ENHANCEMENT OF EMULSIFYING PROPERTIES OF NANOCELLULOSE FROM DEFATTED RICE BRAN THROUGH ULTRASONIC-ASSISTED ENZYMATIC TREATMENT

NURUL NAJIHAH BINTI ILIAS

UNIVERSITI SAINS MALAYSIA

2025

ENHANCEMENT OF EMULSIFYING PROPERTIES OF NANOCELLULOSE FROM DEFATTED RICE BRAN THROUGH ULTRASONIC-ASSISTED ENZYMATIC TREATMENT

by

NURUL NAJIHAH BINTI ILIAS

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2025

ACKNOWLEDGEMENT

In the name of Allah, the Most Merciful and the Most Compassionate. Firstly, praise is to Allah for His blessing that I have finally finished writing the report for my PhD thesis. I would like to express my deepest gratitude to my supervisor, Dr. Norazatul Hanim bt Mohd Rozalli, for her endless support, guidance and advice throughout the years. I appreciated her effort in patiently providing me with all the skills, knowledge and everything I need for the completion of my study. I am also indebted to offer my profound gratitude to my co-supervisor, Assoc. Prof. Dr. Mohamad Haafiz bin Mohamad Kassim for his support and advice throughout the period of the study.

Most importantly, thank you to my husband, Mohd Rizal bin Razak for his patience and perseverance and for always being with me in any hardships. Not to forget, my gratitude to all my beloved children, Arissa, Aydan and Aqeef for the motivation and countless sacrifices in helping your Mom to finish her study. I hope I make all of you proud. Special thanks are also dedicated to my parents for their continuous support and encouragement.

As for my colleagues, thank you for always sharing knowledge and helping me whenever I am having a hard time during the completion of this study. I am also indebted to thank Ministry of Higher Education (MOHE) Malaysia for funding this project and providing financial support throughout the study. Lastly, I would like to thank all lecturers, staff and other postgraduate students who are involved directly or indirectly, for continuously guiding me in completing my laboratory work and also the final report for my PhD study. Without such a team behind me, I doubt I will be finishing my PhD study successfully.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

θ	Angular displacement (theta)
η	Apparent viscosity
β	Beta
°	Degree
°C	Degree Celsius
δ	Delta
ρ	Density
<	Less than
μ	Micro
>	More than
%	Percentage

LIST OF ABBREVIATIONS

AFM	Atomic force microscopy
ANOVA	Analysis of variance
BNC	Bacterial nanocellulose
C	Surface coverage
cm^2	Square centimetre
CNC	Cellulose nanocrystals
CNF	Cellulose nanofibres
CrI	Crystallinity Index
($D_{3,2}$)	Sauter mean diameter
($D_{4,3}$)	Volume mean diameter
D_i	The droplet diameter for droplet fraction i
DLS	Dynamic Light Scattering
DRB	Defatted rice bran
DTG	Derivative thermogravimetric
EI	Emulsification index
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
h	Height of cellulosic nanofibres
H_2SO_4	Sulphuric acid
H_C	Height of the cream layer
HIU	High-intensity ultrasonic
H_T	Total height of the emulsion
I_{am}	Amorphous material taken at 2θ around 18°

I_{cry}	Crystalline region at a 2θ angle around 22°
KBr	Potassium bromide
kHz	Kilohertz
KOH	Potassium hydroxide
L/D	Length/Diameter (Aspect ratio)
LCNC	Lignocellulosic nanocrystals
LCNF	Lignocellulosic nanofibres
LIU	Low-intensity ultrasonic
m_p	Mass of cellulosic nanofibres
MARDI	Malaysian Agricultural Research and Development Institute
MHz	Megahertz
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
mN/m	Milinewton per metre
MPa	Megapascal
NaOH	Sodium hydroxide
N_i	The number of the droplet with size D_i
nm	Nanometre
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
RBN	Rice bran nanofibres
SEM	Scanning electron microscopy
T_{max}	Maximum degradation temperature

$T_{10\%}$	Temperature at 10% mass loss from the initial mass of the sample
$T_{20\%}$	Temperature at 20% mass loss from the initial mass of the sample
T_{on}	Onset temperature
TAPPI	Technical Association of Pulp and Paper Industry
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric analysis
UA	Ultrasonication amplitude
UT	Ultrasonication time
µm	Micrometre
V_{oil}	Volume of oil
W	Watt
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water
w/v	Weight over volume
w/w	Weight over weight
XRD	X-ray diffraction

LIST OF APPENDICES

PENINGKATAN SIFAT-SIFAT EMULSIFIKASI OLEH NANOSELULOSA DARIPADA DEDAK BERAS YANG TELAH DINYAHLEMAK MELALUI RAWATAN ENZIM BERBANTU ULTRASONIK

ABSTRAK

Dedak padi yang dinyahlemak (DRB) adalah sisa pertanian yang mempunyai potensi ketara dalam industri makanan. Ia juga dianggap sebagai sumber terbaik serat nanoselulosa berasaskan biologi kerana kandungan selulosanya yang tinggi. Penggunaan bahan toksik dalam pengekstrakan gentian nanoselulosa dan penggunaan pengemulsi sintetik dalam penyediaan emulsi sedia ada boleh menjejaskan pengguna dan alam sekitar. Kajian ini menyiasat penggunaan inovatif kaedah mesra alam iaitu rawatan enzimatik berbantu ultrasonik untuk mengasingkan sejenis gentian nano lignoselulosa (LCNF) daripada dedak padi yang dinyahlemak (DRB), dinamakan gentian nano dedak padi (RBN) sebagai pengemulsi berkesan untuk menstabilkan emulsi Pickering. Proses saringan mendapati bahawa DRB daripada varieti MR 219 (DRB 219) berpotensi untuk bertindak sebagai stok suapan yang sangat baik untuk menghasilkan RBN kerana ia mempunyai komposisi hemiselulosa dan lignin yang paling rendah dengan kehabluran (11.55%) dan kestabilan terma (307°C) yang baik berbanding DRB 297 dan DRB 220 CL2. Keputusan selanjutnya menunjukkan bahawa aktiviti hidrolitik oleh enzim selulase dan pendeligninan oleh enzim lakase telah menyumbang kepada peningkatan sifat RBN berbanding DRB yang tidak dirawat (DRB-UT). Peningkatan ini dibuktikan dengan kehadiran struktur gentian hujung terbuka dalam sampel yang dirawat selulase (RBN-AC), disebabkan oleh pengurangan saiz gentian nano oleh hidrolisis berenzim. Sebaliknya, sampel yang dirawat dengan lakase (RBN-AL) mempamerkan penyingkiran lignin yang berkesan (penyingkiran

