Valorization of Sugarcane Bagasse as Novel Emulsifiers by Hydrothermal Liquefaction Technology

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Abstract

As natural resources continue to be depleted and rise in greenhouse emissions, biomass valorizations are significant research interests for most governments and research institutes. Biomass resources are forestry residue, agricultural residues, marine products, and municipal solid, to name a few. As agricultural residue bagasse is the most abundant agro-industrial by-product and is composed of bagasse is 40–45% of cellulose, 20–30% lignin, 30–35% hemicelluloses and minor amounts of extractives and inorganic compounds. Cellulose is a linear homopolymer composed of d-glucopyranose units linked by β-1,4-glycosidic bonds. It is associated with hemicellulose molecule via hydrogen bonds and is linked to lignin through covalent bonds. Hemicelluloses are branched polymers of low molecular weight with a degree of polymerization, with xylans being the most abundant hemicellulosic polymers with enormous potential for industrial applications.

Bagasse is a highly productive candidate feedstock for valorization because it has a faster growth cycle and has a high yield compared to sorghum or switchgrass. With cell walls composition having high proportions of pentoses in the wall polysaccharides such as arabinoxylan, disintegrating the complex structure is a limitation. From an industrial viewpoint, thermochemical conversions such as combustion, gasification, pyrolysis and hydrothermal liquefaction (HTL) or a combination of each has been gaining interest for their robustness to treat biomasses. In hydrothermal liquefaction, water is an important reactant and catalyst, as a region of operation is between 150 to 370 °C and between 4 to 25 MPa. Water properties such as the high value of the ionic product of water (K_W) in the

subcritical range means that high levels of H^+ and OH^- will accelerate biomass hydrolysis. Therefore, biomass not usually soluble at room temperature are potentially soluble in these conditions. Batch reactors are the most common type of reactor set up and eventually developed into a more advanced semi-continuous or continuous reactors. Also, HTL treatment is a much quicker, more efficient technique to solubilize and achieve complexation to compounds that have poor water solubility. As technologies continue to evolve, HTL is breaking into territories instead of being regarding as just a pretreatment technique such as it uses on emulsion systems. As a thermodynamically unstable system of two immiscible liquids, an emulsion is achieved by an emulsifier and a homogenization step. As much as synthetic additives dominate the food additives application, natural alternatives are more appealing, yet its availability and functionality are debatable. The work presented herein mainly focuses on the HTL treatment of bagasse and characterization as a natural emulsifier in Oil-water-emulsion.

Firstly, in a HTL reactor, 3% (w/w) grounded bagasse (500 μm) was treated at 160 °C, 30 min, and 1 MPa. For the 3% initial solid concentration used in this study, 47.4% of bagasse was solubilized, and 52.6% remained as insoluble solids. The composition of the bagasse extracts mainly composed of carbohydrates at 51.0%, with approximately 45% of the bagasse extracts existing as the higher molecular weight oligosaccharides other than xylotriose and xylobiose. Together with the presence of organic acids, phenolics compounds contributed to a lower pH of 3.9 detected after HTL. The use of such a treatment technique is an effort to promote more eco-friendly technologies with less dependency on the use of solvents.

Secondly, the liquefied portion separated and freeze-dried before re-dissolving it in 5 mM Phosphate buffer solution (0.5 - 4 wt%) as a continuous phase. Polytron (10,000 rpm, 5 min) and high-pressure homogenization (100 MPa, 4 passes) achieved O/W emulsions with soybean oil before storage at 25 °C. 3 wt%, bagasse extract from HTL was able to stabilize emulsion for 11 days at 25 °C with a d_{av} of 0.79 μm. Likely, the adsorbed layers of the lignin-carbohydrate complex from the bagasse extracts stabilized the O/W emulsion through steric repulsion, and further studies are needed to characterize the potential surface-active macromolecular complexes in bagasse extract.

Lastly, investigating the mechanism stability of the bagasse extract-stabilized emulsions. Three samples, raw bagasse (RB), pith (P), and rind (R), all derived from different parts of the bagasse were treated with HTL and applied as emulsion the different bagasse extracts were able to stabilize emulsion using the high-speed polytron and high-pressure homogenizer. In comparison to RB-stabilized emulsion with 8.0 μm, both P and R-stabilized emulsion achieved 7.7 and 7.9 μm droplet sizes, respectively. The phenolic content in the RB-stabilized emulsion at the interface was 0.13 mg/g extract, while the content in the continuous phase was 0.12 mg/g with the stability of emulsions attributed to the high concentration of carbohydrate that sterically congregate at the aqueous phase

Bagasse extracts containing both hemicellulose and lignified compounds can be valorized using eco-friendly HTL treatment. Therefore, an agro-industrial by-product has potential for application in emulsion such as low-fat salad dressings but, more importantly, adding value through treatment and its applications.

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List of Abbreviation

AP	Aqueous phase
BE	Bagasse extract
$BE_{160-220}$	Bagass extracts from HTL treatment at 160-220 °C
D0	Day 0
d_{av}	Average Droplet diameter
GA	Gum arabic
HLB	Hydrophile-lipophile balance
HPH	High Pressure Homogenizer
HPLC	High Performance Liquid Chromatography
HTL	Hydrothermal liquefaction
O/W	Oil-in-water emulsions
OP	Oil phase
P	Pith
PDI	Polydispersity index
PI	Isoelectric point
PT	Polytron
R	Rind
RB	Raw Bagasse
TCC	Total Carbohydrate Content
TPC	Total Phenolic Content

Chapter 1 Introduction

1.1 Background

With the current pandemic such as the Coronavirus Disease 2019 (COVID-19) being a global health crisis and many United Nations agencies such as WHO, FAO playing a role in assessing and responding to its potential impacts on people's lives and livelihoods, global food trade, markets, and food supply chains is a great challenge. FAO defines that food security exists when "all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy". There are approximately 795 million people in the world that are undernourished in 2014-16, according to FAO, and to be able to feed an increasing world population is a challenge. Although agricultural production can be intensified, it may not be able to meet global food demands.

Food processing is necessary for the availability of food and increased shelf life and thereby reducing losses. It can be defined as any deliberate change that occurs before it is available (Acharya *et al*., 2017). Innovation for sustainable intensification and new food sources should be the focus (Ingram *et al*., 2013). Food processing converts naturally toxic materials into more useful and palatable foods or beverages. Processes such as milling, cooling/freezing, smoking, heating, canning, fermentation, drying, extrusion are just a few examples of the conventional practices used in the industrial scale of food processing.

In contrast, research interest in eco-friendly processes and non-toxic additives has risen in the last decade, especially in both the food and pharmaceutical industries. So, the incorporation of natural preservatives and the phasing out of synthetic chemical additives

have been the trend recently. With a shift in research on food towards organic labeling, delivery systems such as nanoencapsulation and packaging are also being developed as an alternative (Augustin *et al.*, 2016). While traditional food processing plays a significant role in providing food for people, it is expected that there will be an increasing role for the application of novel and emerging food processing technology to improve the quality of food and processing efficiency. Therefore, the potential use of agro-industrial by-products such as sugarcane bagasse in food through additives would be an appealing choice of eco-friendly practices. The focus of this dissertation is on the part of innovative and sustainable primary production systems and food processing in addressing challenges in food security.

1.2 Bagasse

1.2.1 Background

An increasing number of research and development activities and efforts have been focused on the conversion of waste, biomass, and various residues into energy, fuels, and other useful materials, a concept known as valorization. Due to the natural resources being depleted, increasing greenhouse emissions, the trend towards the transformation of waste/biomass to valuable materials and energy has been gaining many research interests. Biomass resources mainly include wood and forestry residues by-products, crops and crop residues, marine products, municipal solid, and waste bio-renewable resources.

Sugarcane is the perennial grass that is native to Southeast Asia or the South Pacific. It was domesticated in New Guinea as far back as 12,000 years ago, and Indians have been recorded as far back as 800 B.C. Today sugar products include brown sugar, granulated sugar, confectionery sugar, caramel, molasses, sugar cubes, syrup. The by-product of these sugar processing is a fibrous material called bagasse. Bagasse is a relevant agro-industrial waste product as it is conventionally used as a burning material in sugar processing industries to produce thermal energy that is eventually converted to electricity. Brazil is the world's largest producer of sugarcane, generated about 93.6–156 million tons of bagasse in the 2010/2011 harvest of which 150–250 kg bagasse produced from 1 ton of sugarcane (Vallejos *et al*., 2012).

The composition of bagasse is 40–45% of cellulose, 20–30% lignin, 30–35% hemicelluloses and minor amounts of extractives and inorganic compounds (Novo *et al*., 2011; Rocha *et al*., 2015). Cellulose is a linear homopolymer composed of d-glucopyranose units linked by β-1,4-lycosidic bonds ($C_{6n}H_{10n+2}O_{5n+1}$) whereby n is the degree of polymerization of glucose (Abdul Khalil *et al*., 2012) as shown in Fig. 1.2A. The global production is estimated to be between 1010 and 1011 tons per year; approximately 6 × 109 tons are processed by industries such as paper, textile, material, and chemical industries(Lavoine *et al*., 2012). Since cellulose materials have high stiffness of the cellulose crystal, it is therefore applied in polymer reinforcement by breaking down the hierarchical structure of the plant into individualized nanofibers of high crystallinity, with a reduction of amorphous parts (Kalia *et al*., 2011). In the lignocellulosic biomass, celluloses are associated with hemicellulose molecule via

hydrogen bonds and physical interactions and are linked to lignin through covalent bonds (Jayapal *et al*., 2013).

Lignin is present in the cellular wall and confers structural support, impermeability, and resistance against microbial attack and oxidative stress. Among the components of lignocellulose, it is the most recalcitrant to biodegradation (Karp *et al*., 2013). Lignin is formed from three precursor alcohols: p-hydroxycinnamyl (coumaryl) alcohol, which forms p-hydroxyphenyl units in the polymer; 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol, the guaiacyl units; and 3,5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol, the syringyl units as depicted in Fig.1.2C. Free radical copolymerization of these alcohols produces the heterogeneous, optically inactive, cross-linked and highly polydisperse polymer (Karp *et al*., 2013)

As with other perennial plants, hemicelluloses are branched polymers of low molecular weight with a degree of polymerization of 80-200. Their general formula is $(C_5H_8O_4)_n$, and they are called pentosans and hexosans, respectively (Peng *et al*., 2009), as shown in Fig. 1.2B. Hemicelluloses are amorphous and therefore, easily extracted from the biomass than cellulose and lignin; hence their potential for conversion into bio-based products has not been developed commercially. Xylans are the most abundant hemicellulosic polymers with an enormous potential for industrial applications. Xylooligosaccharides from xylan rich agro-industrial waste can be used in a wide range of applications, including their conversion to xylose, xylitol, furfural, and other bio-based polymers. Hemicellulose extraction, before the transformation of lignocellulosic materials to high-value bio-based chemicals or materials, could improve the economic

efficiency of such processes. Therefore, this research seeks to exploit the hemicellulose rich extract of bagasse through HTL treatment and emulsion studies.

1.2.2 Valorization potential of bagasse

Due to its exceptional ability to produce biomass, sugarcane will always be necessary for a biomass-dependent economy. Food security could be guaranteed by the use of non-edible feedstock for biofuel production that can be cultivated on marginal land. Initial diversification of sugarcane into bioethanol feedstock occurred in 1930 when Brazil adopted a policy requiring large-scale production of bioethanol as an automotive fuel (de Souza *et al*., 2014). Countries such as the USA and Brazil have been leading the production of ethanol from corn starch and sugarcane sugar, respectively, and the amount of ethanol produced by these two countries together in 2013 was 74 billion liters, accounting for 84% of the world's production in that year. Due to this diversification, India has tied with Brazil as the world leaders in sugar production in the 2019/2020 season (USDA, 2019). According to de Souza *et al*. (2014), bagasse is a highly productive candidate feedstock for valorization in the biofuel industry because it has a faster growth cycle and has a high yield compared to sorghum or switchgrass. With cell walls composition previously described as having a mixture of complex structural and non-structural carbohydrates, chemical hydrolysis is a traditional method used to break open the cell walls of bagasse. That is, cell walls of perennial grasses like sugarcane have high proportions of pentoses as arabinoxylan is the principal compound in hemicellulose (de Souza *et al*., 2014).

From an industrial viewpoint, two main types of technologies for conversion of biomass to biofuels are biochemical and thermochemical processes. Biochemical conversion refers to the use of enzymes with chemical assistance. Thermochemical conversions use higher temperatures and include combustion, gasification, pyrolysis, and hydrothermal liquefaction (HTL). The biomass mentioned above conversion methods and their products are shown in Figure 1.3.

1.3 Hydrothermal Liquefaction

1.3.1 Principle of operation of HTL

In hydrothermal liquefaction, water is an important reactant and catalyst, so the majority of the biomasses can be directly converted without an energy-consuming drying step, as in the case of pyrolysis (Toor *et al.*, 2011). Hydrothermal liquefaction follows the working principle of subcritical water, where the region of operation is between 150 to 370 °C and between 4 to 25 MPa. Fig 1.4. illustrates the phase diagram of pure water in which the solution exists in the liquid phase. Therefore, at conditions close to the critical point, water has several interesting properties such as low viscosity and allowing high solubility of organic substances. This makes subcritical water an excellent medium for fast, homogeneous, and efficient reactions (Toor *et al.*, 2011). Table 1.1 lists some properties of sub- and supercritical water. In the subcritical region, the dielectric constant decreases from 78 Fm^{-1} at 25 °C to 14.07 Fm^{-1} at 350 °C, which gives rise to increased solubility of hydrophobic organic compounds, such as free fatty acids (King *et al.*, 1999).

Another characteristic is the relatively high value of ionic product of water (K_W) in the subcritical range, meaning that high levels of H^+ and OH^- at subcritical conditions will accelerate biomass hydrolysis. With the intermediate density range compared to room temperature, water molecules have a high dissociation constant, favoring ionic reactions such as dehydration of carbohydrates and aldol splitting (Toor *et al.*, 2011). Therefore, biomass not usually soluble at room temperature are potentially soluble in these conditions.

1.3.2 Types of Reactors

1.3.2.1 Batch Reactors

Batch reactors are the most common type of reactor set up and eventually developed into a more advanced semi-continuous or continuous reactors. Often performed in batch mode on small scale reactors, these systems have the advantage of minimizing practical problems typical for the continuous systems, for example clogging of flow control valves, and are therefore to be preferred in the initial screening of an innovative process (Arturi *et al.*, 2017). More specifically, batch systems often require the biomass to be charged in the reactor before heating and pressurization. However, in these cases, the reactor volume is typically tiny and does not allow accurate yield calculations of different product fractions (Arturi *et al.*, 2017). The slow heating time of hydrothermal batch reactors is one of the major drawbacks when operating on lignocellulosic materials, leading to results that are very different from those obtained in continuously stirred tank reactors where fast heating of the feed is achieved. As a consequence, results obtained in

laboratory-scale batch reactors often do not provide an accurate basis for developing continuous flow processes. The aim of the present work is a demonstration of a batch reactor that is on par with continuous reactors with optimal parameters studied were chosen after preliminary experiments (Arturi *et al*., 2017).

1.3.2.2 Continuous Reactors

As HTL gains more interests, especially in algal research, continuous systems have been built and tested by research institutions and private companies over the last decade. These systems vary substantially because each converts a different type of biomass and serves a different purpose. The first continuous systems in the 1970s converted woody biomass as a large-scale demonstration but were dormant in the 1990s and 2000s. The first continuous system was a demonstration-scale plant in Albany, Oregon, which was scaled up from laboratory testing at the Pittsburgh Energy Research Center (PERC) and the Lawrence Berkeley National Laboratory (LBNL) (Elliott, 2011). It was not until a decade later that more research such as Jazrawi *et al*. (2013) and Elliott *et al*. (2015) at the Pacific Northwest National Laboratory (PNNL) employed continuous systems for research purposes of algae conversion. Jazrawi *et al*. (2013) used a HTL system consisting of two pumps, coil in shell heat exchangers for preheating, a tubular coiled reactor submerged in a fluidized sand bath for primary heating and the same heat exchangers for cooling at the University of Sydney. The primary heating reactor consisted of four 316 stainless steel tubing sections, each 16 m in length, with a 9.5 mm OD, 1.65 mm wall thickness and 2 L total reaction volume. The coils were submerged in the sand

bath, which was heated by four 6 kW electric heating elements. It operated successfully; however, it could not process any slurry with greater than 10 wt% algae because the control valve orifice became blocked (Jazrawi *et al*., 2013). However, with more published papers, HTL of algae produced a high biocrude yield (40–60 wt.%) obtained in a continuous-flow reactor. However, more still needs to be studied as the operating conditions are highly strained and system-specific (Elliott, 2011; Jazrawi *et al*., 2013).

Similar to the application of HTL in biomass treatment for biofuel production, its use on food has been thoroughly researched in the last decade. In these cases, temperatures and pressure fall in the lower subcritical region of the water phase. That is, temperature ranges from 100 to 250 °C and pressure from 1 to 15 MPa. Ayala & De Castro (2001) used subcritical water at 2.0 MPa, 125 °C and 1 ml/min for 24 min to extract edible essential oils as compared to hydro distillation. The subcritical water extraction was quicker and more efficient than hydro distillation (3 h) with higher yields from 15 min extraction. However, HTL application has moved from being an extraction technology to being employed as a homogenization process in emulsion systems. It provides an efficient technique to solubilize and achieve complexation to compounds that have poor water solubility and producing the smallest particle size and lowest polydispersity index values (Chen *et al*., 2016; Sayyar & Jafarizadeh-Malmiri, 2020). Therefore, it is a novel strategy for water-insoluble hydrophobic compounds for interfacial delivery in food emulsified systems.

1.4 Emulsion and Natural Emulsifiers

1.4.1 Background

By definition, an emulsion consists of thermodynamically unstable systems of two liquids that are immiscible, such as oil and water, in which one liquid is dispersed in another (McClements, 2015) and being accommodated by an emulsifier. Two emulsion systems, oil-in-water (O/W) and water-in-oil (W/O) emulsions, are well known. Oil is usually dispersed in a continuous water phase for O/W emulsions, whereas water is dispersed in an oil phase for W/O emulsion (Tadros, 2013). Well, known examples of O/W emulsions are milk and mayonnaise while butter and margarines for W/O emulsions with lecithin and caseins as examples of well-known emulsifiers.

Without emulsifiers, most emulsions are unstable. The reason is that they lower the surface tension of an oil and water interface by forming a protective layer around emulsion droplets. In order to achieve a homogenous solution, high pressure homogenizers are employed. High-pressure homogenizer operates by forcing coarse emulsions through a narrow valve and breaking down the large droplets by a combination of intense, disruptive forces (McClements, 2008). With this method, sufficient numbers of emulsifiers fully cover the interfaces of the newly formed droplets resulting in a homogenous emulsion solution.

