CHOLINE CHLORIDE – CITRIC ACID MONOHYDRATE BASED DEEP EUTECTIC SOLVENTS AS ALTERNATIVE MEDIA FOR *AVERRHOA BILIMBI* PECTIN (ABP) EXTRACTION AND AS PLASTICIZERS FOR ABP BIOPLASTIC

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by

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MUHAMMAD HAKIMIN SHAFIE

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LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
κ	Kappa
ρ	Para
%	Percentage
°C	Degree Celsius
&	And
~	Approximately
<	Less than
>	More than
×	Times
+	Plus
±	Plus-minus

LIST OF ABBREVIATIONS

2-D	Two-dimensional
3-D	Three-dimensional
ABP	*Averrhoa bilimbi* pectin
ABrC	Analytical Biochemistry Research Centre
ACN	Acetonitrile
ANOVA	Analysis of variance
Ara	Arabinose
AS	Artificial saliva
ATR	Attenuated total reflectance
BBD	Box-Behnken design
BP	Bioplastic
BP-ABP	*Averrhoa bilimbi* pectin bioplastic
BP-C	Bioplastic prepared without plasticizer
BP-DES	Bioplastic prepared with deep eutectic solvent as a plasticizer
BP-G	Bioplastic prepared with glycerol as a plasticizer
BSA	Bovine serum albumin
BW	Beeswax
^{13}C NMR	Carbon-13 nuclear magnetic resonance
CAM	Citric acid monohydrate
CAPS	Citric acid-modified pea starch
CARS	Citric acid-modified rice starch
CCD	Central composite design
cm	Centimeter

CN	Carbon-nitrogen bond
CV	Coefficient of variation
Da	Dalton
DBP	Di-n-butyl phthalate
DE	Degree of esterification
DEHP	Di(2-ethylhexyl) phthalate
DES	Deep eutectic solvent
DIDP	Diisodecyl phthalate
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
e.g.	*Exempli gratia* (for example)
EA	Emulsifying activity
EDTA	Ethylenediamine tetraacetic acid
ES	Emulsion stability
F-test	Fisher distribution
FC	Foaming capacity
FDA	Food and Drug Administration
FFD	Full factorial design
FRAP	Ferric reducing antioxidant power
FS	Foaming stability
FTIR	Fourier transform infrared
Fuc	Fucose
g	Gram
g	Gravity

GA	Gallic acid
Gal	Galactose
GalA	Galacturonic acid
Glu	Glucose
GluA	Glucuronic acid
^{1}H NMR	Hydrogen-1 or proton nuclear magnetic resonance
h	Hour
HCl	Hydrochloric acid
HG	Homogalacturonan
HPLC	High-performance liquid chromatography
HPMC	Hydroxyl methycellullose
i.e.	*Id est* (that is)
IDF	International Dairy Federation
ISO	International Organization for Standardization
IPharm	Institute of Pharmaceutical and Nutraceuticals
iPROMISE	Integrative Pharmacogenomics Institute
KBr	Potassium bromide
LBW	Low birth weight
kDa	Kilodalton
kg	Kilogram
kN	Kilonewton
kV	Kilovolt
µm	Micrometer
M	Molar
M	Miscible

m	Meter
Man	Mannose
MEM	Eagle minimum essential medium
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
mm	Millimeter
mN	Millinewton
MPa	Megapascal
mPa	Millipascal
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid
MW	Molecular weight
N	Normality
n	Number in a trial or sample
ND	Not detected
N–H	Nitrogen-hydrogen bond
NaOH	Sodium hydroxide
NM	Nonmiscible
nm	Nanometer
NMR	Nuclear magnetic resonance
O/W	Oil-in-water
OHC	Oil holding capacity
p	Probability
Pa	Pascal

pH	Potential/power of hydrogen
PHB	Poly(3-hydroxybutyrate)
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PMP	1-phenyl-3-methyl-5-pyrazolone
POM	Polarized light microscopy
$R–COO^-$	Carboxylate group
R–COOH	Carboxylic group
RG	Rhamnogalacturonan
RG-I	Rhamnogalacturonan I
RH	Relative humidity
Rha	Rhamnose
Rib	Ribose
rpm	Revolutions per minute
RSM	Response surface methodology
s	Second
SEM	Scanning electron microscopic
SGF	Simulated gastric fluid
TPC	Total phenolic content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
T_g	Glass transition temperature
USA	United States of America
UV	Ultraviolet
UV-Vis	Ultraviolet–visible
v/v	Volume over volume
W/O	Water-in-oil

w/w	Weight over weight
w/v	Weight over volume
W	Watt
WHC	Water holding capacity
wt	Weight
WVTR	Water vapor transmission rate
y	response
Xyl	Xylose

KOLIN KLORIDA - ASID SITRIK MONOHIDRAT BERASASKAN PELARUT EUTEKTIK MENDALAM SEBAGAI MEDIA ALTERNATIF UNTUK PENGEKSTRAKAN PEKTIN *AVERRHOA BILIMBI* (ABP) DAN SEBAGAI PEMPLASTIK UNTUK BIOPLASTIK ABP

