EXTRACTION OF ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND MINERALS IN BANANA PEEL

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EXTRACTION OF ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND MINERALS IN BANANA PEEL

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A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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DECEMBER 2010

I declare that this thesis entitled **"**Extraction of Antioxidant Activity, Phenolic Content and Minerals in Banana Peel" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special for my parent and family members
for their care, love and support.

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ABSTRACT

The objective of this research project is to establish the optimal conditions for obtaining the banana peel extract with high antioxidant activity, phenolic content and minerals. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. The banana peel extraction process was optimized by varying different parameters such as drying temperature for banana peels, types of solvent used, various type of solvents ratio, temperatures of extraction and time of extraction. The drying process was done at temperatures of 50^{o}C - 70^{o}C for 24 hours. The ground banana peel samples were extracted with different solvents (methanol, acetone and distilled water) with different solvents ratio (50%, 70% and 90%). The extraction was performed in a water bath at 40^{o}C-60^{o}C for 1- 120 min. UV-visible spectrophotometer was used to determine the antioxidant activity and phenolic content. High Performance Liquid Chromatography (HPLC) used to verify the existence of vitamin C. The minerals composition was determined by using atomic absorption spectrophotometer. The result showed that, optimal condition for the extraction of antioxidant activity, phenolic content and minerals in banana peel were drying at 60^{o}C, extracted by using 70% acetone solvent in the water bath for 2 hours at 55^{o}C. Concentration of antioxidant activity and phenolic content at this optimal extraction condition was 1061.33±0.03μmol/g and 1474.17±0.02 mg/L respectively.

ABSTRAK

Tujuan projek ini adalah untuk mendapatkan keadaan optimum untuk mendapatkan ekstrak kulit pisang dengan aktiviti antioksidan, kandungan fenolik dan mineral yang tinggi. Bahan buangan utama dari industri pemprosesan pisang adalah kulit pisang, yang mewakili sekitar 30% dari buah. Proses ekstraksi kulit pisang adalah dioptimumkan oleh pelbagai parameter yang berbeza seperti suhu pengeringan kulit pisang, jenis pelarut yang digunakan, nisbah pelarut, suhu ekstraksi dan masa ekstraksi. Proses pengeringan dilakukan pada suhu 50^{o}C - 70^{o}C selama 24 jam. Sampel pisang tersebut diekstrak dengan mengunakan pelarut yang berbeza (metanol, aseton dan air suling) dengan perbandingan pelarut yang berbeza (50%, 70% dan 90%). Ekstraksi dilakukan dalam penangas air pada suhu 40oC-60oC selama 1 - 120 minit. Spektrofotometer UV digunakan untuk menentukan aktiviti antioksidan dan kadar fenolik. Kromatografi (HPLC) digunakan untuk membuktikan kewujudan vitamin C. Komposisi mineral ditentukan dengan menggunakan spektrofotometer serapan atom. Keputusan kajian menunjukkan bahawa, keadaan optimum untuk ekstraksi aktiviti antioksidan, kandungan fenolik dan mineral dalam kulit pisang adalah pengeringan 60oC, diekstraksi dengan menggunakan pelarut aseton 70% dalam air selama 2 jam pada 55^{o}C. Konsentrasi aktiviti antioksidan dan kadar fenolik pada keadaan ekstraksi yang optimum adalah 1.061,33 ± 0.03μmol / g dan 1474.17 ± 0.02 mg / L masing-masing.

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LIST OF SYMBOLS

^{o}C	Degrees Celsius
%	Percentage
µM	MicroMolar
µL	Micro liter
µmol/g	micromol per gram
HCl	Hydrocloric Acid
m	Meter
m^3	Cubic meter
mg	miligram
mg/l	miligram per Liter
min	minute
mL	mililiter
mL/min	milliliter per minutes
mM	miliMolar
mol/g	mol per gram
nm	nanometer
O_2	Oxygen
rpm	rotation per minutes
ppm	part per million
w/v	Weight per Volume
g	gram
h	hour

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CHAPTER 1

INTRODUCTION

1.1 Research Background

According to several authors, banana peel recorded stronger antioxidant activity, pooled more quantity of phenolic compounds (Someya *et al.*, 2002), greater range of phenolics composition and higher in minerals content than banana pulp. Banana pulp had been reported as having various antioxidants such as vitamins (A, B and E), β – carotene (Kanazawa & Sakakibara, 2000) and phenolic compounds like catechin, epicatechin, lignin, tannin (Someya *et al.*, 2002), gallocatechin and anthocyanins like peonidin and malvidine.

There several type of antioxidant such as vitamin C, E, A, beta-carotene, lycopene and also other substances. Antioxidants are abundant in fruits and vegetables, as well as in nuts, grains, and some meats, and fish. Combination of vitamin C with other antioxidants, including vitamin E, b-carotene, and selenium, provides a synergistic antihypertensive effect. Experimental evidences prove that antioxidants can protect human body from free radicals and reactive oxygen species (ROS) effects.

Antioxidants is substance that can prevent or slow the oxidative damage process toward our body. Besides, antioxidant also protect cells from the damage that may caused by free radicals. Free radicals are unstable molecules or highly reactive chemical that often contains oxygen and is produced during oxidation. The defensive effect of natural antioxidant in fruit and vegetable are related to its major group, which are vitamin, phenolic and carotenoid.

Ascorbic acid and phenolic and are known as the hydrophilic antioxidant while carotenoid was known as lipophilic antioxidant (Halliwell., 1996). Exposure to various environmental factors will lead to free radical formation such as tobacco smoke and radiation. Free radicals can damage important cellular molecules such as DNA or lipids or other parts of the cell and may lead to cancer.

Banana peel also demonstrated the presence of various phenolic compounds such as gallocatechin and anthocyanins like peonidin and malvidin. Phenolic compounds are secondary metabolites, which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur & Kapoor, 2001). Extraction of phenolic compounds in plant materials is influenced by many factors including the extraction method employed, types of solvent polarity used, storage time, presence of interfering substances, sample particle size and conditions as well as their chemical nature.

Bananas were enriched with minerals like potassium, phosphorus, magnesium and calcium. Banana peel could be a good source of carbohydrates and fiber. The high fiber content also indicates that the peels could help treat constipation and improve general health and well being (Anhwange, B. A. *et al.,* 2009*).*

Natural antioxidant are compound from plant or animals sources that retard oxidative rancidity of oil, fats and fat soluble components, thus protecting them while delaying the development of unpleasant flavors and odors resulting from oxidation process.

Antioxidants are present naturally in most raw food sources. Processing of the fruit can remove or trigger the degradation of this antioxidant.

Nowadays, the most widely used antioxidant were synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyanisole (TBHQ), and propyl gallate (PG). Figure 1.1 shows the molecular structure of the available synthetic antioxidant.

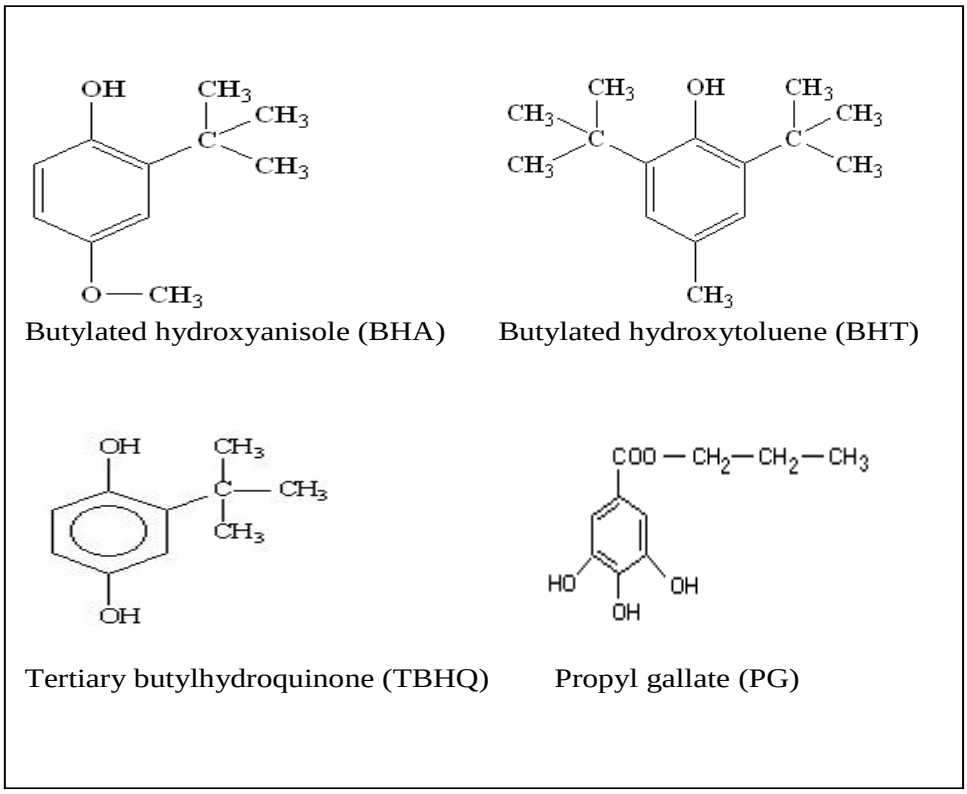

Figure 1.1: Synthetic antioxidants

1.2 Problem Statement

Doubt on the safety of the synthetic arose first in the 1960s and led to an increased interest and broad research on natural antioxidant. Natural antioxidant particularly found

in fruit and vegetables have gain interest among consumer and the scientific community because epidemiological studies have indicate that frequent consumption of natural antioxidant is associate with the lower risk of cardiovascular and cancer (Renoud et al.1998).