1.4.2 Emulsion Stability

Both physical and chemical forces affect the stability of emulsions as it is thermodynamically unstable (McClements, 2005). Some of the known instabilities are

Ostwald ripening, creaming, flocculation, and coalescence. The growth of large droplets by the diffusion of emulsified monomers from small droplets to larger droplets through the continuous phase is called Ostwald ripening. On the other hand, creaming refers to the separation of emulsion droplets as a cream layer separates from the continuous aqueous phase due to gravity. Creaming can also be suppressed by increasing the viscosity of the continuous phase or the volume fraction of the dispersed phase to limit the movements of droplets (McClements, 2005). Lastly, flocculation is the aggregation of droplets caused by the frequent encounter of droplet particles in liquid by Brownian motion. In close packing structures, droplets rearrange their positions after they come in contact with each other, producing flocculates that have a more compact structure with less continuous phase entrapped (McClements, 2008; Walstra, 1996).

1.4.3 Types of Emulsifiers

The hydrophile-lipophile balance (HLB) value can be used to classify different emulsifiers based on their affinity for oil or water phases. An emulsifier with a HLB value of 7 indicates it is equally soluble in water and oil. Less than 7 means an emulsifier is more soluble in oil, whereas a HLB value higher than 7 is an indication that the emulsifier is more soluble (Walstra, 1996). According to Bancroft's rule, water-soluble emulsifier should be used in O/W emulsions, and a soluble oil emulsifier should be used in W/O emulsions according to their HLB value (McClements, 2005). Most emulsifiers used in the food industry can also be divided, based on their charge, into three groups, nonionic, ionic and zwitterionic (McClements, 2005).

short-range repulsive forces, such as steric repulsion, hydration, and thermal fluctuation interactions, are mechanisms of nonionic emulsifiers to stabilize emulsions. Emulsions stabilized by nonionic surfactants are less sensitive to pH and ionic strength, while ionic emulsifiers stabilize emulsions by electrostatic repulsion emulsion through the same surface charges (McClements, 2005). Proteins being dominant as emulsifying agents in the food industry, stabilize emulsion droplets by a viscoelastic layer formation at the oil and water interface with the non-polar portions facing the aqueous phase and the polar portions facing the oil phase (McClements, 2005; Wilde *et al.*, 2004). The major mechanism stabilizing an emulsion system is the electrostatic repulsion between droplets. In contrast, the magnitude of the surface charge on emulsion droplets is determined by the type of emulsifier and the surrounding aqueous conditions.

With McClements *et al*. (2017) stating the increase in natural alternatives in the formulation of new emulsion-based products, Tween 80 is one of the most used synthetic emulsifiers in the preparation of conventional emulsions while lecithin is the most widely used natural emulsifying agent in the food industry (Arancibia *et al*., 2017).

Since synthetic emulsifiers are well established, naturally derived emulsifiers need to meet the requirements in order to be considered a potential emulsifier, as shown in Fig 1.5. That is, they can facilitate the emulsification, and promote physical stability by adsorbing at the oil-water interface, reducing the interfacial tension and improving the protection of droplet from aggregation (McClements *et al*., 2017).

Lecithin is a phospholipid that has appreciable non-polar and polar regions within the same molecule making it amphiphilic molecules that can adsorb to oil-water interfaces

and stabilize lipid droplets (McClements *et al*., 2017). Biosurfactant is another type that has dominant amphiphilic that hydrophilic regions and hydrophobic groups such as phenolic groups distributed within a single molecule (McClements *et al*., 2017; Mitra & Dungan, 1997). Proteins, as described previously and depicted in Fig 1.6, have been well documented through its structure and electrostatic repulsion mechanism; however, those that are derived from plant sources such as soy, bean, and canola are receiving much of attention as natural emulsifiers. Lastly, polysaccharides such as Gum arabic is another natural emulsifier type. Perennial grasses such as bagasse with high composition of xylose sugars that are hydrophilic and not a preferable choice as a stabilizer/emulsifier. However, modifications have allowed for polymers to be amphiphilic and stabilize emulsions mainly through steric repulsions. Pectins on the other hand have anionic hydrophilic polysaccharide with hydrophobic protein or phenolic groups attached (McClements *et al*., 2017).

1.5 Objective and overall structure of thesis

The objective of this thesis understands and investigated the HTL system through batch experiments at a laboratory scale that would potentially be used to provide insightful knowledge for future experiments. The work presented herein mainly focuses on the development and characterization of bagasse extract stabilized by natural emulsifiers. The objectives the study in this dissertation are listed as follows:

1. To extract bagasse using HTL and characterize the extracts obtained after treatment.

2. To be able to apply the bagasse soluble extract from HTL treatment in emulsion systems as an emulsifier by formulation and stability analysis.
3. To elucidate the mechanism of formulation, stability and mechanism of bagasse extract in stabilizing O/W emulsion

The thesis is organized in the following manner:

1. Chapter 2 presents the HTL treatment of bagasse. It introduces the procedures and parameters of HTL treatment. Next, the resulting soluble and insoluble portions are analyzed and discussed separately.
2. Chapter 3 reports introduces the emulsion systems used. Briefly describes the formulation and stability tests used. Discussion about the possible mechanisms of stability are proposed.,
3. Chapter 4 gives the experiment design of the bagasse and HTL treatment to better understand the mechanism of O/W emulsion stability described in Chapter 3.
4. Chapter 5 is a brief summary and future outlook with recommendations.

Table 1. 1 Properties of water at various conditions, adapted from Toor *et al*. (2011).

	Normal water	**Subcritical water**		**Supercritical water**	
Temp. (°C)	25	250	350	400	400
Pressure (MPa)	0.1	5	25	25	50
Density, ρ (g cm^{-3})	1	0.80	0.6	0.17	0.58
Dielectric constant, ε (F m^{-1})	78.5	27.1	14.07	5.9	10.5
Ionic product, pK_w	14.0	11.2	12	19.4	11.9
Heat capacity C_p (kJ $kg^{-1}K^{-1}$)	4.22	4.86	10.1	13.0	6.8
Dynamic viscosity, η (mPa s)	0.89	0.11	0.064	0.03	0.07

Table 1. 2 Summary of various homogeneous and heterogeneous catalysts under HTL conditions (Toor *et al*., 2011).

Catalyst	**Feedstock**	***T* (°C)**	***P* (MPa)**	**Catalytic effect**
Na_2CO_3	Corn stalk	276–376	25	Increased oil yield
K_2CO_3	Wood biomass	280	N/A	Less solid residue
Na_2CO_3, Ni	Cellulose	200–350	N/A	Char reduction
K_2CO_3, Ni	Glucose	350	30	Water–gas shift
K_2CO_3	Glucose	400–500	30–50	Water–gas shift
KOH, K_2CO_3	Wet biomass, organic wastes	550–600	25	Water–gas shift
NaOH, KOH, ZrO_2	Stearic acid	400	25	Enhanced decarboxylisation
NaOH, H_2SO_4, TiO_2, ZrO_2	Glucose	200	N/A	Increased isomerization of glucose
Ni	Cellulose	350	18	Enhanced H_2 yield
Ni, Ru	Waste materials	350	21	Enhanced CH_4 yield

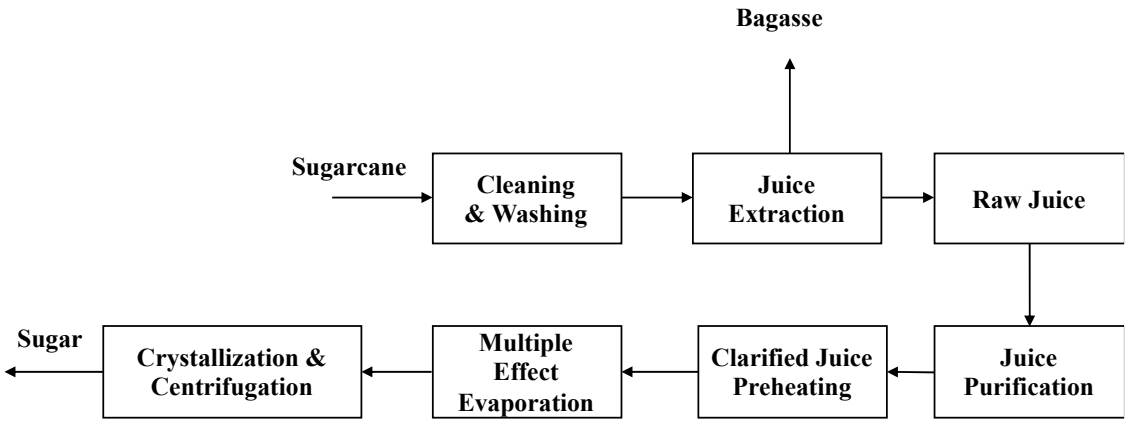

Figure 1. 1 A simplified process diagram for the generation of sugarcane bagasse.

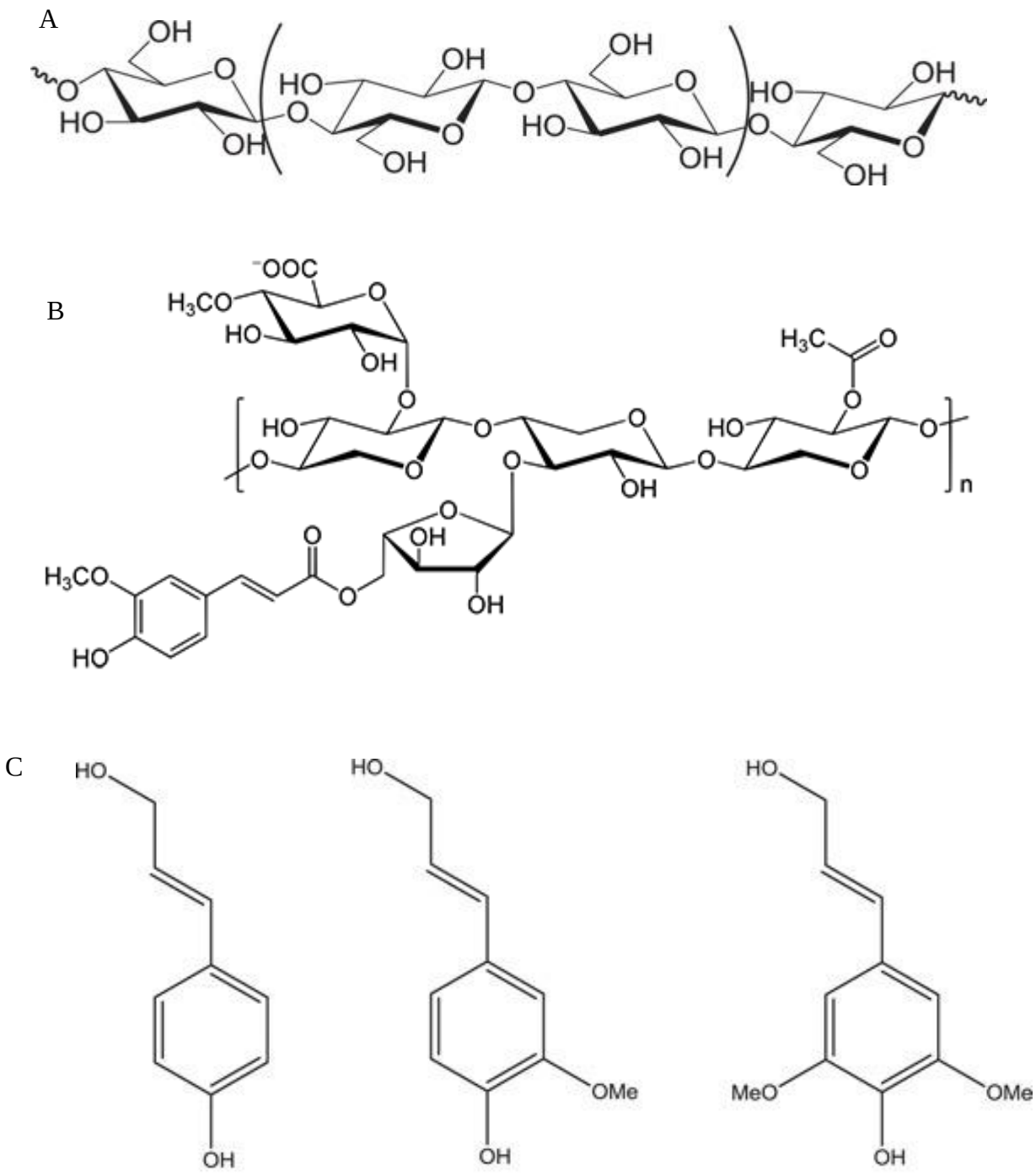

Figure 1. 2. Composition of sugarcane bagasse: A- cellulose, B- hemicellulose, C- lignin.

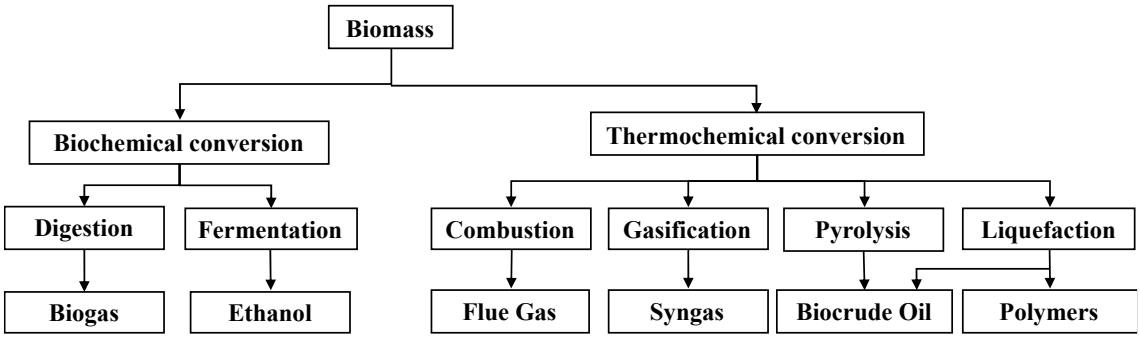

Figure 1. 3 Biomass conversion methods.

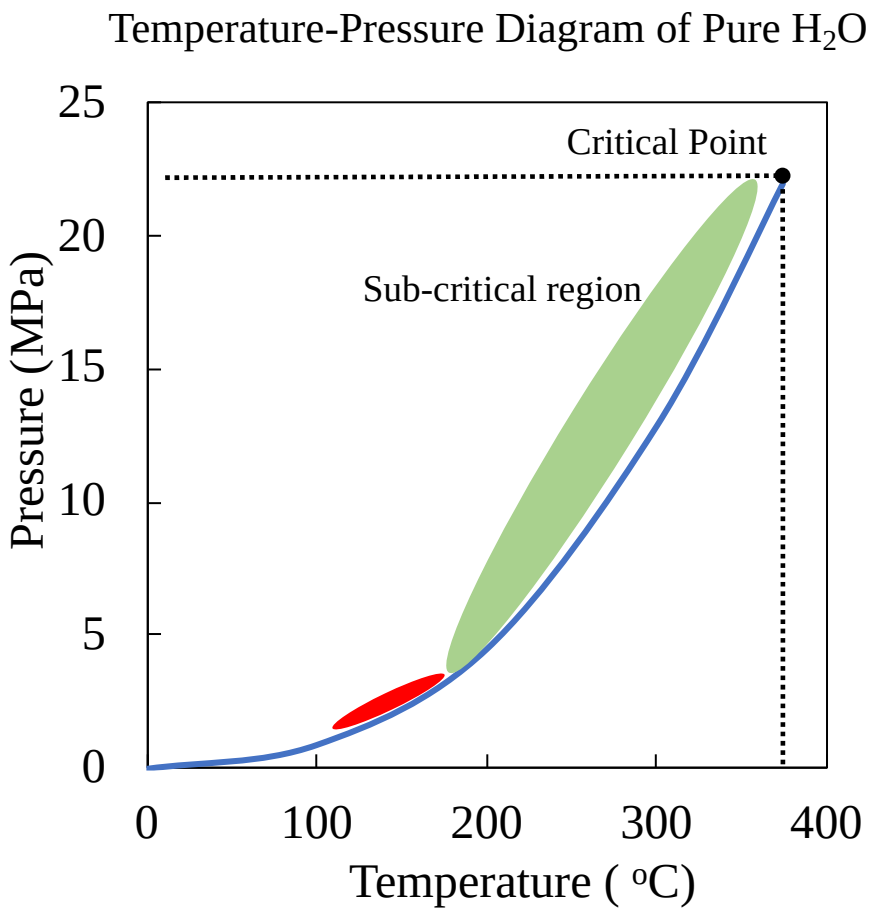

Figure 1. 4 Temperature and pressure profile of pure water under subcritical conditions with the red-colored region indicating the HTL parameters used in this study.

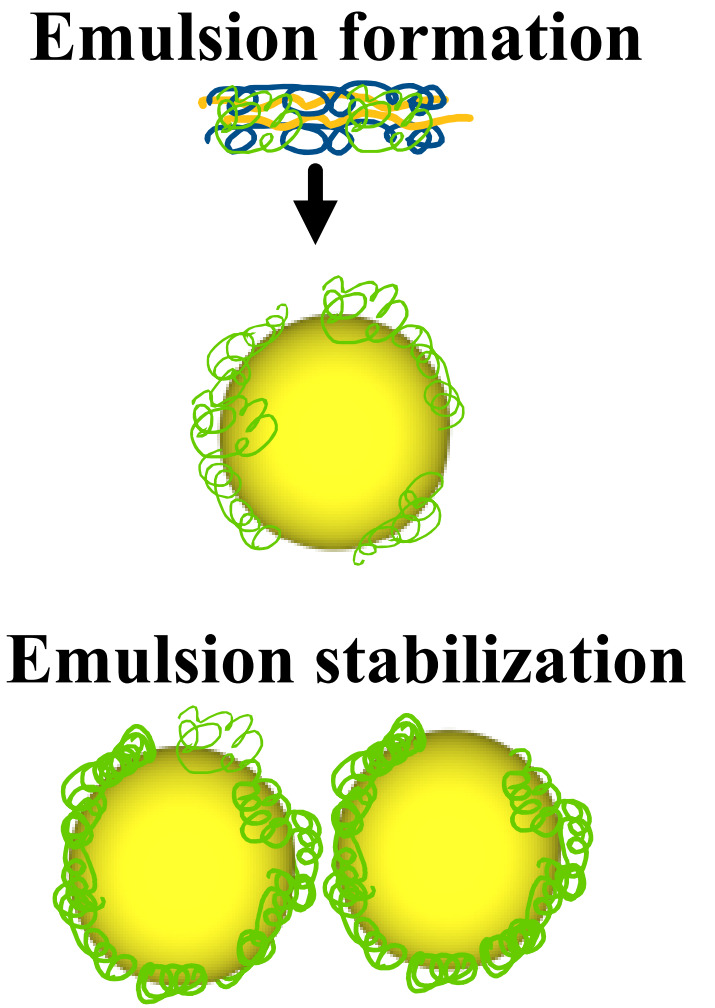

Figure 1. 5 Emulsifier formation and stabilization (McClements *et al*., 2017).

