MECHANICAL EXTRACTION OF FERULIC ACID FROM BANANA STEM WASTE

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MECHANICAL EXTRACTION OF FERULIC ACID FROM BANANA STEM WASTE

SITI NATRAH BINTI ISMAIL

Thesis submitted in fulfill of the requirements
For the award of degree of
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DEDICATION

My humble effort I dedicate to my sweet and loving

My beloved parents and family

The reason of what I become today,
Thanks for your great support and continuous care

My supervisor

I am really grateful to you
Who gave me encouragement and became my inspiration

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In the name of Allah, the most gracious and merciful;

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ABSTRACT

The present of ferulic acid (FA) in plants attract the attention of many researchers, especially in food, pharmaceutical and cosmetic industries. Banana stem waste (BSW) was generated from banana plantation. In this study, FA was extracted from BSW by using sugarcane press machine. There were three objectives in this study. The first objective was to characterize the compositions of BSW and extracted BSW juice (EBJ). The characterization of BSW was done based on the compositions of moisture, lignin, cellulose and hemicellulose. For the EBJ, it was characterized based on the compositions of total phenolic and glucose. The second objective was to analyze factors affecting FA extraction from BSW. In factorial analysis, there were five factors were selected based on the preliminary study. The factors were part of stem, ultraviolet (UV) light pre-treatment, pre-treatment temperature, cycle of extraction and storage time of EBJ. Design Expert software was used for the experimental design. Two-level factorial design was applied for the factorial analysis. The FA was analyzed by using high performance liquid chromatography (HPLC). The third objective was to optimize the extraction of FA from BSW. The experimental table for the optimization was constructed by using central composite design (CCD). There were two factors chosen from the factorial analysis which were pre-treatment temperature and storage time of EBJ. The suggested optimum conditions that obtained during optimization were validated by using validation experiment at pre-treatment temperature 25 °C and storage time of EBJ at 24 h. The result that obtained concluded that the optimum FA yield was obtained at the suggested conditions. Therefore, the application of Design Expert software wascapable to obtain the optimum conditions and to improve the extraction of FA.

ABSTRAK

Kewujudan asid ferulik (FA) di dalam tumbuhan menarik perhatian ramai penyelidik terutamanaya dalam industri makanan, farmasi dan kosmetik.Sisa batang pisang (BSW) dihasilkan daripada kebun pisang.Dalam kajian ini, FA diekstrak daripada batang pisang dengan menggunakan mesin tebu.Terdapat tiga objektif dalam kajian ini.Objektif pertama adalah menentukan komposisi kelembapan, lignin, selulosa dan hemiselulosa.Untuk jus BSW yang telah diektrak (EBJ), ianya ditentukan berdasarkan komposisi jumlah fenolik dan glukosa.Objektif kedua kajian ini adalah untuk menganalisa faktor-faktor yang mempengaruhi pengekstrakan FA daripada BSW. Di dalam analisis faktoran, terdapat lima faktor yang dipilih berdasarkan kajian awal. Faktor-faktor ini dikaji adalah bahagian batang, pra-rawatan dengan cahaya ultraungu (UV), suhu pra-rawatan, kitaran perahan dan masa penyimpanan bagi EBJ.Perisian Design Expert telah digunakan untuk rekabentuk ekperimen.Ujikaji faktorial dua peringkat digunakan untuk analisis faktoran.FA dianalisa dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC).Objektif yang ketiga adalah untuk mengoptimumkan pengeluaran FA daripada BSW. Bagi proses pengoptimuman, jadual ekperimen dibina dengan menggunakan rekabentuk komposit berpusat (CCD). Terdapat dua faktor yang telah dipilih daripada analisis faktoran.Faktor-faktor ini telah dikaji pada nilai yang berbeza; suhu pra-rawatan dan masa penyimpanan. Keadaan optimum yang disarankan semasa proses pengoptimuman disahkan dengan menjalankan experiment pengesahan pada suhu pra-rawatan pada 25 °C dan masa penyimpanan EBJ pada 24 h. Keputusan yang diperolehi dapat disimpulkan bahawa hasil FA yang optimum dapat diperolehi pada keadaan yang disarankan. Oleh itu, aplikasi Perisian Design Expert berupaya untuk mendapatkan keadaan yang optimum and untuk meningkatkan jumlah pengektrakan FA.

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LIST OF SYMBOLS

%	Percentage
°C	Celsius
β_0	Constant coefficient
β_i	Linear coefficient
β_{ii}	Quadratic coefficients
β_{ij}	Interaction coefficients
cm	Centimeter
G	Gram
g/g	Gram per gram
g/l	Gram per liter
h	Hours
kg	Kilogram
min	Minutes
mg/g	Milligram per gram
mg GAE/g	Milligram Gallic acid per gram
mg/l	Milligram per liter
mg/ml	Milligram per milliliter
ml	Milliliter
ml/min	Milliliter per minutes
mm	Millimeter
nm	Nanometer
rpm	Revolution per minutes
x_i	Independent variable
x_j	Independent variable

w	Watt
y	Predicted response
µl	Microliter
µm	Micrometer

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
BSW	Banana stem waste
C	Carbon
CCD	Central composite design
DAD	Diode array
Df	Degree of freedom
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic colorimetric
EBJ	Extracted BSW juice
FA	Ferulic acid
FAEs	Feruloyl esterases
FAOs	Food and organization
FAOSTAT	Food and agriculture organization of the united nations
FFD	Full factorial design
HCL	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
H_3PO_4	Phosphoric acid
H_2SO_4	Sulphuric acid
IR	Infrared
LCC	Lignin-carbohydrate complex
MAE	Microwave-assisted extraction

NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
NDF	Neutral detergent fiber
OPF	Oil palm frond
PAL	Phenylalanine Ammonia-lyase
PHWE	Pressurized hot water extraction
PLPWE	Pressurized low-polarity water extraction
R^2	R-square
RSM	Response surface methodology
UE	Ultrasonic extraction
USA	United States of America
UV	Ultraviolet
p-CA	*p*-coumaric acid

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Ferulic acid (FA) is a common phenolic acid that have many applications in food, pharmaceutical and cosmetic industries. It becomes an important ingredient because of their fragrant, aromatic and antioxidant properties. Normally, FA was recovered from the biomass such as plants and agriculture crop residues. It usually presents in plant cell walls as a cross linking agent between polysaccharide and lignin compounds (Shin et al., 2006). The agriculture waste such as banana stem waste (BSW), flax shives, sugar beet pulp, wheat and corn cob contained FA in their cell wall (Oliveira et al., 2006; Buranov and Mazza, 2009; Buranov and Mazza, 2008; Torre et al., 2008).

Malaysia has the potential to produce FA from the agriculture waste because it rich with the agriculture activity. BSW is one of the agriculture wastes that highly produced in Malaysia. This type of waste was generated from the banana plant. Banana plant is the common name for the herbaceous plants of the genus Musa. It was cultivated in more than 120 countries. Banana was produced from this plant and it was an important fruit in the tropical and subtropical regions. The banana rank as fourth major crops after rice, wheat and maize (Jain, 2010). According to the food and organization (FAOs) statistics database, the total production of banana in Malaysia was more than 290,479 metric tons (FAOSTAT, 2013). Banana plant is normally tall and fairly sturdy. The height of banana plant is from 0.8 m to more than 7.5 m (Tock et al., 2010). This plant only produces one bunch of bananas and after that, it will lose its ability to produce the fruit anymore. Usually, it will be cut down and the whole plant including the stem, leaves and the rhizome will become waste. This banana waste has been used for different purpose depending on the parts of plant. BSW consisted of cylinder leaf-petiole sheaths that composed of long fiber and

overlapping called pseudo stem. BSW usually used as handicraft works, livestock feed or even as natural manure on the soil (Pereira et al., 2010). Besides that, natural fiber known as banana fiber also produced from BSW. Banana fiber is a lingo-cellulosic fiber that have relatively good mechanical properties (Mukhopadhyay et al., 2008). However, these types of alternatives are rarely applied. Normally, this waste were just thrown away without caution and care about the environment. In order to prevent it from happen, BSW was used as the alternative raw material to extract the FA.

There was several extraction methods used to recover and isolate FA from the agriculture waste. To date, researchers were able to find out three types of extraction methods which were enzymatic, chemical and mechanical extractions (Tilay et al., 2008). Enzymatic extraction was commonly used to produce FA by using the fermentation and synthesis by the bacteria or microorganism. According to Shin and Chen (2006), microorganisms such as *Fusarium proliferatum*, *Fusarium verticillioides* and *Mucor circinelloides* were the three strains that capable to produce feruloyl esterases (FAEs). FAEs degraded the structure of plant cell wall by hydrolyzing the ferulate ester groups involved in the cross-linking between hemicellulose and lignin (Fazary and Ju, 2008). For the chemical extraction method, the used of acid or alkali solution that hydrolyze the ether and ester bonds in agriculture waste in order to release FA. The used of an alkaline solution dissolve the lignin by cleavage of ester linkages in lignin–polysaccharide complexes, thus releasing the FA (Torre et al., 2008). Finally, the mechanical extraction was applied to provide shear force to the cell wall in order to free the FA (Grimi et al., 2007). In this study, sugarcane press machine was chosen as the mechanical extractor that used to extract FA from BSW.

1.2 PROBLEM STATEMENT

The increasing demands for the application of FA shift the researchers to prefer the new type of raw materials and methods for their production. From the previous study, rice bran, wheat bran, sugarcane bagasse and corn cob were used in the production of FA and from the past findings the corn cob gave the highest amount of FA(Huang et al., 2011). However, raw materials from corn were limited in Malaysia since corns are not our main crops. In order to overcome this problem, this study used BSW as the alternative raw material. BSW was used to prevent it remained idle and to prevent the environmental pollution in order to dispose it.

For the extraction of FA, the mechanical extraction was chosen instead of chemical and enzymatic extraction. The mechanical extraction by using sugarcane press machine has a lot of advantages such as the extraction time was short and low cost. The cost was the main focus on industrial production to ensure each product that produced increased the company profits. Reducing the cost of raw material and chemical has substantial impact on the production cost. Moreover, the mechanical extraction is simpler method compared to others without perform the purification step. This extraction method also does not require other chemicals as the solvent.

The extraction of FA from the agriculture waste became a challenging process due to the complex structure of the plant cell wall. In order to optimize the extraction of FA, response surface methodology (RSM) has been widely used. By established the mathematical model, RSM capable to evaluate multiple factors and their interactions using quantitative data and optimize the extraction statistically (Wang et al., 2013). This method has the advantages of identifying and isolating the significant factors while minimizing the number of experiments without neglecting the interaction effect between the factors (Chan et al., 2009).

1.3 OBJECTIVES OF STUDY

The objectives of this study were:

i. To characterize the chemical composition of raw BSW and extracted BSW juice (EBJ).
ii. To identify the most significant factors that affect the FA extraction from BSW.
iii. To optimize the significant factors that affects the FA extraction from BSW.

1.4 SCOPE OF STUDY

To achieve the following objectives, the scopes of this study were:

i. To characterize the compositions of raw BSW based on the compositions of lignin, cellulose, hemicellulose and moisture.
ii. To characterize the compositions of total phenolic and glucose in EBJ.
iii. To screen and analyze the following factors which affecting the FA extraction using two-level factorial design:
    a) Part of stem
    b) Pre-treatment temperature
    c) Ultraviolet (UV) light pre-treatment
    d) Cycle of extraction
    e) Storage time for EBJ
iv. To optimize the values of factors which affect the FA extraction using central composite design (CCD).
v. To utilize RSM by using Design Expert Software (Version 7.1.3, Stat-Ease, Inc., Minneapolis, MN) program.
vi. To determine the FA concentration by using high performance liquid chromatography (HPLC).
vii. To validate the suggested optimum condition for the extraction of FA from BSW.

1.5 OVERVIEW OF THE THESIS

The content and all the information in this thesis were presented in five chapters. In Chapter One, it consists of the introduction of FA extraction, BSW potential as FA source and it current statuses. Besides that, this chapter also provided the objectives and scopes of this study.

Chapter Two introduces some literature review and the fundamental concept on the extraction of FA from BSW by using sugarcane press machine. Briefly, this chapter was divided into eight subchapters; introduction, FA, FA content in plant, FA extraction from natural resource, factors affecting FA extraction, FA analysis, RSM and chapter summary. The in-depth information on FA was explained in the first subchapter. This subchapter introduced the source and application of FA in food, medical and cosmetic industries. It also discussed about the chemical and physical properties of FA. The used of raw material in this study was introduced in second subchapter. BSW was chosen as the raw material in this study. The third subchapter explained the type of extraction method that used to extract FA from natural resources. In this study, mechanical extraction by using sugarcane press machine was chosen for the extraction of FA from BSW. The next subchapter explained the factors that affect FA extraction. In this subchapter, various factors were discussed such as type of extraction, pre-treatment categories and effect of storage condition to FA yield. The analysis of FA after the extraction was discussed in fifth subchapter. In this study, HPLC was used to analyse the FA yield. For the last subchapter, it discussed the use of RSM. For factorial analysis, two-level factorial design was applied while in optimization it used CCD. Besides, it also discussed the application of RSM in extraction process.

Chapter Three provided the materials and methods that used in this study. It presented the steps and procedures required in order to optimize the extraction of FA from BSW. It divided into eight subchapters where the introduction, overall methodology, raw material (BSW), sugarcane press machine, preliminary study for FA extraction, factorial analysis method, optimization method and analysis of sample. The last subchapter concluded all the methods used in order to achieve the objectives of this study.

Chapter Four provided and discussed the data and results that obtained in this study. This chapter was divided into eight subchapters. The first subchapter introduced the overview of chapter four. Second subchapter discussed the compositions of lignin, cellulose, hemicellulose and moisture in BSW. The third subchapter discussed the preliminary study in order to determine the factors that affect the extraction. From this subchapter, the selections of factors with their range were determined in order to use in forth subchapter. The forth subchapter discussed and analyzed the factors affecting the FA extraction by using two-level factorial design. From factorial analysis, two factors were chosen for the optimization. The fifth subchapter discussed the use of CCD to obtain the optimum condition for FA extraction from BSW. The compositions of total phenolic and glucose in EBJ were explained in sixth subchapter. For the seventh subchapter, it discussed the comparison of the extraction of FA with other researchers. In the last subchapter, it concluded the results and discussions that obtained in this study.

Finally, Chapter Five summarized all the works for this study and provided a brief conclusion on the thesis. Besides, it also provided some recommendations in order to improve this study and increased the FA yield.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter covers the literature review and fundamental concept on the ferulic acid (FA) extraction from banana stem waste (BSW). The literature reviews are divided into six subtopics covering the FA, FA content in plant, factor affecting FA extraction, FA analysis, response surface methodology (RSM) and chapter summary.

2.2 FERULIC ACID

2.2.1 Source and application of ferulic acid

Ferulic acid (FA) is a phenolic acid that commonly find in many staple foods, such as vegetables, fruits, cereals, coffee, and herbs (Chung and Champagne, 2011). It also found in monocotyledons plants such as paddy (Salleh et al., 2011), wheat (Sarangi and Sahoo, 2010) and sugarcane (Xu et al., 2005). In plants cell wall, FA exists in a free form or covalently linked to lignin and other polymers. FA is a very important component in cell wall for the growth and reproduction of the plants. It also acts as defense mechanism against pathogen infections at injured sites.

FA offers good anti-oxidizing properties and commonly used in food, medical and cosmetic industries. FA can exhibits anti-oxidant activity by donating one hydrogen atom from phenolic hydroxyl group (Kumar and Pruthi, 2014). In food industry, FA used to

prevent the discoloration of food such as to maintain the colour tone of green peas, discoloration of green tea, and oxidation of banana turning black color (Ferulic Acid Catalogue, 2009). Besides, FA also transformed into vanillin to use as flavoring agent (Kaur et al., 2013; Gallage and Moller, 2015).

The health benefit of FA is gaining a lot of attention in medical industry. It has potential to exhibit the biological effects such as anti-viral, anti-carcinogenic, anti-bacterial, anti-inflammatory, anti-allergic, hepatoprotective, and anti-thrombotic actions (Srinivasan et al., 2007). FA can act as strong antioxidant in humans and it is effective against skin cancer, skin disorders, fatigue, ageing, flu cold and influenza. The antioxidant of FA also serve as protective function against neurodegenerative disease such as Alzheimer's disease and stroke (Sarangi et al., 2011 ; Szwajgier and Jakubczyk, 2010). It prevent brain and neuronal cells from injury (Koh, 2015). Besides, FA also increased antioxidant activity in the plasma, liver, heart and alleviate late stage of diabetes (Song et al., 2014).

In cosmetic industries, the FA is use in anti-aging product to help protect the skin against sun rays or ultraviolet (UV) light. The exposure of skin to UV light promote erythema, inflammation, premature skin ageing, immunodepression and photocarcinogenesis (Srinivasan et al., 2007). The present of FA in cosmetic product protect the skin and maintain the youthful appearance. It has the ability to protect the skin against the free radicals from the surrounding that lead to the oxidation of the cell.

2.2.2 Chemical and physical properties of ferulic acid

FA ($C_{10}H_{10}O_4$) also known as ferulate is a hydroxycinnamic acid derived from phytochemical phenolic compounds. FA present in cell wall polysaccharides via an ester linkage between the carboxylic acid group and the primary alcohol on the C5 carbon of the arabinose side chain of arabinoxylans (Buanafina, 2009). The distinctive structures of FA contributed to the antioxidant activity. It capable to eliminate reactive forms of oxygen and free radicals (mostly superoxide, hydroxyl and hydroxyl peroxides) (Urbaniak et al., 2013). The benzene ring with the presence of electron donating groups gives the additional

property of terminating free radical chain reactions. The carboxylic acid group in FA with an adjacent unsaturated C=C double bond provide additional attack sites for free radicals (Itagaki et al., 2009). According to Jankovska et al. (1996), FA consisted of two stereoisomers form which were trans and cis isomer. The trans isomer of FA presented in white crystalline while cis isomer in yellowish liquid substance (Fazary and Ju, 2007). The molecular structure for trans and cis isomer of FA showed in Figure 2.1.

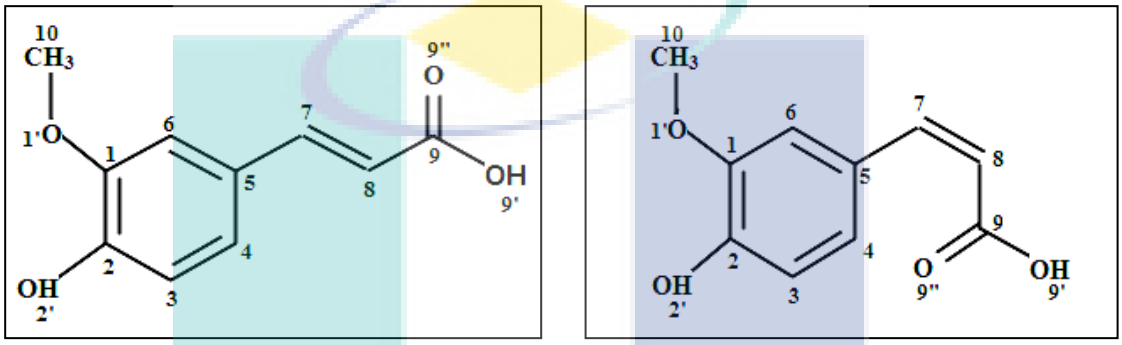

Figure 2.1: Molecular structure (a) trans-FA and (b) cis-FA

Source: Urbaniak et al. (2013)

2.3 FERULIC ACID CONTENT IN PLANT

Ferulic acid is one of the phenolic compounds that present in plants. It widely distributed in tissue, cellular and subcellular of the plants. The compositions of phenolic compounds are not uniform for every type of plants. At the subcellular, the phenolic compounds mainly located in the vacuoles, small amounts of it in the free space and none in the cytoplasm (Robards et al., 1999). The phenolic compounds was classified into two categories which were insoluble and soluble phenolic (Naczk and Shahidi, 2006). The insoluble phenolic was found in cell walls while the soluble present within the plant cell vacuoles. Table 2.1 shows the relative concentration of phenolic compounds in plant tissue.

Table 2.1: Relative concentration of phenolic compounds in plant tissue

Tissue	Relative Concentration
Fruit	Cinnamic acids > catechins ≈ leucoanthocyanins (flavan-3,4-diols) > flavonols
Leaf	Flavonols ≈ cinnamic acids > catechins ≈ leucoanthocyanins
Wood	Catechins ≈ leucoanthocyanins > flavanols > cinnamic acids
Bark	Catechins ≈ leucoanthocyanins > flavanols > cinnamic acids

Source: Robards et al., 1999

FA is a hydroxycinnamic acid and it is one of the major phenolic compound that contain in the cell wall. It is most abundant in the epidermis, sclerenchyma, bundle sheaths and xylem vessels (de O Buanafina, 2009). The structures of FA have both carboxylic acid and hydroxyl groups. This structure make it capable to form both ester and ether bonds with other compounds and form the linkages with cell wall polysaccharides (Madhujith and Shahidi, 2009). FA shuttled into wall matrices and attached to the lignin and hemicellulose via ether and ester bonds (Sun et al., 2002). These bonds act as bridges and form lignin/phenolic-carbohydrate complexes (LCC). The attachment of FA was different between monocot and dicot plants. In monocots plants, FA was attached to cell wall by ether bonds to lignin. The hydroxyl group was covalently linkage to lignin or by ester bonds through its carboxylic acid group with the C5 hydroxyl of a-L-arabinosyl side chains of xylans (de O Buanafina, 2009). In dicot plants, FA is associated with pectic polysaccharides via ester linkages to C6 hydroxyl group of galactopyranose residues or to the C2 hydroxyl group of arabinofuranose (de O Buanafina, 2009). Figure 2.2 shows the structure of LCC where FA attaches to lignin with ether bond and carbohydrates with ester bond.