Natural antioxidants are perceived safe, less toxic and beneficial for human health. However, natural antioxidant is very expensive and has been not widely commercialized. Recently, the demand for natural antioxidants has increased, due to consumer concerns about the safety of synthetic antioxidants (S. Okonogi et al., 2007).

There had been an explosive interest in studying antioxidants of some fruits due to their health promoting properties. A large number of studies have been demonstrate either on the effect of extraction time and extraction temperature or drying temperature toward antioxidant activity, phenolic content and minerals in banana peels.

Antioxidant activity could influence by geographical origin, cultivar and harvest storage time. *(*C. Guo *et al., 2003).* These statements show that different origin of banana peel will have different value of antioxidant. This study is focusing on the banana peel that originally harvest in Malaysia because banana peel from Malaysia origin is rarely being studied before.

1.3 Objective

The objective of this research project is to establish the optimal condition for obtaining the banana peel extract with high antioxidant activity, phenolic content and minerals by using solvent extraction.

1.4 Research Scope

Following tasks will be undertaken as a part of the proposed research:-

- Different drying temperatures of 50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C and 70^{o}C used to dry the sample. 70% acetone was use as the extracting solvent.
- The most optimum drying temperature sample was extract by using different type of solvent (methanol, acetone and water) with different solvent ratio (90%, 70%, 50%). The extraction was done at 50^{o}C for 1 hour in the water bath.
- Time of extraction vary from 1min, 30min, 60min, 90min and 120min was used to determined the effect of extraction time on antioxidant activity and phenolic content. Optimum drying temperature of the banana peel sample and optimum solvent ratio was used in the extraction process.
- Effect of different extraction temperatures of 40^{o}C, 50^{o}C, 55^{o}C and 60^{o}C were analyst where optimal conditions that obtained in the drying temperature, type of solvent, solvent ratio and time of extraction were used in the extraction process.
- Antioxidant activity and phenolic content was analyst by using Ferric Reducing Antioxidant Power (FRAP) assay and Folin-Ciocalteu's calorimetric (FC) respectively. The detection of the products was performed by using UV-visible Spectrophotometer.
- Verification of the Ascorbic acid existence in the banana peel extract at optimal conditions was performs using High Performance Liquid Chromatography (HPLC) unit.
- Sodium(Na), Magnesium(Mg) and Calcium(Ca) compositions in banana peel extracted at optimal condition were investigate by using Atomic Absorption Spectrophotometer (AAS) unit.

1.5 Rationale and Significance

Banana peels are waste from banana fruit and they contain high amount of antioxidant, phenolic content and mineral. This peel is biodegradable and it will produce environmental problem due to it nitrogen and phosphorus quantity. Therefore, extracting the banana peel will be the best solution in order to protect human being, gaining some profit and creating waste to wealth. Banana peel also can be commercialize because it qualitative and quantitatively contain more antioxidant than it pulp. It also will have does not compete with banana pulp in producing end product especially in the food industry. Besides, banana fruit is easy obtains because it is not a seasonal fruit and it grow well in Malaysia. These ensure that, banana can continuously act as the natural antioxidant source.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

2.1.1 Banana (Banana Peel)

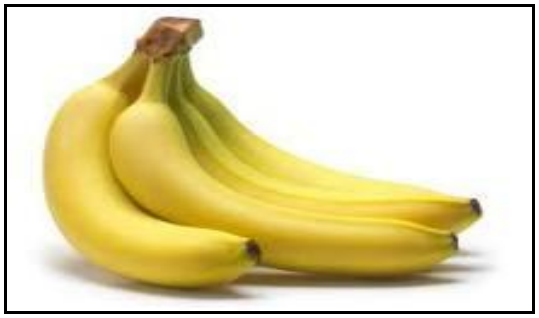

Figure 2.1: Banana Fruit (*Musa sapientum)*

Banana is genus of *Musa* and family of *Musaceae.* It was originally from Malaysia and are now cultivated all over the tropical and sub-tropical continents. Banana plants are the world's biggest herbs, grown abundantly in many developing countries (G. Aurore et al., 2009). Musa sapientum trees are best cultivated in a highly organic soil with pH 5.5-7.0. This plant need a lot of water is to grow and yield bananas.

After 9-12 month of planting the banana plant, flower start to develop. The underground stem initiates the development of the flower. The stem is also known as pseudostem. After producing single bunch of banana, this pseudostem will die and replace with new pseudostem. Banana plant can grow up to the height of 2-8m. Fruit maturation take an about 60 - 90 days after flowers first appear. The banana fruit grows in hanging cluster, with twenty fruits to a tier and 3 - 20 tiers to a bunch. The fruit is protected by its peel which is discarded as waste after the inner fleshy portion is eaten (Anhwange, B. A. et al., 2009).

The primary reason for the cultivation of banana plant is its fruits. Banana fruits are widely available and they had been used as food without apparent toxic affect. In some countries, banana fruit and its peel are considered to be the golden fruit of nature because they do helps to promote natural beauty by providing the body with essential nutrients and also healthy digestion. It have been reported that banana fruit do help preventing anaemia, cure the heart burns stress, strokes, ulcers and many other minor illness. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease (Someya, S., Y. Yoshiki and K. Okubo, 2002).

Peels are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their unknown benefit of commercial application. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. This by-product constitutes an environmental problem because it contains large quantities of nitrogen and phosphorus and its high water content makes it susceptible to modification by microorganisms (R. González-Montelongo et al., 2010). Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions

The banana peel could be a potential being the source of antioxidant and antimicrobial activities. Banana peel is rich in phytochemical compounds, mainly

antioxidants (R. González-Montelongo et al., 2010). Banana peels are commonly used as a home remedy for several skin problems including allergies, bruises and skin irritation. It can reduced several skin problems conditions including treating acne, treating poison ivy rashes irritation from mosquito bites, reducing bruises, getting rid of warts and also managing wrinkles. Banana peel will not be instantly effective to manage the wrinkles, but it helps the skin to look more radiant, fresher and healthier.

Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre (50% on a dry matter (DW) basis), proteins (7% DW), essential amino acids, polyunsaturated fatty acids and potassium. Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana peel ranges from 0.90 to 3.0 g/100 g DW .

Someya et al. (2002) identified gallocatechin at a concentration of 160 mg/100 g DW in the banana peel. Ripe banana peel also contains other compounds, such as the anthocyanins delphinidin and cyaniding, and catecholamines. Furthermore, carotenoids, such as b-carotene, a-carotene and different xanthophylls, have been identified in banana peel in the range of 300–400 lg lutein equivalents/100 g, as well as sterols and triterpenes. To date, only Someya et al. (2002) have evaluated the antioxidant activity in banana peel, measured as the effect on lipid autoxidation in relation to its gallocatechin content. (R. González-Montelongo et al., 2010)

Banana peels have been effectively used for the synthesis of silver nanoparticles. Currently, silver nanoparticles is use in the production of antibacterial and antifungal agents biotechnology and bioengineering, textile engineering, water treatment, and silver-based consumer products. It can be synthesized by several chemical, physical and biological method. Nanoparticle are one of the effective medium in against the fungal and bacteria culture such as C. albicans and E. coli, Staphylococcus aureus, Bacillus anthracis and Proteus mirabilis. These in turn, could be applied in the fields of microelectronics, biodiagnostics, sensing, and imaging as well as in designing newer drugs (A. Bankar et al.,*2010).*

The commonly used of banana peel in the silver nanoparticles production is due to the its composition. Banana peels are inherently rich in polymers such as lignin, hemicellulose and pectins that contribute to the synthesis of silver nanoparticles. (A. Bankar et al.,2010). Therefore, banana peel is a potential new generation for the production antimicrobials products due to displayed by of antimicrobial activities silver nanoparticles that produced from the banana peels.

2.2 Antioxidant Activity And Phenolic Content

Bananas peel are a good source of natural antioxidants, which include vitamins and beta carotene**.** It is contains a number of antioxidants and minerals that can help the skin restore itself naturally. Natural antioxidant are primarily phenolic compound that may occur in all part of a plant. They are multifunction and can react as free radical terminators, metals chelators and single oxygen quenchers. The common plant phenolic antioxidant are topopherols, flavonoids and other related compound such as phenolics acid.

Antioxidants react as free radical scavengers by protecting the cell from damage by free radicals .Antioxidants may also enhance immune defense. In humans, the most common form of free radicals is oxygen. Oxygen molecule (O_2) steals electrons from other molecules when it is electrically charged. This may caused damage to the DNA and other molecules. Over time, such damage may become irreversible and lead to disease including cancer.

Free radicals can attack any various substrates of interest in the body,. Therefore, it will contribute to chronic disease development such as oxidatively modified LDL. Oxidatively modified LDL has been hypothesized to be a causative agent in the

development of cardiovascular disease . Oxidatively modified DNA may also play an important role in human carcinogenesis. Many nutritional factor are widely considered to be critical for human health. Among them, free radicals have been of concern as one of the factors contributing to chronic degenerative disease (O. Patthamakanokporn *et al.*, 2008).

Antioxidants protect fats and lipids in foodstuffs. Most of these act through reaction with free radicals. Formation of these free radical from lipids is the first step in their oxidative deterioration. Other antioxidants in food such as ascorbic acid which lower the potential of the foodstuffs to which they are added. Oxidation reaction has deleterious effect on the antioxidant activity where this oxidation level is influenced by temperature, light, air, physicochemical as well as the presence of catalyst (Frankel&Meyer, 2000)

Phenolic compounds are in the category of natural antioxidants and are the most abundant antioxidants in human diet. Phenolic make an important contribution to the flavor to the fruits It is well known that fruits contain various antioxidants, such as vitamin C, vitamin E, and b-carotene. Phenolic compounds are naturally and commonly found in both edible and inedible plants. The phenolic content and composition in plants depend on genetic and environmental factors, as well as post harvest processing and storage conditions. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. This prove that solvent polarity will play a key role in increasing phenolic solubility.

