different letters (a, b, c) indicate that the oil removal (%) values significantly differ ($p<0.05$) with the same sample.

To relate the salinity concentration with the oil removal result, the solubility for all the samples in different saline concentrations was tested. Based on Figure 5-8, at 1% (*w/v*) of surfactants concentration, adding NaCl to 1% (*w/v*), the solubility for all samples was decreased ($p>0.05$). However, when the concentration of NaCl increased up to 7% (*w/v*), the solubility decreased ($p<0.05$). At 2% (*w/v*) of surfactants concentration, a similar trend was also observed. The solubility decreased when NaCl was added up to 7% (*w/v*) ($p<0.05$) in Tween 20, MDE4 and MDE13, but not in MDE16.5 ($p>0.05$). Increase in the salt concentration causes the dissociation of the salt where the sodium chlorine ions unite with the water molecules forming strong bonds, hence the phase separation becomes easier, the interactions between the surfactant and water become increasingly smaller, and these ultimately lead to decreased solubility (de Lemos Araújo et al., 2015).

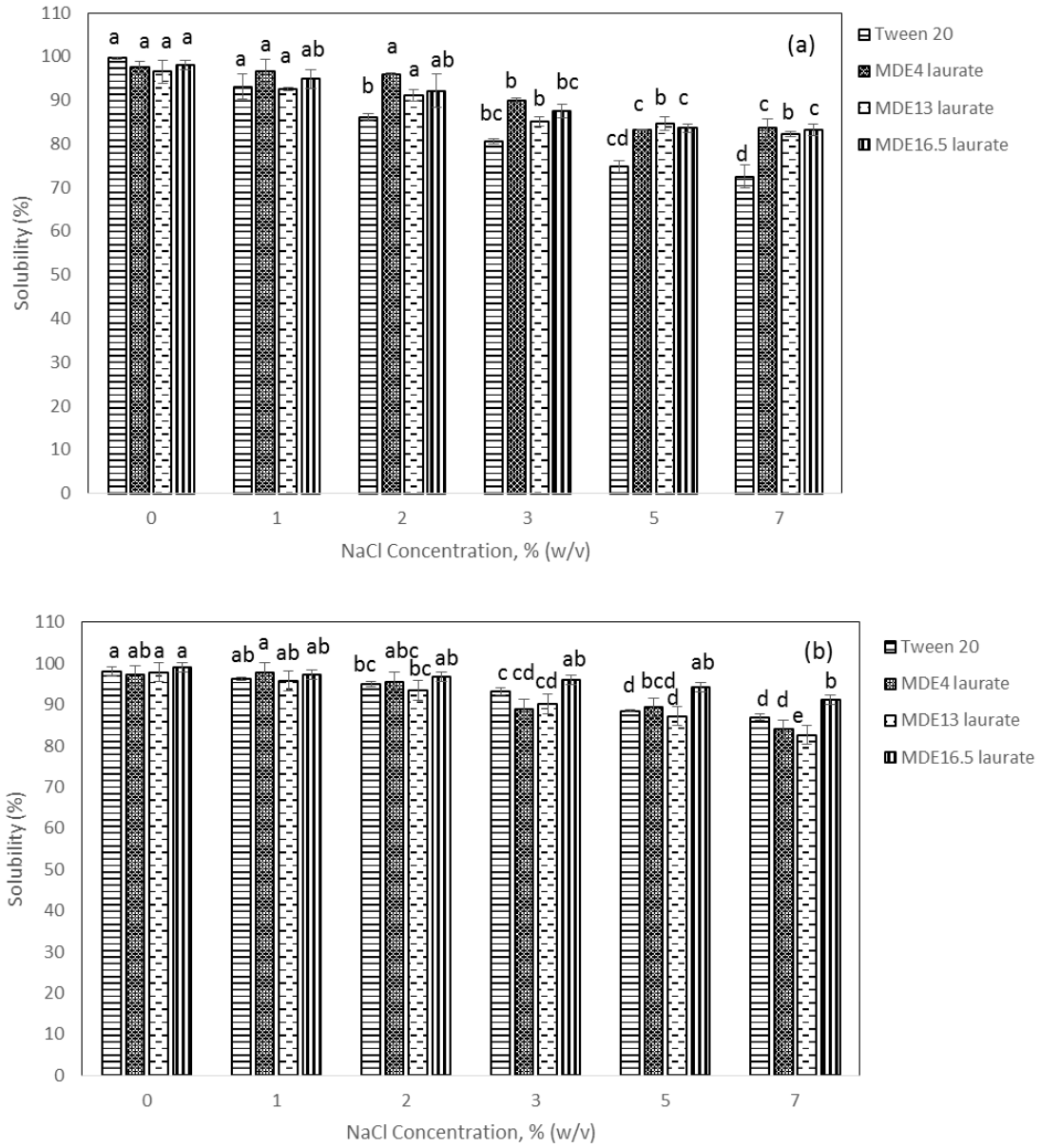

Figure 5-8: Effect of surfactants concentration and salinity on the solubility of the surfactant in washing solutions with (a) 1% (*w/v*) of surfactants and (b) 2% (*w/v*) of surfactants in 0.1 M Trizma buffer at pH 9 after incubation at 37 °C for 1 h. Each value represents the mean ± sd of two experiments. Different letters (a, b, c) indicate that the solubility (%) values significantly differ ($p<0.05$) with the same sample.

Figure 5-9 shows the influence of the surfactant concentration and salinity to the surface tension. In solutions containing 1% (*w/v*) surfactant, the increase of NaCl concentrations up to 7% (*w/v*) reduced the surface tension of Tween 20 and maltodextrin

laurate from 31.3-35.8 and 54.0-68.1 mN/m ($p<0.05$). At 2% (*w/v*) of Tween 20 and maltodextrin laurate, the surface tension also reduced from 28.1-37.7 and ~ 54.0-68.1 mN/m when the salinity increased up to 7% (*w/v*) ($p<0.05$). de Luna et al. (2009) reported that the surface tension of the cell-free broth of *Candida glabrata* containing the biosurfactant was stable, and independent of the concentration of added salt. Increasing the electrolyte concentration causes the surfactant solution to become more hydrophobic and thus segregate more towards the oil-water interface, thereby reducing the surfactant surface tension and interfacial tension. This phenomenon is mainly observed for ionic surfactants, however, it has been also reported for non-ionic surfactants (Do et al., 2009).

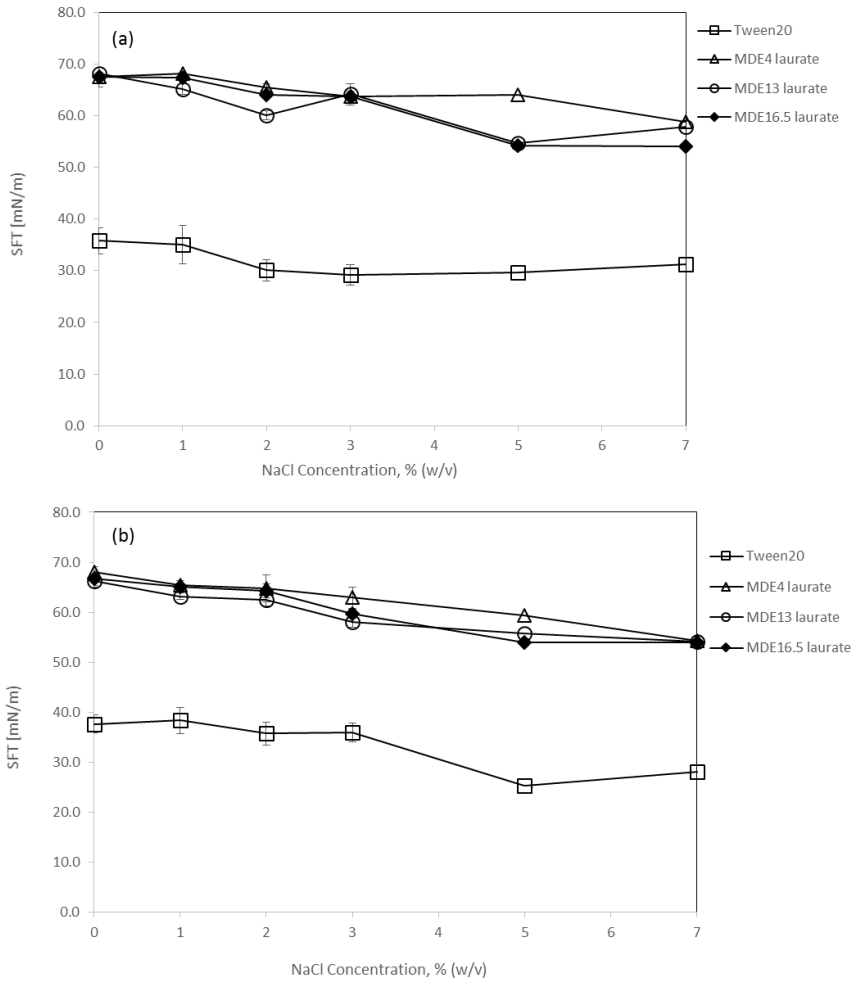

Figure 5-9: Effect of concentration of the surfactants and salinity of the 0.1M Trizma Buffer pH9 on the surface tension in (a) 1% (*w/v*) surfactants and (b) 2% (*w/v*) surfactants. Each value represents the mean ± sd of three experiments.

5.3.6 Effect of temperature of the washing solutions on the oil removal and solubility of surfactants.

The effect of temperature of the washing solutions on the oil removal was investigated by measuring the detergent efficiency at three different temperatures (25, 37 and 60 °C) (Figure 5-10). Figure 5-10 (a) shows at 1% (*w/v*) of surfactant solution, the increase in the temperature from 25 to 37 °C greatly enhanced the oil removal in Tween 20 from 60-86%, MDE4 laurate from 63-74%, MDE13 laurate from 51-75% and MDE16.5

laurate from 66-72% ($p<0.05$). However, the oil removal decreased when the washing temperature increased from 37 to 60 °C (statistical significantly for Tween 20 and MDE16.5 ($p<0.05$) but not for MDE4 and MDE13 laurate ($p>0.05$)). The trend is clear and indicates that the optimal detergency temperature is around 37 °C.

However, at higher concentration of surfactants, 2% (*w/w*) the optimal detergency temperature was higher at 60 °C (Figure 5-10 (b)). MDE13 and MDE16.5 laurate showed a significant increase in oil removal when the detergency temperature increased from 37 to 60 °C, with the highest oil removal being equal to 82%, *w/w* and 80%, *w/w* respectively ($p<0.05$). For non-ionic surfactants, increasing the temperature causes the surfactant solution to become more hydrophobic and segregate more towards the oil-water interface (Do et al., 2009). Therefore, these findings suggest that the current washing solution formulation can work efficiently over a wide range of temperatures.

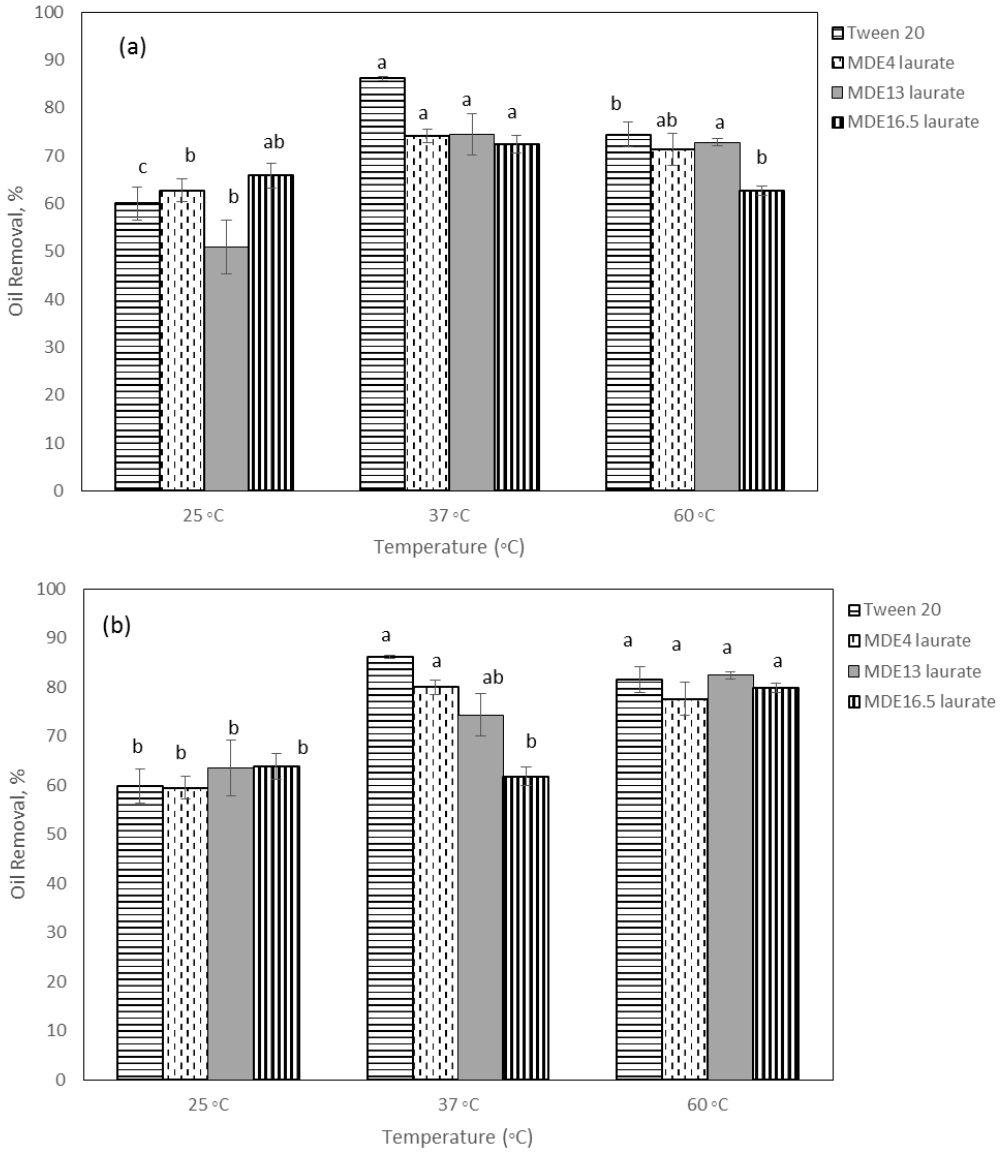

Figure 5-10: Effect of detergency temperature on the wash performance (a) 1% (*w/v*) surfactants (b) 2% (*w/v*) surfactants in 0.1M Trizma Buffer at pH9 with 1% (*w/v*) of NaCl after incubation for 1 h. Each value represents the mean ± sd of four experiments. Different letters (a, b, c) indicate that the oil removal (%) values significantly differ ($p<0.05$) with the same sample.

Furthermore, the effect of the temperature of the washing solution to the solubility of the surfactants was investigated in detail. As shown in Figure 5-11, at 1% (*w/v*) and 2% (*w/v*) of surfactants concentration, the solubility did not change significantly when the detergency temperature increased from 25 to 60 °C ($p>0.0.05$). Thus, it can be stated that

the solubility of maltodextrin laurate was not significantly affected by temperature ($p>0.05$) (as indicated previously in Section 3.3.2).

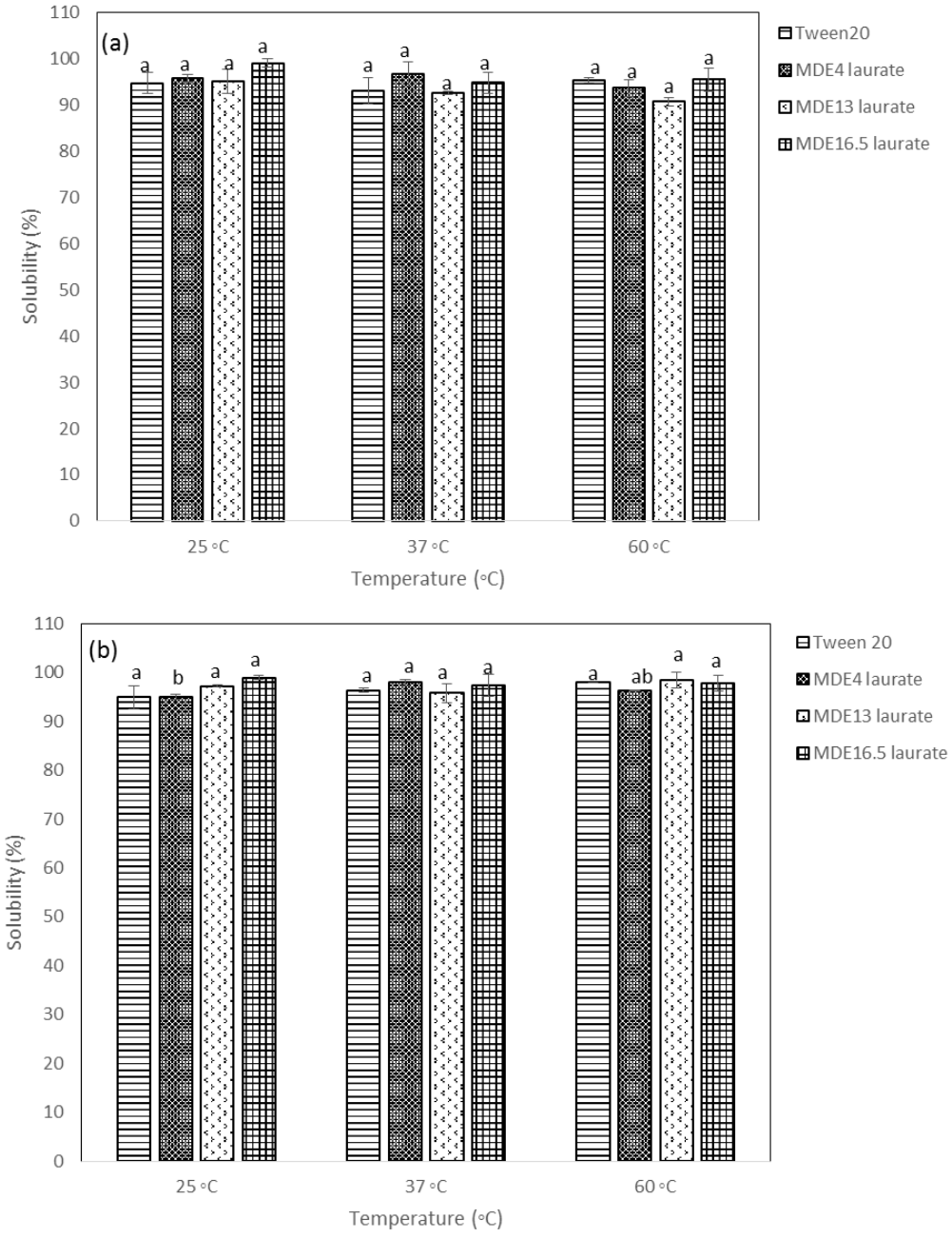

Figure 5-11: Effect of temperature on the solubility of the surfactant in washing solutions with (a) 1% (*w/v*) of surfactants and (b) 2% (*w/v*) of surfactants in 0.1M Trizma buffer with 1% (*w/v*) NaCl at pH 9 after incubation for 1 h. Each value represents the mean ± sd of three experiments. Different letters (a, b, c) indicate that the solubility (%) values significantly differ ($p<0.05$) with the same sample.