sebanyak 29.6%) disebabkan oleh tindakan pendeligninan oleh enzim ini. Perubahan struktur dan komposisi ini seterusnya disokong oleh peningkatan kandungan selulosa dalam kedua-dua sampel RBN-AC dan RBN-AL yang juga memperbaiki sifat fizikokimia dan haba mereka berbanding DRB-UT dan DRB-A. Kajian tentang keupayaan pengemulsi mereka menunjukkan RBN-AL mempunyai ciri-ciri pengemulsi yang sangat baik dengan emulsi paling stabil (indeks pengemulsi; 98.3%) dan saiz titisan terkecil (20.57 µm) selepas 30 hari penyimpanan. Kajian pengoptimuman yang dijalankan ke atas parameter enzimatik dan ultrasonik dengan menggunakan pengoptimuman faktor tunggal menunjukkan bahawa sifat terbaik RBN boleh dihasilkan dalam keadaan berikut: 3% kepekatan lakase, 1% penggunaan substrat, dan 15 minit masa ultrasonikasi pada 30% amplitud. Dengan menggunakan parameter ini, pengurangan lignin yang lebih tinggi (73.5%), potensi zeta (-44.8 mV), dan kehabluran (49.91%) telah diperolehi dalam RBN yang terhasil. RBN juga menunjukkan pengurangan 40% dalam ketegangan antara muka dinamik dengan sudut sentuhan hampir 90° (89.34°), membuktikan potensinya sebagai pengemulsi yang baik. Tambahan pula, kestabilan emulsi telah bertambah baik dengan ketara pada kepekatan RBN yang lebih tinggi (>1%) menggunakan nisbah 1:9 minyak kepada air. Selain itu, variasi yang dibuat kepada parameter ultrasonikasi semasa pengemulsian mengesahkan bahawa penggunaan kaedah gabungan (penghomogen pemegun rotor dan ultrasonik) boleh meningkatkan kestabilan emulsi dengan ketara, terutamanya pada 6 minit masa ultrasonikasi. Pengoptimuman selanjutnya pada amplitud ultrasonikasi yang lebih tinggi (40, 60, 80%) menunjukkan peningkatan yang luar biasa dalam kestabilan emulsi dengan pengurangan saiz titisan ($D_{3,2}$), peningkatan kelikatan, luas permukaan spesifik dan liputan permukaan yang mencukupi. Analisis reologi mendedahkan bahawa emulsi yang diperoleh menunjukkan sifat penjarangan

ricih dengan sifat seperti gel yang tertinggi didapati apabila amplitud 80% digunakan. Secara keseluruhannya, rawatan lakase dibantu dengan ultrasonik berjaya meningkatkan sifat pengemulsi RBN daripada DRB, seperti yang ditunjukkan oleh emulsi Pickering minyak dalam air yang stabil selepas 14 hari penyimpanan.

ENHANCEMENT OF EMULSIFYING PROPERTIES OF NANOCELLULOSE FROM DEFATTED RICE BRAN THROUGH ULTRASONIC-ASSISTED ENZYMATIC TREATMENT

ABSTRACT

Defatted rice bran (DRB) is an agricultural waste that has notable potential in the food industry. It is also considered an excellent source of bio-based cellulosic nanofibres due to its high cellulose content. The use of toxic materials in the extraction of cellulosic nanofibres can affect both consumers and the environment. This study investigates the innovative use of an environmentally friendly method that is the ultrasonic-assisted enzymatic treatment, to isolate a type of lignocellulosic nanofibres (LCNF) from defatted rice bran (DRB), named rice bran nanofibres (RBN), as potent emulsifiers for stabilizing Pickering emulsions. It was found from the screening process that DRB from the MR 219 variety (DRB 219) has the potential to act as an excellent feedstock to produce RBN, as it has the lowest composition of hemicellulose and lignin with good crystallinity (11.55%) and thermal stability (307°C) compared to DRB 297 and DRB 220 CL2. The results further demonstrated that the hydrolytic and delignification activities of cellulase and laccase enzymes, respectively, contributed to the enhancement of RBN properties compared to the untreated DRB (DRB-UT). This improvement is evidenced by the presence of open-end fibre structures in the cellulase-treated sample (RBN-AC), attributed to the reduction in nanofibre size by enzymatic hydrolysis. In contrast, the laccase-treated sample (RBN-AL) exhibited effective lignin removal (29.6% removal) due to the delignifying action of the enzyme. These structural and compositional changes are further supported by an increase in cellulose content in both RBN-AC and RBN-AL samples, which also improves their

physicochemical and thermal properties compared to DRB-UT and DRB-A. The study on their emulsifying ability revealed excellent emulsifying characteristics of RBN-AL with the most stable emulsion (emulsification index: 98.3%) and the smallest droplet size (20.57 μm) after 30 days of storage. An optimization study conducted on the enzymatic and ultrasonication parameters using single-factor optimization showed that the best properties of RBN can be produced under the following conditions: 3% (w/w) laccase concentration, 1% (w/v) substrate loading and 15 min of ultrasonication time at 30% amplitude. By using these parameters, a higher lignin reduction (73.5%), zeta potential (-44.8 mV), and crystallinity (49.91%) were obtained in the resultant RBN. The RBN also demonstrated a 40% reduction in dynamic interfacial tension with a contact angle close to 90° (89.34°), confirming its potential as a good emulsifier. Furthermore, the emulsion stability was markedly improved at higher RBN concentrations (>1% w/v) using a 1:9 ratio of oil to water. Additionally, variations made to the ultrasonication parameters during emulsification confirmed that the combined method (rotor-stator homogenizer and ultrasonication) could significantly increase the emulsion stability, especially at 6 min of ultrasonication time. Further optimization at higher ultrasound amplitudes (40, 60, 80%) showed remarkable improvement in the emulsion stability by the reduction in the droplet size ($D_{3,2}$), increase in viscosity, specific surface area and sufficient surface coverage. Rheology analysis revealed that the obtained emulsions exhibit a typical shear-thinning behaviour, with the strongest gel-like properties found when 80% amplitude was used. Overall, the ultrasonic-assisted laccase treatment is successful in enhancing the emulsifying properties of RBN from DRB, as shown by a stable oil-in-water Pickering emulsion after 14 days of storage.

CHAPTER 1

INTRODUCTION

1.1 Research Background

A wide variety of food products exists in a way that oil and water are present as two immiscible phases. To address this problem, a system known as emulsion is used in which a phase is dispersed in another in the presence of an emulsifier. An effective emulsifier reduces interfacial tension and prevents droplet coalescence (Tian et al., 2022). While small surfactant molecules are effective, they offer short-term stability and raise health concerns, leading to interest in Pickering emulsions as an alternative (Rousta et al., 2021). Pickering emulsions are stabilized by solid particles that adsorb at the oil/water interface, providing better stability and environmental compatibility compared to conventional emulsifiers. Pickering emulsions can be stabilized by various types of solid particles, including clay, metal oxides, protein isolates and nanofibres. The demand for natural, plant-based stabilizers has led to research on cellulose-containing nanofibers (cellulosic nanofibers), such as cellulose nanocrystals (CNC), cellulose nanofibres (CNF), and lignocellulosic nanofibres (LCNF) derived from agricultural waste (Cui et al., 2023). Furthermore, the amphiphilic character of cellulosic nanofibres allows these materials to be preferentially wetted by water and oil phases (Teo et al., 2022).

