# EXTRACTION OF NATURAL PIGMENTS FROM FRUITS AND VEGETABLES WASTES TO REDUCE FOOD WASTE PROBLEM

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# EXTRACTION OF NATURAL PIGMENTS FROM FRUITS AND VEGETABLES WASTES TO REDUCE FOOD WASTE PROBLEM

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**A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Chemical Engineering**

**Lee Kong Chian Faculty of Engineering and Science**
**Universiti Tunku Abdul Rahman**

**May 2022**

# DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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**APPROVAL FOR SUBMISSION**

I certify that this project report entitled **"EXTRACTION OF NATURAL PIGMENTS FROM FRUITS AND VEGETABLES WASTES TO REDUCE FOOD WASTE PROBLEM"** was prepared by **WONG KWONG LONG** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Chemical Engineering at Universiti Tunku Abdul Rahman.

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# ABSTRACT

Increased food wastage problem during COVID-19 pandemic, limitations on the conventional food waste management approaches and consumers' concerns on artificial colourants have induced the urge to recover valuable components from food waste as an approach for food waste valorisation practice. Therefore, recovery of natural pigments from fruits and vegetables wastes was introduced to address these issues. This study aims to analyse the factor that affects the stability of the pigments and compare the novel extraction techniques and determine the possibility of implementing green extraction techniques. The targeted pigments include water soluble pigments (anthocyanins and betalains) and lipid soluble pigments (carotenoids), whereas the novel extraction technologies being compared are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed-electric field (PEF) extraction, supercritical fluid extraction (SFE) and enzyme-assisted extraction (EAE). The stability assessment of the pigments consists of four main factors; pH, temperature, light exposure and oxygen concentration. The outcomes of stability assessment showed that anthocyanins were highly sensitive to pH change, betalains were thermolabile and carotenoids were highly destructed under strong light exposure and oxygen-rich atmosphere. The results of comparison between the novel extraction techniques have manifested that PEF extraction was the best technique used to recover water soluble pigments due to its highest extraction yield of anthocyanins and betalains, non-thermal approach and insignificant environmental impacts. In contrast, SFE exhibited the highest potential in extracting lipid soluble pigments due to its non-polar nature, greatest extraction yield of carotenoids and created no environmental impacts owing to the fact that supercritical carbon dioxide is a green solvent. To achieve the best extraction yield, sufficient induction time should be established for PEF extraction and adequate static and dynamic extraction time should be allocated for SFE. The outcomes of the green assessment have manifested that MAE, UAE, PEF and EAE could employ green extraction by using green solvent such as oil and water to replace the use of organic and petroleum-based solvents.

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| | |
|---|---|
| $T_c$ | critical temperature, K |
| $p_c$ | critical pressure, atm |
| | |
| COVID-19 | Coronavirus disease |
| FAO | Food and Agriculture Organization |
| TLC | Thin-layer Chromatography |
| HPLC | High Performance Liquid Chromatography |
| FTIR | Fourier-Transform Infrared |
| MAE | microwave-assisted extraction |
| MHG | microwave hydro-diffusion and gravity |
| NPMAE | nitrogen-protected microwave-assisted extraction |
| VMAE | vacuum microwave-assisted extraction |
| UAE | ultrasound-assisted extraction |
| PEF | pulse-electric field |
| SFE | supercritical fluid extraction |
| EAE | enzyme-assisted extraction |

# CHAPTER 1

# INTRODUCTION

## 1.1 General Introduction

During the COVID-19 epidemic, food waste, particularly household food waste, was reported to have increased significantly. This was the consequences of the movement restrictions, consumers' behaviour on buying food such as panic buying and food hoarding and the inappropriate management for food waste during the COVID-19 pandemic. The Food and Agriculture Organization (FAO) claimed that the largest percentage of food waste in 2020 was generated from fruits and vegetables, accounted for 45%, followed by meat, seafood and dairy for 35% and cereals for 30% (Seberini, 2020).

The conventional food waste management has overlooked the potentials for these food wastes to be valorised and converted into enhanced value products like natural pigments, enzymes, concentrates, as well as to be applied in the generation and recovery of energy through treatment like incineration and anaerobic digestion. Various types of foods, on the other hand, have different shelf lifes. Ndraha, et al. (2020) claimed that fruits and vegetables have a longer shelf life than other perishable food like meats, seafoods and dairy as bacteria growth could happen quickly in perishable food if they are not stored properly. As a result, perishable foods that have a shorter shelf life will get spoiled and decayed easily, making them unfit to be consumed. This statement is supported by the Shelf-Life Guide published by the Los Angeles Regional food bank. According to the Shelf-Life Guide, frozen fruits and vegetables normally have a shelf life of 8 months whereas frozen fish and meat have a shelf life of 2 months (Los Angeles Regional food bank, 2018). This serves as an indication that fruits and vegetables have a higher potential for being valorised in the production of highly value-added products compared to meat and seafood wastes which cannot withstand chemical and microbial spoilage.

In the era of green technology, reduction of food wastes is a significant consideration. As a result, various researchers have proposed

different solutions to minimise the food waste problem by finding a balance between the traditional food waste management and the emerging food waste valorisation techniques. Jainudin, et al. (2017) suggested that the concept of green technology is to develop and apply products, equipment and systems to conserve the environment in the attempts to minimise the negative impacts on environment from human activities. Conventional food waste management is different with the valorisation techniques. In conventional food waste management, landfilling, animal feeding, composting and thermal treatment such as incineration were the common approaches opted. The concept of conventional food waste management contradicted the concept of green technology where no valuable products are recovered from the treatment process. In contrast to conventional treatments, emerging valorisation techniques involved extraction, chemical conversion, biological conversion, and synthesis of other materials, which matched the goals of green technology (Esparza, et al., 2020).

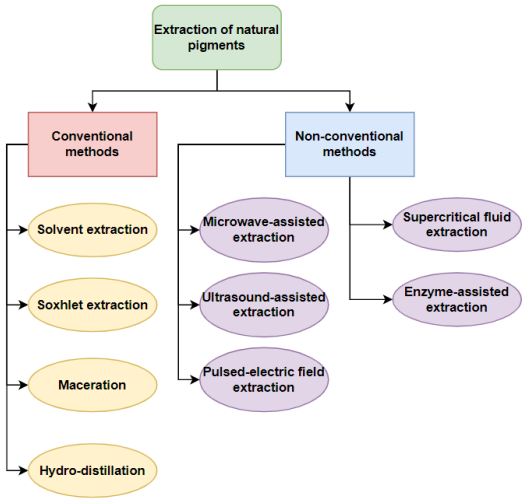


Figure 1.1: Conventional and non-conventional extraction techniques of natural pigments.

The valorisation approaches can synthesis valuable products as well as can conserve the environment. One of the most common valuable products that can be extracted from the fruits and vegetables wastes is the natural pigments. There are four major types of natural pigments, which are anthocyanins, betalains, carotenoids and chlorophylls. For different types of

natural pigments, there are different extraction methods available, such as conventional extraction technologies and non-conventional extraction technologies, as illustrated in Figure 1.1. However, characterization of the extracted natural pigments is essential to ensure its quality to be used as natural colourants for different industries. Besides, the factors that affect the stability of extracted pigments need to be investigated in order to preserve the extracted pigments by employing the suitable pre-treatments and post-treatments (Sharma, et al., 2021).

Natural pigments have found the niche market for different technological applications. For instance, natural pigments have been utilised in food processing industries, cosmetic industries, pharmaceutical industries, and textile industries (Muhamad, et al., 2018). This niche market of natural pigments existed because of the awareness of consumers and the increased concerns about the potential adverse health effects and environmental damage caused by artificial colourants (Luzardo-Ocampo, et al., 2021). Therefore, there are considerations to replace the use of artificial colourants by natural colourants as the application of artificial colourants disobeyed the principle of green technology as it was found that artificial colourants will create health impacts on humans as well as negative impacts on the environment. For instance, textile dyes which are composed of synthetic or artificial colourants are toxic in nature, when the wastewater that contains the textile dyes was discharged from the factory, it might trigger water pollution and this poses a challenge to environmentalists to handle the wastewater (Manzoor and Sharma, 2019).

To wrap out, COVID-19 pandemic triggered the significant increment in food wastes generation, and the emerging valorisation approaches should be employed to recover valuable products from food wastes such as natural pigment because natural pigment has the potential to replace the use of artificial colourants.

## 1.2 Importance of the Study

It was found that the extraction of natural pigments from fruits and vegetables wastes was efficient in reducing the increased food wastes problem created during the COVID-19 pandemic. This is because the

extraction of natural pigments is not necessary to be conducted at industrial scale or laboratory scale but can be done at home. Thus, this paper can help to educate the public to conduct extraction of natural pigments such as maceration that can be done at home. Apart from that, this study also highlights the importance of applying natural colourants to replace the use of synthetic colourants in different industries especially food processing industry, cosmetic industry, and textile industry to reduce the impacts on human health and environment. Hence, it is important to identify the extraction of natural pigments from fruits and vegetables to bring down the food waste problem as well as to minimise the use of synthetic colourants in different industries.

## 1.3 Problem Statement

It was reported that the food waste problem has increased by approximately 12% during COVID-19 pandemic (Das, et al., 2021). As consequences of panic buying practice, food hoarding and stockpiling ignited by the fear of scarcity among consumers due to the outbreak of COVID-19 pandemic, consumers tend to buy groceries, grains, fishes, meats in a large proportion. This phenomenon has contributed to the increase of food waste problem as food had shelf life especially for perishable food such as fishes, meats, vegetables, and fruits that have shorter shelf life (Wang and Hao, 2020). Therefore, a possible solution was developed to valorise the food waste to reduce the food waste problem. For example, the fruit peels of fruit wastes could be potential raw materials that served for natural pigment extractions, which served as a better solution as compared to landfilling options.

In the second place, conventional food waste treatment involved animal feeding, landfilling, composting and thermal treatment. However, the conventional practices did not recover the valuable components from the food waste (Esparza, et al., 2020). It is important to take into consideration the valorisation approaches which can be applied in the recovery of value-added products and the remaining waste can be treated conveniently. These valorisation practices were gaining high attention from economic, environmental, and natural resources consumption issues associated with their profitability and sustainability. To address this issue, a possible solution

was suggested to apply a valorisation approach such as extraction technology to recover valuable products like pigments instead of practising the conventional food waste treatment.

Not least for all, it was claimed that artificial food colorings are hazardous and dangerous to consumers as it could potentially trigger health impacts, and thus there were parties calling for the government to ban the common dyes used in food colorings processing (Dafallah, et al., 2015). There were studies proven that consumers who consumed more artificial colored food manifested more risks of having health problems like sleeplessness, irritability, hyperactivity, memory loss, depression and severe cases reported that some inorganic pigments were carcinogenic (Okafor, Obonga and Ezeokonkwo, 2016). In the attempts to minimise the side effects triggered by artificial colourants, a possible mitigation was established to replace artificial food colorings with natural pigments.

## 1.4 Aim and Objectives

The main aim of this study is to explore and compare the available extraction technologies of natural pigments from fruit and vegetable wastes. The specific objectives of this study include:

i. To study the characteristics of targeted natural pigments.
ii. To analyse the factors that affect the stability of the natural pigments.
iii. To compare the novel technologies available for the extraction of natural pigments from fruits and vegetables wastes.
iv. To determine whether green extraction can be employed for the extraction methods.

## 1.5 Scope and Limitation of the Study

In this study, the first scope is to define the targeted pigments and their respective characteristics. The characteristics of the targeted pigments, conventional and novel pigments extraction techniques will be identified through literature review. Before the comparison between the novel pigments extraction methods is carried out, the factors that affect the stability of the

recovered natural pigments will be evaluated. In closing, green assessment of the pigment extraction techniques will be conducted to determine whether that particular extraction method fulfils the standards and requirements of green extraction.

In the meanwhile, there are several limitations for this study that should be taken into consideration. First of all, the targeted pigments include anthocyanins, betalains and carotenoids only, other pigments will not be considered to narrow down the scopes of the study. In the second place, the extraction of pigments will be analysed from fruits and vegetables wastes only, other food wastes such as meat, seafood or cereals will not be taken into consideration. Furthermore, only the novel extraction methods will be compared, whereas the comparison between the conventional extraction methods will not be performed owing to the fact that it was no point to compare the conventional extraction methods as these methods are less frequent to be used nowadays. Instead, the comparison between the novel extraction techniques is an issue that is gaining attention, especially green novel extraction to fulfil the concepts of green extraction. Aside from that, the main factors that affect the stability of the pigments will only include pH, heat, light and oxygen, other factors will not be considered. Not least for all, this is a review based paper where all the information is based on previous research studies conducted and therefore some of the information may not be readily available. Thus, there will be some own analysis and personal judgements to be included in this study.

## 1.6 Contribution of the Study

Recovery of natural pigments from fruits and vegetables wastes was proposed to serve as a valorisation approach that reduce the food waste problem and urge to replace the use of hazardous artificial colourants in different industries. In this study, the parameters that determine the efficiency of the extraction technique such as stability of the recovered pigments, extraction yield, extraction temperature, extraction time, complexity of separation process, quality of recovered pigments and green assessment of the extracted pigments were discussed to figure out the best extraction technique and optimum extraction condition (such as pigments' thermal

degradation temperature, pH, presence of light, oxygen concentration) for water soluble and lipid soluble pigments. This contributed to the future experimental studies in extracting natural pigments from fruits and vegetables samples, to minimise the wastage of energy and a better protocol can be established before the experiment is carried out. For example, an individual can gain a clear understanding to determine the most appropriate extraction technique to be used on the targeted pigments to be recovered by knowing the characteristics of the targeted pigments in the literature review, and propose the best technique based on the outcomes of analysis of results and discussions in this study.

## 1.7 Outline of the Report

As the first outline of this study, a general introduction on the food waste problem, natural pigment and its current demand and side effects of artificial colourants are highlighted in Chapter 1. In the meanwhile, the food waste problem, types, sources and characteristics of the targeted natural pigments, conventional and novel pigments extraction technologies are reviewed in Chapter 2. The methodology of this review paper is discussed in Chapter 3. In Chapter 4, stability assessment of the natural pigments is conducted to aid the decision in selecting the most appropriate extraction approach for that particular approach. Apart from that, comparison between the novel extraction technologies is made based on extraction yield, extraction temperature, extraction time, complexity of the separation process involved, and the quality of the extracted pigments. Not least for all, green assessment is carried out to examine whether the extraction techniques used fulfils the concepts of green extraction. At the end, conclusion on the study is made with some recommendations provided in Chapter 5.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Review on the food waste problem

In the 21st century, the food waste problem has gained momentum and raised the attention of different parties. The Food and Agriculture Organization (FAO) declared that one third of the world's food was degraded or discarded. The figures of food waste were extremely significant, contributing for 1.3 billion tons per year and incurred a loss of approximately 990 billion USD (Seberini, 2020). There were two important terms in the discussion of the food waste problem, which are food loss and food waste. Food loss could be defined as the unintentionally reduction or deterioration in the quality or quantity of food whereas food waste could be interpreted as any food or inedible parts of food that are removed from the food supply chain that undergo recovery or being disposed (Jain, et al., 2018).

Table 2.1 showed the composition and percentage of food waste generated all around the world in 2020. The conventional practices to treat the food wastes include composting, anaerobic digestion, bio-energy production, co-generation, incineration, landfilling, discarded to sea and valorisation (Aschemann-Witzel, et al., 2015). However, it was claimed that these conventional food waste practices neglected the potential of food wastes that could be valorised to generate valued products (Carpentieri, et al., 2021).

Table 2.1: Composition and percentage of food waste generated all around the world in 2020 (Seberini, 2020).

| Types of food waste | Percentage (%) |
|---|---|
| Fruits and vegetables | 45 |
| Meat, seafood, dairy | 35 |
| Cereals | 30 |

Food hoarding was found to be prevalent during the COVID-19 pandemic. Thus, the food waste problem was reported to have increased significantly during COVID-19 pandemic, especially for household food

waste. This was due to the panic buying and food hoarding behaviour of consumers, which distorted the regular market because of the emptying action of stores and shelves as well as creating panic to the society. In this scenario, food and other household essentials were on the top of consumers' buying lists (Wang and Hao, 2020). A conclusion was drawn in which the COVID-19 pandemic triggered the movement restrictions of people, shutdown of food production facilities, constricted food trade policies, changes in the behaviour of consumers and creating financial pressures on the food supply chain.

Therefore, strategies and approaches should be developed to improve the Food Supply Chain during COVID-19 pandemic. In order to enhance food security and improve the food supply chain efficiency, adequate food loss and waste (FLW) management was introduced. Valorisation of food waste such as extraction of natural pigments from fruits and vegetables wastes was one of the approaches applied in FLW management (Aldaco, et al., 2020).

## 2.2 Natural pigments

Pigments could be defined as compounds that produce colours and present in all living organisms or matter which were important in the development of organisms apart from providing attractive colours (Shetty and Geethalekshmi, 2017). In general, pigments can be classified by their origin, the chemical structure of the chromophore and the structural characteristics of the pigments (Swami and Ghgare, 2020).

The origin of pigments could be categorised as natural, synthetic, organic, or inorganic. Natural pigments were normally derived from the kingdoms of plants, animals or even fungi as well as from some simplest prokaryotic organisms such as cyanobacteria whereas synthetic pigments could be obtained from laboratories' works, and inorganic pigments like titanium oxide and metallic pigments are obtained from natural minerals or ores (Mortensen, 2006). Figure 2.1 depicted that natural pigments could be derived from plants (fruits, vegetables, and flowers) and microorganisms (bacteria, fungi, and algae), however this study only focused on the extraction of natural pigments from fruits and vegetables.

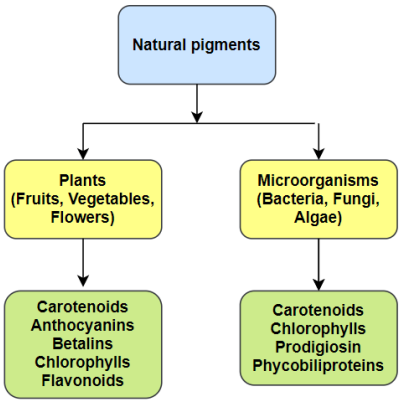


Figure 2.1: Sources of natural pigments (Muthusamy, et al., 2020).

The second criterion used to classify pigments was the chemical structure of the chromophore. Chromophores are chemical groups in molecules or atomic configurations that are responsible to alter the energy in the delocalised systems (Carle and Schweiggert, 2016). Owing to the fact that chromophore configurations often existed as multiple units, having conjugated double bonds due to the interaction between the double bonds, partial delocalisation of the electrons were induced in the bonds. As a result, the colour of the pigments appears when a molecule absorbs, transmits or reflects others energy when the chromophore undergoes conformational change when hit by light or radiation (Rajesh, et al., 2017). Thus, different atomic configurations of chromophores containing different electron-conjugated systems would give different colours, which aided the categorization of natural pigments by using the colour differences (Shetty and Geethalekshmi, 2017).

### 2.2.1 Types of natural pigments

Natural pigments could be categorised into four major groups, which are anthocyanins, betalains, carotenoids and chlorophyll. However, only three main pigments were reviewed in this study, including anthocyanins, betalains and carotenoids. There were two systems discovered by scientists to classify natural pigments. In the first place, natural pigments could be sorted based on the topics of structural affinities. Under this classification, natural pigments could be further categorised into six structural classes, which

involved quinones, tetrapyrroles, tetra-terpenoids, metallo-proteins, *N*-heterocyclic and *O*-heterocyclic.