5.3.7 Visual observations

Figure 5-12 shows the washing solutions after the wash steps using two surfactants concentrations, 1% and 2 % (*w/v*) at 60 °C for 1 h. These washing conditions were chosen as an example of visual observation of the efficacy of the surfactants due to the higher oil removal (> 90%). The removed oil by the Tween 20 solution was found to suspend in the washing solution with much smaller particles compared to the maltodextrin laurate solutions. Among the maltodextrin laurate samples, the removed oil was found dominantly in MDE4 laurate solution with a relative mixture of large and small oil droplets floating on the surface. Surfactants that can produce small liquid droplets tend to remove the oil more effectively compared to those that produce large droplets.

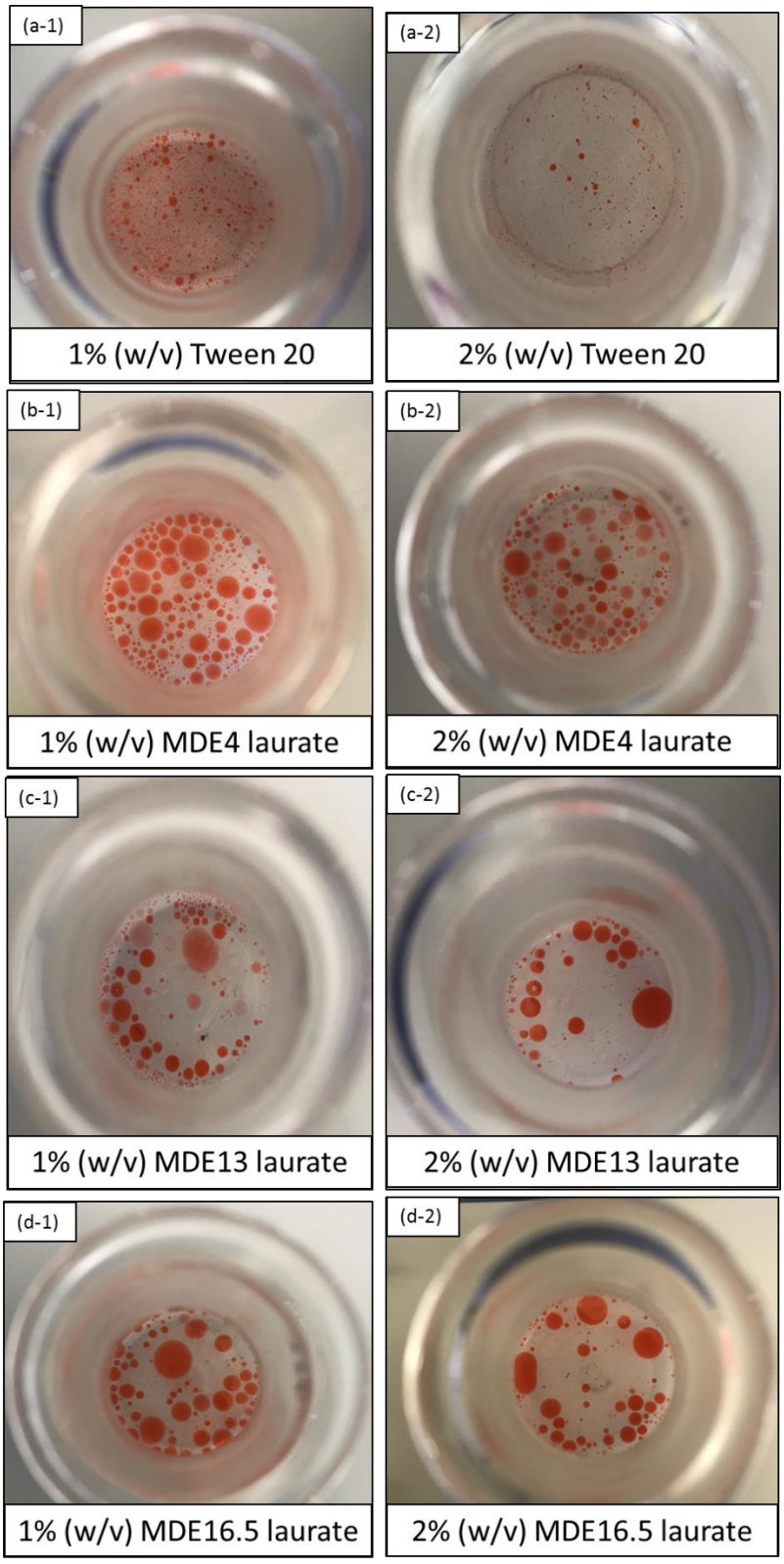

Figure 5-12: Photographs of washing solutions of the cotton cloth stained with Sudan red dyed rapeseed oil after the wash step (a-1) 1% (*w/v*) of Tween 20 (a-2) 2% (*w/v*) Tween 20 (b-1) 1% (*w/v*) MDE4 laurate (b-2) 2% (*w/v*) MDE4 laurate (c-1) 1% (*w/v*) MDE13 laurate (c-2) 2% (*w/v*) MDE13 laurate (d-1) 1% (*w/v*) MDE16.5 laurate (d-2) 2% (*w/v*) MDE16.5 laurate with 1% (*w/v*) of NaCl at 60°C for 1 h.

5.4 Conclusions

CFAE could be potentially desirable components of detergents, based on the findings of this study in terms of their compatibility, stability and efficiency and can be used in conjuction with commercial non-bio laundry detergents, to improve the washing performance. The improved performance of washing solutions containing maltodextrin laurate, buffer and saline (NaCl) and the studies investigating the effect of temperature, gave useful insights of the surfactants' potential in detergent formulations. The results of the study show that the future use of CFAE as eco-friendly laundry detergents is highly promising.

Acknowledgement

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Supplementary materials

Supplementary Table 5-3: Influence of the pH on surface tension reduction property of Tween 20 and maltodextrin laurate.

	SFT [mN/m]			
pH	7	8	9	10
Control	70.2 ± 1.32^{aA}	70.5 ± 0.85^{aA}	69.4 ± 2.12^{bA}	69.0 ± 0.96^{bA}
Tween 20	38.5 ± 2.33^{aD}	31.0 ± 1.41^{cE}	35.8 ± 2.56^{bC}	31.2 ± 3.87^{cD}
MDE4 laurate	68.3 ± 1.62^{bB}	67.6 ± 1.40^{cB}	67.5 ± 0.98^{cB}	69.0 ± 0.70^{aA}
MDE13 laurate	63.4 ± 1.42^{cC}	61.3 ± 0.99^{dD}	68.1 ± 1.22^{aB}	64.2 ± 2.40^{bB}
MDE 16.5 laurate	62.8 ± 1.56^{bC}	62.5 ± 1.37^{bC}	67.5 ± 1.85^{aB}	61.1 ± 1.25^{cC}

All experiments were conducted two times with calculating the mean ± standard deviation. Different letters (a, b, c) in the same row indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same sample. Different letters (A, B, C) in the same column indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same pH of the wash solution.

Supplementary Table 5-4: Influence of the surfactant concentration on surface tension reduction property of Tween 20 and maltodextrin laurate.

% (w/v)	SFT [mN/m]					
Surfactants	0.25	0.50	1.00	1.50	2.00	3.00
Tween 20	49.3 ± 1.75^{aC}	44.6 ± 1.16^{bC}	36.0 ± 2.12^{dB}	38.4 ± 2.00^{cC}	37.7 ± 1.81^{cC}	37.3 ± 1.43^{cdD}
MDE4 laurate	69.1 ± 2.55^{aA}	69.3 ± 0.68^{aA}	67.5 ± 0.98^{bA}	67.3 ± 0.85^{bcA}	68.1 ± 0.97^{bA}	66.4 ± 2.37^{cA}
MDE13 laurate	68.3 ± 0.55^{aB}	67.8 ± 1.73^{aB}	68.1 ± 1.22^{aA}	66.3 ± 0.95^{bB}	66.2 ± 0.83^{bB}	64.1 ± 1.35^{cB}
MDE 16.5 laurate	68.2 ± 0.95^{bB}	69.3 ± 1.66^{aA}	67.5 ± 1.85^{bcA}	66.5 ± 2.04^{dB}	66.7 ± 1.71^{cdB}	62.7 ± 1.25^{eC}

All experiments were conducted two times with calculating the mean ± standard deviation. Different letters (a, b, c) in the same row indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same sample. Different letters (A, B, C) in the same column indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same surfactant concentration of the wash solution.

Supplementary Table 5-5: Influence of the surfactant concentration 1% (*w/v*) and NaCl concentration on surface tension reduction property of Tween 20 and maltodextrin laurate.

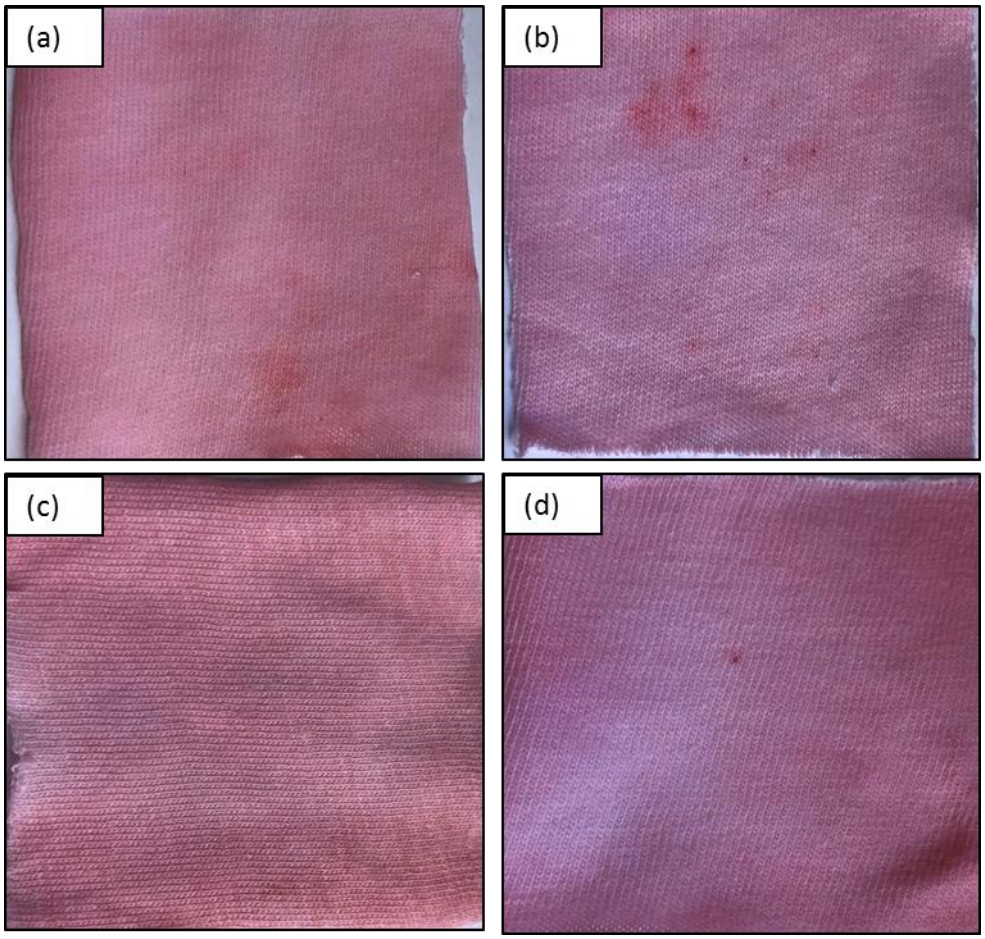
Surfactants	NaCl Concentration, % (*w/v*)					
	0	1	2	3	5	7
Tween20	35.8 ± 2.56^{aB}	35.1 ± 3.73^{aC}	30.1 ± 2.11^{bA}	29.2 ± 1.92^{bB}	29.6 ± 1.81^{bC}	31.3 ± 2.14^{bD}
MDE4 laurate	67.5 ± 0.98^{bA}	68.1 ± 0.36^{aA}	65.5 ± 0.80^{cA}	63.7 ± 0.64^{dA}	64.1 ± 1.31^{dA}	58.9 ± 0.92^{eA}
MDE13 laurate	68.1 ± 1.22^{aA}	65.2 ± 1.02^{bB}	60.1 ± 0.90^{dC}	64.2 ± 2.02^{cA}	54.7 ± 2.42^{fB}	57.9 ± 1.52^{eB}
MDE16.5 laurate	67.5 ± 1.85^{aA}	67.5 ± 0.49^{aA}	64.0 ± 0.46^{bB}	63.7 ± 1.12^{bA}	54.3 ± 0.40^{cB}	54.0 ± 0.63^{cC}

All experiments were conducted two times with calculating the mean ± standard deviation. Different letters (a, b, c) in the same row indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same sample. Different letters (A, B, C) in the same column indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same NaCl concentration of the wash solution.

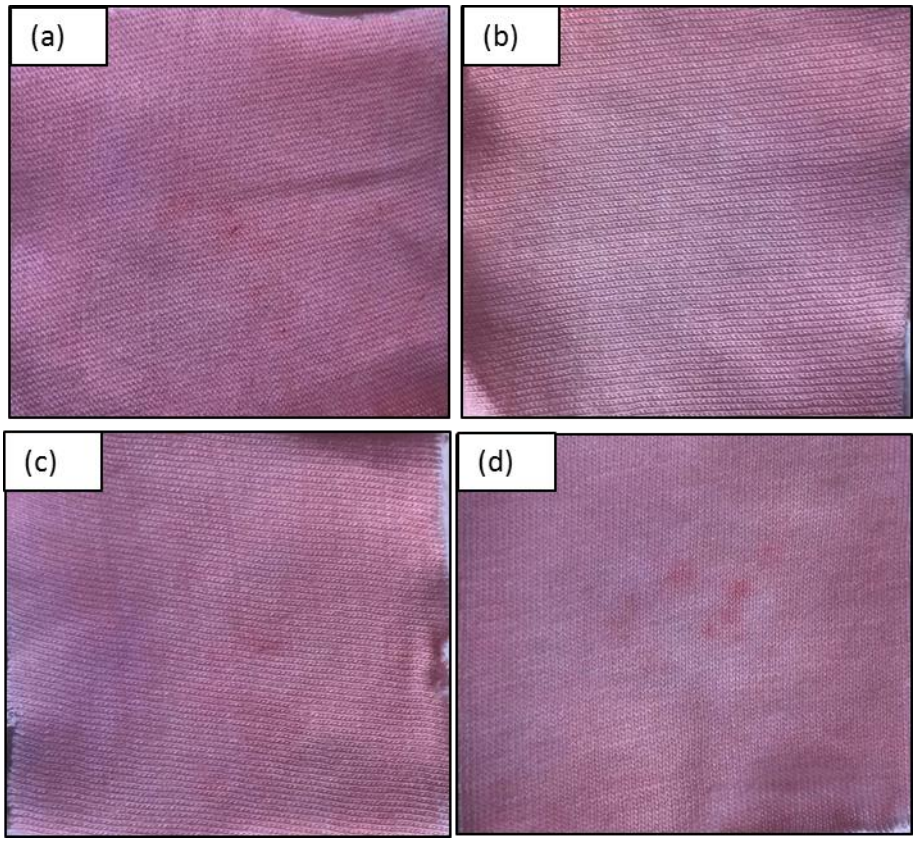
Supplementary Table 5-6: Influence of the surfactant concentration 2% (*w/v*) and NaCl concentration on surface tension reduction property of Tween 20 and maltodextrin laurate.