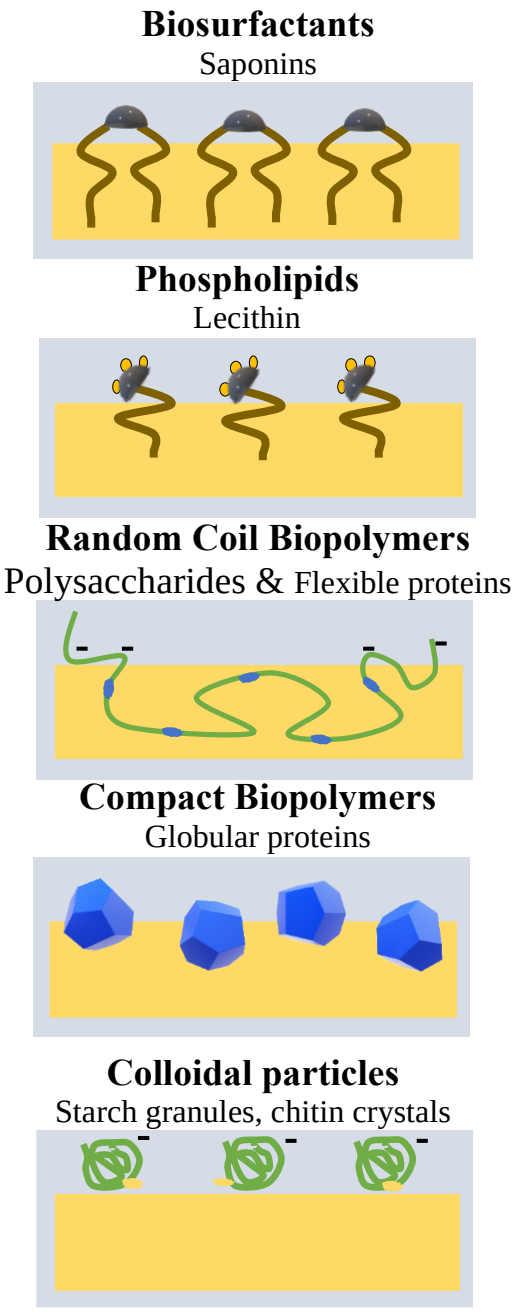

Figure 1. 6 Examples of natural emulsifiers (McClements *et al.*, 2017).

Chapter 2 HTL Treatment of Bagasse

2.1 Introduction

Biomass has many advantages compared with other renewable resources and it is a promising candidate to take place of fossil fuels in the future. However, due to low caloric value, low energy density, high volatile content and high hydrophobicity, it is defined as a low-grade fuel. Therefore, the biomass such as bagasse needs a pre-treatment to upgrade its valorization potential by convert to other valuable chemicals with sustainable qualities before utilization.

Bagasse, is the by-product from sugarcane industries and up to 280 kg is produced from 1 ton of sugarcane (Rabelo *et al.*, 2011). It had a global production of 279 million tons but only 50% left unutilized after burning to produce electrical energy (Chandel *et al.*, 2012; Pandey *et al.*, 2000). Major compositions are cellulose of 40-50%, hemicellulose at 25-35% and lignin from 7- 24%. Minerals, waxes and other compounds exist but to a lesser content. HTL as a pre-treatment method is mostly towards woody biomass and algae valorization for biorefineries (Caili *et al.*, 2015; Karagöz *et al.*, 2005; Dărăban *et al.*, 2015; Caporgno *et al.*, 2016; Jin *et al.*, 2014) while application on food is largely unexplored. Various methods employed in combination to breakdown the heterogeneity structure of biomass include chemicals, acid hydrolysis or alkaline, biological such as enzymatic hydrolysis and subcritical water treatment.

Acid or alkaline treatment h that is conventionally used for biomass treatment process using concentrated acid or alkaline. They are corrosive and the process by-products disposal is a very expensive and detrimental to the environment. It also has many

drawbacks, such as requirement of corrosion resistance equipment, low concentration of sugar, formation of inhibitory by-product and requirement of a pH neutralization process.

Enzymatic hydrolysis is one of the unit operations on the biomass conversion process that utilization of enzymes or microorganisms to depolymerize biomass to produce reducing sugars or other value-added chemicals. However, this process requires a long retention time for hydrolysis and biomass pre-treatment, enzyme production as well as enzyme recovery makes this process economically unfeasible.

Hydrothermal liquefaction are sometimes labelled autohydrolysis and hot water extraction basically operate under subcritical water condition. Two main parameters are temperature with a range from 150 –350 °C and pressures from 0.4 – 20 MPa. Water has a lower density and dielectric constant in these conditions allowing it to act as a reactant and hydrolyze water-insoluble biomasses. Use of HTL as a pre-treatment method have dominated recent publications in the last decade. For example, it has been used in lignin pre-treatment (Watanabe *et al*., 2018), sorghum (Qiu *et al*., 2017), water hyacinth (Singh *et al*., 2015) and switchgrass (Yu *et al*., 2016). HTL treatment as a green technology very much appeals as a promising route for conversion technology with focus on alternate, clean and cheap technologies being a global challenge. Therefore, the objective of this study was to treat bagasse using HTL and characterize the soluble extracts before application in foods as an emulsifier as described in Chapter 3.

2.2 Materials and Methods

2.2.1 Materials

Raw bagasse was obtained from Fiji Sugar Co. Ltd (Lautoka, Fiji), dried (105 °C, 4 h), fragmented to uniform sizes using a blender (Vita-Mix, Olmsted Township, Ohio, USA) and sieved (500 μm Sanpo I.S.O, Tokyo). Composition of raw bagasse was determined according to NREL method as described in the High-Performance Liquid Chromatography (HPLC) analysis below (Sluiter *et al.*, 2008) using H_2SO_4 and autoclaving. Chemicals used were purchased from Wako Pure Chemical Industries and Sigma-Aldrich.

2.2.2 Hydrothermal liquefaction treatment of bagasse

The treatment of the prepared bagasse samples was carried out in a batch reactor as depicted in Fig. 1.1. In each batch around 6 g of prepared bagasse and 194 g of distilled water were added in a stainless-steel vessel. This was then placed in a reactor before enclosing it with a heating mantle. Purging was done at first for 30 secs before pressurizing to 1 MPa. The reactor was heated externally from 25 °C to the set temperature of 160 ± 10 °C for 30 min. The reactor was cooled to room temperature before collecting the treated bagasse. The slurry solution was then separated to 2 portions of the soluble and insoluble. This was achieved through vacuum filtration using Whatman® glass microfiber filters with 100 mL of distilled water used for washing. It

was then freeze-dried using Eyela FDU-2110 Freeze Dryer (Tokyo Rikakikai Co., Ltd, Tokyo, Japan) for 48 h, before characterization analyses.

To understand the effect of HTL treatment, model compounds, starch and microcrystalline cellulose were treated with the bagasse samples following the described method above but at 220 ± 10 °C for 30 min.

Another process parameter, different temperatures was also employed using only bagasse as the sample. The method already described were treated at four different temperatures, 160, 180, 200, 220 ± 10 °C for 30 min.

2.2.3 Analysis of the bagasse extracts from HTL treatment

2.2.3.1 Liquefaction yield

The yield of the soluble (liquid) and insoluble residue (solid) after HTL treatment was calculated according to the equation below (Bi *et al*., 2017):

$$\%\ Yield = \frac{Product\ Weight\ on\ Dry\ Weight\ basis(g)}{Bagasse\ Weight\ on\ Dry\ Weight\ basis\ (g)} \times 100$$

2.2.3.2 Scanning electron microscopy (SEM)

The morphology of the samples was observed using SEM (TM-1000 Miniscope, Hitachi High Technologies, Tokyo, Japan). About 0.5 g of the freeze-dried samples was placed on the sample holding unit, placed in the sample chamber before applying the vacuum and had the images captured as in Fig. 2.

2.2.3.3 Ash

The ash content of the raw bagasse and the bagasse extract from HTL was determined by heating 6 g of the samples at 575 °C for 24 h in a muffle furnace.

2.2.3.4 Total carbohydrate content

Using Phenol-sulphuric acid method adapted from Ford (1981), total carbohydrate in the extract was analyzed. Briefly, 15 ml tube, 0.2 ml of the extract was made to 1 ml with distilled water before adding 1 ml of 5% Phenol and 5 ml of concentrated H_2SO_4. After 10 min the tubes were placed in a water bath at 27 °C for 15 min. It was read at 490 nm using the UV-Vis Spectrophotometer (V-570, JASCO Co., Ltd. Hachioji, Japan) after cooling to room temperature. An external calibration curve was prepared with 0.1 – 4 mg/ml xylose as standards.

2.2.3.5 Total phenolic content

Following Sacchetti *et al*. (2009), phenolic contents were determined Folin-Ciocalteu reagent. 0.1 ml of the sample and 0.5 ml of Folin-Ciocalteu reagent were mixed. After 3 min, 1.5 ml of 25% Na_2CO_3 solution were added and mixed before filled to 10 ml with water. Absorbance was read at 725 nm using the UV-Vis Spectrophotometer after keeping in the dark for 60 min at 25 °C. Gallic acid was used as standard.

2.2.3.6 Soluble lignin

Soluble lignin was determined using the equation below adapted from Sluiter *et al*. (2008):

$$\%\ Soluble\ lignin = \frac{UVabs\ x\ volume\ x\ df\ x\ 100}{\varepsilon\ x\ weight\ x\ pathlength}$$

where UVabs = average UV-Vis absorbance

Volume = volume of bagasse extract

DF = Dilution Factor

ε = Absorptivity of biomass at 240 nm

Weight = Weight of extract in mg

Pathlength = pathlength of UV-Vis cell in cm.

2.2.3.7 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography was done according to Sluiter *et al*. (2008) with few modifications. Bagasse extracts from HTL was filtered using a 0.45 μm hydrophilic PVDF syringe filter unit. The HPLC system (JASCO) employed a Shodex Sugar KS-801 column and a refractive index detector. Distilled water was used as the eluent at a flow rate of 0.3 ml/min and oven temperature set to 80 °C. Compound concentrations of the bagasse extracts from HTL were calculated based on the peak areas

from the HPLC chromatograms. Cellobiose, xylobiose, xylotriose and 5-Hydromethyl furfural (5-HMF) was used as standards with known 0.5 – 3 mg/ml.

The pH of the bagasse extracts from HTL was measured using a Metrohm 827 digital pH meter equipped with a glass electrode (Metrohm A.G., Herisau, Switzerland) which was calibrated before analysis.

2.2.3.8 Elemental analysis

Organic elemental analysis for Carbon, Hydrogen and Nitrogen of raw bagasse and extracts from HTL were conducted using a Perkin-Elmer instrument (2400 II CHN Elemental Analyzer) whereby approximately 5 g of the samples were produced for analysis. Each analysis was repeated in triplicates and the average results were taken.

2.2.3.9 Fourier transform infrared spectroscopy (FT-IR)

Pure KBr (FT-IR grade) pellets were prepared into a disc and then analyzed as a reference. Then 1.5 - 2.0 mg of lyophilized bagasse from HTL treatment were grounded and mixed with approximately 200 mg of KBr to form a mixture. Later the mixture was molded into a disc that was analyzed by FT-IR spectrometer (FT/IR-300, JASCO Co., Ltd. Hachioji, Japan) from 400 - 4000 cm^{-1} scanning range.

2.2.4 Statistical analysis

HTL experiments only were duplicated whereas other analyses were conducted in triplicates with data reported as mean ± standard deviation. VassarStats website was used to carry out one-way analysis of variance (ANOVA) with Tukey HSD tests ($p < 0.05$) were used for comparisons of mean group differences.

2.3 Results and discussion

2.3.1 Effect of HTL treatment on different Cellulose, Starch and Bagasse

In order to understand the effect of HTL treatment using only water, different samples were employed in comparison with bagasse. 3 wt% of each sample was treated at 220 °C for 30 min and 1 MPa initial pressure. As observed in Fig. 2.2, the liquid yield of cellulose, starch and bagasse was at 25.8, 87.0 and 68.7% respectively. On the other hand, the solid yield was at 73.0% for pure cellulose, 9.3% for starch and 27.7% for bagasse. The effect of HTL on different samples can be attributed to the chemical structure of the different samples. For cellulose, it has rigid and microcrystalline structure due to the β-1,4-glycosidic linkages which makes it harder to be hydrolyzed (Rogalinski *et al.*, 2008). However, at temperatures above 200 °C microcrystalline solubility has been shown to be more water soluble with more than 50% converted at 300 °C (Tolonen *et al*., 2011; Yu & Wu, 2010). In addition, Kumar & Gupta, (2008) reported about 65% of cellulose were converted to oligomers at 335 - 354 °C. For starch, the β-1,6- glyosidic linkages is more vulnerable to temperature. It has amorphous structure and is easily susceptible to

temperature changes. As a carbohydrate containing amylopectin, temperature from HTL treatment have shown that majority can be converted to the liquid yield. The yield for starch in this study have been reportedly similar to values obtained in previous studies. Yoshida *et al*., (2010) in their study reported 75.2% of carbohydrates from corn starch treated using 220 °C. With HTL batch reactors, approximately 45- 63% of starch have been also reported with major products being glucose, maltose, fructose and degradation products such as furfurals even with only 30 mins (Nagamori & Funazukuri, 2004; Orozco *et al*., 2012). Bagasse contains cellulose with a complex heterogeneity structure with hemicellulose and lignin. It has shown that at temperature ranges from 160- 220 °C there are slight differences which will be discussed in the next section. As a biomass, it follows similar liquefaction yield reported at similar parameters obtained in this study. That is, 30.4% of palm fruit bunch were liquefied using HTL with only water at 270 °C , 20 min and 2 MPa (Akhtar *et al*., 2010). Prado *et al*. (2014) reported 95% liquefaction at 251 °C with only 5.6 % of monosaccharides and oligosaccharides obtained at 213 °C HTL treatment.

With carbohydrates most likely undergoing degradation reaction at higher temperatures, organic acids would result in addition to phenolic compounds also produced. This can be supported by the analyzed pH after treatment as shown in Table 2.1. Ando *et al*. (2000) in their study of bamboo, chinquapin and Japanese cedar with hot compressed water reported that the pH of the aqueous solution decreased as the product yield increases. This is in agreement with the results obtained in this study as Cellulose with the highest liquid yield produced the lowest pH and pure microcrystalline cellulose

having a high pH. Similarly, treatment of oil palm biomass from 150 - 190 °C also reported a drastic decrease in pH from 6.5 in untreated samples to pH of 3.8. These reduced pH can be mostly be attributed to both formic and acetic acids and lignin derived phenolic compounds (Ando *et al*., 2000; Pourali *et al*., 2009).

Under hydrothermal conditions, it can be observed that all polymeric compounds such as cellulose, starch and bagasse can all be hydrolyzed into the soluble. However, depending on the chemical structure of the compound, liquefaction at 220 °C, 1 MPa for 30 min was restricted for cellulose but more pronounced for starch hydrolysis because of their β-1,4- and β-1,6- glycosidic linkages respectively.

2.3.2 Effect of different HTL temperature on bagasse liquefaction

After observing the effect of HTL on model compounds mentioned in previous section, the effect of different temperature was studied to understand temperature as a function of hydrothermal liquefaction treatment. With lower subcritical water conditions utilized in this study, using only water has been shown to be an effective catalyst since it can act as both the extractant and catalyst (Pourali *et al.*, 2009). As more research focus on HTL technology advances, more parameters are being optimized such as the use of catalyst, flowrate for continuous reactors, time, solid to liquid ratio, pressure, and the list goes on. However, since HTL is based on the working principle of subcritical water primarily temperature and pressure, it was therefore important to study the best temperature that can be used to hydrolyze functional extracts to be employed in food industries such as emulsifiers without employing any chemical or catalyst in the process.

The different temperatures used to treat 3 % (w/w) of bagasse produced different liquefaction results. That is, 180 - 220 °C treatment significantly liquefied solid bagasse into soluble extracts or liquid (Fig. 2.3). 47.8% of the initial 6 g were solubilized into the liquid portion at 160 °C whereas 180, 200, and 220 °C converted 65.9, 68.2, and 68.6% respectively. The liquefaction data reported in this study is in agreement with previous studies. Previous studies have shown that treatment at 300 °C treatment converted 61.8% (Bi *et al*., 2017) and more than 65% converted with water but 90% when tetralin was used in the treatment of bagasse from 200 - 320 °C (Li *et al*., 2015). It can also be observed that as temperature increases, the solid residue yield decreases as it is hydrolyzed to the mixtures of poly-, oligo-, di-, and monosaccharides in the water soluble portion (Pourali *et al*., 2009).

However, HTL is an important pretreatment method basically used in majority of the biorefineries for the production of value-added products from wood as well as other perennial grasses. Catalysts have been employed in combination with other chemicals to optimized extraction conditions. As previously mentioned, depending on the targeted polymer and their use, certain conditions might be preferable over others. In this study, 180 - 220 °C would be a more preferable condition since it converted above 50% of the initial bagasse however in terms of functionality as an emulsifier, the extracts produced from the treatment at these conditions did not satisfy surface active properties which will be discussed in Chapter 3. As a target of extraction, the higher liquefaction yields are more preferable as this can translate to reduced input factors such as heat or electrical energy to produce extracts and also improves the efficiency of the treatment method.

Since the objective of this research is towards identifying the functionality of the extracts as an emulsifier and not so much on optimizing the HTL treatment where quantification of the saccharides at each temperature would be of major interest.

2.3.3 Hydrothermal liquefaction treatment of bagasse at 160 °C

From the treatments presented in earlier sections, HTL at 160 °C provided the chosen condition for further analysis as it showed the best characteristics for further application in the Chapter 3. So the next few sections will solely discussion and characterization of extracts from bagasse at 160 °C. The temperature-pressure profile as shown in Fig. 1.2 where it took 36 min for the temperature to reach 150 °C from room temperature. The timer automatically recorded 30 min from this point as the heater maintained the temperature inside the reaction vessel at 160 ± 10 °C. After 30 min, the heater was turned off and it took approximately 4.7 h to cool to room temperature. On the other hand, the pressure was dependent on the set temperature during the HTL treatment and in this study, 1 MPa was initially set inside the reaction vessel and it reached a maximum pressure of 1.9 MPa after 62 min. Therefore, hydrolysis may have started at 100 °C and continued after the heater was turned off since elevated temperatures above 100 °C and pressure above 1 MPa have been known to hydrolyze the lower solubility sugars. Therefore, it exposed the intact lignin-cellulose structure for further hydrolysis during the treatment.

For the 3% initial solid concentration used in this study, 47.4% of bagasse was solubilized and 52.6% remained as insoluble solids. Santucci *et al.* (2015) extracted only 27% of the initial mass at 160 °C which was low while others have contrasting findings

with 60 – 85% extracted after HTL treatment (Carvalho *et al*., 2015; Ju *et al*., 2011). HTL as an appealing eco-friendly technology consists of a lot of parameters needed for effective liquefaction. Temperature and reaction time are the two most common parameters studied in HTL treatment while liquid to solid ratio is another parameter that affects liquefaction Usually, there is combination of different solvents such as black liquor, alkaline or ethanol (Kosinkova *et al*., 2016; Long *et al*., 2015; Yuan *et al*., 2007) for conversion into biofuel production but with our research targeted towards emulsion studies, water was used instead to minimize neutralization or by-products disposal.