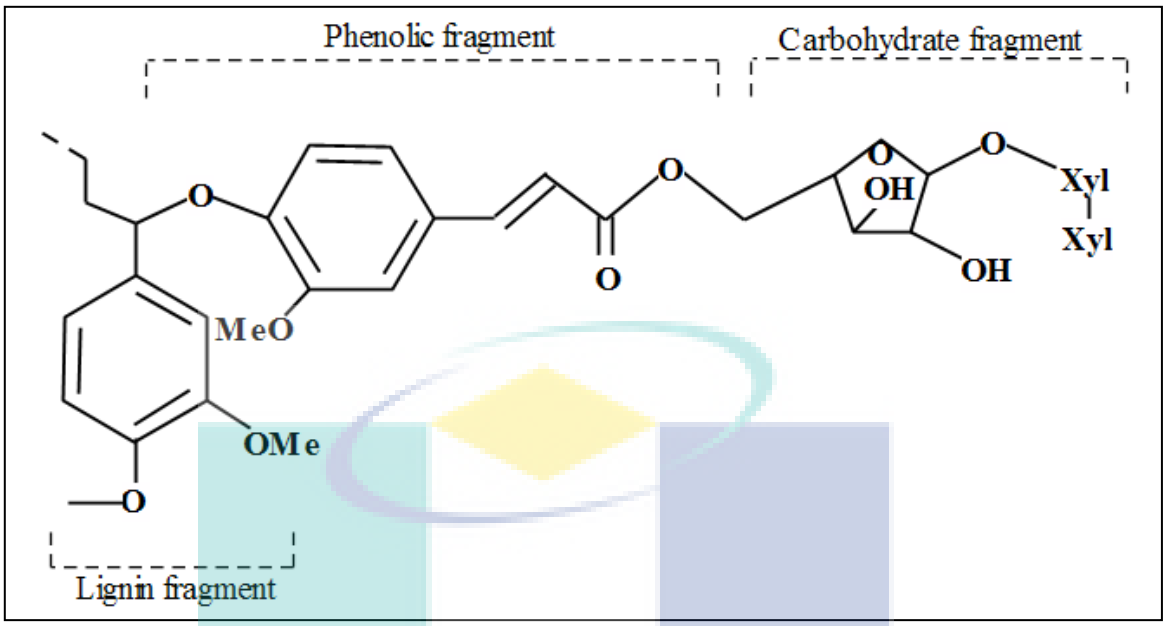

Figure 2.2: Molecular structure of LCC

Source: Buranov and Mazza, 2008

The FA was released from the plants by using chemical and enzymatic treatment. The chemical treatment was used to break the ester and ether bonds between FA and LCC. The ester bond was broken by using alkaline treatment due to its alkaline-labile while the ether bond was broke by using acidic treatment due to its acid labile. From studies conducted by Torre et al. (2008), FA was released from the LCC by dissolving the lignin and cleavage the ester bond using the alkaline treatments. Hosseinian and Mazza (2009) also studied the used of sodium hydroxide (NaOH) to break the ester and ether bonds and released FA from LCC. Besides, the ether bonds between lignin and FA also broken by using steam, hot water and dilute acid (Buranov and Mazza, 2008).

Grains such as corn, wheat, oats and rice contains phenolic compounds include derivatives of benzoic and cinnamic acids. About 74% and 69% of the phenolic presented in rice and corn respectively. It presented in the insoluble form with the FA being the major compound (Adom and Liu, 2002). Among these grains, corn had the highest FA

yield followed by wheat, oats, and rice. The FA production from the corn cobs was achieved until 1.1 g/l (Torre et al., 2008). From the research conducted by Adom and Liu (2002), FA yield was the highest in corn (0.176 mg/g of grain), followed by wheat (0.065 mg/g of grain), oats (0.036 mg/g of grain) and rice had the lowest FA yield (0.029 mg/g of grain).

Figure 2.3 shows the phenolic acid in flax and wheat straws. From the figure, there were four phenolic acids content in both straw which were FA, *p*–coumaric (*p*-CA), vanillic acid, and vanillin. The wheat straw contained more FA, *p*-CA, and vanillin than flax straw. The wheat straw contained more lignin and the alkali extractable phenolic acid compared to flax straw (Tapin et al., 2006). The studied conducted by Buranov and Mazza (2008) also found the wheat straw contained higher FA and *p*-CA (~3.6mg/g) than the flax straw (~0.2 mg/g).

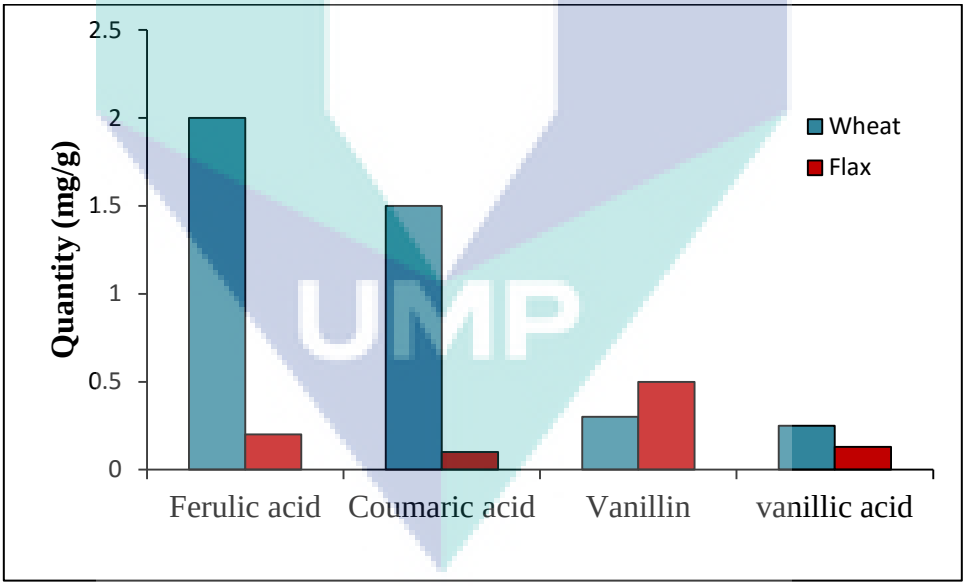

Figure 2.3: Phenolic acid in wheat and flax straws

Source: Tapin et al., 2006

Besides, the phenolic compounds also widely distributed in several parts of the banana plant. From studies conducted by Sulaiman et al. (2011), it revealed that both banana peel and pulp contains phenolic compounds such as catechin, epicatechin, lignin, tannins and anthocyanins. The phenolic compound in pulp (2.32 mg/g dry wt.) was lower than the peel (9.07 mg/g dry weight) (Someya et al., 2002; Sulaiman et al., 2011). According to Fatemeh et al. (2012), the banana peel contained about 9 to 30 mg/g dry weight of the phenolic compounds. Banana plant also presence others phenolic compounds such as tannin, catechic, catechin, protocatechuic, gallic, caffeic, FA, and cinnamic acids (Ewane et al., 2012). Table 2.2 shows the phenolic compound that identified in banana plant tissue.

Table 2.2: Phenolic compounds in banana plant tissue

Phenolic Compounds	Tissues	Class (Subclass)
Gallic acid, Vanillin	Fruit	Phenolic acids Hydroxybenzoic acids
Chlorogenic, caffeic, FA, p-CA, vanillic and synaptic acids	Fruit, roots	Hydroxycinnamic acid and derivatives
Catechin	Fruit	Simple phenols
Gallocatechin		Flavan-3-ol
Rutin, naringin	Fruit	Flavonoids, Flavonols,
Anthocyanidins	Leaves, roots	Flavanones, Flavonoids derivatives
Tannin catechic,	Fruit	Tannins
Proanthocyanidins	Roots	Hydrolysable tannins Condensed tannins
Lignin	Roots	Lignin

Source: Ewane et al., 2012

From Table 2.2, it shows the phenolic compound mostly present in the fruits and roots of the banana plant. From the studied conducted by Kondo et al. (2005) and Lim et al.

(2007), banana fruit contained antioxidant such as polyphenolic and vitamins. The antioxidants have the role in the scavenging of free radicals and give the antioxidant properties. Furthermore, phenolic acids such as FA and *p*-CA presented in the roots of the banana plant (de Ascensao and Dubery, 2003). Other than fruits and roots, the pseudo stem and foliage of banana plant also contained the phenolic acids such as FA and *p*-CA. This phenolic acid represents 75% of aromatic fraction in the foliage of the banana plant (Oliveira et al., 2006). This also has been supported by a study that conducted by Oliveira et al. (2009) that found the FA and p-CA also contained in stalk lignin of banana plant.

2.4 FACTORS AFFECTING FERULIC ACID EXTRACTION

The extraction is a very important stage in the isolation as well as identification of phenolic compounds. Many authors have studied the factors that influenced the effectiveness of the extraction process. Normally, the extraction depends on the raw materials and type of extraction used. The pre-treatment process before the extraction of the plant biomass also influenced the content of phenolic compounds. Besides that, the storage condition of the extracted product also affected the phenolic compounds content.

2.4.1 Type of extraction

The production of FA was done by using two different methods which were chemical synthesis, and extraction from the natural resources. The chemical synthesis of FA involved the condensation reaction of vanillin with malonic acid catalyzed by piperidine (Prakash and Bhathena, 2008). The other method was the extraction of FA from natural resources. There were several type of extraction was used such as enzymatic, chemical and mechanical extractions (Tilay et al., 2008). The chemical synthesis of FA was not an option to apply in industrial and as commercial used. This was due to the reaction and chemical synthesis to produce FA take about three weeks to complete (Tilay et al., 2008). So, the extraction of FA from natural resources was selected to produce in large quantities and for commercial used.

Extraction is a technique for the isolation of plant constituents. The extraction is one of the most important unit operations in industry. The extraction of phenolic acid from the plants is influenced by the extraction method, chemical nature, particle size of the sample and the presence of interfering substances (Stalikas, 2007).

Enzymatic extraction

The feruloyl esterase (FAEs) is an enzyme that represents subclass of carboxyl esterase. FAEs is use to release the phenolic acids, such as FA or other cinnamic acids from plant cell wall (Li et al., 2011). This enzyme is a biochemical tools that catalyze the cleavage reactions between the phenolic compounds in the cell walls. FAEs were produced from the microorganisms such as *Aspergillus niger, Fusarium proliferatum, Neosartorya spinosa* (Ou et al., 2011; Shin and Chen, 2006; Shin et al., 2006). Although there was extensive research on the preparation of FA by using FAEs, this method was rarely used in the commercial production. This was due to the limited availability of the microorganism that produce the enzyme (Choi et al., 2008). Besides that, the production of enzyme by microorganism also required high cost and long reaction time to hydrolyze the FA bound (Fazary and Ju, 2007).

Chemical extraction

The chemical extraction was applied in the extraction of phenolic acid from the plant cell wall. The acid and alkali solutions were used in the chemical extraction to hydrolyze the ether and ester bonds between the phenolic acid with other compounds. The chemical hydrolysis of phenolic acid depended on the concentration of acid or alkali solutions, temperature and the reaction times (Mussatto et al., 2007). The yield and the profile of phenolic acids were affected by the type of the extraction method used (Kim et al., 2006). In the extraction of FA, the used of acid solution broke the ether bonds between the lignin and hydroxycinnamic acid (Buranov and Mazza, 2008).

In the chemical extraction, the used of alkaline solution was better that acid solution. This was due to the alkaline solution gave a better hydrolysis to extract the phenolic acids (Kim et al., 2006). The extraction of phenolic acid by using the alkaline solution gave nearly twice than the acid solution (Verma et al., 2009). The alkaline solution dissolved the lignin by cleavage the ester links in lignin-carbohydrate complex (LCC) and released the phenolic acid (Torre et al., 2008). From the previous studies, the alkaline solution was applied to extract the FA from the plants such as wheat bran, corn bran, paddy straw and flax shives (Salleh et al., 2011; Buranov and Mazza, 2009). The hydrolysis by using alkaline solution easily dissolved the lignin and it already proved to completely utilized the lignocellulosics (Xiao et al., 2001).

Mechanical extraction

Mechanical extraction also one of the extraction methods used in the extraction of phenolic acid from the plants. It is widely used because this method is easy to use with high efficiency and wide range applications. The mechanical extractor is capable to extract the active compounds from the plants faster than other methods. It capable to disrupt the cell membranes by the shear forces or local heating and released the phenolic acids. The mechanical extractions that commonly used were pressurized hot water extraction (PHWE), microwave-assisted extraction (MAE), ultrasonic extraction (UE) and pressing extraction (Khan et al., 2005).

Pressurized hot water extraction (PHWE) also known as pressurized low-polarity water extraction (PLPWE). This extraction method was done by modified the properties of water. The water was heated at the temperature above 100 $^{\circ}$C and at high pressure (Buranov and Mazza, 2009). It was done in order to maintain the liquid state of the water and to improve its extraction capability. This method also lowers the viscosity, dielectric constant and the surface tension of the water. It allowed the extraction of low-polar and nonpolar phenolic compounds from the plants. Based on previous research, PHWE was used to extract the phenolic compounds such as syringic acid, vanillic acid, vanillin, acetovanillone, and FA from the plants (Kim and Mazza, 2006). This type of extraction has

higher selectivity, quickly extract the phenolic compounds and save the cost in term of material and energy consumptions.

Microwave-assisted extraction (MAE) was used to extract the phenolic compounds from plant materials with the faster heating (Alupului et al., 2012). This method introduced the kinetic energy thought heating process. The kinetic energy that provided through heating process propagates in the whole mass of the liquid and increasing the diffusion rate (Alupului et al., 2009). This process heated the solvent that contacted with the sample by using microwave energy or non-ionizing electromagnetic waves. The interested phenolic compounds from the sample matrix were released into the solvent. This method dissolved and broke the cell wall and released their phenolic compounds. From the previous study, the MAE was applied to extract the FA from the *Radix Angelicae sinensis* with the presence of ethanol as the solvent (Liu et al., 2006).

Ultrasonic extraction (UE) also used to extract phenolic compounds from the plant. The application of UE in the extraction process was done by disrupt the cell wall using high intensities of the liquid and improved the extractability. The high intensities of the liquid were provided during the sonication process. The ultrasonic was used in the extraction of phenolic compounds by provided the kinetic energy. The kinetic energy was introduced by the collapsed of cavitations bubble at or near the cell wall or the interfaces (Alupului et al., 2009). This process improved the mass transfer across the solid liquid interface. The kinetic energy that provided during the extraction disrupted the cell wall matrix and released the phenolic compounds. This type of extraction was used in the extraction of FA from the root of *Ligusticum chuanxiong* and tropical forages (Sun and Wang, 2008; Santos, 2011).

Besides that, pressing extraction also one of the mechanical extraction. During this type of extraction, the mechanical presser was used to extract the phenolic compounds that contained in the plants. This method was commonly used in the production of sugar, fruit juice and wine. This method also used in the production of oil from the palm oil industries. The phenolic compounds in plants were surrounded by the membranes and enclosed by the cell walls. The cell walls provided a rigid wall to prevent easy damage of the membrane

(Grimi et al., 2007). The presser was used in this type of extraction in order to disrupt the cell membranes and cell walls. The pressing extraction was applied in the extraction of phenolic compounds due to economical and environmentally friendly compared to the enzymatic and chemical extraction methods. Other extraction methods required the used of large amounts of the organic solvent and longer extraction times (Luengo et al., 2013). The pressing extraction were used in the previous studies to extract the juice and phenolic compounds from the fruits such as apple, carrot, grape, banana and orange (Bazhal et al, 2001; Grimi et al., 2007; Grimi et al., 2009; Kasozi and Kasisira, 2005; Aye and Ashwe, 2012).

Selection of the extraction method

The extraction of phenolic compounds from the plant cell depends on the extraction method applied and the target product. In this study, pressing extraction was chosen in the FA extraction from BSW. Pressing extraction was chosen because it became a good alternative method compared to enzymatic and chemical extraction. This was due to its high efficiency, low energy and water consumption. The use of sugarcane press machine was considered as pressing extraction. The sugarcane press machine is a well establish equipment in the processing of plant material, particularly to extract the sugarcane to get the juice and sugar content (Eshtiaghi and Yoswathana, 2012). Besides that, sugarcane press machine also used in the extraction of juice from the fresh oil palm frond (OPF) in order to get the renewable sugar (Zahari et al., 2012). This method was successfully used to extract bioactive compounds from the plants.

The application of sugarcane press machine was a good approach to extract FA from BSW. This type of extraction method neither required harsh pre-treatment steps and the used of enzyme nor the chemical solvent. The method chosen need to be cost effective and sustainable to support in biotechnology industries. In this study, the extraction of FA from BSW was done by pressing the fresh BSW to obtain the juice.

2.4.2 Type of pre-treatment

Generally, phenolic compounds in plant are bonded to dietary fiber, proteins or two sugars to form the complex structures. The bond needs to be broken in order to release the phenolic compounds during the extraction process. Pre-treatment is often used on plants biomass before the extraction in order to increase the extraction rate. Pre-treatment methods were divided into different types such as biological, physical, chemical, physicochemical and photochemical (Kumar et al., 2009). The biomass pre-treatment depended on the combination of several parameters that effected biomass such as temperature, acidity and duration of pre-treatment (Agbor et al., 2011). However, process for the pre-treatment of biomass also one of the challenging process. It is use to maximize the extraction yield while minimizing the cost.

Biological pre-treatment

The biological pre-treatment is performing by using various types of fungi. Fungi is capable to produce enzymes that can degrade cellulose, lignin and polyphenols that contained in plants (Agbor et al., 2011). White, brown and soft rot-fungi are three types of fungi that commonly use for the pre-treatment. According to Sun and Cheng (2002), brown rots is capable to attack cellulose while white and soft rots are able to attack cellulose and lignin (Sun and Cheng, 2002). The use of fungi in biological pre-treatment is safe and environmental friendly. This type of pre-treatment remove the lignin from the lignocellulosic biomass without using high amount of energy (Kumar et al., 2009). However, the reaction time for the biological pre-treatment was longer than other pre-treatment methods. This is due to the reaction rate for biological hydrolysis is low and need more time to complete.

Physical pre-treatment

Physical pre-treatment is performing by reducing size of the sample. The efficiency of phenolic compounds extraction is increase with the decreasing of the sample size. The

pre-treatment are done by using several methods such as chopping, milling and grinding (Harmsen et al., 2010). The reduction of sample size will ensure the sample easier to handle and increase the surface area. The increasing of the surface area increased and speeds up the reaction rate. Higher surface area allowing more contact and increase the collision between particles. Besides, subdividing the large sample into smaller size increase the amount of damage cells at the slice surface (Grimi et al., 2007). So, the extraction processes are more efficient thus increase the quantity of the phenolic compounds.

Chemical pre-treatment

The chemical solvent is use in order to perform the chemical pre-treatment on the plant biomass before the extraction process. The use of chemical solvent initiates the chemical reactions to disrupt the biomass structure. There are several type of solvent that use in pre-treatment such as acid, alkali, organic and ionic liquid solvents. All the solvents have a significant effect on the native structure of lignocellulosic biomass (Agbor et al., 2011).

Acid pre-treatment involve the use of dilute or concentrated acids to break the rigid structure of lignocellulosic biomass. Acid pre-treatment is predominantly affect the hemicellulose while only give a little impact on lignin degradation (Silverstein et al., 2007). It hydrolyzed the hemicellulose to its monomeric unit and make cellulose more available. The most commonly acid that use for the pre-treatment are sulphuric acid (H_2SO_4), hydrochloric acid (HCL), phosphoric acid (H_3PO_4) and nitric acid (HNO_3) (Brodeur et al., 2011).

The application of alkaline solution to treat plant biomass is a substituted to acid solution. An alkaline solution is use for pre-treatment of lignocellulosic biomass and the effect depends on the lignin content. It capable to disrupt the lignin structure and brake the linkage between lignin and the carbohydrate fractions (Agbor et al., 2011). It also increase the porosity of biomass by breaking the ester cross-linking between lignin and xylan

(Silverstein et al., 2007). The alkaline solutions that commonly use are sodium, potassium, calcium and ammonium hydroxide (Kumar et al., 2009).

Physiochemical pre-treatment

Physiochemical pre-treatment are the combination of physical and chemical methods. This type of pre-treatment use heat to manipulate the temperature surrounds the plant cell which affects the content of phenolic compounds. The heat provides thermal energy that disrupts the cell membranes and cell walls of the plants. It hydrolyzes the bonds to release the phytochemicals from the insoluble portion (Roy et al., 2009). This type of pre-treatment increases the phenolic compounds and their availability during the extraction process.

The pre-treatment with heat is performing by using blanching method to increase the content of phenolic compounds. The blanching method described as the process of heating at high temperature to destroy the enzymes that presented in plant tissues (Oboh, 2005). But, this process showed the different effects on the different type of plants with some showed the increasing of phenolic compounds while other decreased (Wen et al., 2010). There are two types of blanching which are water and steam blanching. The use of steam is better and more economic compare to the water blanching. This is due to the steam is relatively inexpensive and retain more minerals and water soluble components. The use of steam blanching brake more cellular constituents and release the bound phenolic compounds. It also effective to deactivates oxidative and hydrolytic enzymes to protects the loss of phenolic compounds from the activity of enzymes in fresh products (Roy et al., 2009). During pre-treatment, the application of high temperature lead to the breakdown of complex compounds into simple compounds.

The pre-treatment by using high temperature commonly used in the juice manufacturing to obtain higher extracted juice and reduced the processing time (Grimi et al., 2011). In the study conducted by Eshtiaghi and Yoswathana (2012), sugarcane was used to study the effect of temperature to sugar extraction. The sugarcane was immersed in

water at different temperatures before the extraction process. The extraction of sugar was increased with the increasing temperature and the result was showed in Figure 2.4.

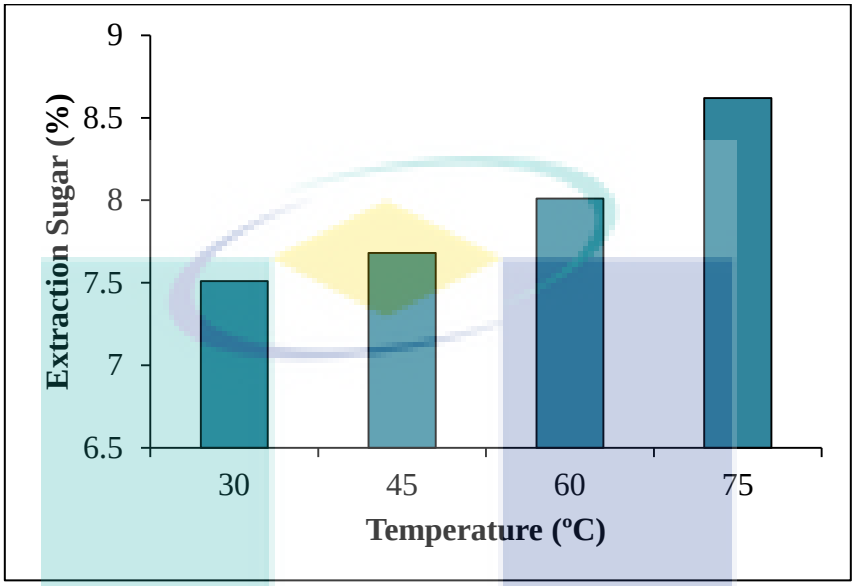

Figure 2.4: Effect of temperature on sugar yield

Source: Eshtiaghi and Yoswathana (2012)

Besides that, the dehydration of sample through a drying process also contributed to the successive extraction of the phenolic compounds (Sulaiman et al., 2011). The drying process was done using an oven to change the surrounding temperature and to dehydrate or dry the sample. Toor and Savage (2006) reported a significant increased in the content of phenolic compounds when tomatoes were dried at high temperature. The vacuoles in the cellular structure collapsed during the dehydration process. The number of free hydroxyl phenol groups were increased due to the hydrolysis of flavonoid glycoside and the released of phenolic compounds from the cell walls. The availability of phenolic compounds also increased due to the non-enzymatic inter-conversion between the phenolic molecules (Vega-galvez et al., 2009).

Photochemical pre-treatment

Photochemical pre-treatment is the treatment that initiated by the absorption of energy in the form of light such as ultraviolet (UV) light. UV light is an invisible form of electromagnetic radiation that has a shorter wavelength than the light humans can see. UV light is divide into three parts: UV-A (315-400 mμ), UV-B (280-315 mμ) and UV-C (less than 280 mμ), ranked from long to shorter wavelengths (Hollosy, 2002). UV-A represent about 6.3% of the solar radiation and this spectrum is the less hazardous part of UV radiation. UV-B represents only 1.5% but this type of spectrum can induce variety of damaging effect to plants while UV-C is extremely harmful to the organisms. UV light carries more energy than visible light that capable to break the bonds between the atoms and molecules. It also is altering the chemistry of materials when exposed to it. According to Kovacs and Keresztes (2002), the UV photon destroyed the chemical bonds and causing the photochemical reaction. It also damaged the deoxyribonucleic acid (DNA) and physiological processes of the organisms (Hollosy, 2002).