ABSTRAK

Buah *Averrhoa bilimbi* dihasilkan dengan banyak dan kurang digunakan. Pengekstrakan pektin menggunakan asid yang kuat dan asid mineral masing-masing menghasilkan sisa buangan yang berbahaya dan hasil yang rendah. Oleh itu, buah *Averrhoa bilimbi* disarankan sebagai sumber pektin yang baik manakala pelarut eutektik mendalam (DES) dicadangkan sebagai media mesra alam dan sangat berkesan untuk pengekstrakan pektin *A. bilimbi* (ABP) dan sebagai pemplastik alternatif untuk penghasilan bioplastik (BP). DES berasaskan kolin klorida – asid sitrik monohidrat yang berbeza nisbah molar (iaitu 3:1, 2:1, 1:1, 1:2 dan 1:3) berjaya disintesis dan DES 1:1 didapati mencapai titik eutektik. Hasil kajian menunjukkan bahawa nilai kelikatan, tegangan permukaan dan ketumpatan adalah lebih tinggi dengan peningkatan nisbah molar asid sitrik monohidrat dalam DES. Seterusnya, DES yang terhasil digunakan sebagai medium pengekstrakan ABP. Hasil kajian menunjukkan bahawa proses pengekstrakan dengan bantuan DES memerlukan masa yang lebih pendek (kurang 30 minit) daripada asid sitrik monohidrat dan hasilnya lebih tinggi (4.96% lebih banyak) dengan keadaan optima pada 3.74% (w/v) DES, suhu pengekstrakan 80 °C dan masa pengekstrakan 2.5 jam. Selain itu, ABP-CAM didapati kurang linear dengan saiz cabang pektin yang lebih besar berbanding ABP-DES. Penggunaan DES sebagai media pengekstrakan juga dilihat dapat menambah baik kapasiti pegangan air dan minyak (masing-masing 4.25 dan 101.69%), kapasiti berbuih (6.19%), dan aktiviti penghapus sisa 2,2-diphenyl-1-picrylhydrazyl (DPPH) (43.56%) berbanding ABP-CAM. Hasil kajian juga menunjukkan bahawa nisbah molar komponen DES yang berbeza mempengaruhi sifat struktur ABP yang diekstrak (iaitu lineariti pektin dan saiz cabang). Oleh yang demikian, ia mempengaruhi sifat berfungsi struktur ABP. Potensi ABP sebagai sumber biopolimer untuk pengeluaran BP juga dikaji. BP-ABP3:1 yang dihasilkan daripada ABP dengan saiz cabang yang besar menunjukkan nilai suhu lebur (175.30 °C), tekanan tegangan (7.32 MPa) dan modulus (33.64 MPa) lebih tinggi disebabkan oleh interaksi rantaian pektin yang lebih kuat. BP-ABP3:1 juga menunjukkan sifat penghalang yang lebih baik dengan memperoleh nilai kadar pemindahan wap air (1.10 – 1.18 mg/m^2.s) dan penyerapan kelembapan (2.61 – 32.13%) terendah bergantung kepada kelembapan relatif berbanding dengan BP-ABP lain yang mempunyai struktur linear dengan saiz cabang yang lebih kecil. Selain itu, pengaruh nisbah molar komponen dalam DES yang berbeza sebagai pemplastik dikaji terhadap sifat BP. Perkara yang perlu ditekankan berdasarkan kajian ini adalah DES pada titik eutektik (nisbah molar 1:1) menunjukkan kesan pemplastik yang minimum kerana kolin klorida dan asid sitrik monohidrat berinteraksi sendiri. Kajian ini juga menunjukkan kesan pemplastik meningkat apabila nisbah molar asid sitrik monohidrat lebih tinggi. Selanjutnya, BP-DES1:1 yang dipilih menunjukkan potensi aplikasi sebagai bahan kapsul kerana kemampuannya untuk menahan pencernaan dalam air liur buatan dan larut sepenuhnya dalam cairan gastrik simulasi, tidak sitotoksik serta melambatkan proses pengoksidaan dalam minyak ikan. Kesimpulannya, DES ini adalah alternatif yang baik untuk pengekstrakan pektin dan sebagai pemplastik untuk penghasilan BP. Penemuan ini menunjukkan bahawa pektin yang diekstrak dari *A. bilimbi* boleh diterokai sebagai farmakologi biopolimer semulajadi yang baharu dan/atau berpotensi sebagai bahan makanan berfungsi.

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ABSTRACT

Averrhoa bilimbi is an abundance and underutilized fruit. The extraction of pectin using strong acids and mineral acids produce hazardous effluents and low yield, respectively. Therefore, *A. bilimbi* fruit was suggested to be a good source of pectin whereas the deep eutectic solvent (DES) was suggested to be an environmental friendly and highly efficient medium for *A. bilimbi* pectin (ABP) extraction and as an alternative plasticizer for bioplastic (BP) production. The choline chloride – citric acid monohydrate based DES at different molar ratios (*i.e.* 3:1, 2:1, 1:1, 1:2 and 1:3) were successfully synthesized and DES 1:1 was found to meet the eutectic point. Results showed that increasing the molar ratio of citric acid monohydrate in DES, higher values of viscosity, surface tension and density were observed. Subsequently, these DESs were used as a medium for extraction of ABP. The result showed that DES-assisted extraction process required shorter time (30 minutes less) than citric acid monohydrate and the yield was higher (4.96% more) with optimal condition of 3.74% (w/v) of DES, an extraction temperature of 80 °C, and extraction time of 2.5 h. Apart from that, ABP-CAM was found lower in linearity with higher branch size of the pectin than ABP-DES. It was also observed that using DES as the extraction medium had improved the water and oil holding capacities (4.25 and 101.69%, respectively), foaming capacity (6.19%) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (43.56%) compared to ABP-CAM. Results also demonstrated that different

molar ratios of DES components had significantly affected the structural properties of the extracted ABPs (*i.e.* linearity of pectin and branch size). Thus, affecting the functional properties. The potential of ABP as a source of biopolymer for BP production was also explored. The BP-ABP3:1, which was produced from ABP with large branch size, showed a higher value of melting temperature (175.30 °C), tensile stress (7.32 MPa) and modulus (33.64 MPa) due to the interaction of pectin chains was stronger. The BP-ABP3:1 also showed better barrier properties by obtaining the lowest water vapor transmission rates (1.10 – 1.18 $mg/m^2.s$) and moisture absorption values (2.61 – 32.13%) depending on the relative humidity compared to other BP-ABPs that have linear structure with smaller branch size. Besides that, the influence of different molar ratios of components in DES as a plasticizer was investigated on the BP properties. It should be highlighted that DES at its eutectic point (molar ratio 1:1) exhibited minimal plasticizing effect due to the self-interaction of choline chloride and citric acid monohydrate. Also, the plasticizing effect increased when the molar ratio of citric acid monohydrate was higher. Furthermore, the selected BP-DES1:1 showed potential application as a capsule material by exhibiting its capability to withstand digestion in artificial saliva and fully dissolved in simulated gastric fluid, not cytotoxic, as well as provide delayed action in the fish oil oxidation process. In conclusion, this DES is a good alternative for pectin extraction, and as a plasticizer for BP production. These findings demonstrated that pectin extracted from *A. bilimbi* could be explored as novel promising natural pharmacological biopolymer and/or potential functional food ingredient.

CHAPTER 1

INTRODUCTION

This chapter introduces the current research topic in a general manner and divided into four sections. Section 1.1 gives an overview on the background of the current study. Section 1.2 discusses the problem statements in order to alert the need of conducting this study. Section 1.3 highlights the importance of this study and its contribution. Section 1.4 points out the objectives of this research project one by one to resolve the stated problems.