2.3.4 Characterization of bagasse extracts from HTL at 160 °C

2.3.4.1 Morphological characterization of the bagasse extracts from HTL

Bagasse extracts from HTL treatment (Fig.1.3B) appeared to be powdery and fluffy as compared to raw bagasse (Fig. 1.3A). The SEM images of the bagasse extract from HTL shown in Fig. 1.3D was observed after freeze-drying. Similar smooth surface was in agreement with Kumar *et al*. (2014) in their delignification study of bagasse with acidified sodium chlorite. It had oval and longitudinal shapes after internal water evaporation during freeze-drying. Duchesne *et al*. (2001) on their study of hemicellulose of kraft pulp with a high content of hemicellulose contained a porous surface while low hemicellulose content had a more compacted surface structure. HTL treatment of bagasse hydrolyses the rigid glycosidic bonds between saccharides resulting in a thin and curved morphology of the liquefied product characteristic of an amorphous polymer. Raw

bagasse SEM image (Fig. 1.3C) showed a more intact structure with rigidity but the effect of fragmentation during bagasse preparation into uniform sizes was evident, posing more surface area for effective hydrolysis during HTL treatment.

2.3.4.2 Extracts obtained from HTL Treatment of Bagasse at 160 °C

The major components of bagasse extracts from HTL treatment are carbohydrates and their derivatives in soluble forms such as sugars or organic acids. Recent researchers have shown that HTL treatment of bagasse at 160 °C produces hemicellulosic sugars such as xylose or xylooligosaccharides from xylan which have low molecular weight hemicellulose abundant in bagasse (Neves *et al*., 2016; Yu *et al*., 2013). The composition of the bagasse extracts mainly composed of carbohydrates at 510.3 mg/g with detected saccharides in decreasing order are xylotriose > cellobiose > xylobiose. This amount corresponded to 51% of the bagasse extracts (Table 1) which may be attributed to the higher molecular weight oligosaccharides other than xylotriose and xylobiose, that were hydrolyzed during lower subcritical parameters of HTL. Yu *et al*. (2016) characterized 33.0 - 55.9% of xylooligomers (xylose to xylohexose) after liquid hot-water treatment of three hybrid switchgrass but 44.1- 67.0% were unidentified and labelled as other xylooligomeric compounds. On the other hand Szczerbowski *et al*. (2014) reported lower concentration of unidentified cellulose derivative, anhydrohexose, at 1% after acid treatment. In our study, the unidentified compounds at 49% of the bagasse extract may be attributed to water-soluble polysaccharides, lignin-carbohydrates complexes (Tarasov

et al., 2018), acetyl groups derived from xylan (Carvalho *et al*., 2015) and organic acids. Interestingly, lignin-carbohydrate complexes are molecules with a hemicellulose backbone covalently bonded to small lignin fragments from plant cell (Martínez-Abad *et al*., 2018). Lehtonen *et al*. (2016) reported that these unidentified compounds were most likely flavonols. Therefore, these soluble fragments in bagasse extracts are common in biomass treatment at subcritical water conditions.

In perennial plants such as bagasse, xylose is usually the major component of the hemicellulosic fraction. There is a high solubility of hemicellulose sugars at subcritical temperatures below 180 °C because of their amorphous structure (Ju *et al*., 2011). In this study at 160 °C, xylotriose was the dominant derivative detected due to the degradation of xylan. Santucci *et al*. (2015) found that 94.5% of hemicellulosic sugars were removed during HTL (190 °C, 67 min) as compared to 20% of cellulose and 35% of lignin.

Cellulose is made up of amorphous and crystallized regions whereby the solubility of the crystallized cellulose usually occurs at temperatures above 275 °C (Kumar & Gupta, 2008). Zhang & Wu (2013) indicated higher solubilization of glucose as compared to xylose during their CO_2-assisted HTL treatment of bagasse where 67% of xylose and 94% of glucose were extracted. Cellobiose in the extract at 0.9 µg/g is a result of cellulose dehydration before degraded to acetic acid, ketones, aldehydes, and other derivatives (Gao *et al*., 2011). Xylobiose at 0.1 µg/g was negligible, as xylan was present as higher molecular weight oligosaccharides since the extracts particle size measured 443 nm

(Table 1). With higher temperatures and longer time, degradation would be dominant over hydrolysis resulting in low molecular weight monomeric sugars. Lü & Saka (2010) reported that Japanese beech at subcritical temperatures, xylose was present at 290 °C but xylooligosaccharides were not when using similar Shodex KS-801 column.

However, with approximately 20 - 35% of lignin present in bagasse, soluble lignin detected in the bagasse extract was at 7.1% (Table 1). Qiu *et al*. (2012) reported that bagasse treated with water only solubilized 2.3% of the initial lignin. In pressurized reactors, lignin are depolymerized to phenols (Yoshikawa *et al*., 2013) and with HTL treatment at 160 °C, the majority remained in the insoluble residue. The phenolic content of the bagasse extract was 0.5 mg/g, lower than 3.65 mg/g previously reported by Zheng *et al*. (2017).

Phenolic content in bagasse are derived from cell walls and consist of gallic acid, ferulic acid, *p*-coumaric acid and vanillin acid (Xu *et al*., 2005; Zhao *et al*., 2015). The pH of the bagasse extract was at 3.9 and in agreement with Sasaki *et al*. (1998) because lignin fractions and cellulose are converted to acetic acid or organic acids. 5-HMF is a result of cellulose degradation but it can be further degraded to levulinic acid and formic acid (Larsson *et al*., 1999). For this reason, HTL treatment conditions should be well optimized to recover biomass extracts with less acidity that can be optimized further in the food industry or pharmaceutical industries.

2.3.4.3 Fourier-transform infrared spectroscopy and elemental analysis

Raw bagasse was analyzed separately with the bagasse extract in order to compare the surface functional groups converted as a result of HTL treatment (Fig. 1.5A). With a lower transmittance and a slight shift in wavenumber, raw bagasse which may be due to its intact structure as compared to the exposed structure of bagasse extract from HTL. The broad bands at 3333 cm^{-1} are typical of O-H stretching as observed in most carbohydrates. C-H stretching in hemicellulose and cellulose occurs at 2908 cm^{-1} and bands at 1606 cm^{-1} are similar to the carbonyl stretching of *p*-Coumaric acid, a phenolic residue (Sofla *et al*., 2016). Absorbance at 1505 cm^{-1} is characteristic of the aromatic skeletal vibrations with C-O stretching of lignin (Zhang *et al*., 2013). The band observed at 1119.8 cm^{-1} is typical of the aromatic C-H in-plane deformation seen in lignin-carbohydrate complex observed by Singh *et al*. (2005). The glycosidic linkages between glucose units in cellulose can be identified by the spectrum at 826 cm^{-1}. Below 190 °C, HTL usually favors the hydrolysis of amorphous hemicellulose such as xylan but cellulose and lignin are partially hydrolyzed as seen in the FT-IR spectra (Fig. 1.5B).

Bagasse as a biomass is made up of carbon, hydrogen, nitrogen, and oxygen. Carbon contents in raw bagasse was at 48.3 wt% and upon HTL treatment, 50.1 wt% of the carbon (Table 1) was liquefied in the form of soluble saccharides. Previous literature have shown comparable carbon content in the extract with 45.7 wt% detected (Ju *et al*., 2011; Savou *et al*., 2019). Carbons are hydrolyzed from hemicellulosic sugars such as xylose and amorphous cellulose in their oligomeric structures. Nitrogen content, on the other hand,

at 0.2 wt% was minute but HTL at higher temperatures can liquefy the proteinaceous compound from biomass. HTL hydrolyses the peptide bonds and producing amino acids, that are decarboxylated to ammonia or organic acids (Sheehan & Savage, 2016). Ash in bagasse extract was at 2.1% and mostly consists of Si, Ca, K, Na and Mg (Raveendran *et al*., 1995). The reduced mineral content in bagasse may be influenced by processing in raw sugar factories which minimizes any juice left and, in the process, removed the minerals accumulated from harvesting.

2.4 Conclusions

The results presented results validated the applicability to HTL in converting the structural polysaccharides that are not usually soluble at room temperature. HTL treatment have conventionally used as a pre-treatment to disintegrate biomasses before applying chemical or biological hydrolysis. Interestingly, the results showed that lignin-carbohydrate compounds might be produced as intermediates as hydrolysis at the subcritical region usually produce reactive polymer fragments. We have shown that HTL has the potential to liquefy polymeric extracts that can act as natural emulsifiers in emulsion systems in any industry. Bagasse extracts from HTL showed the presence of carbohydrates and phenolics at 51.0 % and 0.05 % respectively as well as the presence of degradation products such as furfurals. Together with the presence of organic acids, phenolics compounds contributed to lower pH of 3.9 detected after HTL. The use of such treatment technique is an effort to promote more eco-friendly technologies with less

dependency on the use solvents. However, depending on the biomass, it might be necessary to use organic solvents in hydrothermal liquefaction for specially hydrolyzing certain lignocellulosic compounds such as phenols or hemicellulose.

Table 2. 1 pH of Cellulose, Starch and Bagasse samples before and after HTL treatment at 220 ℃, 1 MPa, and 30 min.

Sample	pH before HTL	pH after HTL
Cellulose	6.7 ± 0.1^{a}	3.2 ± 0.2^{b}
Starch	6.9 ± 0.1^{a}	2.9 ± 0.3^{b}
Bagasse	6.0 ± 0.1^{a}	3.6 ± 0.3^{b}

Data expressed as mean ± standard deviation (n=3).

Table 2. 2 Summary analysis of raw bagasse and bagasse extract from HTL at 160 ℃, 1 MPa and 30 min.

Properties and composition	**Raw bagasse**	**Bagasse extract from HTL**
Total carbohydrates (%)	63.6 ± 0.1	51.0 ± 0.1
Total phenolics (%)	ND	0.05 ± 0.1
5-HMF (%)	0.01 ± 1.9	ND
Ash (%)	2.7 ± 0.1	2.1 ± 2.7
Soluble lignin (%)	12.5 ± 0.4	7.1 ± 0.4
Protein (%)	1.9 ± 0.1	1.25 ± 0.1
Unknown compound (%)	19.3 ± 0.7	38.6 ± 0.5
Carbon (%)	48.3 ± 0.5	50.1 ± 0.4
Hydrogen (%)	6.2 ± 0.2	6.2 ± 0.1
Nitrogen (%)	0.3 ± 0.1	0.2 ± 0.1

Values were calculated based on dry weight of extracts. Data expressed as mean ± standard deviation (n=3). ND= Not detected.

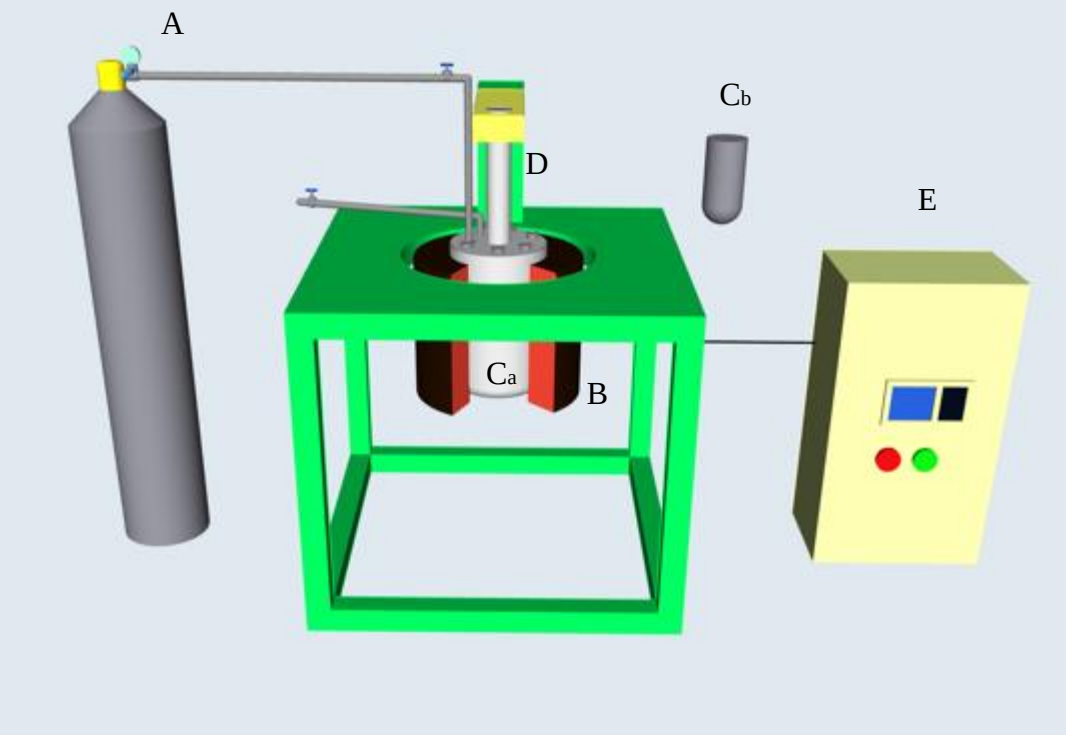

Figure 2. 1 Experimental setup of the hydrothermal liquefaction employed in this study. A: Nitrogen gas cylinder, B: Heater, C_a: Sample reactor, C_b: Reaction sample cell, D: Mechanical stirrer, E: Control panel

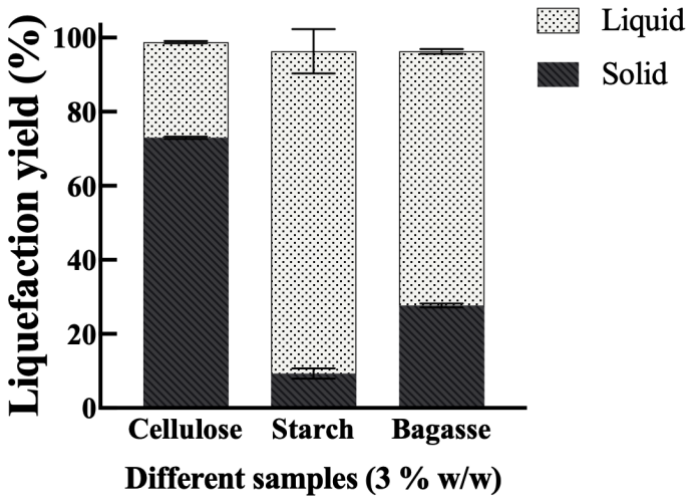

Figure 2. 2 Effect of HTL liquefaction at 220 °C on Cellulose, Starch and Bagasse samples.

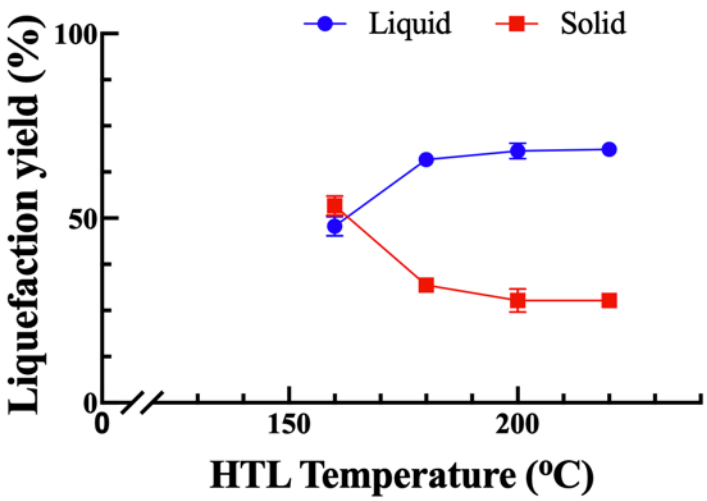

Figure 2. 3 Effect of different HTL temperature on liquefaction yield of 3 % (w/w) bagasse samples.

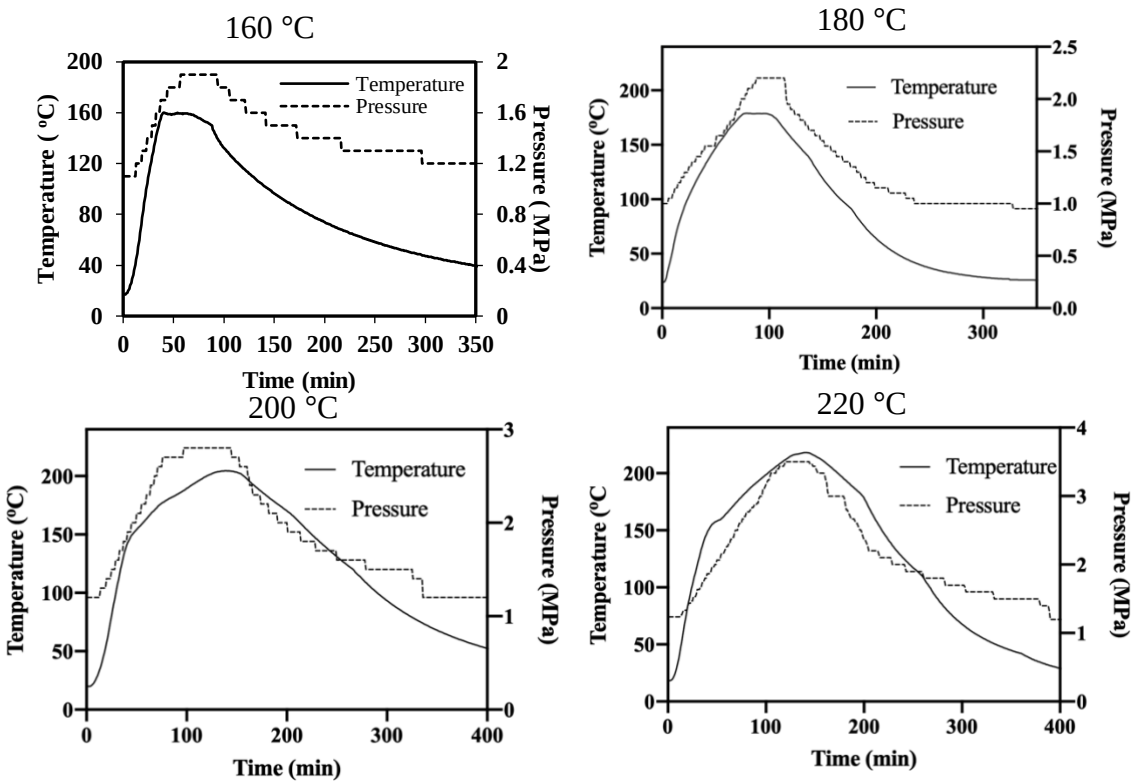

Figure 2. 4 Temperature and pressure profile of HTL treatment of bagasse at 160-220 °C for 1 MPa initial pressure and 30 min reaction time.