UV light stimulated the formation and accumulation of certain phenolic compounds in plants (Treutter, 2010). From studies conducted by Eichholz et al., (2011), the amount of phenolic compounds in blueberries was increased when exposed to UV light. The UV light mediated the stress to increase the phenolpropanoid metabolism that leading to an acceleration of the biosynthesis of phenolic compounds. Interdonato et al., (2011) also found the phenolic compounds in plants increased when exposed to UV light. The phenolic compounds such as FA, *p*-CA and caffeic acid in tomatoes showed approximately 20% higher under UV transmission compared to UV blocking treatment (Luthria et al., 2006).

2.4.3 Effect of storage condition

During the extraction, the cell walls break and release the juice and all nutrients from the plant fibers. When the extracted juice exposed to the surroundings, it affect the stability and amount of nutrients and phenolic compounds. The fresh juice that extracted from the plants has high tendency for spoilage. This was due to the extracted juice was unprotected by cell wall and exposed to the air and microorganisms from the environment (Shamsudin et al., 2014). The nutrients and phenolic compounds that contained in the extracted juices degenerate as they were sensitive to air, light, heat and time. The stability during the storage were essential as its influence the physical appearance, retention of nutritional value and their microbial shelf-life (Suarez-Jacobo et al., 2012). In order to maintain the stability of the extracted juice, the conditions such as temperature and storage time need to be maintained and controlled.

The storage temperature played an important role in order to maintain the content of phenolic compound during the storage times. From the studies conducted by (Ayala-Zavala et al., 2004), the amount of phenolic compounds in strawberry was continuously increased when stored at 5 and 10 $^{\circ}$C while it was maintained when stored at 0 $^{\circ}$C. Besides, the strawberry that was stored at 10 $^{\circ}$C also had higher antioxidant content and antioxidant enzyme activities compared to those that stored at 0 and 5 $^{\circ}$C (Jin et al., 2011). This was due to the levels of the enzyme activities which were increased with the increasing of storage temperature.

The level of phenolic compounds contained in the extracted juice can be prolonged if stored at low temperature during the storage time. The used of low temperature lower the degradation rate of phenolic compounds (Suarez-Jacobo et al., 2012). However, the phenolic compounds in extracted juice were decreased if stored at longer storage time. According to Patthamakanokporn et al. (2008), the phenolic compounds in homogenized guava samples was gradually decreased when stored for more than 30 months. Therefore, there is a limit time to store the phenolic compounds and it must be used within reasonable

time after been extracted. This is to avoid the degradation of the phenolic compounds which reduces their benefit.

2.5 FACTORS SELECTION

The extraction of phenolic compounds depends on the type of extraction, pre-treatment methods and storage condition for the extracted juice. The mechanical extraction by using sugarcane press machine was chosen to extract the FA from BSW. The mechanical extraction was selected because this type of extraction required short extraction time and low cost compared to conventional method. It does not require the use of chemical solvent. Three types of pre-treatment methods were selected in this study which were physical, physiochemical and photochemical pre-treatments. The selection of pre-treatment process was important in order to increase the amount of phenolic compounds. The raw materials in this study were treated before the extraction process. The physical pre-treatment was done by reducing the size of the raw materials. It increased the surface area of the raw materials for the extraction and weaken the plant cell walls (Zuniga et al., 2003). The physiochemical pre-treatment was applied by manipulating the temperature while photochemical pre-treatment was done by exposing the samples to UV light. Previous study proved that the amount of phenolic compounds were increased when treated under high temperature and exposed to UV light (Dewanto et al., 2002; Eichholz et al., 2011). Lastly, the storage time for the extracted juice was chose in order to increase and maintain the amount of phenolic compounds.

2.6 FERULIC ACID ANALYSIS

The purposes of plant extract analysis are to identify and characterize the composition of phenolic compounds. It is one of the most challenging process due to the various combination and different polarities of the phenolic compounds. Phenolic compounds usually present as the minor components in the plant extract. High performance liquid chromatography (HPLC) is one of the analytical equipment to determine the composition of phenolic compounds. HPLC is ideally suited to analyze the various

combination of phenolic compounds (Sasidharan et al., 2011). It also used to classify the phenolic compounds into their specific group or class (Naczk and Shahidi, 2006).

In HPLC, there are several types of detectors used to analyze the composition of phenolic compounds. The detectors that commonly use are refractive index (RI), fluorescence, ultraviolet spectrophotometry (UV), infrared (IR) spectrophotometry and diode array (DAD) (Christie, 1992; Irakli et al., 2012). According to Naczk and Shahidi (2006), the detector that commonly used in the analysis of phenolic compounds were UV and DAD. The UV detector is one of detector that offer high sensitivity and provide good stability (Sasidharan et al., 2011). Meanwhile the DAD detector is capable to analyze the phenolic compounds by using different wavelength in their spectra (Amarowicz and Weidner, 2001).

The separation of phenolic compounds from the sample depended on the movement of the compounds in HPLC column (Sasidharan et al., 2011). It was performed with the injection of the sample in the liquid form from the vial. The sample was injected into the moving stream of liquid that known as mobile phase. There are several types of mobile phase that commonly used such as acetonitrile, methanol, ethyl acetate and water (Kimura and Rodriguez-Amaya, 2002; Watanabe et al., 1998). After the injection, the sample will pass through the column. The column consists of the stationary phase. The selection of stationary phase depends on the separation of phenolic compounds, whether it is done based on adsorption, partition or ion-exchange process. From the previous study, there were several types of columns that used for analysis phenolic compounds as showed in Table 2.3. From the table, it showed different researchers used different type of columns analyze the phenolic compound.

Table 2.3: Type of columns that used by different researchers

Reference	Type of column
Buranov and Mazza, 2009	Zorbax SB-C18 (250 mm x 4.6 mm, 5 µm)
Zhou et al., 2005	Zorbax SB-C18 (250 mm x 4.6 mm, 5 µm)
Sun and Wang, 2008	YMG C18 (250 mm x 4.6 mm, 5 µm)
Musatto et al., 2007	Water Resolve C18 (300 mm x 3.9 mm, 5 µm)
Liu et al., 2006	Fuji Silysia C18 (200 mm x 4.6 mm, 5 µm)

In the analysis of phenolic compounds, the composition of phenolic compound was determined based on the standard calibration curve of the selected compound. It was determined by comparing the retention time of the sample with the standard (Choi et al., 2008). The time was measured from the injected sample until the maximum peak height for the compound was showed. The time taken for the analysis is different based on type of phenolic compounds. Besides that, it also depends on the use of temperature, pressure, type of column and mobile phase.

2.7 RESPONSE SURFACE METHODOLOGY (RSM)

The optimization technique is useful to find the optimum condition. The optimum condition for the factors could either be a maximum or a minimum (Aslan, 2008). The relationships between the factors and their response are complex and difficult to explain. Therefore, the response surface methodology (RSM) was introduced into the process. The application of RSM is able to determine the relationships between the factors and their response and reduce the number of experiments (Alireza et al., 2013). It is also capable to determine the relationship between the factors. This method extensively adopted in industry in order to determine the optimal condition for the factors to attain desired quality of the product. It became one of the important method to increasing the production rates while reducing the production cost (Sinha et al., 2012).

RSM is an effective method that uses to predict the optimum response with good precision for the combination of several factors that influence the process. The experimental design for RSM was done by using Design Expert software (Version 7.1.3, Stat-Ease, Inc., Minneapolis, MN) program. This method consists of the statistical and mathematical techniques that successfully use for developing, improving and optimizing the process (Tabaraki and Nateghi, 2011). The mathematical model is adequate to evaluate multiple factors and their interactions using quantitative data. It also reduced the number of experiment that required to run in the process (Wang et al., 2013).

There were four steps to design the experiment by using RSM (Aslan, 2008). The first step was done by designing the experimental runs based on several factors and the response of interest. The second step was the developing of the mathematical model for the first-order or the second-order model with the best fitting. Then, the third step was the finding of the optimal set for the factors that produce the maximum or minimum value of response. Lastly, the factors that gave direct and interactive effect on the process were represent through two and three-dimensional (3D) plots

2.7.1 Two-level factorial design

Two-level factorial designs are often use in the factorial analysis to give a complete understanding of the aliasing structure of the design (Butler, 2008). The application of this design was capable to determine the effect of the main factors and their interactions. The experimental design is constructed by setting all factors at two levels. The two levels of the factors are low level (-1) and high level (+1) that were used as the limits of the values range (Bingol et al., 2010). The used of this design was able to reduce the number of experimental runs. According to Rozet et al. (2013), this design was used to select factors that have the largest effect to the response. It have great efficiency and flexibility to determine the main and interaction effects (Witek-Krowiak et al., 2014).

The design in two-level factorial may involve a lot of factors. It may consists of 2 to 21 factors in which the main and interaction effects are studied. For an example, the design

with 3 factors generated 8 experimental runs. During the experiment, some error could be occurred. The experiments can be replicated in order to reduce or avoided the errors. The replication of experiments is trustworthy step and commonly applies for designs that have low number of run. However, when a lot of factors involve in a design, it will generate a large number of experimental runs. As for an example, a design with 9 factors generated 512 experimental runs. For this design, the number of runs is high and it quite difficult to do the replication for all the runs. Besides, the replication also requires high cost for the operation and raw material. In order to overcome this problems, the two-level fractional factorial design is applied since it is more efficient and economic (Bezerra et al., 2008). The used of fractional factorial design have less runs compared to a full factorial design (Ou et al., 2013). According to Chang et al. (2011), the fractional factorial design has the advantages to identify and isolate the significant factors without neglecting the interaction effects between factors.

The number of experiments for a design depends on the selection of design resolution. The design resolution describe the degree of estimated main effects are aliased with estimated two-factor interactions or higher interactions (Georgiou, 2007). There are three types of resolutions which are V, IV and III. In factorial analysis, Resolution V represent by green and white colors. It estimates all the main effects and two-factor interactions. In this resolution, there are no main effect and two-factor interaction that aliased to each other. However, there is a possibility for two-factor interactions that aliased with three-factor interactions. For resolution IV, it is represented by yellow color. This resolution indicates the main effect may be aliased with three-factor interactions and two-factor interactions aliased with other two-factor interactions. Lastly, resolution III was represented by the red color. The resolution III indicates the main effects may be aliased with two-factor interactions. Besides, there is also a possibility for two-factor interactions that aliased to each other.

The relationships between the factors and the response in factorial analysis were established by using first-order regression model. The experimental data that obtained from

the factorial analysis was analyzed and fitted into the first-order equation. A general first-order equation is defined as Eq. 2.1:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \in \quad \text{(Eq. 2.1)}$$

Where y represents the predicted response, β_0 is the constant coefficient, β_i is the linear coefficients, x_i is independent variables and $\in$ is the residual associated to the experiments.

2.7.2 Central composite design (CCD)

Central composite design (CCD) is a very useful tool that provide statistical model to understand the interactions between the factors that have been optimized (Nasirizadeh et al., 2012). It provides the information as much as the three-level factorial design but with the smaller number of experiments. The CCD contains two level of fractional points (plus and minus 1), center point and two level of axial points (plus and minus alpha) (Witek-Krowiak et al., 2014). The center point is the point that corresponds to the middle of the factors. The axial points are the points that choose depend on the number of factors and the specific desired properties of the design. The replication of experiments is important in order to estimate the experimental error (Yi et al., 2010). In CCD, the replication of experiments was provided at the center point (Aslan, 2008).

In CCD, the relationships between the factors and response were established by using second-order regression model. The model is significantly improving the optimization process compare to the first order model. The experimental data from the CCD was analyzed and fitted to the second-order equation (Salleh et al., 2011). A general second-order model is defined as Eq. 2.2:-

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j} \sum \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \in \quad \text{(Eq. 2.2)}$$

Where y represent the predicted response, β_0 the constant coefficient, β_i the linear coefficients, β_{ij} the interaction coefficients, β_{ii} the quadratic coefficients and x_i, x_j are independent variables.

The response surface plot is a three dimensional graph that illustrate the relationship between the factors and the response. The graph is plotted based on two factors while the other factors are constant at the optimum points (Tabaraki and Nateghi, 2011). Figure 2.5 shows the example of response surface plot with the response was plotted versus the two factors which are x_1 and x_2. Besides that, the response surface also viewed in two dimensional graphs which is contour line as shown in Figure 2.6. The contour plot shows the contour lines of x_1 and x_2 that response to y value. The shape of contour indicates the interaction between the factors. It may represented by the shaded areas, contour lines, or both. According to Zhao et al. (2012), an elliptical contour plot show the interactions between the factors were significant while a circular contour plot means otherwise.

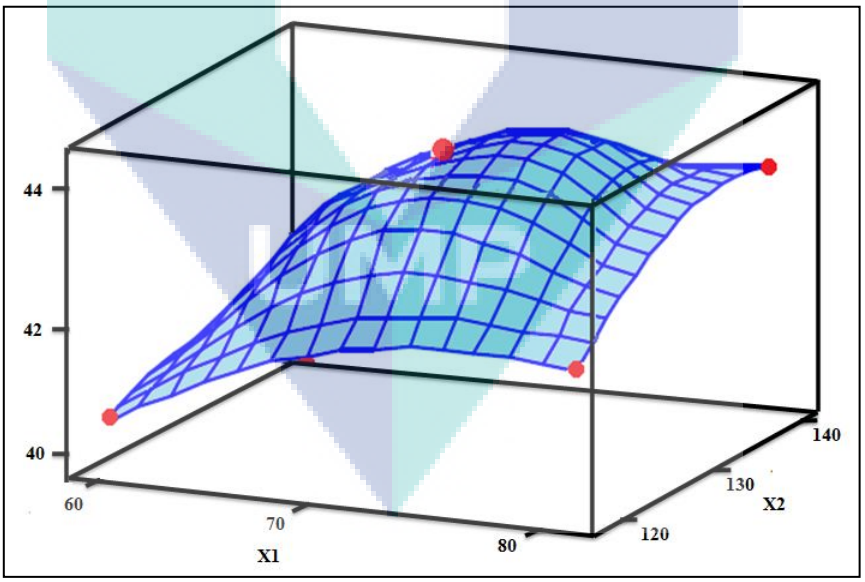

Figure 2.5: Response surface plot

Source: Bradley (2007)

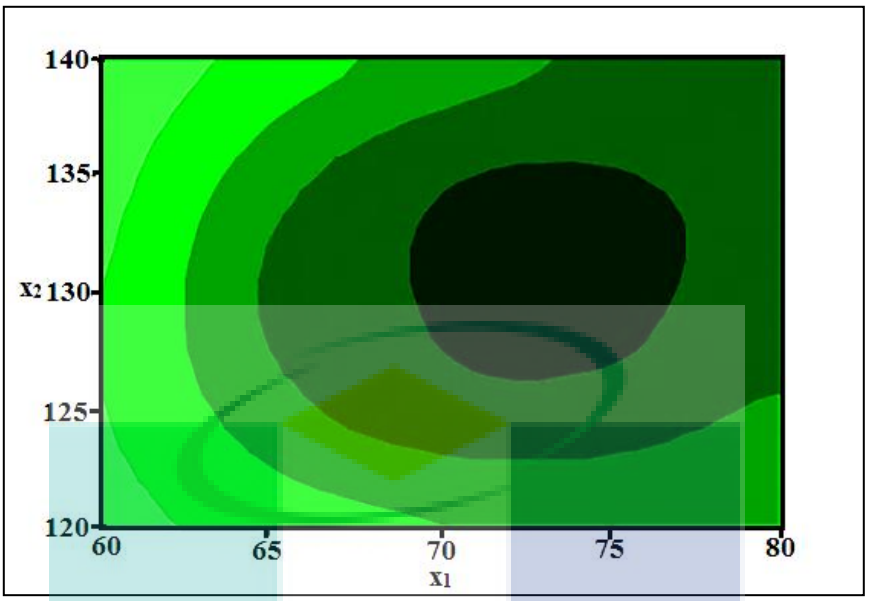

Figure 2.6: Contour plot

Source: Bradley (2007)

2.7.3 Application of RSM in extraction

RSM is an effective tool for optimizing the process when a lot of factors involve. It is widely used to optimize the process, especially in the extraction process. The application of RSM capable to increase the production rate while minimizing the cost. The used of RSM in the extraction process also provide a clear understanding about the interaction between the factors (Salleh et al., 2011). RSM was successfully applied in the optimization of phenolic compounds from natural resources. From the previous studies, RSM was applied in the extraction of phenolic compounds from *Mangifera pajang* (Prasad et al., 2011), onion (Kiassos et al., 2009), wheat (Liyana-Pathirana and Shahidi, 2005), and stink bean (Gan and Latiff, 2011). Besides that, RSM also used to optimize the extraction of FA from agriculture waste. From the research conducted by Salleh et al. (2011) RSM was used to improve the extraction of FA from paddy straw.

2.8 CHAPTER SUMMARY

This chapter covered the literature review and fundamental concept on the extraction of FA from BSW. BSW was chosen as the raw material for the extraction of FA since it abundant in our country. BSW is one of the agriculture waste and the used of this waste is able to prevent it remain idle. The BSW which contain high concentration of phenolic compounds make it a great candidate to use as the raw material. In this study, the extraction of FA from BSW was done by using sugarcane press machine. The extraction by using sugarcane press machine was a new method that offers great result at low cost. This type of extraction method was simple compare to different type of method without using any type of chemical as the solvent. The optimum condition for the factor that effects the extraction of FA was the main objective of this study. The analysis of FA from the extracted BSW juice was determined by using HPLC method. HPLC was chosen to analyze the concentration of FA that produced after the extraction process. Five from all the factors that discussed in this chapter were selected for the factorial analysis. These factors were part of stem, pre-treatment temperature, UV light pre-treatment, cycle of extraction and the storage time for the extracted BSW juice. Two-level factorial design was selected to apply in factorial analysis. As for the optimization process, the CCD was chosen in order to determine the optimum condition for the extraction of FA from BSW.

CHAPTER 3

METHODOLOGY

3.1 OVERAL METHODOLOGY

Figure 3.1 shows the overall procedures used to optimize the extraction of FA from BSW. Firstly, BSW was collected from banana plantation and divided into outer stem and pith. Then, the samples were characterized based on the compositions of moisture, lignin, cellulose and hemicellulose. The characterization was performed in order to fulfill the first objective of this study. After the characterization, the preliminary study was done to determine and choose the factors contribute to the amount of FA that obtained during the extraction process. The selected factors were used in factorial analysis. This analysis was the second objective in this study. The factorial analysis was performed to analyze five factors that were found to be affecting the FA extraction. These factors were part of stem, ultraviolet (UV) light pre-treatment, pre-treatment temperature, cycle of extraction and storage time of extracted BSW juice (EBJ). Two-level factorial design in response surface methodology (RSM) was used to construct the experimental table and to analyze the data.

Central composite design (CCD) in RSM was applied to design the experimental table and analyze the experimental data. In this study, optimization of FA extraction was done to fulfill the requirement of third objective. The suggested optimum condition for FA extraction from BSW was obtained from the optimization. The validation of the experimental data was performed at suggested optimum condition. The sample of the EBJ was analyzed for the compositions of total phenolic and glucose.

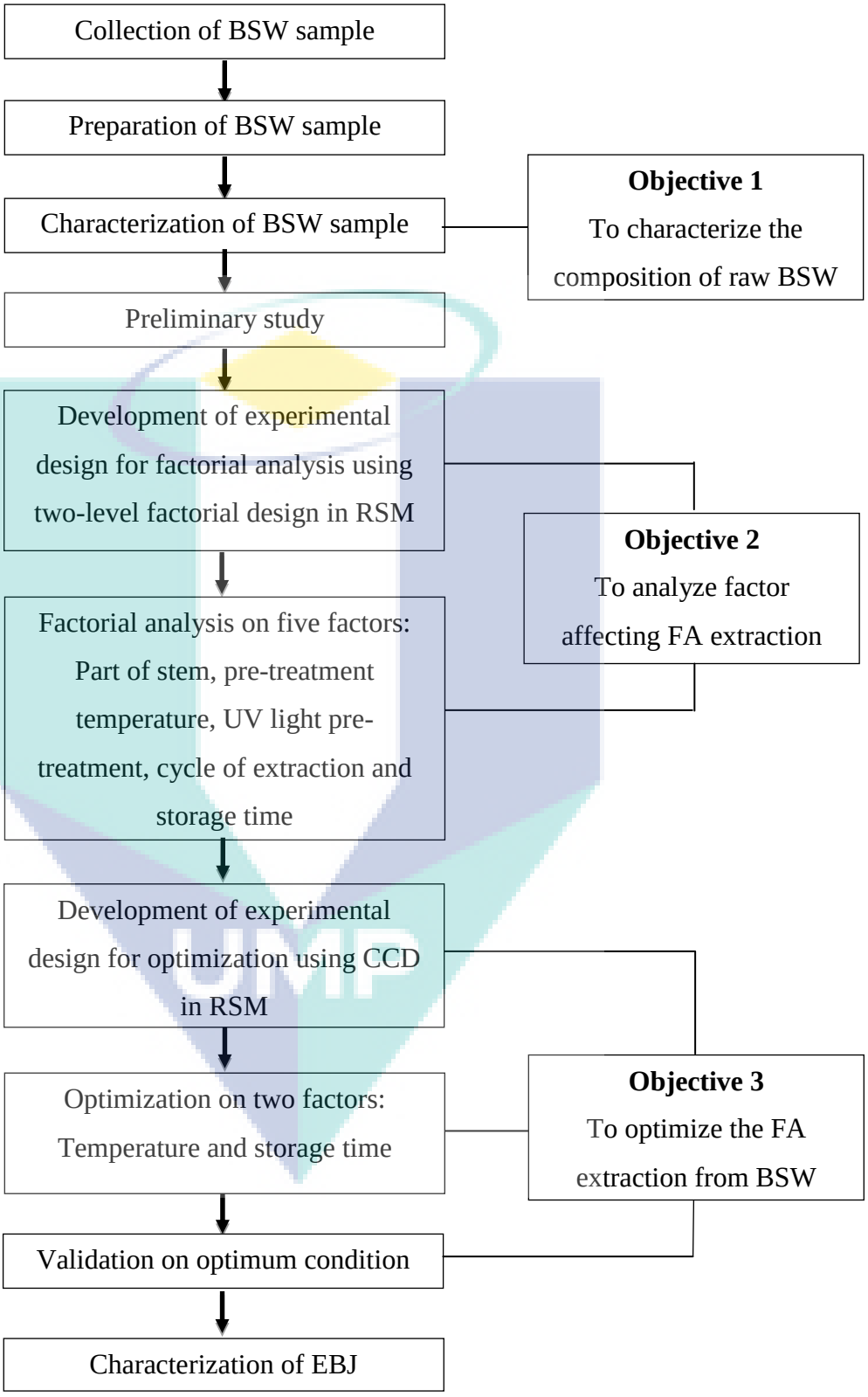

Figure 3.1: Process flow for the experiment

3.2 CHEMICALS AND MATERIALS

3.2.1 Chemicals and reagents

The chemicals in this study were purchased from various suppliers. The list of all the chemical was showed in Appendix A.