Surfactants	NaCl Concentration, % (*w/v*)					
	0	1	2	3	5	7
Tween20	37.7 ± 1.81^{aC}	38.4 ± 2.61^{aC}	35.8 ± 2.30^{bC}	35.9 ± 1.93^{bD}	25.3 ± 1.16^{dD}	28.1 ± 2.36^{cB}
MDE4 laurate	68.1 ± 0.97^{aA}	65.4 ± 0.92^{bA}	64.8 ± 2.69^{bA}	63.0 ± 2.11^{cA}	59.5 ± 0.91^{dA}	54.4 ± 0.83^{eA}
MDE13 laurate	66.2 ± 0.83^{aB}	63.2 ± 0.71^{bB}	62.4 ± 1.11^{cB}	58.1 ± 1.02^{dC}	55.8 ± 1.23^{eB}	54.1 ± 0.90^{fA}
MDE16.5 laurate	66.7 ± 1.71^{aB}	65.1 ± 0.65^{bA}	64.3 ± 1.38^{cA}	59.7 ± 0.89^{dB}	53.9 ± 0.68^{eC}	54.0 ± 0.63^{eA}

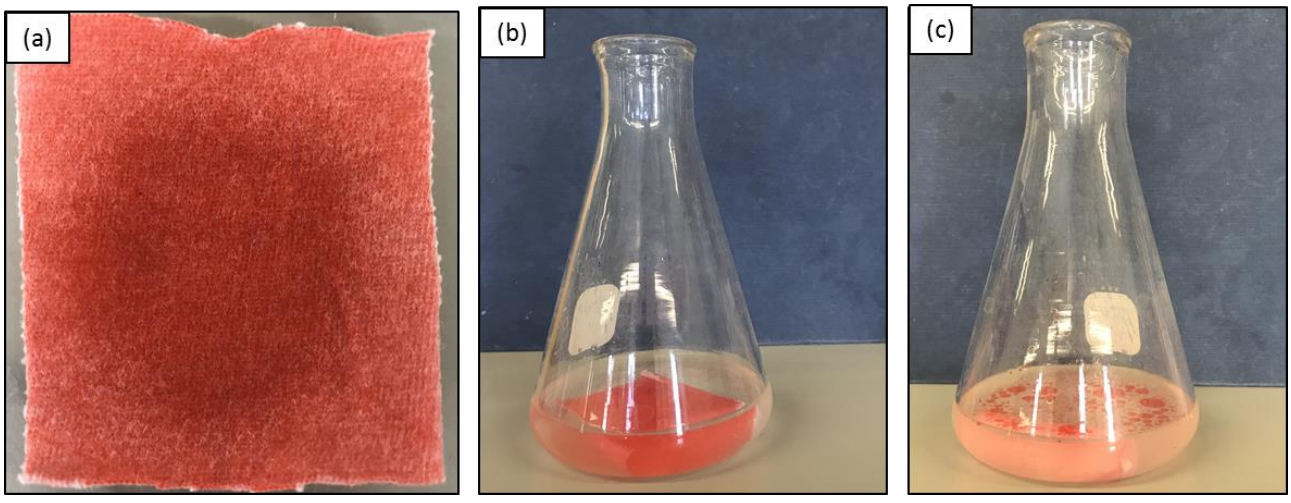
All experiments were conducted two times with calculating the mean ± standard deviation. Different letters (a, b, c) in the same row indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same sample. Different letters (A, B, C) in the same column indicate that the surface tension (mN/m) values significantly differ ($p<0.05$) with the same NaCl concentration of the wash solution.



Supplementary Figure 5-13: Photographs of cotton cloth soiled with Sudan red dyed rapeseed oil after washing with 1% (*w/v*) surfactants solutions (a) Tween 20 (b) MDE4 laurate (c) MDE13 laurate (d) MDE16.5 laurate with 1% (*w/v*) NaCl at 60°C for 1 h.



Supplementary Figure 5-14: Photographs of cotton cloth soiled with Sudan red dyed rapeseed oil after washing with 2% (*w/v*) surfactants solutions (a) Tween 20 (b) MDE4 laurate (c) MDE13 laurate (d) MDE16.5 laurate with 1% (*w/v*) NaCl at 60°C for 1 h.



Supplementary Figure 5-15: Washing step (a) cotton cloth stained with Sudan red dyed rapeseed oil (b) in the surfactant solution (b) after a wash in surfactant solution.

Chapter 6 - General Discussion and Future Perspectives

The market demand for surfactants (surface active materials) is increasing across various industries including food, detergents, pharmaceuticals, cosmetics, water treatment and paints, and is currently in line with the rising socio-economic standards of countries across the globe (Akbari et al., 2018; Karmee, 2008; Khan & Rathod, 2015; Lu et al., 2017; Mahamallik & Pal, 2017). Hence, the production of petrochemical-based surfactants is increasing continuously as the technology around this sector is already available, and as a result of the majority of the industry markets still rely on petrochemicals. Caresana (2012) reported that household detergents and cleaners are the most representative applications of surfactants from a consumer point of view, and the demand from this sector was expected to grow by 2.6% by 2018. Based on this, there is a strong demand from consumers, industry and national and international authorities to find alternative renewable sources that can replace petroleum-based surfactants and can meet the current needs of industrial and domestic applications.

The chemical industry is exploiting the utilisation of renewable materials such as fats, oils and carbohydrates as feedstocks for chemical synthesis of surfactants. The first commercial chemical process for the preparation of sugar fatty acid esters (SFAEs) was realised by Nippon Co., Japan, in 1959 (Karmee, 2008). Today the major producers of sucrose esters are *DaiIchi-Kogyo Seiyaku* and *Mitsubishi* in Japan, *Croda* in the USA, *Sisterna* (a joint venture of *Dai-Ichi* with *Suiker Unie* from The Netherlands) and *Goldschmidt* in Germany (Hill & Rhode, 1999). However, the chemical synthesis of surfactants requires the use of high temperatures (above 100°C), which leads to high-energy cost for the overall production process and requires challenging and multistep separations (refining, drying, pulverizing, sieving) (Neta et al., 2015).

Currently, the attention is on alternative environmentally friendly processes for the production of different types of renewable surfactants. The enzymatic synthesis of carbohydrate fatty acid esters (CFAE) can be considered advantageous as it represents a low energy process (reaction temperatures range between 40-60°C), and typically produces monoesters that are relatively easy to purify and are accompanied by high conversion yields (Neta et al., 2015). Carbohydrates from plant-based renewable materials such as cellulose and starch represent advantageous substrates for enzymatic esterification. To this end, hydrolysed products of starch, namely maltodextrins, with DE lower than 20 are soluble in water and hold potential as raw materials for the enzymatic reaction with medium long-chain saturated fatty acid (e.g. lauric acid). Saturated fatty acids have been reported to form more stable complexes with starch compared to unsaturated fatty acids or mono or di-acylglycerols (Arijaje & Wang, 2017; Tufvesson et al., 2003). The produced amphiphilic polymers may present a good ability for oil emulsification probably due to steric stabilisation with respect to their macromolecular structure (Sadtler et al., 2002). Previously, amphiphilic maltodextrin was produced through esterification reaction using maltodextrin (DE16) and three fatty acids (decanoic acid, lauric acid and palmitic acid). This study using the lipase enzyme obtained from *Thermomyces lanuginosus* resulted in a low degree of substitution (DS) 0.015–0.084 at optimum conditions of 60 °C for 4 h (Udomrati & Gohtani, 2014). This research focused on the effect of amphiphilic maltodextrin with three molecular weight fatty acids; it was found that the produced molecules had emulsifying activities in *n*-hexadecane oil/water (O/W) emulsions with the lower molecular weight fatty acid (highest DS) seemed to be less effective in stabilising emulsions. In this PhD work, an efficient strategy of exploiting maltodextrin and vinyl laurate was demonstrated through the use of transesterification reactions with immobilised lipase from *Candida antarctica* (Novozym® 435). The transesterification reactions with a molar ratio of vinyl laurate/maltodextrin (2:1 to 4:1) and 0.3% (*w/v*) immobilised lipase enzyme (Novozym® 435), at optimum conditions

successfully produced maltodextrin laurate with higher DS (**Chapter 3**). This finding opens up opportunities for using immobilised enzymes in transesterification reactions to produce a range of CFAEs with functional properties. In order to render the process more cost-effective, immobilised enzymes can be used, which can be recycled several times. The commercial immobilised lipase *Candida antartica* lipase B (Novozym® 435) produced by Novozymes is based on the immobilisation of CALB on macroporous acrylic resin support crosslinked with divinylbenzene. With this method, the enzyme can be used over many batches (5-10 times) and remain stable, especially under conditions of high temperature (20-110°C), pressure, and pH, or when using non-conventional media, such as organic solvents (Novozymes, 2016).

In this reaction, a mixture of an organic solvent *ter*-butanol (*t*-BuOH) and dimethylsulfoxide (DMSO) (90:10) was applied in order to increase the solubility of polar and non-polar substrates and the reversibility of the thermodynamic equilibrium of the hydrolysis reaction (Doukyu & Ogino, 2010). In this work, 1g of maltodextrin in 10 ml DMSO was dissolved with the vinyl laurate (at different ratio) in 90ml of *t*-BuOH, this mixture of solvents reduced the solubility of the maltodextrin, and as a result precipitation of the amylose occurred (crystalline complex due to the linear structure of amylose). Thus, the solubility of the substrates (maltodextrin and vinyl laurate) in this mixture of solvents led to low yields of ester production (**Chapter 3**). Thus, in future work, the solubility of the reacting substrates in different ratios of solvent mixtures (increasing DMSO and reducing *t*-BuOH concentration) could be an advantage and should be investigated in order to increase the solubility of maltodextrin in a DMSO/*t*-BuOH mixture solvent and potentially produce higher yields. As a result of the low yield obtained in this work, as it stands this method is more suitable to laboratory-scale as it would be difficult to scale up.

In view of the subsequent downstream processing for the purification of the synthesised esters, it should be noted that there is a requirement for large amounts of solvents such as DMSO and *t*-BuOH, even though they are removed prior to precipitating the product. Therefore, performing the enzymatic process in a large scale would lead to for the need for large amounts of organic solvents, such as DMSO and *t*-BuOH, which are potential environmental pollutants and may pose health hazards. To this end, future work could focus on alternative technologies such as supercritical fluid extraction or ionic liquids, which have emerged as greener solvent systems. In this study, preliminary experiment have been conducted in supercritical fluid carbon dioxide ($SFCO_2$) using immobilised enzyme for transesterification reaction using the optimal conditions obtained in the solvent reaction. This reaction using $SFCO_2$ successfully produced maltodextrin laurate. However, the products produce are not achieve the purity thus the reaction condition need to improve in order to produce the purify products.

The crude product of transesterification contained a mixture of monoesters, diesters as well as residual maltodextrin, vinyl laurate. In this crude maltodextrin laurate product, the solubility observed depends on the DS (**Chapter 3**). As such, in future, it would be interesting to investigate the contribution of the different purified components (monoester, diester) present in the product, to the properties of air/water interfaces stabilised by maltodextrin laurate. Previously, these compounds reported to have completely different surface activities and functional properties compared to the pure components (van Kempen et al., 2013a). Hence, the composition of pure components should affect their performance (e.g. foaming and emulsion stabilisation) (van Kempen et al., 2013a). For example, a diester has a higher hydrophobicity than a monoester which leads to reduced solubility and in turn, low solubility with water will lead to slower adsorption at the interface (Mackie & Wilde,

2005). Therefore, higher surface activity is expected for diesters compared to monoesters (van Kempen et al., 2013a).

In this present study, the formation and stability of maltodextrin laurate esters in o/w emulsion was monitored. Husband et al. (1998) indicated that the crude product of sucrose lauric acid esters improved the foaming properties compared to the pure components of the reaction (sucrose and lauric acid). Investigation of the crude maltodextrin laurate (MDE4, MDE13 and MDE16.5) in the formation and stability of maltodextrin laurate in oil/water emulsion showed that low DE of maltodextrin was stable for a long period of time due to the higher molecular fractions of high molecular weight maltodextrin (**Chapter 4**). Thus, these findings with crude maltodextrin laurate are essential to provide the understanding of the formation, structures, and properties of emulsions, which is necessary to understand the creation and stabilisation of structures in food matrices. The emulsifier must possess suitable functional properties to confer stability against droplet coalescence during shelf-life and it has to be food grade (Partal et al., 1999). Sugar fatty acid esters used in ice cream, soup and mayonnaise, are marked as E 473. Thus, the identification of non-toxic, food-grade surfactants that possess adequate surface activity properties is of great interest to the food industry. Thus, the potential of maltodextrin laurate to be used as emulsifier and stabiliser of O/W food emulsions would need additional studies (e.g., animal model studies) to ensure that the ingredient proposed for use in food has low toxicity (Kralova & Sjo¨blom, 2009). As such, the detection of saturated fatty acid (lauric acid) using gas chromatography could be developed to determine its percentage contribution to the fatty acid profile. The use of supercritical carbon dioxide as a solvent, followed by purification and distillation, could be an appropriate avenue for producing food-grade maltodextrin which would be safe to use as an emulsifier in food systems.

The regulations that currently exist include specification standards for detergents and control the addition of phosphates in order to reduce water pollution, however, they do not provide guidance on the other components of the cleaning formulations. Laundry detergents are popular as they can be used automatically into the washing machine, impart softness, antistaticness, resiliency to fabrics, they are mild to eyes and skin, and demonstrate satisfactory dispersibility in water (Bajpai & Tyagi, 2007). This is an important finding as it demonstrates that enzymatically produced CFAE is suitable for the detergent industry. Hence, the present work explored the application of maltodextrin laurates as laundry detergents. Maltodextrin, a hydrolysed product from starch is a natural resource that a biodegradable; it can be degraded by microorganisms, but this does not necessarily mean that it does not cause damage to the ecosystem (Kogawa et al., 2017). The use of maltodextrin laurates in washing solutions under controlled condition was associated with a high oil removal capability (~80% or more), which render them as promising candidates for laundry detergent formulations (**Chapter 5**). In order to further investigate the performance of maltodextrin laurate esters as detergents, ultimately assessment using full-scale domestic appliances together with quality controls (e.g. ecotoxicity, toxicology, microbiology counts, and biocidal efficiency) are needed in order to test safety and compliance with regulations.

The biotechnology industry will continue to move toward low-cost and highly efficient renewable surfactants for use in industrial applications. Development of the proposed route for the production of CFAE using as basis polysaccharides have considerable potential. The use of low-cost renewable sources as starting materials, combined with a fast enzymatic production process could impact on reducing the total surfactant production costs, leading to the potential commercialisation of such process. Overall, it has been shown through this PhD work that maltodextrin laurate is a good candidate and has potential applications in foods and in detergent formulations.

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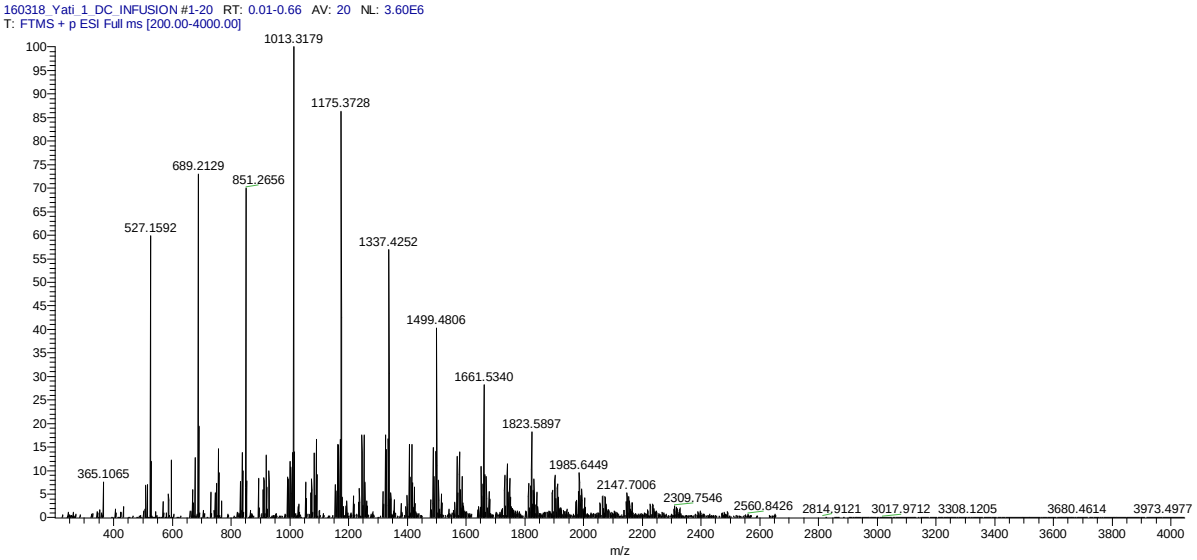
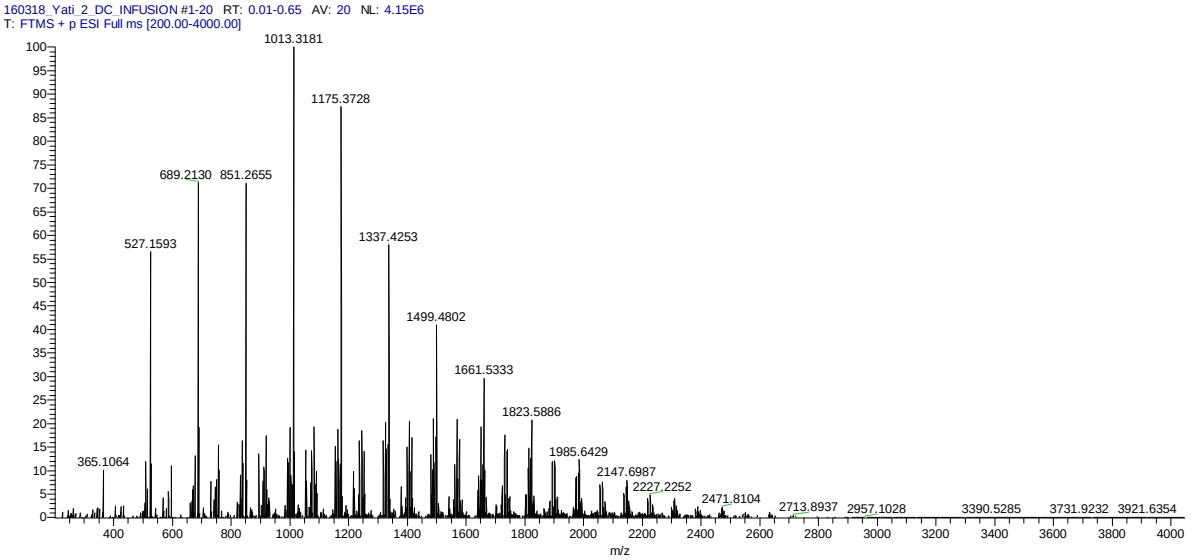
APPENDIX I