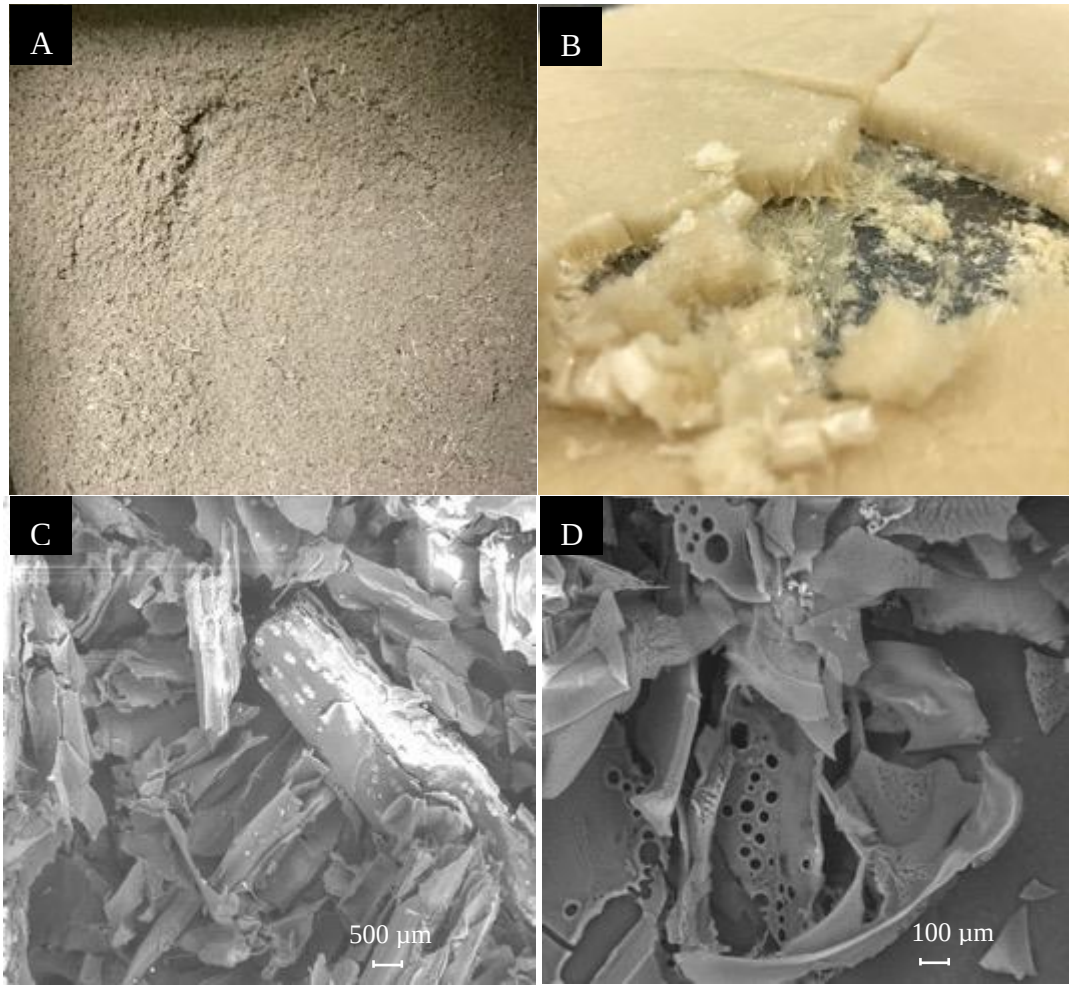

Figure 2. 5 Photo and scanning electron microscopy (SEM) of raw bagasse and bagasse extract from HTL. A: Photo of raw bagasse, B: Photo of bagasse extract from HTL after lyophilization, C: SEM of raw bagasse (200×), D: SEM of bagasse extract from HTL (800×).

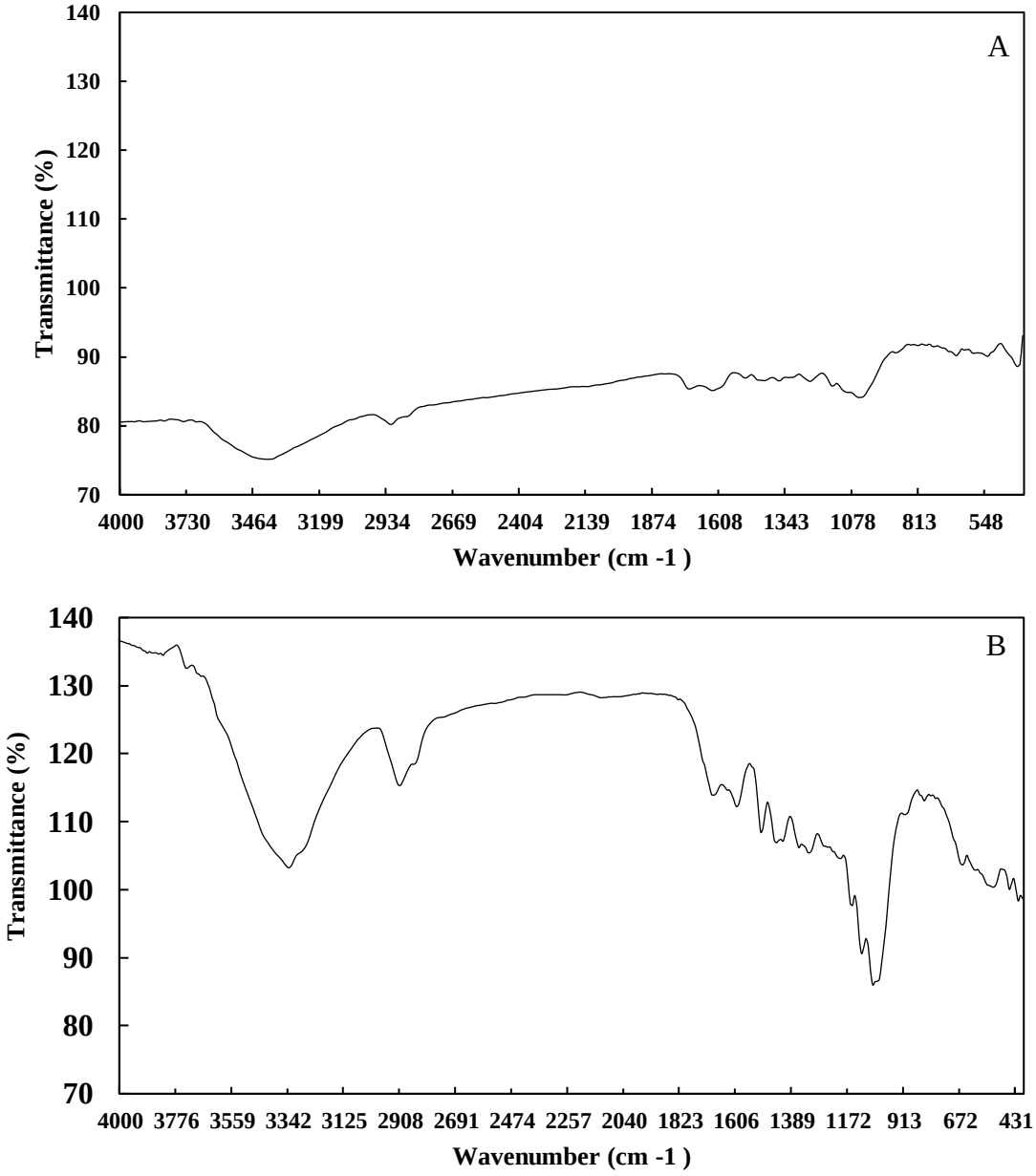

Figure 2. 6 FT-IR spectra for raw bagasse (A) and bagasse extract from HTL (B).

Chapter 3 Emulsion Formulation of Bagasse Extract

3.1 Introduction

With the pandemic such as Coronavirus stretching out the supply demands due to panic buying; food manufacturing industries have to be on standby. This is just a small picture of the long supply chain from farms to food delivery with food processing included. With emulsion commonly used in both pharmaceutical and food industries, more research emphasis are being focused towards these two industries in the current global pandemic. With bagasse being an agro-industrial by-product, a lot of publications revolve around biofuel whereby its application as an emulsifier is hardly studied.

Emulsifiers are compounds that have amphiphilic properties due to their chemical structure which enables them to stabilize two immiscible liquids where one is dispersed in the other and forming an emulsion. It has been a decade since Sangnark & Noomhorm (2004) utilized bagasse as a dietary fiber in comparison with commercial dietary fiber (Solka Floc® 900). They found that bread made by 10 g/100 g of each dietary fiber substitution scored favorable by consumer when sugar ester was added at 1.5 g/100 g as wheat flour. Kim *et al*. (2018) used alkaline-treated bagasse fiber as a meat emulsifier. Their results confirmed that bagasse fiber improved the physicochemical and textural properties of meat emulsion, at 2% addition level. Therefore, the insoluble bagasse fibers have shown potential in meat emulsifiers but HTL treatment can be able to produce soluble fibers/polymers from the insoluble bagasse. In order to be identified as an emulsifiers should rapidly adsorb to the oil droplet surfaces and form a stable protective

layer around the oil droplets that prevents them from aggregation and instability (Dickinson, 2009; McClements, 2015).

Gum arabic (GA) and pectin are two well-known natural emulsifiers widely employed in the food industries. Their stability mechanisms well documented and are attributed to the presence of proteins or by increasing the viscosity of the aqueous phase (Lehtonen *et al.*, 2018). The production of GA has been declining in these countries such as Sudan and Nigeria with 45,000 tons annually. However it cannot meet the global demands due to increased application in confectionaries (Mujawamariya *et al*., 2013). Pectin on the other hand has a global production exceeding 60,000 tons annually with an increasing demand that will soon exceed production (Ciriminna *et al*., 2016). This is because the global food hydrocolloids market is expected to reach US$7.56 billion by this year, 2020 (Lehtonen *et al.*, 2018). It is critical to look at bagasse as a natural alternative provided it is abundant, is cheap and readily available as a polysaccharide source. These natural soluble polymers have the potential to stabilize emulsions instead of burning it in high-pressure boilers. Therefore, in this study, we evaluated bagasse extracts from HTL and formulated an O/W emulsion stabilized by these extracts in comparison with GA.

3.2 Materials and methods

3.2.1 Materials

Raw bagasse extracts from HTL treatment at different temperatures (160-220 °C) was obtained after lyophilization as described in Chapter 2.3.2 and 2.3.3. Chemical

reagents used (analytical grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich.

3.2.2 Preparation of O/W emulsions

3.2.2.1 Interfacial tension

To measure the interfacial tension between the extracts and soybean oil, firstly, 3 wt% of the continuous phase was prepared by dissolving the lyophilized bagasse extracts ($BE_{160\text{-}220}$) in 5 mM Phosphate buffer at 25 °C for 12 h. The interfacial tension between the bagasse extract dispersion and soybean oil was measured using a DM-501 Interfacial Tension Meter (Kyowa Interface Science Co., Ltd. Saitama, Japan) following the pendant drop method.

From the results obtained at different HTL temperatures extracts, BE_{160} was then further prepared (0- 4 wt%) in 5 mM Phosphate buffer at 25 °C for 12 h. A 3 wt% of GA was used in comparison by following similar procedures described above while only 5 mM Phosphate buffer was used as a control.

3.2.2.2 Formulation of O/W emulsions

After preliminary experiments, 3 wt% of bagasse extracts from HTL treatment ($BE_{160\text{-}220}$) was chosen as the continuous phase. The dispersed phase contained soybean oil with a weight fraction of 5 wt%. Initially, pre-mix emulsions were prepared using a Polytron

rotor-stator homogenizer (P) at 10,000 rpm for 5 min. Immediately it was transferred to a high-pressure homogenizer, (NanoVator NV200, Yoshida Kikai, Co., Ltd., Tokyo, Japan) illustrated in Fig 3.2. The parameters used in the homogenization pressure was 100 MPa and 4 passes to achieve a homogenous emulsion. A separate 3 wt% of BE_{160} was chosen for further investigation and homogenized following similar parameters previously described with 3 wt % GA used in comparison.

3.2.2.3 Droplet size distribution of O/W emulsion

Average droplet diameter of 3 wt% BE-stabilized emulsions was determined with a Laser Diffraction Particle Size Analyzer (LS 13320, Beckman Coulter, Brea, USA). Variation in emulsion droplet sizes within 15 days for $BE_{160\text{-}220}$ and 11 days for a separate set of emulsion stabilized by BE_{160} and GA. It was stored at 25 °C with the measure of stability of the emulsions reported as volume mean diameter ($d_{4,3}$) and Sauter mean diameter (d_{32}). The refractive index used in the measurement were 1.471 for oil and 1.333 for the water.

The particle size was expressed as De Brouckere or volume mean diameter (d_{43}) and Sauter mean diameter (d_{32}) as shown in Equation 1 and 2 respectively,

$$d_{43} = \frac{\Sigma(n_i.d_{i4})}{\Sigma(n_i.d_{i3})} \text{..... Equation (1)}$$

$$d_{32} = \frac{\Sigma(n_i.d_{i3})}{\Sigma(n_i.d_{i2})} \text{.....Equation (2)}$$

in which *n* is the number of *d* diameter droplets.

3.2.2.4 Zeta potential

To determine the zeta-potential, 3 wt% of the BE_{160}-stabilized emulsion at different pH was diluted 100 times with the 5 mM Phosphate buffer solution for consistent data reading. It was then transferred into folded capillary cells (DTS1060) that was fitted into a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). Also, particle size measurements of the BE_{160} were analyzed using the dynamic light scattering mode of the Zetasizer Nano ZS. Measurements were done in triplicates and average data reported.

3.2.2.5 Emulsion droplet morphology

A Leica Optical Microscope (Leica Microsystems Inc., Heidelberg, Germany) was used to observe the microstructure of the BE_{160} and GA-stabilized emulsions. Images of representative areas of each emulsion sample were taken using a 40X magnification.

3.2.3 Statistical analysis

Experiments were conducted in triplicates with data reported as mean ± standard deviation. VassarStats website was used to carry out one-way analysis of variance (ANOVA) with Tukey HSD tests ($p < 0.05$) were used for comparisons of mean group differences.

3.3 Results and discussion

3.3.1 O/W emulsions stabilized by bagasse $BE_{160\text{-}220}$ and GA

3.3.1.1 Interfacial properties of the $BE_{160\text{-}220}$

Simple saccharides such as glucose, xylose, and galactose are hydrophilic and do not have lipophilic groups to influence interfacial activity. HTL disintegrates bagasse into high molecular weight oligosaccharides that are water soluble and having interfacial activity properties (Table 3.1). As the temperature of HTL increases, the bagasse extracts produced all showed interfacial activity but BE_{160} was significantly reduced the interfacial tension than $BE_{180\text{-}220}$. This finding indicated that even though HTL at 180-220 °C can liquefy more than 50% of the bagasse, the extracts produced lack the surface-active compounds compared to BE_{160}. It was an indication that at higher temperatures, degradation is the dominant reaction over hydrolysis whereby bagasse extracts produced are slightly less amphiphilic than BE_{160} (Baxter *et al.*, 2014).

Initially, after HTL treatment, bagasse extracts contained 39 μm/g of the detected oligosaccharides (Table 3.2) which were in low concentration and therefore have less contribution towards the interfacial activity of the aqueous phase. However, when concentrating the extract between 0.5- 4 wt% concentration, interfacial tension decreased from 19.8 to 14.0 mN/m with the lowest value achieved by 4 wt%. (Rojas *et al.* (2007) reasoned that for polymers, lignin might contribute to the overall amphiphilic property of the extract. GA was prepared at only one weight concentration (3 wt%) and it reduced the interfacial tension to 12.4 mN/m. Reason for a lower value as compared to the bagasse

extracts is because of the presence of polysaccharide-protein backbone making it amphiphilic as the protein will adsorb into the oil phase. Bagasse extracts, on the other hand, has very negligible protein content after converting the nitrogen content by 6.25 factor. The lignin-carbohydrate complex may contribute to extracts interfacial properties. Most lignin reported, are stabilizers in Pickering emulsions due to their hydrophobic nature (Wei *et al*., 2012) while Liu *et al*. (2016) modified lignin to enable it as an efficient emulsifier. Modification such as the addition of a long alkyl chain to lignin derivatives and drastically improving their interfacial tension to 31 mN/m at 0.1 g /ml concentration have been reported (Homma *et al*., 2010). That is, lignin as a hydrophobic domain and polyethylene glycol diglycidylethers as a hydrophilic domain improved the overall amphiphilic properties and surface activity of the modified lignin derivatives. Mechanism of biomass-derived emulsifiers produced by subcritical water condition has been gaining interest lately as it holds great potential in both food and pharmaceutical industries. Lehtonen *et al*. (2018) reported that phenolic residues in galactoglucomannan as being partly responsible for stabilization of oil-in-water (O/W) emulsions. Therefore, the bagasse extracts from HTL treatment has surface active properties that are comparable to well-known emulsifier such as GA.

3.3.1.2 Physical stability and morphology of $BE_{160\text{-}220}$-stabilized O/W emulsion

The storage stability of emulsion-stabilized by $BE_{160\text{-}220}$ was observed for 15 days of storage at 25 °C as depicted in Fig 3.1. The d_{av} of each sample over the storage period

was 2.8 μm for BE_{160}, 9.765 μm for BE_{180}, and 15.33μm for BE_{200}. The measurement for BE_{220}-stabilized emulsion was discontinued after the Day 3 as the emulsion was unstable with dark colored aqueous phase and phase separation as observed in the photographs in Fig 3.1, in addition to the large droplet sizes. Similarly for 3 wt% of BE_{180} and BE_{200} , even after homogenization (Day 0), the extracts produced an emulsion with a slight brownish appearance which were probably due to the Maillard reaction of the extracts.

From these results, 3 wt% of BE_{160} was then prepared with GA and observed for 11 days of storage at 25 °C as depicted Fig 3.3. The average droplet diameter, d_{av} of the emulsion-stabilized by bagasse extracts was 0.79 μm while GA-stabilized emulsion had a d_{av} of 2.2 μm. The droplet sizes of the emulsion stabilized by bagasse extracts were lower than hemicellulose-stabilized emulsion observed by Qiu *et al*. (2017). They observed a d_{av} of 1.19 μm for 7 days. The emulsions were stable until Day 2 when the bagasse extract-stabilized emulsion formed a light creaming layer and a characteristic light brown color from the solubilized lignin fractions during HTL treatment (Fig 2.2A). The creaming could be attributed to the oil phase as it was less dense and the aqueous phase containing high molecular weight compounds were denser. In terms of size distribution, bagasse extract-stabilized emulsion had bimodal distribution. This was due to varying droplet sizes, but it maintained stability at the emulsion interface and prevented coalescence. It can be observed that there was not much change in particle size distribution between Day 0 and Day 11 (Fig 2.3A). Droplet sizes variation in bagasse extracts was expected since it was not purified into a homogenous sample such as GA.

In the droplet size distribution, a small bubble peak above 1 μm could be attributed to droplet agglomeration. That is, the sufficient attraction between emulsion droplets as it cannot rapidly adsorbed onto the oil phase during the homogenization step. Optical microscopic images of the bagasse extract-stabilized emulsion (Fig 2.3A) after Day 11 showed a reduced droplets diameter. Void spaces observed was a result of creaming as oils and fats floats, displacing the emulsion matrix. GA-stabilized emulsion as seen in Fig 2.3B appeared cloudy white after homogenization however from Day 5, a transparent partial separation from the bottom of the tube. GA-stabilized emulsion had larger d_{av} than bagasse extract-stabilized emulsion however droplet sizes were consistently uniform throughout (Fig 2.3B). This was evident in the optical microscopic image where droplets formed a dense and compact monodispersed particle.

Stability mechanism of GA was due to high viscosity producing steric layers that stabilize around the oil interphase. For bagasse extracts having negligible protein, the lignin-carbohydrate complex may have an effect but not solely responsible. Bhattarai *et al*. (2019) reported a maximum of only 10% polysaccharide being adsorbed into the oil phase when using galactoglucomannans as stabilizers. Molecular weight of oligosaccharides in bagasse extracts ranges from less than 300 g/mol to approximately 3000 g/mol (Szczerbowski *et al*., 2014), so bagasse extracts containing lignin-carbohydrate compounds may have a combined effect on reducing interfacial tension. Boeriu *et al*. (2004) reported that lignin with 0.3- 24.5% of detected total sugars from jute and wheat straw showed emulsifying capacity and stabilized O/W emulsion. The particle

sizes of the bagasse extracts measured using Zetasizer Nano ZS was at 443.8 ± 57.6 nm and the detected xylotriose, cellobiose and xylobiose accounting for only 0.01 % of the carbohydrates. This means that 99% of the extracts were higher molecular weight compounds.