Various methods are employed to produce purified cellulosic nanofibres, including physical (mechanical disruption), physicochemical (steam explosion), chemical (acid hydrolysis), and biological (enzymatic hydrolysis) treatments (Pradhan et al., 2022). While chemical methods require high temperatures, pressures, and chemicals, enzymatic treatment is an eco-friendly alternative. Combining mechanical

pre-treatments such as high-pressure homogenization, ultrafine grinding, ultrasound, and cryocrushing with enzymatic treatments can overcome the slow process of enzyme degradation in an aqueous system (Manasa et al., 2018). Among these mechanical means, ultrasonication appears to be a promising pretreatment as it can enhance the reaction rates and mass transfer, which contributes to the increase in the enzymatic reactions by forming microbubbles during sonication treatments caused by the cavitation effect (Tahir et al., 2022).

The production of cellulosic nanofibres from agricultural by-products is gaining interest. Promising sources include corn cob (Louis & Venkatachalam, 2020), oat hull (Debiagi et al., 2021), and canola straw (Huerta & Saldaña, 2019). Rice bran, a by-product of rice processing, is also a valuable source of cellulosic nanofibres. Rice can be considered the largest produced cereal crop in the world. Rice bran, a by-product of rice processing, is estimated to generate around 8% of the total rice production (MAFI 2017). Defatted rice bran (DRB), produced after rice bran oil extraction, has significant applications in the food industry, such as fiber-enriched bread and low-fat meat products. Beyond food applications, DRB is an excellent source of biobased cellulosic nanofibres due to its lignocellulosic compositions: 20-35% cellulose, 11-55% hemicellulose, and 5-33% lignin (Moreira et al., 2022). Research has explored extracting cellulosic nanomaterials from rice by-products for applications including emulsifiers (Angkuratipakorn et al., 2017), dietary fibre (Yan et al., 2018), and reinforcing agents (Razali et al., 2021). Cellulosic nanofibres from agricultural waste offer sustainability, biodegradability, high aspect ratio, and flexibility (Abdullah et al., 2021; Kellersztein et al., 2019).

Considering the lignocellulosic compositions of DRB, this study focuses on extracting rice bran nanofibres (RBN), a type of lignocellulosic nanofibre (LCNF),

using ultrasonic-assisted enzymatic methods. These RBNs can act as emulsifiers for stabilizing oil-in-water Pickering emulsions. Ultrasonication is also used as one of the emulsification methods that induces cavitation effects by offering significant potential in dispersing and reducing the size of oil droplets, leading to the stability of emulsion (Meirelles et al., 2020). This study explores the potential of ultrasound to enhance both RBN properties and the emulsifying efficiency of the resulting Pickering emulsion.

1.2 Problem statements

Increasing environmental awareness is prompting scientists and manufacturers to develop strategies for green production of nanofibres by using processes and materials with low cost, low energy consumption and low toxicity, together with high biodegradability. Several agricultural wastes have been used to produce cellulosic nanofibres like lignocellulosic nanofibres (LCNF), including corn stover, pineapple leaves, garlic skin and rice bran. Rice bran, as a by-product of rice processing that is abundant but not efficiently utilized, has usually been treated as animal feed or discarded into the surrounding area for open burning, leading to environmental pollution. The abundance of rice bran, as well as its low material cost with a substantial cellulose content, allows it to be considered as a potential biomass in the production of value-added materials like lignocellulosic nanofibre (LCNF). LCNF is an emerging sustainable biomaterial that is extracted from inexpensive renewable cellulosic biomass for its applications as a nanocomposite in food eco-packaging and as emulsifiers for stabilizing food-based Pickering emulsion.

However, the variety of rice bran used as a feedstock also plays a significant role in determining its physicochemical properties. Different rice bran varieties exhibit varying lignocellulosic compositions, which influence the extraction yield, structural

characteristics, and functionality of the resulting LCNF. Without proper screening and standardization, inconsistencies in LCNF properties may arise, leading to variable emulsifying efficiency. Hence, selecting a single rice bran variety for LCNF extraction is essential to ensure uniformity, reproducibility, and optimized emulsification performance.

Nevertheless, due to the adverse effects of chemical treatments, such as acid-based extraction, particularly in the production of cellulosic nanofibres for food applications, various attempts were made to utilize a greener method, such as an enzymatic treatment. Apart from its mild conditions, enzymatic methods eliminate the need for solvents and chemicals, making it both environmentally friendly and economically attractive (Costa et al., 2018). However, enzymatic extraction produces lower nanofibre yields and requires more time than acid hydrolysis due to limited enzyme-substrate interaction. This drawback can be addressed by ultrasonic-assisted extraction, which promotes the disintegration of nanofibres and enhances the reaction rate between the enzyme and the substrates through its cavitation effect. Meanwhile, the selection of enzymes plays a crucial role in the production of LCNF using ultrasonic-assisted enzymatic treatment. Different enzymes perform specific functions, influencing the characteristics of LCNF, which in turn affect its emulsifying properties and emulsion stability. Since Pickering emulsion stability depends on both the particle size and lignocellulosic compositions of the emulsifier, careful screening of hydrolytic (size-reducing) and ligninolytic (lignin-reducing) enzymes is essential to determine which properties contribute most in enhancing the emulsion stability.

The stability of oil-in-water emulsions is a key factor influencing their applicability, particularly in the food and pharmaceutical industries, thus, the usage of an appropriate emulsifier and emulsification technique is essential. The high cost of

conventional emulsifiers, along with their potential negative health and environmental effects, has made solid particle-based Pickering emulsions a promising alternative. The traditional emulsification methods, such as rotor-stator homogenization or high-speed homogenization, have been widely used to produce emulsions with fine droplet sizes and enhanced stability. However, these single emulsification techniques have limitations, including high energy consumption, inefficient droplet size reduction, and limited long-term stability. The reliance on these methods alone may not be sufficient to achieve optimal emulsification, necessitating the exploration of combined techniques to improve emulsifying efficiency and stability, such as by using ultrasonication.

Given these challenges, this study aims to enhance the emulsifying properties of rice bran nanofibre (RBN), a type of LCNF extracted from defatted rice bran, through an ultrasonic-assisted enzymatic treatment. LCNF has been produced previously by using either mechanical methods or hydrolytic enzymes for its function as an emulsifier (Cui et al., 2023; Li et al., 2020). However, to date, limited information is available on the extraction of LCNF from defatted rice bran (DRB) via a combination method of ultrasonication and enzymes to serve its purpose as a food emulsifier in Pickering emulsion. Hence, this proposed technique of producing LCNF would pave the way for a novel approach in achieving sustainable production of natural polymer-based emulsifiers from DRB.

1.3 Aims and Objectives

This research investigates the properties of rice bran nanofibers (RBN), a type of lignocellulosic nanofiber (LCNF) derived from defatted rice bran (DRB), through sequential treatments of alkaline, ultrasonic, and enzymatic treatments. Additionally, it evaluates RBN's potential as an emulsifier for stabilizing solid particle-based Pickering emulsions.