The second classification system was based on the natural occurrence of the natural pigments such as physical characteristics like colour, odour and solubility. On the other hand, chemical properties such as the presence of certain phytochemicals in the natural pigments, pH, and acidity could be applied in differentiating the pigments (Priya and Preetha, 2016). Figure 2.2 illustrated the different types of natural pigments that could be recovered from fruits and vegetables wastes that fall under the three major groups of natural pigments.

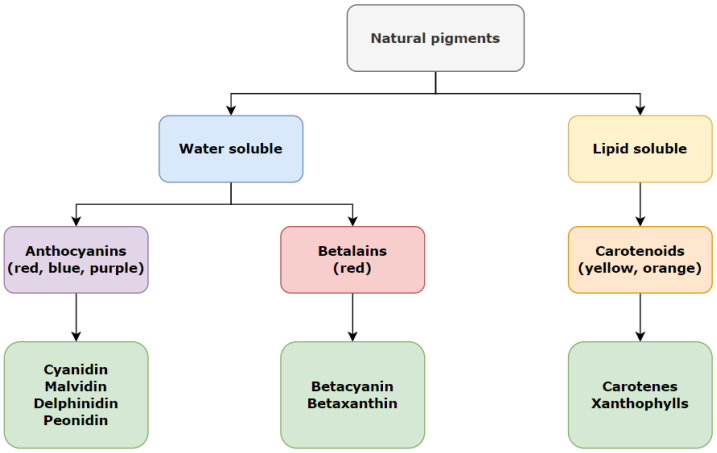


Figure 2.2: Types of natural pigments derived from plants (Sharma, et al., 2021).

### 2.2.2 Sources of natural pigments

As this study focused on the production of natural pigments from fruits and vegetables wastes, the sources of natural pigments were mainly identified from fruits and vegetables. Table 2.2 outlined the types of natural pigments that could be derived from the sources of fruits and vegetables wastes.

Table 2.2: Sources of natural pigments from fruits and vegetal waste (Shetty and Geethalekshmi, 2017).

| Natural pigments | Sources of natural pigments |
|---|---|
| Anthocyanins | Blackberry residues, apple peels, grape pomace, banana peel, eggplant peels, cherry skins, purple potato peels. |
| Betalains | Dragon fruit skins, Beetroot peels, prickly pears peels. |
| Carotenoids | Tomato peels, pomegranate peels, orange peels, carrot peels, pumpkin seeds, paprika leaves, sweet potato wastes. |

### 2.2.3 Characteristics of natural pigments

The characteristics of natural pigments were the important factors that determine the biological properties and commercial activities of the natural pigments like pharmacological activities, food industries, textile industries, cosmetic industries, and other applications in industries (Basri, et al., 2021).

Figure 2.3 outlined that the characteristics of natural pigments could be subdivided into physical properties and chemical properties. Under physical properties, parameters such as colour and solubility were applied to define the characteristics of the pigments. In the meanwhile, chemical properties like the structural formula, structural affinities and arrangement of functional groups were used in the classification for chemical properties.

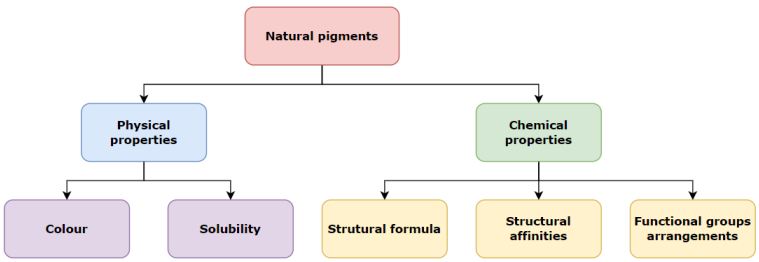


Figure 2.3: Characteristics used to define the natural pigments.

#### 2.2.3.1 Physical properties

Physical properties of the natural pigments could be categorised into colour and solubility. In order to differentiate the types of natural pigments, Sharma, et al. (2021) suggested using colour and solubility to define the physical properties of the natural pigments. Table 2.3 summarised the colour and solubility examined for anthocyanins, betalains and carotenoids pigments.

Table 2.3: Natural pigments and its physical properties (Sharma, et al., 2021).

| Natural pigments | Physical properties | |
|---|---|---|
| | Colour | Solubility |
| Anthocyanins | Red, blue, purple | Hydrophilic |
| Betalains | Red, violet, yellow, orange | Hydrophilic |
| Carotenoids | Yellow, orange, red | Lipophilic |

Colour was the most obvious physical property that can be observed from the physical appearance of the natural pigments. It was disclosed that the basic C6-C3-C6 anthocyanin structure was the main reason that colours were produced and the process involved chemical combination with glycosides and acyl groups (Delgado-Vargas, et al., 2000). Priya and Preetha (2016) provided further justifications for the colour difference in anthocyanins was caused by the structural differences in the hydroxyl groups, the number and the position of the sugar moieties in the anthocyanins pigments. Apart from anthocyanins, the colour of betalains was attributed by the reasoning double bonds in their chemical structure, conjugation of a substituted aromatic nucleus to the chromophore shifts the absorption maximum wavelength from 480.00 nm in yellow betaxanthins to 540.00 nm in red-purple betacyanins (Delgado-Vargas, et al., 2000), thus giving betalains a range of colour. Regarding on the topics of colour for carotenoids, there were published studies proposed that carotenoids were involved in photosynthesis as it would participate in the energy transfer in the presence of chlorophyll in plants, and thus yellow colour can be found in different types of fruits and vegetables (Mortensen, 2006).

Solubility of the natural pigments was extremely important in proposing the most appropriate extraction techniques for the natural pigments

as conventional extraction methods involved the use of the solvent such as water, alcohol or other organic solvents that considered the solubility of the pigments, which utilised the “like dissolve like” rules (Tena and Asuero, 2022). It was found that anthocyanins were highly soluble in water owing to the fact that anthocyanins were accumulated in the plant vacuoles that contained large amounts of water (Burton-Freeman, Sandhu and Edirisinghe, 2016). Same as anthocyanins, betalains was reported as water-soluble nitrogen containing pigments as it also presented in the vacuoles of plants (Sadowska-Bartosz and Bartosz, 2021). Unlike anthocyanins and betalains, carotenoids were lipophilic in nature due to the presence of long unsaturated aliphatic chains in the structure of carotenoids (Tapiero, Townsend and Tew, 2004).

### 2.2.3.2 Chemical properties

The chemical properties of the natural pigments could be viewed from the aspects of structural formula, structural affinities and arrangement of functional groups in that particular pigment.

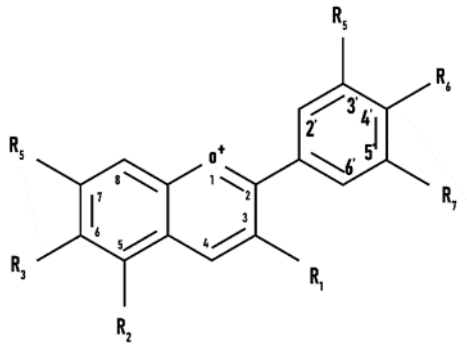


Figure 2.4: The basic structure of anthocyanins pigments (Enaru, et al., 2021).

Figure 2.4 illustrated the basic structure of anthocyanin pigments, in which the $R_x$ group could be replaced by H, (hydroxyl group) OH or (methyl ether/ methoxy group) $OCH_3$, depending on the types of anthocyanin pigments present. The basic structure of anthocyanins was normally known as glycosides of anthocyanidins, which was also called as flavylium cation where the term anthocyanidins were anthocyanins without sugar molecules

(Khoo, et al., 2017). In short, different structures of anthocyanins could exist based on different numbers of hydroxyl groups, methoxy groups, glycoside, and degree of acylation.

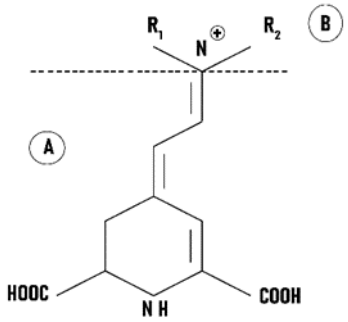


Figure 2.5: The basic structure of betalains pigments (Herbach, Stintzing and Carle, 2006).

Figure 2.5 displayed that there were two major parts found in betalain general formula, a water-soluble nitrogen containing pigment, which are part (A), betalamic acid moiety was present in all betalain molecules, whereas part (B) was the structure which determined whether the betalains would be classified as betacyanin or betaxanthin, depending on $R_1$-*N*-$R_2$ moieties arrangement (Delgado-Vargas, et al., 2000). Thus, betalains were normally known as immonium conjugates of betalamic acid with cyclo-DOPA (cyclo-3,4-dihydroxyphenylalanine residue) and amino groups like amino acids, amines, or other derivatives. The core structure of betalain was represented by the protonated 1,2,4,4,7-pentasubstututed 1,7-diazaheptamethin system, which played an important role in the chromophore configurations. Therefore, betalains classification was commonly based on the substitution of the sugar moieties arrangement (Carrillo-López and Yahia, 2017). Unlike anthocyanins, betalains are indole-derived compounds, which are composed of a benzene ring fused to a five-membered nitrogen containing pyrrole ring. In addition, studies have shown that betalains will never co-occur in plants with anthocyanins (Swami and Ghgare, 2020). Therefore, it made a clear representation that anthocyanins could be differentiated from betalains.

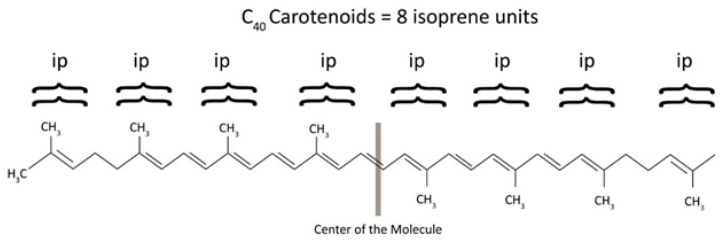


Figure 2.6: The basic structure of carotenoids pigments (Dhan and Charu, 2014).

Figure 2.6 depicted the basic structure of carotenoids pigments. As seen in Figure 2.6, carotenoids were lipophilic isoprenoid compounds, which produced by tail to tail linkage of two $C_{20}$ geranylgeranyl diphosphate molecules, and this will formed a parent $C_{40}$ carbon skeleton, consisted of 40 carbon atoms with eight isoprenoid units (Swami and Ghgare, 2020). It could be observed that the carotenoid backbone contained eight isoprenoid units which are linked covalently to create a symmetrical polyene chain with the presence of multiple conjugated double bonds (Giuliani, Cerretani and Cichelli, 2015). The most notable and distinct features in the carotenoid structures include the reversed order tail to tail linkage at the centre of the molecule and the basic linear and symmetrical skeleton can be modified via different chemical reactions such as cyclization, hydrogenation, dehydrogenation, oxidation, migration of double bonds, chain shortening or extension, which results in a wide array of different structures (Rodriguez-Amaya, 2018). Oxygen fixation, the presence or absence of oxygen atom, was important in the structure of carotenoids because the arrangement of hydroxyl groups gave rise to different structure of isomers, which are *trans*-isomers and *cis*-isomers due to the position of hydroxyl groups. Therefore, carotenoids can be classified into carotenes and xanthophylls depending on the oxygen fixation and the position of hydroxyl groups (Muthusamy, et al., 2020).

## 2.3 Extraction pathways of natural pigments

In the recovery of natural pigments from fruits and vegetables wastes, there was a series of procedures that involved, as depicted in Figure 2.7.

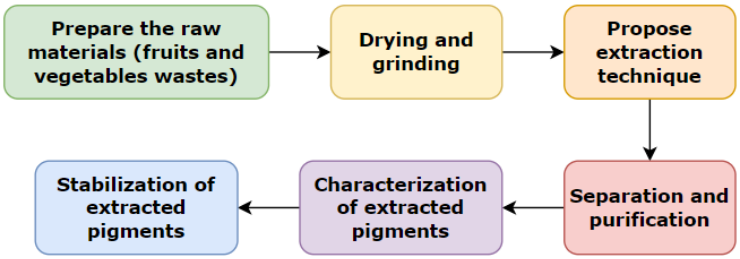


Figure 2.7: Extraction pathways of natural pigments.

The raw materials, which referred to the fruits and vegetables wastes, were prepared first. The next procedure was to dry and grind the materials with the main objectives to create a homogeneous mixture that maximised the contact surface area during the extraction process and remove excessive moisture from the materials to prevent spoiling (Hagerthey, William Louda and Mongkronsri, 2006). The third step was to propose the most appropriate extraction technique for the pigments, considering the nature of the pigments to be extracted. For instance, if water soluble pigments were to be recovered, the solvent used in the extraction process should exhibit excellent polar characteristics to obey the “like dissolve like” rules.

Separation and purification process was the next stage of the extraction pathways. The most commonly utilised techniques include adsorption chromatography, Thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) to isolate the recovered pigments from other substances like solvents, fruits and vegetables waste matrices and bioactive components (Hasbay and Galanakis, 2018). Characterization of natural pigments will be carried out after the separation and purification stage in order to identify and classify the recovery pigments. Frequently used methods in the characterization tests consisted of ultraviolet (UV) spectroscopy and fourier-transform infrared (FTIR) spectroscopy (Yusuf, Shabbir and Mohammad, 2017). As the final stage, stabilisation of the extracted pigments will be conducted to preserve the natural pigments. Wang, et al. (2018) suggested that encapsulation technique was the best approach to store and stabilise the extracted pigments.

## 2.4 Conventional extraction techniques

Conventional extraction techniques referred to the traditional methods utilised in the recovery of natural pigments. Basri, et al. (2021) claimed that conventional extraction technologies often employed thermal operations to recover the targeted pigments. These approaches involved high temperatures, long extraction times and large amount of solvent was required, which was inefficient in the recovery process. Therefore, Manzoor, et al. (2021) claimed that modern extraction approaches such as green extraction technologies were proposed to cope with the limitations of conventional pigments recovery technologies. Chapter 2.4 outlined the approaches utilised in the conventional extraction techniques, including solvent extraction, Soxhlet extraction, maceration and hydro-distillation.

### 2.4.1 Solvent extraction

Depending on the nature and chemical structure of the natural pigment, the selection of solvent was a critical factor as the polarity and the solubility of the solvents should be considered as pigments exhibited different solubility and polarity (Muhamad, et al., 2017). Solid to liquid extraction or liquid to liquid extraction were normally involved in the solvent extraction techniques (Moldoveanu and David, 2015). Solvent extraction involved the use of solvent that provides physical carriers to transfer the molecules in different phases such as solid, liquid and vapour. In solvent extraction, mixing of two phases occurs, referring to the solvent used and the plant matrices involved. The solute in the plant matrices was the targeted pigment to be extracted would come into contact with the solvent until an equilibrium condition was reached (Kultys and Kurek, 2022).

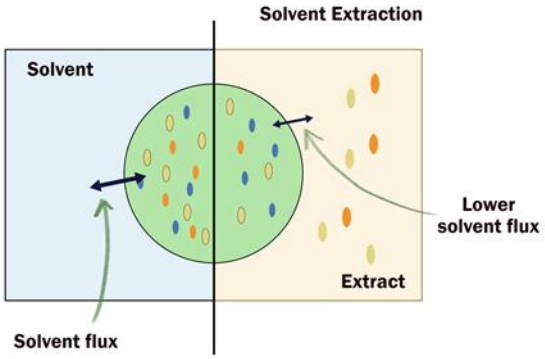


Figure 2.8: Mechanisms involved in solvent extraction (Pagels, et al., 2021).

Figure 2.8 depicted the transfer of pigments from the plant matrices to the solvent, driven by chemical affinities induced by the actions of solvents. As illustrated in Figure 2.8, the organic solvent molecules will be absorbed on the cell wall, triggering the rupture and release of pigments from the cell matrices. The organic solvent will then penetrate into the solid matrix in the plant cells, and the pigment molecules will be dissolved in the solvents. The dissolved pigments together with the solvents were called extract, and will be collected and subjected to downstream separation and purification process to recover the pigments from the extract (Hasbay and Galanakis, 2018).

There were several important factors involved in the solvent extraction process, which include the particle size of the solute, the type of solvent used, the operating temperature and the presence of agitation (Prado, et al., 2015). In order to improve the extraction efficiency and shorten the extraction time, sufficient stirring or mixing can be performed to ensure the mass transfer rate in the solvent extraction process (Gupta, et al., 2019).

The main drawback of solvent extraction was the use of organic solvents for the recovery of pigments that are normally toxic in nature, which will trigger environmental pollution associated with safety hazards (Puri, Sharma and Barrow, 2012). For example, the most commonly used solvent like methanol was flammable and toxic in nature, improper discharge of the solvent will create subsequent impacts to human beings and the environment. Therefore, it was urged to replace the use of organic solvents by implementing the use of green solvent, which could minimise the environmental impacts (Panja, 2018).

### 2.4.2 Soxhlet extraction

Soxhlet extraction was a solid to liquid extraction approach to recover pigments by using thermal nature similar to conventional heating extraction (Basri, et al., 2021). Sagar, et al. (2018) provided further justifications on the suitability of Soxhlet extraction in recovering compounds with low solubility from a solid mixture, indicating that Soxhlet extraction was highly suitable for solid to liquid extraction as mentioned by Basri, et al. (2021). Figure 2.9 depicted a typical Soxhlet apparatus for Soxhlet extraction.

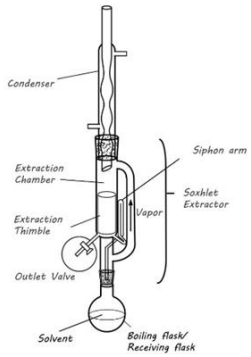


Figure 2.9: Soxhlet apparatus for Soxhlet extraction (Basri, et al., 2021).

By using the heat from the distillation flask, the Soxhlet extraction could maintain a relatively high extraction temperature, and thus this technique was not applicable for thermolabile compounds, especially heat-sensitive pigments such as betalains. In contrast, this technique was highly suitable for the recovery of pigments made up of lipid structures such as carotenoids (Soquetta, Terra and Bastos, 2018).

The main limitations of Soxhlet extraction were the long extraction time and huge consumption of solvent. Therefore, selection of solvent was a critical factor in Soxhlet extraction. It was suggested that the selection of solvent was based on the increasing polarity order such as the order of acetone, ether, ethyl acetate, chloroform, methanol, ethanol and water. Among all the solvents proposed, methanol was claimed to be the most often used solvent due to its semi-polar properties which are able to extract large numbers of pigments and suitable for recovering both hydrophilic and hydrophobic pigments (Kim, Choi and Chung, 2012).

### 2.4.3 Maceration

As one of the conventional pigments extraction techniques, maceration was applied to recover pigments by placing the samples in a liquid or solvent inside an airtight container for a varied duration depending on the types of samples and the solvents used (Muhamad, et al., 2017). Before maceration was conducted, the samples must be grinded into fine particles to increase the surface area during the extraction process. The second step was to add an appropriate quantity of solvent namely menstruum into the container or vessel. Menstruum was a liquid or viscous agent that used to extract constituents from plants through maceration. When the liquid turned colourless, this indicated that maceration was completed and the next stage will be the separation stage by discarding the menstruum with the recovered pigments. After the extraction solvent was discarded, the residues were pressed with mechanical means or centrifugal force to get the extracted pigments and the last step is to filter the pressed extraction solvent to remove impurities (Sagar, et al., 2018). Figure 2.10 depicted the procedures involved in the maceration process.