So, the stability of bagasse extract-stabilized emulsion was probably because of the steric effect where the hydrophobic moiety of lignin-carbohydrate complex co-adsorbs into the oil surface. The hydrophilic heterogenous oligomers then extends into the aqueous phase and stabilizing it (Koshijima *et al*., 1989; Yadav *et al*., 2007). Steric repulsion of the polymers adsorbed at the oil-water interface prevents coalescence and cause partial agglomeration. That is, depletion attraction from high levels of non-adsorbed emulsifiers might not fully cover the oil droplet during the homogenization process (McClements *et al*., 2017; Mikkonen *et al*., 2016). Since bagasse extracts produced from HTL treatment are heterogenous, purification step may lower or disrupt the hydrophobic moiety of lignin-carbohydrate complex concentrations. This could result in an increased hemicellulosic polymer concentration and therefore reducing the surface activity potential of the extract. A study on unpurified gum from distillers' grains showed better emulsifying stability than purified gum due to arabinoxylan–protein complex (Xiang *et al*., 2014). However, lignin-carbohydrate complex in an O/W emulsion needs further concrete analysis and thorough investigation in future studies.

3.3.1.3 Effect of pH on zeta potential and droplet size of the BE_{160} -stabilized O/W emulsion

Zeta potential is a measure of electrostatic forces due to the surface charge and high zeta potential values above 30 mV in absolute value (for a positive or negative charge) are usually considered stable. Emulsion-stabilized by 3 wt% bagasse extract was investigated for its zeta potential values at different pH. At pH 1 and 3, zeta potential was outside the mentioned range whereas emulsions at pH above 5 showed a higher absolute value of negative zeta potential (Fig. 2.5B). The droplet size diameter, $d_{4,3}$ of 3.3 μm was larger at pH 1, as acidic pH is known to disrupt the oligomeric-oligomeric interaction and lignin-carbohydrate interaction. So emulsifiers in crosslinking network usually collapse and then aggregation results in large emulsion droplets sizes (Phyo *et al*., 2019). The droplet diameter was not significantly different for high pH because of the steric repulsion by polymer chains instead of the charge effect. This may contribute to the emulsion stability that has near net-zero charge surface as the lignin-carbohydrate complex coverage promoting steric forces for emulsion stability (Jones *et al*., 2010; Xiang *et al*., 2014). Oligomeric carbohydrates solubilized during HTL treatment of bagasse have potential as an emulsifier in food, cosmetics or pharmaceutical industries because of its versatility as compared to protein-polysaccharide emulsifiers (Dickinson, 2009).

3.4 Conclusions

The results obtained showed that bagasse extracts produced from HTL treatment has surface activity properties. It reduced interfacial tension between soybean oil interphase to 14 mN/m. Bagasse extracts-stabilized emulsion was stored for 11 days at 25 °C and it showed comparable stability with a well-known emulsifier, Gum arabic. It even had a lower droplet size over the 11 days with a d_{av} of 0.79 μm. Stability mechanism was likely to be the adsorbed layers of the lignin-carbohydrate complex. That is, stabilizing O/W emulsion was achieved through steric repulsion, but further studies are needed to validate the potential of bagasse extract as a natural emulsifier. Bagasse as an agro-industrial by-product can be valorized by HTL treatment at lower subcritical regions and utilized as an emulsifier. Thereby opening up new possibilities for biomass residue treatment in the food and pharmaceutical industries.

Table 3. 1 The interfacial tension between soybean oil and bagasse extract from different HTL temperatures.

Emulsifier	**Interfacial tension, γ (mN/m)**
Control*	25.1 ± 2.1^{a}
Bagasse extract from HTL at 160-220 °C at 3 wt%	
BE_{160}	14.7 ± 0.2^{b}
BE_{180}	17.3 ± 0.3^{c}
BE_{200}	18.1 ± 0.2^{d}
BE_{220}	18.3 ± 0.2^{d}

Means with different superscript are significantly different ($P<0.05$). Data expressed as mean ± standard deviation (n=3).

Table 3. 2 The interfacial tension between soybean oil and BE_{160} from HTL at different concentrations (0.5- 4 wt%) and GA.

Emulsifier	**Interfacial tension, γ (mN/m)**
Control*	25.1 ± 2.1^{a}
Bagasse extract from 160 °C (BE_{160})	
0.5 wt%	19.8 ± 0.5^{b}
1 wt%	17.3 ± 0.5^{c}
2 wt%	15.5 ± 0.1^{d}
3 wt%	14.7 ± 0.2^{e}
4 wt%	14.0 ± 0.3^{f}
Gum arabic- 3 wt%	12.4 ± 1.1^{g}

* 5mM Phosphate buffer solution only without any emulsifier

Means with different superscript are significantly different ($P<0.05$). Data expressed as mean ± standard deviation (n=3).

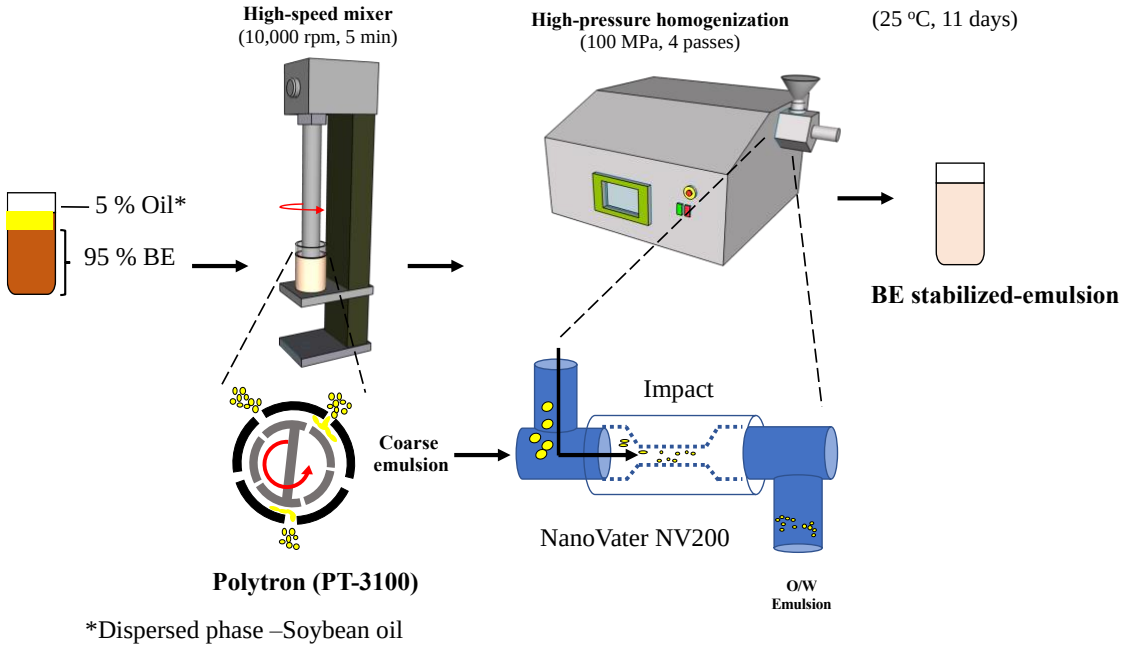

Figure 3. 1 Emulsion preparation by polytron and high-pressure homogenization.

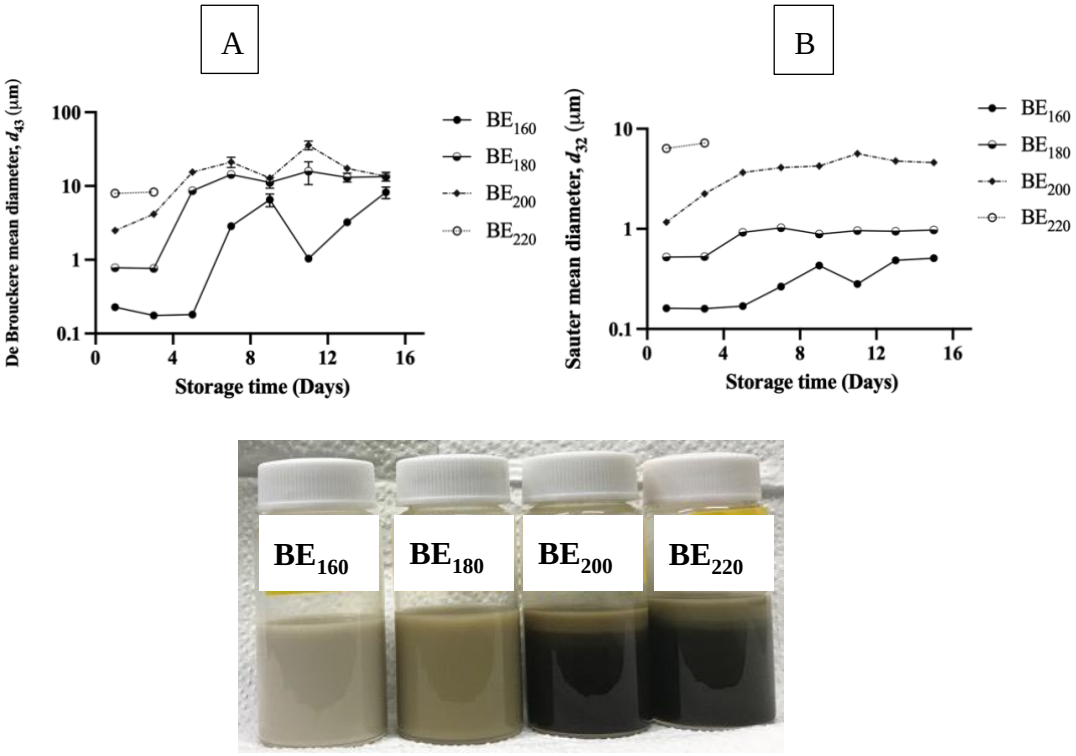

Figure 3. 2 Storage stability of 3 wt % emulsions-stabilized by bagasse extract from HTL at different temperatures for 15 days at 25 °C, [A]-De Brouckere mean diameter (d_{43}) and [B]-Sauter mean diameter (d_{32}). PHOTO: Fresh emulsion-stabilized by bagasse extract from HTL at different temperatures (Day 0).

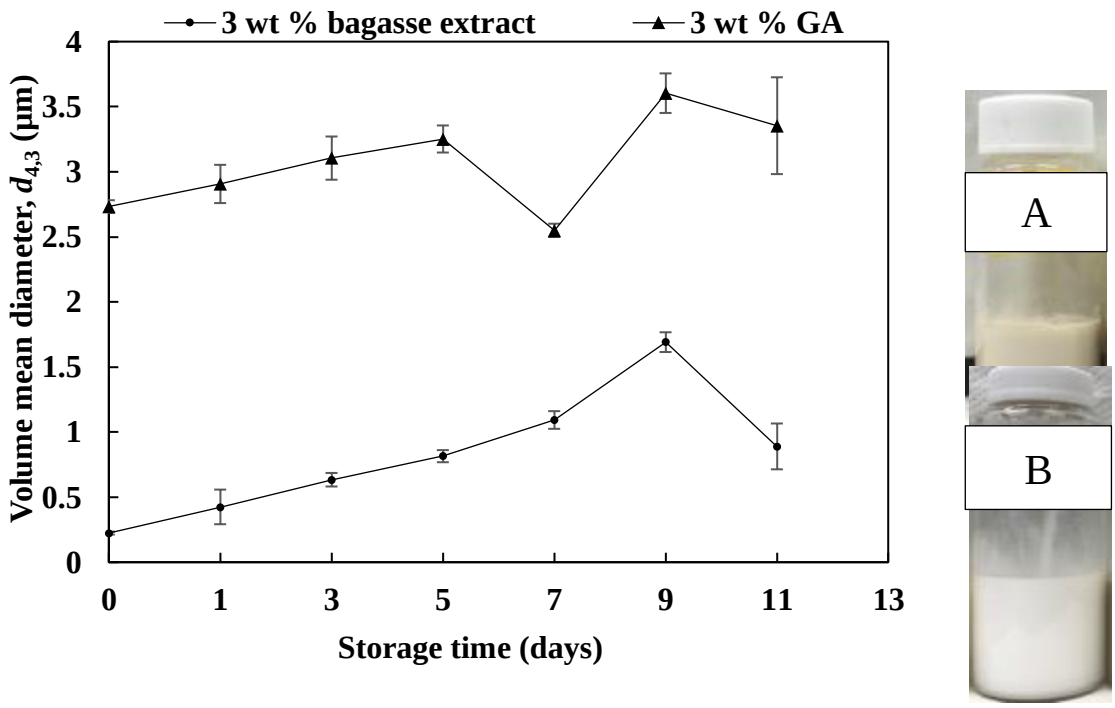

Figure 3. 3 Stability of 3 wt % emulsions-stabilized by bagasse extract from HTL and GA after 11 days at 25 °C. A: Photo of emulsion-stabilized by bagasse extract from HTL after Day 11. B: Photo of emulsion-stabilized by GA after Day 11.

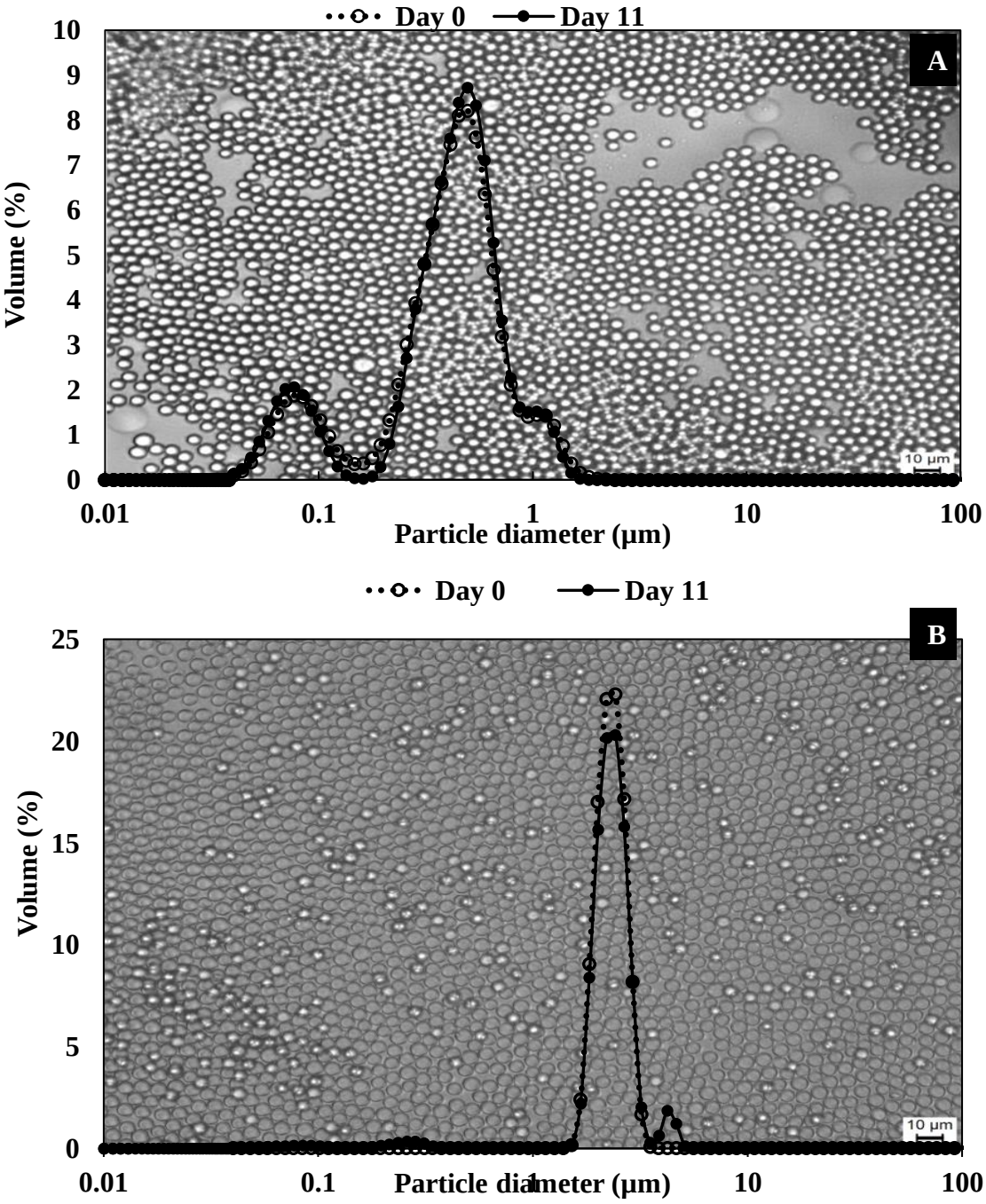

Figure 3. 4 The droplet size distribution and optical microscopic images of O/W emulsions stabilized by bagasse extract from HTL (A) and gum arabic (B). Results are presented as means ± standard deviation (n=3).

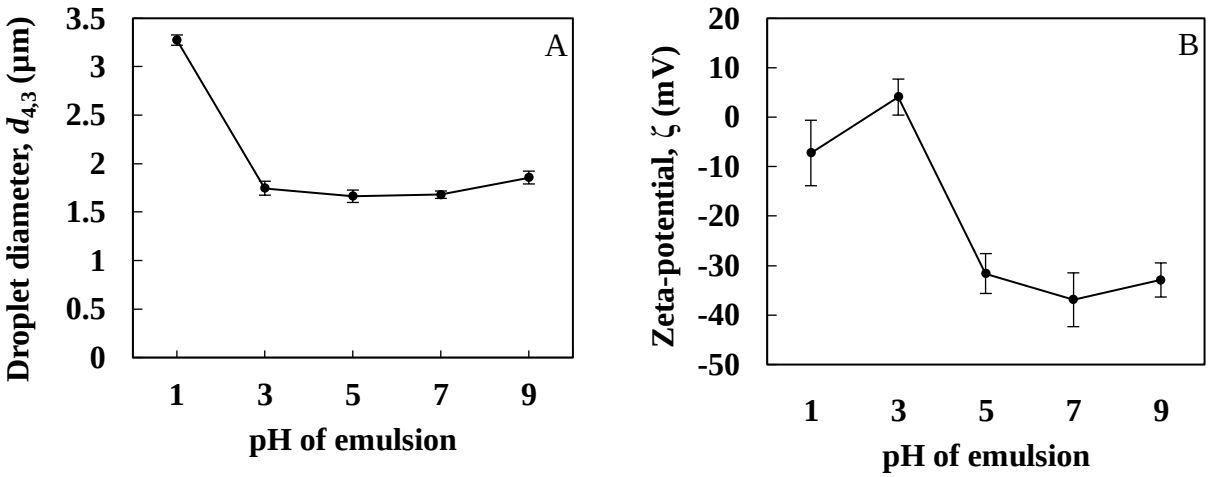

Figure 3. 5 Effect of pH on the stability of fresh emulsions (Day 0) formulated by 3 wt% bagasse extract from HTL (A) and ζ-potential of emulsions (B). Error bars represent standard deviation (SD) for n=3.