1.1 Background of study

This study was to explore the usage of new deep eutectic solvent (DES) as an extraction medium whereas the *Averrhoa bilimbi* fruit was used as the alternative source of pectin. Other applications of DES and the extracted pectin were studied. Therefore, these two components were mainly discussed in this section.

Acids, such as hydrochloric acids or citric acids, are usually used for the extraction of pectic polysaccharides (Sandarani, 2017). However, the extraction yield is lower if a conventional approach is used. DES was therefore suggested to be a suitable extraction medium replacement due to their uniqueness. This solvent is produced when the mixtures arise at the eutectic point, which has a lower melting point. The DES can be classified into four types and the Type III DES has the most applications due to their low cost and low eco-toxicity. This system consists of a cation and an anion from quaternary ammonium salt (*e.g.* choline chloride) and hydrogen bond donating species from a hydrogen bond donor (*e.g.* citric acid monohydrate). However, DES is structurally different from ionic liquids because of DES is not only having cations and anions but also non-ionic species. The quaternary ammonium salt (*e.g.* choline chloride) consists of choline cation ($C_5H_{14}ON^+$) and chloride anion (Cl^-).

Meanwhile, the hydrogen bond donor, such as citric acid monohydrate, is the source of non-ionic species. According to Ghaedi, Ayoub, Su, Shariff, & Lal (2017), DES is easily be prepared, nontoxic and biodegradable. In addition, the authors also reported that DES is now a class of solvents that possess desirable characteristics, such as a wide liquid range, lower melting point than the constituents of the mixture, nonreactive with water, non-volatile, thermally stable, highly conductive and cheaper price compared to ionic liquid. Apart from that, Cunha & Fernandes (2018) stated that DES has the capability to act as an effective medium for the extraction of a wide range of non-polar and polar components. Hence, the potential of DES as a medium for pectin extraction should be explored.

The second major component of this study, *A. bilimbi,* is a plant, which belongs to the family of Oxalidaceae. It can easily be found throughout Asian countries. *A. bilimbi* has been widely well informed for culinary uses and has applied for medicinal purposes. Roy, Rv, & Lakshmi (2011) reported that the paste of the leaves can be used as a curative for itches, swelling, skin eruptions, cough and poisonous sites. Usually, the fruit marmalade is used as a treatment for coughs, beriberi and biliousness. In addition, *A. bilimbi* juice can be taken as a cure for fever, inflammation, alleviate internal hemorrhoids and to stop rectal bleeding. Apart from that, Peris, Singh, & Dsouza, (2013) reported that *A. bilimbi* is a nutrition-packed fruit and known to be a rich source of ascorbic acids, fibers, ashes, proteins, moistures, vitamins and minerals. According to the researchers, it is also known to provide a wide range of health and medicinal benefits, which include anti-infertility, hepato-protective, hypoglycemic, hypotriglyceridemic, antimicrobial and antioxidant activities. Therefore, it was suggested that the pectin from *A. bilimbi* (ABP) could be useful for food industry and it is worth to be investigated.

1.2 Problem statement

According to Sun (2016), the bioactive compounds in plant and animal sources are usually present in low concentrations. Thus, it is of importance to develop more effective and selective extraction method as well as the extraction medium for the recapture of the desired constituents. In addition, the extraction methods and parameters (*i.e.* solvent, temperature, and time) will also affect the yield of the pectin. Currently, chemical method was used to extract the pectin in order to investigate the structural features and their functional properties. As stated by Sandarani (2017), the chemical agents used for pectin extraction are divided into four groups such as water/buffers, calcium-ion chelators, acids and bases. The author also reported that the acid extraction of pectin is the most common method that has been applied because acids are the strongest extraction medium for pectin as they assist the extraction of insoluble pectin that is tightly bound to the cell matrix of the plant material and resulted in higher yields of pectin. Commonly, the acids used were acetic acid, citric acid, lactic acid, malic acid, tartaric acid, hydrochloric acid, nitric acid, oxalic acid, phosphoric acid and sulfuric acid. Nevertheless, some of the above-mentioned acids such as hydrochloric acid and sulfuric acid have an unavoidable weakness. This is because the use of these strong acids produces hazardous effluents that triggers environmental problems (Dominiak, 2014). Furthermore, as mentioned by Huang (1973) and Sandarani (2017), they reported that the extraction of pectin using mineral acid without the addition of resin as a medium gave low yields of extracted from lemon, grapefruit and orange peels.

Apart from that, Malaysia was reported as one of the countries in the world that has an abundant diversity of 500 species of cultivated, underutilized and rare fruit species either growing wild or planted in the villages in Peninsular Malaysia, Sabah and Sarawak (Aziz, 2016). As mentioned by Anuar & Mohd Salleh (2019), underutilized fruits such as *A. bilimbi* has not received attention from the community due to the fact that the fresh fruit is extremely sour (high acidity, which is caused by the presence of oxalic acid). Furthermore, *A. bilimbi* has a short shelf life after harvesting and the fruit must be utilized within 24 h (Berkley, 2010). Thus, wastage of this underutilized of *A. bilimbi* fruit occurred. Therefore, it is necessary to conduct a research on *A. bilimbi* such as extraction of pectin to facilitate the utilization of available resources as well as to value add the *A. bilimbi*.

Other than that, most of the plastics are made from petroleum based hydrocarbons, which are from non-renewable resources (Kuruppalil, 2011). Due to the unrestrained use of plastics in various applications, approximately 265,000,000 tons of plastics are manufactured and used every year (Ginting & Tarigan, 2015). In this context, synthetic plastics have topped the list in the non-recyclable and non-biodegradable material (Bharti & Swetha, 2016). Therefore, this occurrence has elevated a serious issue of plastic waste disposal and pollution, which cause global warming due to the discharge of carbon dioxide and dioxins during the combustion of these plastics (Kale, Deshmukh, Dudhare, & Patil, 2015). Therefore, it is necessary to lead a research for the development of biodegradable bioplastics to solve the issues (Cazon, Velazquez, Ramirez, & Vazquez, 2017). In this study, pectin, that was extracted from *A. bilimbi*, was used as the material for producing bioplastic. Apart from that, the production of bioplastics is depending on the combination of biopolymer and a plasticizer. However, the common plasticizers used in the industry are phthalates. They provide most desirable properties of plasticizers, such as good compatibility, high gelling capacity, relatively low volatility at ambient temperature, water resistant and low cost. According to Sheikh & Beg (2019), world consumption of phthalates as plasticizers in 2017 was about 65%. Some of the examples of phthalate plasticizers are di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP). Among these compounds, DEHP is the most important phthalate and widely used plasticizers in the world, which is produced on a massive scale, due to its decent plasticizing properties. Its short chain offers higher compatibility and plasticizing effect. However, leaching problems occur, especially in a medical device have prohibited its application (Rahman & Brazel, 2004). In addition, DEHP was reported to be poorly biodegradable under anaerobic conditions (Gavala, Alatriste-Mondragon, Iranpour, & Ahring, 2003). It should also be noted that this compound is known to be an endocrine compound that has carcinogenic and mutagenic effects. According to Hartemann, Rodriguez-Farre, & Testai (2016), the phthalate exposures in humans has been linked to effects on testosterone production and semen quality, breast tumours, infants with respiratory failure, low birth weight (LBW), hypospadias and cryptorchidism, decreased anogenital distance, childhood growth and pubertal development, endometriosis as well as toxicity to liver, kidney, and testis. Hence, an alternative plasticizer (DES, which was also used as the extraction medium), that has minimal toxicity, should also be explored.