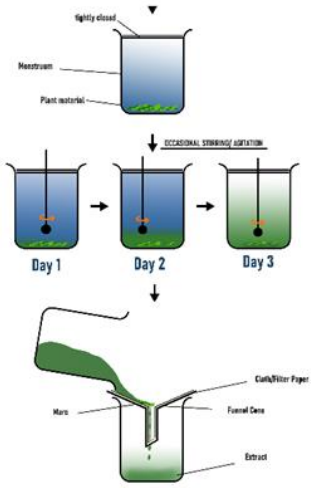


Figure 2.10: Maceration to extract natural pigments from plants (Muhamad, et al., 2017).

During maceration, variables such as sufficient agitation, stirring or occasional shaking were needed to enhance the maceration rate. Apart from

mechanical means such as agitation and stirring, maceration could be performed at room temperature and thus there was no need for any heating actions. Therefore, this approach was appropriate for heat sensitive pigments such as betalains. The advantages of maceration included low costs, simplicity of the technique and could be performed at room temperature. However, the demerits of this method include the long maceration period, the need to replace a large amount of extraction solvent and the need to repeat the extraction until the extraction solvent turns colourless (Ngamwonglumlert, Devahastin and Chiewchan, 2017). The time of maceration was always a major concern as time was a crucial factor in industries. According to a study conducted to investigate the effect of maceration time on the yield of extracts, the longer the time of the extraction agent contact with the sample, the more extracts will be obtained as more contents of the sample will be dissolved in the extraction solvent. Therefore, the outcomes of the study concluded that the longer immersion time of maceration yields a greater extract content (Lidiana, Sulmartiwi and Andriyono, 2018). To come to a point, maceration was claimed to be not applicable when there was a constraint employed on the extraction time.

### 2.4.4 Hydro-distillation

Hydro-distillation was another conventional pigments recovery technique. In hydro-distillation, the samples were packed in a still compartment and sufficient water was added and brought to be boiled. Subsequently, direct steam was injected into the plant sample. The vapour mixture of water and the extracts containing oils, pigments etc were condensed by indirect cooling with water. The condensed mixture is then directed from the condenser to a separator where the oil, pigments, and other bioactive compounds are separated from the water (Hasbay and Galanakis, 2018). Figure 2.11 showed the hydro-distillation Clevenger apparatus.

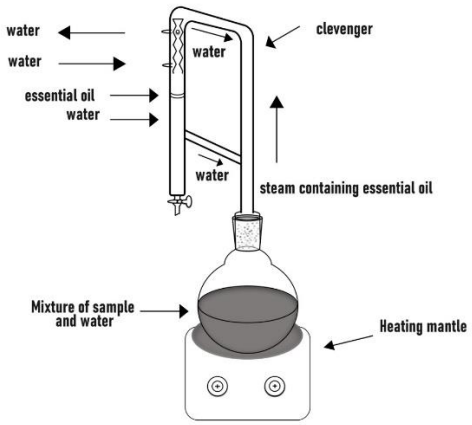


Figure 2.11: A basic set up for hydro-distillation Clevenger apparatus (Samadi, et al., 2017).

The main advantages of employing hydro-distillation include the absence of organic solvents, and there was no need for the dehydration of the plant materials. As water was the only solvent used in this process, the risk of toxic substances being released from the process is greatly reduced and the risk of contamination of the extracts with organic solvents is minimised as organic solvents are not used. However, the demerit of this process include the easy degradation of heat-sensitive pigments as hydro-distillation was conducted at temperature above the boiling point of water, 100.00 ℃, thus this resulted the loss of the desired pigments or any other valuable bioactive compounds (Ngamwonglumlert, Devahastin and Chiewchan, 2017).

## 2.5 Novel extraction technologies

Due to the limitations of the conventional extraction technologies, different emerging or novel extraction technologies were proposed to improve the performance of the pigments extraction process, in terms of extraction yield, shorten the extraction time required and minimise the energy consumption for the extraction process. Figure 2.12 illustrated the novel extraction technologies suggested by different researchers and scientists. Kultys and Kurek (2022) proposed that the novel extraction approaches could be classified into extraction processes driven by electrical force, pressure force and biological force.

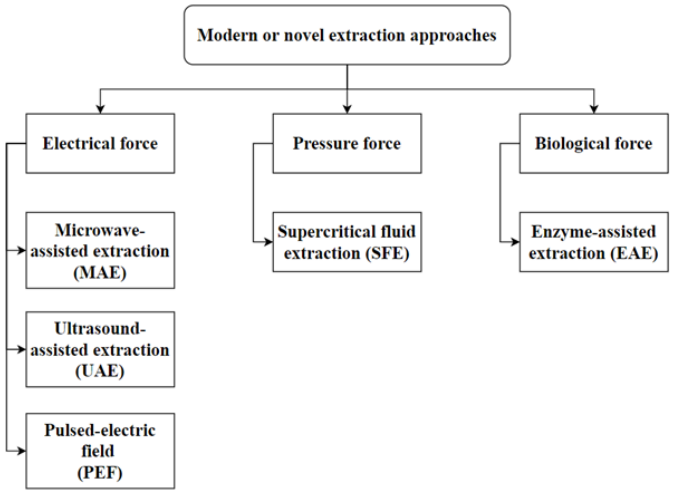


Figure 2.12: Scheme of novel extraction technologies (Kultys and Kurek, 2022).

### 2.5.1 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) employed the principles of microwaves transmission ranging at the frequency between 300.00 MHz to 300.00 GHz (Saini, Panesar and Bera, 2019). In MAE, microwaves were applied to generate heat shock in the medium, as shown in Figure 2.13. This phenomenon produced high heat to vaporise the water present in the internal spaces of the sample matrices. Aside from that, the heat shock triggered the rupture of the cell wall, allowing the solvents to penetrate easily into the cell and favoured the mass transfer from the cell to the solvent (Uzel, 2018).

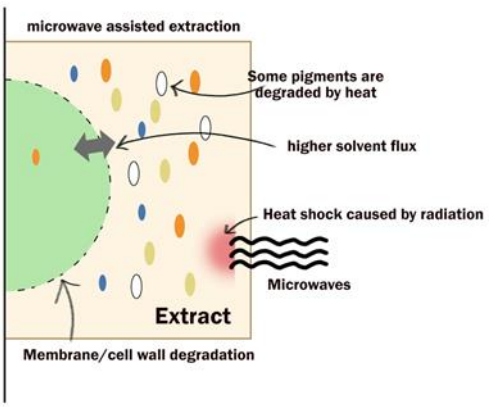


Figure 2.13: Effects of microwaves in the sample matrices (Pagels, et al., 2021).

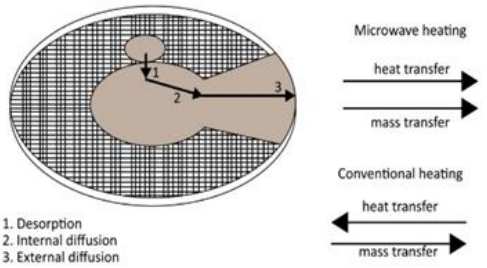


Figure 2.14: Mechanisms of microwave-assisted extraction (Muhamad, et al., 2017).

Figure 2.14 showed the mechanisms involved in MAE where the contents of the plant materials were discharged into the liquid phase solvent when microwave energy is applied to heat the solvent. It was observed that the mass transfer for microwave heating and conventional heating occur in the same direction, but the heat transfer is in the opposite direction. The difference was caused by the volumetric heating effect occuring in the microwave-assisted extraction process, where the heat was dissipated volumetrically inside the irradiated medium (Llompart, et al., 2019). In general, the mechanisms of MAE involved three steps, desorption, internal diffusion, and external diffusion. Desorption was the splitting and separation of solute molecules from the plant materials' matrix due to the increased pressure and temperature in the microwave irradiated medium. Internal diffusion referred to the solvent diffusion alongside the plant materials' matrix. External diffusion involved the release of solutes into the solvent from the plant materials (Muhamad, et al., 2017).

The merits of MAE techniques include the reduction of extraction time and the reduction of solvent volume requirement as the heating of solvent has achieved a faster rate due to the rapid heating by microwave radiation (Ngamwonglumlert, Devahastin and Chiewchan, 2017). Apart from that, it was reported that MAE involves a moderate capital cost as the apparatus set up was simple and efficient. There were studies showed that the yield of extraction in MAE was increased by the increment of extraction time but MAE offered a greater yield by shortening the extraction time, thus saving the cost of operation (Soquetta, Terra and Bastos, 2018). Therefore,

MAE has been popular due to the enhanced productivity, higher yield, reduction of extraction time, less solvent used, simplicity and lower overall costs. However, MAE was found to be not suitable to extract heat-sensitive pigments due to the thermal nature of the process (Saini, Panesar and Bera, 2019). To address the limitations of MAE due to the high thermal nature operations, Tena and Asuero (2022) suggested that nitrogen-protected microwave-assisted extraction (NPMAE) or vacuum microwave-assisted extraction (VMAE) could be employed for the recovery of heat-sensitive pigments such as betalains based on the concepts of using low oxygen atmosphere in the extraction medium to minimise the degradation of thermolabile pigments such as betalains and oxygen-sensitive pigments like carotenoids. However, the operational costs will be increased significantly due to the application of vacuum conditions.

### 2.5.2 Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE), or commonly known as sonication, was generated from elastic mechanical waves at the frequency ranging from 20.00 to 2000.00 kHz (Panda and Manickam, 2019). Ultrasonication was gaining attention in the recovery of pigments on the grounds that ultrasound waves manifested significant impacts on elastic medium like the solvent in liquid phase and the soft tissues of plant compartments. Figure 2.15 demonstrated the effects of ultrasound-assisted extraction in the samples.

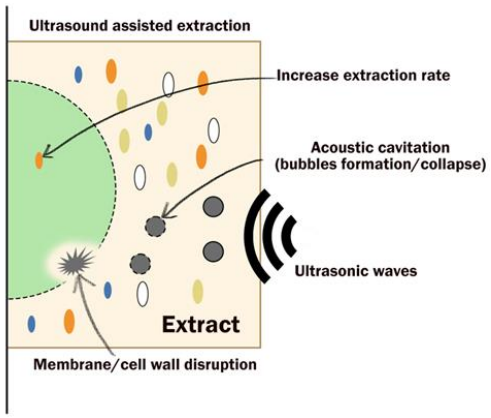


Figure 2.15: Effects of ultrasound-assisted extraction in the samples (Pagels, et al., 2021).

When ultrasound waves were applied, the elastic medium changed according to the shape of the medium as the ultrasound waves travel through the medium (plant tissues) and returns to its original shape when the supply of ultrasound waves is stopped (Calderón-Oliver and Ponce-Alquicira, 2021). When the plant materials were dissolved in the solvent in the presence of ultrasound waves, there were formation of small bubbles in the liquid phase solvent. The small bubbles formed will experience an increment in size and expand until a level the expanded bubbles could not retain their original shape, their cavity collapses and the bubbles will burst, creating high temperature and pressure in the medium. When millions of these small bubbles were formed, expanded and burst, this will create high temperature up to 5000.00 K and pressure up to 1000.00 atm during the extraction process, and thus the extraction efficiency is maximised in a reduced extraction time (Mansour, 2018).

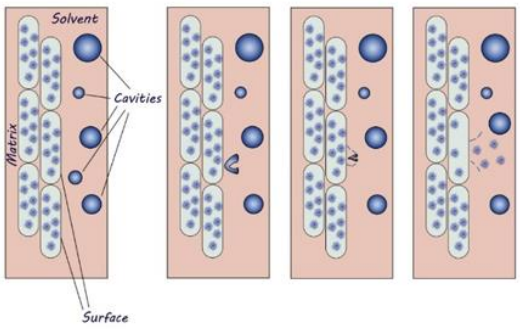


Figure 2.16: Mechanisms involved in ultrasound-assisted extraction (Katsampa, et al., 2015).

Figure 2.16 illustrated the mechanisms of UAE, which was known as the cavitation effect. There were four important steps involved in the cavitation effect. The first stage involved the generation of cavitation bubbles and moving towards the surface of the plant matrix. In the second step, collapse of cavitation bubbles occurred, resulting in a microjet with high localised temperature and pressure towards the surface of the sample matrices. Followed on, the cell walls of the sample matrices ruptured, triggering the collapse of millions of cavitation bubbles and a direct contact between the pigments inside the sample matrices with the solvents was established. Lastly,

the targeted pigments were recovered by the actions of solvents (Katsampa, et al., 2015).

The advantages of applying UAE in the extraction methods include the reduction of extraction time, lower extraction temperature thus less thermal degradation of pigments was expected, lower requirement of solvent, simplicity of the equipment with higher operation efficiency (Saini, Panesar and Bera, 2019). Apart from that, both water and lipid soluble pigments could be extracted via UAE due to its non-thermal approaches. The drawbacks of UAE include the high cost operation and the generation of free radicals from the plant constituents because of the application of ultrasound energy (Muhamad, et al., 2017).

### 2.5.3 Pulsed-electric field (PEF) extraction

As one of the emerging technologies or green extraction methods, pulsed-electric field (PEF) extraction employed short and high voltage electric field to induce pore formation in the cell walls of the samples, and trigger the release of pigments from the sample matrices (Ranjha, et al., 2021).

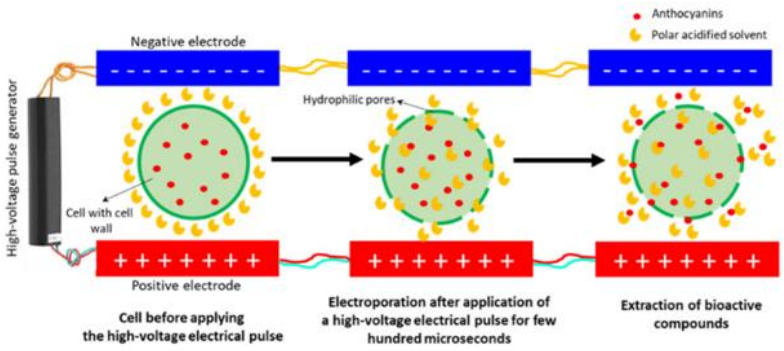


Figure 2.17: Mechanisms of electroporation for pigments extraction (Tena and Asuero, 2022).

Figure 2.17 demonstrated the mechanisms of PEF extraction. Electroporation was interpreted as the formation of hydrophilic pores in the cell membranes of the samples under the application of electric field, which eased the actions of solvent to extract the pigments contained in the internal spaces. Electroporation was the important phenomenon in PEF extraction that enhanced the permeability of cell membranes by an external electrical force

(Putranto, Argo and Wijana, 2014). As seen from Figure 2.17, the damage of cell walls were observed since hydrophilic pores were formed as the electrical charge applied on the cell walls will split the cell walls based on the charges due to their dipole nature. As a result, the pigments molecules will be released from the internal spaces of the sample matrices, carried by the solvent molecules and left the internal sample matrices (Basri, et al., 2021).

Tena and Asuero (2022) claimed that PEF extraction was classified as a non-thermal approach as different published studies have shown that the temperature increment during PEF extraction was lower than 10.00 ℃, indicating that PEF extraction was an ideal technique to recover thermolabile pigments. Apart from that, the operating parameters of PEF extraction such as the electric field strength, pulse duration and number of pulses can be controlled easily (Panja, 2018). In contrast, the main demerit of PEF extraction was that PEF extraction only applicable for the extraction of water-soluble pigments such as anthocyanins and betalains as the solvents used in PEF extraction were mainly polar solvents that contains ions that possess electrical conductivity so that electricity can pass through the plant sample cells. Non-polar solvents did not allow electric fields to pass through as it is an electrical resistant solvent that has limited or negligible conductivity. Thus, PEF extraction was not suitable for the extraction of carotenoids which were lipophilic in nature (Ranjha, et al., 2021). Furthermore, PEF extraction involves the need of a high-power supply equipment and treatment chamber which are rather expensive and thus the PEF based extractor is not constructed at industrial scale (Sampedro, et al., 2014).

### 2.5.4 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) was one of the types of solvent extraction that recovered extracts from solid matrices based on the use of renewable solvents such as carbon dioxide and nitrogen that are above or near their critical temperature and pressure (Carpentieri, et al., 2021). Supercritical stage could be achieved when a fluid was forced to achieve a pressure and temperature above its critical limit. During this stage, the viscosity of the fluid acted like a gas and the density of the fluid exhibited an intermediate diffusivity between liquid and gas. This offers a better solvent status that

allows the solubility of organic compounds in these fluids to be increased so that the bioactive compounds such as pigments can be extracted easily (Wrona, et al., 2017). Figure 2.18 demonstrated the use of supercritical fluid in the extraction process. In SFE, the fluid entered and left the sample matrices as a gas, whereas the fluid acted as liquid during the recovery of pigments inside the sample matrices. This offered excellent pigments recovery actions as insignificant damages on the samples were observed in SFE (Soquetta, Terra and Bastos, 2018).

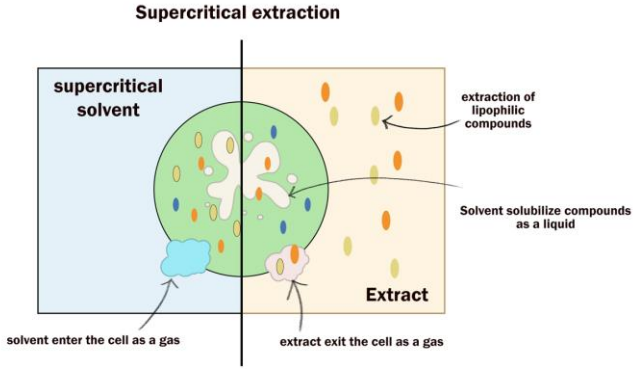


Figure 2.18: Mechanisms of supercritical fluid extraction in sample matrices (Pagels, et al., 2021).

SFE involves two main stages which include the contact of the supercritical fluid with the plant material sample and the separation of the extracts with the supercritical fluid. In the first stage, the dissolution of target pigments present on the solid plant matrices by the supercritical fluid was performed. This could be done by introducing the pressurised and heated supercritical fluid through a solvent pump and a heat exchanger before being subjected to the extraction system. In this step, the supercritical solvent was absorbed by the pigments and promoted the dilatation of the plant matrix structure, therefore enhancing the extraction rate (Moldoveanu and David, 2015). The second stage was to separate the extracted pigments with the supercritical fluid in the separator, in which a simple depressurization system could be applied to produce a solvent-free extract. The extracted pigments can be easily collected whereas the depressurized solvent can be recycled or released to the atmosphere (Nunes, et al., 2022).

Supercritical solvent is a fluid that existed in a supercritical state and manifested the intermediary properties between gases and liquids. There were two important parameters in the formation of supercritical fluid, which were the critical temperature and critical pressure. The critical temperature, $T_c$ is the temperature that liquid phase cannot be formed from gas phase irrespective of high pressure and the critical pressure, $p_c$ is the vapour pressure of the fluid at its critical temperature. There was no liquid-gas phase boundary at supercritical condition. Thus, no surface tension exists as fluid behaves as single phase, at the same time retaining the properties of gas and liquid (Nobre, et al., 2006). Fluids with these properties were effective in the extraction of active components from plant parts as the supercritical fluid is able to diffuse into the plant matrix like a gas and able to dissolve active ingredients like a liquid solvent (Panja, 2018). In short, the fluid will have high diffusivity and low viscosity like gas but possess high solvation power like liquid at supercritical state. The potential solvents that can be used as supercritical solvents were summarised in Table 2.4 with their critical properties.