Folin-Ciocalteu’s calorimetric (FC) method is the method to analyze the phenolic content in the banana peel extracts. For qualitative identification of phenolic compounds, ultraviolet– visible spectrophotometer were considered as a tool for the identification of the phenolic contents.

2.3 Minerals Profile

Minerals play a vital role in proper development and good health of the human body and fruits are considered to be chief source of minerals needed in the human diet. Inadequate intake of mineral has been observed to be a major nutritional problem in our environment.

Banana has been reported to prevent anaemia by stimulating the production of haemoglobin in the blood. Its role to regulate blood pressure has been assosiated eith the high content of potassium. Its peels in conjunction with other substances create a liniment for reducing the acuteness of the arthritis aches and pains**.**

Both banana peel and pulp have impressive potassium content. It is highly recommended by doctors for patients whose potassium is low. B. A. Anhwange (2008) states as in table 1.1 that concentration potassium in the banana was found to be highest (78.10 mg/g) among other minerals compound. The concentration of calcium, sodium, iron and manganese are 19.20, 24.30, 0.61 and 76.20, respectively. The minerals profile are determined by using flame atomization.

Table 2.1: Mineral composition of *Musa sapientum peel*

Element	Concentration (mg g^{-1})
Potassium	78.10±6.58
Calcium	19.20±0.00
Sodium	24.30±0.12
Iron	0.61±0.22
Manganese	76.20±0.00
Bromine	0.04±0.00
Rubidium	0.21±0.05
Strontium	0.03±0.01
Zirconium	0.02±0.00
Niobium	0.02±0.00

2.4 Vitamin C

Another abundant of antioxidant in nature is ascorbic acid. Ascorbic acid or Vitamin C is organic acid with antioxidant properties and also water-soluble compound that fulfills several roles in living systems. It is generally recognized as safe substances by FDA. As a potent antioxidant, ascorbic acid has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant a-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be the substrate for oxalate and tartrate biosynthesis and has a role in stress resistance (Y. Hernández et al., 2006).

Ascorbic acid serves as a reducing agent and may exert a chelating action. Several analytical methods have been reported for the determination of vitamin C using titrimetry, spectrometry and amperometry .Most of these methods may give overestimes due to the presence of oxidizable species other than AA and/or not to measure DHA. For example, the AOAC's official method , based on the titration of AA with 2,6-dichloroindophenol in acidic solution, is not applicable in all the matrices (Y. Hernández et al., 2006). The commonly equipment used to the determined the ascorbic acid composition is by using high performance liquid chromatography (HPLC).

The major benefit of ascorbic acid with regard to cancer is it reacts as anticancer agent. It may be precaution from developing cancer, rather than in therapy. Vitamin C work from inside the cells and protect DNA, the hereditary material in cells from the damage caused by free radicals. It can also reduce the development of nitrosamines from nitrates. Nitrates chemicals that are commonly used for foods processing. Once formed, nitrosamine can become carcinogenic which can cause cancer.

Lea (1992) reported that fresh apple contains up to 100ppm of vitamin C but during the processing into juice it is rapidly lost. The lost of ascorbic acid was also found to be

highest in the medicinal plant dried at 50^{o}C for 9 hours (75.60%) compared to freeze drying(21.13%)(Mahanom et al. 1999). This statement show that, the amount of ascorbic acid exist in the is depend on the it processing method.

2.5 Analysis Method

2.5.1 Solvent Extraction

The most common techniques that were employed to obtain high yield of antioxidant activity is direct by using solvent. The solvent used for the extraction is a major importance for the recovery of the antioxidant component, the coextraction of undesirable substances and the process yield. Selective extraction methods should be practiced since active compounds in plants that exhibit biological activities are usually present in low concentrations.

The type of the solvent used to extract antioxidants from banana peel can affect single electron transfer and hydrogen atom transfer, which are key aspects in the measurements of antioxidant capacity. The polarity of the solvent and that of the different antioxidant compounds affects the efficiency of the extraction and the activity of the obtained extracts. Water, methanol, ethanol, acetone, aqueous solutions of the aforementioned solvents and ethyl acetate are commonly used as extraction solvents (R. González-Montelongo et al., 2010). However acetone is the most commonly used as extraction solvent due to it extensive validation by FDA.

Several studies focused on the efficiency of different organic solvent used in extraction process. Organic solvent with high polarity are more effective in quantitive recovery than nonpolar solvent. For example, the extraction of various aromatic herbs by

hexane, ethyl acetate or ethanol showed that ethanol extract were the most active in retarding the autoxidation process in the lipid substrates(N.Valishlieva & EM. Marinova., 1998). M. Alothman et al., (2009). claimed that acetone (70%) was the most efficient solvent system for the extraction of phenols from pisang mas

The extraction yield is influenced mainly by the extraction condition which during the process of solid- liquid extraction is carried out. Each solid and liquid composition had their own unique structure and composition. Thus, when they are combined with solvents the behaviour of the resulting material-solvent system is unpredictable (R. González-Montelongo et al., 2010). The example of extraction conditions are the sample moisture content (drying effect), type of solvent used, concentration of the selected solvent, and the extraction time. High temperatures are reported to improve the efficiency of extraction due to the enhanced diffusion rate and solubility of analytes in solvents; although elevated temperatures can also affect the activity of the extracts due to the degradation of the phytochemical compounds and losses due to volatilization (R. González-Montelongo et al., 2010). Used of high temperature may be improved the extraction efficiency, but the degradation rate of antioxidant composition are based on the time and the extraction temperature. Therefore, the combination of high temperatures and high extraction times can contribute to greater losses of these compounds (R. González-Montelongo et al., 2010).

2.5.2 Dehydration process (Drying)

Drying is the pretreatment process this banana peel extraction. Drying appears to be a potential food preservation option to extend their storage and shelf-life. It is a complex process involving simultaneous coupled transient heat and mass transfer. Drying or dehydration is the process of water removal which minimized the microbial spoilage and deterioration. A. Kaya et al., (2008). reported that many of the properties of the kiwi fruits, especially the level of vitamin C, are affected by the drying conditions.

The drying process may be done by using tray dryer and also conventional oven with their temperature are controlled. In the drying process, moisture content need to be determine by taking the before and after the drying process. The air temperature, humidity and velocity in convective drying have significant effect not only the drying kinetics but also on the quality of the food product

Most of food materials are heat sensitive. Thus drying at relative high temperature implies structural, organoleptic and nutritional change during the dehydration process. Heating and high oxygen pressure caused an acceleration of chain initiation and propagation of oxidative process and hence a decrease in the oxidation stability or in the activity of present antioxidant (Yanishlieva-Maslarove, 2001).

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Fruit extract contains bio-active compound that are sensitive to thermal or heat application. The heating temperature of the fruit and vegetable beverage depended on the microbial level of the raw material and acidity of the product (Saniah Kormin, 2005). During hot air drying, bio-active compound is introduced to the heat application which can induced the degradation process and undergoes several physical, chemical, organoleptic and nutritional changes that can cause loss in final product quality (M. Miranda et al., 2010).

There are several study on the impact of the drying temperature toward the product quality. Drying temperature of 50, 60, 70, 80 and 90^{o}C was use by M. Miranda et al. (2010) in the determination of the influence of the drying kinetic in the Aloe Vera gel. In this study, the optimum drying temperature was obtained between 60^{o}C and 70^{o}C. T. Kuljarachanan et al. (2009) states in the study of antioxidant compound in the limes residues that drying at 60^{o}C could retain the highest antioxidant activity. In this study, the hot air drying temperature range used is 60^{o}C- 120^{o}C.

2.6 Analysis assays

2.6.1 Antioxidant activity

There are several assays have been frequently used to estimate antioxidant activities in fresh fruits and vegetables such as Ferric Reducing Antioxidant Power (FRAP), Oxygen Radical Absorption Capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (ABTS). These techniques have shown different results among crop species and across laboratories (K. Thaipong et al., 2006).

FRAP assay are the most common method selected in determining the antioxidant activity. In this study, FRAP method was selected because it is relatively simple, easy to standardized and treat samples as reductant in redox- linked calometric method. The FRAP technique showed high reproducibility as it is simple, rapidly performed and showed the highest correlation with both ascorbic acid and total phenolics (K. Thaipong et al., 2006).

The antioxidant activity of banana peel extract is determined by the ability of the antioxidant in this extract to reduce ferric iron to ferrous in FRAP reagent. At low pH, Ferric (Fe III) reduce to ferrous ion (Fe II) and form an intense blue color with an absorption maximum at 593 nm. The FRAP value are obtained by measuring the absorbance change at 593 nm. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma and with solutions containing one antioxidant in purified form (Benzie and Strain, 1996).

FRAP assay was performed according to a modified method described by Benzie and Strain (1999). Approximately, 40 µl of diluted fruit extract was mixed with 3 ml of FRAP reagent. Then, the reaction mixture was incubated at 37^{o}C for 4 min. The FRAP reagent was freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine

(TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM FeCl3. 6H2O and 25 ml of 0.3 M acetate buffer, pH 3.6. The reading were taken by using UV-visible Spectrophotometer at 593nm against distilled water used as blank. Aqueous solutions of known Fe(II) concentration (FeSO4.7H2O) were used for calibration of the FRAP assay and antioxidant power was expressed as lmol/g FRAP (M. Alothman et al., 2009).