1.3 Significant of study

In order to solve the problems aforementioned, DES was suggested as an efficient medium for pectin extraction, and plasticizer for bioplastic production. As an extraction medium, DES could provide additional interaction with the plant material due to the presence of cations, anions and hydrogen donors, and subsequently, more pectin could be extracted out compared to using citric acid monohydrate alone. As mentioned by Zhang & Wang (2017), the DES is capable of donating or accepting exterior electrons or protons to form hydrogen bonds. In this scenario, they can dissolve a wide variety of materials, including salts, proteins, drugs, amino acids, surfactants, sugars and polysaccharides. In the view of these unique features, it was proposed that DES could be used as a highly efficient extraction medium.

With the aim to optimize the utilization of underutilized fruit, *A. bilimbi* can be explored as a suitable candidate to be the source of functional biopolymer (pectin). In this case of study, the *A. bilimbi* fruit contains up to 90% of water due to the presence of pectin that has high water binding activity. Therefore, it is suitable to be used as a pectin source. Apart from that, it could be noted that pectin bioplastic has received a lot of attention due to their applications, especially in active packaging and food preservation (Naqash, Masoodi, Rather, Wani, & Gani, 2017). It was therefore believed that pectin that extracted from *A. bilimbi* fruit could possesses different properties that is suitable to be used for the production of bioplastic that could contribute to food preservation.

In terms of replacing common plasticizer for bioplastic production, DES has been newly introduced as a non-toxic and eco-friendly plasticizer (Hayyan, Looi, Hayyan, Wong, & Hashim, 2015). This DES will provide additional interaction with the pectin, due to the presence of cations, anions and hydrogen bond donors. Thus, it could be more effective compared to other common plasticizers. As reported by Qu (2015), the benefits using DES derived from choline chloride are inexpensive, biodegradable, biocompatible, non-toxic (also known as pro-vitamin B4), and easy to prepare with hydrogen bond donors. Moreover, the use of citric acid monohydrate as a hydrogen bond donor in DES that contains one hydroxyl and three carboxyl groups might improve the interaction between the carboxyl group of citric acid monohydrate, not just with choline cation ($C_5H_{14}ON^+$) of choline chloride but also with hydroxyl group of *A. bilimbi* pectin (ABP). As a result of its multi-carboxylic structure, this interaction could improve the water resistibility due to reducing available hydroxyl groups of ABP (Ghanbarzadeh, Almasi, & Entezami, 2011). In addition, the used of citric acid monohydrate as a hydrogen bond donor that contains hydroxyl and carboxyl molecules might improve the antioxidant of bioplastics. According to Liang & Kitts (2014), the hydroxyl molecules could function as a hydrogen donator to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and neutralized the effect of oxidative stress. Besides, citric acid monohydrate also rated as nutritionally harmless (non-toxic) and it has been approved by the Food and Drug Administration (FDA), United States for using in food formulations. The combination of choline chloride and citric acid monohydrate was therefore suggested to produce a DES (which was also used as an extraction medium in this study) as a plasticizer with unique properties that might improves the properties of bioplastics.

1.4 Objectives

The main aims of this study were to synthesis new DES and to explore its potential applications. *A. bilimbi* fruit (an unexplored fruit) was used as an alternative source of pectin. The DES-assisted extraction process was assessed based on the *A. bilimbi* pectin (ABP) extraction and then the pectin was used to produce bioplastics. The whole study was divided into several specific objectives, as stated below:

i. To synthesize new DES using choline chloride and citric acid monohydrate with different molar ratios as well as to investigate their physicochemical properties.

ii. To optimize the extraction of pectin from *A. bilimbi* fruit using the newly synthesized DES and to investigate the effects of DES molar ratio on the properties of extracted pectin.

iii. To produce bioplastic using the selected pectins extracted from *A. bilimbi* fruit and the selected DESs were used as alternative plasticizers followed by characterization.

iv. To explore the application of bioplastics (potentially as fish oil capsules).

CHAPTER 2

LITERATURE REVIEW

This chapter describes the general literature review of the fundamental knowledge relevant to the subject matter of this research and divided into four sections. Section 2.1 reviews on deep eutectic solvent (DES) in terms of its formation, physicochemical properties and the potential application of DES as an alternative extraction medium and plasticizer. Section 2.2 discusses on pectin and its sources as well as *A. bilimbi* as the sources of pectin with various biological activities. Section 2.3 details on extraction of pectin, effect of parameters on extraction efficiency and the optimization strategy using response surface methodology (RSM) as well as the pectin characteristics in terms of its physicochemical properties, functional properties and bioactivities. Section 2.4 reports the application of pectin as a biopolymer in bioplastic production, defines bioplastics and plasticizer, theory of bioplastics formation as well as the properties of bioplastics.