ESI MASS SPECTRUM MDE4 LAURATE





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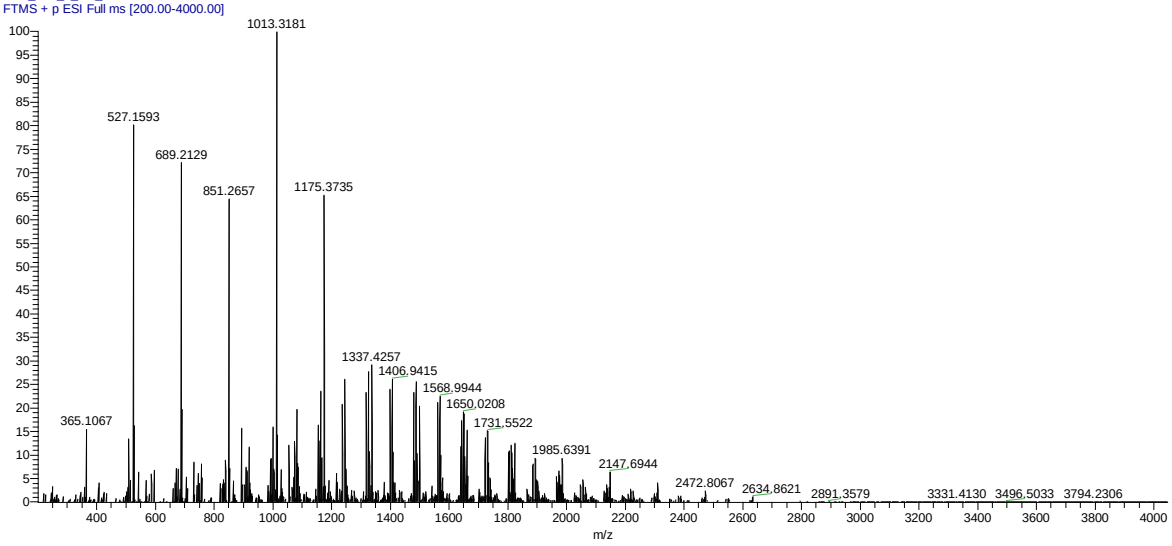

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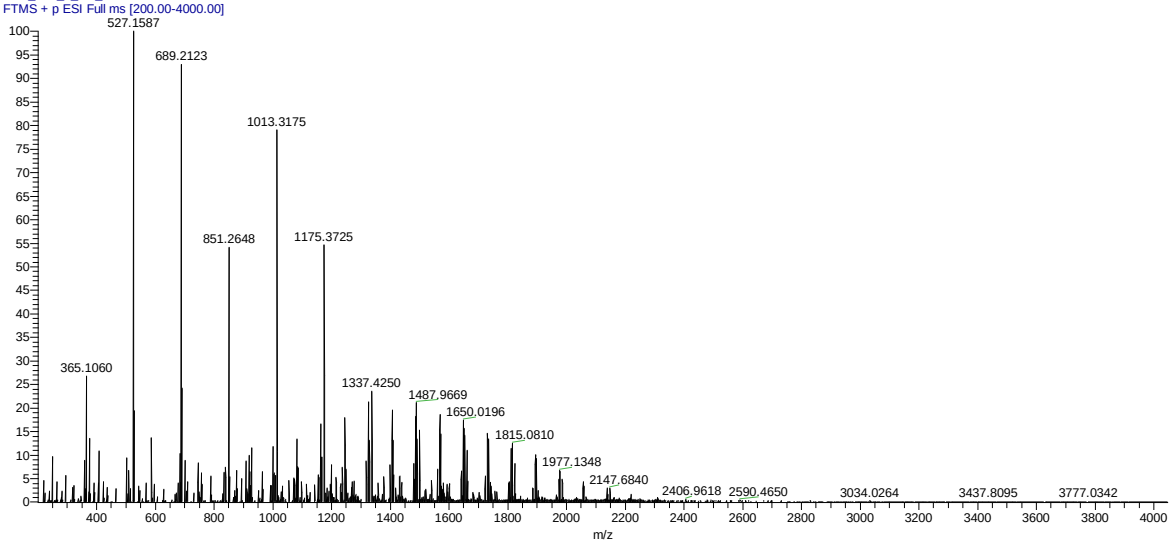

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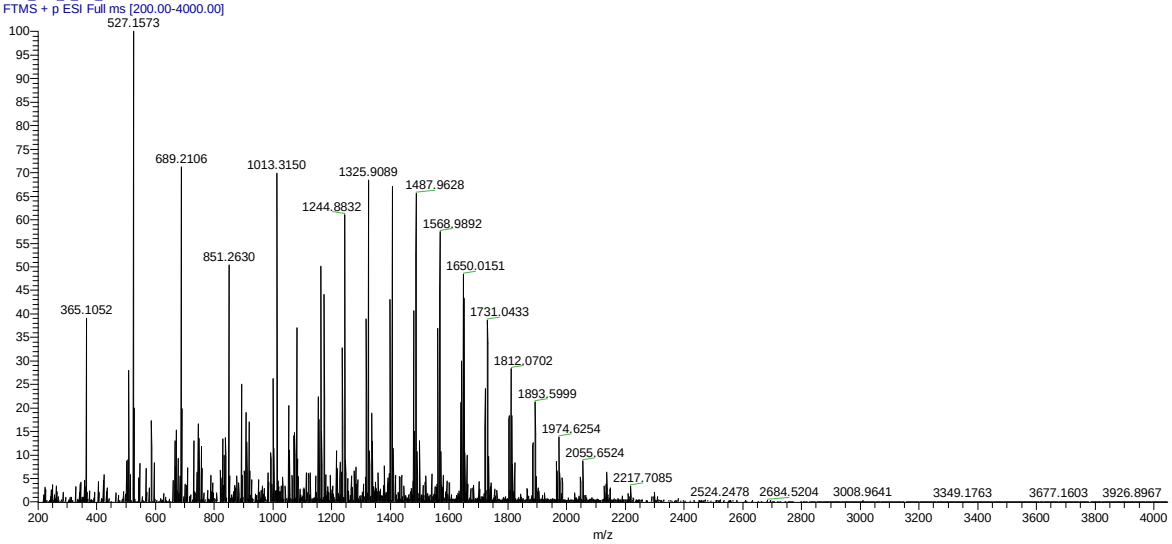

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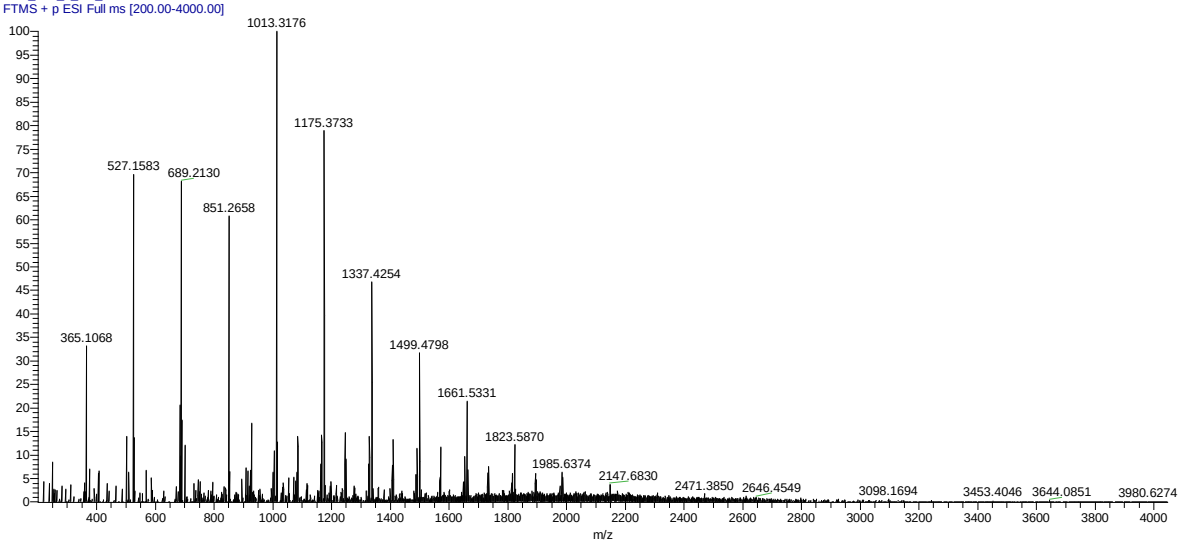

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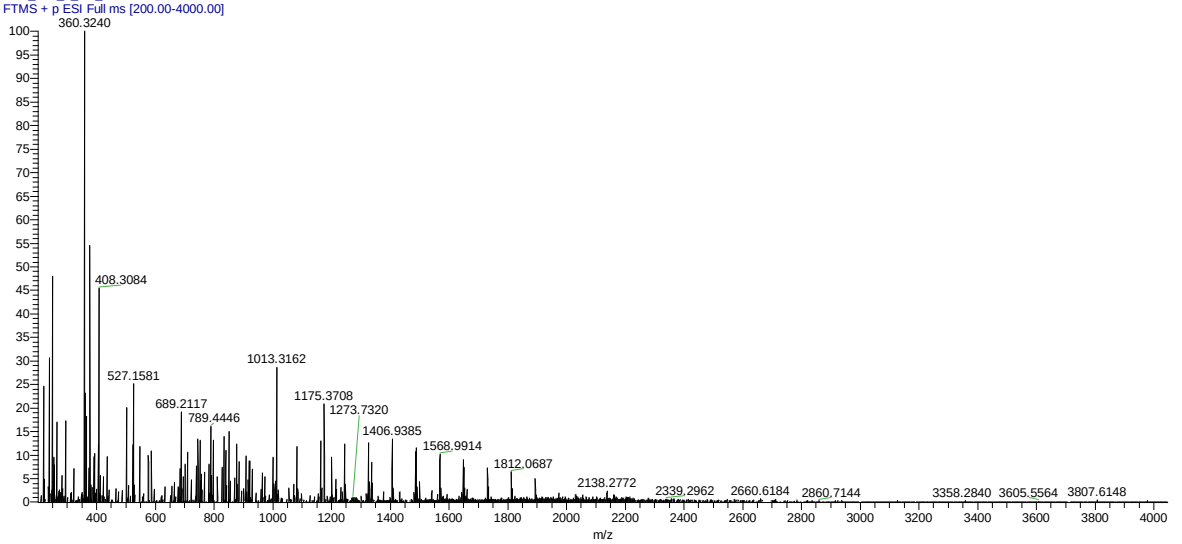

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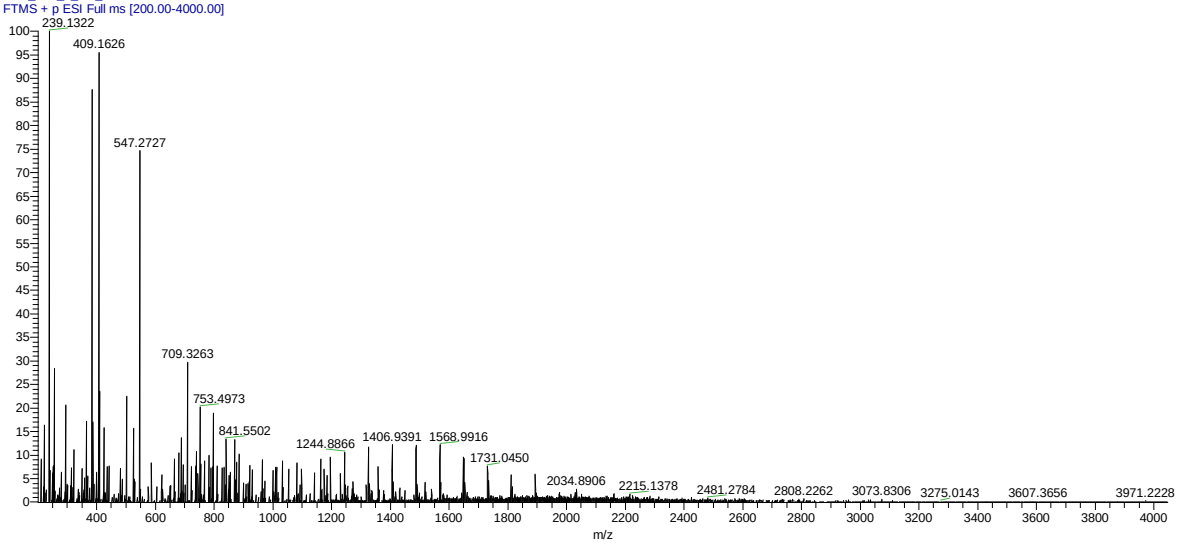

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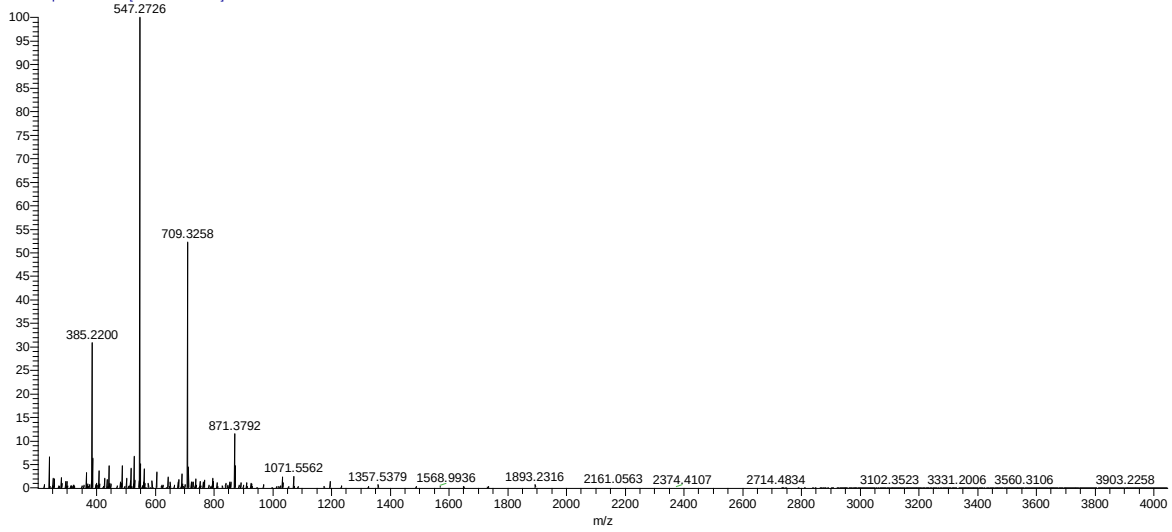

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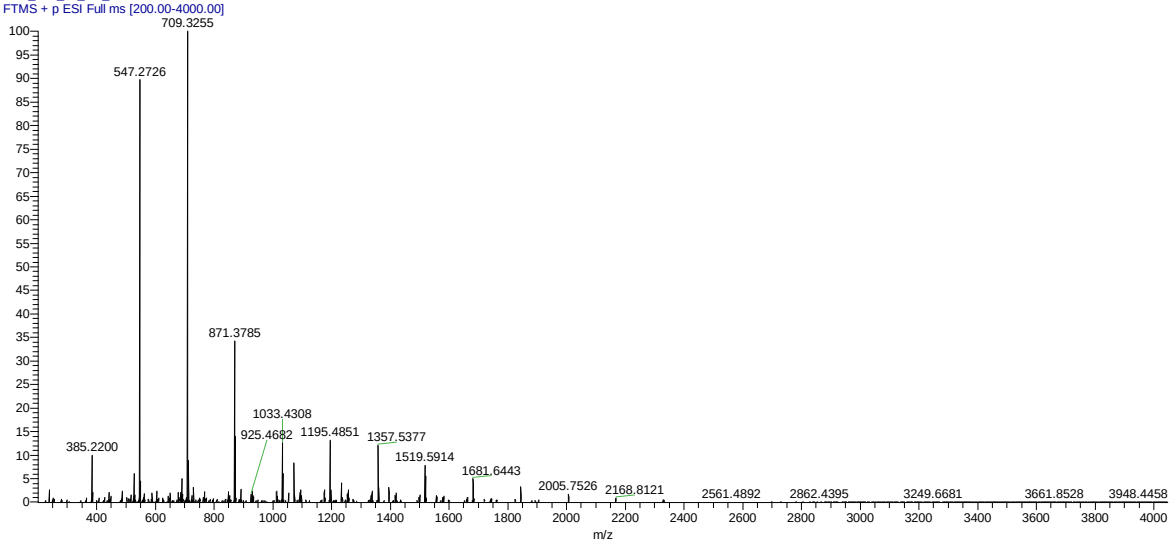