To achieve the aim of this project, research work was conducted based on the following objectives:

1) To screen the high-potential rice bran among three major Malaysian paddy varieties – MR 219, MR 297 and MR 220 CL2, as feedstock for rice bran nanofibres (RBN) production.
2) To evaluate the suitability of two types of enzymes (cellulase and laccase) with different functions for ultrasonic-assisted enzymatic treatment to produce rice bran nanofibres (RBN) that can stabilize oil-in-water Pickering emulsions.
3) To characterize rice bran nanofibres (RBN) derived from defatted rice bran through ultrasonic-assisted enzymatic treatment, utilizing the enzyme obtained from Objective 2, by analyzing their lignocellulosic compositions, morphological, structural and physicochemical properties.
4) To determine the performance of the rice bran nanofibres (RBN) obtained in Objective 3 as a potential emulsifier for oil-in-water Pickering emulsion through the analysis of emulsion stability, including visual appearance, microstructure, droplet size and emulsification index.

CHAPTER 2

LITERATURE REVIEW

2.1 Emulsion

Emulsions are colloidal dispersions of at least two immiscible fluid phases (normally oil and water) with one phase dispersed as small droplets into another. An emulsion essentially consists of a continuous phase, also known as an external phase, where the droplets are distributed, and a dispersed phase, which is defined as an internal phase that appears in the form of droplets (Akbari and Nour, 2018; Tan and McClements, 2021). Emulsions are frequently used in many different industries, including food (Wang et al., 2023), cosmetics (lipstick, cosmetic emulsion) (Guzmán et al., 2022), pharmaceuticals (encapsulation and delivery of drugs) (de Carvalho-Guimarães et al., 2022), and petroleum (Abed et al., 2019). A stable emulsion requires an adequate mechanical force to disperse one phase into another. Several sources of mechanical shearing can be used to form emulsion. Generally, increasing the amount of shear leads to a reduction in the droplet size which contributes to the emulsion stability (Liu et al., 2015). The presence of an emulsifying agent is another important factor in producing a stable emulsion. Appropriate compositions and types of emulsifiers used in stabilizing an emulsion were key factors to ensure that a stable and tight emulsion can be formed (Goodarzi and Zendehboudi, 2019).

Essentially, emulsion can be classified into three types which are oil-in-water (O/W), water-in-oil (W/O) and complex emulsion as demonstrated in Figure 2.1. O/W emulsions are comprised of oil droplets that were dispersed in a water-continuous phase, whereas W/O emulsions are attributed to droplets of water phase in a continuous flow of oil (Zhu et al., 2019). Meanwhile, complex or multiple emulsions involve a more complicated system that contains tiny droplets that are suspended in larger

droplets, that are also dispersed in a continuous phase. Examples of complex emulsions are water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions.

In the food industry, O/W emulsion is commonly used as it can be found in several products like sauces, milk, ice cream, yoghurt and mayonnaise while the W/O concept of the emulsion was used in the making of butter and margarine (Santamaria-Echart et al., 2021). On the other hand, complex emulsions exhibit excellent potential for several applications in the food, cosmetic and pharmaceutical industries (Huynh Mai et al., 2019). For the complex emulsion, W/O/W emulsions are frequently used due to their ability to develop fat-reduced and low sucrose products which contributed to the development of innovative food through double emulsions (Santamaria-Echart et al., 2021).

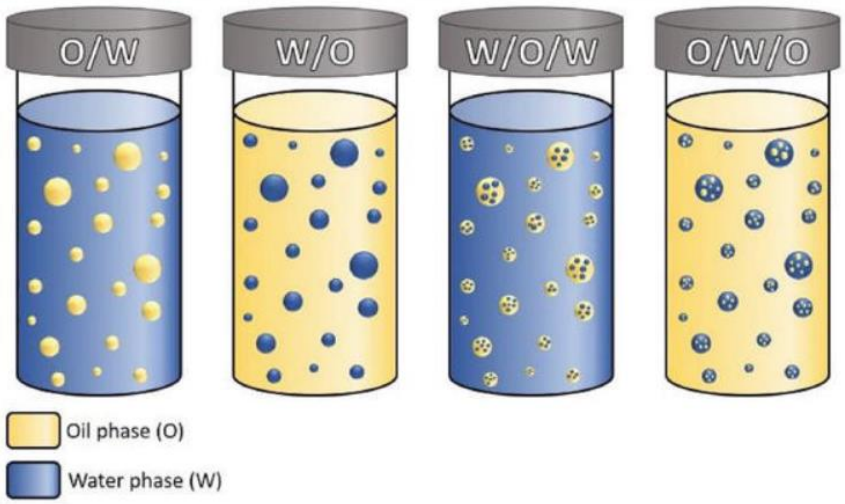

Figure 2.1 Types of emulsion. Source: Santamaria-Echart et al. (2021).

The thermodynamic instability that occurs in the emulsion phase has commonly resulted from the high interfacial energy of the dispersed droplets. Hence, an energy barrier has to be developed through the adsorption of the emulsifying agents at the oil-water interface so that the droplet coalescence can be prevented and

reasonable emulsion stability can be achieved. This substance is usually included in emulsion formulations and can decrease the interfacial tension between the phases (Wu and Ma, 2016). Generally, emulsifying agents can be classified into two types: surface-active agents and finely divided solids (Goodarzi and Zendehboudi, 2019). Conventional emulsion is an emulsion stabilized by biopolymers, like proteins, and low-molecular-weight emulsifiers (LMWEs), such as surfactants, whereas, emulsion stabilized by solid particles is known as Pickering emulsion as shown in Figure 2.2.

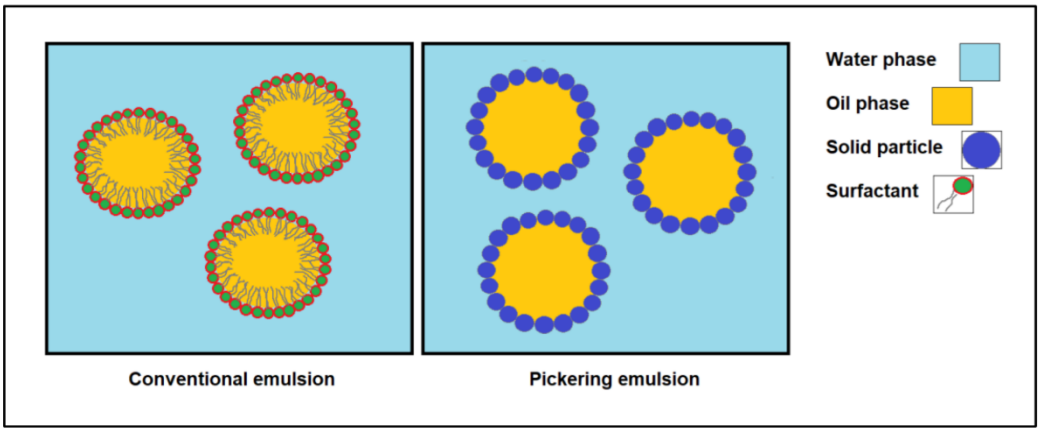

Figure 2.2 Schematic diagram of conventional and Pickering emulsion. Source: Teo et al. (2022).