3.2.2 Raw material (banana stem waste)

BSW was used as raw material in the extraction of FA. BSW was collected from banana plantation. The BSW sample was obtained from banana plantation in Kuantan, Pahang. The banana trees were cut down during the harvesting process of matured fruits. It usually takes about 15 to 17 months for the fruits matured and ready to be harvested. The sample that used in this study was collected in May 2013. There were collected in large quantities during the harvesting time to ensure the quality of raw material constant during experiment.

3.2.3 Sugarcane press machine

Sugarcane press machine is one of the mechanical extractor that commonly used for the extraction of juice (Zahari et al., 2012). In this study, BSW was extracted by using electric sugarcane press machine as showed in Figure 3.2. The model of this machine was KR3176 with the rotating speed was 80 rpm. This semi-automatic machine consists of three stainless steel roller to extract the juice from BSW. The top or driven roller acted as pinion with the lower rollers. The lower rollers function as feed and discharge roller. The rollers were grooved transversely to their axis with V-shaped grooves to increase the surface area of the rolls during pressing.

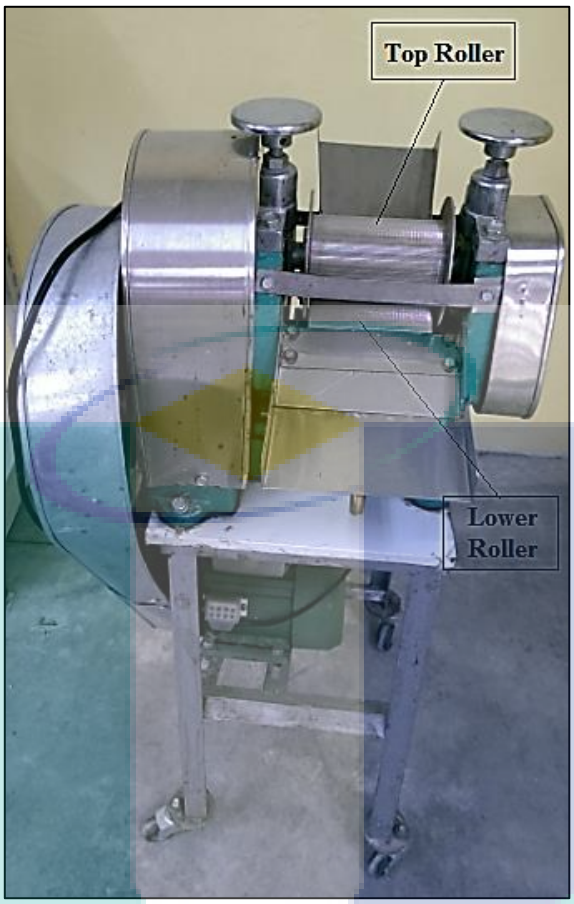

Figure 3.2: Sugarcane press machine

3.3 CHARACTERIZATION OF BSW

The BSW was characterized to determine the composition of moisture, lignin, cellulose and hemicellulose. The composition of moisture was determined based on the water content in BSW. The analysis was performed according to ABNT NBR 9656 (Brazilian association for technical standards). In this test, the sample was put in the oven at temperature 105 $^{\circ}$C for 4 h (Guimaraes et al., 2009). The composition of moisture was determined by calculating the percentage difference between the initial weight of the sample (1.0 g) and after drying as shown in the Eq. (3.2).

$$M_n = \left[\frac{(W_w - W_d)}{w_w}\right] \times 100 \quad (3.2)$$

in which:

M_n = moisture content (%) of sample

W_w = wet weight of the sample, and

W_d = weight of the sample after drying.

The composition of lignin, cellulose and hemicellulose in BSW were analyzed via acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) methods analysis (Omar et al., 2011). NDF analysis was done by weighing and transferred one gram of dried ground BSW samples into hot extraction unit (Fibertech System, USA). 100 ml of neutral detergent solution (Appendix A.2) was added into the extraction unit and the mixture was heated at boiling temperature for an hour. After the heating process, the mixture was cooled and filtered to collect the residue. The collected residue was washed three times with hot distilled water. Then, it was transferred to cold extraction unit (Fibertech System, USA). After that, the residue was washed with acetone for three time and vacuum dried. Further drying was done by putting the residue in oven at 105 °C until the weight become constant. The different weight of residue before and after drying was calculated to determine the composition of NDF. For the ADF analysis, it was done similarly with the method to determine the NDF except for the different solution called acid detergent solution (Appendix A.3).

The ADL analysis was started by covering the residues from ADF analysis with 72% of sulphuric acid solution at 15 °C. It was stirred three times at one hour interval. After washing with hot distilled water and filtered, the residues were oven-dried at 105 °C for 3 h and then cooled. Then, the residue was ignited in 500 °C furnace for 2 h. The hot crucibles was transferred into a 100 °C oven for an hour before cooled in desiccators and then weighed. The differences of the sample weight before and after drying were calculated to determine the composition of ADL. Finally, the composition of lignin, cellulose and

hemicellulose were calculated from the contents of NDF, ADF and lignin. The compositions were calculated by using Eq. (3.3), (3.4) and (3.5):

$$\text{Lignin } (\%) = \text{ADL} \tag{3.2}$$

$$\text{Cellulose } (\%) = \text{ADF} - \text{ADL} \tag{3.3}$$

$$\text{Hemicellulose } (\%) = \text{NDF} - \text{ADF} \tag{3.4}$$

3.4 PRELIMINARY STUDY FOR FERULIC ACID EXTRACTION

Preliminary study was carried out to give an overview and to ensure the chosen factors affected the FA extraction. Since FA was naturally present in BSW, pre-treatment was essential to improve the FA yield. The pre-treatment methods employed in this study were physical, UV light and temperature. Besides, other factors such as the cycle of extraction and storage time for the EBJ also have been studied.

3.4.1 BSW extraction method

The extraction of BSW was carried out by using sugarcane press machine. The BSW was extracted after the pre-treatment process. The BSW was pressed pass through the roller pressers and the bagasse was thrown away. For the extracted BSW juice, it was collected and centrifuge at 15,000 rpm for 15 min at 4 $^{\circ}$C (Thermo Fisher Scientific, NC, USA). After centrifuged, the EBJ was filtered using Whatman No. 41 filter paper (20–25 μm) to remove all the remaining debris. Then, the juice was stored at -20 $^{\circ}$C before the analysis.

3.4.2 Physical pre-treatment of BSW

In this study, the physical pre-treatment was done by reducing the size of BSW sample to enhance the availability of FA during the extraction process. The BSW was collected from banana plantation in bulk quantities and the samples were separated into two parts which were outer stem and pith. Both parts were cut into the size approximately

2.5±0.5 cm long and 2.5±0.5 cm width. The prepared samples were stored at 4 °C before undergo the pre-treatment process with the temperature and UV light.

3.4.3 UV light pre-treatment of BSW

The UV light pre-treatment of BSW was done to increase the availability of FA during extraction. The ultraviolet (UV) light was used for the pre-treatment as showed in Figure 3.3. The amount of phenolic compounds in plants was affected with the exposure of UV light (Treutter, 2010). In this study, 100 g of BSW was put in the tray and placed in the laminar air flow. The BSW was exposed into UV light for 30 min. After the pre-treatment, the BSW was extracted by using sugarcane press machine.

Figure 3.3: BSW that exposed into UV light

3.4.4 Pre-treatment temperature of BSW

The pre-treatment temperature of BSW was performed to increase the FA yield during the extraction. It was done by manipulating the temperature during pre-treatment. According to Roy et al. (2009), the availability of phenolic compounds were affected with

the variation of temperature. In this study, water bath was used to control the pre-treatment temperature. 100 g of BSW was weighed and put in the beaker. The beaker was immersed in water bath for 30 min at different temperatures between 20 to 90 °C.

3.4.5 Effect number of extraction cycle

100 g of BSW was pressed pass through the three stainless steel roller pressers as showed in Figure 3.4. The pressing process repeated one to three cycle (Eshtiaghi and Yoswathana, 2012). This study was done to determine the effect of pressing cycle to FA yield.

Figure 3.4: BSW press by using sugarcane press machine

3.4.6 Effect of storage time for EBJ

The FA yield also affected by the storage condition of EBJ. According to Ayala-Zavala et al. (2004), temperature and time during storage influenced the amount of phenolic compounds in extracted juice. The samples of EBJ were collected and stored in universal bottle at room temperature for 0 to 36 h. Then, the samples were collected for every 12 h and stored at -20 °C for further analysis

3.5 FACTORIAL ANALYSIS METHOD

The experimental design for factorial analysis was performed using Design Expert software (Version 7.1.3, Stat-Ease, Inc., Minneapolis, MN) program. The experimental table was constructed in two-level factorial design of response surface methodology (RSM). Two-level factorial design was used to determine the influence of several factors on the response (Golshani et al., 2013). A lot of information was provided with minimum run of experiment (Rozet et al., 2013). Besides, non-significant variables were eliminated in the process. In factorial analysis, five factors were studied with 36 runs of experiment. The main factors and the interactions between the factors were determined in factorial analysis. Information about the most contribution factors influenced the extraction of FA from BSW also provided in this analysis.

3.5.1 Experimental setup for factorial analysis

The experimental trials were performed at the Bio-process Laboratory of Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang. There were five factors studied in factorial analysis. Two types of factors involved in categorical factor and the other three were numerical factor. The categorical factors (part of stem and UV light pre-treatment) were studied at two fixed level. Part of stem was categorized by separating the BSW into two parts which were outer stem and mixed stem. The UV light pre-treatment by using UV light was controlled by exposed the sample in laminar air flow for 30 minutes. The three numeric factors (pre-treatment temperature, cycle of extraction

and storage time of EBJ) were studied based on the pre-determined values. The temperature for the pre-treatment of BSW was set between 25 to 90 °C. The temperature was controlled by using water bath. In this study, BSW was immersed in water bath for 30 minutes. The BSW was extracted by using sugarcane press machine. The number of extraction was varied from one to three cycles. The EBJ was collected and stored at room temperature between 0 to 24 h. Then, the EBJ was stored at -20 °C before analyzed. Table 3.1 shows the level for each factor for in factorial analysis.

Table 3.1: Level for each factor in factorial analysis

Factors	Symbols	Units	Type of Factor	Level		
				α = -1	**α = 0**	**α = +1**
Part of stem	A		Categorical	Outer	-	Mixed
UV light pre-treatment	B		Categorical	Yes	-	No
Pre-treatment temperature	C	°C	Numerical	25	57.5	90
Cycle of extraction	D		Numerical	1	2	3
Storage time of EBJ	E	Hours	Numerical	0	12	24

3.5.2 Validation experiment for factorial analysis

The validation experiment for factorial analysis was done to validate the predicted values from the model. It also determined the suitability of the model generated in this study to obtain the optimum of FA extraction. The condition for the validation was obtained from the predicted best condition generated from two-level factorial design. All factors for validation were controlled in a similar way as the factorial analysis. The condition for each of the factor was showed in Table 3.2. The validity of the model was determined by comparing the predicted and experimental values. The percentage errors between these values were calculated using Eq. (3.5).

Table 3.2: Condition factors for validation of factorial analysis

No	Type of Stem	UV light Pre-treatment	Pre-treatment temperature (°C)	Cycle of extraction	Storage time of EBJ (h)	Predicted Value (mg/g)
1	Outer	Yes	25	1	24	0.0959
2	Outer	No	25	1	24	0.0757

$$\%\, Error = \frac{(Actualvalue - Predictedvalue)}{Actualvalue} \times 100\% \quad (3.5)$$

3.6 OPTIMIZATION METHOD

The experimental design for optimization was done by using Design Expert software (Version 7.1.3, Stat-Ease, Inc., Minneapolis, MN). The experimental table was constructed by using central composite design (CCD) in response surface methodology (RSM). CCD was applied to identify the relationship between the factors and their response. In this study, mechanical extraction of FA from BSW was optimized by using CCD. According to Salleh et al. (2011), CCD was used to optimize the extraction of FA from paddy straw. The selection and range of two factors for the optimization were chosen from the factorial analysis. Based on these two factors, 13 runs of experiments were generated.

3.6.1 Experimental setup for optimization

The experimental trials for the optimization of FA from BSW were performed at Bio-process Laboratory. Two factors which were pre-treatment and storage time of EBJ were studied for the optimization. The pre-treatment temperature was set between 20 to 30 °C and controlled using water bath. After the extraction, the EBJ was collected and stored at room temperature between 20 to 28 h. The other factors were fixed using outer part for the part of stem, without UV light pre-treatment, and single pressing cycle. The experimental table for optimization was constructed with five levels of numeric factors. The five levels consisted of plus and minus alpha (axial point), plus and minus 1 (factorial points), and the center point. Table 3.3 show the level for each factor in optimization.

Table 3.3: Level of factor tested in optimization

Factors	Symbols	Units	Level (α)				
			-2	-1	0	+1	+2
Pre-treatment temperature	A	°C	20	22.5	25	27.5	30
Storage time of EBJ	B	Hours	20	22	24	26	28

3.6.2 Validation experiment for optimization

The predicted values from the model were validated based on validation experiment. The validation condition was obtained from the suggested optimum condition that generated from CCD. Table 3.4 shows the condition for each of the factors that was used in this experiment. The predicted and experimental values were compared to determine the validity of the model. Eq. (3.5) was used to calculate the percentage error between the values.

Table 3.4: Condition factors for validation of optimization

Type of Stem	UV light pre-treatment	Pre-treatment temperature (°C)	Cycle of extraction	Storage time of EBJ (h)	Predicted Value (mg/g)
Outer	No	25	1	24	0.1969

3.7 ANALYSIS OF SAMPLE

Analysis of samples was done to determine the FA yield and composition of EBJ. The identical analysis method was used for both factorial analysis and optimization study to determine the FA yield in EBJ. The analysis of FA was done by using high performance liquid chromatography (HPLC). Lastly, the analysis of EBJ was performed to determine the compositions of total phenolic and glucose.

3.7.1 Analysis of FA by using HPLC

The concentrations of FA in samples were analyzed by using high performance liquid chromatography (HPLC). The analytical HPLC system employed consisted of an Agilent 1100 HPLC equipped with a diode array detector (DAD). The HPLC pumps, column oven, auto sampler, and DAD system were monitored and controlled using the HP Chem Station computer program. The separation and analysis of FA were carried out using a 5 µm Zorbax SB-C18 (250 mm x 4.6 mm, Agilent Technologies, Palo Alto, CA) (Buranov and Mazza, 2009). The temperature of the column was set at 30 °C. The mobile phase consists of water (eluent A) and acetonitrile (eluent B). The gradients of mobile phase were 45 % of water and 55 % of acetonitrile. The mobile phase was pumped with the flow rate at 1.0 ml/min by a quaternary gradient pump (G1311A Quat Pump, Agilent). The injection volume was 10 µl.

The FA yields in the samples were identified by comparing the retention times with the FA standards. It was determined under analytical conditions and quantified by the external standard method. The stock solution of FA was prepared with the concentration 0.5 g/l. It was prepared by 0.05 g of accurately weighed FA in water. The FA was put into volumetric flask and dissolved with 100 ml of water. 8, 6, 4 and 2 ml of stock solutions were pipette out and transferred into four volumetric flasks. Then, the volume was made up to 10 ml by adding the required volume of distilled water to get a concentration of 0.1, 0.2, 0.3 and 0.4 g/l. This solution was used for calibration curve during the analysis of FA using HPLC. The solution was filtered through 0.45 µm nylon syringe filter (Millipore). 10 µl of

solution was automatically injected into the HPLC equipment. From the analysis, the calibration curve was constructed with five different concentrations of FA. This curve should pass through or very near to the origin. The correlation coefficient for the calibration curve should be higher than 0.95 (Michaelevski et al., 2010)

3.7.3 Characterization of extracted BSW juice

The analysis of EBJ was done to determine the compositions of total phenolic and glucose. The sample was obtained from the optimization study. The composition of total phenolic was analyzed by using Folin-Ciocalteus reagent (Aziz et al., 2011).This method relied on the transferred of electrons from phenolic compounds to the Folin–Ciocalteu reagent in alkaline medium (Fu et al., 2011). 0.5 ml of the samples was inserted into different test tube and mixed thoroughly with 2.5 ml Folin-Ciocalteu reagent (previously pre-dilute 10 times with distilled water). 2 ml of 7.5 % sodium carbonate (Na_2CO_3) was added and allowed to react for 30 min at room temperature. The absorbance was measured at 765 nm using UV-VIS spectrophotometer (Bucic-Kojic et al., 2011). The composition of total phenolic was determined by comparing the absorbance with the standard of Gallic acid. The concentration of Gallic acid was prepared at 0.05, 0.10, 0.15, 0.20 and 0.20 mg/l in 80% methanol solution (Maoulainine et al., 2012). The composition of total phenolic per extract was expressed as Gallic acid equivalent (GAE).

The composition of glucose in the EBJ was determined by using dinitrosalicylic **(**DNS) test. This method determined the presence of reducing sugars with the free carbonyl group (C=O). During DNS test, the functional sugar group was oxidized while DNS was reduced to 3-amino-5-nitrosalicylic acid (Teixera et al., 2012). This reactions were occurred at alkaline condition with the presence of heat (Wood et al., 2012). The equation that involved in this reaction was showed in Eq. (4.6).

3,5-Dinitrosalicylic acid+Reducing sugar→3-amino-5-nitrosalicylic acid+Oxidized sugar (4.6)

3 ml of the DNS solution (Appendix B.3) and sample were added into test tube. The solution was mixed properly. The test tube was covered and placed in a boiling water bath for 10 min. Then, the sample was cooled down to room temperature and the absorbance was measured at 575 nm by using UV-VIS spectrophotometer. The composition of glucose in the sample was determined by comparing the absorbance with the standard of glucose. The concentration of glucose was prepared at 0.05, 0.10, 0.15, 0.20 and 0.25 mg/l. The calibration curve was showed in appendix B4.

3.8 CHAPTER SUMMARY

This chapter describes the methods that were used in this study. In characterization of BSW, four tests were performed to determine the compositions of moisture, lignin, cellulose and hemicellulose. The characterization was done to fulfill the requirement of first objective. The composition of moisture in BSW was done according to ABNT NBR 9656. The compositions of lignin, cellulose and hemicellulose were determined via ADF, NDF and ADL methods analysis.

The preliminary study was performed to study the factors affecting the extraction of FA from BSW. Three type of pre-treatment methods employed in this study which were physical, UV light and temperature. The other two factors which were cycle of extraction and storage time for the EBJ also have been studied. From this study, five factors were chosen for factorial analysis. The factors were part of stem, pre-treatment temperature, UV light pre-treatment, number of extraction cycle and storage time of EBJ. The experimental data was analyzed using two-level factorial design. The factorial analysis was performed to achieve the second objective. For the third objective, the optimization of FA extraction was done by using CCD in RSM. Two factors which were pre-treatment temperature and storage time of EBJ were chosen from the factorial analysis. The validation experiment was done to validate the predicted value. The validation was performed based on the suggested optimum condition that generated from CCD.

Lastly, the compositions of total phenolic and glucose were determined using the samples that obtained from the optimization study. The composition of total phenolic was analyzed by using Folin-Ciocalteus reagent while glucose was determined by using DNS test.

CHAPTER 4

RESULT AND DISCUSSION

4.1 CHARACTERIZATION OF RAW BANANA STEM WASTE

The BSW is a pseudo stem that consists of clustered, cylindrical aggregation of leaf stalk bases. They are complex structure and consisting of cellulose micro fibrils that held together by lignin and hemicellulose (Mukhopadhyay et al., 2008). The characterization of the waste was done to determine the composition of the stem. As explained in chapter 3, the characterization of BSW was done based on the compositions of moisture, lignin, cellulose and hemicelluloses. Besides that, the compositions of ash and extractive that contained in BSW also discussed in this chapter.

The analysis of the composition of moisture in BSW was determined according to ABNT NBR 9656 (Guimaraes et al., 2009). The composition of moisture was 92.52%. The data showed BSW contained high composition of moisture. From the study conducted by Li et al. (2010), the fresh banana stem contains about 96% of moisture from the original weight. The differences between this study and study conducted by Li et al. (2010) was 3.76%. The composition of moisture in BSW was different from other researchers due to different test procedure and the ambient temperature during the test.

The compositions of lignin, cellulose, hemicelluloses ash and extractive that contained in BSW were showed in Table 4.1. The compositions of BSW were at 13.02% lignin, 25.92% cellulose and 19.29% hemicellulose. The composition of lignin was lower than the cellulose and hemicellulose. According to Saraiva et al. (2012), the composition of

lignin in BSW was lower than the cellulose because cellulose is an important structural component of the primary cell wall of the plants. From the Table 4.1, it also showed the composition of ash and extractive that present in BSW based on the studied that conducted by Pereira et al. (2010).

Table 4.1: Compositions of raw banana stem waste (BSW)

Components	Composition (%)	Error (%)
Lignin	13.02	2.27
Cellulose	25.92	4.44
Hemicellulose	19.29	1.28
Ash (Pereira et al., 2010)	13.54	0.32
Extractive (Pereira et al., 2010)	23.14	8.30

As shows in Table 4.2, the composition of lignin, cellulose, hemicellulose ash and extractive in this study was slightly different compared to study done by Tripathi et al. (2013), Oliveira et al. (2007) and Bilba et al. (2007). This was due to the used of different type of banana species and the morphological origin of BSW (Max et al., 2010).

Table 4.2: Compositions of banana stem waste (BSW) from other researchers

Research	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Ash (%)	Extractive (%)
This research	13.02	25.92	19.29	13.54	23.14
Tripathi et al., 2013	12.40	53.70	15.5	5.80	1.80
Olieveira et al., 2007	13.30	37.30	7.20	19.00	12.60
Bilba et al., 2007	15.07	31.27	14.98	8.65	4.46

Lignin is a composition for secondary cell wall and the main fraction after cellulose and hemicellulose. It also the phenolic polymer that formed by the oxidative coupling of

phenolic compounds such as coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol (Max et al., 2009). Besides, phenolic acids such as hydroxycinnamic and hydroxybenzoic acids also highly present in cell walls. These acids covalently bond with polysaccharide components by ester bonds and with lignin by ether bonds (Max et al., 2010). The well-known phenolic acids such as FA, *p*-CA, vanillic acid, gallic acid, *p*-hydroxybenzoic acid and vanillin can be found in biomass (Akpinar and Usal, 2014). The FA can be released from biomass by breaking the ester bond in lignin/phenolic-carbohydrate complex (LCC) (Torre et al., 2008). FA was associated with lignin by ether bond and hemicellulose by ester bond (Xu et al., 2005).

4.3 PRELIMINARY STUDY

In this study, BSW was chosen as raw material to extract the FA from the cell wall. FA is a hydroxycinnamic acid that present in the plants cell wall. It has a bifunctional structure that can form ester and ether bonds by the reaction of carboxyl and phenolic groups (Jeffrie, 1990). It shuttled into wall matrices and attached to the lignin and hemicellulose via ether and ester bonds as bridges and form LCC(Sun et al., 2002). Preliminary study was done to study the factors that can affect the mechanical extraction of FA from BSW. Some pre-treatments were used to release the FA from the bonds and increased the FA yield during the extraction. It also can disrupt the complex structure of lignin, cellulose and hemicellulose in BSW. In this study, physical, temperature and UV light pre-treatments were applied to treat the BSW before the extraction. Besides, the effects of cycle of extraction and storage time of EBJ also have been studied.