Table 2.4: The critical properties of extraction fluids (Moldoveanu and David, 2015).

| **Solvent** | **Critical temperature, $T_c$ (°C)** | **Critical pressure, $p_c$ (atm)** |
|---|---|---|
| Carbon dioxide ($CO_2$) | 31.10 | 72.90 |
| Nitrous oxide ($N_2O$) | 36.50 | 71.70 |
| $CHClF_2$ (Freon-22) | 96.00 | 48.50 |
| $CHF_3$ (Freon-23) | 25.90 | 46.90 |
| $CH_3OH$ (Methanol) | 240.00 | 78.50 |
| Water ($H_2O$) | 374.10 | 218.30 |

Table 2.4 proposed the candidates for supercritical solvents. However, the right candidate solvent must be screened from various aspects such as energy requirement, operability, safety issues etc before it is proposed as a supercritical solvent because it would be energy intensive if a solvent with high critical temperature is selected as this implies that a lot of energy is required to raise the solvent to its critical points. Based on Table 2.4, it can be observed that carbon dioxide was considered as an ideal candidate as its

critical points were relatively low compared to other solvents, thus it was commonly used as the supercritical solvent in the extraction process. Aside from that, supercritical carbon dioxide was recognized as Generally Recognised As Safe (GRAS) solvent type as it could selectively extract the targeted pigments from the fruits and vegetables wastes matrices without leaving any toxic residues in the extracts (Razi Parjikolaei, et al., 2015). On the other hand, flammable solvents like methanol should not be employed as it should be avoided at high temperature due to safety issues. Apart from that, in terms of energy cost, water is not considered as a suitable candidate as it is energy intensive to raise the temperature and pressure of water to its critical points and the high critical temperature, 374.10 ℃ will cause the thermal degradation of heat sensitive pigments (Mięku̇s, et al., 2019).

Supercritical solvent extraction was effective and relatively fast because of the low viscosity, high diffusivity, and tunable power of the supercritical fluid. Furthermore, the use of organic solvent was at a minimum level, thus the risk of contamination of the extracts with volatile organic solvent can be minimised. Besides, the supercritical solvent extraction is considered as safe and green technology as the solvent used is renewable such as carbon dioxide (Prado, et al., 2015). Besides, the SFE method was suitable for lipid-soluble pigments like carotenoids and chlorophylls as most of the extraction solvents used in SPF are non-polar. However, the supercritical solvent extraction involved high capital and operating costs as there was a need of high pressure for the operation. (Ngamwonglumlert, Devahastin and Chiewchan, 2017). However, it was stated that the higher energy consumption for the supercritical fluid extraction was compensated by the lower solvent costs (Ludwig, et al., 2021).

### 2.5.5 Enzyme-assisted extraction (EAE)

Enzyme-assisted extraction (EAE) involves the use of enzymes in the extraction of bioactive compounds from plant materials. As fruits and vegetables wastes tissues were made up of cellulose, starches and pectin as binding materials, this gave plant cells a rigid and strong structure (Mansour, 2018).

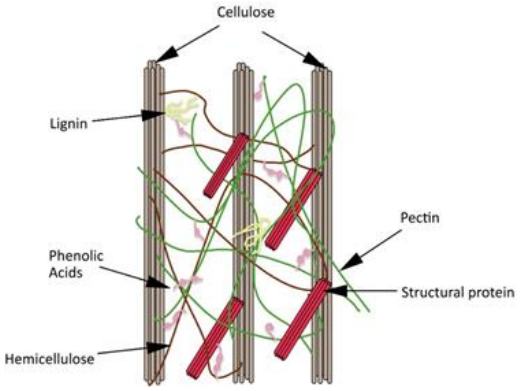


Figure 2.19: Structures of cell walls of plant materials (Nadar, Rao and Rathod, 2018).

As shown in Figure 2.19, biomolecules trapped inside a plant cell were present in insoluble or soluble conjugate forms. Thus, enzyme was added into the extraction medium to enhance the recovery of bioactive compounds from the plant materials by breaking and softening the cell walls of the plant materials (Ricarte, et al., 2020). This allowed the penetration of the solvent to trap the pigments contained inside the sample matrices once the cell wall is broken because the bound or dispersed phytochemicals inside the cell or on the cell walls are difficult to be extracted by normal or conventional solvent extraction. Thus, enzymes help to digest the surrounding materials on the cell walls so that the ingredients inside the plant cell can be released (Lotfi, et al., 2015). Examples of enzymes can be used to hydrolyse the plant cellulosic cell walls include pectinase, cellulase, and hemicellulose to facilitate the extraction process. The overall mechanisms involved in enzyme-assisted extraction was illustrated in Figure 2.20.

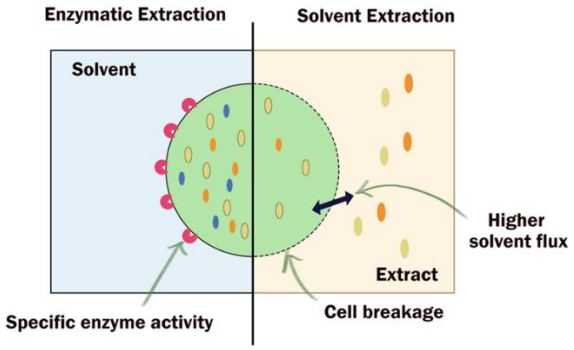


Figure 2.20: Mechanisms involved in enzyme-assisted extraction (Pagels, et al., 2021).

The main principle involved in the enzyme-assisted extraction was enzymatic hydrolysis, in which the mixture of the plant samples, enzyme and the solvent is incubated at low temperature (35.00 to 50.00 ℃) with proper adjusted pH as the enzyme works best in acidic medium. The hydrolysis process will be stopped as enzymes will be deactivated at high temperatures of 80.00 to 90.00 ℃ (Panja, 2018).

The benefits of employing enzyme-assisted extraction include less solvent consumption as enzyme helps to facilitate the hydrolysis of cell walls, lower toxicity as water was mainly used as the solvent and there were no, or less organic solvents used. Apart from that, enzyme-assisted extraction involves non-thermal nature, which indicated that it was suitable for the extraction of heat-sensitive pigments (Saini, Panesar and Bera, 2019). The main demerits of enzyme-assisted extraction was the long extraction time which could be varied from 1 hour to 48 hours and the extracts need to undergo separation by filtration or centrifugation (Sagar, et al., 2018).

# CHAPTER 3

# METHODOLOGY AND WORK PLAN

## 3.1 Introduction

This study was a review based study, therefore literature review played an important role as the data and information gathered for the results and discussion sections were solely experimental results obtained from other published studies.

Literature review was generally defined as a collection of summaries from papers or bibliography of different research manuscripts. Other parties recognized literature review as the application of ideas in the justification of a particular approach towards a topic and the demonstration in contributing something new (Levy and Ellis, 2006). Sometimes, literature review was called the “Cinderella” of a study or research as it was often related poorly to the primary research. However, a good literature review could help other researchers to develop and synthesise new ideas from the previous sources. Thus, in the academic aspect, literature reviews were mainly classified as the proposals for funding in research articles, or serves as guidelines for professional and evidence-based practice to satisfy public and personal curiosity (Bolderston, 2008).

Basically, there were four types of literature reviews. The first type of literature review was known as narrative review that attempts to do a summary based on the published research works on a topic of interest. The second type of literature review was developmental review, in which new concepts, research models, theories, frameworks or methodological approaches were proposed. The third type of literature review was cumulative review, which compiled the empirical evidence to connect with the literature in order to draw an overall conclusion for the particular topic of interest. The last type of literature review was aggregative review that binded the prior findings and the specific research hypotheses or propositions (Templier and Paré, 2015).

## 3.2 Framework for the methodology

A framework was important in establishing the methodology of a review based study because all the empirical studies such as the qualitative, quantitative, or mixed methods must be connected to the literature, or the supportive concepts needed for the study (Rocco and Plakhotnik, 2009).

The establishment of a framework for literature review normally involved identification of the problem statements, documentation of the data gathered, screening for inclusion, interpretation of the data and the transferring of data, synthesising of new ideas and lastly the evaluation of the data gathered and synthesised (Levy and Ellis, 2006). In short, literature review serves a main purpose to identify the data involved in the research field, collect the main ideas, context, and theories for the study and explore the relationship between the studies conducted by different researchers to identify the gaps between the literature and current situation. Figure 3.1 provided a general approach to conduct a review based study.

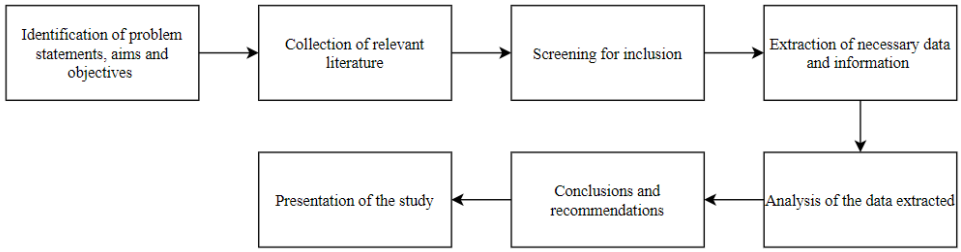


Figure 3.1: Overall flowchart for the systematic approach to conduct a review based study.

### 3.2.1 Identification and formulation of problem statements, aims and objectives

The kick-off step for a literature review was to identify and formulate all the problem statements, aims and objectives involved in the study. This step was extremely important and must be conducted before the literature review was started owing to the fact that identification and formulation of relevant problem statements, aims and objectives could provide a general and clear direction to collect the appropriate literature to support the formulated

problem statements, aims and objectives that are needed for the study (Templier and Paré, 2015).

In this study, the identification and formulation of problem statements, aims and objectives were done by discussion with the project supervisors, ChM. Ts. Dr. Tee Shiau Foon and Ts. Dr. Shuit Siew Hoong beforehand to gain a better understanding of the background of this literature review works. In this scenario, the discussion was conducted on the basis to study the available extraction methods for natural pigments from fruits and vegetable wastes to solve the current food waste problems. All the outcomes of the discussions such as problem statements identified, aims and objectives established were covered in Chapter 1.3 and Chapter 1.4.

### 3.2.2 Collection of relevant literature

The following step was the collection of relevant literature for the study. During the data collection phase, all the necessary information and data that were related to the research topic were gathered. Therefore, the techniques involved in the collection of data should be smart and intelligent. As the range of the information sources was relatively large, the keywords for the data collection are crucial to avoid wastage of time in looking for appropriate information and data needed for the study. When conducting data collection, keywords such as “Natural pigments”, “Extraction of natural pigments”, “Food waste problem” and “Stability of natural pigments” were most commonly used to look for the relevant literature pertinent to the review. The main resources used for this review were obtained from channels that offered online articles, journals, or e-books. The online sources that have been utilised for this review to obtain the relevant journals related to the topic of extraction of natural pigments, food waste problems, natural pigments, stability of natural pigments include:

i. ScienceDirect (https://www.sciencedirect.com/)
ii. ResearchGate (https://www.researchgate.net/)
iii. SpringerLink (https://link.springer.com/)
iv. SAGE Journals (https://journals.sagepub.com/)
v. ACS Publications (https://pubs.acs.org/)

vi. UTAR Library E-Journals Website (https://library.utar.edu.my)

vii. National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/)

The online website of the UTAR library provided a list of reliable databases which could be browsed by subjects. In this study, the subject of "Engineering" was selected to shorten the list of relevant databases, as shown in Figure 3.2.

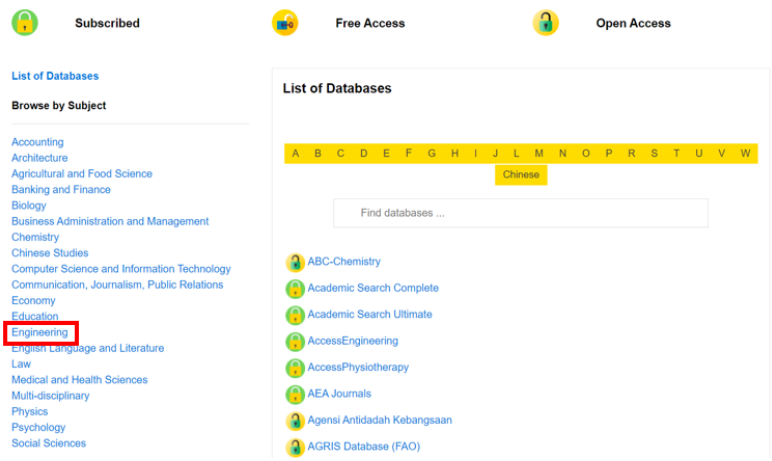


Figure 3.2: Selection of subjects in the database searching.

### 3.2.3 Screening for inclusion by evaluating the quality of literature

Screening process was extremely crucial after gathering all the necessary and relevant literature for the study. This action involved the decision to include or exclude the information gathered for the review work by evaluating their level of relevance to the study. One of the important tips to be taken into consideration during the screening process was to ensure and maintain the objectivity throughout the screening process to avoid mistakes or biases when writing the literature (Snyder, 2019).

In the stage of literature review, the collected or gathered literature was organised using the Mendeley software. Mendeley software eased the process in citing and creating references. The screening process of the grouped literatures can be conducted easily as all the literatures obtained were screened through the year of publication. Normally, the literature

published in recent years showed a higher level of relevance as the data and information were not outdated. Figure 3.3 displayed the use of Mendeley process in organising the grouped literatures as highlighted in Figure 3.3 using red colour boxes. This software showed useful information like the authors' name, year published and the abstracts of the journals. This helped to serve as the first level for the screening process to exclude those unreliable and uncited literatures.

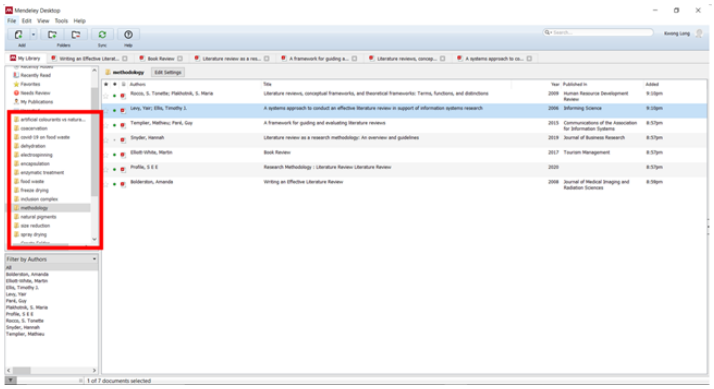

Figure 3.3: The use of Mendeley software for screening process.

### 3.2.4 Extraction of necessary data and information

After the screening process is completed, extraction of relevant data and information was conducted. However, only useful and valued information were extracted. The process of data extraction was conducted in the form of descriptive information that involved the description of types of study and or findings. The second form of data extraction carried out was in the form of conceptualizations that consist of theories and ideas (Snyder, 2019). In this study, both descriptive and conceptualization extraction methods were employed. In the data extraction stage, the ideas, theories, and concepts discussed in the selected literatures were classified and grouped into each category consisting of different topics. The information and data extracted including the qualitative analysis for the natural pigments such as quality of the recovered pigments, methods employed to extract natural pigments, the comparative analysis between the novel extraction technologies and other relevant information needed for this review based study.

### 3.2.5 Analysis of the data extracted

The analysis or interpretation of the data was crucial. This involved the actions to categorise, summarise, organise and compare the findings and ideas extracted from the former researchers. Apart from that, any similarities or correlations between the findings of the research topics were identified and discussed respectively to reduce the gaps in the research. It was reported that apart from the four main types of literature review such as narrative, developmental, cumulative and aggregative, there are other types of literature review proposed by different researchers such as critical, comprehensive, systemic or meta-analysis (Templier and Paré, 2015).

In the analysis stage, narrative review was mainly be employed on the research for the topic of interest that was published by former researchers. This involved the actions to bind, summarise and integrate the concepts and theories proposed by former researchers. For instance, the experimental results extracted from different experiments will be analysed and grouped into subsequent categories, as depicted in Figure 3.4. As an example, the stability assessment of natural pigments considered pH, temperature, light exposure and oxygen concentration conducted by different researchers were reviewed and compared. This helped to form a critical evaluation which identified whether the claims in the articles were credible by giving the supportive statements and further justifications derived from other published studies.

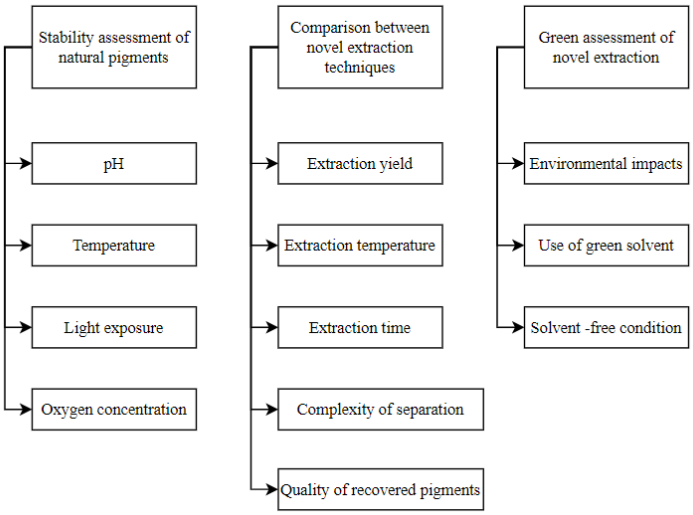


Figure 3.4: Categories and subcategories of the study.

### 3.2.6 Conclusions and recommendations

After the analysis of results and discussion, conclusions statements were made based on the outcomes of results and discussion. This was done by analysing the most appropriate extraction technique for the targeted pigments based on the stability assessment conducted previously. For instance, heat-sensitive pigments will be recovered by using non-thermal approaches to minimise potential thermal degradation of the thermolabile pigments. Aside from that, recommendations for future work were provided to improve the existing extraction technologies.

### 3.2.7 Presentation of the study

As the last step, the review based study was presented by compiling, evaluating, organising, summarising, and comparing all the empirical studies suggested by the former researchers. The presentation of the study should be clear, concise, specific and precise to allow the readers to gain the understanding easier.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Stability assessment of the extracted natural pigments

The stability assessment of the natural pigments was important as it helped to determine the most suitable approach that could be used in the extraction process of that particular pigment. It was revealed that the stability of pigments not only influenced by the structural features such as the number of hydroxyl and methoxy groups, degree of acylation, but also affected by the four major factors including pH, heat, light, oxygen (Jurić, et al., 2020).

### 4.1.1 Effects of pH change on the stability of natural pigments

In terms of pH stability, anthocyanins were found to be more stable at lower pH condition as the higher pH value will trigger colour fading of the anthocyanins pigments, leading to irreversible transformation of the structure of anthocyanins. The reason behind the colour fading was the deprotonation of the anthocyanins' polar structure when exposed to high pH medium (Wahyuningsih, et al., 2017). Jenshi, et al. (2011) suggested that the anthocyanin extract was found to be more stable at pH ranges from 5.10 to 6.00 whereas Torskangerpoll and Andersen (2005) stated that anthocyanin pigments exhibit excellent stability under low pH environment between 4.10 and 5.10. It could be seen that the stable pH range for anthocyanins was relatively narrow. Therefore, it can be concluded that anthocyanins pigments were stable under low pH or acidic conditions since alkaline environment was believed to induce the colour loss phenomenon in recovered anthocyanins pigment as declared by Wahyuningsih, et al. (2017).