2.1.1 Conventional emulsion

Generally, stabilization of emulsion can be achieved by the incorporation of amphiphilic emulsifying agents which position themselves at the oil-water interface and reduce the interfacial tension of liquid-liquid interfaces, thus preventing or delaying the aggregation process (Taha et al., 2020). Among the frequently used emulsifying agents in conventional emulsions are surfactants, proteins, phospholipids and polysaccharides (Tan and McClements, 2021; Wu and Ma, 2016). The strong cohesive forces between the water molecules have resulted in the increment of surface

tension of water. Owing to their amphiphilic nature, surfactants absorb at the oil-water interface by aligning themselves so that the hydrophobic tail of the emulsifier remains enclosed in the oil phase, while the hydrophilic part is typically found in the aqueous phase which resulted in the decrement of the interfacial surface tension (Iborra et al., 2018; Santamaria-Echart et al., 2021). Figure 2.3 shows the mechanism of the stabilization of emulsion using surfactants.

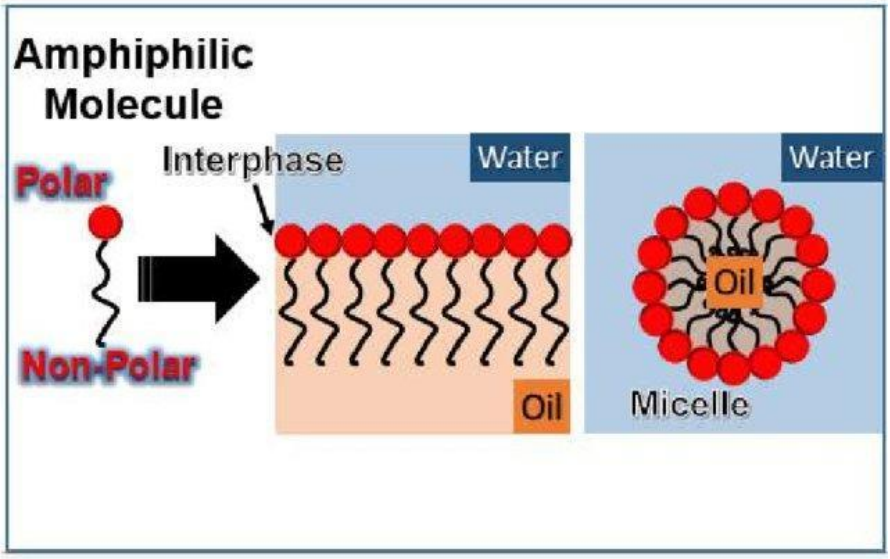

Figure 2.3 Schematic representation of a surfactant and its behaviour at the oil-water interface or micelle formation. Source: Iborra et al. (2018).

Meanwhile, proteins, which an amphiphilic biopolymer are among the commonly used emulsifiers in the food industry. The surface activity of proteins and, subsequently, their tendency to either adsorb to the oil-water interface, solubilize, or self-aggregate, are determined by the relative balance of polar and non-polar groups exposed on their surfaces (Tang et al., 2015). Among others, the use of protein as a food emulsifier especially in the form of dairy proteins and plant-derived proteins (Kim et al., 2020) is common. Protein structure and flexibility are two key factors that

influence the physical properties (eg: thickness, mechanical resistance, viscoelasticity) of protein-based interfacial films in emulsions.

Phospholipids are molecules that are made from the combination of a phosphoric acid (H_3PO_4) hydrophilic head and a hydrophobic tail that consists of one or two non-polar fatty acids. Phospholipids are commonly derived from natural sources like vegetable oil (rapeseed, sunflower and soybean), meat, fish, egg and milk (Ali et al., 2019). Due to their amphiphilic features, these compounds not only can stabilize emulsion but are also helpful as texturizing agents, which enhance the organoleptic attributes of the final product (Perazzo et al., 2021). On the other hand, large molecules like polysaccharides which are composed of monosaccharide units (eg: glucose, fructose and mannose) bonded by glycosidic bonds have much slower adsorption than the smaller surface-active agents. The presence of hydrophobic (glycolipids) and hydrophilic (hydroxyls) groups governed their amphiphilicity as it is being adsorbed at the interface to form a thick stabilizing layer. Examples of common polysaccharides used are pectins, gum Arabic, galactomannans and modified starch and cellulose (Santamaria-Echart et al., 2021). Their functional properties like solubility, rheology and amphiphilic character were influenced by the structural rearrangement including type and number of monosaccharides, type of glycosidic bonds, electrical charge, branching degree, molecular weight, hydrophobicity and the presence of other groups (carboxylate, sulfate or phosphate) (McClements and Gumus, 2016).

2.1.1(a) Limitation of conventional emulsion

Apart from the high cost of surfactants, some of them are partially biodegradable such as surfactants used in detergent formulations, resulting in potential negative environmental impact (Badmus et al., 2021). Small surfactant molecules are

well-known as good emulsifiers. However, it has short-term stability due to its presence in dynamic equilibrium with the bulk medium, which limits its application and practicality (Costa et al., 2021). The tendency of emulsion breakdown was also higher in conventional emulsions as a result of physical instability mechanisms, like droplet aggregation and gravitational separation (Tan and McClements, 2021).

In the food industry, limitations in using conventional emulsions can be found in certain applications. For instance, the use of protein as an emulsifying agent was greatly influenced by several environmental factors like temperature, ionic strength and pH. Besides that, the large molecular weight and poor solubility of food proteins, especially those that were derived from plants could negatively impact the stability of the protein-stabilized food emulsion (Liu et al., 2017; Nishinari et al., 2014). At present, the use of two common synthetic dietary emulsifiers namely carboxymethylcellulose (CMC) and polysorbate-80 (P80) has been reported to increase the risk of obesity and negatively affect the human gut (Aponso et al., 2017; Chassaing et al., 2022). Both CMC and P80 were found to induce lasting detrimental impacts on the intestinal microbiota in ways suggested to cause various inflammatory diseases (Naimi et al., 2021). Other than that, organic emulsifying agents like egg yolk, milk and soybean could contain residual allergens that might promote severe allergic responses (Verhoeckx et al., 2015). In this context, attention has been shifted to the exploration of more advanced emulsion systems as a solution for all limitations of using conventional one which is believed to improve the functional attributes and increase the shelf-life of the emulsion.

2.1.2 Pickering emulsion

In 1903 and 1907, the ability of solid particles to adsorb at the oil-water interface and stabilize oil and water emulsions was found by W. Ramsden and S.U.

Pickering (Liu et al., 2019). Walter Ramsden initially discovered the adsorption of soft solid particles known as "proteids" at air-water interfaces. However, this type of emulsion was named Pickering emulsion after S.U. Pickering further investigated the ability of solid particles to stabilize a mixture of paraffin oil-in-water (o/w) by its adsorption onto the interface. Thus, in general, Pickering emulsion is an emulsion formed due to the droplet coating and stabilization by a layer of adsorbed solid particles onto the oil-water interface (Berton-Carabin and Schroën, 2015; Meirelles et al., 2020).