The ORAC assay is said to be more relevant because it utilizes a biologically relevant radical source. These techniques have shown different results among crop species and across laboratories (K. Thaipong et al., 2006). The main disadvantage of ORAC techniques comparing to other method is the analysis equipment used. ORAC technique require expensive equipment while other method required only a simple Spectrophotometer which commonly available in most laboratory.

The ORAC assay is based on the scavenging of peroxyl radicals generated by 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH). AAPH prevent the degradation of the fluorescein probe and consequently prevent the loss of fluorescence of the probe. The ORAC procedure used an automated plate reader with 96-well plates (Prior et al., 2003). Analyses were conducted in phosphate buffer pH 7.4 at 37^{o}C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was freshly prepared for each run. ORAC values were expressed as μmol Trolox equivalents/g of plant extract using the standard curve that established (Dudonne´ et al., 2009).

DPPH is a crystalline powder form that composed of stable free-radical molecules. DPPH absorbance method is usually applied for laboratory research such as determination of antioxidant activity and degree of oxidative in the fruit extract. The DPPH assay was based on the reduction of the stable radical DPPH• to yellow colored diphenylpicrylhydrazine in the presence of a hydrogen donor(N.Loganayaki et al., 2010). This stable free radical has an unpaired valence electron at one atom of nitrogen bridge(O.P. Sharma & T.K. Bhat. 2009).

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20^{o}C until needed. The working solution was then prepared by mixing fruit extracts (150 mL) with 2850 mL of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM TE/g fresh mass. High concentrations of DPPH in the reaction mixture give absorbance beyond the accuracy of spectrophotometric measurements (Sloane & William, 1977).

ABTS assay are performed followed the method of Arnao et al. (2001) with some modifications. The freshly prepared stock solutions included 7.4mM ABTS solution and 2.6mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark condition. The solution was then diluted by mixing 1mL ABTS solution with 60mL methanol. Fruit extracts (150 mL) were allowed to react with 2850 mL of the ABTS solution for 2 h in a dark condition. Then the absorbance was taken at 734nm using the spectrophotometer. The standard curve was linear between 25 and 600 mM Trolox. Results are expressed in mM Trolox equivalents (TE)/g fresh mass. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve(K. Thaipong et al., 2006).

2.6.2 Phenolic content

The very common assay in analyzing phenolic content is Folin Ciocalteu (FC) assay. It also known as Gallic Acid Equivalence method (GAE). It works by measuring the amount of the substance that inhibits the oxidation of the reagent.

Phenolic content of fruit extract is determine by Folin Ciocalteu assay which described by Singleton and Rossi(1965). 40 µL of the extract was mixed with 1.8 ml FC reagent that was pre-diluted 10 times with distilled water. The mixture was allowed to react for 5 min before a solution of 1.2mL sodium carbonate (7.5% w/v) added. Then, the mixture was allowed to stand at room temperature for 1 h. The absorbance was then measured at 765 nm using UV-visible Spectrophotometer. A calibration curve was prepared by using gallic acid as the standard at 20-100 mg/l, $R^2 = 0.989$) (M. Alothman et al., 2009). The phenolic content obtains in this banana peel extract was 72.2±2.03µmol Fe II/g fresh weight. The Folin-Ciocalteu reagent is not specific and detect all phenolic group compound founds in the extract. A disadvantages of this assay is the interference of reducind substances such as ascorbic acid with the determination.

Different method was used by R. González-Montelongo et al. (2010) in determining the phenolic content in banana peel extract. In this study, phenolic compound content was estimated by mixing 200µL of deionized water with 50µL of extract diluted by factor of 5 and 50µL of FC reagent. After 6 minutes, 7% sodium carbonate solution added into the mixture. The mixture volume then adjusted to 1.3 ml with deionized water and allowed to stand at room temperature for 1 hour. The absorbance was read at 765nm by using UV-visible spectrophotometer. Gallic acid ranging from 15-250mg/l was used to construct the calibration curve. The result obtained was 116±4mg trolox equivalent/100g DW.

2.7 Analysis equipment

There is several analytical technique that available to determine the antioxidant activity and phenolic compound in the fruit extract. The instrumental equipment are categorized as spectroscopic and chromatographic.

2.7.1 Spectroscopic techniques

Spectroscopic is based on the in teraction matter with electromagnetic radiation. Interaction can take the form of absorption and emission and can be detected by using emission, transmission and reflection designs. Commonly Spectroscopic equipment used in the study antioxidant activity and phenolic content are UV visible spectrophotometer and Atomic absorption Spectrophotometer.

UV visible spectrophotometer measured absorbed radiant and has been used in the used in the laboratories for many years. A food component that absorb in the ultraviolet or visible range may be analyzed at its characteristic wavelength in a UV-visible spectrophotometer as long as there are no interfering compounds.

Atomic Absorption spectroscopy (AAS) is based on the absorption of UV-Vis radiation by atomic minerals. Flame AAS are relatively simple to operate and can be use to determine many nutritional elements and some toxic element at level interest in food. Analytical solutions are aspirated into a flame that is usually air acetylene or nitrous oxide –acetylene, depending on the element being determined. Analytical solutions are prepared by either wet digestion or dry ashing. Flame AAS, sample are atomizer by nebulizer and burner. It measure trace metal concentration in complex matrices with excellent precision and accuracy.

2.7.2 Chromatographic techniques

Chromatography is based on the distribution or partition of sample solute between stationary and mobile phase. Chromatographic technique that commonly used in food

analysis is high-performance liquid chromatography (HPLC) and gas chromatography (GC).

HPLC was develop in the 2960s as an improvement over column liquid chromatography and has been used to measure non volatile food component. Normal phase HPLC, in which the stationary phase is a polar absorbent and the mobile phase is nonpolar solvent is often used for fat soluble vitamin and carbohydrates. Reversed phase HPLC, with a nonpolar stationary phase and polar mobile phase, is more popular because of its wider application. Ion exchange HPLC, with functionalized organic resin as packing material is used for detection of inorganic ion and analysis of carbohydrate and amino acid.

The sample to be analyzed in HPLC is introduced in small volume to the stream of mobile phase. The solution movement through the column is slowed by specific chemical or physical interactions with the stationary phase present within the column. The flow rate of the solution moves is depends on the nature of the sample and on the compositions of the stationary (column) phase. Retention time is the time at which a specific sample elutes. Retention time under particular conditions is considered an identifying characteristic of the given sample.

GC was introduced in the 1950s and had been applied to a wide range of food. It is applicable to volatile substances that are thermally stable. GC is useful for the analysis of nonpolar compounds. Isolation of the analyte from the sample matrix is particularly important in GC to avoid false response from matrix degradation products.

CHAPTER 3

METHODOLOGY

3.1 Introduction

Figure 3.1 show the summary of overall process that applied in this study. The process starts with the preparation of banana peel sample where banana peel sample undergo drying process for 24 hours. Secondly, solvent extraction step was done by extracting dried banana peel sample with different type of solvent ratio. Then, each extract obtained was purified by using rotary evaporator to remove solvent content in the extract. Lastly, the analysis was performed. The analysis process was divided into four type of analysis which is analysis of antioxidant activity, phenolic contents, mineral composition and verification of ascorbic acid existence.

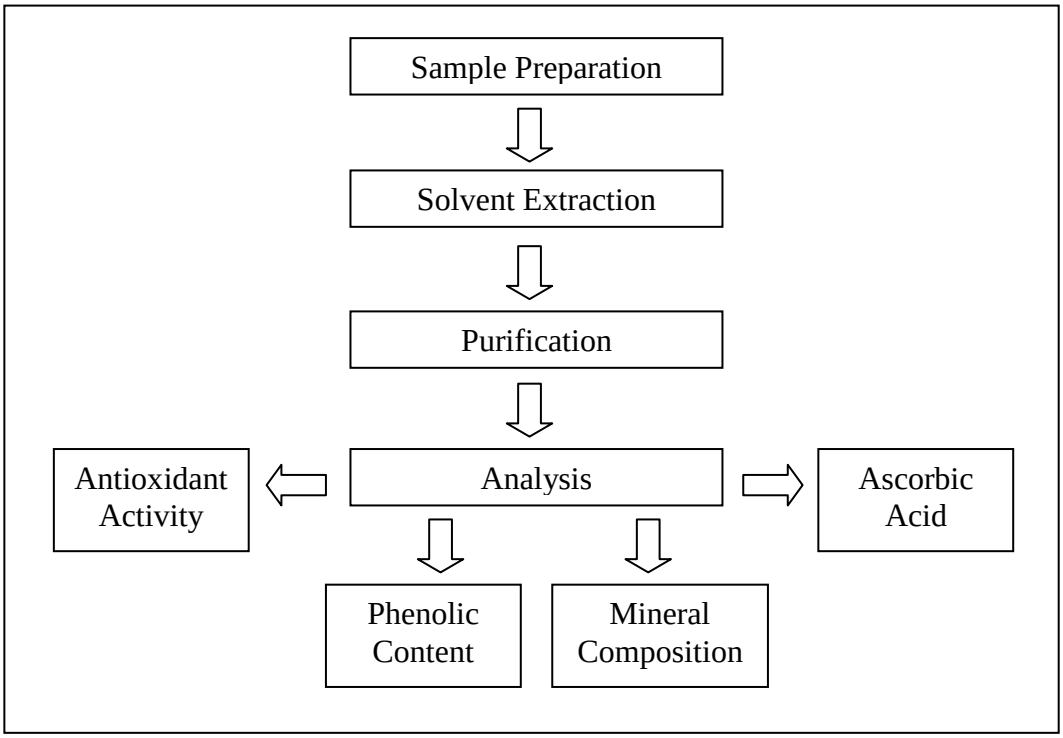

Figure 3.1: Summary for overall process

3.2 Chemicals and reagents

Acetone, water, methanol, 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ), FeCl3. 6H2O, acetate buffer, FeSO4. 7H2O, Folin Ciocalteu reagent, sodium carbonate, Gallic acid, potassium phosphate dibasic solution and orthophosphoric acid were used in this extraction process

3.3 Apparatus

The apparatus used in this study including oven, blender, freezer, water bath, refrigerated centrifuged, UV- visible spectrophotometer, Atomic Absorption Spectrophotometer(AAS), and High Performance Liquid Chromatography (HPLC). Detail explanation on the each apparatus are provided in Section 3.3.1 , 3.3.2, 3.3.3, 3.3.4, 3.3.5, 3.3.6 and 3.3.7.