Chapter 4 Mechanism of Stability Studies

4.1 Introduction

Given that cellulose, the most abundant organic polymer is well researched and documented, hemicellulose and lignin have lately appeal to researchers focus of study. Lignin the second most abundant biopolymer after cellulose have been thoroughly research in the past few years but are mostly for low-value by-products. Bagasse as a by-product with a composition of cellulose, hemicellulose and lignin is considered a second-generation biofuel production. It is however the most abundant agro-industrial by-product with approximately 279 million tons per year (Chandel *et al*., 2012).

Hemicelluloses having an average molar mass of 1000–10,000 g/mol and a notable fraction of phenolic co-components, have been identified as effective novel bio-based emulsifiers for oil-in-water emulsion stabilization (Lehtonen *et al*., 2018; Valoppi *et al.*, 2019). Phenolic residues have been particularly responsible for hemicelluloses' amphiphilic characteristics and superior stabilizing capacity against emulsion droplet breakdown and creaming compared to other commonly used biopolymers such as gum arabic or synthetic surfactants (Lehtonen *et al.*, 2018; McClements *et al*., 2017). Mikkonen *et al.* (2019) recently reported a highly promising new technical alkyd paint emulsion systems stabilized with hardwood glucuronoxylans and softwood galactoglucomannans. Lignin particles on the other hand have mostly been used as stabilizers in Pickering emulsion (Bertolo *et al*., 2019). With both hemicellulose and lignin being shown to have interfacial properties, HTL treatment of biomass have particularly shown to produce both. That is hemicellulose sugars rich in xylose and

usually phenols are produced at lower subcritical regions as described in Chapter 2 and 3.

However the bagasse extract described in previous chapter is a complex mixture of different chemical compounds, including hemicelluloses, free and hemicellulose-bound phenolics, and residual lignin (Valoppi *et al*., 2019). However most of the described galactoglucomannans are derived and obtained from wood industries (Lehtonen *et al*., 2018;) however there is no real background information available for bagasse extracts that are derived from HTL treatment as an application in emulsion as emulsifiers. The HTL method described in this chapter employs not a single catalytic solvent but water to fractionate hemicelluloses and lignin components in comparison to raw bagasse. Raw bagasse is made up of different compositions. The outer epidermis layer of cellulose usually termed the rind (R) and the inner soft tissues called pith (P). The composition of pith and rind are not significantly different. Mostly the pith and rind contain similar compositions of cellulose, hemicellulose and lignin. Brienzo *et al*. (2016) mentioned that at different portions of the sugarcane stalk, lignin and cellulose do not differentiate much. That is, similar contents of cellulose, hemicellulose, and lignin, but the pretreatments applied would define the compositions of extracts. If acidic medium is used then mostly cellulose will be abundant in the extracts while alkaline solution mostly affects the lignin or delignification (Brienzo *et al*., 2016). The physical properties of the pith and rind allows textural as well as surface active properties. The database for surface active properties from bagasse is not readily available however other perennial grasses have been reported such as sorghum, wheat or other agro-industrial byproducts. Most of these

biomasses have been largely been identified because of its composition and have always revealed a common factor in the presence of lignin-carbohydrate complexes. Lignin-carbohydrates complexes have always led to some research questions that allows it to be identified as a responsible compound for stability. However structural elucidation is attentively pursued, and each structure is a complex heterogenous compound with estimation still an undergoing process through proper machineries (Zhang *et al*., 2020).

Thus, this study is aimed to evaluate the mechanism of stability of O/W emulsions formulated with different types of bagasse extracts in order to understand the stability mechanism. Besides, the structure of the emulsions was analyzed microscopically through images and particle size distribution.

4.2 Materials and Methods

Raw bagasse (RB) and chemicals were obtained as previously described in Section 2.2.1 (Chapter 2). In order to compare and differentiate the materials responsible for stability mechanism, the preparation of raw bagasse powder was separated into Pith (P) and Rind (R) fractions as depicted in Fig 4.1. Raw bagasse was sieved and the retained portion that did not passed through the 500 µm sieve was labelled as R whereas the sieved portion that passed through was the named as the P portion (Fig 4.2). The portions, P and R were then fragmented to uniform sizes using a blender (Vita-Mix, Olmsted Township, Ohio, USA) and sieved (500 µm Sanpo I.S.O, Tokyo).

4.2.1 HTL treatment of bagasse

The samples (RB, P, R) treatment was carried out according to the presented method in Section 2.2.2 (Chapter 2).

4.2.2 Oil-in-water emulsions

4.2.2.1 Interfacial tension

Interfacial tension between the samples (RB, P, R) and soybean oil was carried out according to the presented method in Section 3.2.2.1 (Chapter 3).

4.2.2.2 Preparation of O/W emulsions

Oil-in-water emulsions were prepared using the extracts from the samples (RB, P, R) according to the presented method in Section 3.2.2.2 (Chapter 3) with modifications. Briefly, the HTL extract obtained after HTL was used directly as an aqueous phase without lyophilization. The extracts from the different from RB, P and R samples were then homogenized with PT and HPH. The parameters used in HPH was 10,000 rpm for 5 min and HPH was 50 MPa for 4 passes.

4.2.2.3 Droplet size distribution of O/W emulsion

Emulsions (RB, P, R) treatment was carried out according to the presented method in Section 3.2.2.3 (Chapter 3). In order to understand the stability mechanism of the different extracts, the effect of high-speed homogenizer on the emulsion was observed. That is, the

premix emulsions (Fig 4.4) were prepared using Polytron at a speed of 10,000 rpm for 5 min was analyzed for droplet size using the Beckman and Optical Microscope.

4.2.2.4 Emulsion Partitioning

Emulsions were partitioned into aqueous and creamed phases by centrifugation following method described by (Lehtonen *et al*. 2018) with few modifications (Fig 4.3). That is, 20 g of emulsion was centrifuged at 20 000 g at RT for 15 min. The creamed phase was collected, while the residue was centrifuged for additional 5 min. The second creamed phase was combined with the first one for the analysis of adsorbed RB, P, R fraction. 1 mL of continuous phase was collected for the analysis of the aqueous phase (RB, P, R) fraction. The total collected cream was weighed and mixed with 1% SDS solution in cream: SDS solution ratio of 1:10. The mixture was stirred overnight with a magnetic stirrer and centrifuged again for 30 min at 20,000 g. Distribution and changes occurring in oil phase (OP) and aqeous phase (AP) of RB, P, R were determined in order to evaluate their possible contribution to emulsion stability.

4.2.2.5 Total carbohydrate analysis

The total carbohydrate content of the separated portion from emulsion (AP and OP) was carried out according to the presented method in Section 2.2.3.4 (Chapter 2). Similarly, the extracts from HTL were analyzed following the same procedure.

4.2.2.6 Total phenolic analysis

The total phenolic content of the separated portion from emulsion (AP and OP) was carried out according to the presented method in Section 2.2.3.5 (Chapter 2). Similarly, the extracts from HTL were analyzed following the same procedure.

4.2.2.7 Emulsion droplet morphology

Emulsion droplet morphology between the samples (RB, P, R) was carried out according to the presented method in Section 3.2.2.5 (Chapter 3).

4.2.3 Statistical analysis

Experiments were at least duplicated with data reported as mean ± standard deviation. All calculations were done using GraphPad Prism^{TM}, Version 8.0 (San Diego, USA).

4.3 Results and Discussion

4.3.1 Preparation of RB, P and R extracts- stabilized emulsions

Oil-in-water emulsions were prepared by homogenizing the bagasse extracts from different parts of bagasse such as RB, P and R portion with soybean oil. In previous chapter, it was found that bagasse extracts were able to stabilize emulsion using the high speed polytron (PT) and high-pressure homogenizer (HPH).

Figure 4.5 shows droplet size of O/W emulsions stabilized by RB, using PT. The average size of droplets generated by was 14.7 μm. Both P and R-stabilized emulsion

achieved 15.6 and 19.4 µm droplet sizes respectively. The droplet size distribution can be observed in Fig 4.5, having monomodal size distribution with morphology shown in Fig 4.6. This shows that both P and R extracts can achieve smaller droplets sizes with PT while RB-stabilized emulsion cannot completely cover the oil droplet with this method of homogenization. After leaving at room temperature for 1 day, the pre-emulsion could be observed to have phase separation for the P and R-stabilized emulsions whereas RB-stabilized emulsion was not as severe. This can be seen in Fig 4.4 where Day 0 samples are at the top and Day 1 at the bottom. Conventionally, standard procedures allow for the successive samples to be conducted as soon as high speed is done without being observed in between. However, it is necessary to understand the changes in droplet size distribution and emulsifier coverage in between each process.

Usually when using HPH at 50 MPa, most emulsions with smaller droplet sizes were produced. A high-pressure homogenizer is capable of producing stable submicron emulsions by breaking down the oil droplets to the submicron scale with a narrow size distribution. However, in this study, it was true for only the RB-stabilized emulsion as the droplets size drastically reduced from 20.8 to 2.6 µm. The emulsion droplet size distribution seen in Fig 4.5 clearly shows the shift in distribution from the wide distribution of the high speed to the narrow distribution of the HPH. This can be attributed to the fact that RB extracts from HTL treatment contains polymers with both hydrophilic and hydrophobic moieties as hypothesized in Chapter 3. P and R-stabilized emulsions also had reduced droplets sizes. That is for P, droplet sizes decreased to 4.7 µm while R-stabilized emulsions, it increased drastically to 4.2 µm. To compare between the effects

of different homogenization techniques, the observed size droplet size distribution in Fig 4.5 is in agreement with the droplet data mentioned. For P-stabilized emulsion, there was not much difference except for the bimodal size attributing to its chemical nature as an extract that was not purified. R-stabilized emulsion showed similar bimodal distribution however the homogenizer might be effective in the hydrophobic moieties most probably from the phenolic groups being adsorbed resulting in increased coverage and smaller droplets. Similar results were observed by Juttulapa *et al*. (2017) when observing emulsions containing pectin and zein where HPH reduced droplet sizes of the internal phase by forcing macro-emulsion through narrow gaps by imposing a higher pressure.

4.3.2 Emulsion partitioning

To understand how phenolic residues in different extracts affect emulsion stability, their distribution between the OP and AP was determined. OP is basically the adsorbed phenols or carbohydrates after separation though the accelerated tests. The aqueous phase (AP) represents the free extracts not adsorbed due to its hydrophilicity or free phenols and carbohydrates. The content of OP carbohydrate content in RB-stabilized emulsion was 18 mg/g while 69 mg/g was detected in the aqueous phase. The effect of high-pressure homogenization on the change in emulsion droplet size suggested it could cause the partial deformation leading to a network formation or rearrangement (Floury *et al.*, 2000). P-stabilized emulsion contained total carbohydrate content of 49.7 and 51.6 mg/g emulsion in the OP and AP respectively. The results demonstrated that the stability of emulsions was due to the high concentration of carbohydrate that sterically congregate at

the aqueous phase that while the adsorbed hemicellulose extracts were minute. Since the P extracts mainly contained amorphous carbohydrate polymers that were water soluble, high pressure homogenization allowed for proper oil surface coverage as discussed in Section 4.3.1. R-stabilized emulsion with total carbohydrate content of 12.6 mg/g in AP while 20.5 mg/g present at the OP as shown in Fig 4.8. The free carbohydrates in the aqueous phase however typically showed a higher concentration than those detected at the interface. Since the cell wall structures of bagasse have interwoven arrangement of cellulose, hemicellulose and lignin, the carbohydrate content was comparable to the more carbohydrate-rich extracts of RB and P.

The phenolic content in the RB-stabilized emulsion at the OP was 0.13 mg/g while the content in the AP was 0.12 mg/g of the dried extracts after centrifugation (Fig 4.8). Lehtonen et al. (2016) reported similar results stating that majority of the adsorbed phenolic residues (77%) were ester bound to soluble galactoglucomannan fractions, but some of the free or weakly associated phenols (21%) were located at the interface. In both the P and R-stabilized emulsion, the content of interfacial phenolics could not be detected which agrees previous research on hemicellulose-rich galactoglucomannan. That is, emulsion was stabilized through steric repulsion with minor contribution from the phenolic residues as majority were detected in the aqueous phase (Lehtonen *et al*., 2016; Mikkonen *et al*., 2016).

4.3.3 Storage stability of RB, HC and L-stabilized emulsions

The physical stability of O/W emulsions was also investigated in this study and could be reflected by droplet size of emulsion after storage at room temperature for 11 days. Fig 4.7 shows the droplet size of the emulsion after analyzing using the Dynamic Light Scattering technique through the Beckman Coulter analyzer. The emulsions prepared with RB had a d_{43} of 8.0 µm and a Sauter mean diameter, d_{32} of 2.5 µm. The emulsions showed good physical stability, with no sign of phase separation after 14 days. Mikkonen *et al.* (2019) stated that short extraction time at low temperatures is expected to release a low yield of hemicelluloses with high molar mass and a small number of co-components HTL treatment at 160 °C for 30 min. These polymers as similar to the one described bagasse extract in Chapter 3 may contain polysaccharide backbone length and acetyl groups that are able to adsorb into the oil phase and sterically stabilize the emulsion.

The emulsions prepared with P had mean droplet diameter, d_{43} of 7.7 µm and a Sauter mean diameter, d_{32} of 2.8 µm. The emulsions stabilized showed good physical stability in the first week before emulsion separation. It did not drastically change as the droplet sizes slowly increased indicating instability after 11 days. R-stabilized emulsions had similar trend as the P-stabilized emulsion with increasing droplet sizes after 8 days. It had a droplet size, d_{43} of 7.9 µm and a d_{32} of 0.9 µm. The emulsions stabilized showed did not show good physical stability with minor phase separation immediately observed after Day 1. This is in agreement with the hypothesis that when both the pith (P) and phenolic-rich (R) components of bagasse are separated and hydrolyzed through HTL, it loses it amphiphilic potential. On the other hand, other researches results showed that even by

removing phenolic residues through ethanol extraction, the hemicellulose rich emulsifiers exhibited excellent performance.

4.3.4 Mechanism of stability

This study clearly demonstrates that RB containing phenolic residues are more efficient emulsifiers than P or R extract alone. The composition of residual phenolic compounds in RB define their ability to adsorb to the oil-water interface and stabilize it. Although only a small portion of phenolic residues adsorbed at the interface, they had a remarkable effect on emulsion morphology and stability which similar observed in Lehtonen *et al*. (2016) study of galactoglucomannan as emulsion stabilizer. From the influence of different homogenization methods, chemical composition at the interface and storage stability it lead us to hypothesize either that the size of bagasse extract is of importance or that these larger molecules contain other functionalities, most likely phenolic residues, which aid their anchoring at the interface (Lehtonen *et al*., 2016).

4.4 Conclusions

Bagasse extracts containing both hemicellulose and lignified compounds were solubilized using only water through the eco-friendly HTL treatment. The different samples of bagasse utilized showed that all have interfacial activity properties to efficiently stabilize O/W emulsions. As biopolymers extracted using HTL treatment from an agro-industrial by-product, bagasse is hardly viewed as a potential candidate for versatile emulsifiers showing emulsification and physical stabilization functions. It

therefore provides an understanding for application of emulsions stabilized using bagasse extracts from HTL treatment. With the milky brown color, low-fat salad dressings could be a potential application or provided that additional additives are comparable. Therefore, it leaves a lot to be thought of and optimized for bagasse using HTL treatment to be fully realized to its potential through eco-friendly practices.

Table 4. 1 The interfacial tension between soybean oil and RB, P and R extracts from HTL. Data expressed as mean ± standard deviation (n=3).

Emulsifier	**Interfacial tension, γ (mN/m)**
Control*	25.20 ± 0.5^{a}
Different bagasse extract from HTL at 160 °C	
Raw Bagasse (RB)	14.8 ± 0.0^{b}
Pith (P)	15.6 ± 0.3^{c}
Rind (R)	14.9 ± 0.1^{b}

Table 4. 2 pH of Raw bagasse, Pith and Rind samples before and after HTL treatment at 160 °C, 1 MPa, and 30 min. Data expressed as mean ± standard deviation (n=3).

Sample	pH before HTL	pH after HTL
Raw bagasse (RB)	6.8 ± 0.1^{a}	4.4 ± 0.2^{b}
Pith (P)	6.5 ± 0.0^{a}	4.9 ± 0.1^{b}
Rind (R)	6.5 ± 0.0^{a}	4.2 ± 0.1^{b}

Figure 4. 1 Diagrammatic representation of the sample preparation for the different portions of the bagasse samples into RB, P and R before HTL treatment.

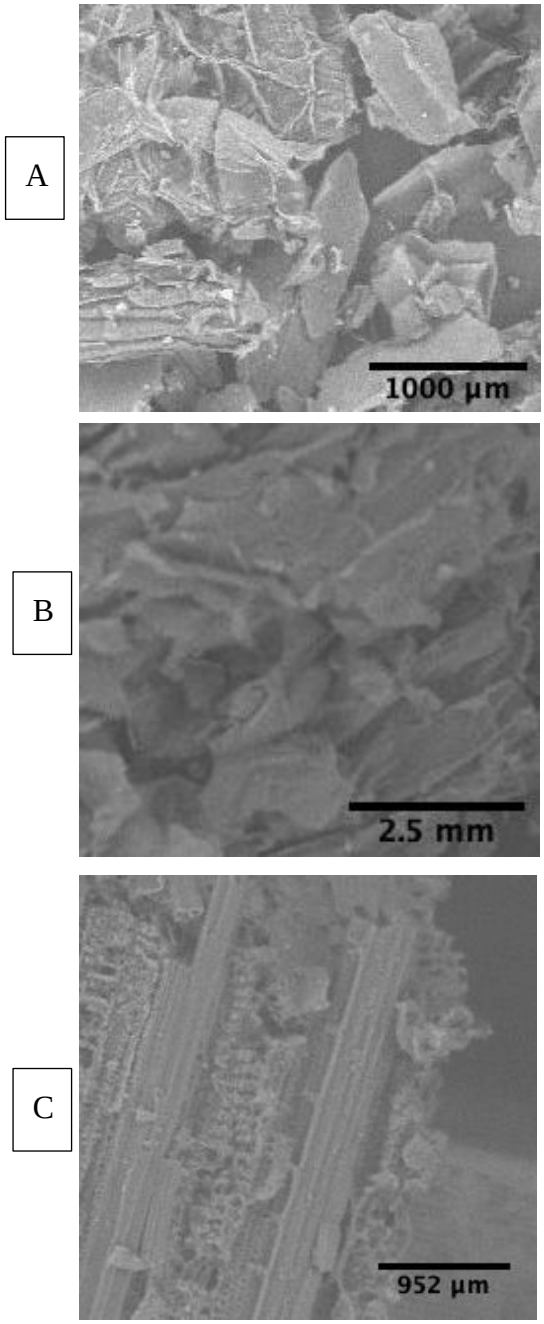

Figure 4. 2 Scanning electron microscopy (SEM) of Raw bagasse, Pith and Rind after different preparation before HTL treatment. A: SEM of raw bagasse (200×), B: SEM of Pith (80×), C: SEM of Rind (200×).