2.1 Deep eutectic solvent (DES)

The DES was introduced in 2003 by Abbott, Capper, Davies, Rasheed, & Tambyrajah. The term “deep eutectic solvent” refers to the liquids near to the eutectic composition of two or more compound mixtures which formed a new homogeneous liquid phase with a lower freezing point or melting point than their individual components upon stirring at 80 °C. The formation of DES requires at least two compounds that consist of a quaternary ammonium salt and hydrogen bond donor in a certain ratio (Abbott, Boothby, Capper, Davies, & Rasheed, 2004a). Based on Figure 2.1, the eutectic point for the molar composition of components can be defined as the lowest eutectic temperature observed at a specific molar ratio of component A and component B. According to their uniqueness such as low vapor pressure, low flammability, good recyclability, high thermal stability and a wide range of solubility, DES received a lot of attention (Smith, Abbott, & Ryder, 2012). Therefore, they were used for a wide range of applications, such as metal oxide processing (Abbott, Capper, Davies, Mckenzie, & Obi, 2006), biodiesel purification (Shahbaz, Mjalli, Hashim, & AlNashef, 2010), extractions (Saravana, Cho, Woo, & Chun, 2018), electrochemistry (Xu *et al.*, 2018a), nanomaterials (Xu *et al.*, 2016), carbon dioxide absorption (Zhang, Vigier, Sebastien, & Jerome, 2012), organic synthesis (Hu *et al*., 2018), biochemistry (Li *et al*., 2016), biocatalysis (Gotor-fernández & Paul, 2019) and polymer synthesis (Pereira & Andrade, 2017).

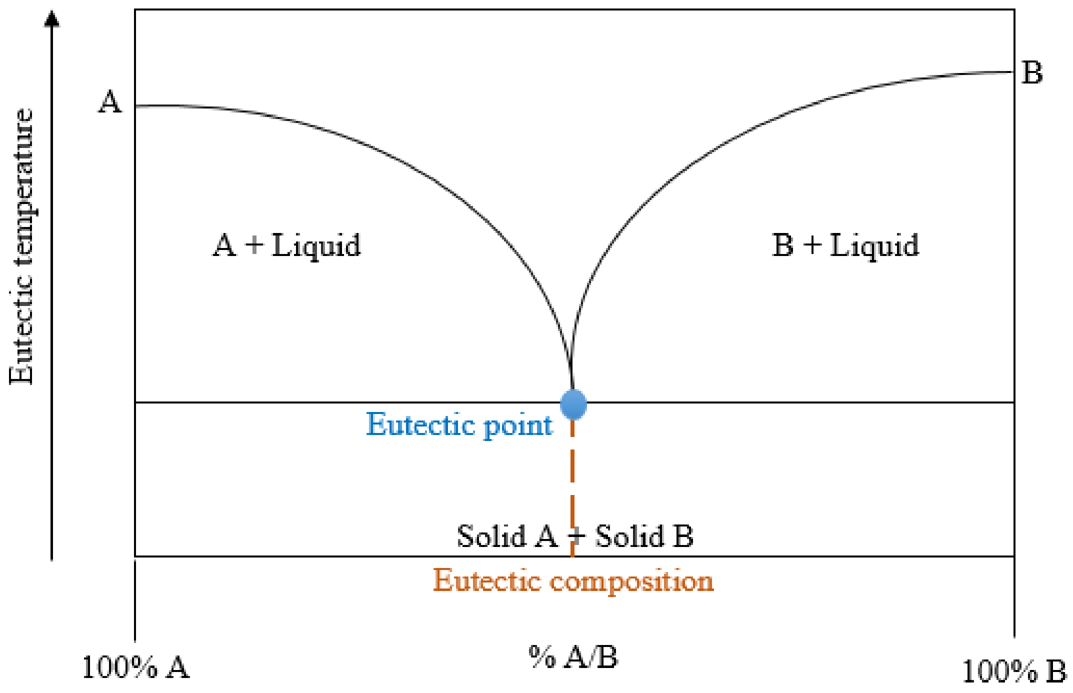

Figure 2.1 Phase diagram for a eutectic mixture (Adapted from Abolibda, 2015).

2.2 Formation of DES

DES are consisting a mixture of nonsymmetrical cation and a metallic salt or hydrogen bond donor. Nonsymmetrical quaternary ammonium salt, such as choline chloride, is the type of cationic component that commonly used because it is very cheap, easily accessible, non-toxic, biocompatible and biodegradable, which is also used nowadays as additive in animal feed and human nutrition (Delgado-mellado *et al.*, 2018). The hydrogen bond interaction occurs between the choline chloride and hydrogen bond donor commonly involved a charge delocalization between the chloride ion (Cl^-) and the hydrogen atoms from the hydrogen bond donor (Figure 2.2).

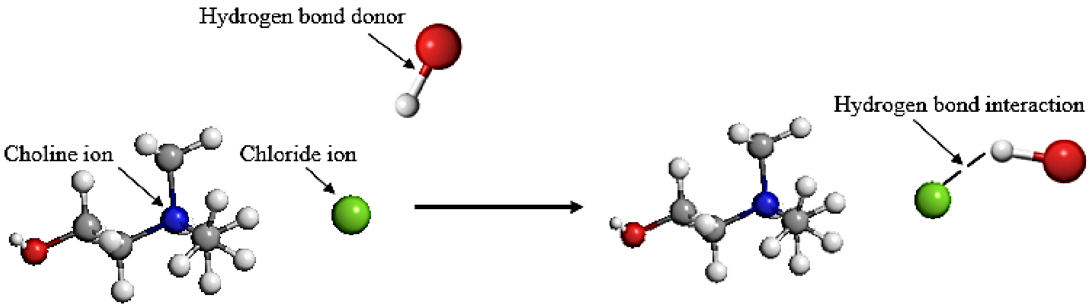

Figure 2.2 The illustration of hydrogen bond interaction between a quaternary ammonium salt and hydrogen bond donor.

DES can be classified into four types (Table 2.1) based on the components used in their preparation. Type I DES is synthesized by mixing metal salt, such as zinc chloride ($ZnCl_2$) and tin dichloride ($SnCl_2$), with a quaternary ammonium salt. Frequently, the Type I DES is used as a potential medium for conducting liquid in an electrochemical application. It was reported that Type I DES has a lower production cost compared to imidazolium based ionic liquids and the higher stability towards moisture (Yusof, 2016). As reported by Abbott *et al.* (2001), the acidity of these DES could be adjusted by changing the molar ratio of metal salt. Table 2.2 shows the examples of Type I DES that had been successfully produced in the past by other researchers.

Table 2.1 The classification of DES (Adapted from Abbott, Harris, & Ryder, 2007 and Abolibda, 2015).

Type of DES	**Mixtures**
Type I	Metal salt + Quaternary ammonium salt
Type II	Metal salt hydrate + Quaternary ammonium salt
Type III	Quaternary ammonium salt + Hydrogen bond donor
Type IV	Metal salt hydrate + Hydrogen bond donor

Table 2.2 Examples of Type I DES.