The study on the food-grade Pickering emulsion is drawing much attention these years due to its favourable features like lower cost, lower toxicity and a simpler recovery. It is characterized by a long-term stability feature and can be considered an environmentally friendly method due to the absence of synthetic emulsifiers that comply with the recent trends in the food industry which emphasize more on healthy and sustainable technologies (Tan and McClements, 2021). Aligned with the application of natural emulsifiers, several food-grade Pickering emulsions have been studied recently for various purposes in the food industry.

In the production of dairy products such as pasteurized milk, ice cream and yoghurt, the instability of these products due to several external conditions during the production and storage process can be overcome by incorporating natural emulsifiers like whey protein and casein. In the research conducted by Jiao et al. (2022), a mixed protein consisting of whey protein isolate and sodium caseinate (WPI/SC) was found to be able to keep the emulsion with desired stability at a temperature of 25°C and 50°C by showing more uniform droplet distribution compared to the usage of WPI and SC alone. Chevallier et al. (2018) also reported a heat-stable emulsion in the presence of casein with whey protein microgel (WPM) as an emulsifier.

Functional food pertains to food that incorporates specific functional ingredients and active constituents like protein, dietary fibre, vitamins and bioactive substances. These elements help in the regulation of body functions and offer preventive measures against diseases including anti-inflammatory and anticancer properties (Li et al., 2020). However, these ingredients exhibit lower solubility in water and instability in external environments. Thus, the special features of Pickering emulsion can be used to enhance the process stability and bio-accessibility by encapsulating these active ingredients so that they can be effectively absorbed by the body. Lu and Huang et al. (2020) in their study found that cellulose nanofibre (CNF) obtained from sigmacell cellulose type through media-milling treatment can produce a stable emulsion over 30 days of storage when curcumin was encapsulated in Pickering emulsion as the bioactive compound.

Pickering emulsion is also used in the food industry as a fat substitute. It is well known that trans-fat in processed food can severely cause cardiovascular diseases (Zhao et al., 2020). Hence, high internal phase Pickering emulsion (HIPE) can be one of the promising approaches which can be an alternative liquid oil to the use of partially hydrogenated oils (PHO) and crystalline triacylglycerol (TAG) that lead to cardiovascular diseases. This can reduce fat intake and benefit consumers' health. Costa et al. (2020) reported that CNF obtained from banana peel bran was excellent in the inhibition of lipid digestion through the strong adsorption of particles onto the oil droplet interface as well as the presence of large oil droplets which can reduce the number of TAG molecules exposed to lipase action. Pickering emulsion also finds applicability in various food items such as sauces, soups, gluten-free rice bread, and similar products. In these cases, Pickering emulsion can serve as an alternative for

certain ingredients, thereby enhancing the quality of the food and extending its shelf life.

2.1.2(a) Stabilizing mechanism

In comparison to conventional emulsifiers (surfactants and biopolymers), solid particles provide different stability mechanisms as carefully explained by Binks (2002) and Tcholakova et al. (2008). The adsorption of solid particles on the oil-water interface forms a mechanical barrier that prevents the destabilization of emulsion droplets (Bai et al., 2017). This adsorption mechanism was started by solid particles that have been partially wetted by oil and water. Depending on the relative affinity for both phases, these particles will position themselves at the oil-water interface which is denoted by a contact angle, θ, as shown in Figure 2.4.

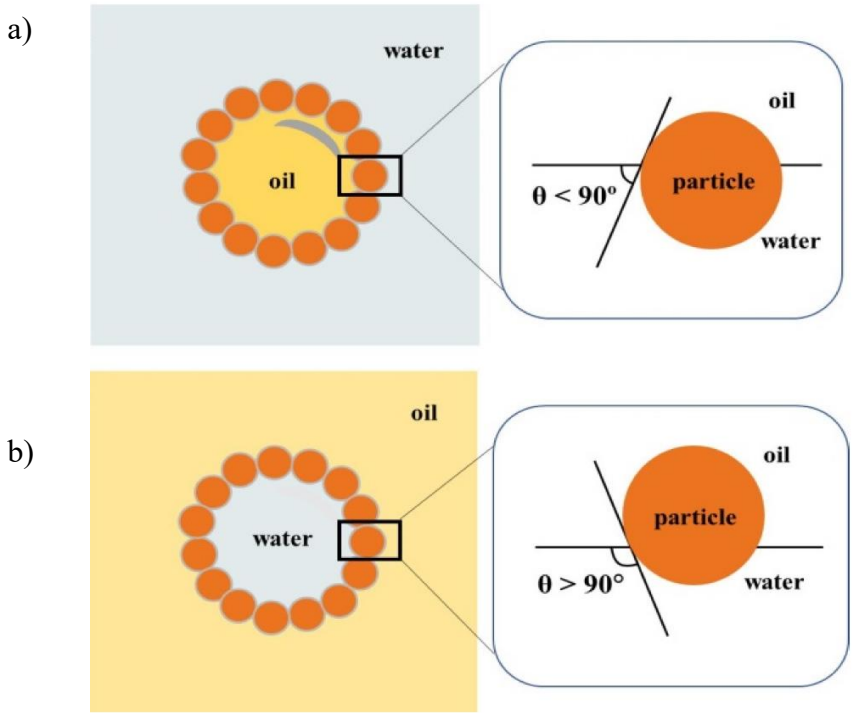

Figure 2.4 Schematic representation of adsorption of solid particles with a contact angle of a) θ < 90° showing O/W emulsion and b) θ > 90° showing W/O emulsion. Source: de Carvalho-Guimarães et al. (2022).

As a result, these particles will cause the interface to bend towards the phase that has a lower affinity in a way that particles with θ below 90° are suitable in the preparation of O/W emulsion, while θ greater than 90° indicates its suitability in forming W/O emulsions. Particles with θ = 90° showed an equal immersion in both phases (Cui et al., 2021; Santamaria-Echart et al., 2021). Nevertheless, it is reported that to emulsify a Pickering emulsion, it is best to have θ relatively closer to 90° as particles are prone to remain dispersed at low θ (too hydrophobic) or high θ (too hydrophobic), causing instability of the emulsion (Yang et al., 2017). Thus, it led to the requirement of certain particles to undergo modification using different molecules or to different degrees to make them more amphiphilic (Xu et al., 2022; Zhou et al., 2023).

2.1.2(b) Advantages over conventional emulsion

Comparing the emulsions stabilized by solid particles with surfactant-stabilized emulsions, the former is more favourable due to its greater stability against coalescence. The high physical and energy barrier provided by the irreversibly adsorbed and densely packed particle layer at the oil-water interface can prevent droplet flocculation and coalescence that reduce the creaming or sedimentation rate (Berton-Carabin and Schroën, 2015; Li et al., 2019). Consequently, the high surface energy of the oil-water interface was reduced and contributed to the emulsion stability.