4.3.1 Physical pre-treatment of BSW

The physical pre-treatment of BSW was done by reducing the size of samples in order to increase the FA yield during extraction. The outer stem and pith of BSW sample was separated from each other. The samples were diced into small size approximately 2.5 cm (length) x 2.5 cm (width) in order to increase the surface area per unit mass (Luthria, 2012). Figure 4.1 shows the FA yield in different part of stem. From the figure, it shows the

FA yield in the outer stem was slightly higher than the pith. The FA yield in the pith was 0.0127 mg/g while in the outer stem was 0.0138 mg/g. It showed the FA yield in outer stem was 8.47% higher than the pith. The differences between both parts were due to their lignin composition because the FA was esterified with lignin and polysaccharide in herbaceous plants (Oliveira et al., 2006).

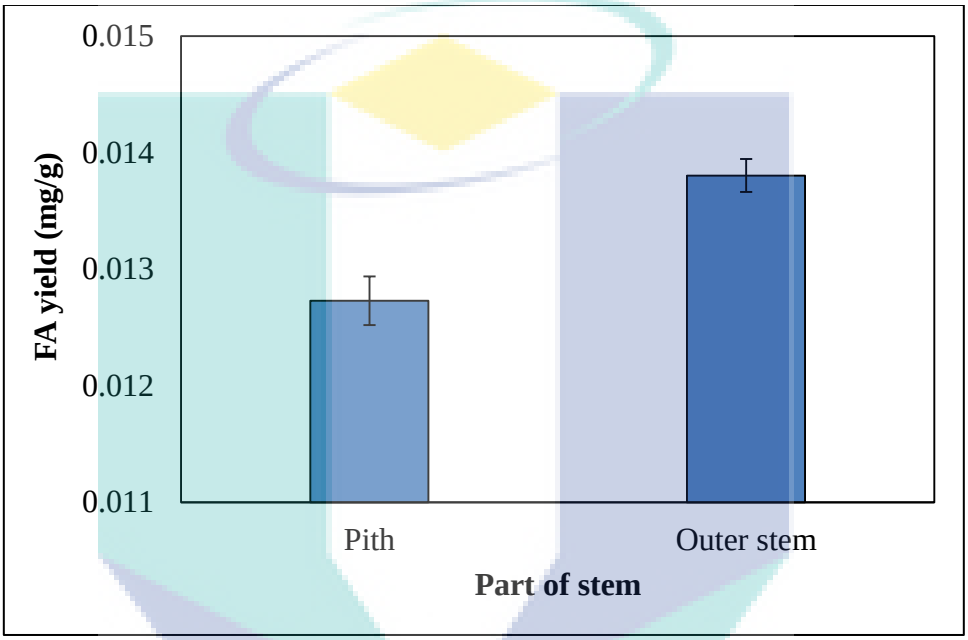

Figure 4.1: FA yield in different part of stem

4.3.2 Pre-treatment temperature of BSW

The pre-treatment temperature of BSW was done to increase the FA yield during the extraction. In this study, the pre-treatment temperature of BSW was done at different temperature between 20 to 90 °C. Figure 4.2 shows the FA yield at was increased from 0.0140 to 0.0153 mg/g when the temperature increased from 20 to 30 °C. The present of heat during pre-treatment can enhanced the recovery of phenolic compounds (Silva et al., 2007). The heat can soften the plant tissues and weaken the phenol-protein and phenol-polysaccharide interactions (Al-Farsi and Lee, 2008). It also can break the polyphenol

bounds and cellular constituents of plants cells (Thoo et al., 2010). So, the availability of phenolic compounds increased with the increasing of temperature (Wang et al., 2008).

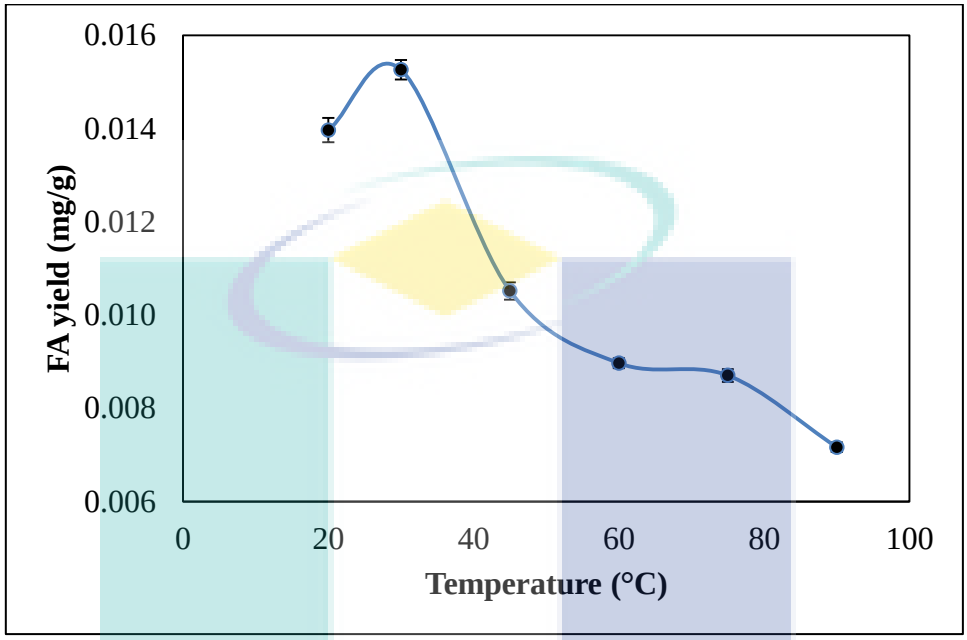

Figure 4.2: FA yield at different pre-treatment temperature

However, the FA yield was decreased when the temperature increased from 30 to 90 °C. The FA yield was decreased from 0.0153 to 0.0072 mg/g. When the temperature increased beyond 25 °C, the decomposition of phenolic compounds which were already mobilized at lower temperature also increased (Liyana-Pathirana and Shahidi, 2005). Besides, it also can broke down the phenolic compounds that still remained in the plants matrix into smaller phenolic compounds (Chan et al., 2009).

4.3.3 UV light pre-treatment of BSW

The pre-treatment of BSW was done by using UV light. BSW was exposed to UV light for 30 min in laminar air flow. Figure 4.3 shows the FA yield without and with photochemical pre-treatment. From the figure, the FA yield was increased when BSW was exposed to UV light. The FA yield when the BSW was exposed into UV light was 0.0149

mg/g while without exposed into UV light was 0.0138 mg/g. It showed the FA yield was 8.12% higher when the pre-treatment with the UV light was done to the BSW than that without the pre-treatment. UV light can stimulated the formation and accumulation of certain phenolic compounds in the plants. This was due to the induction of phenylalanine ammonia-lyase (PAL) activity by UV light which mediated the synthesis of the coumaryl CoA (Bakhshi and Arakawa, 2006). Besides, the photon from UV light also provided enough energy to destroy the chemical bonds in cell walls (Kovacs and Keresztes, 2002).

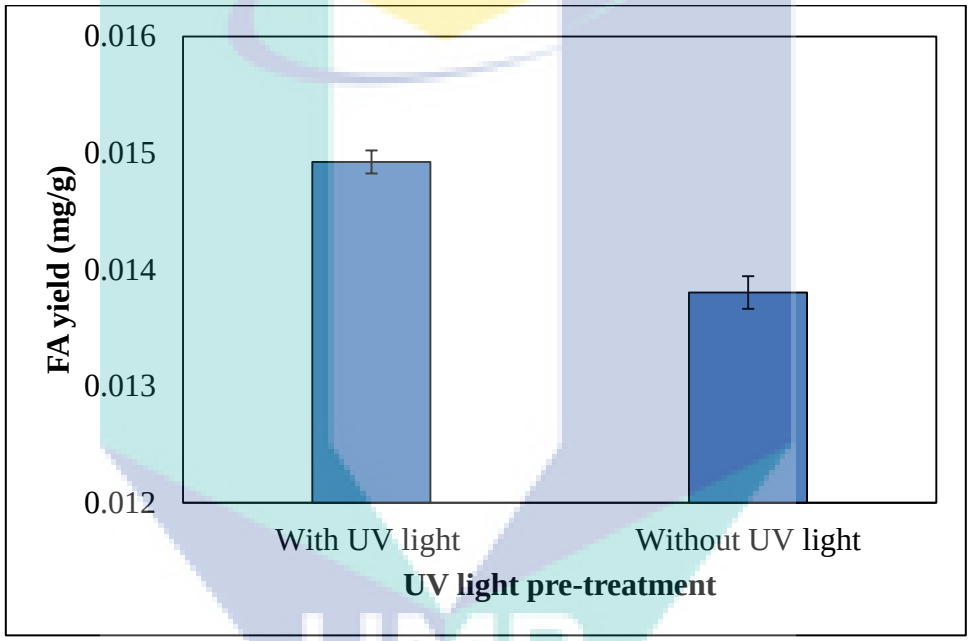

Figure 4.3: FA yield with and without photochemical pre-treatment

4.3.4 Effect number of extraction cycle

BSW was extracted by using sugarcane press machine with rotating cylinder rolls. The extraction of BSW was repeated for three times. The extracted juice for each pressing were collected and measured separately as showed in Figure 4.4. The BSW was extracted for three times to make sure the juice was fully extracted. During the extraction, the shear stress between the cylinders rolls increased the destruction of BSW tissues. It can ruptured

the plant tissues and affected their properties (Moelants et al., 2014). It also can release the intracellular materials that contain in the cell walls.

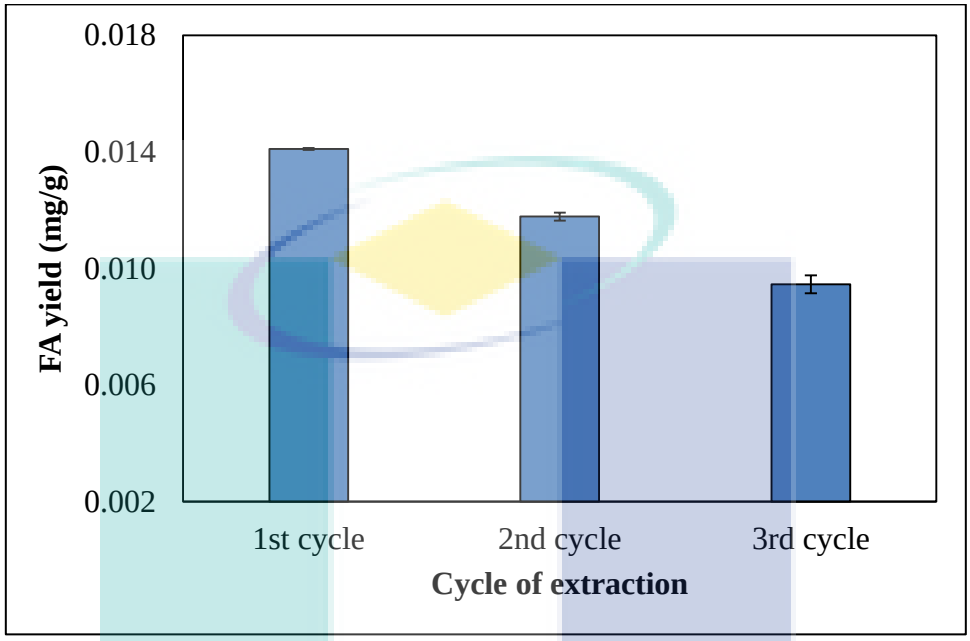

Figure 4.4: FA yield with different number cycle of extraction

From Figure 4.4, the FA yield that obtained during the first cycle was higher than the second and third cycles. The FA yield for the first cycle was 0.0141 mg/g while for the second and third cycles were 0.0118 and 0.0095 mg/g respectively. During the extraction, the three rollers that attached to the sugarcane press machine was applied the pressure and crushed the BSW. The FA yield in extracted BSW juice still available after the first pressing. This was due to the cellular structures of the plant cells did not completely disrupted during the pressing period and the FA still remained in the cells (Bazhal et al., 2001). The BSW needs to be pressed several times due to the complex structure of cell walls and the cellulosic biomass were more difficult to break down (Abramson et al., 2010).

4.3.5 Effect of storage time for extracted BSW juice at ambient temperature

The sample of BSW was cut and extracted by using sugarcane press machine with rotating cylinder rolls. The EBJ was collected after the extraction process. The EBJ was stored at ambient temperature to determine the effect of storage time to the FA yield. It was stored for 72 h at room temperature (25 ± 0.5 °C) and the samples were taken for every 24 h. The result of FA yields in extracted BSW juice at different storage times showed in Figure 4.5.

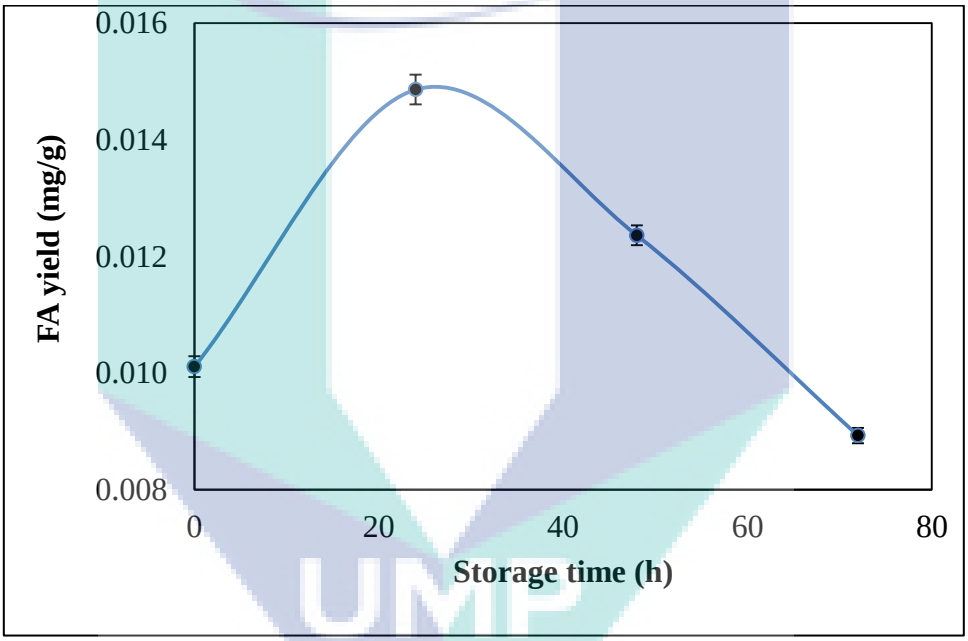

Figure 4.5: FA yield at different storage time of EBJ

From Figure 4.5, FA yield was increased with the increasing of storage time from 0 to 24 h. It showed the FA yield was increased from 0.0101 to 0.0149 mg/g. It was increased due to the released of free acids from their bounds during the storage time (Klimczak et al., 2007). The FA was sensitive to the presence of oxygen at ambient temperature (Vicente et al., 2014). Based on research conducted by Klimczak et al. (2007), the FA yield in orange juice also increased when stored at room temperature. However, the FA yield was decreased when the storage time was longer than 24 h. This was due to the oxidation

reaction of FA that occur during processing and storage (Robards et al., 1999). The oxidation reaction can be reduced by storing the juice at temperature -20 °C (Jeusti Bof et al., 2012). At low temperature, the biochemical process such as production of ethylene, respiration and enzyme activity in plants also decreased (Serea et al., 2014).

4.3.6 Selection of factors for factorial analysis

The selection of factors for factorial analysis was done from the result of preliminary study. These factors were selected based on their effect to FA extraction from BSW. There were five factors were chosen and this factors was divided to categorical and numerical factor. There were two categorical factors which were part of stem and photochemical pre-treatment with UV light. Other three factors were numerical factors which were physiochemical pre-treatment at different temperature, cycle of extraction and storage time of EBJ. Table 4.3 shows the factors and ranges that were chosen for factorial analysis.

Table 4.3: The factors and ranges for factorial analysis

Factors	Symbol	Type of Factor	Level	
			α=-1	α=+1
Part of stem	A	Categorical	Outer	Mixed
UV light pre-treatment	B	Categorical	No	Yes
Pre-treatment temperature	C	Numerical	25	90
Cycle of extraction	D	Numerical	1	3
Storage time of EBJ	E	Numerical	0	24

4.4 FACTORIAL ANALYSIS OF FERULIC ACID EXTRACTION

4.4.1 Design of experiment for factorial analysis

The experimental design for factorial analysis was done by using Design Expert software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, Version 7.1.6). The two-level factorial design was used in this study. This method was used to analyze five factors that were found to be affecting the mechanical extraction of FA from BSW. These factors were part of stem (A), UV light pre-treatment (B), pre-treatment temperature (C), cycle of extraction (D) and storage time of EBJ (E). Factorial analysis was done to fulfill the second objective. The objectives were to determine the factors affecting FA extraction and the interaction between factors. This factorial analysis was carried out at certain ranges of value that obtained from the preliminary study. The design of experiment was applied at 2^5 full factorial design (FFD). This design suited for factorial analysis because it allowed the investigation of large number of factors at the initial of experiment. In FFD, it has the advantages to identify the significant factors with minimum number of experiments (Chang et al., 2011). It also can determined the effect for the main factors and the interactions effect between the factors (Golshani et al., 2013). From the design, 36 runs of experiments were generated. The sequence of experiments was randomized in order to minimize the experimental error and the effects of uncontrolled factors. Table 4.4 shows the experimental design and FA yield for factorial analysis.

Table 4.4: Experimental design and FA yield for factorial analysis

Run	A	B	C	D	E	FA yield (mg/g)
1	-1	+1	-1	-1	-1	0.0337
2	+1	+1	-1	-1	-1	0
3	-1	-1	-1	-1	-1	0.0033
4	+1	-1	-1	-1	-1	0.0054
5	-1	+1	+1	-1	-1	0.0972
6	+1	+1	+1	-1	-1	0.0041
7	-1	-1	+1	-1	-1	0.0037
8	+1	-1	+1	-1	-1	0.0016
9	-1	+1	-1	+1	-1	0.1126
10	+1	+1	-1	+1	-1	0.0059
11	-1	-1	-1	+1	-1	0.0044
12	+1	-1	-1	+1	-1	0.0024
13	-1	+1	+1	+1	-1	0.0575
14	+1	+1	+1	+1	-1	0.0041
15	-1	-1	+1	+1	-1	0.0041
16	+1	-1	+1	+1	-1	0.0042
17	-1	+1	-1	-1	+1	0.0057
18	+1	+1	-1	-1	+1	0.0033
19	-1	-1	-1	-1	+1	0.0035
20	+1	-1	-1	-1	+1	0.0015
21	-1	+1	+1	-1	+1	0.0010
22	+1	+1	+1	-1	+1	0
23	-1	-1	+1	-1	+1	0.0704
24	+1	-1	+1	-1	+1	0.0015
25	-1	+1	-1	+1	+1	0.0043
26	+1	+1	-1	+1	+1	0.0033
27	-1	-1	-1	+1	+1	0.0663
28	+1	-1	-1	+1	+1	0.0047
29	-1	+1	+1	+1	+1	0.0021
30	+1	+1	+1	+1	+1	0.0019
31	-1	-1	+1	+1	+1	0.1204
32	+1	-1	+1	+1	+1	0.0022
33	-1	+1	0	0	0	0.0042
34	+1	+1	0	0	0	0.0040
35	-1	-1	0	0	0	0.0036
36	+1	-1	0	0	0	0.0040

4.4.2 Statistical modeling and ANOVA for factorial analysis

The independent and dependent variables were analyzed to obtain the regression model for linear equation. From the equation, the factors can be determined whether it gave positive or negative effect to the FA yield. There were four equations that obtained from this analysis as showed in Eq. (4.1) to Eq. (4.4). The equations were generated based on two categorical factors which were part of stem and UV light. Each of the factors generated two equations. The equations were used to calculate the predicted values of FA yields in EBJ. Each equation depended on the level of both categorical factors which were outer or mixed stem for part of stem and yes or no for the pre-treatment with UV light.

Part of stem: Outer stem;
UV light pre-treatment: Yes;

$$FA = 0.0106 + 9.8077x10^{-6}C + 0.0310D + 4.8821x10^{-3}E - 3.7510x10^{-4}CD - 4.6763x10^{-5}CE - 1.3499x10^{-3}DE + 1.1402x10^{-5}CDE \quad (4.1)$$

Part of stem: Outer stem;
UV light pre-treatment: No;

$$FA = -0.0245 + 9.8077x10^{-6}C + 0.0457D + 4.8821x10^{-3}E - 3.7510x10^{-4}CD - 4.6763x10^{-5}CE - 1.3499x10^{-3}DE + 1.1402x10^{-5}CDE \quad (4.2)$$

Part of stem: Mixed stem;
UV light pre-treatment: Yes;

$$FA = -0.0135 + 2.7211x10^{-4}C + 7.7570x10^{-3}D + 1.4855x10^{-3}E - 1.2990x10^{-4}CD - 2.4022x10^{-5}CE - 6.7646x10^{-4}DE + 1.1402x10^{-5}CDE \quad (4.3)$$

Part of stem: Mixed stem;
UV light pre-treatment: No;

$$FA = -0.0152 + 2.721x10^{-4}C + 8.0819x10^{-3}D + 1.4854x10^{-3}E - 1.2990x10^{-4}CD - 2.4022x10^{-5}CE - 6.7646x10^{-4}DE + 1.1402x10^{-5}CDE \quad (4.4)$$

Where A, was the part of stem, B was UV light pre-treatment, C was pre-treatment temperature, D was the cycle of extraction and E was the storage time of EBJ. A, B, C, D and E were referred as the main effects while CD, CE, DE and CDE were the interaction effects involves in the extraction process. When the coefficient of main factors gave the positive values, it showed positive impact on FA yield while negative values showed the negative impact (Chang et al., 2011). In this study, the factors which were part of stem, pre-treatment temperature and UV light pre-treatment gave negative effect on the FA yield. The FA yield was decreased when the factor changed from low to high level. However, the cycle of extraction and storage time of EBJ showed the positive effects.

The analysis of variance (ANOVA) was done to determine the significance of the model. Table 4.5 shows the results of ANOVA. The significance of a regression equations was checked by using F-values while the p-values was used to check the significance of each coefficients (Wang et al., 2012). The p-value tests the null hypothesis that data from the experiment with the identical means. If the p-value was less than 0.05, the null hypothesis was rejected. The null hypothesis was failed to reject when the p-value higher than 0.05. From the ANOVA, the F-value for the model was 6.1793 and the p-value was 0.0002. The F-value for the model only showed at 0.02% chance the value occurred due to noise. The corresponding coefficient was more significant when the p-value is small (Zou et al., 2011). Besides, the p-value for A (part of stem), C (pre-treatment temperature) and AC (part of stem - pre-treatment temperature) showed the value less than 0.05. It indicated the contribution of the model was significant (Wang et al., 2012). The F-value for A, C and AC were higher than other factors. It showed these factors gave the strong effect on the mechanical extraction of FA from BSW. The R-squared (R^2) from the ANOVA was used to indicate how close the data to the fitted regression line. R^2 should more than 80% for a

good fitting model (Karazhiyan et al., 2011). The R^2 obtained from factorial analysis was 0.8673 which shows that the model was a good fit.