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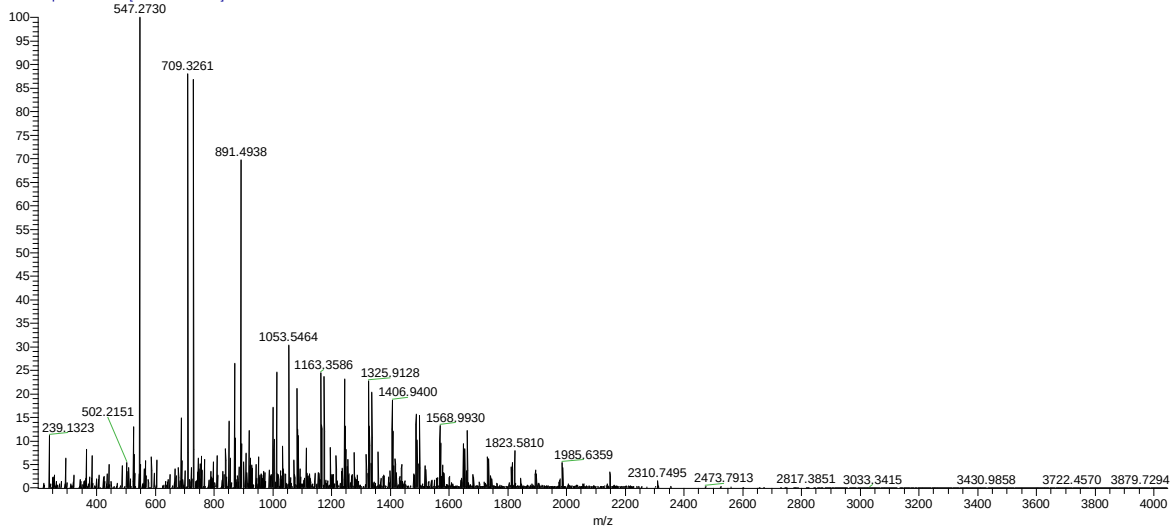

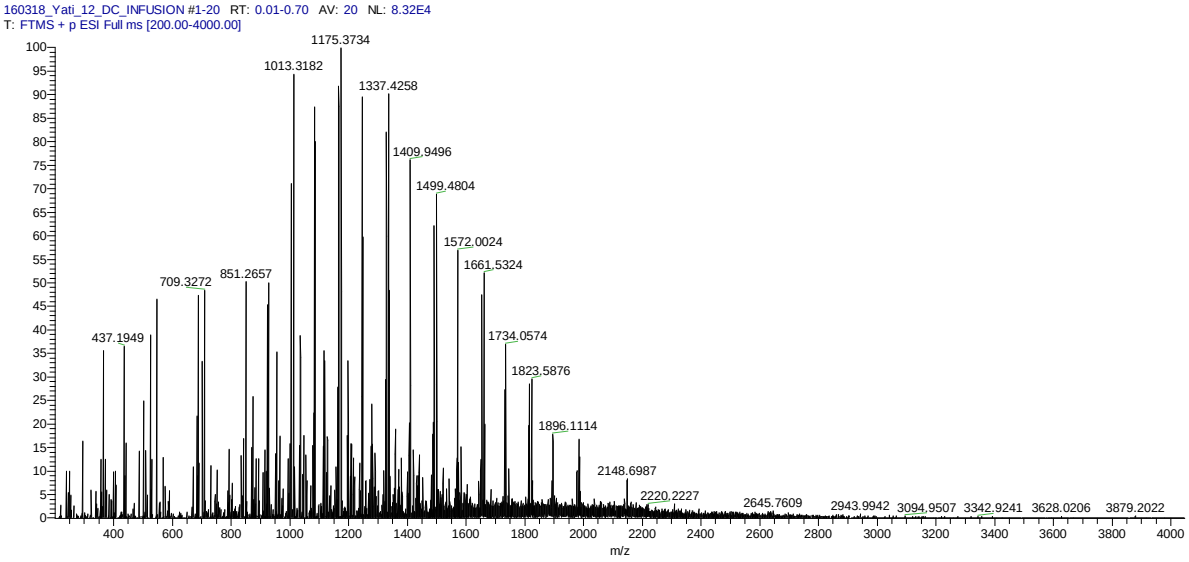
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1896.1114
2148.6987
2220.2227
2645.7609
2943.9942
3094.9507
3342.9241
3628.0206
3879.2022
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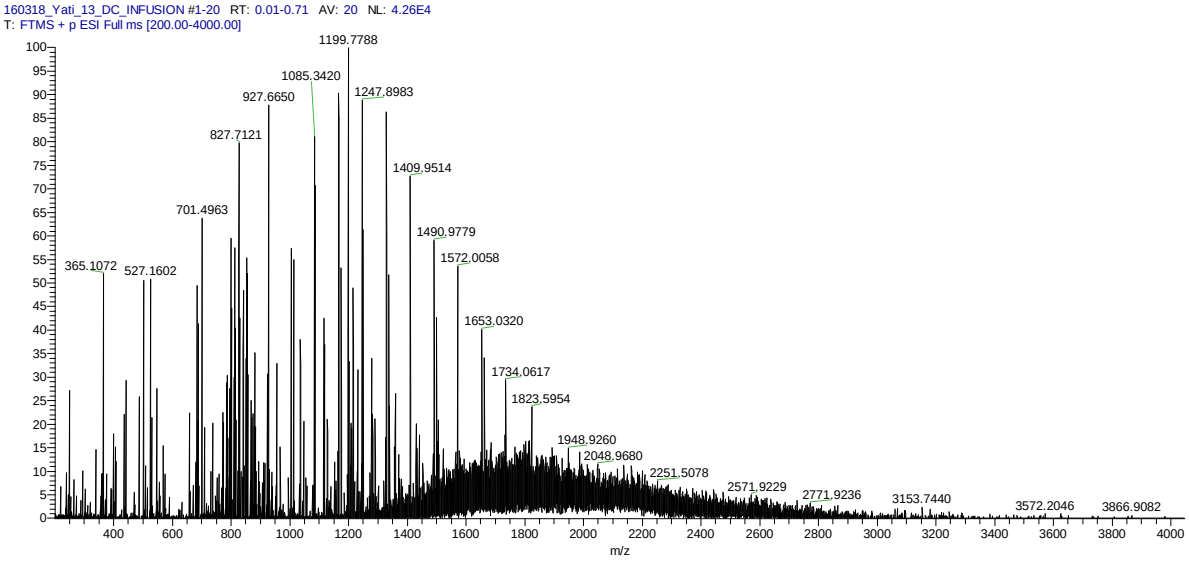
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1085.3420
1247.8983
927.6650
827.7121
1409.9514
701.4963
1490.9779
1572.0058
365.1072
527.1602
1653.0320
1734.0617
1823.5954
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2048.9680
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2771.9236
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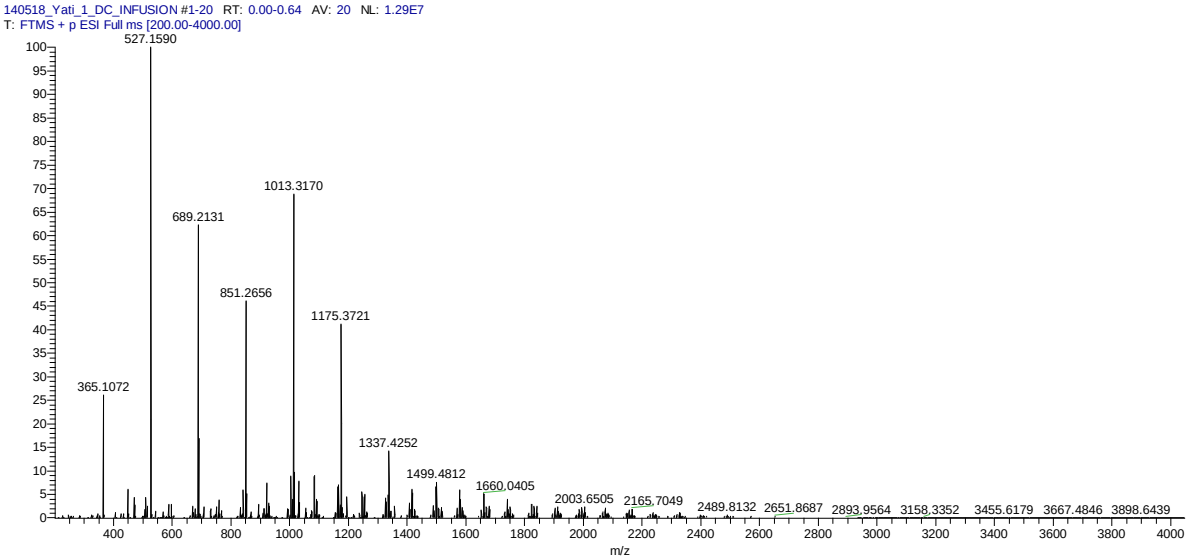
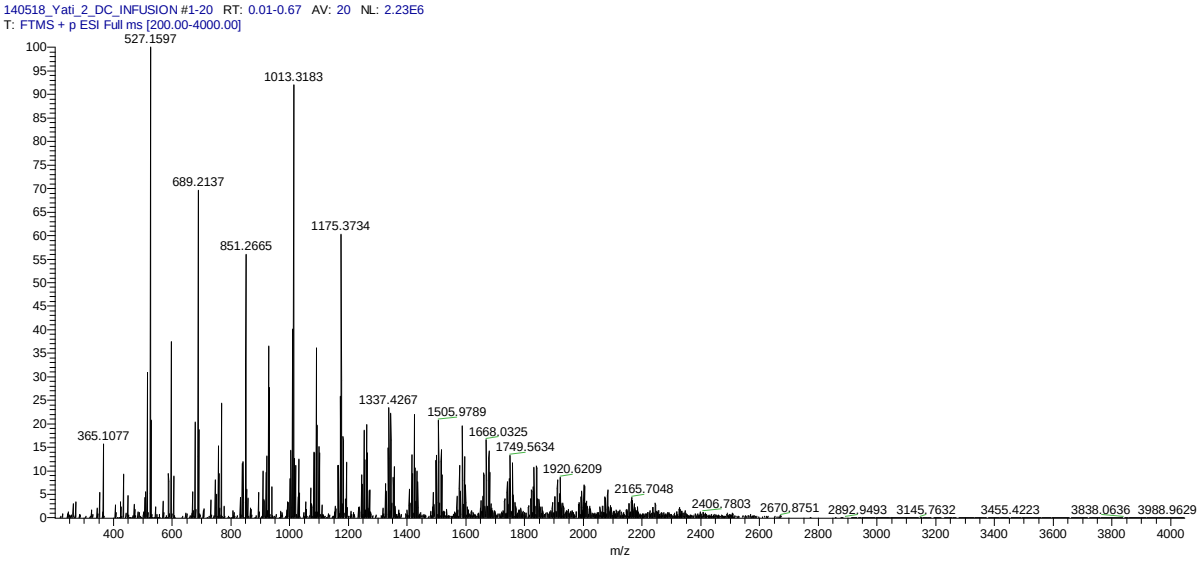
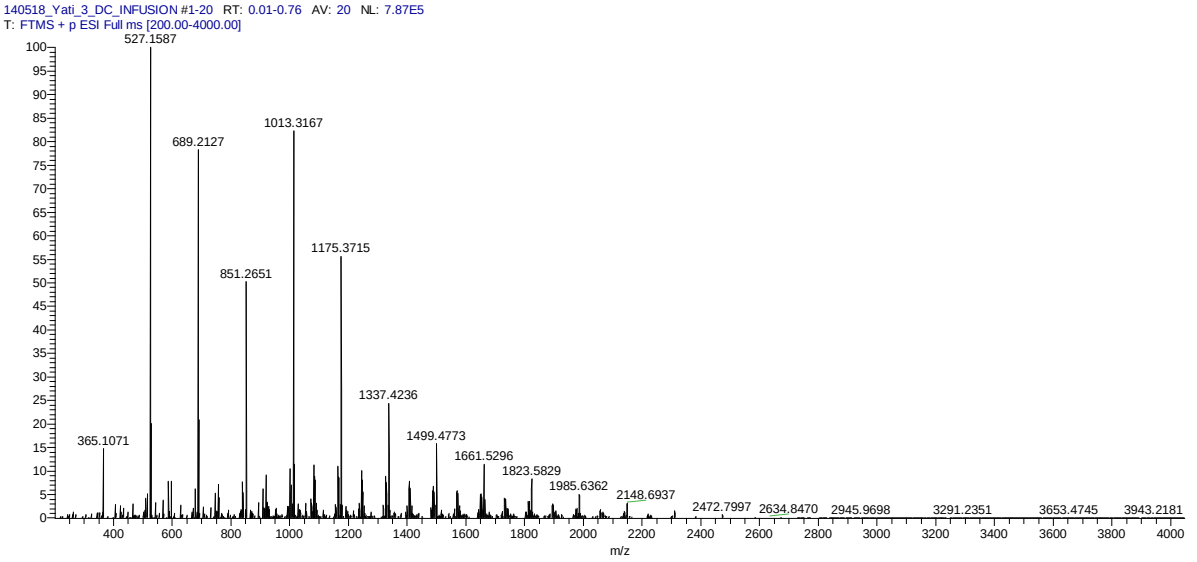
ESI MASS SPECTRUM MDE13 LAURATE