Betalains were found to exhibit high stability to pH change as betalains were most stable in a pH range of 3.00 to 7.00, which showed a broad range of stable pH as compared to anthocyanins (Cejudo-Bastante, et al., 2016). When the pH condition was brought below 3.00, the structure of betalains will be converted from red to blue-violet whereas pH above 7.00 will lead to aldimine bond hydrolysis, that caused rapid degradation of betalains into betalamic acid and cyclodopa-5-*O*-glucoside and the colour of

the degradation product change to yellow-brown colour (Herbach, Stintzing and Carle, 2006).

According to a study conducted to investigate the effect of pH on the chemical stability of carotenoids, it was discovered that the observed carotenoids degradation was relatively small when carotenoids were subjected to pH change, and the most stable pH range was found in between 3.00 and 6.00 (Bell, Alamzad and Graf, 2016). This claim was verified by Ordoñez-Santos, Martínez-Girón and Villamizar-Vargas (2018) based on a pH stability study conducted to recover carotenoids from peach palm and the outcomes showed that degradation of carotenoids are insignificant when subjected to pH changes from 3.00 to 11.00, supported by the experimental outcomes that fluctuations of carotenoids retention rate was less than 5.00 %. However, Qian, et al. (2012) stated that significant carotenoids degradation was observed when the pH of the medium is less than 3.00. This was because carotenoids manifested a good pH-stability because carotenoids were generally lipophilic and there were coatings of lipid droplets by a relatively thick layer of hydrophilic polysaccharide molecules (Anarjan, et al., 2014).

Table 4.1: Stable pH range for anthocyanins, betalains and carotenoids.

| Pigments | Stable pH range | References |
|---|---|---|
| Anthocyanins | 5.10 to 6.00 | Jenshi, et al., 2011 |
| | 4.10 to 5.10 (Narrow range) | Torskangerpoll and Andersen, 2005 |
| Betalains | 3.00 to 7.00 (Broad range) | Cejudo-Bastante, et al., 2016 |
| Carotenoids | 3.00 to 6.00 (Broad range) | Bell, Alamzad and Graf, 2016 |

Table 4.1 made an effort to summarise the findings of the stable pH range suggested for anthocyanins, betalains and carotenoids. It could be seen that the stable pH range for anthocyanins was relatively narrower as compared to betalains and carotenoids, indicating that pH change was a critical factor that affect the stability of anthocyanins. Overall, it can be said that anthocyanins showed lowest stability towards pH change, followed by

betalains whereas carotenoids displayed the highest stability in relation to the pH change.

### 4.1.2 Effects of temperature on the stability of natural pigments

Temperature remained as a crucial factor to determine the thermal stability of the natural pigments as it would significantly affect the types of extraction technologies that could be applied on the recovery of that particular pigment, whether thermal or non-thermal approaches.

It was figured out that anthocyanins pigments exhibited excellent stability to heat treatment because of the complex patterns of glycosylation and acylation (Rodriguez-Amaya, 2018). Anthocyanin pigments showed a high stability to heat as the degradation of anthocyanins only occured when the heating temperature reached 80.00 ℃ or above as there was a study showed that an increase in temperature triggered an increase in the extraction of anthocyanins, however a drastic decrease in anthocyanins extraction was observed at 80.00 ℃, indicating that anthocyanins will degrade at 80.00 ℃ (Lasunon, et al., 2018). The thermal degradation involved the formation of colourless carbinol pseudobase and the subsequent opening of the pyrylium ring to produce chalcone, and finally turned into a brown product (Delgado-Vargas, et al., 2000).

Betalains exhibited low stability to heat, this claim was further confirmed by other studies that suggested that heat was the most critical factor that gave rise to the degradation of betalains, and heat will affect other factors that influence the stability of betalains such as oxygen. It was because heat would induce oxidation in betalains, aldimine bond hydrolysis and the decarboxylation of betalains that caused colour change to orange-yellow. The degradation temperature of betalains was reported at 50.00 ℃ by Santos, et al. (2018). When the operating temperature of the extraction process exceeded 50.00 ℃, thermal degradation of betalains occured drastically (Carrillo-López and Yahia, 2017).

Unlike betalains and anthocyanins, carotenoids were found to be more heat resistant as thermal degradation of carotenoids was observed at particularly high thermal temperature, 90.00 ℃ (Jirasatid, Chaikham and Nopharatana, 2018). This statement was proven by Gheonea, et al. (2020)

that the thermal degradation temperature of carotenoids happen over 100.00 ℃ because the *trans* structure of carotenoids would be altered to the *cis* structure, leading to the reduction in biological activity and colour changes of carotenoids. The reason that carotenoids could withstand higher temperature than anthocyanins and betalains was because of the fact that carotenoids were made up of a system of conjugated double bonds which makes carotenoids vulnerable to heat (Bonnie and Choo, 1999).

Table 4.2: Thermal degradation temperature of anthocyanins, betalains and carotenoids.

| Pigments | Degradation temperature (℃) | Heat stabiliy | References |
|---|---|---|---|
| Anthocyanins | 80.00 | Moderate | Lasunon, et al., 2018 |
| Betalains | 50.00 | Low | Santos, et al., 2018 |
| Carotenoids | 100.00 | High | Gheonea, et al., 2020 |

To give an outline of the effects of temperature on the stability of pigments, it could be summarised that carotenoids exhibited the greatest ability to withstand high thermal temperature, followed by anthocyanins. However, betalains were found to be extremely heat sensitive due to the lowest degradation temperature. Table 4.2 presented the results of thermal degradation temperature for anthocyanins, betalains and carotenoids.

### 4.1.3 Effects of light exposure on the stability of natural pigments

According to a study conducted to investigate the effect of presence or absence of light on the stability of anthocyanin, it was reported that 50.00 % of anthocyanins experienced destruction in the presence of light whereas the absence of light, dark environment also contributes to 30.00 % destruction of the pigments (Bakhshayeshi, et al., 2006). This showed that light exposure would create certain destruction in the anthocyanins content, but the destruction percentage was comparatively small. This claim was supported by the experimental results which claimed that no significant decrease in the anthocyanins pigments retention on the light exposure as the percentage of destruction observed was less than 2.00 % (Sipahli, Mohanlall and Mellem, 2017).

In terms of light exposure, degradation of betalains followed a first-order kinetic and betalains are most stable in dark conditions, which is the absence of light (Carrillo-López and Yahia, 2017). In another study to investigate the effects of light on betalains stability, it was proved that light contributes to the degradation of betalains, but the degradation was not significant (Wong and Siow, 2015). Table 4.3 showed the light destruction percentage for anthocyanins and betalains pigments. Based on Table 4.3, it could be seen that the light destruction percentage for both anthocyanins and betalains pigments were relatively small, not more than 10.00 %, which showed strong agreement to the claims that anthocyanins and betalains were less likely to degrade or destruct on light exposure.

Table 4.3: Effects of light on anthocyanins and betalains pigments.

| **Pigments** | **Percentage of pigments retained (%)** | | **References** |
|---|---|---|---|
| | **Light present** | **Light absent** | |
| Anthocyanins | 72.06 | 79.04 | Laleh, et al., 2006 |
| Betalains | 73.00 | 83.00 | El-Ashry, El-Bahr and Gabr, 2020 |

It was suggested that carotenoids displayed the weakest stability towards oxygen and light exposure. This was due to the chemical structure of carotenoids, in which most of the carotenoids had bilaterally symmetric and varied numbers of conjugated double bonds. This gave carotenoids a strong ability to harvest light and undergo oxidation-reduction reaction due to the presence of large numbers of conjugated double bonds (Lu, et al., 2021). On top of that, strong light would trigger negative effects on the synthesis of carotenoids, as it would induce the isomerization of *trans* structure of carotenoids into *cis* structure (Yue, Wang and Yang, 2021). This was further verified by the experimental results contributed by Atencio, et al. (2022), as shown in Figure 4.1 in which the retention percentage of *trans* β-carotene experienced drastic decrement when the light dosage increased from a small percentage. This showed that carotenoids were extremely light sensitive due to the isomerisation of carotenoids which will be induced easily by the action of light.

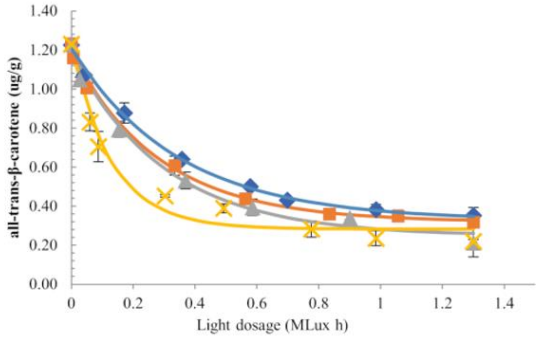


Figure 4.1: Destruction curve for carotenoids on light exposure (Atencio, et al., 2022).

In closing, it was found that anthocyanins and betalains showed the greatest stability towards light exposure, however carotenoids were extremely sensitive to the action of light.

### 4.1.4 Effects of oxygen concentration on the stability of natural pigments

There were experimental results manifested that an oxygen-rich environment was able to improve the anthocyanins contents owing the fact that the remarkable antioxidant properties exhibited by anthocyanins was able to withstand elevated oxygen environment as it could inhibit the oxidation process (Khoo, et al., 2017).

Table 4.4: Changes in the anthocyanins contents in strawberries under different oxygen concentrations (Zheng, et al., 2007).

| **Duration (days)** | **Total anthocyanins (mg/ 100 g)** | | |
|---|---|---|---|
| | **Air** | **60.00 % $O_2$** | **100.00 % $O_2$** |
| 0 | 20.07 | - | - |
| 3 | 20.18 | 21.67 | 22.34 |
| 7 | 20.70 | 22.13 | 24.23 |
| 10 | 23.68 | 20.49 | 20.08 |
| 14 | 20.12 | 18.52 | 18.97 |

In the report on the effects of high-oxygen atmospheres on blueberry anthocyanins, it was discovered that elevated oxygen concentration could increase the total anthocyanins in the blueberries (Zheng, et al., 2003). This hypothesis was verified by the experimental outcomes provided in Table 4.4.

As depicted in Table 4.4, the total anthocyanins in the strawberries increased with the elevated oxygen environment for the first 7 days. However, prolonged treatment could potentially trigger the oxidation of the anthocyanins, which resulted in the loss of anthocyanins content, but the decrement observed was relatively small, indicating that anthocyanins showed strong stability to withstand high oxygen atmosphere.

In the presence of oxygen, betalains were reported to experience degradation lineary with the increasing concentration of oxygen (Herbach, Stintzing and Carle, 2006). The oxidation of betalains tends to occur due to the chemical structure of betalains as substrates for oxidation, and the formation of brown polymers will be observed at the end of the degradation process due to oxidation (Pedreño and Escribano, 2000). Czapski (1985) performed an experimental study to explore the influence of oxygen on the retention rate of betalains. From Figure 4.2, it was obvious that betalains experienced degradation at different storage conditions in the presence of oxygen. Therefore, a conclusion could be drawn that oxidation was one of the possible degradation causes of betalains.

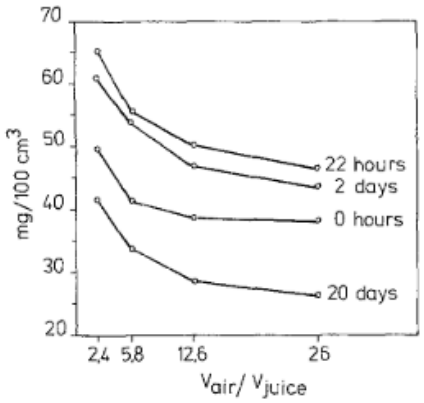


Figure 4.2: Influence of oxygen on the retention rate of betalains (Czapski, 1985).

Due to the highly unsaturated chemical structures of carotenoids, carotenoids are highly sensitive to oxygen rich atmospheres. Owing to the fact that carotenoids could be oxidised easily by the presence of conjugated polyene chains that made carotenoids susceptible to oxidative degradation (Boon, et al., 2010). It was outlined that the oxidative alteration and

degradation of carotenoids followed different pathways, including epoxidation, isomerization, oxidative cleavage and hydrolysis (Krinsky and Deneke, 1982). Henry, et al. (2000) carried out an experiment to determine the percentage remaining carotenoid under different atmospheres. Figure 4.3 depicted that degradation of carotenoids displayed significant decrement as the steepness of the slope for oxygen exposure was comparatively larger than the slope for nitrogen exposure. This indicated that oxygen was an agent to accelerate the degradation of carotenoids.

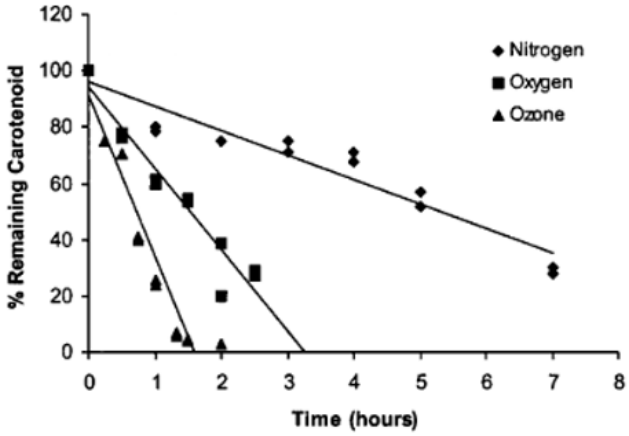


Figure 4.3: Stability plots of carotenoids after nitrogen, oxygen and ozone exposures (Henry, et al., 2000).

To come to the point, carotenoids were extremely prone to oxidation with massive destruction of carotenoids observed under oxygen exposure, followed by betalains with lower degradation rate under treatment of oxygen. On the other hand, anthocyanins revealed a completely different pattern, with oxygen treatment, increasing anthocyanins concentration was observed for a particular period of time, but sustained treatment might induce oxidation of anthocyanins, though the deterioration of anthocyanins was insignificant under oxygen treatment.

### 4.1.5 Summary of the stability assessment of natural pigments

To put in a nutshell, the stability assessment of natural pigments was presented in Table 4.5.

Table 4.5: Stability assessment of different natural pigments.

| Natural pigments | Stability of natural pigments | | | |
|---|---|---|---|---|
| | **pH change** | **Heat** | **Light** | **Oxygen** |
| Anthocyanins | Low | Moderate | High | High |
| Betalains | High | Low | High | Moderate |
| Carotenoids | High | High | Low | Low |

Table 4.5 was useful to determine the appropriate extraction approaches for the particular pigments. By way of illustration, anthocyanins showed low stability towards pH change, followed by betalains and carotenoids. This demonstrated that treatment that involved extreme pH conditions such as enzyme-assisted extraction might not be suitable to extract anthocyanins as anthocyanins was extremely sensitive towards pH change. Betalains and carotenoids, on the other hand, could be recovered by using enzyme-assisted extraction due to their high stability to pH change.

Aside from that, the heat stability assessment could help in the selection of extraction technology selection. For instance, pigment recovery processes that applied thermal methods like microwave-assisted extraction should be avoided for betalains due to its heat sensitive properties. In contrast, carotenoids which can withstand high heat could be subjected to thermal extraction technologies.

In terms of light and oxygen exposure, anthocyanins and betalains displayed remarkable stability towards light and oxygen treatment. In contrast, carotenoids were extremely susceptible to light and oxygen treatment. It was revealed that the presence of light and oxygen could initiate the degradation of carotenoids. Therefore, it was advised that carotenoids should be extracted in a dark environment and oxygen-limited atmosphere to reduce the amount of carotenoids lost. Otherwise, an inert atmosphere created by nitrogen gas or carbon dioxide atmosphere could be employed during the recovery of carotenoids to minimise the oxidation of carotenoids.

## 4.2 Comparison of novel extraction technologies based on classical topics

Figure 4.4 depicted the classical topics used to compare novel pigments extraction technologies, including extraction yield, extraction temperature, extraction time, complexity of separation process and lastly quality of the recovered pigments. In the classical topics, operating pressure and pH medium were not considered. This was because only supercritical fluid extraction involved pressurised fluid that was used to extract pigments from the fruits and vegetables wastes matrices, in which other methods were conducted under atmospheric pressure. On the other hand, pH of the extraction medium will be kept constant during the extraction process for all the methods apart from enzyme-assisted extraction. Therefore, it was difficult to establish a comparison for the operating pressure and pH of the extraction medium.

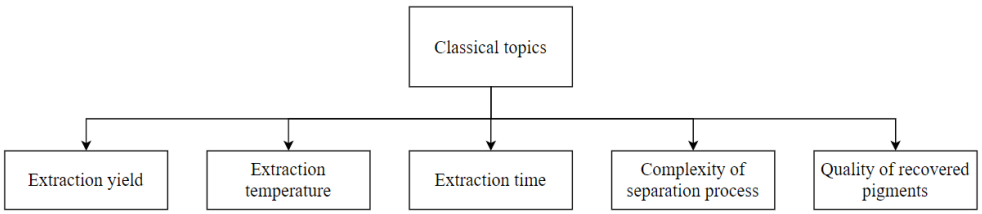


Figure 4.4: Classical topics discussed in terms of comparison between different novel pigment recovery technologies.

Table 4.6 showed the extraction yield, extraction temperature and extraction time for different novel pigment extraction approaches.