Table 4.5: ANOVA for factorial analysis

Source	Sum of Square	Df	Mean square	F Value	p-value Prob>F	
Model	0.0338	18	0.0019	6.1793	0.0002	significant
A	0.0082	1	0.0082	27.0341	< 0.0001	
B	0.0001	1	0.0001	0.2694	0.6104	
C	0.0090	1	0.0090	29.5033	< 0.0001	
D	0.0006	1	0.0006	1.9607	0.1794	
E	0.0009	1	0.0009	2.8501	0.1096	
AB	0.0000	1	0.0000	0.1355	0.7174	
AC	0.0089	1	0.0089	29.2395	< 0.0001	
AD	0.0005	1	0.0005	1.7832	0.1994	
AE	0.0006	1	0.0006	2.0876	0.1667	
BD	0.0005	1	0.0005	1.4928	0.2385	
CD	0.0005	1	0.0005	1.4878	0.2392	
CE	0.0008	1	0.0008	2.5373	0.1296	
DE	0.0006	1	0.0006	1.9381	0.1818	
ABD	0.0004	1	0.0004	1.3667	0.2585	
ACD	0.0005	1	0.0005	1.6713	0.2134	
ACE	0.0006	1	0.0006	2.0701	0.1684	
ADE	0.0005	1	0.0005	1.7188	0.2073	
CDE	0.0006	1	0.0006	2.0818	0.1672	
Residual	0.0052	17	0.0003			
Cor Total	0.0390	35				

Values of "prob>F" less than 0.05 indicate model are significant.

4.4.3 Main effect for factor analysis

One of the aspects that were studied in the factorial analysis was the main effect analysis. This analysis was studied in order to determine the factors that most contributed to the mechanical extraction of FA from BSW. Table 4.6 shows the contribution of each main factor to FA extraction.

Table 4.6: The contribution of main factors to FA extraction

Factor	Contribution (%)
A	21.62
B	0.22
C	23.59
D	1.57
E	2.28

From Table 4.6, factor C (pre-treatment temperature) proved to be most contributing factor with 23.59%. This seems legit as temperature play important part for the pre-treatment of BSW. In BSW, FA was attached to the lignin and hemicellulose via ester and ether bonds as bridges and formed LCC structure (Sun et al., 2002). The ether bonds between lignin and hydroxycinnamic acid were broken by using steam, hot water and dilute acid (Buranov and Mazza, 2008). So, heat was used in this study to increase the FA yield by breaking the ether and ester bonds. From the study conducted by Vanbeneden et al. (2008), the released of FA yield increased when the pre-treatment temperature increased due to the additional of chemical hydrolysis by enzyme activity. But, when the temperature increased beyond certain values, it may promote the decomposition of phenolic acids. It was supported by Mcmurrough et al. (1996) that stated when the temperature was increased above 65 $^{\circ}$C, the FA yield decreased due to the enzyme that released FA from the bonds almost completely denatured. The pre-treatment at different temperature influenced the released of phenolic acids from the cell (Xu et al., 2005). This indicated that this factor was important to determine the phenolic acids

Another factor that showed high contribution was factor A (part of stem) with 21.62 % contribution. This factor was proven to be one of the major factors that influence the FA extraction. The FA yield from outer stem was higher than the mixed stem that consisted of outer stem and pith. The pith in the form of rolled cylinder was located in the middle of the stem. In pith, FA acted as nucleation sites for lignin formation during early stages of

lignification (Buranov and Mazza, 2008). Besides, Aziz et al. (2011) also found that the outer layers of the BSW have greater concentration of phenolic acids than the pith

Factor E (storage time of EBJ) showed low contribution with 2.28%. The storage condition has been reported can change the concentration of certain phenolic acids (Carbone et al., 2011). It also has been supported by Begic-Akagic et al. (2011) that found the phenols content in apple juice was different after storage at a certain period of time. Factor D (cycle of extraction) showed a contribution as much as 1.57% to the FA extraction. It gave less effect on FA extraction. Out of all factors involved, factor B (UV light pre-treatment) showed the lowest contribution on FA extraction with only 0.22% contribution.

4.4.4 Interaction between factor for factorial analysis

The interaction effect plot was generated to represent the results of the regression analysis. It was represented the deviations of the average between the high and low levels for each factors. The effect for the factor was positive when the FA yield increased as the factor change from low to high level. However, it was negative when the FA yield decreased from low to high level. When the lines of two factors were unparalleled, the factors were interacting. On the contrary, when the lines were parallel to each other, it shows there was no interaction between the factors (Chang et al., 2011). The significant interactions between the factors were showed in Figure 4.6 and 4.7.

Figure 4.6 shows the interaction plot between part of stem (A) and pre-treatment temperature (C). These plot clearly indicated that the interaction between part of stem and pre-treatment temperature (AC) was stronger than others interaction. The interaction between AC gave higher contribution for the FA extraction which at 23.38%. The amount of FA yields that extracted from the outer stem was higher at temperature 25 $^{\circ}$C than at 90 $^{\circ}$C. It showed the FA yield decreased with the increasing of pre-treatment temperature. From the study conducted by Humberstone and Briggs (2000), FA yield was decreased at high temperature due to the denatured of enzyme that released FA from the cell walls.

However, FA yield from mixed stem did not showed any different when the temperature increased. The mixed stem was the combination of outer stem and pith. The FA yield that contained in pith was lower than the outer stem (Aziz et al., 2011). With the presence of pith, it causes low FA yield in the mixed stem. So, the pre-treatment of BSW at temperature 25 °C did not contributed to the increasing of FA yield.

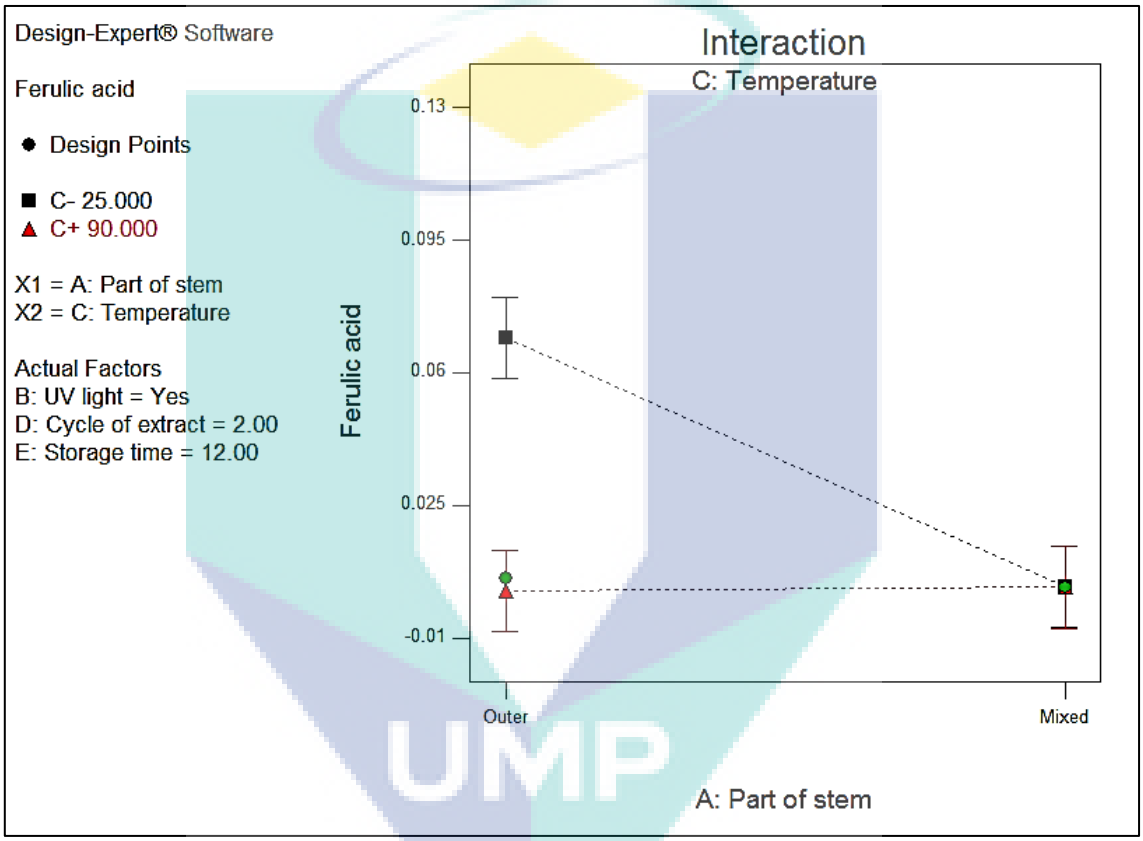

Figure 4.6: Interaction effect plot between A (part of stem) and C (pre-treatment temperature)

Figure 4.7 shows the second interaction which involved the factor C (pre-treatment temperature) and factor E (storage time of EBJ). As explained earlier, the temperature greatly affected the FA extraction from BSW. Besides, storage time also discovered to be crucial in this interaction. At the longer storage time, the FA yield also increased. This factor has significantly improved the FA yield during the analysis. At temperature 25 °C,

the change of storage time showed an increased in FA yield. However, when the temperature was increased to 90 °C, the FA yield was decreased.

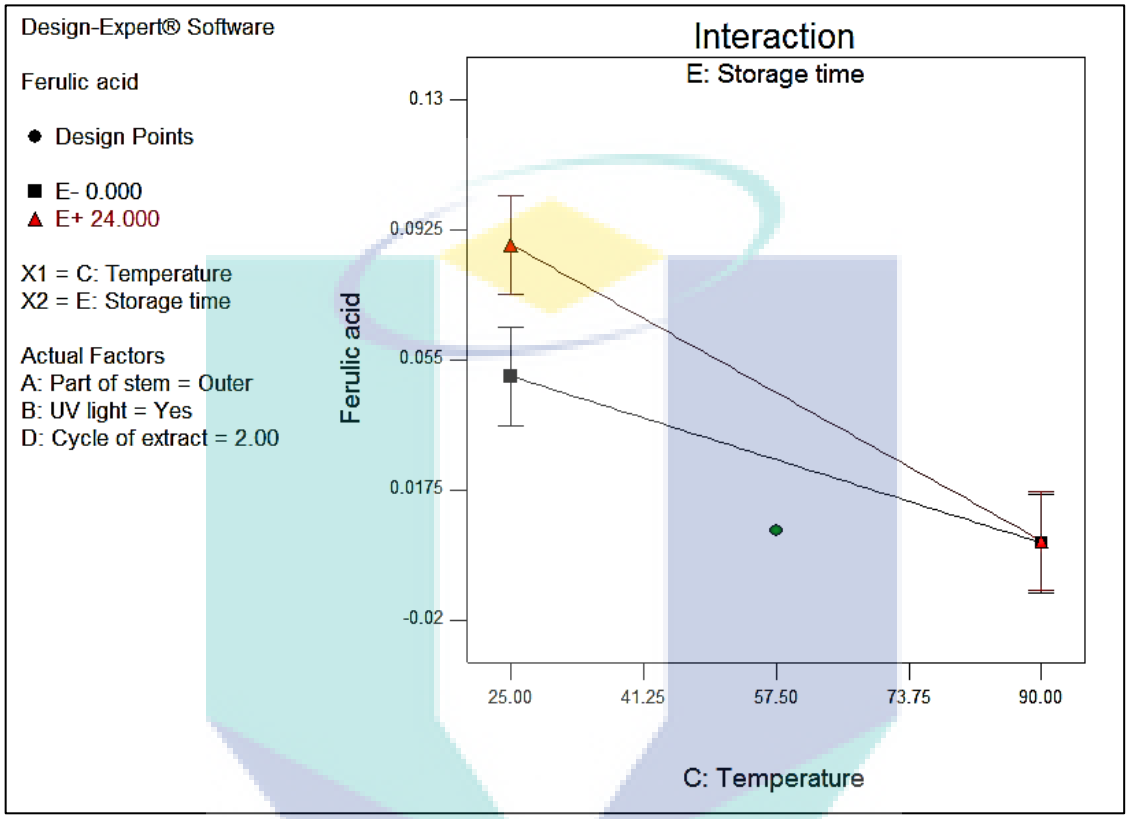

Figure 4.7: Interaction effect plot between C (pre-treatment temperature) and E (storage time of EBJ)

4.4.5 Validation of factorial analysis

Validation experiment for factorial analysis was performed at the selected best conditions. It was performed using the conditions as showed in Table 4.7. The experimental values that obtained in this validation were compared with the predicted values. These two values were compared to calculate the percentage error. The percentage error between the experimental and predicted values of FA yield were calculated based on Eq. (3.1).From the data, the experimental values were reasonable close to the predicted values. It confirmed

the validity and adequacy of the predicted models. The percentage errors were 10.49% and 9.39% for run 1 and 2 respectively.

Table 4.7: Predicted and experimental values for factorial analysis

Run	A	B	C	D	E	Predicted value (mg/g)	Experimental value (mg/g)	Percentage error (%)
1	Outer	Yes	25	1	24	0.0959	0.0859	10.49
2	Outer	No	25	1	24	0.0757	0.0828	9.39

4.5 OPTIMIZATION OF FERULIC ACID EXTRACTION

4.5.1 Design of experiment for optimization

The experimental design for optimization was done by using Design Expert software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, Version 7.1.6). CCD was used in this study. CCD was used to optimize the process and product by constructing proper equation to correlate the interaction between input and output variable (Tong et al., 2011). It also used to provide a clear understanding of the interactions involved between the factors during the extraction (Salleh et al., 2011). This method is widely used to analyze and optimize the factors affecting the extractions from natural resources (Wang et al., 2013). The optimization was done to fulfill the third objective of this study. The objective was to optimize the FA extraction from BSW.

Two factors from factorial analysis were selected as independent factors for optimization. These factors were pre-treatment temperature and storage time of EBJ. The pre-treatment temperature was selected for optimization as it was one of the most important factors affecting the extraction of FA from BSW. During factorial analysis, pre-treatment temperature gave high contribution on FA yield. However, it gave negative effect on FA yield when the temperature increased. So, temperature was selected in order to determine the optimum temperature for pre-treatment of BSW. For storage time of EBJ, it was

selected because it gave positive effect on FA yield. In factorial analysis, FA yield was increased when the storage time increased. The other three factors which were part of stem, UV light pre-treatment and cycle of extraction were fixed at outer stem, no UV light pre-treatment and one time respectively. For the part of stem, the outer stem was used due to the highest FA yield compared to the mixed stem. The pre-treatment with UV light was ignored due to low contribution. The cycle of extraction was fixed since the extractions that were done at one or three times only had slightly difference on FA yields. For two factors, the recommended number of experiments at the center point was five. Hence the total number of experiments for the optimization was 13 runs. Table 4.8 shows the experimental design and FA yield for optimization.

Table 4.8: Experimental design and FA yield for optimization

Run	Pre-treatment temperature (°C)	Storage Time of EBJ (h)	FA yield (mg/g)
1	-1	-1	0.1404
2	+1	-1	0.1735
3	-1	+1	0.1916
4	+1	+1	0.1481
5	-2	0	0.1370
6	+2	0	0.1254
7	0	-2	0.1101
8	0	+2	0.1164
9	0	0	0.1915
10	0	0	0.2274
11	0	0	0.1848
12	0	0	0.1826
13	0	0	0.1922

4.5.2 Statistical modeling and ANOVA for optimization

A regression analysis was performed to fit the experimental data based on the second order equation (Salleh et al., 2011). In this study, the regression model was showed in Eq. (4.5). Where F was referred as the response of FA yield, A was the pre-treatment temperature and B was the storage time of EBJ. The variable of AB was the interaction

between pre-treatment temperature and storage time of EBJ. The presence of curvature in the model was presented by A^2 and B^2. This equation shows the relationship between temperature, storage time and FA yield.

$$FA = -6.6735 + 0.2191\,A + 0.3438B - 3.83x10^{-3}AB - 2.5678x10^{-3}A^2 - 5.134x10^{-3}B^2 \quad (4.5)$$

The ANOVA was done to analyze the experimental data. The second order polynomial model for responses was determined by using ANOVA as showed in Table 4.9. From the ANOVA, the F-value for the model was 13.5320 and the P-value was less than 0.05. The model was significant and there was only 0.017% chance that the F-value for the model occurred due to noise. The F-value for lack-of-fit was 0.1187 and p-value was 0.9445. The p-value of lack-of-fit was more than 0.05. The lack-of-fit was not significant which indicated the suitability of model to predict the variation accurately (Prasad et al., 2011). It also showed the model was adequately fits the data well (Salleh et al., 2011). The R^2 for this model was 0.906 (90.6%) which presented only 9.4% of variability in response.

Table 4.9: ANOVA analysis for central composite design (CCD)

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob>F	
Model	0.0139	5	0.0028	13.5320	0.0017	significant
A	0.0001	1	0.0001	0.4563	0.5210	
B	0.0001	1	0.0001	0.5960	0.4654	
AB	0.0015	1	0.0015	7.1152	0.0321	
A^2	0.0059	1	0.0059	28.6254	0.0011	
B^2	0.0097	1	0.0097	46.8722	0.0002	
Residual	0.0014	7	0.0002			
Lack of Fit	0.0001	3	0.0000	0.1187	0.9445	not significant
Pure Error	0.0013	4	0.0003			
Cor Total	0.0154	12				

Values of "Prob>F" less than 0.05 indicate model are significant.

4.5.3 Response surface plot for optimization

In this study, response surface plot showed the relationship between the response and independent factors (Bas and Boyac, 2007). Figure 4.8 shows the effect of two factors which were pre-treatment temperature and storage time of EBJ on FA yield. The plot shows approximately symmetrical in shape with circular contours. It shows clear peak, implying that the optimum condition for maximum value of FA yield were attributed by temperature and storage time. Besides, this plot also indicated the maximum point for FA yield was located inside the experimental region (Bezerra et al., 2008).

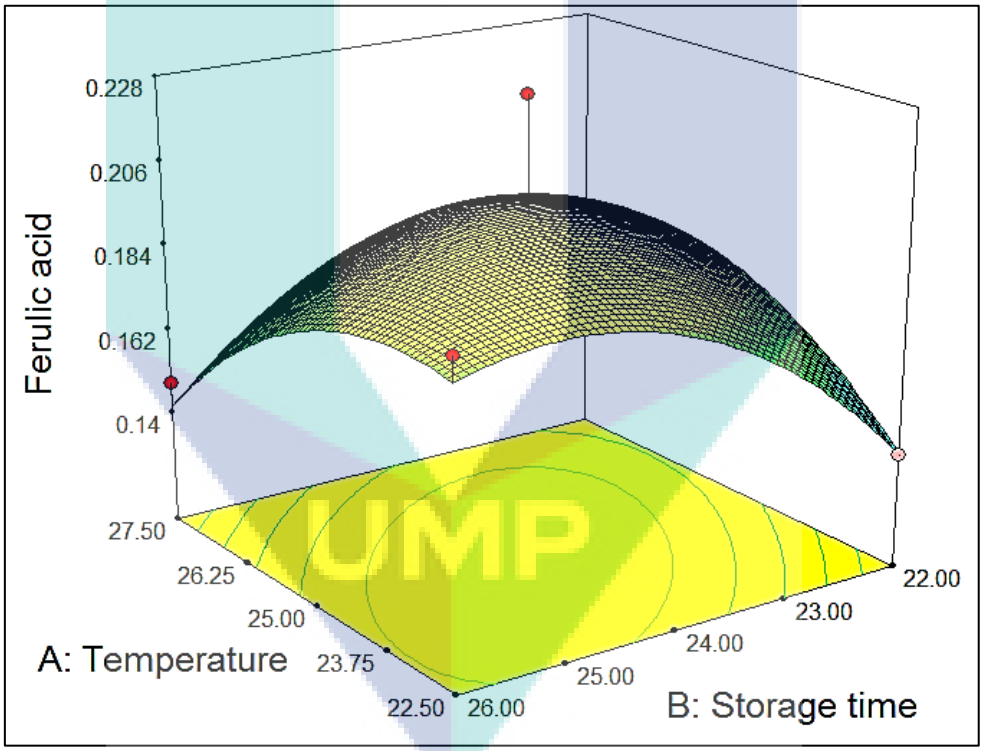

Figure 4.8: Response surface plot of the interaction between pre-treatment temperature (A) and storage time of EBJ (B) and their interaction on FA yield

From Figure 4.8, it shows the FA yield increased and reaches the maximum level at pre-treatment temperature 25 °C and storage time of EBJ at 24 h. The FA yield was increased when the temperature increased from 22.5 to 25 °C. However, FA yield was

decreased when the temperature increased from 25 to 27.5 °C. This was due to higher temperature may promoted the decomposition of FA which were already mobilized at lower temperatures (Chan et al., 2009).

4.5.4 Pertubation and interaction plots between factors for optimization

Pertubation plot in this study was used to compare the effect of all factors at a particular point (Chow and Yap, 2008). The steepest slope or curvature that exist in perturbation plot indicated the sensitiveness to the specific factor (Solanki et al., 2015). Figure 4.9 shows the perturbation plot for FA extraction from BSW at pre-treatment temperature at 25 °C and storage time of EBJ at 24 h.

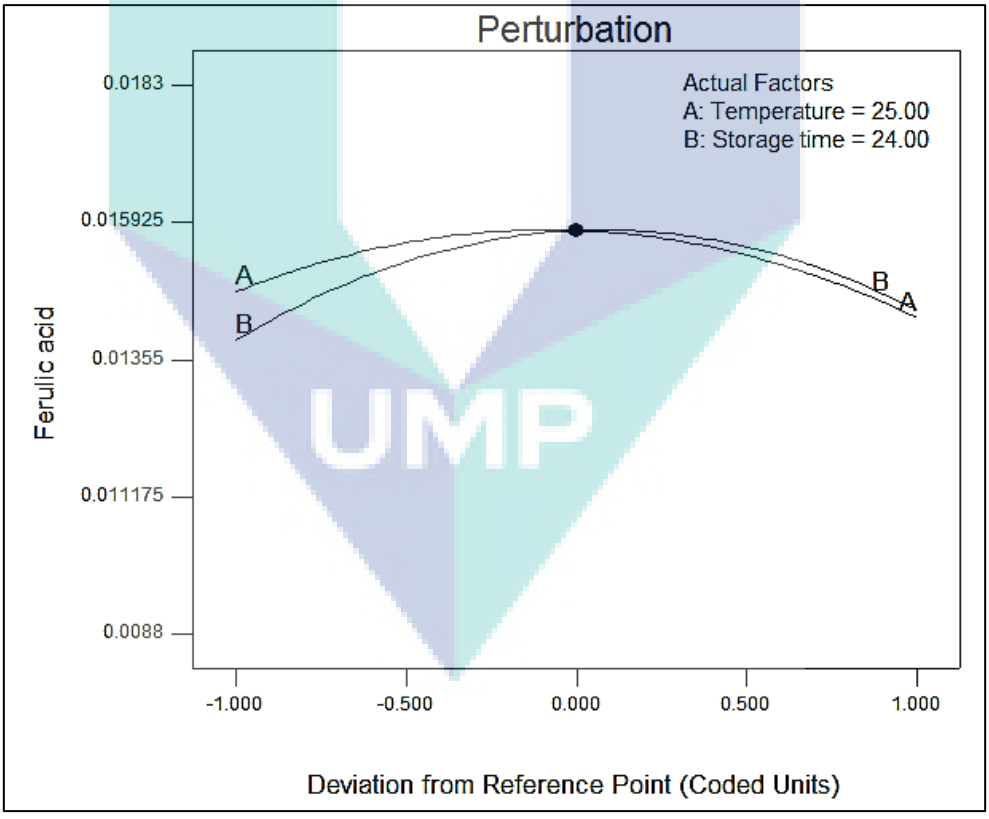

Figure 4.9: Pertubation plot for pre-treatment temperature (A) and storage time of EBJ (B)

In this study, storage time gave greater influenced to FA extraction compared to temperature. The deviation in perturbation curve for storage time was steeper slope than the temperature. The storage condition strongly affect the phenolic content in extracted BSW juice since it can undergo modifications during storage due to the hydrolysis, oxidation and complexations (Zafrilla et al., 2003).