3.3.1 Oven

Ovens that commonly used in laboratory is the ovens for high-forced volume thermal convection applications. These ovens generally provide uniform temperatures throughout. In this study, the oven was used to dry the banana peel sample. Typical sizes for oven are from one cubic foot to 32 cubic feet (0.91 m^3) with temperatures that can be over 340 degrees Celsius.

Figure 3.2: Oven

3.3.2 Blender

Blender is the equipment designed for blending, mixing puree and crumbs the sample. In this study, blender was used to ground the dried banana peel sample. There are no addition of water is required during the banana peel sample blending process.

Figure 3.3: Warring Blender

3.3.3 Shaking Water Bath

Shaking water bath is also known as laboratory water bath which is a tool to maintained very stable operation temperature much like incubator. Instead of maintaining the stable temperature, it is also can be used to give continuous stable shaking process. In this study, extraction was performed in the shaking water bath. Therefore stable temperature can be achieved throughout the extraction process.

Figure 3.4: Shaking water bath

3.3.4 Refrigerated Centrifuge

Refrigerated centrifuged is one of the equipment use to separate in between two component. It is generally driven by an electric motor where an object is placed in the rotation around a fixed axis and a force was applied perpendicular to the axis. Centrifuge operation follow the sedimentation principle, where the centripetal acceleration causes more dense substances to separate at the bottom and lighter substances to move to the tops of the sample.

Figure 3.5: Refrigerated Centrifuge

3.3.5 UV-Visible Spectrophotometer

UV - Visible spectrophotometer model Hitachi U-1800 was used in the quantitative analysis of antioxidant activity and phenolic content of the banana peel extract exposed with various parameter discussed in this study. The device is operate base on the theory of Beer-Lambert law that stated the absorbance of solution is directly proportional to the absorbing material in the sample. The sample was placed in a cuvette with internal width of 1cm and distilled water was used as blank. The wavelength was set to be 593nm and 765nm for FRAP assay and Follin-Ciocalteu assay respectively.

Figure 3.6: UV-Visible Spectrophotometer

3.3.6 Atomic Absorption Spectrophotometer (AAS)

Atomic absorption spectrophotometer is a spectroanalytical procedure use for measuring mineral compositions in the sample by employing the absorption of optical radiation (light) by free atoms in the gaseous state. Standards with known analyte concentration are required to establish the relation between the analyte concentration and the sample. This equipment is operate based on the Beer-Lambert Law.

In short period of time, the electrons of the atoms in the atomizer is promoted to higher orbitals by absorbing a defined quantity of energy. This amount of energy is proportional to the wavelength which specific to a particular electron transition in a particular element. A detector was used to measure the radiation flux either with a sample or without a sample in the atomizer. The ratio the between two absorbance is converted to analyte concentration or mass using Beer-Lambert Law.

Figure 3.7: Atomic Absorption Spectrophotometer(AAS)

3.3.7 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a chromatographic technique that used to separate a mixture of compounds. It is commonly used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. Each sample will has their specific retention times. Retention time is the time at which a specific sample elutes. Characteristic of the given sample can be analyst by comparing to retention time. In this study, HPLC unit was used in order to verify the existence of ascorbic acid in the banana peel extract.

Figure 3.8: High Performance Liquid Chromatography (HPLC)

3.4 Sample Preparation (pretreatment)

Ripe banana (Figure 3.8) was bought from Tunas Mart, Gambang, Pahang. These Bananas then were washed 3 times by using distilled water. The peels were manually separated and dried in the oven at temperatures of 50^{o}C - 70^{o}C for 24 hours. The dried banana peel then was ground to a fine powder and packed in the sealed polyethylene bags. (M. Miranda e*t al*., 2009). All the dried grounded samples were stored at -20^{o}C until the extraction were carried out. The moisture content of the sample was calculated.

Figure 3.9: Fresh Banana sample

Figure 3.10: Dried grounded sample at different drying temperature

3.5 Solvent Extraction

The dried banana peel sample (0.15 g) was extracted with 3mL of different solvent (acetone, methanol, water) with different water ratio (90%, 70%, and 50%). The extractions were carried out in the sealed tube in the in a water bath at 40^{o}C, 50, 55^{o}C and 60^{o}C for 1min- 120 min. Precaution was taken in order to performed the extraction in the reduced light condition by covered up the sealed tubes with foil paper. The extracts then centrifuged at 6000rpm for 15 min in a refrigerated centrifuge. The supernatant were collected and concentrated by removing solvent from the extract using rotary evaporator (BücHi Rotavapor R-200). The extracts obtained were stored at -40^{o}C for less than three days, at which time the antioxidant potential, extraction yield and bioactive compounds were estimated. The sample was for the analysis of antioxidant activities, phenolic contents, minerals compounds and ascorbic acid existence. Each of extraction process is done in triplicate.

Figure 3.11: Banana peel extract

3.6 Analysis

3.6.1 Antioxidant Activities

FRAP assay was done according to the method described by Benzie and Strain (1999) with some modification. 40µL of banana peel extract was mixed with 3 ml of FRAP reagent. The mixture then was incubated at 37^{o}C for 4 min. Absorbance was determined at 593 nm with distilled water used as blank in the UV-visible Spectrophotometer Hitachi U-1800 unit. FRAP reagent should be pre-warmed at 37^{o}C and should always be freshly prepared. A calibration curve was prepared by using an aqueous solution of ferrous sulphateFeSO4. 7H2O (200, 400, 600, 800 and 1000 µM, R^2 = 0.997).

3.6.2 Phenolic content

The phenolic content was measured using the Folin Ciocalteu assay that was described by Singleton and Rossi (1965). 40 µL of the extract was mixed with 1.8 ml FC reagent that was pre-diluted 10 times with distilled water. The mixture was allowed to react for 5 min before a solution of 1.2mL sodium carbonate (7.5% w/v) was added. The mixtures were homogenized by using vortex. Then, the mixture was allowed to stand at room temperature for 1 h. The absorbance was then measured at 765 nm using UV-visible Spectrophotometer. A calibration curve was prepared by using gallic acid as the standard (20, 40, 60, 80 and 100 mg/l, R^2 = 0.989).

3.6.3 Minerals

Compositions of each mineral were determined after digestion of the sample with nitric acid (65%, Suprapur grade, Merck, Germany). Standard solution of each mineral was prepared (1-30ppm) and the ultrapure water MilliQ Plus, Millipore (USA) was used as blank and for dilution of the standard solutions. An atomic absorption spectrophotometer Model z-5000 series, Polarized Zeeman, HITACHI was used to estimate the sodium, magnesium and calcium composition. Calcium, magnesium and sodium were calibrated against known standards of 1.0 ppm, 5.0 ppm, 10.0 ppm and 30.0ppm.

3.6.4 Ascorbic Acid Content

Ascorbic acid in the extracts was determined using High Performance Liquid Chromatography (HPLC) Agilent Technology 1200 series. Ascentis RP-amide (Alltech) column (5 cm x 4.6mm I.D., 5µm particles) was used in this analysis. A 25mM potassium phosphate dibasic solution in ultra-purified water was used as mobile phase (pH adjusted to 3.5 with orthophosphoric acid) at a flow rate of 1 ml/min at 30^{o}C. Detection wavelength for the detector was set at 230nm.

Figure 3.12: Ascorbic acid standard solution and samples for HPLC

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The result for this studies were divided into three parts which are the effects of drying temperature, solvent ratio, extraction time and extraction temperature on antioxidant activity and phenolic content in the banana peel extract. The verification of ascorbic acid existence was obtained by using HPLC unit. Magnesium (Mg), Calcium (Ca), and Sodium (Na) composition were investigated by using AAS unit.