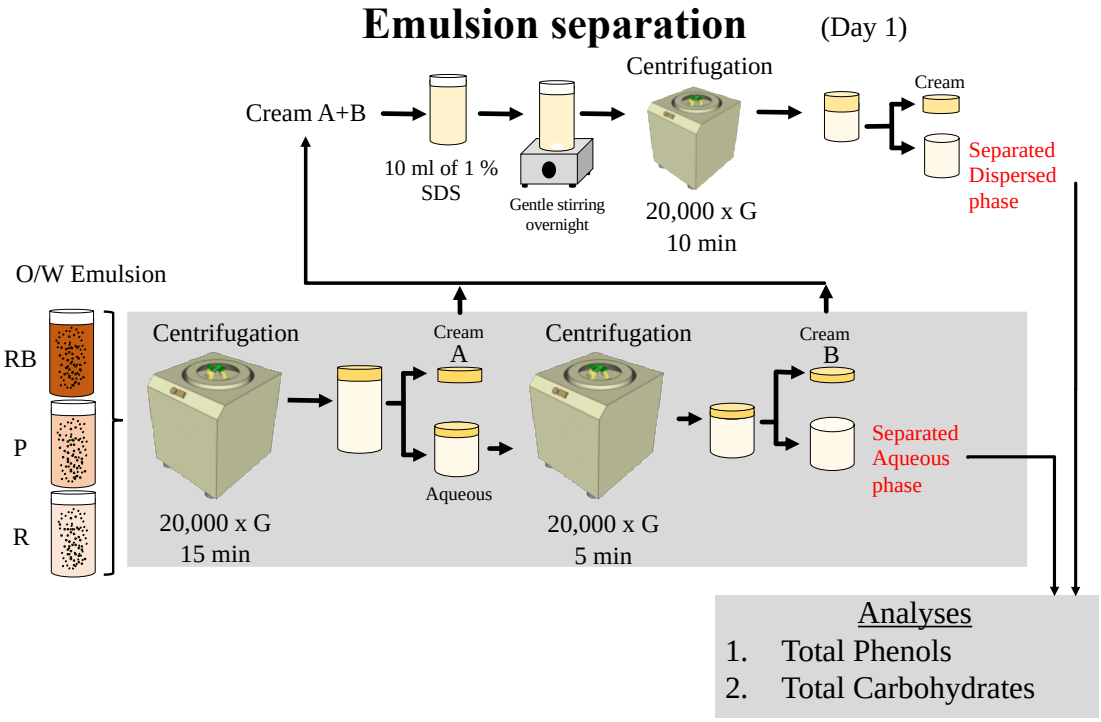

Figure 4. 3 Diagrammatic representation of the emulsion separation before analysis of Total Phenols and Total Carbohydrates.

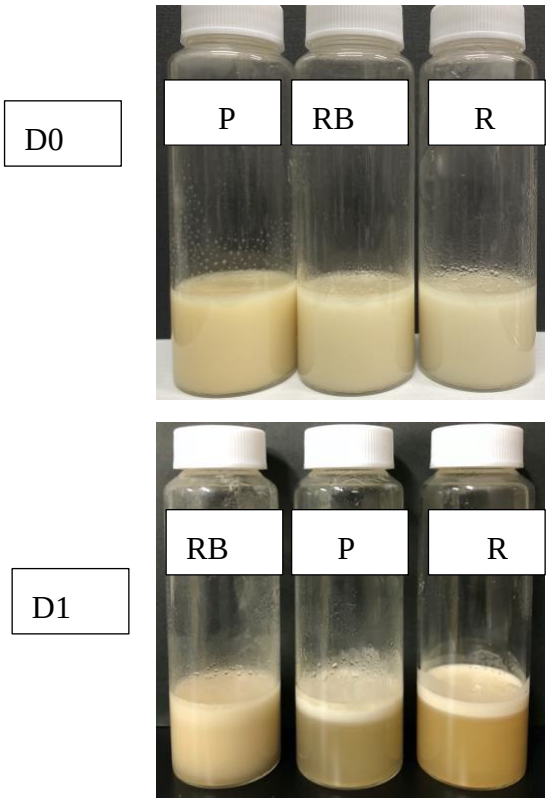

Figure 4. 4 Photograph of RB, P and R-stabilized O/W emulsions prepared by Polytron (PT) only at D0 (Day 0) and D1 (Day 1).

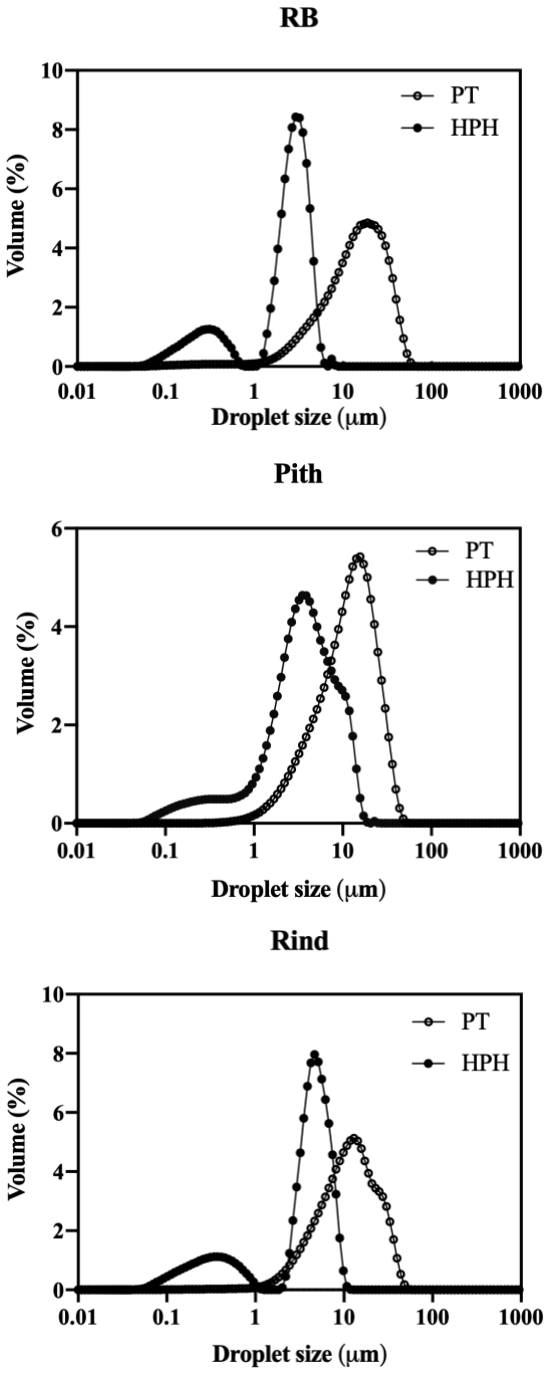

Figure 4. 5 Droplet size distribution of RB, P, and R-stabilized fresh (Day 0) O/W emulsions prepared by Polytron (PT) and High-Pressure Homogenizer (HPH) . Conditions for PT was 10,000 rpm for 5 min and HPH was 50 MPa for 4 passes.

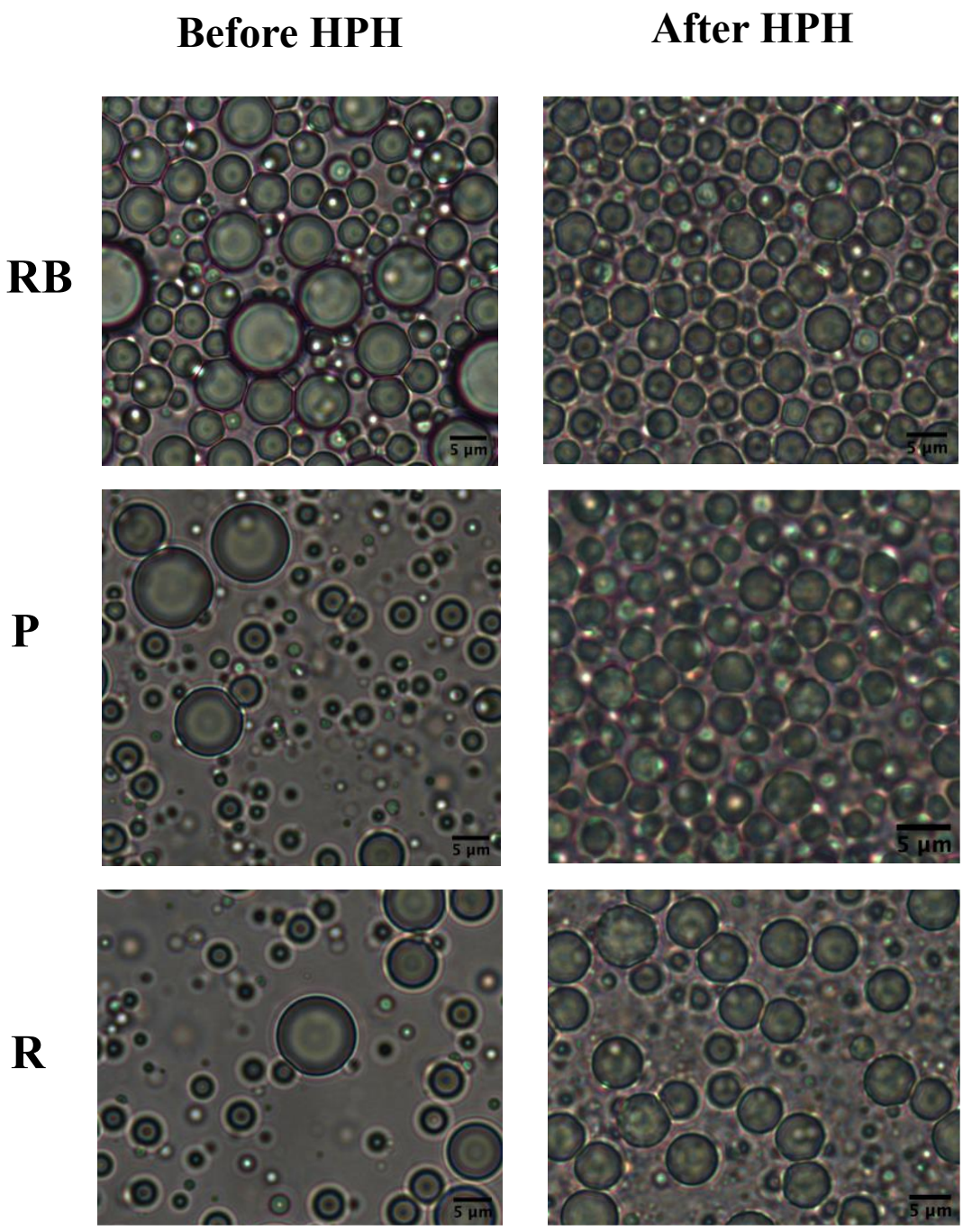

Figure 4. 6 Optical microscopic images of the emulsion before HPH (PT) and after HPH at Day 0 (D0).

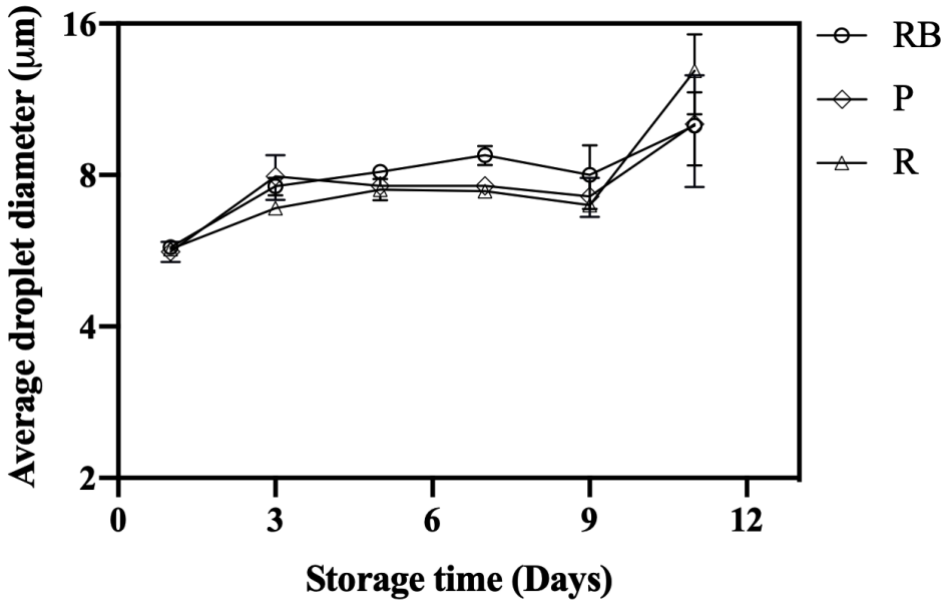

Figure 4. 7 Storage stability of RB, P and R-stabilized O/W emulsions stored at 25 °C for 11 days.

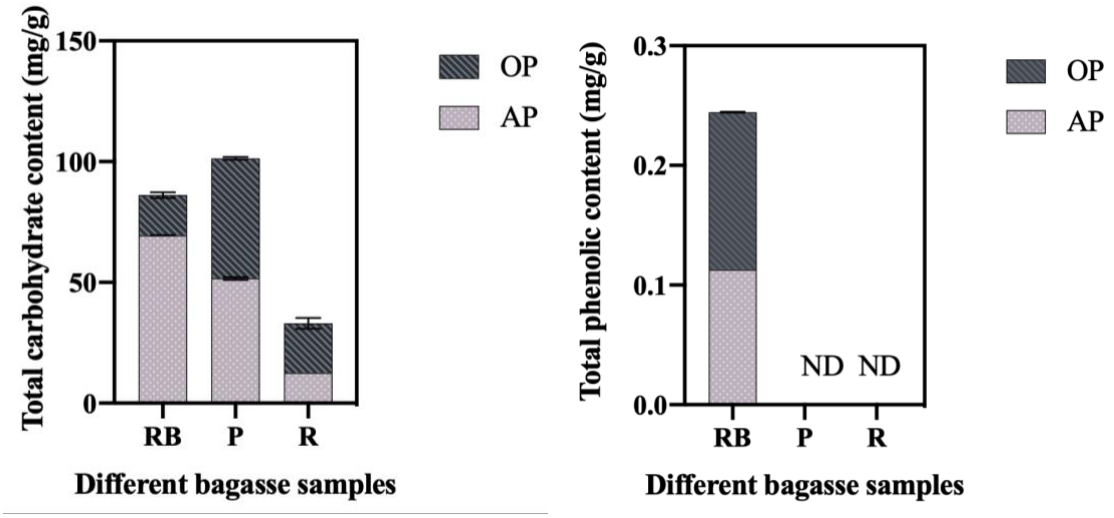

Figure 4. 8. Composition of carbohydrates and phenols at the interfaces of RB, HC and L -stabilized O/W emulsions stored at 25 °C for 1day.

Chapter 5 General conclusions and future perspective

5.1 Summary of each chapter

5.1.1 Introduction

Basically, most of the fundamentals are introduced in this section. The sample, bagasse being utilized and how important it is. The basic terminology about the treatment used which is HTL and finally applying it through emulsion system as an emulsifier. The current trends and brief history of HTL reactors was discussed. Basic definition and stability studies of emulsion was discussed in length, as well as, the potential of natural emulsifiers in the food market.

5.1.2 HTL Treatment of Bagasse

In this chapter, the effect of HTL treatment on bagasse was investigated. The effects of HTL treatment on different model compounds (Cellulose and Starch) in comparison to bagasse, the effect of temperature (160 - 220 °C) and finally the characterization of the extracts from 160 °C. The results showed bagasse extracts from HTL at 160 °C had 51.0% of carbohydrates, phenols, as well as the presence of furfurals, a degradation product from cellulose.

5.1.3 Emulsion Formulation of Bagasse Extract

The bagasse extract from different temperatures of HTL treatment were prepared by polytron and high-pressure homogenization in this section. The results showed that all

bagasse extracts produced from different temperatures of HTL treatment had surface activity properties. The extract from HTL treatment at 160 °C reduced the interfacial tension between soybean oil interphase to 14 mN/m. Bagasse extracts-stabilized emulsion was stored for 11 days at 25 °C and it showed comparable stability with a well-known emulsifier, Gum arabic. It even had a lower droplet size over the 11 days with a d_{av} of 0.79 µm. Stability mechanism was likely to be the adsorbed layers of the lignin-carbohydrate complex.

5.1.4 Mechanism of Stability Studies

In this part, the stability mechanism of bagasse extracts was investigated by separating the bagasse into 2 parts, Pith and Rind. The extracts from the different samples of bagasse all indicate stability in the period of storage. The stability of each extract showed that there was not any significant difference among each sample but the d_{av} from lowest to highest was P < R < RB. Phenolic content was not responsible, but carbohydrates indicated its presence in both AP and OP for RB extract only while phenolics could not be detected in P and R-stabilized emulsions.

5.2 Future perspectives

In this study, we focused on the potential of bagasse extracts from HTL as an emulsifier in emulsions that has potential to be utilized in food, chemical or pharmaceutical industries.

For future work, it would be ideal to apply HTL on other soft wood biomass instead of only bagasse as it would be a great research area utilizing the availability of hemicellulose and lignin compositions.

Subsequent HTL treatments of a sample would also be an interesting focus of study provided HTL is still in batch scale whereby low solubility compounds will be liquefied first. Parameters can be varied or not depending on the sample before the second round of HTL treatment.

Application can be focused on emulsion but not solely on food depending on the nature and chemistry of the sample. It can be used even as a homogenization step in an emulsion system for water insoluble compounds.

Therefore, it would be meaningful to understand the mechanism and kinetics of stabilization and the rheological studies to enhance its applicability and label as naturally derived emulsifier from a bagasse, an agro-byproduct. We are confident that by providing this data, it would broaden the interest and appeal to future studies regarding the employment of green technology such as HTL treatment in converting agro-based by-products.

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List of publications

This thesis is based on the following publications, which will be referred to in the text by their roman numerals.

I. Vodo, Sekove, Noamane Taarji, Meryem Bouhoute, Lorena de Oliveira Felipe, Marcos A. Neves, Isao Kobayashi, Kunihiko Uemura, and Mitsutoshi Nakajima. "Potential of bagasse obtained using hydrothermal liquefaction pre-treatment as a natural emulsifier." International Journal of Food Science & Technology (2020).

II. Bouhoute, Meryem, Noamane Taarji, Sekove Vodo, Isao Kobayashi, Mohamed Zahar, Hiroko Isoda, Mitsutoshi Nakajima, and Marcos A. Neves. "Formation and stability of emulsions using crude extracts as natural emulsifiers from Argan shells." Colloids and Surfaces A: Physicochemical and Engineering Aspects 591 (2020): 124536.

III. Taarji, Noamane, Sekove Vodo, Meryem Bouhoute, Nauman Khalid, Abdellatif Hafidi, Isao Kobayashi, Marcos A. Neves, Hiroko Isoda, and Mitsutoshi Nakajima. "Preparation of monodisperse O/W emulsions using a crude surface-active extract from argan by-products in microchannel emulsification." Colloids and Surfaces A: Physicochemical and Engineering Aspects 585 (2020): 124050.

IV. Vodo, Sekove, Marcos A. Neves, and Mitsutoshi Nakajima. "Stability mechanism of bagasse extract from HTL treatment in O/W emulsion''. *In preparation*