In CCD, interaction plot shows the interaction between factors that affected FA extraction from BSW. Figure 4.10 shows the interaction plot between pre-treatment temperature and storage time of EBJ.

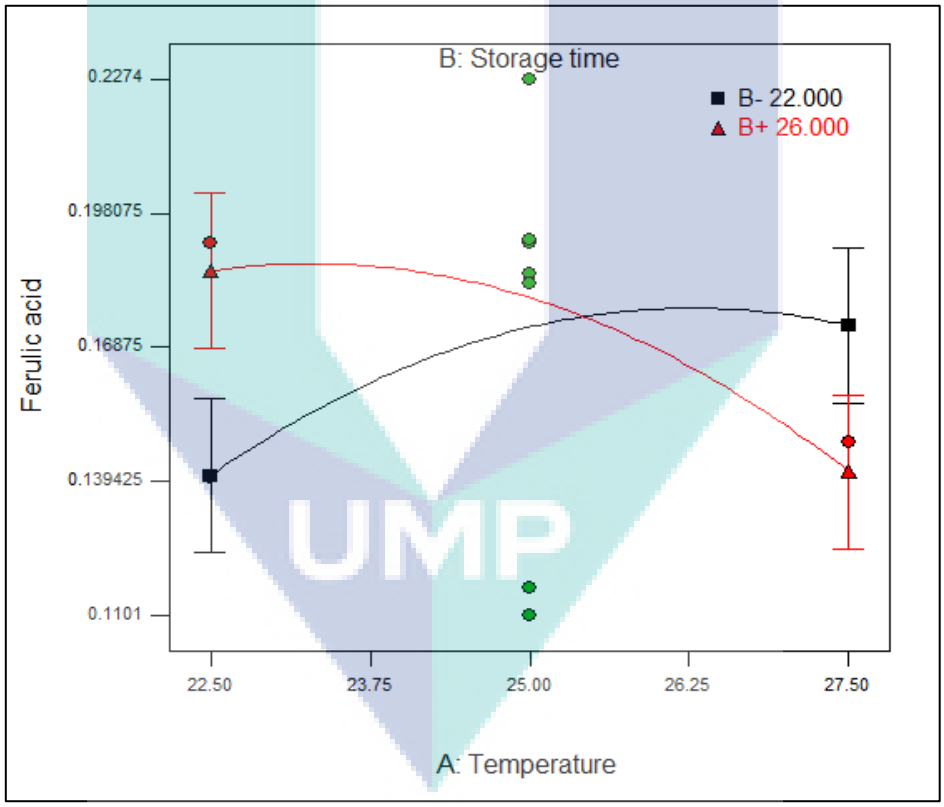

Figure 4.10: Interaction plot between pre-treatment temperature (A) and storage time (B)

Similar to factorial analysis, the optimization proved that the interaction between these two factors affected the FA extraction from BSW. The FA yield that extracted from

BSW was affected by the temperature during the pre-treatment. From the graph, at pre-treatment temperature below 25 °C, longer storage time of EBJ proved to increase the FA yield. However, the FA yield was decreased when the temperature exceed 25 °C. According to Recamales et al. (2006), the temperature has a significant influence on the FA yield. This was due to FA was sensitive and susceptible to oxidize when exposed to heat (Li et al., 2009). This interaction already been discussed in the factorial analysis at subchapter 4.4.4.

4.5.5 Validation for optimization

Validation experiment for optimization was performed at suggested optimum condition by Design Expert software as showed in Table 4.10. The FA yield obtained from the experiment was compared with the predicted value to calculate the percentage error. It was calculated based on Eq. (3.1). From Table 4.10, it shows the experimental value of FA yields were reasonably close to the predicted values (error less than 10%). So, this validated the predicted values, and the suggested model from this study was adequate to obtain an optimal FA yield from the extraction of BSW.

Table 4.10: Predicted and experimental values for optimization

Run	Temperature (°C)	Storage time of EBJ (h)	Predicted value (mg/g)	Experimental value (mg/g)	Percentage error (%)
1	25	24	0.1969	0.1875	4.79
2	25	24	0.1969	0.1859	5.60
3	25	24	0.1969	0.1846	6.26

4.6 CHARACTERIZATION OF THE EXTRACTED BANANA STEM WASTE (BSW) JUICE

The characterization of EBJ was done to determine the compositions of total phenolic and glucose. As explained in chapter 3, the characterization of juice was done with the sample of EBJ from the optimization study.

4.6.1 Total phenolic in extracted BSW juice

Phenolic compounds widely distributed in tissue, cellular and subcellular of the plants. The structure of phenolic compounds consisted of an aromatic ring and bearing one or more hydroxyl substituent (Balasundram et al., 2006). The phenolic compounds that contained in the plants can be determined by total phenolic. The composition of total phenolic in EBJ was determined by using Folin-Ciocalteus colorimetric method. In this study, the part of BSW used was outer stem. The total phenolic in EBJ was 22.61 mg GAE/g of dry weight as showed in Table 4.11. The value was different compared to other researchers. From the study conducted by Anusuya et al., (2013) and Aziz et al. (2011), the total phenolic contained about 79.92 and 65.32 mg GAE/g of dry weight respectively, which higher than this study. However, the study conducted by Loganayaki et al, (2010) obtained lower than other researchers which only 12.00 mg GAE/g of dry weight. The composition of total phenolic in EBJ was different from other researchers due to the different type of extraction used (Sulaiman et al., 2011). In this study, BSW was pressed using mechanical extraction by using sugarcane press machine to extract the juice while other researchers used chemical extraction with the solvents such as water, methanol, acetone, and chloroform (Anusuya et al., 2013; Aziz et al., 2011; Loganayaki et al., 2010; Sulaiman et al., 2011).

Table 4.11: Compositions of total phenolic from other researchers

Research	Type of extraction	Part of banana plant	Total phenolic (mg GAE/ g dry weight)
This research	Mechanical extraction	Outer stem	22.61
Anusuya et al., 2013	Solvent extraction	Outer stem	79.92
Aziz et al., 2011	Solvent extraction	Outer stem	65.32
		Pith	12.45
Loganayaki et al., 2010	Solvent extraction	Outer stem	12.00
		Flower	14.00
Sulaiman et al., 2011	Sequential extraction	Pulp	20.47
		Peel	8.08

GAE = Gallic acid equivalent

Different total phenolic also depend on the part of banana plants. From the study conducted by Aziz et al. (2011), the composition of total phenolic in outer stem and pith were showed in Table 4.11. Total phenolic in outer stem was higher than the pith. The difference in composition of total phenolic depends on the type of banana plants (Zheng and Wang, 2001). Total phenolic in plants can be classified into different classes from the simpler molecules such as simple phenol to highly polymeric compounds such as condensed tannins. Phenolic acids which were hydroxycinnamic and hydroxybenzoic acids also included in the composition of total phenolic (Dai and Mumper, 2010).

Besides, Loganayaki et al, (2010) also studied the composition of total phenolic in outer stem and flower. The total phenolic contained in flower was higher than the outer stem. This was due to the accumulation of secondary metabolites in flower tissues (Maoulainine et al., 2012). The pulp and peel also contained phenolic compounds. The total phenolic in pulp and peel were 20.47 and 8.08 mg GAE/g of dry weight respectively (Sulaiman et al., 2011). From this, it showed that the total phenolic contained in outer stem was higher than the other part of the plants.

4.6.2 Glucose in extracted BSW juice

Glucose was a simple sugar that abundant in most of the plants. The composition of glucose in EBJ was determined by using dinitrosalicylic acid (DNS) colorimetric method (Ingale et al., 2014). According to Oliveira et al. (2007), glucose was range from 9 to 24% of total sugar in BSW. It also supported by (Li et al., 2010) that glucose was a predominant monomer. In this study, the composition of glucose was 0.46 mg/ml as showed in Table 4.12.

Table 4.12: Composition of glucose from other researchers

Research	Part of banana plant	Glucose content (mg/ml)
This research	Outer stem	0.46
Souza et al., 2014	Outer stem	0.60
Balg et al., 2004	Outer stem	1.34

The composition of glucose in this study was lower than the other researcher. According to Souza et al., (2014) and Baig et al., (2004), the composition of glucose were 0.60 and 1.34 mg/ml respectively. This was due to the different type of banana species used. The composition of glucose in plants also depends on the compositions of cellulose and hemicellulose. In plant, cellulose and hemicellulose was hydrolyzed become simple sugar and increased the composition of glucose (Vasquez et al., 2011). According to Idrees et al. (2013), the pre-treatment of BSW increased the accessible of glucose by altering the cellulose and hemicellulose structures. Besides, the FA yield also increased when the pre-treatment and extraction of BSW were done. The bond between FA and hemicellulose was broken and increased their availability (Torre et al., 2008).

4.7 COMPARISON WITH OTHER RESEARCHERS

Table 4.13 shows the comparison of FA yield in this study with other researchers. The FA yields from other researches depend on the type of extractions and raw materials used. In this study, FA yield that extracted from BSW was 0.1875 mg/g meanwhile from a study conducted by Oliveira et al. (2006) was 0.0360 mg/g. Although the same raw material used, the FA yields were different due to the used of different method of extraction. In research conducted by Oliveira et al. (2006), chemical extraction using Soxhlet extraction was used with dichloromethane as the solvent while in this study the BSW was physically extracted by sugarcane press machine. The used of Soxhlet extraction will require more time compared to sugarcane press machine. The extraction by using sugarcane press machine only takes a few minutes and without using any chemicals. The Soxhlet extraction used high temperature and it became one of the disadvantages due to thermal degradation of phenolic compounds when exposed to high temperature (Ince et al., 2013). The used of sugarcane press machine also become well established equipment to extract bioactive compound such as sugar from plants (Eshtiaghi and Yoswathana, 2012; Zahari et al., 2012).

The studies conducted by Mussatto et al. (2007) and Xiros et al. (2009) also used same raw material but different extraction methods. They used Brewer's spent grain to extract the FA. The methods used were alkaline hydrolysis and enzyme extraction by Mussatto et al. (2007) and Xiros et al. (2009) respectively. From studies conducted by Mussatto et al. (2007), Brewer's spent grain produced 0.5100 mg/g of FA yield while Xiros et al. (2009) only produced 0.4267 mg/g and this shows large difference. There were variations on FA yields when different extraction methods used for the same raw material. The used of solvent and enzyme for the extraction affected the FA yield. During alkaline hydrolysis, the concentration of solvent affect the released of FA from the plant cell wall (Choi et al., 2008). While FA yield from enzyme hydrolysis depended on the production of enzyme by microorganism and the reaction time (Fazary and Ju, 2007). In this study, the FA was extracted by using sugarcane press machine. The application of sugarcane press machine reduced the chemical cost and the removal of chemical solvent can be ignored

during the purification step. Besides, type of raw material used also affects the FA yield produced. The studies conducted by Max et al. (2009) and Salleh et al. (2011) used Vine shoots prunnings and paddy straws respectively. Both studies extracted the FA by using the alkaline hydrolysis but there were variation on FA yields. This was due to the FA content in Vine shoots prunnings and paddy straws were different to each other.

Table 4.13: Comparison with different type of extraction and raw material with other researchers

Research	Type of extraction	Raw material	FA yield (mg/g)
This research	Mechanical extraction	BSW	0.1875
Olieveira et al. (2006)	Soxhlet extraction	BSW	0.0360
Musatto et al. (2007)	Alkaline hydrolysis	Brewer's spent grain	0.5100
Xiros et al. (2009)	Enzyme extraction	Brewer's spent grain	0.4267
Max et al. (2009)	Alkaline hydrolysis	Vine shoots prunnings	0.0376
Adom and Liu (2002)	Solvent extraction	Corn	0.1760
		Rice	0.0648
		Oats	0.0359
		Wheat	0.0298
Buranov and Mazza (2009)	Pressurized low-polaritywater (PLPW) extraction	Flax shives	0.2500
		Corn bran	0.2510
Salleh et al. (2011)	Alkaline hydrolysis	Paddy straw	8.1700

FA can also be extracted from agriculture waste such flax shives and corn bran (Buranov and Mazza, 2009) and grain such as corn, rice, oats and wheat (Adom and Liu, 2002). The FA yields obtained were different depending on the raw materials as showed in Table 4.13. The difference due to the variation of FA contents in every plant. The FA yield

that contained in BSW was lower than other sources due to low composition of lignin and hemicellulose available in BSW (Guimaraes et al., 2009). The composition of lignin and hemicellulose affect the FA yield because FA acts as bridges in LCC (Buranov and Mazza, 2008). Even though the FA yield in BSW was lower than others raw materials, but the availability of this BSW was abundant in Malaysia.

The extraction of FA from BSW has been statistically improved through the optimization by using CCD. The FA yield was increased from 0.0859 to 0.1875 mg/g. It showed the FA yield that obtained during optimization was increased about 118.28% than the factorial analysis. From the study conducted by Salleh et al. (2011), the FA yield also showed an improvement from 5.1810 to 8.1700 mg/g when using CCD to optimize the alkaline hydrolysis of FA from paddy straw. This study showed the FA yield was increased about 57.72%. Therefore, the application of CCD in optimization was suitable to analyze the experimental data and to suggest the optimum condition for FA extraction.

4.8 CHAPTER SUMMARY

The characterization of outer stem and pith were done to fulfill the first objective. The compositions of BSW were 92.52% moisture, 13.02% lignin, 25.92% cellulose and 19.29% hemicellulose. The composition of stem was closely related to the FA yield. This was due to the FA yield was associated with lignin and hemicellulose to form LCC.

For the second objective, the factorial analysis was performed to determine the factors affecting FA extraction and the interaction between factors. Five factors were analyzed in factorial analyses which were part of stem, UV light pre-treatment, pre-treatment temperature, cycle of extraction and storage time of EBJ. From the factorial analysis, two factors which were pre-treatment temperature and storage time of EBJ were selected for optimization. The optimization study was done to fulfill the third objective of this study. The optimization of FA extraction has increased the FA yield from 0.0859 to 0.1875 mg/g. The suggested optimum condition for pre-treatment temperature was 25 $^{\circ}$C and storage time of EBJ was 24 h. Then, the validation experiment was performed to

compare the experimental and predicted values. The FA yield that obtained from the experiment was 0.1875 mg/g while the predicted value was 0.1969 mg/g. The percentage error between the predicted and experimental values was 4.79%.

Besides, the EBJ also were characterized by the compositions of total phenolic and glucose. From the analysis, the composition of total phenolic in EBJ was 22.61 mg GAE/g of dry weight and glucose was 0.46 mg/ml. The FA yield in EBJ also related with the composition of total phenolic. This was due to the FA is one of the phenolic acid that included in the composition of total phenolic. Besides, the composition of glucose also affected the FA yield. The compositions of glucose depend on the hydrolysis of cellulose and hemicellulose in BSW. The cellulose and hemicellulose was hydrolyzed during pre-treatment and increased the composition of glucose.

As a conclusion, FA can be extracted from BSW by using sugarcane press machine. The temperature and storage time of EBJ were two factors that influence the FA yield. The FA yield obtained from this study was different than the other researchers. The variation on the FA yield depended on the raw material and method of extraction.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This chapter was designed to conclude the whole thesis based on the objectives of this study. In this study, it consisted of three objectives and each of the objectives was divided into several scopes. These scopes were specified in order to achieve all the objectives.

5.1.1 Characterization of banana stem waste (BSW)

In this study, the first objective was to characterize the compositions of BSW and EBJ. The compositions of BSW were 92.52% moisture, 13.02% lignin, 25.92% cellulose and 19.29% hemicellulose. The characterizations of the EBJ were done with the compositions of total phenolic was 22.61 mg GAE/g of dry weight and glucose was 0.4568 mg/ml.

5.1.2 Factors affecting ferulic acid extraction

The second objective of this study was to analyze factors affecting ferulic acid (FA) extraction from BSW. Five factors were analyzed in factorial analysis which was part of stem, UV light pre-treatment, pre-treatment temperature, cycle of extraction and storage time of EBJ. These factors were studied for their effect and contribution to the FA extraction. The concentration of FA was determined by using high performance liquid

chromatography (HPLC) method. In factorial analysis, Design Expert software was used to analyze the contribution of the factors. From the result, pre-treatment temperature gave the highest contribution at 23.59%, followed by the part of stem at 21.62% and storage time of EBJ at 2.28%. The cycle of extraction and UV light pre-treatment gave low contributions with 1.57% and 0.22% respectively. In term of the interaction, part of stem and pre-treatment temperature gave the highest contribution for the FA extraction which at 23.38%. Two of the factors from factorial analysis were selected for the optimization process. From the analysis of variance (ANOVA), the R-square (R^2) was 0.8673. It showed the model equation that obtained in factorial analysis was reliable to represent the experimental data.

5.1.3 Optimization of pre-treatment temperature and storage time of EBJ on ferulic acid extraction

The third objective was to optimize the extraction of FA from BSW. Two factors from the factorial analysis were selected to study in optimization by using central composite design (CCD). The suggested optimum condition for pre-treatment temperature was 25 $^{\circ}$C and storage time of EBJ was 24 h. From ANOVA, the R^2 for this model was 0.906 which presented only 9.4% of variability in response. The FA yield that obtained in optimization was higher than factorial analysis. The yield was increased from 0.1204 to 0.1875 mg/g. In validation experiment, the FA yield that obtained during the experiment was compared with the predicted values. The FA yield that obtained from the experiment was 0.1875 mg/g while the predicted value was 0.1969 mg/g. There was 4.79% of error between the predicted and experimental values.

5.2 RECOMMENDATION

Several recommendations were proposed in this chapter in order to improve the extraction and to increase the stability of FA. The recommendations are listed below.

5.2.1 Kinetic study of ferulic acid extraction

Kinetic study is one of the methods that use to clarify the reaction mechanism of a process. It is commonly apply after the optimization study. Kinetic study consists of a series elementary process which explains the overall reaction process. In this study, it will develop the mathematical model that can be used to study the influence of the several factors to the extraction rate and the product yield. The determination of kinetic parameters would allow the application of the extraction at another level especially in scale up process.

5.2.2 Chemical stability and degradation mechanism of ferulic acid

The study of the chemical stability and degradation mechanism are important in order to develop the analytical method and to control the quality of FA. FA receives significant interest in the industry. However, the application of FA in industry is limited due to the pH and temperature instability. So, the study of the chemical stability and degradation mechanism would allow and improve the application of FA in the industry.

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APPENDIX A

A.1 List of Chemicals

List of chemicals

No	List of chemicals	Manufactures	Country origin
1	Acetonitrile	Fisher Chemicals	United Kingdom
2	Cetyl trimethylammonium bromide	Acros Organic	United States
3	Disodium hydrogen phosphate	Merck Chemicals	United States
4	EDTA	Sigma Aldrich	United States
5	Ferulic acid	Acros Organic	United States
6	Folin Ciocalteu Reagent	Sigma Aldrich	United States
7	Gallic Acid	Acros Organic	United States
8	Potassium sodium tartrate tetrahydrate	Merck Chemicals	United States
9	Sodium borate decahydrate	Merck Chemicals	United States
10	Sodium carbonate	Sigma Aldrich	United States
11	Sodium hydroxide	Merck Chemicals	United States
12	Sodium lauryl sulfate	Fisher Chemicals	United Kingdom
13	Sulfuric acid	Fisher Chemicals	United Kingdom
14	Triethylene glycol	Sigma Aldrich	United States
15	3,5-dinitrosalicylic acid (DNS)	R&M Chemicals	United Kingdom
16	2-ethoxy ethanol	Acros Organic	United States

A.2 Preparation of neutral detergent solution

The chemicals listed below were dissolved in 1000 ml distilled water.

1. 30 g sodium lauryl sulphate
2. 18.61 g EDTA
3. 6.81 g sodium borate decahydrate
4. 4.56 g disodium hydrogen phosphate
5. 10 ml 2-ethoxy ethanol

Neutral detergent solution consisted of sodium hydroxide, EDTA, disodium hydrogen phosphate, sodium borate decahydrate and sodium lauryl sulfate, all of which were dissolved in distilled water. Triethylene glycol was then added into the solution to suppress the formation of foam, and the pH (6.95 – 7.05) of the solution was determined.

A.3 Preparation of acid detergent solution

1. 20 g cetyl trimethylammonium bromide was dissolved in 700 ml distilled water.
2. 27.56 ml of 96.7% acid sulphuric was then added to the solution and the topped up to 1000 ml with distilled water.

A.4 Preparation of DNS solution

DNS dilution was prepared by heating gently 500 ml of water to dissolved 300 g of potassium sodium tartrate tetrahydrate and 16 g of sodium hydroxide in 1000 ml conical flask. After the solution was clear, 10 g of 3,5-dinitrosalicylic acid (DNS) was added slowly. The solution was cooled to room temperature and distilled water was added until the solution become 1000 ml..