4.2 Effect of drying temperature

4.2.1 Effect of drying temperature on antioxidant activity

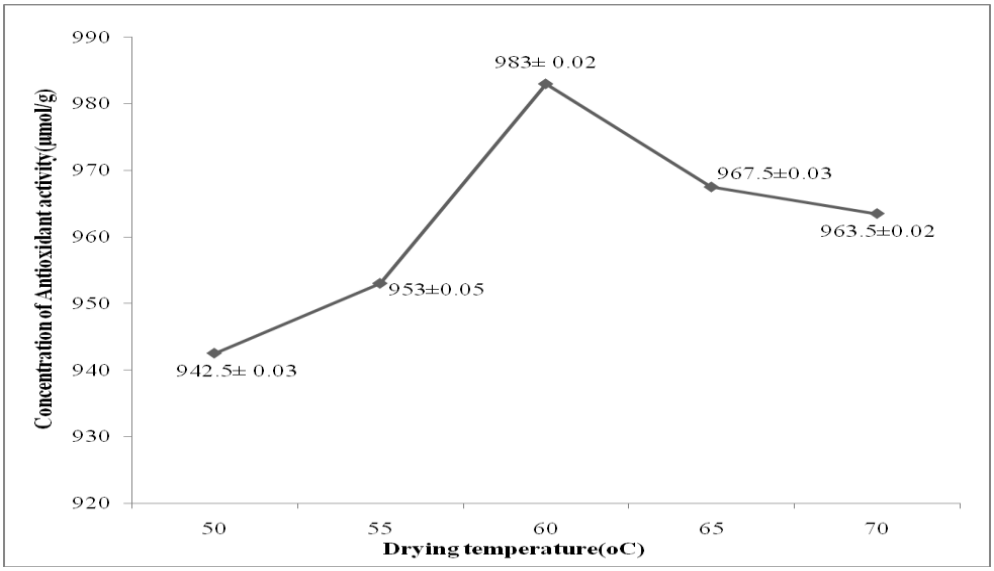

Figure 4.1: Effect of drying temperature on antioxidant activity

Figure 4.1 showed that, drying temperature at 60^{o}C with yield of 983±0.02µmol/g exhibited high FRAP value, which was interpreted as the highest antioxidant activity than other drying temperature in the banana peel extract. The antioxidant activity concentration was increased from 50^{o}C to 60^{o}C. Decreasing in the antioxidant activity occur at 65^{o}C to 70^{o}C with concentration of 967.5±0.03µmol/g and 963.5±0.02 µmol/g respectively. Increasing the drying temperature would increase the reaction rate of the extract over a certain period. Polyphenol oxidase was the enzyme that responsible in the conversion of phenolic content and antioxidant activity. However, the longer the drying time at high drying temperature may cause the degradation of the extract chemical composition.

4.2.2 Effect of drying temperature on phenolic content

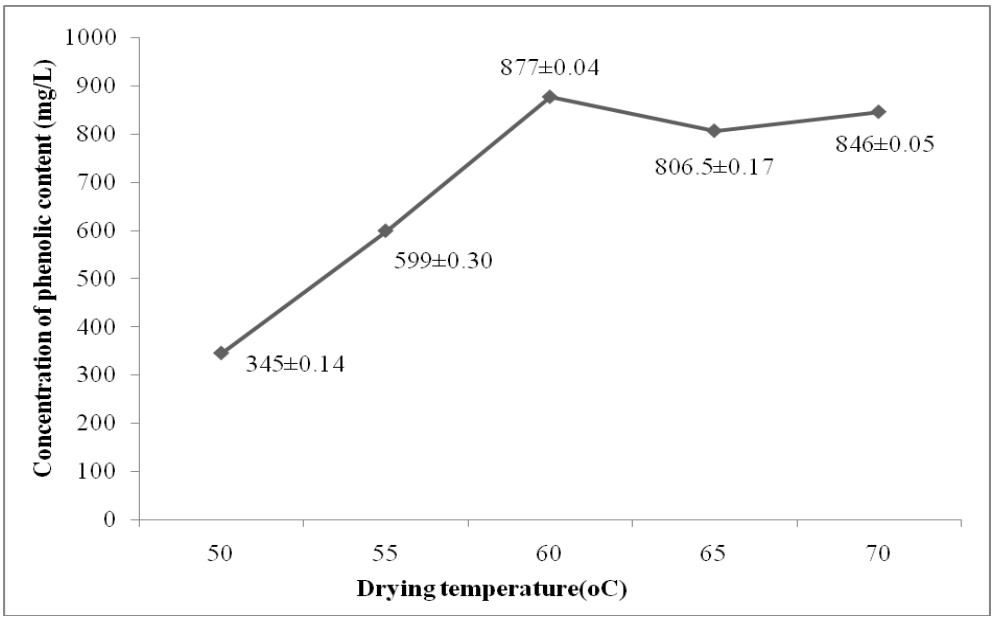

Figure 4.2: Effect of drying temperature on phenolic content

From Figure 4.2, the phenolic content concentration rose steadily from 50^{o}C until 60^{o}C which exhibit as the highest level of phenolic content concentration of 877±0.04 mg/L. These results agree with T.Kuljarachanan et al.'s (2009) statement whose saying in the 'study on antioxidant in lime residue' that drying at 60^{o}C could retain the highest amount of total phenolic compound. The phenolic content concentrations then decrease and slightly fluctuate in between 65^{o}C and 70^{o}C of drying temperature. The decreasing in the phenolic content may be due to the binding of polyphenol component toward other compound such as protein in the extract. High drying temperature would alter the polyphenol chemical structure. This agreed with M. Miranda *et.al*, (2010) who stated that in the study of 'influence of drying kinetic toward antioxidant capacity in Aloe Vera' that low phenolic concentration was obtained because alteration of the chemical structure would cause an incomplete extraction process and could not be determined by available method.

4.3 Effect of solvent ratio

4.3.1 Effect of solvent ratio on antioxidant activity

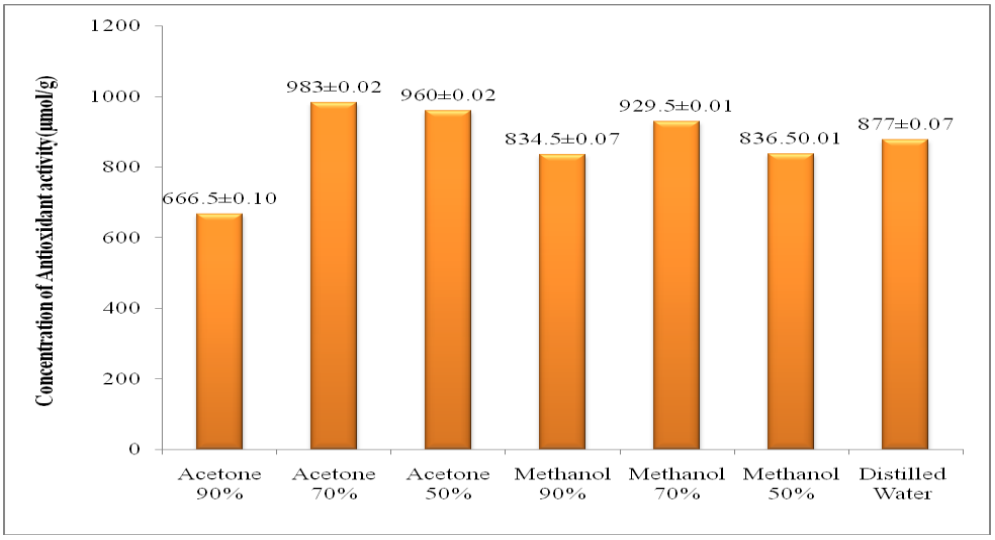

Figure 4.3: Effect of solvent ratio on antioxidant activity

The yield of antioxidant activity concentration of sample extract by different solvent water ratio were presented in the Figure 4.3. Among the various solvent ratio used, acetone 70% show the highest antioxidant activity of the extract. This agree with R.Gonzalez-Montelongo et al.(2010) who stated in the 'study on antioxidant activity in the banana peel extract' that acetone:water had the highest antioxidant activity compared with the other solvent used in the assay. In this study, acetone 50% exihibit as the second higher solvent ratio that produce 960±0.02µmol/g of antioxidant activity. This followed by methanol 70% with concentration of 929.5±0.01µmol/g. Cocentration of 877±0.07 µmol/g and 836.5±0.01 µmol/g of antioxidant activity were found in the distilled water and methanol 50% respectively. The second lower antioxidant activity of the extract was obtain in methanol 90% with concentration of 834.5±0.07µmol/g. Acetone 90% give the lowest yield with 666±0.10 µmol/g of antioxidant activity. This variation in the yield of various extract were caused by polarities of different compound in the banana peel.

The higher the polarity of the solvent will produce higher antioxidant activity. The choice of solvent used are based on their polarities relative to the single electron transfer and hydrogen atom transfer. Natural antioxidant are polar compound thus, polar solvent was suitable to extract antioxidant activity from banana peel. Both acetone and methanol have the same polarity index of 5.1 while polarity index of water is 9. This explain on why acetone and methanol give higher value of antioxidant activity than water. Acetone give higher value of antioxidant than methanol may be due to their viscosity. Acetone and methanol had viscosity of 0.32cp and 0.6cp respectively. Therefore, acetone will easily diffuse into the the particle and react to produce higher extraction efficiently than methanol (S. Hemwimol *et al*. 2006). Decreasing in solvent ratio will increase the mixture viscosity. Thus, increasing in the mixture viscosity improved the mass transfer rate of the solvent into the sample. However, the product recovery with high diluted value will be low caused by water content in the mixture such as in 50% solvent ratio. This low in product recovery because the solvent ratio not any longer favorable for the extration of antioxidant activity. This may be explain on why acetone 70% produce highest antioxidant activity in the banana peels.