Quaternary ammonium salt	**Metal salt**	**Molar ratio**	**References**
choline chloride	zinc bromide	1:2	Abbott, Bell, Handa, & Stoddart (2005)
choline chloride	tin dichloride	1:2	
choline chloride	zinc chloride	1:2	Preethi, Padmapriya, Abarna, & Rajarajeswari (2017)

On the other hand, Type II DES is synthesized by mixing metal salt hydrate, such as chromium (III) chloride hexahydrate ($CrCl_3.6H_2O$), with quaternary ammonium salt. Usually, type II DES exhibits lower value of viscosity than Type I DES because of a higher interrelated water in the mixture (Abbott, Capper, Davies, & Rashees, 2004b). These DES were used in an application of electrodeposition, which produce a thick, adherent and crack free chromium film. Table 2.3 shows some examples of Type II DES that reported by other researchers.

Table 2.3 Examples of Type II DES.

Quaternary ammonium salt	Metal salt hydrate	Molar ratio	References
choline chloride	chromium (III) chloride hexahydrate	1:3	Abbott *et al*. (2004b)
choline chloride	calcium chloride hexahydrate	1:2	Mallakpour & Dinari (2012)
choline chloride	magnesium chloride hexahydrate	1:2	
choline chloride	cobalt (II) chloride hexahydrate	1:2	
choline chloride	copper chloride hexahydrate	1:2	
choline chloride	lanthanum chloride hexahydrate	1:2	Chandran *et al*. (2018)

The mixture of quaternary ammonium salt with a hydrogen bond donor forms Type III DES. This type of DES was the focus of this study because it has a wide range of applications, low cost and low eco-toxicity. As reported by Abbott *et al.* (2003), the combination of high and low melting temperature components such as choline chloride (302 °C) and urea (133 °C) at the molar ratio of 1:2 depressed the eutectic melting point to 12 °C. Choline chloride or also known as a vitamin B4 is widely used as a quaternary ammonium salt due to its production in large scale for using as an additive in chicken feed and abundance. Nowadays, choline chloride was combined with various hydrogen bond donors such as alcohols, carboxylic acids, and urea derivatives to form a DES. Table 2.4 shows the examples of Type III DES that produced the mixture of choline chloride with different hydrogen bond donors. It can be seen that, the used of hydrogen bond donor in the year 2000 were urea derivatives (*i.e.* thiourea, acetamide, benzamide and tertramethyl), sugar acids (*i.e.* succinic acid, malonic acid and citric acid) and sugar alcohols (*i.e.* ethylene glycol and glycerol). Nonetheless, monosaccharides (*i.e.* glucose and fructose) were used as a hydrogen bond donor in 2013. After that, more hydrogen bond donor was proposed based on the applications of the DES. Other commonly synthesized DES using different type of a quaternary ammonium salt and hydrogen bond donor are shown in Table 2.5.

Table 2.4 Examples of choline chloride based Type III DES.

Hydrogen bond donors	Molar ratio of choline chloride with hydrogen bond donor	References
urea	2:1	Abbott *et al.* (2003)
1-methyl urea	2:1	
1, 3-dimethyl urea	2:1	
1, 1-dimethyl urea	2:1	
thiourea	2:1	
acetamide	2:1	
benzamide	2:1	
tetramethyl urea	2:1	
phenylpropionic	1:2	Abbott *et al.* (2004a)
phenylacetic acid	1:2	
oxalic acid	1:1	
malonic acid	1:1	
succinic acid	1:1	
tricarballylic acid	2:1	
glycerol	1:3	Harris (2008)
ethylene glycol	1:3	
1, 4-butanediol	1:4	
1, 6-hexanediol	1:4	
1, 3-butanediol	1:5	
1, 2-butanediol	1:5	
2, 3-butanediol	1:5	
lactic acid	1:1.3 to 1:15	Francisco, van den Bruinhorst, & Kroon (2012)
malic acid	1.2:1 to 1:1.2	
oxalic acid (dihydrate)	1:1	
levulinic acid	1:2	Maugeri & Dominguez de Maria (2012)
itaconic acid	1:1	
itaconic acid	1:0.5	
xylitol	1:1	
D-sorbitol	1:1	
L-(+)-tartaric acid	1:0.5	
D-isosorbide	1:2	
4-hyroxybenzoic acid	1:0.5	
caffeic acid	1:0.5	
p-coumaric acid	1:0.5	
trans-cinnamic acid	1:1	
suberic acid	1:1	
gallic acid	1:0.5	
phenol	1:2 to 1:4	Li, Deng, Chen, Shan, & Ai (2014)
diethylene glycol	1:3	

triethylene glycol	1:4	
p-toluenesulfonic acid	1:2	Cui *et al.* (2017)
trichloroacetic acid	1:2	
monochloroacetic acid	1:2	
propionic acid	1:2	
1,3-dimethylurea	1:2	Troter *et al.* (2018)
thiourea	1:2	
propylene glycol	1:2	
ethylene glycol	1:2	
glycerol	1:2	
xylose	1:1	Aroso, Paiva, Reis, & Duarte (2017)
glucose	1:1	
sucrose	1:1	

Table 2.5 Examples of Type III of DES that formed from various types of quaternary ammonium salts.

Quaternary ammonium salts	Hydrogen bond donor	Molar ratio	References
choline acetate	glycerol	1:1 to 1:3	Zhoa, Baker, & Holmas (2011)
choline acetate	ethylene glycol	1:2	
choline acetate	urea	1:2	
choline acetate	1-propanol	1:1.5	
ethylammonium chloride	urea	1:2	Durand *et al.* (2012)
ethylammonium chloride	glycerol	1:2	
alanine	lactic acid	1:9	Francisco *et al.* (2012)
betaine	lactic acid	1:2	
glycine	lactic acid	1:9	
histidine	lactic acid	1:5 to 1:9	
proline	lactic acid	1:1 to 1:4	
alanine	malic acid	1:1	
betaine	malic acid	1:1	
glycine	malic acid	1:1	
histidine	malic acid	1:1 to 1:2	
proline	malic acid	3:1 to 1:3	
nicotinic acid	malic acid	1:9	
ethylammonium chloride	urea	1:1.5	Cunha & Fernandes (2018)
ethylammonium chloride	acetamide	1:1.5	
tetramethylammonium chloride	ethylene glycol	1:3	
fructose	citric acid	1:1	

Other than that, Type IV DES is composed of metal salt hydrate with hydrogen bond donors. Type IV DES is simply a hybrid between Type I and Type III DES because these eutectic solvents are only formed from a limited number of metal salts and an even narrower range of donors. These DES was introduced by Abbott *et al.* in 2007, when they successfully found that zinc chloride ($ZnCl_2$) forms eutectic mixtures with different hydrogen bond donor such as urea, acetamide, ethylene glycol and 1,6-hexanediol with molar ratio of 1:3.5, 1:4, 1:4 and 1:3, respectively. According to the authors, these DES have similar physical properties to other ionic liquids and can be used effectively to deposit dense zinc layers on an electrode surface.