In addition to this, Pickering emulsions provide a more stable response to stimuli due to the fixed position of the functional particles at the oil-water interface (Cho et al., 2018; Yang et al., 2017). This type of emulsion also offers good biocompatibility and low toxicity due to the absence of surfactant which presents greater advantages over conventional emulsions. Additionally, polymerization via Pickering emulsion was facilitated due to its lower toxicity, high mechanical strength

and controlled size which increase its preferential in various applications (Zhu et al., 2015).

2.1.2(c) Formation of emulsion using mechanical methods

Emulsification is a process of emulsion formation that involves a dynamic and non-spontaneous process whereby energy is required to form the emulsion droplets. A sufficient amount of mechanical energy is usually required to disperse one liquid in the form of small droplets in the continuous phase. Among the common methods of emulsification include high-speed homogenization by using a rotor-stator, high-pressure homogenization and sonication with an ultrasonic processor (Costa et al., 2018; Luo et al., 2021; Taha et al., 2020). Every emulsification technique comes with its own set of strengths and weaknesses. The mechanism of action as well as the advantages and disadvantages of each technique have been explained in Table 2.1.

The choice of an appropriate emulsification method depends on factors such as the volume and composition of the initial mixture, the intended physicochemical attributes of the final emulsion, and the economic considerations associated with the emulsification procedure, as outlined by Taha et al. (2020). Table 2.2 provides a concise overview of the emulsification methods used previously to form a Pickering emulsion and their effect on the emulsion droplet size. Considering its ability to produce a cavitation effect and induce smaller droplet size ($D_{3,2}$ = <10 μm) compared to other methods (as shown in Table 2.2), ultrasonication was frequently selected as the mechanical treatment in preparing a stable emulsion (Lee et al., 2019; Meirelles et al., 2020; Ramos et al., 2023).

Table 2.1 Mechanisms of action, advantages and disadvantages of mechanical methods used for emulsification.

Emulsification technique	Mechanisms	Influencing factors	Advantages	Disadvantages	References
Rotor stator homogenizer	The mixing head induces rotational, longitudinal and radial velocity slopes within the system, leading to the fragmentation of larger droplets into smaller ones	Rotating speed Homogenization time	- a small amount of liquid needed - large distribution of droplet size obtained - high shear rate occurring between the rotor and the stator - allow the preparation of large volumes of emulsion - low investment costs - quick process - ease of handling and cleaning - easy installation	- limited pumping capacity - larger droplet size obtained - high mechanical shear led to the breakdown of particle aggregates	Wu and Ma, (2016) Gazolu-Rusanova et al., (2020) Modarres-Gheisari et al., (2019)
High-pressure homogenization	Severe turbulent flows were generated by the pump that compresses a course emulsion, leading to the formation of fine emulsions with droplet sizes from 0.1 to 1 μm.	Pressure value Homogenization cycle Homogenization time	- continuous process - produce small and uniform droplets.	- high maintenance cost - require high energy - prone to droplet degradation during the process - limited function (can only produce diluted emulsions) - produce droplets with high poly-dispersity	Modarres-Gheisari et al., (2019) McClements et al. (2022) Pereira et al. (2021)

Table 2-1. Continued

Emulsification technique	Mechanisms	Influencing factors	Advantages	Disadvantages	References
Ultrasonication	Droplet disruption occurs due to the cavitation phenomenon which is a rapid formation and collapse of air bubbles in the liquid system.	Ultrasound frequency, power, temperature, Surface tension or viscosity of the medium	- droplet size of the emulsion decreases according to the sonification time - produce smaller droplet size - produce emulsion with greater stability - high energy efficiency - ease of handling and cleaning	- high energy consumption - noise and heat generation - low nanofibres yield	Taha et al. (2020)

Table 2.2 Effects of using different emulsification methods on the emulsion droplet size.

Type of emulsion	Oil type	Emulsification methods		Droplet size ($D_{3,2}$)	References
		Course emulsion	Fine emulsion		
o/w	Sunflower oil	Rotor-stator (10,000 rpm, 2 min)	High-pressure homogenizer	4 µm (Day 6)	Costa et al. (2018)
			Ultrasonication (750 W, 20 kHz)	2 µm (Day 6)	
	Dodecane	High shear blender (11,000 rpm, 4 min)	Homogenizer	10.94 µm (Day 7)	Li et al. (2019)
	Olive oil	-	High shear blender (800 W, 12,000 rpm for 2 min)	9.5 µm	Luo et al. (2021)
	Light mineral oil	Homogenizer (2000 rpm, 1 min)	Homogenizer (10,000 rpm, 1 min)	75 µm	Miao et al. (2020)
	Flaxseed oil	Rotor-stator (10,000 rpm, 3 min)	Rotor-stator (3 min, 10,000 rpm)	3.42 µm (Day 7)	Meirelles et al. (2020)
			Ultrasonication (4 min, 525 W and 20 kHz	1.21 µm (Day 7)	
	Paraffin oil	Rotor-stator (13,500 rpm, 3 min)	-	50-70 µm	Ramos et al. (2023)
			Ultrasonication (120 W, 10 min)	4-20 µm	

Table 2-2. Continued

Type of emulsion	Oil type	Emulsification methods		Droplet size ($D_{3,2}$)	References
		Course emulsion	Fine emulsion		
w/o	Corn oil	High-speed blender (20,000 rpm)	-	50-225 µm ($D_{4,3}$) (Day 7)	Zhou et al. (2023)
	Soybean oil	High speed homogenizer (13,400 rpm, 2 min)	High-pressure homogenizer (300 ± 20 bar)	13-17 µm (Day 21)	Zembyla et al. (2019)
	Dodecane	Rotor/stator homogenizer (25,000 min^{-1}, 2-5 min)	-	10-50 µm	Kempin et al. (2020)
	Dodecane	-	High-speed homogenizer (10,000 rpm, 5 min)	6 µm	Skale et al. (2016)
			Ultrasonic homogenizer (200W, 50%, 5min)	13 µm	
w/o/w	Soybean oil	High-speed homogenizer (13,000 rpm, 2 min)	High-speed homogenizer (8000 rpm, 1 min)	26.7 - 33.8 µm	Xiao et al. (2016)
	Sunflower oil	High-speed homogenizer (16,000 rpm, 10 min)	Ultrasonic homogenizer (120 kHz, 50% pulse, 10 min)	2.94-8.53 µm	Yildirim et al. (2017)
o/w/o	Isopropyl myristate (IPM) oil	Couette cell (100 µm gap, 680 rpm, 14 280 s^{-1})	-	0.7-5.1 µm ($D_{4,3}$)	Stasse et al. (2020)
	Toluene	Vortex mixer (2700 rpm for 30 s)	Vortex mixer (1000 rpm, 30 s)	150-200 µm	Guan et al., (2023)

Ultrasonic devices were reported to have better energy efficiency as compared to high-pressure homogenizers (HPH) as the former could lead to the formation of nanoemulsions through lesser consumption of electrical energy than HPH (Li et al., 2017). This could be a result of the combination of chemical and physical effects exerted on the emulsion samples by ultrasound devices during emulsification. Besides, from the table, it is noteworthy that a single step of emulsification produced a larger droplet size (low emulsion stability) compared to the utilization of course and fine emulsification steps in producing emulsion. Ramos et al. (2023) and Zhou et al. (2023) in their study recorded large droplet sizes ($D_{3,2}$ = >50 μm) when single-step emulsification using a rotor-stator and high-speed blender was used, respectively.