140518_Yati_3_DC_INFUSION #1-20 RT: 0.01-0.76 AV: 20 NL: 7.87E5
T: FTMS + p ESI Full ms [200.00-4000.00]
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689.2127
1013.3167
851.2651
1175.3715
1337.4236
365.1071
1499.4773
1661.5296
1823.5829
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2472.7997
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2945.9698
3291.2351
3653.4745
3943.2181
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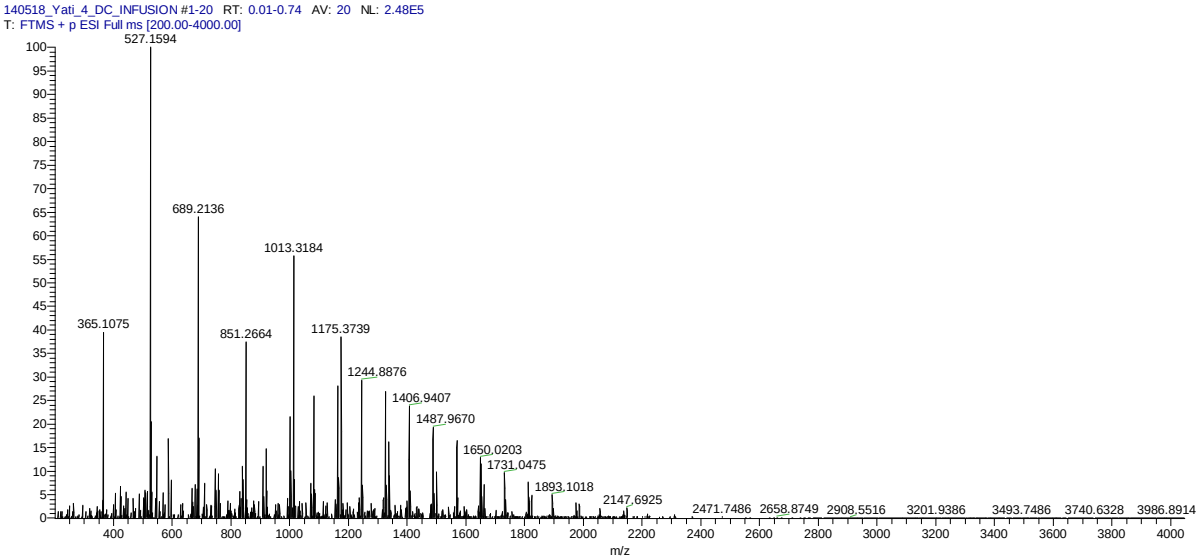
140518_Yati_4_DC_INFUSION #1-20 RT: 0.01-0.74 AV: 20 NL: 2.48E5
T: FTMS + p ESI Full ms [200.00-4000.00]
527.1594
689.2136
1013.3184
365.1075
851.2664
1175.3739
1244.8876
1406.9407
1487.9670
1650.0203
1731.0475
1893.1018
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3493.7486
3740.6328
3986.8914
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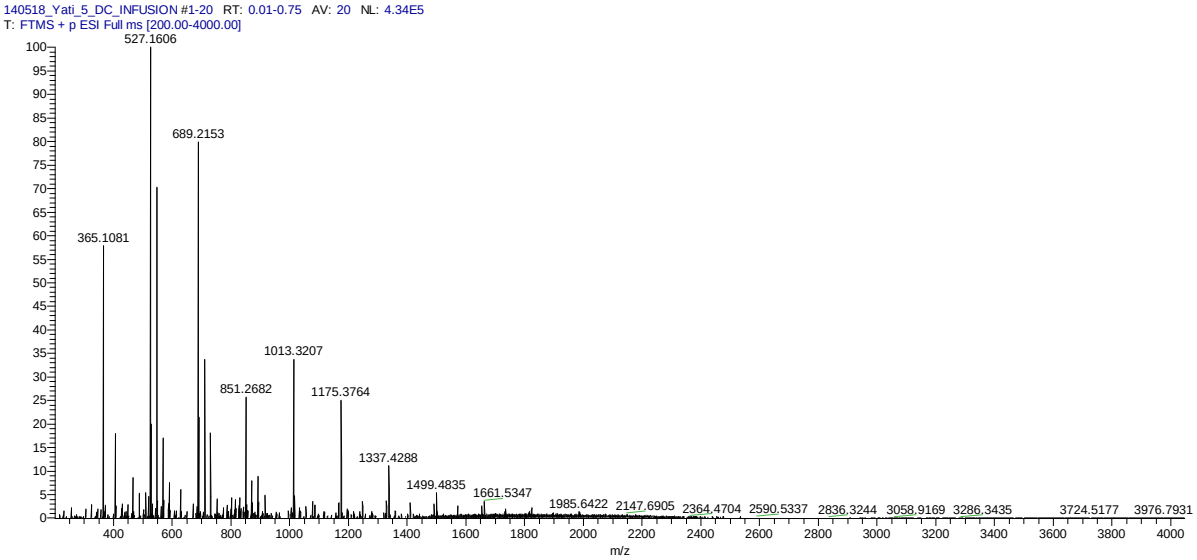
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T: FTMS + p ESI Full ms [200.00-4000.00]
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689.2153
365.1081
1013.3207
851.2682
1175.3764
1337.4288
1499.4835
1661.5347
1985.6422
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2590.5337
2836.3244
3058.9169
3286.3435
3724.5177
3976.7931
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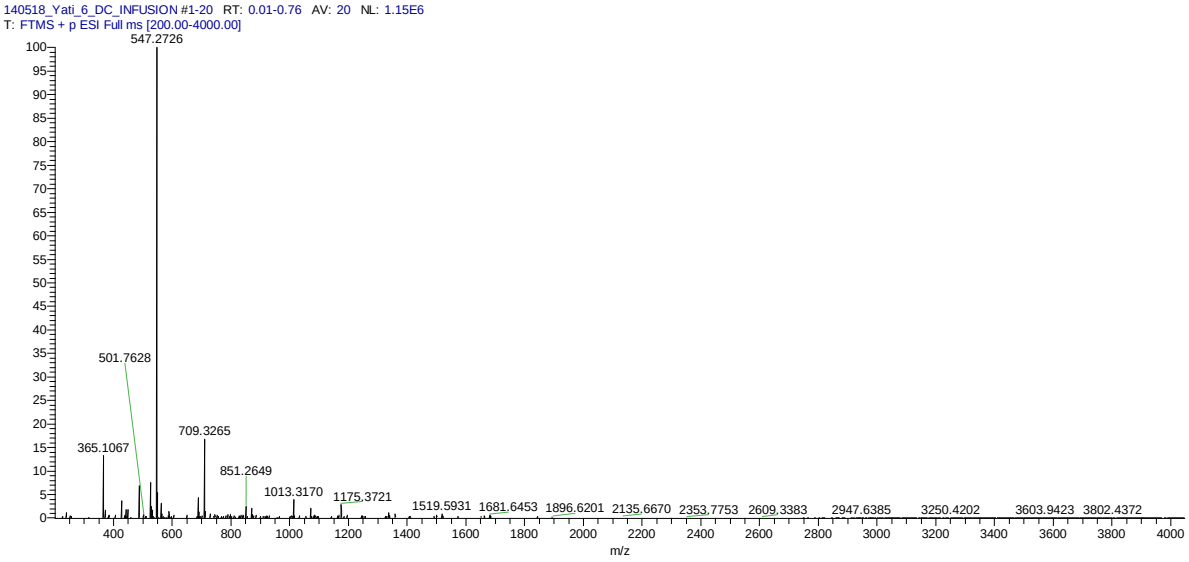
140518_Yati_6_DC_INFUSION #1-20 RT: 0.01-0.76 AV: 20 NL: 1.15E6
T: FTMS + p ESI Full ms [200.00-4000.00]
547.2726
501.7628
709.3265
365.1067
851.2649
1013.3170
1175.3721
1519.5931
1681.6453
1896.6201
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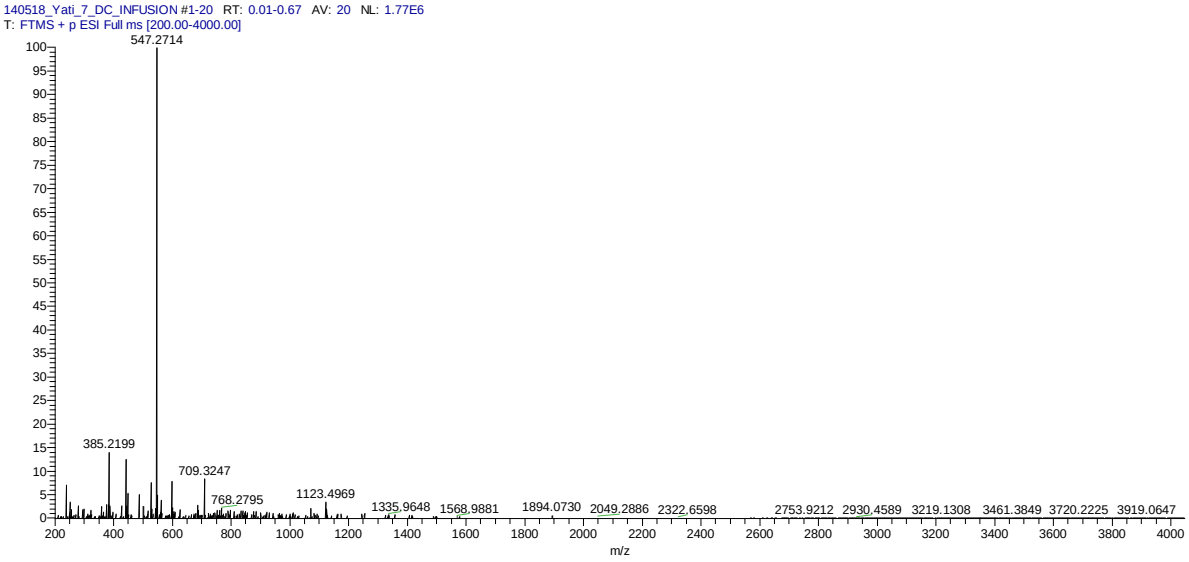
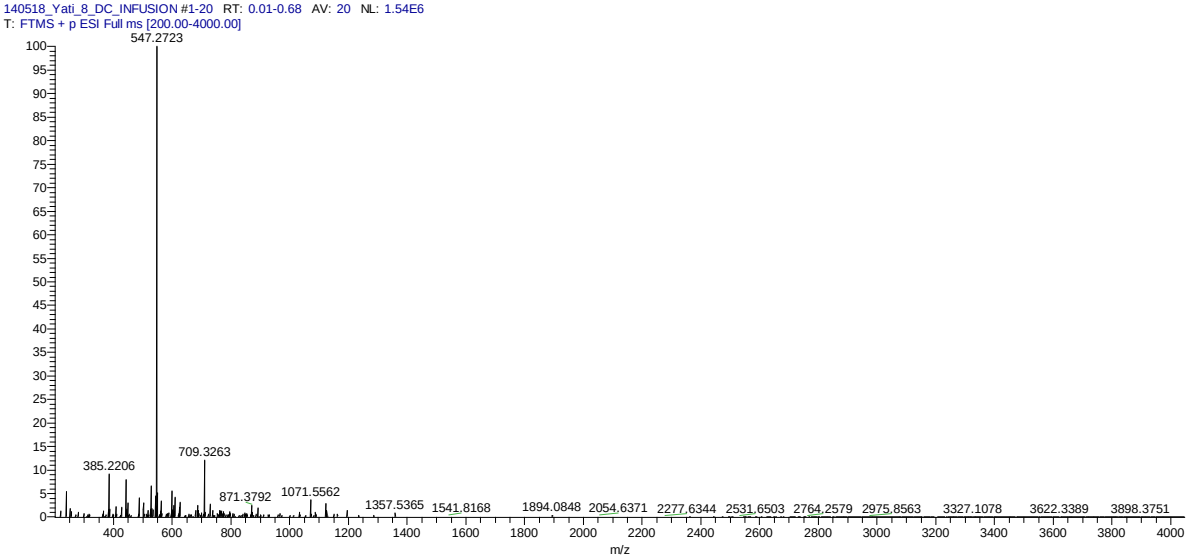
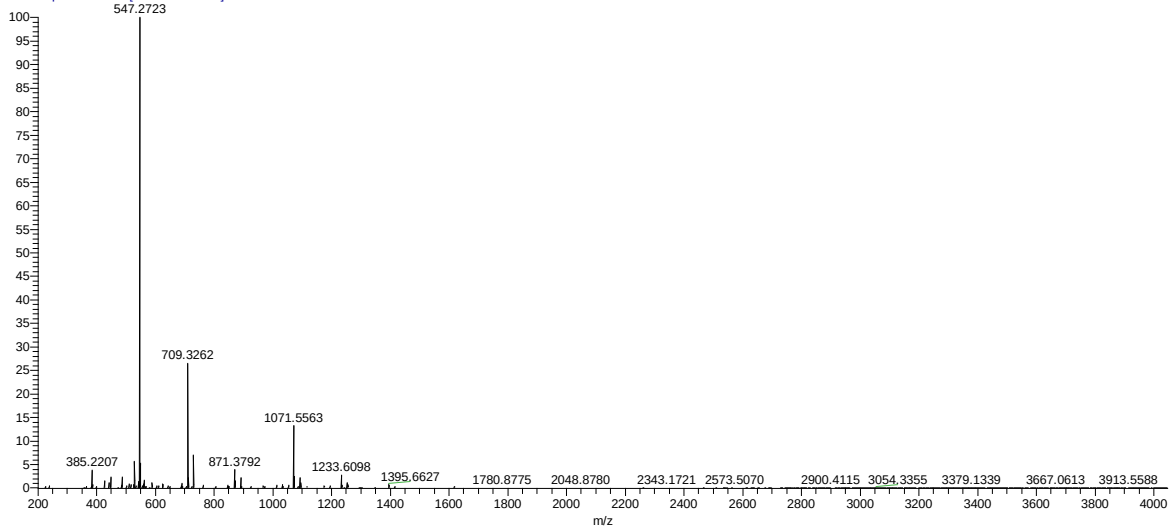
2353.7753
2609.3383
2947.6385
3250.4202
3603.9423
3802.4372
m/z

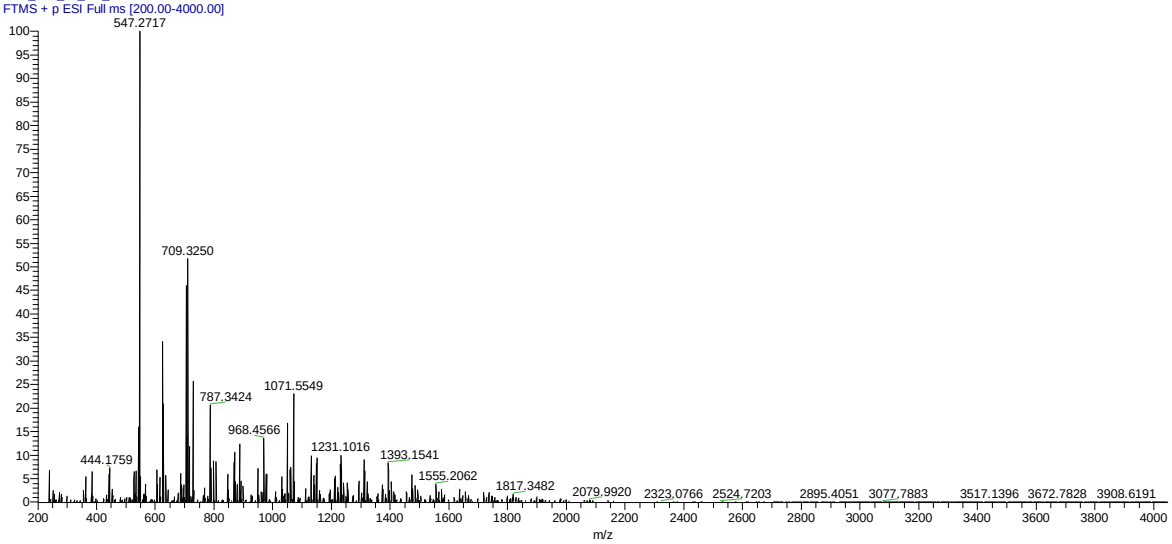

140518_Yati_7_DC_INFUSION #1-20 RT: 0.01-0.67 AV: 20 NL: 1.77E6
T: FTMS + p ESI Full ms [200.00-4000.00]
547.2714
385.2199
709.3247
768.2795
1123.4969
1335.9648
1568.9881
1894.0730
2049.2886
2322.6598
2753.9212
2930.4589
3219.1308
3461.3849
3720.2225
3919.0647
m/z


140518_Yati_8_DC_INFUSION #1-20 RT: 0.01-0.68 AV: 20 NL: 1.54E6
T: FTMS + p ESI Full ms [200.00-4000.00]
547.2723
385.2206
709.3263
871.3792
1071.5562
1357.5365
1541.8168
1894.0848
2054.6371
2277.6344
2531.6503
2764.2579
2975.8563
3327.1078
3622.3389
3898.3751
m/z

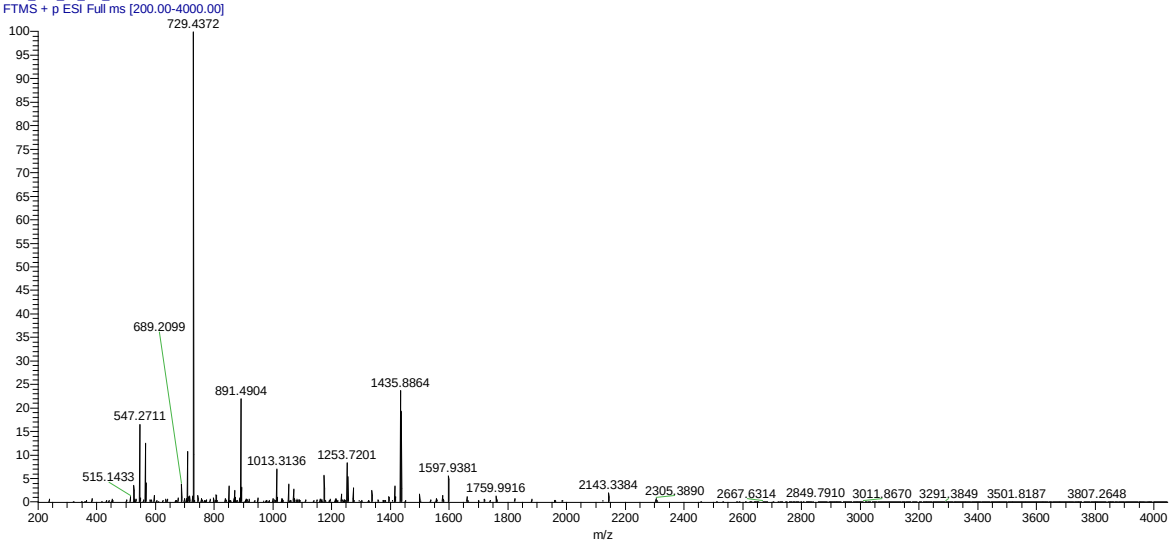
140518_Yati_9_DC_INFUSION #1-20 RT: 0.01-0.90 AV: 20 NL: 4.02E6
T: FTMS + p ESI Full ms [200.00-4000.00]