Apart from that, the molar ratio of a quaternary ammonium salt and hydrogen bond donor also plays an important role in the formation and stability of DES. The formation of hydrogen bond within DES required the favorable molar ratio of a quaternary ammonium salt and hydrogen bond donor to form a eutectic mixture as well as to remain as a liquid at room temperature. As mentioned by Abbott *et al*. (2004a), the presence of more quaternary ammonium salt or hydrogen bond donor causes more formation of hydrogen bond interaction between hydrogen donating species with anion in a quaternary ammonium salt. The authors also stated that the molar ratio of a quaternary ammonium salt and hydrogen bond donor affect the properties of DES. Table 2.6 shows the physicochemical properties of DES are affected by the molar ratio of hydrogen bond donor in the formation of DES. It could be observed that different molar ratios affect the properties of DES (*e.g.* thermal properties, viscosity, density and surface tension). Hence, it is required to investigate when new DES is produced.

Table 2.6 The physicochemical properties of DES that were produced using different molar ratios.

Quaternary ammonium salts (A)	Hydrogen bond donor (B)	Molar ratio (A:B)	Physicochemical properties of DES				References
			Melting point (°C)	Density (g/mL)	Viscosity (mPa.s)	Surface tension (mN/m)	
choline chloride	D-fructose	1:1	20.15	1.337	14347.4	70.4	Hayyan *et al.* (2013)
		1.5:1	12.16	1.304	12772.5	73.6	
		2:1	10.15	1.278	17645.5	74.0	
		1.5:1	37.15	1.259	200.0	75.0	
choline chloride	D-glucose	1:1	32.15	1.298	9037.1	73.1	Hayyan *et al.* (2013)
		1.5:1	24.15	1.268	8000.0	72.7	
		2:1	15.15	1.242	8045.1	71.7	
		2.5:1	44.15	1.252	10910.0	75.0	
tetrabutylammonium bromide	ethylene glycol	1:2	-	1.066	151.2	-	Yusof (2016)
		1:3	-	1.070	76.5	-	
		1:4	-	1.073	45.6	-	
		1:5	-	1.074	22.6	-	
		1:6	-	1.076	19.7	-	
tetrabutylammonium bromide	1,3-propanediol	1:2	-	1.055	219.0	-	Yusof (2016)
		1:3	-	1.054	135.4	-	
		1:4	-	1.053	95.2	-	
		1:5	-	1.053	76.2	-	
		1:6	-	1.052	69.8	-	

tetrabutylammonium bromide	1,5-propanediol	1:2	-	1.029	302.2	-	Yusof (2016)
		1:3	-	1.022	182.8	-	
		1:4	-	1.019	124.8	-	
		1:5	-	1.012	122.8	-	
		1:6	-	1.011	115.4	-	
tetrabutylammonium bromide	glycerol	1:2	-	1.136	467.2	-	Yusof (2016)
		1:3	-	1.150	442.4	-	
		1:4	-	1.161	430.8	-	
		1:5	-	1.165	407.2	-	
		1:6	-	1.187	377.6	-	
choline chloride	monoethanolamine	1:5	3.95	1.077	48.5	48.2	Mjalli, Murshid, Al-Zakwani, & Hayyan (2017)
		1:6	3.84	1.069	36.7	48.7	
		1:7	3.69	1.065	37.7	49.2	
		1:8	5.23	1.063	32.7	49.6	
choline chloride	diethanolamine	1:4	8.03	1.106	400.0	-	Murshid, Mjalli, Naser, Al-Zakwani, & Hayyan (2018)
		1:5	18.15	1.103	265.0	-	
		1:6	19.22	1.099	128.0	-	

2.3 Physicochemical properties of DES

The viscosity, density, surface tension and solubility of DES are important in industrial applications. Therefore, it is important to study the physicochemical properties of DES prior to any applications.

2.3.1 Viscosity

The viscosity can be defined as the measurement of fluidity and it is an important parameter for pectin extraction medium. The viscosity of DES is strongly influenced by the hydrogen bond interactions within DES. Mostly, the selection of the hydrogen bond donor affected the viscosity of DES. As stated by Abbott *et al.* (2003), the mixture of choline chloride and urea (120 mPa.s) had a higher viscosity than the mixture of choline chloride and glycerol (79 mPa.s) at molar ratio of 1:2. This is because the presence of functional group in hydrogen bond donor affects the hydrogen bond interaction occur between hydrogen donating species with anion in quaternary ammonium salt. For example, glycerol consists of the hydroxyl group of hydrogen donating species that causes the DES are less viscous compared to urea, which consist of an amine group of hydrogen donating species. The viscosity of the mixture choline chloride and xylitol (5230 mPa.s) are less viscous compared to those the mixture of choline chloride and D-mannose (~12000 mPa.s), which characterized by high viscosity at molar ratio of 1:1 (Maugeri & Dominguez de Maria, 2012 and Florindo, Oliveira, Branco, & Marrucho, 2017). This is because the presence of aldehyde groups of hydrogen donating species in D-mannose causes the DES more viscous compared with hydroxyl groups of hydrogen donating species in xylitol. Nevertheless, the viscosity of DES depends on temperature applied, and even low fluidity of DES can be less viscous at elevated temperature. The viscosity of DES (choline chloride –

glucose) at molar ratio of 1:1 decreased (34400 to 560 mPa.s) with increasing temperature (50 to 100 °C) as reported by Maugeri & Dominguez de Maria (2012). The presence of the moisture (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013), addition of glycerol (Maugeri & Dominguez de Maria, 2012) or impurities as well as the method preparation of DES (Florindo *et al.*, 2017) will also alter the viscosity of DES.