Table 4.6: Extraction yield, extraction temperature and extraction time for microwave-assisted and ultrasound-assisted extraction.

| Extraction approaches | Types of pigments | Materials | Extraction yield | Extraction temperature (℃) | Extraction time (minutes) | References |
|---|---|---|---|---|---|---|
| Microwave-assisted extraction | Anthocyanins | Grape skins | 1.86 mg/g | 100.00 | 5.00 | Liazid, et al., 2011 |
| | Anthocyanins | Black currant marc | 20.40 mg/g | 80.00 | 10.00 | Pap, et al., 2013 |
| | Anthocyanins | Clitoria Ternatea | 49.97 % | 60.00 | 15.00 | Izirwan, et al., 2020 |
| | Betalains | Dragon fruit skins | 9.00 mg/ L | 35.00 | 8.00 | Thirugnanasambandham and Sivakumar, 2017 |
| | Betalains | Beetroot peels | 22.00 % | 30.00 | 2.50 | Zin and Bánvölgyi, 2021 |
| | Carotenoids | Carrot wastes | 77.48 % | 80.00 | 9.39 | Elik, Yanık and Göğüş, 2020 |
| Ultrasound-assisted extraction | Anthocyanins | Red raspberries | 78.13 % | 40.00 | 3.33 | Chen, et al., 2007 |
| | Anthocyanins | Blackberries wastes | 107.81 mg/ g | 30.00 | 5.00 | Oancea, et al., 2013 |
| | Betalains | Prince’s-feather | 86.00 % | 41.80 | 12.00 | Tabio-García, et al., 2021 |
| | Betalains | Red beet | 75.00 mg/ L | 30.00 | 30.00 | Silva, et al., 2020 |
| | Carotenoids | Carrot wastes | 51.00 % | 29.00 | 16.00 | Umair, et al., 2021 |
| | Carotenoids | Sunflower seed wastes | 334.75 mg/ L | 40.00 | 20.00 | Li, Fabiano-Tixier and Tomao, et al., 2013 |

Table 4.7: Extraction yield, extraction temperature and extraction time for pulsed-electric field extraction, supercritical fluid extraction and enzyme-assisted extraction.

| Extraction approaches | Types of pigments | Materials | Extraction yield | Extraction temperature (°C) | Extraction time (minutes) | References |
|---|---|---|---|---|---|---|
| Pulsed-electric field extraction | Anthocyanins | Blueberries wastes | 223.13 mg/ L | 25.00 | 60.00 | Zhou, Zhao and Huang, 2015 |
| | Anthocyanins | Sweet cherries wastes | 80.00 % | 25.00 | 35.80 | Pataro, et al., 2017 |
| | Anthocyanins | Purple fleshed potatoes | 65.80 % | 40.00 | 60.00 | Puértolas, et al., 2013 |
| | Betalains | Red beetroot | 70.00 % | 22.00 | 60.00 | Visockis, et al., 2021 |
| | Betalains | Red beetroot | 90.00 % | 30.00 | 35.00 | López, et al., 2009 |
| | Carotenoids | Carrot pomace | 19.60 µg/ g | 52.20 | 49.40 | Roohinejad, et al., 2014 |
| | Carotenoids | Tomato peels | 39.00 % | 25.00 | 90.00 | Luengo, Álvarez and Raso, 2014 |
| Supercritical fluid extraction | Anthocyanins | Grape peels | 12.31 % | 46.00 | 30.00 | Ghafoor, Park and Choi, 2010 |
| | Betalains | Red Pitaya fruit | 4.09 % | 45.00 | 90.00 | Fathordoobady, et al., 2019 |
| | Carotenoids | Bitter melon | 90.12 % | 70.00 | 90.00 | Patel, et al., 2019 |
| | Carotenoids | Vegetables waste | 90.00 % | 59.00 | 30.00 | Andrade Lima et al., 2019 |
| Eznyme-assisted extraction | Anthocyanins | Grape pomace | 63.60 % | 40.00 | 120.00 | Maier, et al., 2008 |
| | Anthocyanins | Bird cherry | 62.90 % | 45.60 | 238.80 | Swer, et al., 2018 |
| | Betalains | Red beets | 25.00 % | 25.00 | 240.00 | Lombardelli, et al., 2021 |
| | Carotenoids | Pumpkin | 61.75 % | 42.54 | 91.58 | Sharma and Sogi, 2022 |
| | Carotenoids | Tomato peels | 55.15 mg/ g | 50.00 | 240.00 | Prokopov, et al., 2017 |

### 4.2.1 Extraction yield

The pigments extraction yield for different extraction technologies presented in Table 4.6 and Table 4.7 were presented in Figure 4.5.

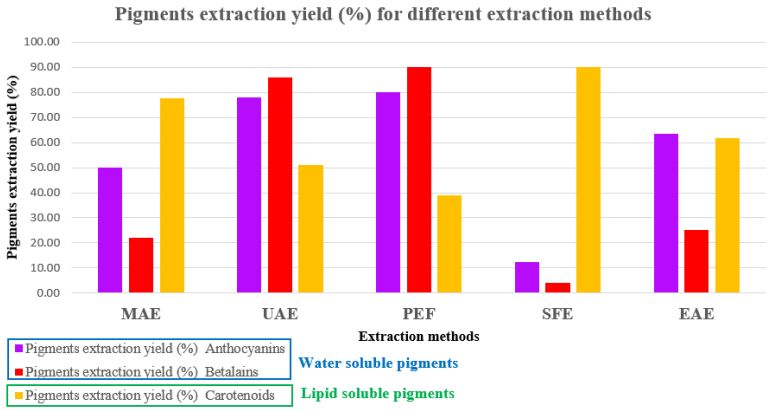


Figure 4.5: Extraction yield for different novel extraction technologies.

There were a few observations that could be drawn from Figure 4.5. In the first place, the recovery of water soluble pigments (anthocyanins and betalains) was the highest in pulsed-electric field extraction, in which the recovery rate of anthocyanins and betalains achieved the maximum value of 80.00 % and 90.00 % respectively. This signified that pulsed-electric field treatment was highly suitable to extract water soluble pigments. This hypothesis was verified by the statement that hydrophilic bioactive compounds such as anthocyanins and betalains could be recovered easily using pulsed-electric field extraction due to the polarity and presence of ions in these compounds worked well with the environment in pulsed-electric field conditions (Gachovska, et al., 2010). On the flip side, recovery rate of lipid soluble pigments in pulsed-electric field extraction was the lowest among all the techniques, reflecting the fact that non-polar pigments extraction was not suitable in pulsed-electric field extraction due to the absence of ions and low dielectric constant of non-polar pigments (Raveendran, Ikushima and Wallen, 2005).

In contrast, supercritical fluid extraction was found to be not applicable in extracting water soluble pigments as the extraction yield of anthocyanins and betalains were comparatively low, less than 15.00 %, indicating that supercritical carbon dioxide was not an ideal solvent for the

extraction of water soluble pigments because supercritical carbon dioxide was a non-polar solvent with low dielectric constant and zero molecular dipole moment, which made it not compatible with polar and hydrophilic compounds (Raveendran, Ikushima and Wallen, 2005). On the grounds that supercritical carbon dioxide was non-polar, with low critical temperature and pressure, making it an ideal solvent for the extraction of lipid soluble components such as carotenoids (Durante, Lenucci and Mita, 2014). This statement is further supported by the claims that supercritical fluid extraction technique was well suited for carotenoid recovery from carotenoid-rich fruits and vegetables wastes due to the critical properties exhibited by supercritical solvent (Andrade Lima, et al., 2019). Therefore, in order to enhance the solvation power of supercritical carbon dioxide in recovering water soluble pigments, co-solvents as modifiers such as ethanol-water mixture, methanol, aniline, toluene and diethylamine were often introduced to increase the extraction yield (Radzali, Markom and Saleh, 2020).

Aside from that, microwave-assisted extraction manifested a greater potential to isolate lipid soluble pigments than water soluble pigments. This phenomenon could be explained by the thermal nature of the microwaves radiations, which gave rise to possible thermal degradation of the recovered pigments, especially heat-sensitive pigments like betalains and anthocyanins (Sadowska-Bartosz and Bartosz, 2021). Therefore, thermal degradation was more likely to occur in water soluble pigments, resulting in the decreased extraction yield of anthocyanins and betalains. Unlike water soluble pigments, carotenoids which showed better heat resistant properties was able to withstand thermal treatment owing the fact that carotenoids was composed of a system of conjugated double bonds which makes carotenoids stable to heat or thermal treatment (Bonnie and Choo, 1999), thus the extraction yield of carotenoids were relatively higher than anthocyanins and betalains in microwave-assisted extraction.

Different from pulsed-electric field extraction, supercritical fluid extraction and microwave-assisted extraction that exhibited distinct trends in the extraction yield, ultrasound-assisted extraction showed a different pattern. In accordance to Figure 4.5, ultrasound-assisted extraction was capable of recovering both water soluble and lipid soluble pigments, but the recovery

rate was not as good as pulsed-electric field treatment for water soluble pigments and supercritical fluid extraction for lipid soluble pigments. It was suggested that ultrasound-assisted extraction was applicable for the recovery of both water soluble and lipid soluble pigments because of the non-thermal nature and the potential of using substitute solvent as the medium to extract pigments (Carreira-Casais, et al., 2021). This claim was upheld by the hypothesis that cellular effects induced by ultrasonic waves were generally classified as non-thermal effects associated with the formation of cavitation effects (Katsampa, et al., 2015). On the other hand, ultrasonic waves could be applied to recover the polar and non-polar compounds from the materials because polarity was not the preliminary factor in determining the extraction efficiency of ultrasound-assisted extraction as ultrasonic waves were able to penetrate through polar and non-polar medium to selectively recover the targeted compounds (Mijangos Ricárdez, et al., 2011).

Not least for all, it was revealed that enzyme-assisted extraction could be applied to recover both water soluble and lipid soluble pigments, however, the extraction yield was fairly lower than other extraction approaches. The fact that enzymes are applicable in polar and non-polar medium is because enzyme were made up of different amino acids molecules, in which amino acids molecules could be polar and non-polar depending on the structure and composition of the enzyme molecules (Dongmo Foumthuim, et al., 2020). In the discussion of low extraction yield found in the enzyme-assisted extraction, it was proposed that sufficient treatment time should be allocated for the actions of enzymes to digest and breakdown the rigid structures of the cell walls of fruits and vegetables wastes (Nadar, Rao and Rathod, 2018). However, time constraint was always a major concern in the extraction process, therefore insufficient extraction time would lead to lower extraction yield in enzyme-assisted extraction.

To present a conclusion in terms of extraction yield, it was figured out that pulsed-electric field extraction should be proposed to recover water soluble pigments due to its excellent recovery rate and non-thermal nature that minimise the thermal degradation of water soluble pigments. On top of that, supercritical fluid extraction was believed as the best extraction technology to isolate lipid soluble pigments from the fruits and vegetables

wastes owing the fact that supercritical carbon dioxide used was non-polar in nature and the critical conditions of the solvent offered excellent carotenoids recovery rate.

### 4.2.2 Extraction temperature

The range of extraction temperature listed in Table 4.6 and Table 4.7 were extracted and organised as Figure 4.6. This chapter aimed to determine the relationship between the extraction temperature, the extraction kinetics and the thermal degradation of pigments. In general, when the extraction kinetics will increase linearly with the extraction temperature, however, treatments involving strong heat and high temperature might trigger thermal degradation of pigments. Therefore, there were non-thermal approaches that did not rely on high extraction temperature introduced to address the issues regarding thermal degradation of the pigments. These non-thermal extraction techniques did not involve the use of heat energy, instead electrical force (ultrasound-assisted extraction and pulsed-electric field extraction), pressure force (supercritical fluid extraction) and biological force (enzyme-assisted extraction) were introduced to replace the use of thermal energy to minimise the loss of pigments during the recovery stage.

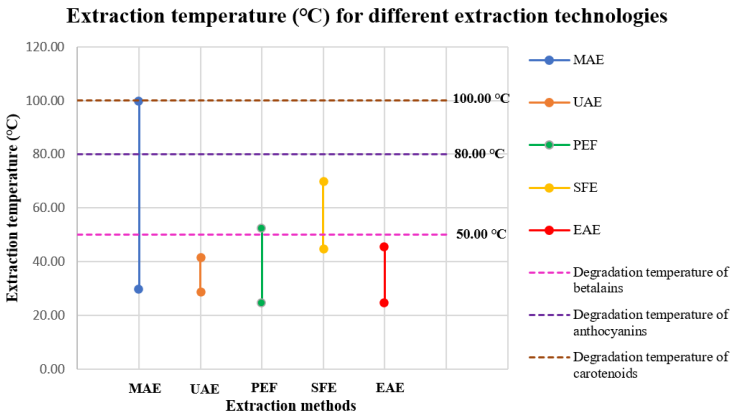


Figure 4.6: Extraction temperature for different novel extraction technologies.

There were several important pieces of information that could be retrieved from Figure 4.6, including the degradation temperature of anthocyanins, betalains and carotenoids as well as the extraction temperature suggested to be employed for different pigments recovery technologies. In

the meanwhile, the extraction temperature displayed a close relationship with the heat stability assessment performed in Chapter 4.1.2 as the heat stability assessment of pigments aided the selection of the most appropriate technique used to extract that particular pigment.

In the attempts to investigate the thermal effects on the extraction of anthocyanins, there was a study showed that an increase in temperature would trigger an increase in the extraction of anthocyanins, however a drastic decrease in anthocyanins extraction was observed at 80.00 ℃ (Lasunon, et al., 2018). For betalains, there was a thermal stability test conducted and it showed that betalains started to degrade from 50.00 ℃ and this statement is supported by the results observed that when the temperature was increased, the half-life of betalains decreased (Santos, et al., 2018). On the other hand, it was found that thermal degradation of carotenoids occur at temperatures over 100.00 ℃ because the *trans* structure of carotenoids will be altered to *cis* structure, leading to the reduction in biological activity and colour changes of carotenoids (Gheonea, et al., 2020).

As presented in Figure 4.6, there were several interpretations that could be made. Primarily, microwave-assisted extraction covered a broad range of extraction temperature, ranging from 30.00 ℃ to 100.00 ℃, and the extraction temperature exceeded all the degradation temperature of pigments. This suggested that potential thermal degradation of pigments will be triggered during the pigments recovery process, leading to loss and deterioration of the pigments. However, extraction of heat-sensitive pigments like belatains could be conducted by using microwave-assisted extraction on the grounds that the extraction temperature could be reduced below the degradation temperature of betalains (50.00 ℃). Thirugnanasambandham and Sivakumar (2017) demonstrated the use of microwave radiations in the recovery of betalains from beetroot peels, but the extraction yield was unsatisfactory, only 22.00 % of betalains could be recovered using this approach, indicating that microwave-assisted extraction might not be a suitable technique to be used in the recovery of betalains. The reason that the extraction yield for betalains and anthocyanins were comparatively lower than for carotenoids by using microwave-assisted extraction was due to the kinetics of extraction. In microwave-assisted extraction, microwaves were

applied to heat the solvents to induce release of pigments as results of cellular breakdown. When the extraction temperature is lowered, the extraction kinetics will be brought down as well, leading to inefficient extraction process and poor extraction yield (Llompart, et al., 2019). Another hypothesis that could be drawn from Figure 4.6 was that microwave-assisted extraction is an extraction technique that involves thermal natures. This hypothesis was confirmed by the microwave theory which stated that microwaves involving heating of plant matrices were induced as results of the interactions between the microwaves and the solvents, resulting in the temperature elevation in the extraction medium (Uzel, 2018).

Dissimilar to microwave-assisted extraction, other extraction technologies were non-thermal in nature. This statement was supported by the fact that the extraction temperature for other technologies fell below the degradation temperature of the most heat-sensitive pigments, betalains, except for pulsed-electric field extraction and supercritical fluid extraction. However, as shown in Table 4.7, the extraction temperature of pulsed-electric field extraction was normally below 40.00 ℃ for anthocyanins and betalains, only extraction of carotenoids involved the highest extraction temperature at 52.20 ℃, which was below the degradation temperature of carotenoids. Therefore, it could be said that thermal degradations of pigments was less likely to occur in pulsed-electric field extraction. This assumption was upheld by the fact that pulsed-electric field extraction was non-thermal in nature as this technique was dependent on the use of short electricity pulses to induce electroporation of plant matrices that trigger cell disintegration and rupture to release the pigments from the plant matrices, with no temperature elevation and thus no degradation of pigments due to thermal effects could be observed (Ranjha, et al., 2021).

Same goes to supercritical fluid extraction, it was known that supercritical fluid extraction was not well suited for the extraction of betalains and anthocyanins due to the extremely low extracted yield resulted, therefore the extraction of carotenoids was preferred in supercritical fluid extraction and the degradation temperature of carotenoids falled above the extraction temperature of supercritical fluid extraction, reflecting the fact that thermal degradation of carotenoids would not occur in supercritical fluid

extraction on the grounds that supercritical fluid extraction involved slight changes in the temperature within the extraction medium, therefore no significant temperature fluctuations during the extraction process and thus thermal degradation of pigments could be minimised (Wrona, et al., 2017).

For ultrasound-assisted and enzyme-assisted extractions, the extraction temperatures were relatively low and close to ambient temperature, as illustrated in Figure 4.6. This was because both approaches did not rely on the extraction kinetics induced by increased extraction temperature. It was stated that ultrasound-assisted extraction did not use any heat as most of the extraction process could be maintained at ambient temperature, 25.00 ℃ (Pawliszyn, et al., 2012). The reason that enzyme-assisted extraction was non-thermal in nature in the view of the fact that high heat or high temperature will disrupt and alter the shape of the active sites of enzymes, reducing its biological activity and triggered the denaturation of enzyme (Calderón-Oliver and Ponce-Alquicira, 2021).

Table 4.8: Summary of the extraction temperature analysis.

| **Extraction technologies** | **Nature** | **Potential thermal degradation of pigments** | | |
|---|---|---|---|---|
| | | **Anthocyanins** | **Betalains** | **Carotenoids** |
| MAE | Thermal | Yes | Yes | Yes |
| UAE | Non-thermal | No | No | No |
| PEF | Non-thermal | No | No | No |
| SFE | Non-thermal | No | No | No |
| EAE | Non-thermal | No | No | No |

Table 4.8 made an effort to summarise the analysis of extraction temperature for different extraction technologies based on the nature of the extraction methods (thermal or non-thermal) and potential thermal degradation of pigments during extraction. In a nutshell, microwave-assisted extraction was the only approach that involved the use of thermal energy as the extraction temperature displayed a linear relationship with the extraction kinetics, leading to potential thermal degradation of pigments during the recovery of pigments. In contrast, ultrasound-assisted extraction, pulsed-electric field extraction, supercritical fluid extraction and enzyme-assisted extraction were non-thermal approaches that were independent on the extraction kinetics owing the fact that these non-thermal approaches could

enhance mass transfer rates by increasing the cell permeability and diffusion, resulting in higher extraction yields at room temperature, with thermolabile pigments structures preservations, therefore there was no significant or lower effects on the structures of the recovered pigments (Moreira, et al., 2019).

### 4.2.3 Extraction time

Extraction time is another important parameter in the comparison between the extraction approaches used apart from extraction yield and extraction temperature. The extraction time required in the extraction technologies were summarised and presented as Figure 4.7.

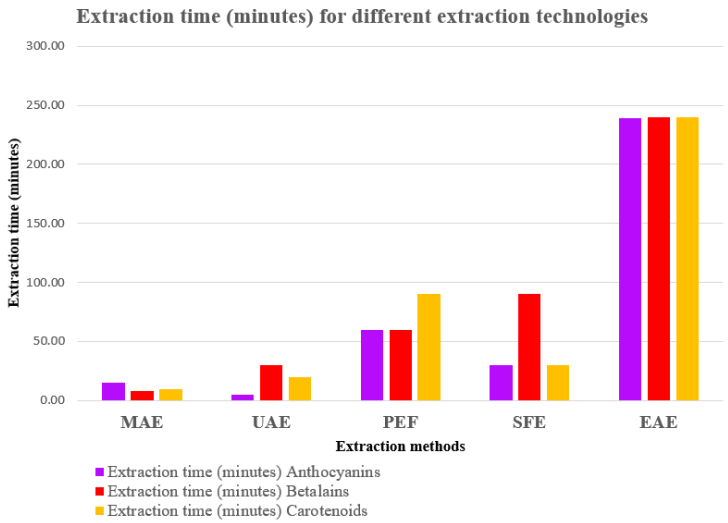


Figure 4.7: Extraction time required for different extraction technologies.

The first distinct observation that could be retrieved from Figure 4.7 was biological force extraction (enzyme-assisted extraction) consumed the longest extraction time, generally 240.00 minutes (2 hours) as compared to other extraction methods. This might be due to the complex structures of the fruits and vegetables matrices that cause enzymatic action requiring a longer time to digest the structures such as cell walls before the pigments contained inside the matrices released to the solvent (Streimikyte and Viskelis, 2022). Sharma and Sogi (2022) suggested long incubation period up to 4 hours should be allocated for the action of enzymes molecules to achieve complete cellular removal and to maximise the recovery rate of carotenoids from pumpkin wastes, however the maximum extraction yield was not as high as

the expected results, only 61.75 % of carotenoids was recovered at the end of the process. This manifested that long extraction time did not reflect the fact that greater extraction yield could be achieved in a long incubation period.