Table 4.1: Solvent properties

solvent	Polarity Index	Viscosity(cp)
Acetone	5.1	0.32
Methanol	5.1	0.6
Water	9	

4.3.2 Effect of solvent ratio on phenolic content

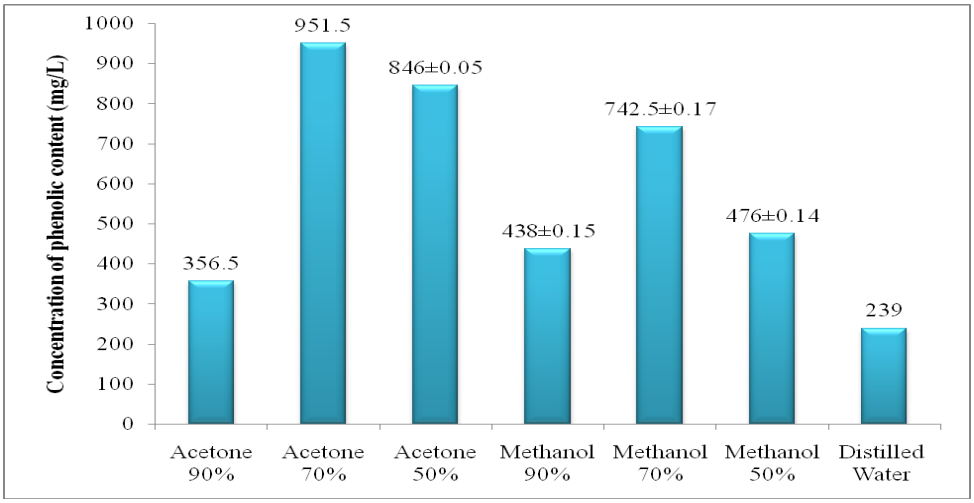

Figure 4.4: Effect of solvent ratio on phenolic content

Phenolic content concentration of the banana peel extract varied significantly in the different solvent extract show in the Figure 4.4. However, 70% acetone show the highest phenolic content among those extract. This agree with M.Alothman et al. who state in the 'study of antioxidant capacity and phenolic content in fruit extracted with different solvent' that acetone 70% could recover the highest yield of total phenolic in the pisang mas extract compared with all other system. The antioxidant activity concentration value resulting from this study was much higher than the value of antioxidant activity that stated by M. Alothman et al. The trends obtain in the effect of antioxidant activity and phenolic content were almost the same except the for distilled water product recovery. In the figure, distilled water produce the lowest yield of 239mg/L phenolic content. This lowest recovery of phenolic content was obtain by extraction with 100% distilled water. This could be caused by the oxidation of phenolic compound by polyphenol oxidase which enzyme where in methanol and acetone the ezyme is inactive (R.Gonzalez-Montelongo et al. 2010). Polyphenol oxidase is the enzyme that found in the plants. Polyphenol oxidase is responsible for browning of freshly peeled fruit or vegetables.

4.4 Effect of time of extraction

4.4.1 Effect of time of extraction on antioxidant activity

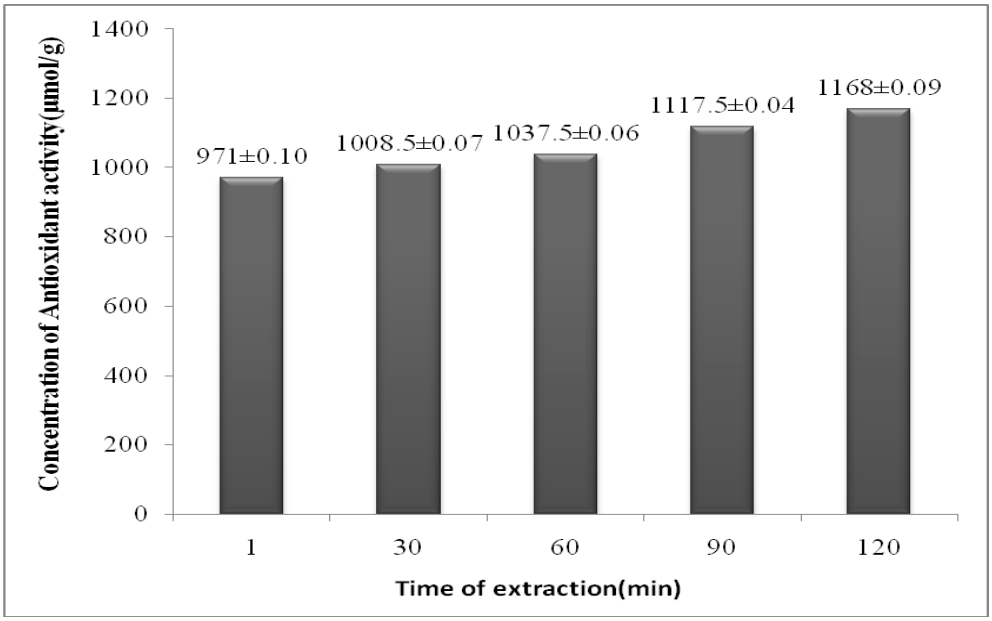

Figure 4.5: Effect of time of extraction on antioxidant activity

Figure 4.5 show that, 70% acetone-water ratio was used in determining the effect of time of extraction. The antioxidant activity was increased steadily from minute 1 to minute 120 as shown in the Figure 4.5. Time of extraction at 120 minutes produced the highest antioxidant activity with 1168±0.09 µmol/g. The lowest antioxidant activity was obtained at 1 minute of extraction with concentration of 971±0.10 µmol/g. This result may due to time taken for the conversion of the antioxidant activity in the extract. The higher the time of extraction, the higher the time for converting the antioxidant activity(R.Gonzalez-Montelongo et al. 2010). Therefore, increasing in the product conversion time would resulting more yield of antioxidant activity.

4.4.2 Effect of extraction time on phenolic content

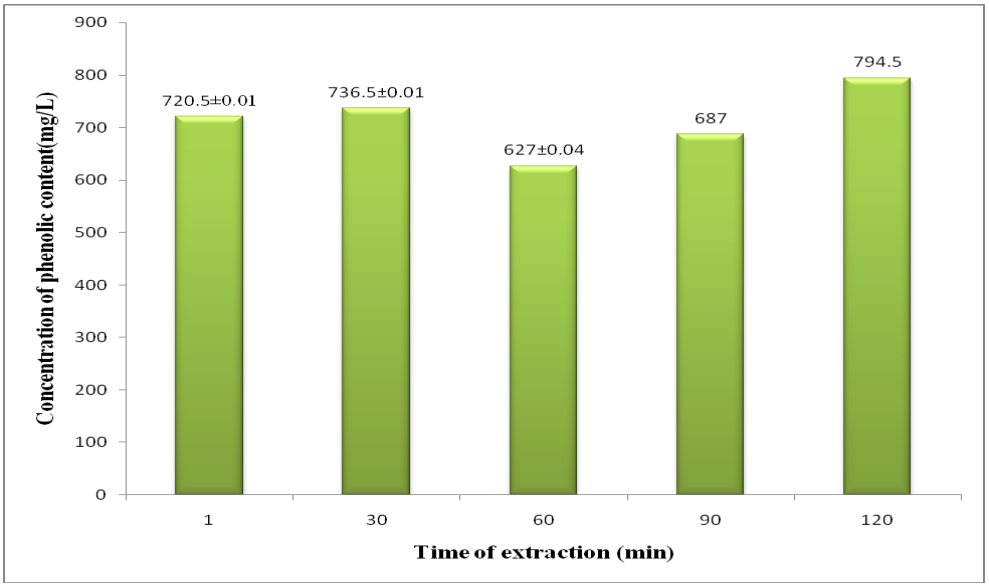

Figure 4.6: Effect of time of extraction on phenolic content

From Figure 4.6, increasing in the time of extraction from 1 min to 30 min produce slightly increasing in the phenolic content with concentration of 720.5±0.01 mg/L and 763.5±0.01mg/L respectively. The phenolic concentration decreased to 627±0.04 at 60 minutes of extraction. At 90 minutes of extraction, the phenolic concentration obtained was 687 which increase the concentration is higher than 60 min of extraction. Highest phenolic concentration was peaked at 120 min of extraction in the water bath with yield of 794.5mg/l. The graph obtain were slightly fluctuate from 1 min of extraction to 90 min. This may be due in the extract that occurs during the phenolic content conversion. This variation of the phenolic content in this result may due to the enzymatic reaction of polyphenol oxidase that occurs during the storage of the dried banana peel sample.

4.5 Effect of temperature of extraction

4.5.1 Effect of temperature of extraction on antioxidant activity

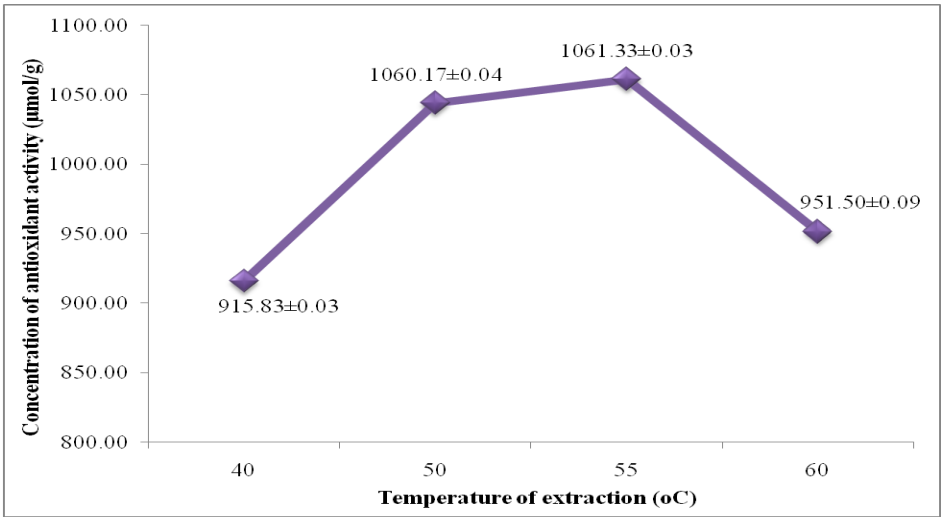

Figure 4.7: Effect of temperature of extraction on antioxidant activity

This effect of extraction temperature was analyst by using 70% acetone for two hours of extraction time. These parameters were selected due to the result obtained in this study. The trend in the Figure 4.7 shows that the antioxidant activity was increase sharply at temperature of 40^{o}C to 50^{o}C with concentration of 915.83±0.03µmol/g and 1060.17±0.04µmol/g respectively. Then the antioxidant activity was gradually increased to 55^{o}C, which exhibit as the highest peak with concentration of 1061.33±0.03µmol/g. This caused by the solubility and diffusion rate of the analytes in the solvent that enhance by increasing in the extraction temperature. However, R.Gonzalez-Montelongo et al.(2010) claims that at higher temperature of extraction the bioactive compound in the extract would interact with others plant material component. This statement explains on the antioxidant activity concentration that decreased from 55^{o}C to 60^{o}C. Extraction temperature at 60^{o}C yields the lowest antioxidant activity with concentration of 951.50±0.09µmol/g.