2.3.2 Density

The density of DES is defined as its mass per unit volume and it is important to gain more understanding of the thermodynamic as well as transport properties of DES (Yusof, 2016). The arrangement of ions is based on the combination of components in DES and it is also affecting the density of DES. A research conducted by D'Agostino, Harris, Abbott, Gladden, & Mantle (2011), reported that the density of the choline chloride based DES with ethaline, glyceline, and reline were 1.12 g/mL, 1.18 g/mL, and 1.25 g/mL, respectively. Abbott *et al*. (2007) also reported that the density of zinc chloride based DES with acetamide and urea were 1.36 g/mL and 1.63 g/mL, respectively. Many studies have discussed the causes that alter the density of DES such as molecular structure (Abbott *et al.*, 2007), cations and anions (Sanchez, Espel, Onink, Meindersma, & de Haan, 2009), concentration (Wu *et al*., 2010) and temperature (Ghatee, Zare, Moosavi, & Zolghadr, 2010). It should be noted that the density of DES could influence the extraction rate when they are used as an extraction medium because the solubility of bioactive compound depends on the solvent density. As mentioned by Roy, Sasaki, & Goto (2006), the low density of solvents caused a higher mass transfer rate of bioactive compound in extraction medium, thus resulting in a higher extraction rate.

2.3.3 Surface tension

The surface tension of DES is affected by the attraction of the quaternary ammonium salts and hydrogen bond donors in the surface layer of the liquid, thus minimize the surface area of DES. According to Abbott *et al.* (2006), they reported that the high surface tension of DES are resulting from the strong hydrogen bond interaction within DES and also the type of hydrogen bond donor has an effect on the surface tension of DES. The surface tension of choline chloride with different hydrogen bond donors, such as glycerol and ethylene glycol at molar ratio of 1:1 were 57. 24 mN/m and 48.91 mN/m, respectively (Shahbaz, Mjalli, Hashim, & AlNashef, 2012). In addition, Hayyan *et al*., (2013) found that the molar ratio of a quaternary ammonium salts and hydrogen bond donors also influenced the surface tension of DES. The mixture of choline chloride and D-glucose at a molar ratio of 1:2.5 (~75 mN/m) had a higher surface tension in comparison with molar ratio of 1:1 (~73 mN/m). A research by Mjalli, Naser, Jibril, Alizadeh & Gano (2014) reported that the surface tension can be altered by changing the molar ratio of a quaternary ammonium salts or hydrogen bond donor. For example, the surface tension of tetrabutylammonium chloride based DES was increased (~39 to ~41 mN/m) with the increasing molar ratio of ethylene glycol (2 to 4) as a hydrogen bond donor. Furthermore, the surface tension was reported to decrease with the increasing temperature (Abbott *et al*., 2011 and Mjalli *et al*., 2014). This is because the kinetic energy was increased with increasing the temperature as well as the cohesive forces between molecules reduces, hence the hydrogen bond interactions in DES are weakening.

2.3.4 Solubility

The wide range solubility of DES increases the interest for most of the researchers. The solubility of DES depends on the presence of hydrophobic or hydrophilic components. According to Moniruzzaman, Nakashima, Kamiya, & Goto (2010), the properties of DES can be changed by selecting different combinations of cation and anion. Previous research has proven that the structure of hydrogen bond donor has effects on the hydrophobicity of DES. As reported by Florindo, Oliveira, Branco, & Marrucho (2014), the presence of methyl groups in levulinic acid resulted in a lower water absorption (9.88 %wt) in comparison with other hydrogen bond donors, such as oxalic acid (19.40 %wt), malonic acid (16.16 %wt), glutaric acid (17.38 %wt) and glycolic acid (14.50 %wt), hence increased hydrophobicity of DES. According to Singh *et al.* (2013), the immiscibility of DES is also a great benefit for certain purposes, such as separation, as the DES can be easily isolated. In the case of this study, the miscibility of DES with water could lead to a higher extraction rate of pectin due to higher mass transfer rate in the miscible extraction medium.

2.4 DES as an alternative extraction medium and plasticizer

Due to the unique properties of DES, it arises interest in many fields of applications such as electrochemistry, biochemistry, deoxyribonucleic acid (DNA) salvation, extraction and polymer. Numerous studies have proven the capability and efficiency of DES as an extraction medium for bioactive compounds from plant materials, thus arise the attention as an eco-friendly and their potential as an alternative to conventional solvents. A research conducted by Krizek *et al*. (2018), reported that the mixture of menthol and acetic acid at molar ratio of 1:1 showed the greatest extraction efficiency with ~8.5% (w/w) percentage yield of tetrahydrocannabinolic acid compared to a methanol (~7.0% w/w), ethanol (~7.3% w/w) and methanol – chloroform mixture (~6.5% w/w). Similar to Vieira *et al.* (2018), the authors stated that the extraction of phenolic compound from *Juglans regia* L. also proven the efficiency of DES as an extraction medium when DES of choline chloride – phenylpropionic acid at molar ratio 1:2 gave a higher percentage yield (~3.5% w/w) compared to ethanol (~2.5% w/w). Furthermore, the application of DES as a medium for extraction of κ-carrageenan form *Kappaphycus alvarezii* was applied by Das, Sharma, Mondal, & Prasad (2016). The results showed that 10% (w/v) hydrated DES of choline chloride – glycerol at molar ratio 1:2 exhibited higher percentage yield (60.25% w/w) than the conventional method using 0.5% calcium hydroxide solution (36.58% w/w). Hence, it was proposed that DES could be used as a highly efficient extraction medium for pectin.

Based on previous studies, Guolin, Jeffrey, Kai, & Xiolan (2012) had extracted pectin from lemon peels using microwave extraction and ionic liquids as an alternative solvent. An optimum pectin yield of 24.68% (w/w) was obtained at 88 °C, liquid-solid ratio of 22.7 mL/g and extraction time of 9.6 min. Liu, Qiao, Gu, Yang, & Yang (2017a) also used the same technique to extract pectin from pomelo peels. The optimal yield was 29.1% (w/w) under the condition of ionic liquid concentration of 10 mM, liquid-solid ratio of 26 mL/g, microwave irradiation time and power of 15 min and 331 W, respectively. On the other hand, Liew, Ngoh, Yusoff, & Teoh (2018a) extracted pectin from pomelo (*Citrus grandis* (L.) *Osbeck*) peels using DES and without the microwave technique. The yield obtained was 23.04% (w/w) under the following conditions: extraction time of 141 min, temperature of 88 °C and liquid-solid ratio of 29 mL/g. From these results, it could be suggested that both ionic liquids and DES have the similar extraction efficiency since the yields obtained were similar even