Another notable feature that could be observed from Figure 4.7 was that microwave-assisted consumed the shortest extraction time, less than 20.00 minutes among all the extraction methods. This could be explained by the action of microwave radiation promoting faster heating and release of the pigments from the plant matrices to the solvent, therefore significantly reducing the extraction time needed (Llompart, et al., 2019). This stand was upheld by the theory which proposed that the energy carried by the microwaves enabled deep solvent penetrations into the sample matrices, leading to the increase of extraction rate due to the increase in the kinetics of extraction, therefore speeding up the extraction process (Sanchez-Prado, et al., 2015).

On the other hand, ultrasonic-assisted extraction was found to be the second extraction method that promoted the shortest extraction time, typically less than 40.00 minutes. Ultrasound-assisted extraction promoted faster extraction period on the grounds that the cavitation effect and mass transfer enhancement induced by ultrasonic waves were able to create more effective mixing and micromixing in the extraction process, therefore the extraction time could be decreased significantly (Chemat, et al., 2017). This scientific claim was further strengthened by the argument that suggested that the propagation of ultrasonic waves could ensure greater solvent penetration into the matrices of the samples, therefore maximising the contacts between the pigments molecules and the solvent molecules, which led to drastic increase in mass transfer rates and reduction of working times (Yolmeh, Habibi Najafi and Farhoosh, 2014).

For pulsed-electric field extraction, it was found that at least 60.00 minutes extraction time should be allocated for the recovery of water soluble pigments, whereas for lipid soluble pigments, the extraction time required was increased to approximately 90.00 minutes. This could be due to the fact that pulsed-electric field extraction was more appropriate in extracting water soluble pigments, therefore the extraction time required was lesser. Same as microwave-assisted extraction and ultrasound-assisted extraction, pulsed-

electric field extraction was a technique that relied on electrical force, however the extraction time required for pulsed-electric field extraction was longer than the other two electrical force extraction approaches. Pappas, et al. (2021) pointed out the reason that pulsed-electric field extraction consumed a longer extraction time was due to the requirement of induction time required for pulsed-electric field treatment. Induction time referred to the period of time that was necessary to initiate a reaction and allowed the reaction to reach a necessary stage (Pappas, et al., 2021). In pulsed-electric field treatment, a variety of electrochemical reactions took place simultaneously, enhancing the mass transfer and diffusion rate of the pigments molecules and solvent molecules, therefore an induction period of 30.00 minutes should be allocated the treatments involved pulsed-electric field applications (Putnik, et al., 2018). Although the extraction time required was longer, the extraction yield recorded the greatest results for both anthocyanins and betalains in pulsed-electric field treatment, approximately 80.00 % and 90.00 % respectively.

Apart from that, pressure force-assisted extraction (supercritical fluid extraction) showed a similar trend as pulsed-electric field extraction as the extraction time required was longer than microwave-assisted and ultrasound-assisted extraction, ranging from 30.00 minutes to 90.00 minutes. Beckman (2004) suggested that supercritical fluid extraction consumed greater extraction time due to the allocation for static extraction time and dynamic extraction time. It was claimed that 20.00 minutes dynamic extraction time and 10.00 minutes static extraction time should be allocated for the supercritical fluid extraction process (Yilmaz, 2020). Besides, the extraction time for carotenoids in supercritical fluid extraction process was generally less than recovery in water soluble pigments, 30.00 minutes. This might be due to the fact that supercritical fluid extraction was highly suitable for the recovery of carotenoids due to the non-polar nature of supercritical carbon dioxide, reaching a maximum yield of 90.12 %.

To come to the point, it was figured out that enzyme-assisted extraction took up the longest extraction time, comparatively longer than other approaches. However, longer extraction time did not guarantee greater extraction yield as reflected in Table 4.7 as the extraction yield for all the pigments were generally lower than other approaches. In contrast,

microwave-assisted and ultrasound-assisted extraction consumed the shortest extraction time due to the mechanisms involved in these two approaches able to shorten the extraction time efficiently, but the extraction yield was lower than pulsed-electric field extraction and supercritical fluid extraction. For pulsed-electric field and supercritical fluid extraction, sufficient induction time, static extraction time and dynamic extraction were encouraged to ensure the maximum recovery of pigments. In conclusion, pulsed-electric field extraction and supercritical fluid extraction manifested the best performance in the recovery of pigments in terms of moderate extraction time involved and maximum pigments recovery. Table 4.9 summarize the analysis of extraction time needed in different extraction technologies.

Table 4.9: Summary of the extraction time analysis for different extraction technologies.

| Extraction technologies | Extraction time required | Descriptions |
|---|---|---|
| MAE | Short (Less than 15 minutes) | Rapid mass transfer occurred as results of increased extraction kinetics due to heating effects induced in the extraction. |
| UAE | Short (Less than 30 minutes) | Fast extraction process due to cavitation effects and effective micromixing. |
| PEF | Moderate (35 to 90 minutes) | Sufficient induction time for the electrochemical reactions was required to achieve maximum pigment recovery. |
| SFE | Moderate (30 to 90 minutes) | Allocation of static and dynamic extraction time was necessary to ensure optimum extraction of pigments. |
| EAE | Long (240 minutes) | Biological breakdown of cellular structures of plant matrices was time consuming. |

### 4.2.4 Complexity of the separation process

In general, for assisted solvent extraction method, such as microwave-assisted extraction, ultrasonic-assisted extraction and pulsed-electric field extraction, the solvents, together with the extracted pigments will first be separated by gravity means, and the extracted pigments will be recovered

through filtration, centrifugation, or evaporation of the solvents under controlled conditions. However, for different extraction approaches, the complexity of the separation process will be varied since the types of solvent, medium and technologies used in the extraction are different.

In microwave-assisted, ultrasound-assisted and pulsed-electric field extraction of pigments, the medium used to extract the solvent involve a solvent and electrical force, without addition of any external solvents or other materials. Hence, the separation of the extracted pigments will only involved the isolation of the pigments from the solvent and the other impurities such as fruits and vegetables waste matrices, which will be subjected to further purification processes (Chemat, Vian and Cravotto, 2012).

The pigments extracted by using supercritical carbon dioxide as solvent could be easily separated by simply depressuriaing (Kitada, et al., 2009). This was because the separation of the extracted pigments from the supercritical $CO_2$ could be conducted in a separator by using pressure reduction approaches to yield a solvent-free extract. In the meanwhile, the depressurized $CO_2$ can be recycled or released to the atmosphere since supercritical $CO_2$ is a green solvent recognised by the European Food Safety Authority and the United States Food and Drug Administration (Nunes, et al., 2022). There was another claim which verified that a supercritical fluid could be separated from the extracts by simply releasing the pressure of the fluid, leaving almost no trace and yielded a pure residue (Ibáñez, Mendiola and Castro-Puyana, 2015). This showed that the supercritical fluid extraction involved least separation process due to the nature of the extraction solvent that can be easily separated from the extracted pigments by using rapid depressurisation, and thus the separation process was simple and short.

In the enzyme-assisted extraction, in most cases, not a single type of enzyme was to be applied, instead a combination of different enzymes were used to selectively extract the pigments from the fruits and vegetables matrices (Sowbhagya and Chitra, 2010). Unlike the other solvents that were used in the extraction, enzymes were normally composed of biological large molecules such as proteins. Like other protein molecules, enzymes can be separated on the basis of polarity such as their net charge, charge density and hydrophobic interactions. When it came to the separation of extract resulting

from enzyme-assisted extraction, the extract was made up of the recovered pigments, fruits and vegetables waste matrices, enzyme mixture and the solvent solution. The cost of enzymes was relatively high, therefore it is necessary to separate the enzyme from the extract so that the enzyme solution can be recycled and reused again in the extraction process. Therefore, it was suggested that the separation process involved in the enzyme-assisted extraction will be longer and more complicated than other extraction methods due to the need to separate a greater number of enzyme mixtures and solvent.

To conclude, supercritical fluid extraction required the least steps for the separation of extracted pigments as the supercritical solvent can be separated from the pigments by simply depressurizing action, followed by microwave-assisted, ultrasound-assisted and pulsed-electric field extraction which involves the main separation between the solvent and the recovered pigments. Lastly, enzyme-assisted extraction involved the largest number of steps in the separation process as the recovered pigments were required to be isolated from the enzyme and solvent mixture. Table 4.10 summarised the analysis of the complexity of separation process required for different extraction technologies.

Table 4.10: Summary of the complexity of separation process involved in different extraction technologies.

| Extraction methods | Complexity of separation process | Reason |
|---|---|---|
| MAE | Moderate | Separation of solvent with the extracts. |
| UAE | Moderate | Separation of solvent with the extracts. |
| PEF | Moderate | Separation of solvent with the extracts. |
| SFE | Low | No separation of solvent was needed. |
| EAE | High | Separation of enzyme solutions, solvents and the extracts. Complicated procedures involved to recycle the enzyme solution. |

### 4.2.5 Quality of the extracted pigments

The quality of the pigments extracted from the extraction process was very much dependent on the operating conditions of the extraction process such as temperature, pH, light exposure and oxygen concentration. During the

extraction process, undesired products loss, deterioration and degradation were possible to occur due to the extreme conditions involved.

In all the extraction technologies, the variables such as light exposure and oxygen concentration were normally fixed and controlled, the manipulated variables were the extraction temperature and the pH of the medium. In microwave-assisted extraction, thermal treatment was involved, and the presence of high heat tends to trigger thermal degradation of the pigments, especially for the heat-sensitive pigments such as betelains. This was because the extraction temperature for microwave-assisted extraction normally ranged from 30.00 ℃ to 100.00 ℃, which exceeded the degradation temperature of all the pigments studied. This signified that thermal degradation of anthocyanins, betalains and carotenoids tend to occur as the extraction temperature was more than the degradation temperature of pigments. For other extraction approaches, the extraction temperature was normally less than 80.00 ℃, therefore it was proposed that thermal degradation was less likely to occur in other extraction methods due to non-thermal nature.

Apart from operating temperature, pH was another critical factor that determined the stability and quality of the recovered pigments. Among all the extraction technologies, only enzyme-assisted extraction involved extreme pH conditions on the grounds that different enzymes had different optimum pH. Some specific enzymes only worked well in extremely acidic (pH less than 5.00) or alkaline (pH more than 9.00) conditions (Lin, Chen and Ding, 2013). Below or beyond the stable pH range, destruction of pigments will occur, which trigger colour changes of the extracted pigments. Thus, it can be said that enzyme-assisted extraction has the potential to cause deterioration of pigments.

For other extraction technologies, it was claimed that supercritical fluid extraction yielded the best quality of extracts due to the nature of supercritical solvent used. The supercritical fluid extraction involved non-thermal nature and no extreme pH conditions was found, and the supercritical solvent could be separated easily from the extracts, leaving a pure residue (Ibáñez, Mendiola and Castro-Puyana, 2015). Therefore, it was expected that the pigments recovered from supercritical fluid extraction displayed the

greatest quality. Unlike supercritical fluid extraction, isolation of pigments from the solvent remained as the largest issue for ultrasound-assisted and pulsed-electric field extraction. Both ultrasound-assisted and pulsed-electric field extraction utilised electrical means to induce the release of pigments from the internal spaces of the plant matrices, with no extreme temperature and pH conditions involved. However, potential degradation of pigments might occur during the separation process as certain separation processes such as distillation might affect the quality of the recovered pigments (Moreira, et al., 2019). Thus, it could be concluded that the quality of the recovered pigments from ultrasound-assisted and pulsed-electric field extraction were better than microwave-assisted and enzymatic-assisted extraction, but lower as compared with the pigments recovered from supercritical fluid extraction.

In a nutshell, microwave-assisted extraction was able to trigger thermal degradation of the extracted pigments and enzyme-assisted extraction displayed the potential to induce deterioration of pigments under extreme pH conditions. On the flip side, possible degradation of pigments might occur during the separation stages involved in pulsed-electric field extraction and ultrasound-assisted extraction. In the meanwhile, supercritical fluid extraction was believed to yield the best quality of the recovered pigments due to the nature of supercritical fluid used. Table 4.11 depicted the summary for the analysis on the quality of the recovered pigments from different extraction methods.

Table 4.11: Summary for the analysis on the quality of recovered pigments from different extraction technologies.

| **Extraction methods** | **Quality of pigments** | **Explanations** |
|---|---|---|
| MAE | Low | Potential thermal degradation of pigments. |
| UAE | Moderate | Possible deterioration of pigments during separation stages. |
| PEF | Moderate | Possible deterioration of pigments during separation stages. |
| SFE | High | No separation stage required, non-thermal approach and no extreme pH conditions found. |
| EAE | Low | Potential degradation of pigments due to extreme pH working conditions. |

## 4.3 Comparison of novel extraction technologies based on green assessment

For green assessment, the environmental impacts resulting from the technologies used was always a major concern. The environmental impacts arised from the technologies should be considered as the improper discharge of the solvents used in the extraction such as methanol, acetone or hexane which are toxic in nature will trigger environmental pollution. Therefore, green extraction was gaining attention in this area as it helped to reduce the environmental impacts caused by the extraction process by allowing the use of green solvents which were more environmentally friendly and renewable natural products such as fruits and vegetable wastes to produce highly value added and safe product (Chemat, Vian and Cravotto, 2012). Figure 4.8 illustrated the green assessment to be studied in Chapter 4.3.

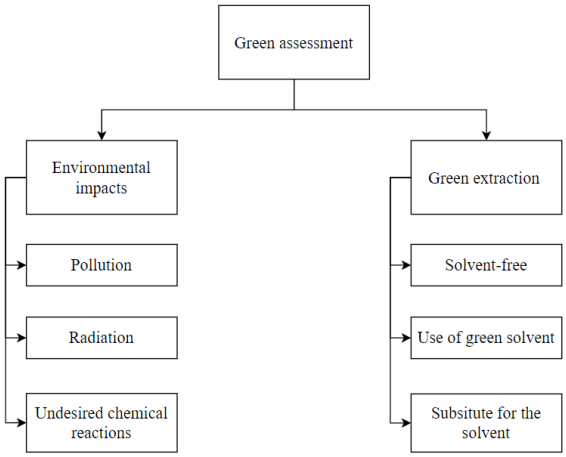


Figure 4.8: Criteria involved in green assessment of extraction technologies.

### 4.3.1 Environmental impacts

In the efforts to investigate the potential environmental impacts arised from the extraction technologies, criteria such as the release of greenhouse gases, generation of free radicals, potential hazardous chemical reactions, environmental pollution and other relevant factors should be taken into consideration.

In fact, scientists conducted numerous experiments to investigate the effects of microwaves on the environment, and some findings confirmed that adverse effects of microwave radiation exist. For example, it was found that

the application of microwaves will release millions of tons of carbon monoxide into the environment every year (Jayasanka and Asaeda, 2014). However, the environmental impacts created by microwave radiation was greatly dependent on the frequency of the microwaves used. In the microwave-assisted pigment extraction, the most commonly used frequency reported was 2450.00 MHz and it was stated that this frequency is less likely to create environmental impacts (Gallego-Schmid, Mendoza and Azapagic, 2018). Thus, it could be said that a typical microwave-assisted pigments extraction process would not create significant damages to the environment under well controlled conditions.

In the meanwhile, ultrasonication was found to cause lower environmental impacts as compared with conventional pigments extraction methods due to the elimination of toxic chemicals used in the extraction process (Pham, et al., 2009). However, cavitation events tend to occur rapidly in ultrasound-assisted pigment extraction. The collapse of cavities inside the bubbles will generate highly free radicals as results of homolytic cleavage of water molecules and dissolved gases like oxygen (Panda and Manickam, 2019). The radicals generated could act as primary reagents to produce hazardous compounds such as hydrogen peroxide ($H_2O_2$), nitrous acid ($HNO_2$) and nitric acid ($HNO_3$) as consequences of the association and rearrangement of hydroxyl radicals reacted with the dissolved nitrogen and oxygen when ultrasonication was conducted (Korn, et al., 2003). This signified that release of free radicals was possible to happen during the ultrasound-assisted extraction.

Pulsed-electric field extraction involved the use of high voltage pulses across two electrodes. However, a very limited number of published studies had addressed the effects of the pulsed-electric field extraction process on the environment. It was no doubt that in the pulsed-electric field treatments, unavoidable electrochemical reactions might take place accompanying the flow of electric current through the treatment chamber and these electrochemical reactions could cause harmful effects to the environment and human beings. In order to overcome this problem, a carbon electrode was suggested to be used to replace the stainless steel electrode and

application of shorter pulses or switching systems without leak current to minimise the hazards (Kumar, Patel and Kumar, 2015).

Unlike other hazardous organic solvents, supercritical carbon dioxide was widely used as a green solvent. It was unquestionably that carbon dioxide was a greenhouse gas but if carbon dioxide was withdrawn from the environment and used in the extraction process, and returned to the environment, this will not contribute to any greenhouse effect (Beckman, 2004). As a matter of fact, supercritical carbon dioxide was widely known as a Generally Recognised As Safe (GRAS) solvent type as it could selectively extract the targeted pigments from the fruits and vegetables wastes matrices without leaving any toxic residues in the extracts (Razi Parjikolaei, et al., 2015). Nevertheless, the nature of supercritical carbon dioxide made it a green solvent as it was odourless, chemically inert and stable, non-flammable, non-toxic, non-explosive and easily handled fluid (Promila and Madan, 2018). Therefore, it could be said that extraction of pigments from fruits and vegetables wastes using supercritical carbon dioxide was less likely to cause environmental impacts.

Enzyme solutions, which were mainly composed of proteins, were readily and ultimately biodegradable in the environment (Becker, Lütz and Rosenthal, 2021). Enzyme solution manifested the potential to replace the use of toxic and hazardous solvents such as methanol in the extraction of pigments from fruits and vegetables matrices owing the fact that various combinations of enzymes can be used to loosen the structural integrity of the fruits and vegetables cell walls structures thereby enhancing the extraction of the pigments. The enzyme-assisted pigment extraction process was green as no hazardous residues or by-products were generated during the extraction process, and the used enzyme solutions could be discharged to the environment due to its biodegradable properties (Sowbhagya and Chitra, 2010).

To sum up, it was found that microwave-assisted extraction, ultrasonic-assisted extraction and pulsed-electric field extraction demonstrated the possibility to trigger environmental impacts whereas supercritical carbon dioxide extraction and enzyme-assisted extraction were environmental friendly approaches that were less likely to cause side effects

on environment and human being. Table 4.12 gave an outline on the summary for the environmental impacts analysis on different extraction technologies.

Table 4.12: Summary of the environmental impacts analysis on different extraction technologies.

| Extraction methods | Environmental impacts | Descriptions |
|---|---|---|
| MAE | Low | Potential release of carbon monoxide gas but the impact was insignificant under well-controlled conditions. |
| UAE | High | Possible generation of free radicals and hazardous compounds such as hydrogen peroxide and nitrous acid. |
| PEF | Low | Harmful electrochemical reactions might cause harm to the environment and human beings. |
| SFE | No | Supercritical carbon dioxide was recognised as a green solvent which creates no environmental impacts. |
| EAE | No | Enzyme solutions were readily biodegradable and environmentally friendly. |

### 4.3.2 Green extraction assessment

In the context of green chemistry, green extraction could be interpreted as the design, invention and application of products and processes to minimise or to fully wipe out the use and generation of hazardous substances. There were a few principles involved in green extraction. Primarily, the materials selected for green extraction should be renewable resources such as fruits and vegetables wastes or agricultural wastes. In the second place, alternative solvents, principally water, agro-solvents or eco-solvents should be used instead of hazardous and toxic organic solvents. Not least of all, the extraction process should aim for a non-denatured and biodegradable extract (Chemat, Vian and Cravotto, 2012).