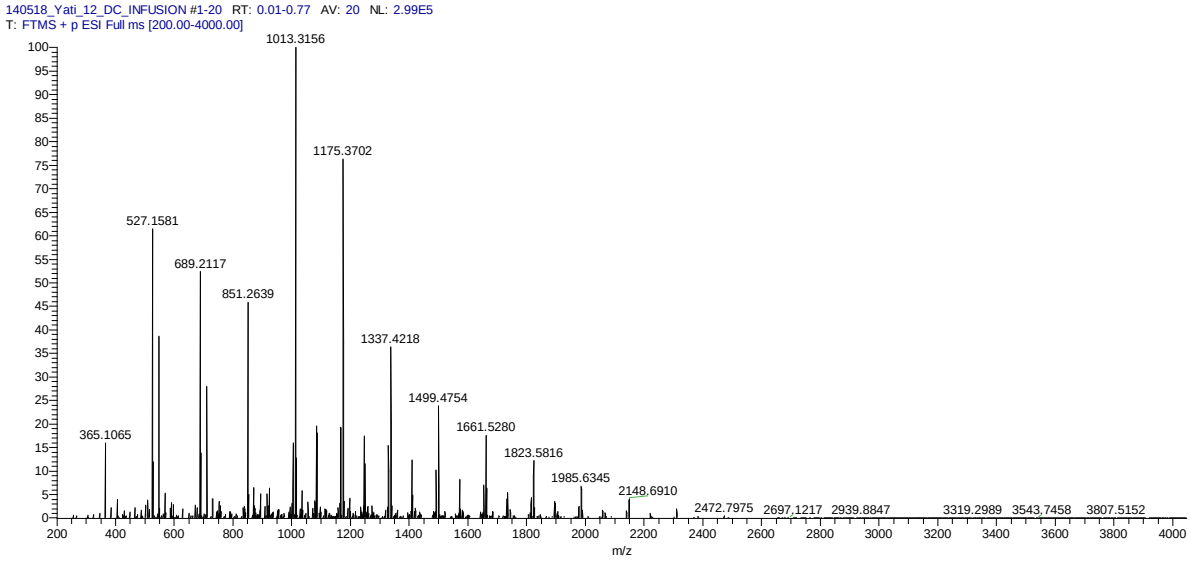


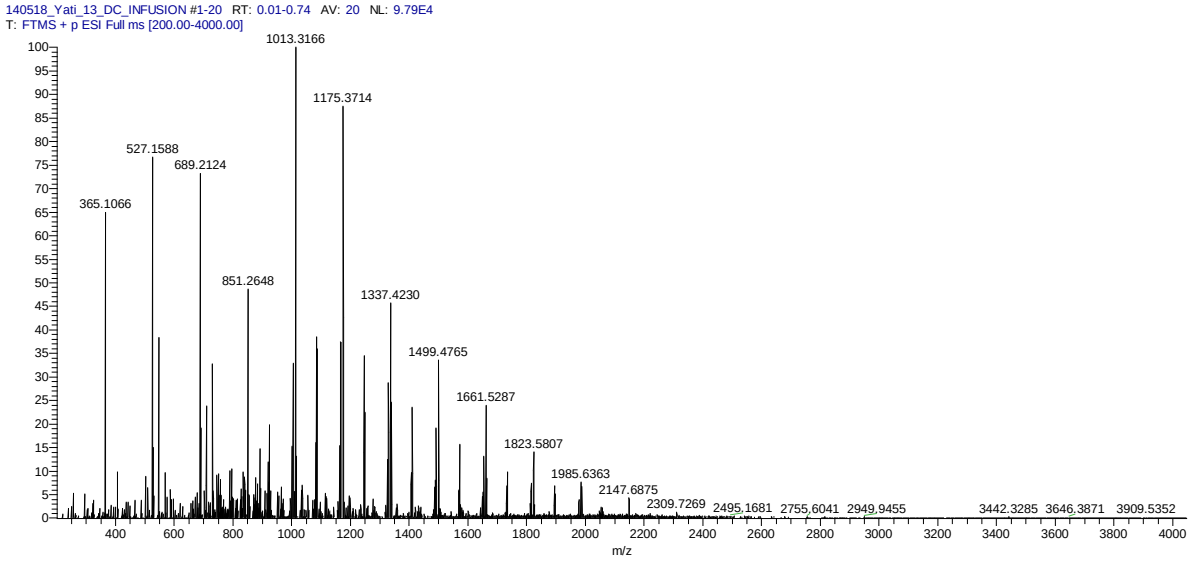
140518_Yati_10_DC_INFUSION #1-20 RT: 0.01-0.64 AV: 20 NL: 1.12E7
T: FTMS + p ESI Full ms [200.00-4000.00]



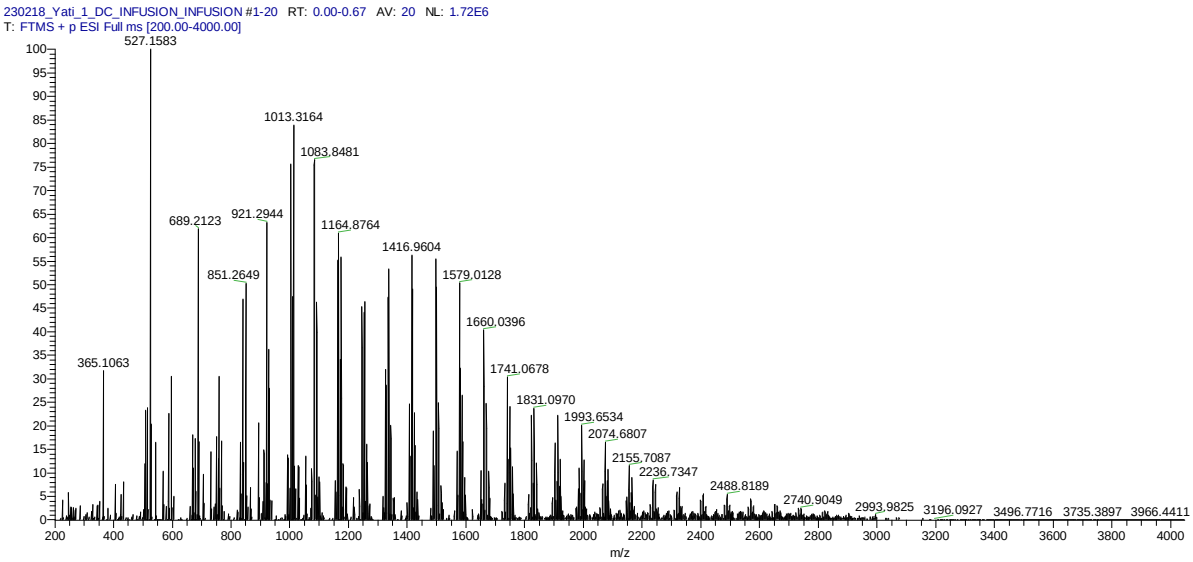
140518_Yati_11_DC_INFUSION #1-20 RT: 0.01-0.66 AV: 20 NL: 1.19E7
T: FTMS + p ESI Full ms [200.00-4000.00]

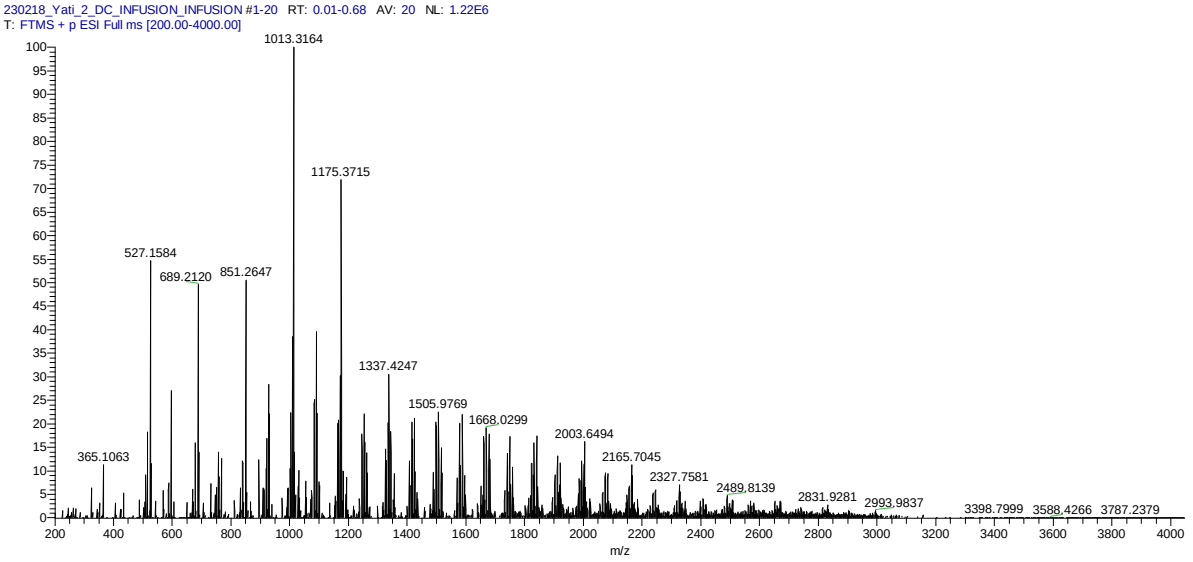


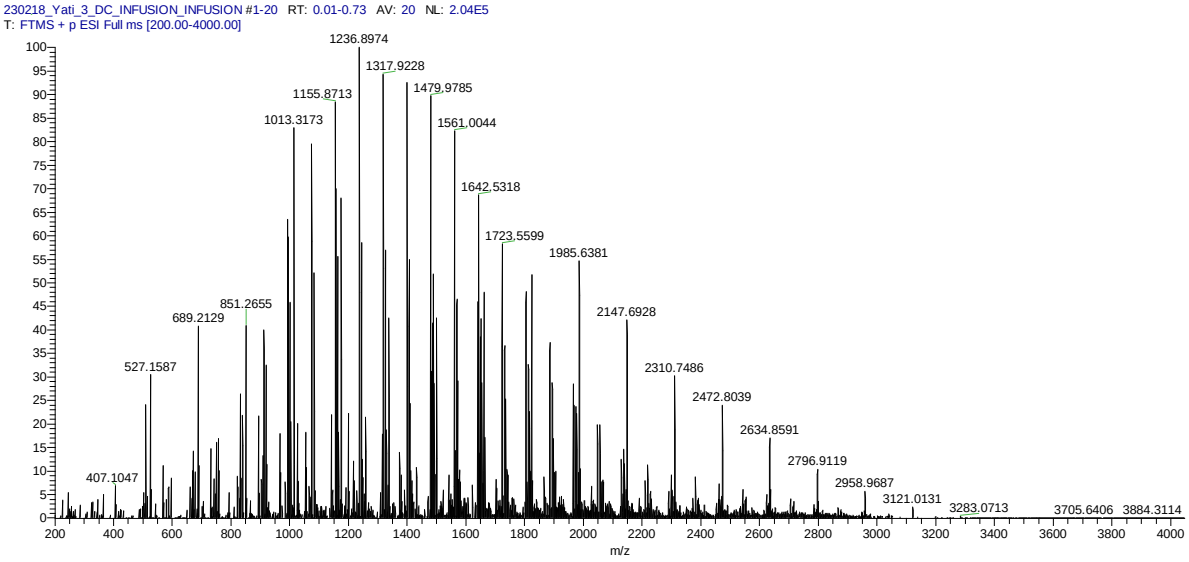

140518_Yati_12_DC_INFUSION #1-20 RT: 0.01-0.77 AV: 20 NL: 2.99E5
T: FTMS + p ESI Full ms [200.00-4000.00]
1013.3156
1175.3702
527.1581
689.2117
851.2639
1337.4218
1499.4754
1661.5280
365.1065
1823.5816
1985.6345
2148.6910
2472.7975
2697.1217
2939.8847
3319.2989
3543.7458
3807.5152
m/z


140518_Yati_13_DC_INFUSION #1-20 RT: 0.01-0.74 AV: 20 NL: 9.79E4
T: FTMS + p ESI Full ms [200.00-4000.00]
1013.3166
1175.3714
527.1588
689.2124
365.1066
851.2648
1337.4230
1499.4765
1661.5287
1823.5807
1985.6363
2147.6875
2309.7269
2495.1681
2755.6041
2949.9455
3442.3285
3646.3871
3909.5352
m/z

ESI MASS SPECTRUM MDE16.5 LAURATE






230218_Yati_3_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.73 AV: 20 NL: 2.04E5
T: FTMS + p ESI Full ms [200.00-4000.00]
1236.8974
1317.9228
1155.8713
1479.9785
1013.3173
1561.0044
1642.5318
1723.5599
1985.6381
851.2655
689.2129
2147.6928
527.1587
2310.7486
2472.8039
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3884.3114
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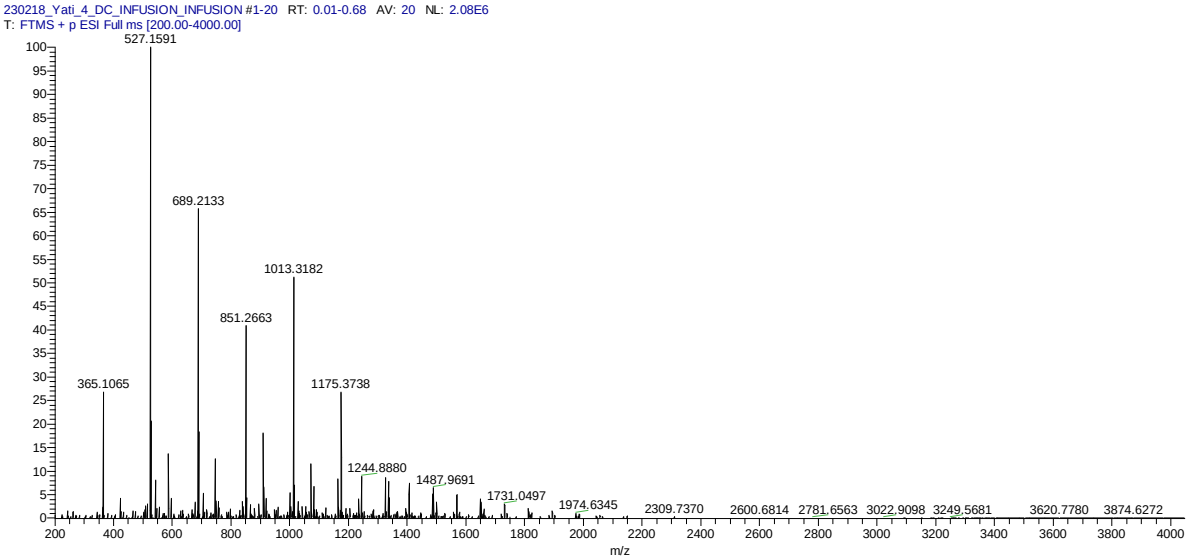
230218_Yati_4_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.68 AV: 20 NL: 2.08E6
T: FTMS + p ESI Full ms [200.00-4000.00]
527.1591
689.2133
1013.3182
851.2663
365.1065
1175.3738
1244.8880
1487.9691
1731.0497
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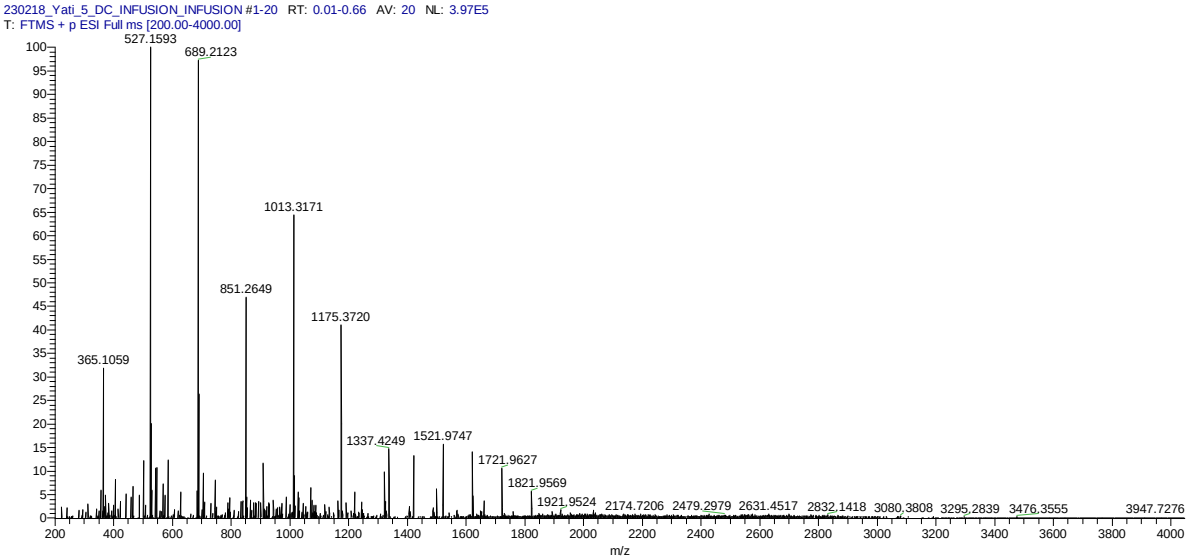
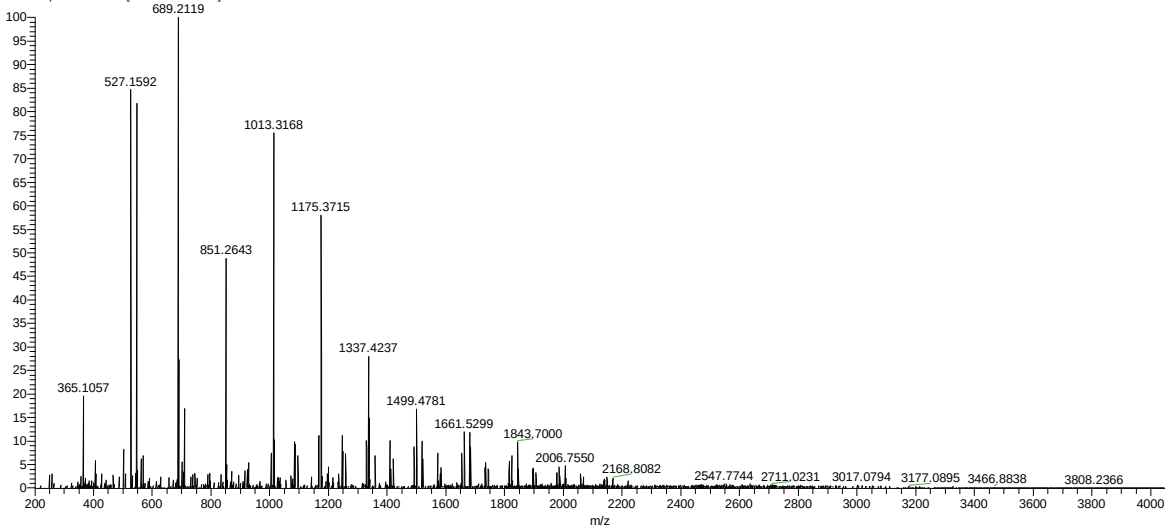
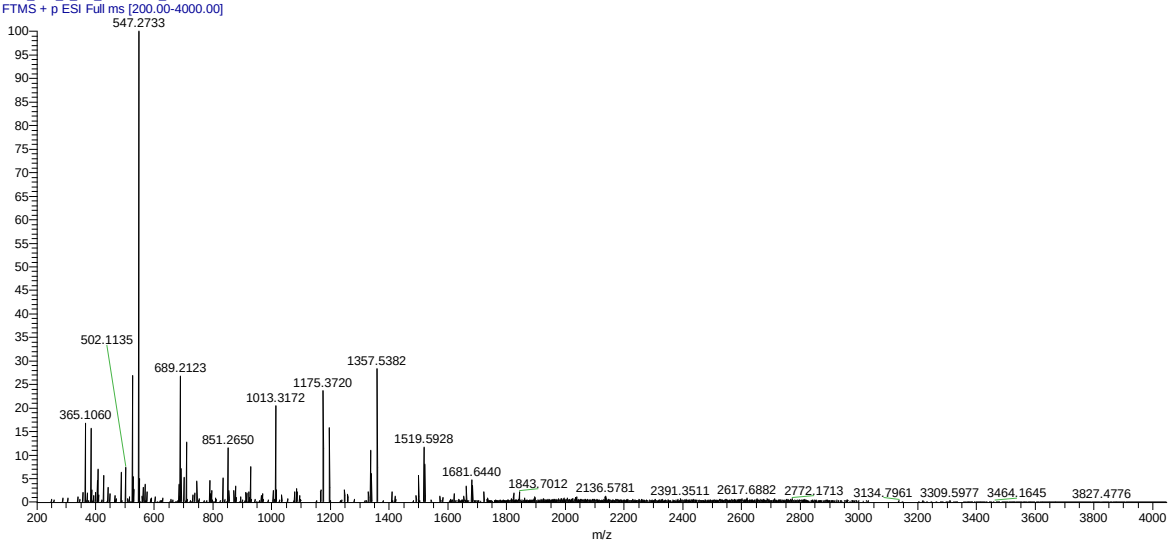