Ultrasound has been widely used for the emulsification of various types of emulsion (He et al., 2023; Shojaeiarani et al., 2020). This method offers several advantages over other emulsification techniques including high energy efficiency, the ability to produce emulsion with good stability and ease of handling and cleaning the device (Li et al., 2017; Taha et al., 2020). Ultrasound refers to a mechanical wave that has a frequency of more than 20 kHz. It is commonly divided into high-intensity ultrasound (HIU, 10-1000 W cm^{-2}, 20-100 kHz) and low-intensity ultrasound (LIU, <1 W cm^{-2}, 100 kHz to 1 MHz) (Bhangu and Ashokkumar, 2016). In the case of emulsification, several researchers reported that emulsification can be best prepared using HIU as it can produce stable emulsion due to the production of smaller droplet size that improves the stability of emulsion (Gul et al., 2018; Costa et al., 2018).

It is reported that the acoustic cavitation produced by the ultrasound device is the most important impact of this method on emulsion (Zhou et al., 2021). Acoustic cavitation occurs due to the formation and collapse of the air bubbles in the system and it can be used to promote chemical and physical reactions. A chemical reaction is

generated as a result of the compression of air bubbles which facilitate the release of reactive radicals while physical effects like high temperature and pressure, high-speed liquid jet, shock waves, turbulence and extreme physical shearing could be generated in the medium through the oscillation and collapse of acoustic cavitation bubbles (Ashokkumar, 2015). Physical effects are normally required for emulsification and extraction while both chemical and physical effects are needed when polymerization of emulsion is the target. By using HIU, two main steps can be used to prepare an emulsion, as summarized in Figure 2.5: 1) formation of large droplets of the dispersed phase in the continuous phase and 2) gradual disruption of the large droplets due to the continuous physical shearing and acoustic cavitation generated in the medium (Leong et al., 2018).

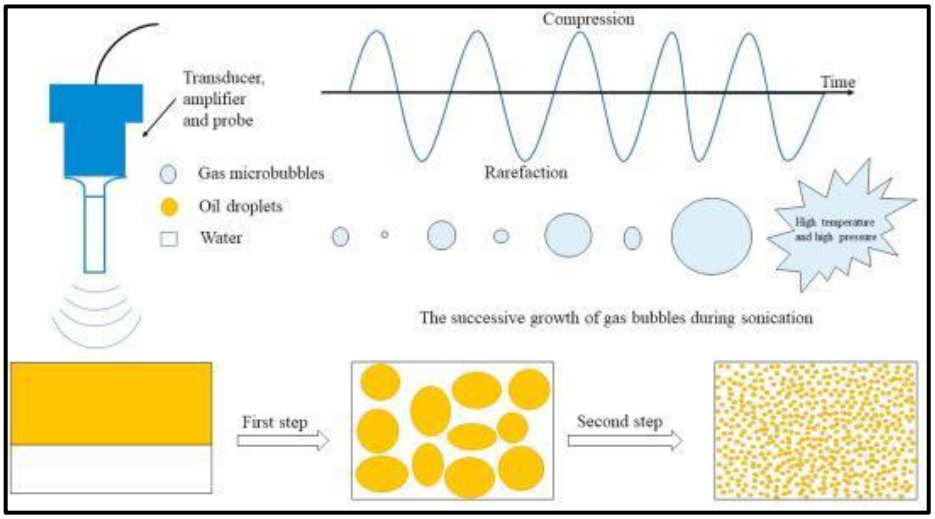

Figure 2.5 The schematic representation of the acoustic cavitation process and emulsification step using high-intensity ultrasonic (HIU). Source: Zhou et al. (2021).

2.1.2(d) Chemical demulsification of Pickering emulsion

The term "emulsion stability" typically denotes an emulsion's capacity to withstand changes in its physicochemical attributes as time progresses (de Carvalho-Guimarães et al., 2022). This equilibrium pertains to the ability of the emulsion to

sustain the stability of its physical characteristics, including shape, size, morphology, rheological properties, and others, for an extended duration. The required stability for food emulsions varies depending on the final applications. For instance, intermediate food like ice cream and cake batter requires short-term emulsion stability from minutes to hours while food with longer shelf life such as salad dressing and mayonnaise needs long-term stability.

Similar to the conventional emulsion, various mechanisms of instability such as gravitational separation (sedimentation/ creaming), flocculation, coalescence and phase separation can also be observed for Pickering emulsion as illustrated in Figure 2.6. To evaluate the quality of the Pickering emulsion, several factors are accessed, including droplet size distribution, the morphology of emulsion phase droplets (via optical microscopy), wettability, rheological properties, shear stress, apparent viscosity, and stability after the centrifugation step was subjected (de Carvalho-Guimarães et al., 2022).

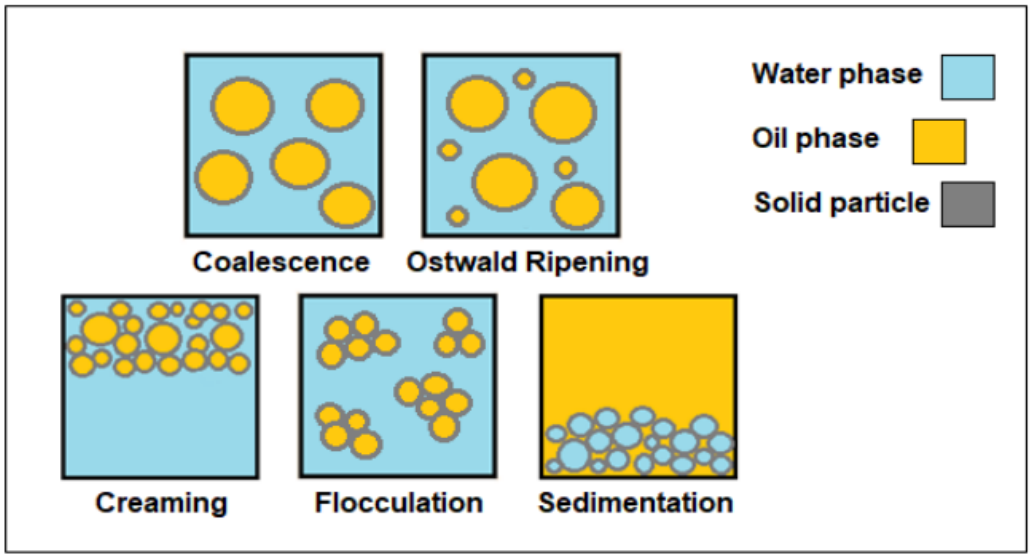

Figure 2.6 Mechanisms of physical instability of Pickering emulsion. Source: Teo et al. (2022).