In order to judge whether the extraction process was green or sustainable, the solvent used in the extraction process was always a main issue to be discussed. By way of an illustration, the polar solvent used in the extraction of hydrophilic pigments such as methanol, ethanol, acetone was highly toxic, if it was discharged to the environment without proper handling,

it might cause environmental pollution. Instead, green solvent such as water or oil should be applied to substitute the use of hazardous solvent. This phenomenon accelerated the invention of green extraction and the concepts of green extraction are widely used nowadays. On the other hand, it could be possible that extraction of pigments can be conducted in a solvent-free condition. This implied that the separation of solvents from the recovered pigments could be avoided, which substantially reduced the complexity of the separation and purification processes.

Green solvents such as water and vegetable oil manifested the ability to substitute organic or petroleum-based solvents in pigments extraction. Sharma and Bhat (2012) conducted an experiment to extract carotenoids pigments from pumpkin peel and pulp by using corn oil as solvent in the presence of microwave heating as an innovative green extraction approach. The outcomes showed amazing improvements in which the yield of carotenoids was almost doubled on employing corn oil as solvent (33.78 µg/ g) when compared to conventional extraction that uses hexane as solvent (16.21 µg/ g). The similar pattern could also be found in the pulsed-electric field extraction that utilised olive oil as solvent to replace the use of ethanol in the recovery of carotenoids from carrot wastes, in which the extraction yield was increased from 15.61 µg/ g to 34.16 µg/ g (Putranto, Argo and Wijana, 2014). In the meanwhile, the extraction time could be shortened effectively when green solvent is used to replace the organic solvent. Li, Fabiano-Tixier, and Tomao, et al. (2013) proven that the extraction time could be reduced from 60.00 minutes to 20.00 minutes when hexane solvent was substituted by sunflower oil in an ultrasound-assisted extraction. On the other hand, enzyme-assisted extraction also demonstrated the possibility of using green solvent such as mixture of mint oils and hydrophobic acids which are readily biodegradable to replace the use of harmful organic solvent *n*-hexane in the extraction of carotenoids from sunflower wastes, and the results showed satisfying outcomes in which the total carotenoids obtained at the end of the extraction process using sunflower oil is 1449.00 mg/ 100g, which was 2.30 times greater than the extraction results using *n*-hexane as solvent (Ricarte, et al., 2020). For supercritical fluid extraction, the supercritical fluid used in the pigments recovery process such as supercritical carbon dioxide

was actually a green solvent recognized as Generally Recognised As Safe (GRAS) solvent type. Therefore, supercritical fluid extraction was also called as green extraction in different published studies. Table 4.13 gave an outline on the potentials of green solvent to be employed during the extraction process to replace the use of petroleum-based or organic solvents for microwave-assisted, ultrasound-assisted, enzyme-assisted and pulsed-electric field extraction.

Table 4.13: Alternative solvents or green solvents that could be applied in different novel extraction technologies.

| Extraction methods | Pigments recovered | Materials | Alternative or Green Solvents used | Remarks | References |
|---|---|---|---|---|---|
| MAE | Carotenoids | Pumpkin waste | Corn oil | The extraction yield was doubled when using corn oil to replace organic solvent, from 16.21 µg/ g to 33.78 µg/ g. | Sharma and Bhat, 2021 |
| UAE | Anthocyanins | Purple sweet potatoes skins | Water | The maximum extraction yield of anthocyanins is 4.72 mg/ 100g. | Zhu, et al., 2016 |
| | Carotenoids | Pomegranate wastes | Vegetable oils | Extraction with vegetable oils using ultrasounds could reach maximum yield up to 93.80 %. | Goula, et al., 2017 |
| | Carotenoids | Carrot wastes | Sunflower oil | The highest carotenoids yield (334.75 mg/L) was obtained in 20 minutes treatment by using sunflower oil, while the maximum carotenoids yield (321.35 mg/L) using hexane required 60 minutes treatment. | Li, Fabiano-Tixier, and Tomao et al., 2013 |
| PEF | Anthocyanins | Purple fleshed potatoes | Water | The extraction yield of using water (65.8 mg/ 100g) was higher than using ethanol as solvent (63.90 mg/100 g). | Puértolas, et al., 2013 |
| | Carotenoids | Carrot pulp | Olive oil | The maximum yield obtained using olive oil as solvent is 104.64 µg/ g, which was 6 times higher than the extraction yield using ethanol as solvent (15.61 µg/ g). | Putranto, Argo and Wijana, 2014 |
| EAE | Carotenoids | Sunflower wastes | Mint oils and hydrophobic acid mixture | The enzyme-assisted extraction using hydrophobic green solvent achieved a recovery of carotenoids (1449.00 mg/ 100 g) 2.30 times higher than the standard solvent *n*-hexane. | Ricarte, et al., 2020 |

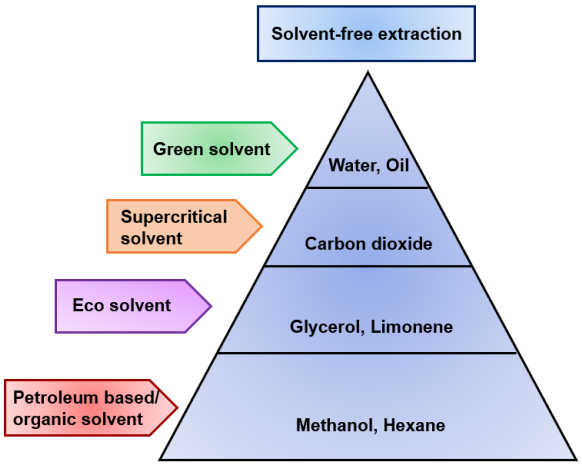


Figure 4.9: Development of alternative solvents to solvent-free extraction (Chemat, et al., 2015).

Due to decreasing fossil resources, increasing energy prices and objectives to reduce the greenhouse gases emission, scientists and researchers were being challenged to figure out new technologies to reduce or fully eliminate the use of solvent in order to minimise the energy consumption as well as to meet the legal requirements on the emissions process during extraction. Figure 4.9 depicted the efforts to find alternative solvents for petroleum based or organic solvents by introducing eco solvent, supercritical solvent and green solvent, finally solvent-free extraction is developed (Chemat, et al., 2015). Solvent-free extraction found a niche market in the pigments extraction process because it helped to eliminate the use of solvent, which subsequently minimised the risk of medium contamination during the extraction process. Apart from that, solvent-free extraction is found to be a sustainable process, as no supplementary energy is required in the separation phases as well as the cost of solvent was eliminated, leading to a more economic process.

In order to meet the goal of green extraction, solvent-free microwave extraction was first proposed by Zill-e-Huma, et al. (2009) to recover bioactive compounds such as pigments, antioxidants and flavonoids from onion wastes. This effort was continued by Périno-Issartier, et al. (2011) by extracting β-carotene from sea buckthorn fruit wastes. This showed that solvent-free microwave extraction could eliminate the use of solvent in the extraction process. The principle of solvent-free microwave extraction was

based on the integration of microwave heating and earth gravity under atmospheric pressure, therefore this technique was also known as microwave hydrodiffusion and gravity (MHG) (Li, Fabiano-Tixier and Vian, et al., 2013).

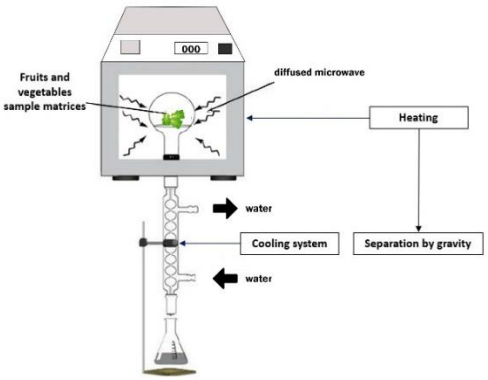


Figure 4.10: Microwave hydrodiffusion and gravity technique (Vian, et al., 2008).

In the microwave hydrodiffusion and gravity extraction, the fruits and vegetables waste sample will be placed in a microwave reactor without addition of any solvent or water. By heating the internal water present in the sample matrices, the plant cells will rupture and disintegrate to release the bioactive compounds contained in the sample matrices. The extracts will be directed to drop out of the microwave reactor under the action of gravity and pass through a cooling system outside the reactor through a perforated Pyrex disc (Vian, et al., 2008) , as shown in Figure 4.10. Figure 4.11 depicted the internal mechanisms occuring in the sample matrices during the extraction process. The proposed solvent-free microwave extraction technique was proven to be a sustainable process due to the elimination of large quantities of solvent used and voluminous extraction vessels.

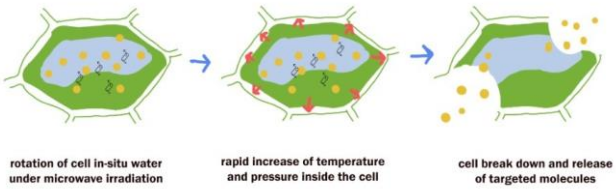


Figure 4.11: Internal mechanisms during microwave hydrodiffusion and gravity technique (Li, Fabiano-Tixier and Vian, et al., 2013).

In the attempts to develop a solvent-free extraction strategy, it was crucial to gain a clear idea in which not every extraction technique could be conducted in a solvent-free condition. In particular cases such as supercritical fluid extraction, the use of solvent was compulsory as the supercritical solvent served as the carrier to extract the pigments enclosed in the sample matrices. Same goes as enzyme-assisted extraction, solvent-free condition was not applicable as enzyme was responsible to break and digest the rigid cell wall structures of the sample matrices, and the pigments released should be carried by the solvent instead of the enzyme mixture. Unlike the microwave radiation, ultrasonic waves and electric fields could not induce internal heating effects in the plant matrices, therefore the solvent-free condition was also not applicable in ultrasound-assisted extraction and pulsed-electric field extraction.

In closing, the use of alternative solvents or green solvents in the recovery of pigments were proven to exhibit remarkable improvements on the extraction process in terms of extraction yield, reduction of extraction time and reduction of solvent costs. This was because renewable green solvents such as water and oils were relatively cheaper and more abundant than petroleum-based or organic solvents. Apart from that, a solvent-free extraction process could be introduced for microwave-assisted extraction to fully wipe out the use of extraction solvents, but not applicable for the other extraction methods.

## 4.4 Summary of the comparison for the novel extraction technologies

Table 4.14: Summary of the comparison between novel extraction technologies.

| Parameters | MAE | UAE | PEF | SFE | EAE |
|---|---|---|---|---|---|
| Extraction yield of anthocyanins | Moderate | High | High | Low | Moderate |
| Extraction yield of betalains | Low | High | High | Low | Low |
| Extraction yield of carotenoids | High | Low | Low | High | Moderate |
| Recovery of water soluble pigments | Low | High | High | Low | Moderate |
| Recovery of lipid soluble pigments | High | Low | Low | High | Moderate |
| Extraction temperature (°C) | 30.00 to 100.00 | 29.00 to 40.00 | 22.00 to 52.20 | 45.00 to 70.00 | 25.00 to 50.00 |
| Extraction time (minutes) | Less than 20.00 | Less than 40.00 | Less than 90.00 | Less than 90.00 | Generally 240.00 or more |
| Involvement of thermal approach | Yes | No | No | No | No |
| Involvement of extreme pH | No | No | No | No | Yes |
| Complexity of separation process | Moderate | Moderate | Moderate | Low | High |
| Quality of the recovered pigments | Low | Moderate | Moderate | High | Low |
| Environmental impacts | Emission of carbon monoxide | Generation of free radicals | Electrochemical reactions | No | No |
| Potential use of green solvent | Yes | Yes | Yes | Not required | Yes |
| Ecological safety | High | Low | Moderate | High | High |
| Potential to employ solvent-free extraction | Yes | No | No | No | No |

# CHAPTER 5

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

In short, parameters such as colour, solubility, structural formula, structural affinities and functional groups arrangements were used to study the characteristics of the targeted pigments. Anthocyanins were normally found in red, blue or purple form, which were hydrophilic in nature due to the presence of flavylium cations which gave anthocyanins a polar nature. Besides, the notable functional groups and bonds that present in anthocyanins were hydroxyl group, methoxy group and glycosidic bonds. In the meanwhile, betalains normally existed in red, violet, yellow or orange colour, with hydrophilic properties as polar nitrogenous components existed in betalains such as amino groups and aldimine bonds. As opposed to anthocyanins and betalains, carotenoids were hydrophobic in nature and existed mainly in yellow or orange colours. The highly unsaturated structure of carotenoids with the presence of multiple conjugated double bonds gave carotenoids a non-polar characteristic. The important functional groups and bonds involved in carotenoids include polyene chain and unsaturated double bonds.

To wrap up, the stability assessment of the natural pigments demonstrated that anthocyanins were extremely sensitive to pH change, this signified that treatments that involved extreme pH conditions such as enzyme-assisted extraction should be avoided in the recovery of anthocyanins. Furthermore, betalains could not tolerate thermal or high heat operations, which suggested that extraction technologies that utilised thermal approaches might not be applicable to recover betalains as thermal degradation of betalains was more likely to occur. Not least for all, carotenoids displayed significant degradation under strong light exposure and oxygen-rich atmosphere, indicating that the extraction of carotenoids should be conducted under a dark environment and oxygen-limited atmosphere such as an atmosphere filled with inert gases to reduce the deterioration of carotenoids.

Another conclusion that could be drawn from this study was the high encouragement to use pulsed-electric field (PEF) extraction in the recovery of water soluble pigments (anthocyanins and betalains) owing to the fact that PEF treatment was highly suitable to extract heat-sensitive water soluble pigments since non-thermal natures and polar medium were practised in PEF. This was reflected in the highest extraction yield of water soluble pigments (ranging from 80.00 % to 90.00 %) during PEF extraction. In terms of the recovery of lipid soluble pigments (carotenoids), supercritical fluid extraction (SFE) manifested remarkable performance in which the recorded maximum yield was 90.12 %. Apart from that, SFE was recognised as a non-thermal approach due to the mild extraction temperature applied and thus thermal degradation of carotenoids was less likely to occur.

In the analysis of extraction temperature, MAE was classified as the only technique that involves the use of thermal approaches due to the broad range covered by microwave extraction. On the flip side, other extraction technologies were categorised as non-thermal approaches as the extraction temperature applied was generally lower than the thermal degradation temperataure of the natural pigments. Therefore, it could be said that thermal degradation of pigments was more likely to occur in MAE as compared to other extraction technologies.

The outcomes of extraction time analysis manifested that biological force-assisted extraction (enzyme-assisted extraction) took up the longest extraction time, followed by pressure force-assisted extraction (supercritical fluid extraction) and electrical force-assisted extraction (pulsed-electric field extraction, ultrasound-assisted extraction and microwave-assisted extraction). This sufficient induction time was required to be allocated for PEF treatment in order to reach the phase that yields the maximum extraction yield. Concurrently, SFE consumed reasonable extraction time as static extraction time and dynamic extraction time need to be fulfilled in SFE. Lastly, it was discovered that longer extraction time did not guarantee greater extraction yield as reflected in the extraction yield of EAE that required 240.00 minutes or more in the extraction process but the extraction yield obtained at the end was unsatisfactory.

Apart from that, the results of complexity of separation stage analysis manifested that SFE was the best candidate as there was no need for the separation process due to the separation of supercritical solvents could be achieved by simply depressurisation. Owing to the fact that SFE involved the least separation process, it was suggested that the quality of the recovered pigments in SFE was the highest as potential loss of pigments during the extraction and separation stages were minimised.

Not least for all, the green extraction assessment performed in the study revealed that SFE was the best candidate in terms of green extraction due to the nature of the supercritical solvent used. In general, supercritical carbon dioxide was used in SFE and it was recognised as a green solvent due to its nature that would not trigger any undesired environmental impacts. Over and above that, it was found that green solvents such as water and oils could be employed for all the extraction methods to replace the use of toxic and hazardous organic solvents except for SFE as SFE have utilised a green solvent in nature. Other than that, solvent-free condition could be established for microwave-assisted extraction (MAE) to fully wipe out the use of solvent, however solvent-free condition was found to be not applicable for other extraction technologies due to the limitations that solvent must be present as the carrier of recovered pigments for the extraction process.

## 5.2 Recommendations for future work

There were different research analyses conducted to improve the existing extraction technologies to cope with the limitations and constraints faced in the current extraction techniques such as low extraction yield, long extraction time and others.

The issues regarding the polarity of the pigments and the extraction medium were always the major issues encountered in the extraction process. In pulsed-electric field (PEF) extraction that relied on the high-polarity medium in the extraction process, recovery of carotenoids using PEF was an obstacle due to its non-polar nature as there were limited published research works carried out on the extraction of lipid soluble pigments using PEF treatments. Puranto, Agro and Wijiana (2014) demonstrated the potential of

extracting carotenoids using olive oil as solvent to maximise the extraction yield of carotenoids from carrot pulps via PEF. The use of olive oil was suggested since electroporation on the pulps was formed, the olive oil molecules were expected to bind with the carotenoids pigments regardless from inside the cell owing to the fact that olive oil has the same polarity as carotenoids. This statement was strengthened by the extraction yield of carotenoids using olive oil as solvent was 104.64 µg/ g, which was 6 times greater then the extraction yield using hexane as solvent, 15.61 µg/ g, indicating that use of lipid soluble solvent was applicable in PEF treatments.

Same as PEF treatments, SFE encountered similar challenges in terms of polarity of the solvent used. As pure supercritical carbon dioxide was completely non-polar in nature, water soluble pigments such as anthocyanins and betalains exhibited low solubilities in supercritical carbon dioxide. To address this problem, it was recommended that addition of polar entrainers could potentially raise the solubility of water soluble pigments in the supercritical carbon dioxide (Radzali, et al., 2014). Examples of polar entrainers that could be used include water, methanol, ethanol, acetonitrile and dimethyl sulfoxide. An experimental study was conducted to compare the efficiency of polar entrainers by comparing the use of water, methanol and dimethyl sulfoxide and it was revealed that the use of water as entrainers led to a significant increase in terms of the recovery of water soluble pigments from 1000.00 mg/ 100 g sample to 2000.00 mg/ 100 g sample (Casas, et al., 2007). Aside from that, another published study showed similar trend as the extraction efficiency of water soluble pigments from Bixa Orellana seeds was increased from 1.00 % to 45.00 % by introducing 5.00 mol % of ethanol as modifier in the supercritical carbon dioxide (Nobre, et al., 2006). This implicated that the addition of polar entrainers could increase the extraction yield and efficiency of water soluble pigments in SFE.

Apart from the polarity issues, there were improvements suggested by combining the novel extraction technologies to increase the extraction yield and efficiency. Manzoor, et al. (2019) proposed an experiment to investigate the effects of combining PEF extraction and UAE in the recovery of anthocyanins from almond extract. The results showed improvements in

terms of the total anthocyanins extracted. The total anthocyanins obtained by using PEF and UAE were 1.05 mg/ L and 1.10 mg/ L respectively, but increment was observed by combining PEF and UAE, 1.28 mg/ L of total anthocyanins was recovered by using combination treatments of PEF-UAE (Manzoor, et al., 2019).

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