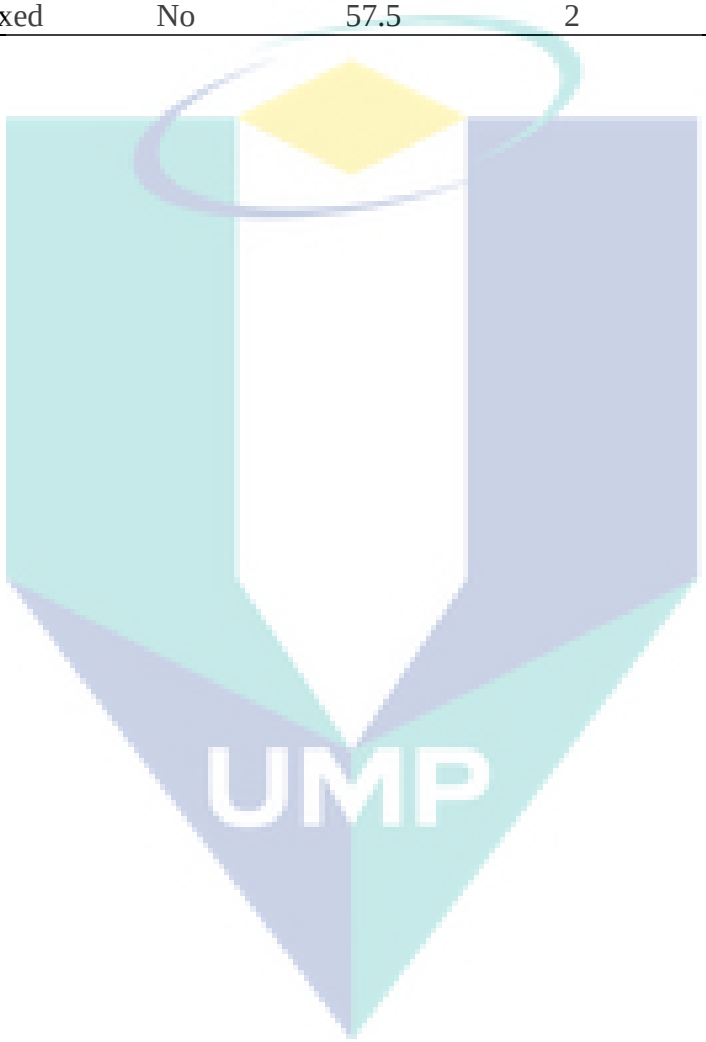
APPENDIX B

B.1 Factorial Analysis

Experimental results for factorial analysis

Run	Part of stem	UV light pre-treatment	Pre-treatment temperature (°C)	Cycle of extraction	Storage time of EBJ (h)	FA yield (mg/g)
1	Outer	Yes	25	1	0	0.0337
2	Mixed	Yes	25	1	0	0
3	Outer	No	25	1	0	0.0033
4	Mixed	No	25	1	0	0.0054
5	Outer	Yes	90	1	0	0.0972
6	Mixed	Yes	90	1	0	0.0041
7	Outer	No	90	1	0	0.0037
8	Mixed	No	90	1	0	0.0016
9	Outer	Yes	25	3	0	0.1126
10	Mixed	Yes	25	3	0	0.0059
11	Outer	No	25	3	0	0.0044
12	Mixed	No	25	3	0	0.0024
13	Outer	Yes	90	3	0	0.0575
14	Mixed	Yes	90	3	0	0.0041
15	Outer	No	90	3	0	0.0041
16	Mixed	No	90	3	0	0.0042
17	Outer	Yes	25	1	24	0.0057
18	Mixed	Yes	25	1	24	0.0033
19	Outer	No	25	1	24	0.0035
20	Mixed	No	25	1	24	0.0015
21	Outer	Yes	90	1	24	0.0010
22	Mixed	Yes	90	1	24	0
23	Outer	No	90	1	24	0.0704
24	Mixed	No	90	1	24	0.0015
25	Outer	Yes	25	3	24	0.0043
26	Mixed	Yes	25	3	24	0.0033
27	Outer	No	25	3	24	0.0663
28	Mixed	No	25	3	24	0.0047

29	Outer	Yes	90	3	24	0.0021
30	Mixed	Yes	90	3	24	0.0019
31	Outer	No	90	3	24	0.1204
32	Mixed	No	90	3	24	0.0022
33	Outer	Yes	57.5	2	12	0.0042
34	Mixed	Yes	57.5	2	12	0.0040
35	Outer	No	57.5	2	12	0.0036
36	Mixed	No	57.5	2	12	0.0040



Main effect list for factorial analysis taken from Design Expert software

Term	Stdized Effects	Sum of Squares	% Contribution
Intercept			
A-Part of stem	-0.030	8.217E-003	21.62
B-UV light	-3.017E-003	8.190E-005	0.22
C-Temperature	-0.032	8.968E-003	23.59
D-Cycle of extract	8.138E-003	5.960E-004	1.57
E-Storage time	9.811E-003	8.663E-004	2.28
AB	2.231E-003	3.983E-005	0.10
AC	0.031	8.888E-003	23.38
AD	-7.760E-003	5.420E-004	1.43
AE	-8.397E-003	6.346E-004	1.67
BC	2.269E-003	4.118E-005	0.11
BD	7.101E-003	4.538E-004	1.19
BE	4.631E-003	1.716E-004	0.45
CD	-7.089E-003	4.523E-004	1.19
CE	-9.257E-003	7.713E-004	2.03
DE	-8.090E-003	5.891E-004	1.55
ABC	-2.294E-003	4.209E-005	0.11
ABD	-6.794E-003	4.154E-004	1.09
ABE	-4.506E-003	1.625E-004	0.43
ACD	7.513E-003	5.080E-004	1.34
ACE	8.362E-003	6.292E-004	1.66
ADE	7.619E-003	5.225E-004	1.37
BCD	-5.944E-003	2.826E-004	0.74
BCE	-4.319E-003	1.492E-004	0.39
BDE	6.369E-003	3.245E-004	0.85
CDE	8.385E-003	6.328E-004	1.66
ABCD	7.469E-003	4.463E-004	1.17
ABCE	4.944E-003	1.955E-004	0.51
ABDE	-6.794E-003	3.692E-004	0.97
ACDE	-7.294E-003	4.256E-004	1.12
BCDE	-7.281E-003	4.241E-004	1.12
ABCDE	6.006E-003	2.886E-004	0.76
Curvature		8.809E-004	2.32
Lenth's ME	0.024		
Lenth's SME	0.045		

ANOVA for factorial analysis from Design Expert software

Response 1 Ferulic acid

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.034	18	1.878E-003	6.18	0.0002	significant
A-Part of stem	*8.217E-003*	*1*	*8.217E-003*	*27.03*	*< 0.0001*	
B-UV light	*8.190E-005*	*1*	*8.190E-005*	*0.27*	*0.6104*	
C-Temperature	*8.968E-003*	*1*	*8.968E-003*	*29.50*	*< 0.0001*	
D-Cycle of ext	*5.960E-004*	*1*	*5.960E-004*	*1.96*	*0.1794*	
E-Storage time	*8.663E-004*	*1*	*8.663E-004*	*2.85*	*0.1096*	
AB	*4.117E-005*	*1*	*4.117E-005*	*0.14*	*0.7174*	
AC	*8.888E-003*	*1*	*8.888E-003*	*29.24*	*< 0.0001*	
AD	*5.420E-004*	*1*	*5.420E-004*	*1.78*	*0.1994*	
AE	*6.346E-004*	*1*	*6.346E-004*	*2.09*	*0.1667*	
BD	*4.538E-004*	*1*	*4.538E-004*	*1.49*	*0.2385*	
CD	*4.523E-004*	*1*	*4.523E-004*	*1.49*	*0.2392*	
CE	*7.713E-004*	*1*	*7.713E-004*	*2.54*	*0.1296*	
DE	*5.891E-004*	*1*	*5.891E-004*	*1.94*	*0.1818*	
ABD	*4.154E-004*	*1*	*4.154E-004*	*1.37*	*0.2585*	
ACD	*5.080E-004*	*1*	*5.080E-004*	*1.67*	*0.2134*	
ACE	*6.292E-004*	*1*	*6.292E-004*	*2.07*	*0.1684*	
ADE	*5.225E-004*	*1*	*5.225E-004*	*1.72*	*0.2073*	
CDE	*6.328E-004*	*1*	*6.328E-004*	*2.08*	*0.1672*	
Residual	5.167E-003	17	3.040E-004			
Cor Total	0.039	35				

ANOVA for factorial analysis from Design Expert software continued

Final Equation in Terms of Actual Factors:

Part of stem	Outer
UV light	Yes
Ferulic acid	=
+0.010555	
+9.80769E-006	* Temperature
+0.030962	* Cycle of extract
+4.88209E-003	* Storage time
-3.75096E-004	* Temperature * Cycle of extract
-4.67628E-005	* Temperature * Storage time
-1.34990E-003	* Cycle of extract * Storage time
+1.14022E-005	* Temperature * Cycle of extract * Storage time

Part of stem	Mixed
UV light	Yes
Ferulic acid	=
-0.013509	
+2.72115E-004	* Temperature
+7.75697E-003	* Cycle of extract
+1.48546E-003	* Storage time
-1.29904E-004	* Temperature * Cycle of extract
-2.40224E-005	* Temperature * Storage time
-6.76462E-004	* Cycle of extract * Storage time
+1.14022E-005	* Temperature * Cycle of extract * Storage time

ANOVA for factorial analysis from Design Expert software continued

Part of stem	Outer
UV light	No
Ferulic acid	=
-0.024458	
+9.80769E-006	* Temperature
+0.045699	* Cycle of extract
+4.88209E-003	* Storage time
-3.75096E-004	* Temperature * Cycle of extract
-4.67628E-005	* Temperature * Storage time
-1.34990E-003	* Cycle of extract * Storage time
+1.14022E-005	* Temperature * Cycle of extract * Storage time

Part of stem	Mixed
UV light	No
Ferulic acid	=
-0.015234	
+2.72115E-004	* Temperature
+8.08197E-003	* Cycle of extract
+1.48546E-003	* Storage time
-1.29904E-004	* Temperature * Cycle of extract
-2.40224E-005	* Temperature * Storage time
-6.76462E-004	* Cycle of extract * Storage time
+1.14022E-005	* Temperature * Cycle of extract * Storage time

B.2 Optimization

Experimental results for optimization

Run	Pre-treatment temperature (°C)	Storage Time of EBJ (h)	FA yield (g/g)
1	22.5	22	0.1404
2	27.5	22	0.1735
3	22.5	26	0.1916
4	27.5	26	0.1481
5	20	24	0.1370
6	30	24	0.1254
7	25	20	0.1101
8	25	28	0.1164
9	25	24	0.1915
10	25	24	0.2274
11	25	24	0.1848
12	25	24	0.1826
13	25	24	0.1922

Fit summary for Optimization taken from Design Expert software

Response 1 Ferulic acid Transform: None

***** WARNING: The Cubic Model is Aliased! *****

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	0.35	1	0.35			
Linear vs Mean	2.170E-004	2	1.085E-004	0.071	0.9315	
2FI vs Linear	1.467E-003	1	1.467E-003	0.96	0.3521	
Quadratic vs 2FI	0.012	2	6.133E-003	29.75	0.0004	Suggested
Cubic vs Quadra	6.361E-005	2	3.181E-005	0.12	0.8934	Aliased
Residual	1.380E-003	5	2.759E-004			
Total	0.36	13	0.028			

*"Sequential Model Sum of Squares [Type I]": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	0.014	6	2.308E-003	6.97	0.0406	
2FI	0.012	5	2.477E-003	7.48	0.0370	
Quadratic	1.179E-004	3	3.931E-005	0.12	0.9445	Suggested
Cubic	5.432E-005	1	5.432E-005	0.16	0.7063	Aliased
Pure Error	1.325E-003	4	3.313E-004			

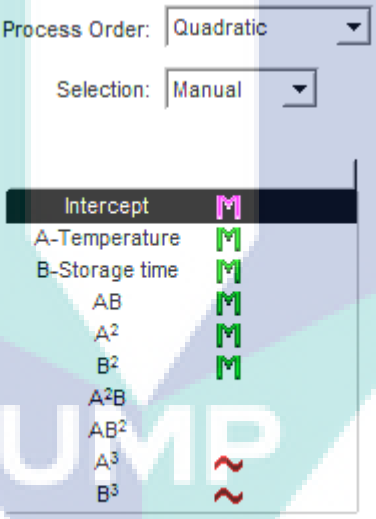
*"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Fit summary for Optimization taken from Design Expert software continued

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.039	0.0141	-0.1831	-0.9921	0.031	
2FI	0.039	0.1094	-0.1875	-0.8315	0.028	
Quadratic	0.014	0.9062	0.8393	0.8082	2.953E-003	Suggested
Cubic	0.017	0.9104	0.7849	0.4642	8.247E-003	Aliased

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".



ANOVA for Optimization from Design Expert software

Use your mouse to right click on individual cells for definitions.

Response 1 Ferulic acid

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.014	5	2.790E-003	13.53	0.0017	significant
A-Temperature	*9.408E-005*	*1*	*9.408E-005*	*0.46*	*0.5210*	
B-Storage time	*1.229E-004*	*1*	*1.229E-004*	*0.60*	*0.4654*	
AB	*1.467E-003*	*1*	*1.467E-003*	*7.12*	*0.0321*	
A^2	*5.901E-003*	*1*	*5.901E-003*	*28.63*	*0.0011*	
B^2	*9.663E-003*	*1*	*9.663E-003*	*46.87*	*0.0002*	
Residual	1.443E-003	7	2.062E-004			
Lack of Fit	*1.179E-004*	*3*	*3.931E-005*	*0.12*	*0.9445*	*not significant*
Pure Error	*1.325E-003*	*4*	*3.313E-004*			
Cor Total	0.015	12				

Final Equation in Terms of Actual Factors:

Ferulic acid =

-6.67351

+0.21919 * Temperature

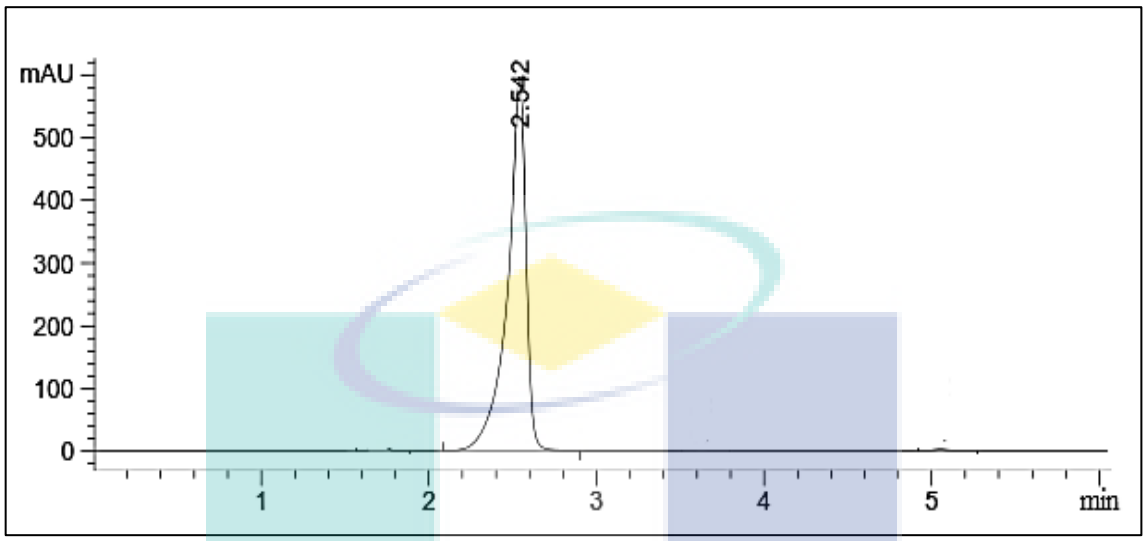
+0.34378 * Storage time

-3.83000E-003 * Temperature * Storage time

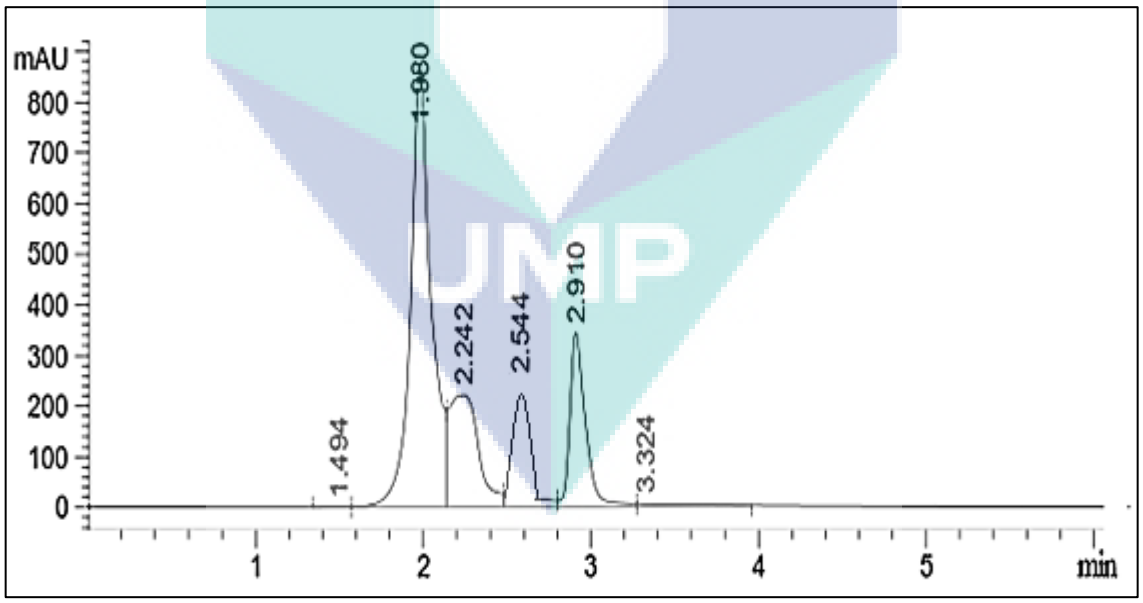
-2.56776E-003 * Temperature^2

-5.13400E-003 * Storage time^2

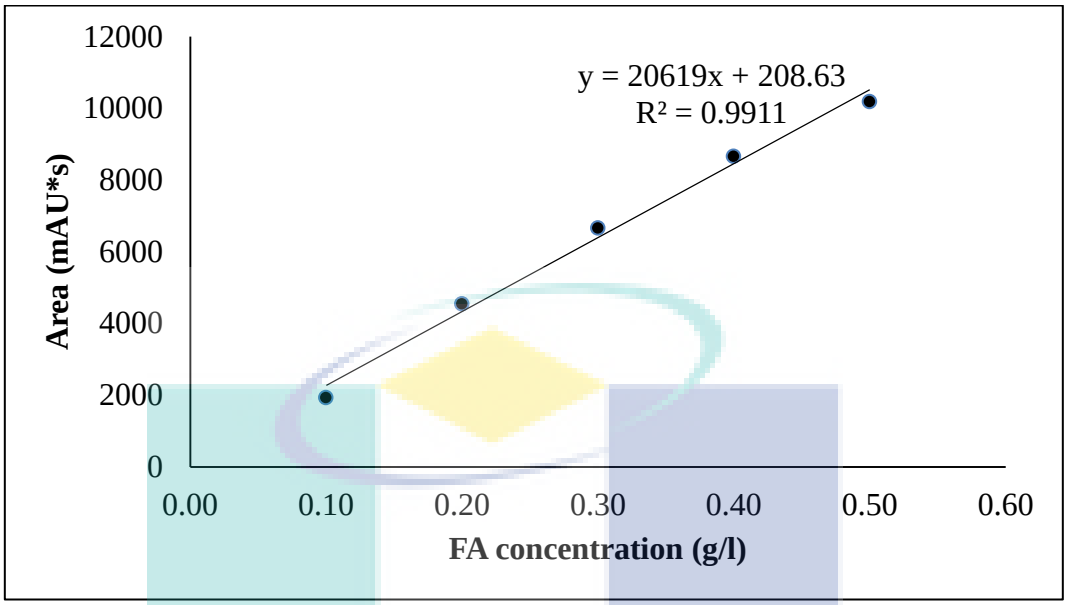
B3 Ferulic acid yield calculation



HPLC chromatogram of FA standard



HPLC chromatogram of the sample



Standard calibration curve of FA

Calculation for FA yield

Area that obtained from HPLC analysis = 551.1012 mAU*s

Dry weight of BSW = 8.02 g

Volume of EBJ = 40 ml

By using the equation from the standard graph:

$y = 20619x + 208.63$

$x = \frac{551.1012}{20619} - 208.63$

$x = 0.0166\ g/l$

Convert the FA concentration from g/l to mg/ml:

$FA\ concentration = 0.0166\ g/l$

$= 0.0166 \frac{g}{l} \times \frac{1l}{1000ml} \times \frac{1000mg}{1g}$

$= 0.0166\ mg/ml$

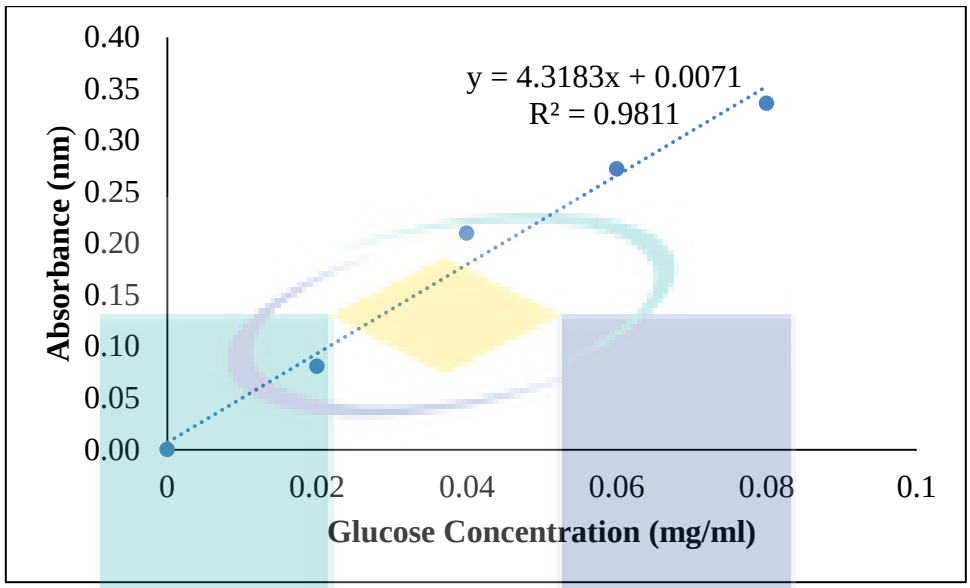
Convert FA concentration form mg/ml to FA yield mg/g:

$= 0.0166 \frac{mg}{ml} \times 40\ ml$

$= 0.6644\ mg \div 8.02\ g$

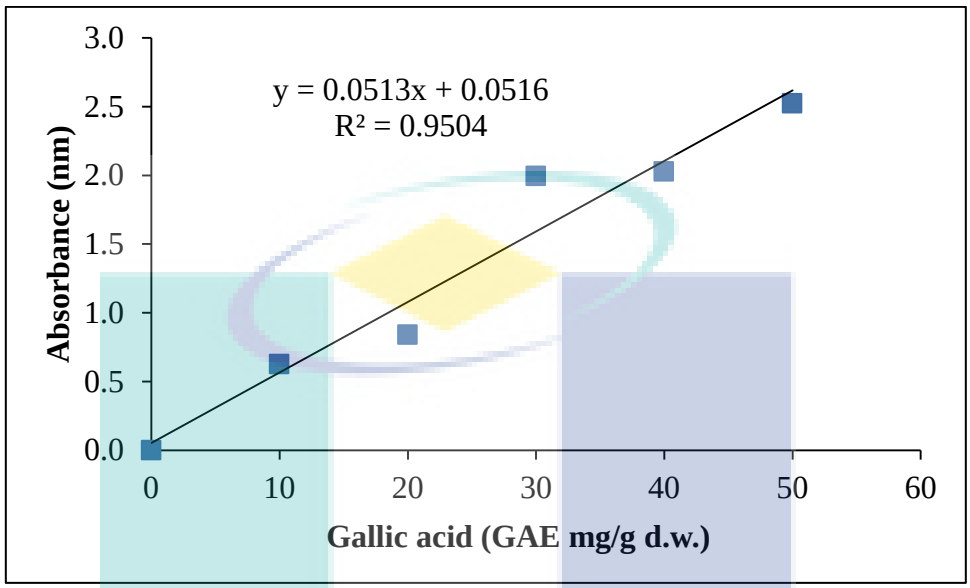
$= 0.0828\ mg/g$

B4 Standard calibration curve of glucose concentration



Standard calibration curve of glucose concentration

B5 Standard calibration curve of Gallic acid



Standard calibration curve of Gallic acid

APPENDIX C

List of Publication

1. Ismail, S. N. and Zainol, N. (2014). Factorial Analysis of Ferulic Acid Extraction from Banana Stem Waste. *International Journal of Applied Engineering Research*. **9**(20): 6823-6833.

2. Ismail, S. N. and Zainol, N. (2014). Optimization of Ferulic Acid Extraction from Banana Stem Waste. *Asian Journal of Microbiology, Biotechnology Environmental Sciences*. **16**(3): 479-484.