230218_Yati_5_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.66 AV: 20 NL: 3.97E5
T: FTMS + p ESI Full ms [200.00-4000.00]
527.1593
689.2123
1013.3171
851.2649
1175.3720
365.1059
1337.4249
1521.9747
1721.9627
1821.9569
1921.9524
2174.7206
2479.2979
2631.4517
2832.1418
3080.3808
3295.2839
3476.3555
3947.7276
m/z

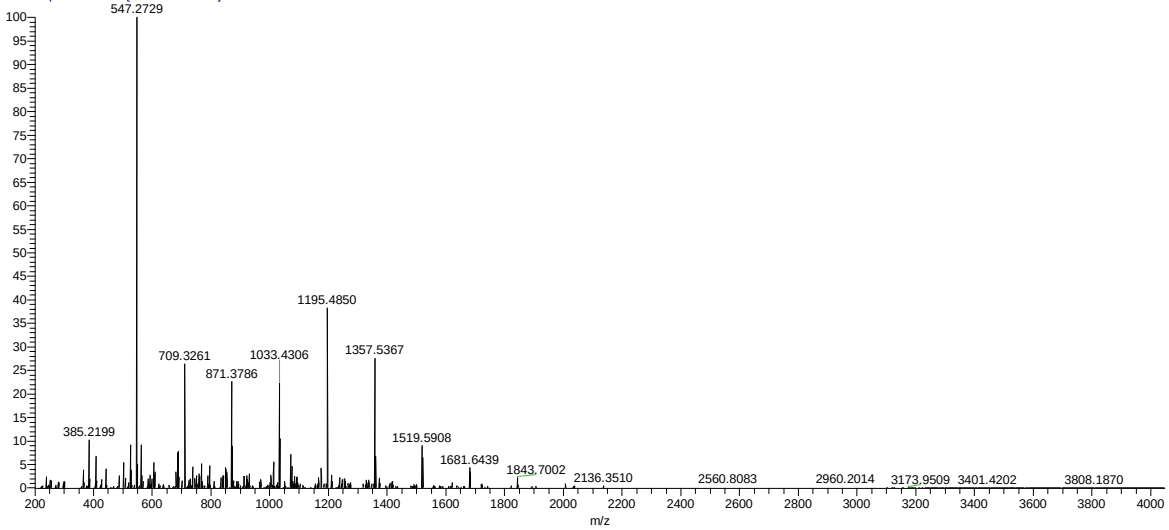
230218_Yati_6_DC_INFUSION_INFUSION #1-20 RT: 0.00-0.68 AV: 20 NL: 2.82E5
T: FTMS + p ESI Full ms [200.00-4000.00]



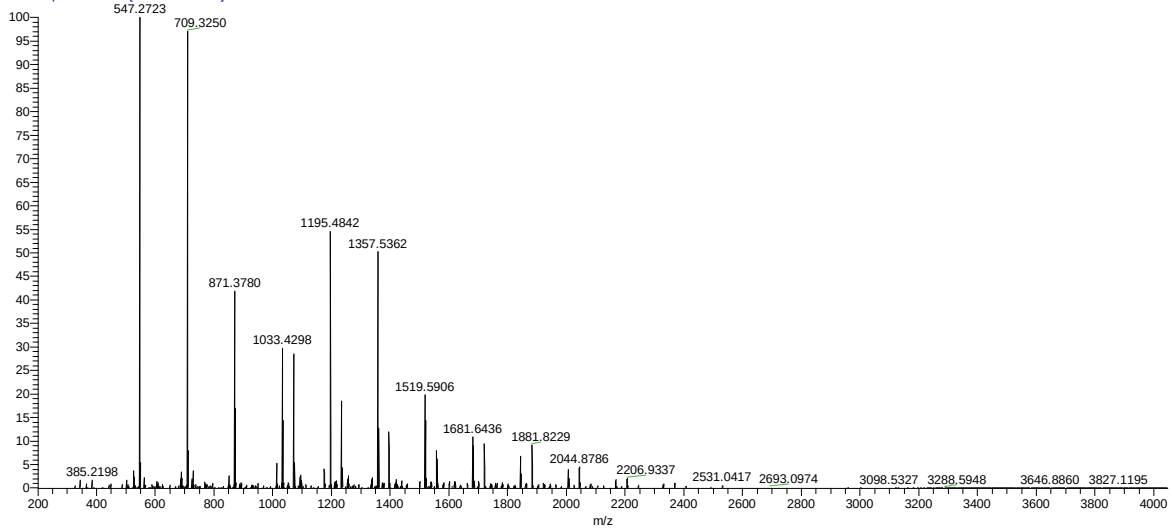
230218_Yati_7_DC_INFUSION_INFUSION #1-20 RT: 0.00-0.70 AV: 20 NL: 2.51E5
T: FTMS + p ESI Full ms [200.00-4000.00]



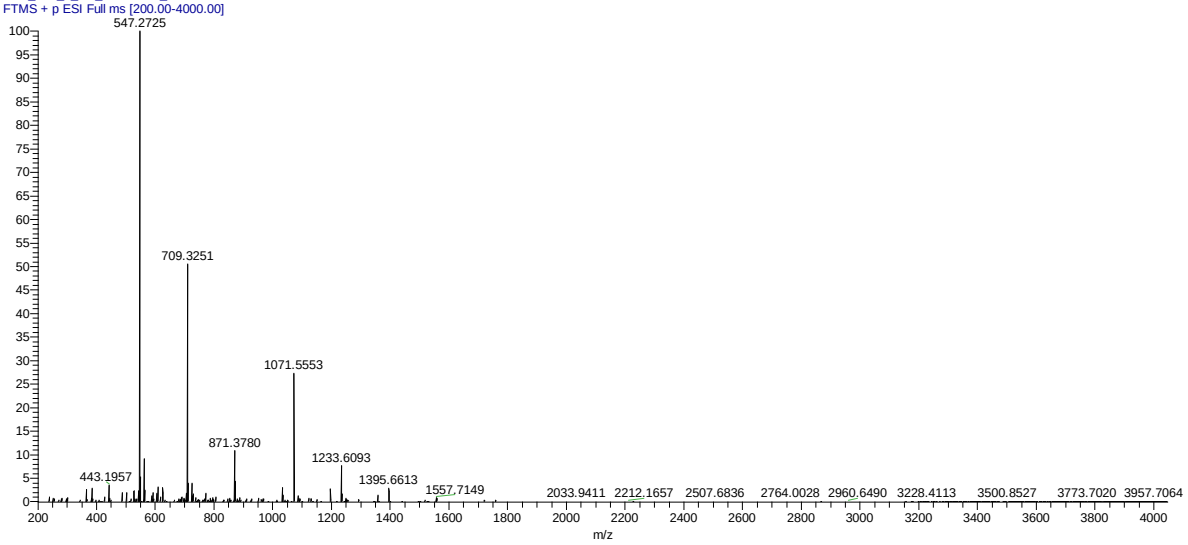
230218_Yati_8_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.67 AV: 20 NL: 7.82E5
T: FTMS + p ESI Full ms [200.00-4000.00]



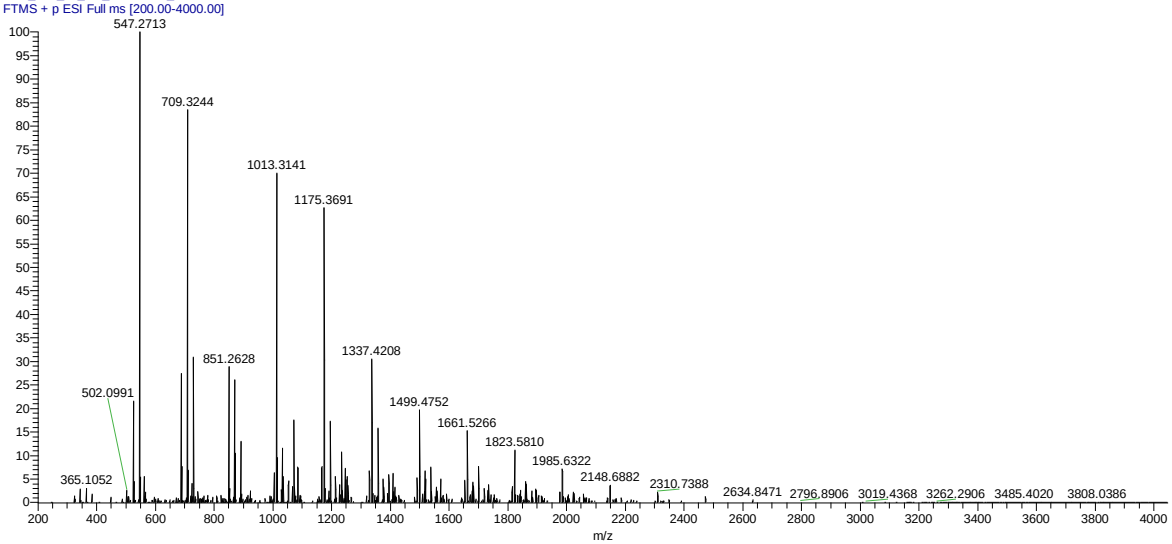
230218_Yati_10_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.66 AV: 20 NL: 2.69E6
T: FTMS + p ESI Full ms [200.00-4000.00]

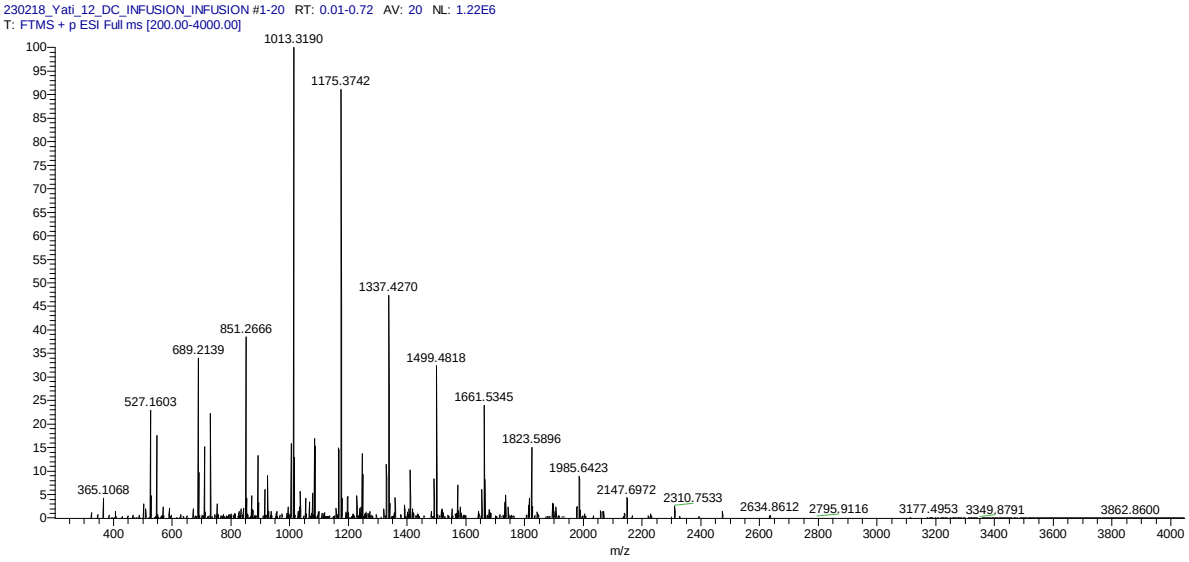


230218_Yati_9_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.67 AV: 20 NL: 2.44E6
T: FTMS + p ESI Full ms [200.00-4000.00]



230218_Yati_11_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.66 AV: 20 NL: 2.06E6
T: FTMS + p ESI Full ms [200.00-4000.00]




230218_Yati_12_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.72 AV: 20 NL: 1.22E6
T: FTMS + p ESI Full ms [200.00-4000.00]
1013.3190
1175.3742
1337.4270
851.2666
689.2139
1499.4818
527.1603
1661.5345
1823.5896
1985.6423
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2310.7533
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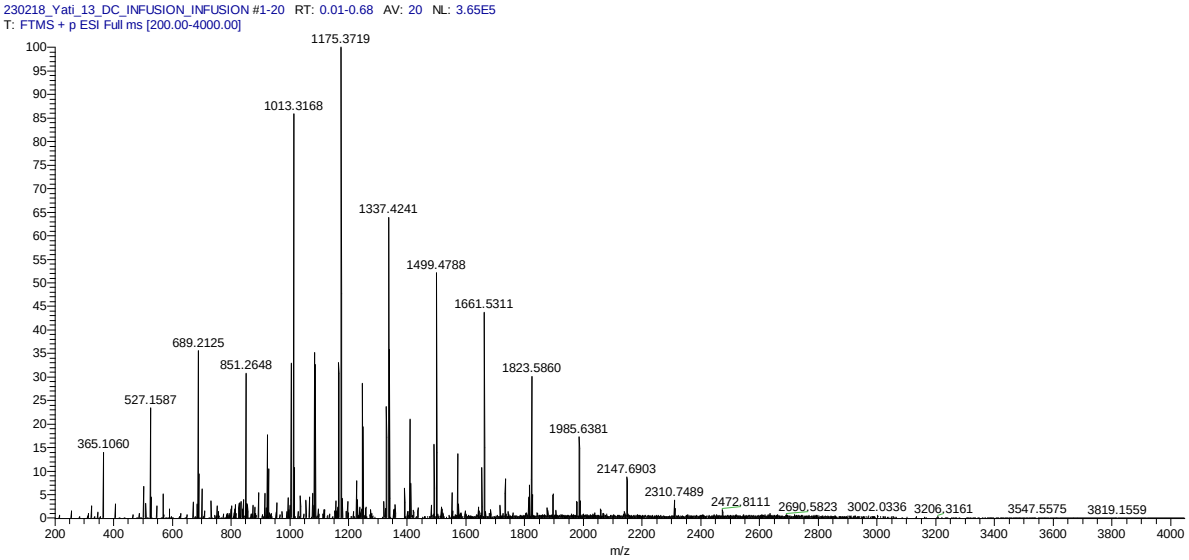
2795.9116
3177.4953
3349.8791
3862.8600
m/z


230218_Yati_13_DC_INFUSION_INFUSION #1-20 RT: 0.01-0.68 AV: 20 NL: 3.65E5
T: FTMS + p ESI Full ms [200.00-4000.00]
1175.3719
1013.3168
1337.4241
1499.4788
1661.5311
689.2125
851.2648
1823.5860
527.1587
1985.6381
365.1060
2147.6903
2310.7489
2472.8111
2690.5823
3002.0336
3206.3161
3547.5575
3819.1559
m/z

APPENDIX II

List of conferences/seminars, rewards/awards, professional memberships and publications during PhD program:

A. Conferences/ Seminars:

1. Oral Presentation at Department of Food and Nutritional Sciences Seminar on 07th June 2017 in University of Reading, under title "Enzymatic transesterification of maltodextrin lauric acid ester".

2. Poster Presentation at 3rd UK Hydrocolloid Symposium Conference on 13th September 2017 at Sutton Bonington Campus at the University of Nottingham under title "Optimization of enzymatic transesterification reaction for the production of maltodextrin fatty acid esters".

3. Oral Presentation at 3rd International Conference of Agriculture and Food Chemistry on 23rd-24th July 2018 in Rome, Italy, under title "Transesterification reaction of maltodextrin lauric acid esters using immobilised lipase from C*andida antarctica* (Novozym® 435)".

4. Oral Presentation at 1st FNS Research Symposium on 21st February 2019 in University of Reading, under title "Emulsifying properties of maltodextrin ester in comparison with Tween 20".

5. Oral Presentation at Department of Food and Nutritional Sciences Seminar on 5th June 2019 in University of Reading, under title "Evaluation of oil-in-water emulsion of maltodextrin lauric acid ester".

B. Rewards/ Awards

1. Bursary Fund for attending a Poster presentation at 3rd Hydrocolloids Symposium in University of Nottingham on 13rd September 2017.

2. Graduate School Travel Grant, for attending an oral presentation at 3rd International Conference of Agriculture and Food Chemistry on 23rd-24th July 2018 in Rome, Italy.