4.5.2 Effect of temperature of extraction on phenolic content

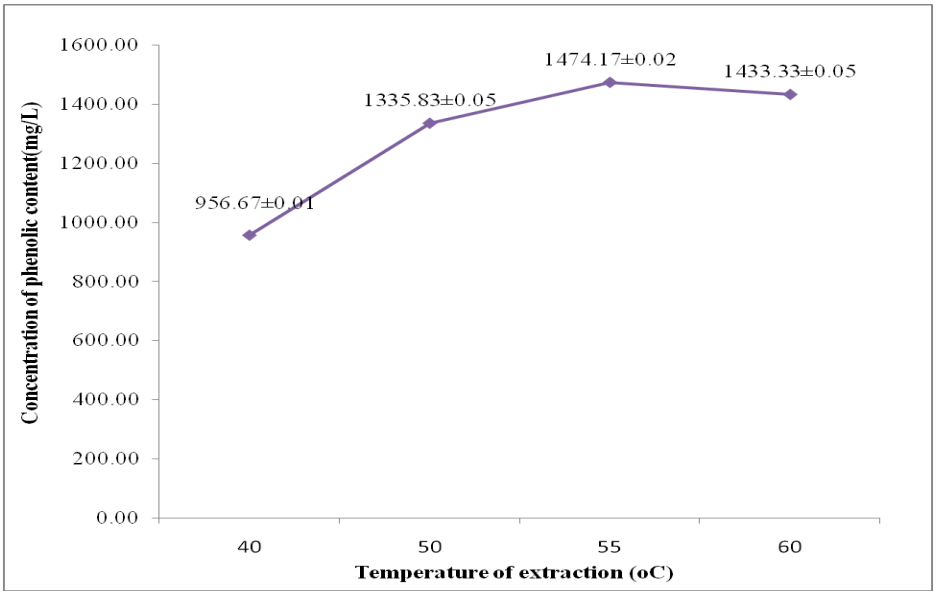

Figure 4.8: Effect of temperature of extraction on phenolic content

The trends in the Figure 4.8 show that the highest peak of phenolic concentration was obtain at 55^{o}C with the yield of 1474.17±0.02 mg/L. The concentrations were rose gradually from 40^{o}C to 55^{o}C with concentration of 956.67±0.01mg/L and 1335.83±0.05mg/L respectively. The phenolic content then fell at temperature of 60^{o}C with concentration of 1433.33±0.05 mg/L. Besides, the liquid viscosity and density will decreased with increasing in the extraction temperature and thus enhanced the mass transfer and the reaction to produce antioxidant activity. Extraction temperature will affect the stability of phenolic compound due to chemical degradation by thermal decomposition (R.Gonzalez-Montelongo et al., 2010).

4.6 Minerals profile in banana peel extract

Table 4.2: Minerals composition in the banana peel extract.

Minerals	Concentration (ppm)
Sodium (Na)	0.85
Magnesium (Mg)	65.34
Calcium (Ca)	3.21

Table 4.2 show the mineral content of dehydrated banana peel which magnesium(Mg) were the predominant mineral element among the mineral being analyst with the yield of 65.34ppm(part per million). Calcium showed as the second dominant minerals and followed by sodium with amount of 0.85ppm. This minerals value may vary widely among type of bananas and other fruits according to several factors such as storage time, ripeness of the fruits, temperature, storage conditions and cultivar. Besides, fresh banana peel and dried banana peel at different drying temperature would have the different in the mineral content. M. Miranda et al. (2010) prove that dehydrated sample showed a considerable decreased in the mineral content with respect to the to the fresh sample due to the differenced in solubility of the inorganic compound into the rehydration water.

4.7 Ascorbic Acid

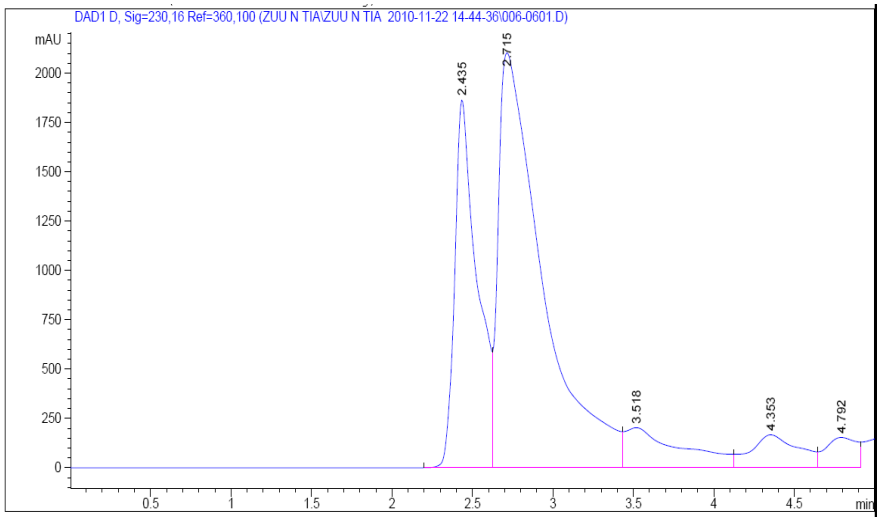

Figure 4.9: Amount of ascorbic acid

The drying temperature 60^{o}C and 2hours of extraction at 55^{o}C were used to extract the ascorbic acid in this study. This parameters were selected according to the result that had been optimized in this study. From the standard obtained, ascorbic acid existence would be detected at the retention time of 3.885 min. Figure 4.9 show that, the ascorbic acid concentration was too low. Therefore, it could not be detected by the system at this extraction condition used in this study. Several peaks were detected at minutes of 2.435 and 2.715. This peaks may related to unknown other component contains in the extract. The low existence of ascorbic acid in the extract may be cause by the degradation of ascorbic acid during the drying process. Besides, the ascorbic acid content also might be degrade during the removal of solvent content in the extract by using rotary evaporator. Application heat to the extract induced the degradation of ascorbic acid in the extract. Ascorbic acid was easily destroyed by oxidation process especially at higher temperature and during washing, processing and storage. Ascorbic acid would degrade by active oxygen in the system.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Banana peel has great potential to be utilized as the antioxidant source. The graph that obtained in this study provide the comparison between the effect of several parameter used such as drying temperature, different type of solvent with different solvent ratio, extraction temperature and extraction time on the antioxidant activity, phenolic content and minerals in the banana peel extract.

The result showed that, optimal condition for the extraction of antioxidant activity, phenolic content and minerals in banana peel were drying at 60^{o}C, extracted by using 70% acetone solvent in the water bath for 2 hours at 55^{o}C. Concentration of antioxidant activity and phenolic content at this optimal extraction condition was 1061.33±0.03μmol/g and 1474.17±0.02 mg/L respectively. For minerals composition in banana peel extract, magnesium (Mg) was found to be the predominant mineral element among the mineral being analyst with the yield of 65.34ppm (part per million). Calcium showed as the second dominant minerals and followed by sodium with amount of 3.21ppm and 0.85ppm respectively. The result show that, ascorbic acid could not be detected by the system at this

extraction condition used in this study because the there was too little amount of ascorbic acid concentration exist in this extract.

5.2 Recommendations

For futher research, the effect of the different parameter toward antioxidant capacity and antioxidant activity should be investigated to obtain the correlation between both antioxidant capacity and activity.

Recent studies have shown that. Ultrasound-assisted extraction and enzyme-assisted extraction process enhanced the efficiency of extraction by shortening the time of extraction. Therefore for further research ultrasonic-assisted extraction and enzyme-assisted extraction process should be carried out to decrease the time of extractionand obtain more yield of antioxidant acitivity and phenolic contents in the extract.

Drying process are the crucial part in the preparing the sample the extraction process. Before analysis step, the extract needs to be purified by using thermal application. Knowing that, at low temperature of drying, less bioactive component would be degraded. These bioactive compounds are sensitive to heat application. Thus, further research should be focused on the drying temperature by wider the range of the drying temperature and the effect of purification process toward antioxidant a ·· ··y, phenolic content and minerals in the extract.

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APPENDICES A

EFFECT OF DRYING TEMPERATURE

Appendix A.1: Effect of drying temperature on antioxidant activity

Temperature (oC)	Absorbance	Concentration (μmol/g)	Standard deviation
50	2.187	942.5	0.03
55	2.208	953	0.05
60	2.268	983	0.02
65	2.237	967.5	0.03
70	2.229	963.5	0.02

Appendix A.2: Effect of drying temperature on phenolic content

Temperature	Absorbance	Concentration	Standard

(oC)		(mg/L)	deviation
50	0.614	345	0.14
55	1.122	599	0.3
60	1.678	877	0.04
65	1.537	806.5	0.17
70	1.616	846	0.05

APPENDICES B

EFFECT OF SOLVENT RATIO

Appendix B.1: Effect of solvent ratio on antioxidant activity

Types of Solvent	Water Ratio	Absorbance	Concentration (μmol/g)	Standard deviation
Acetone	90	1.635	666.5	0.10
	70	2.268	983	0.02
	50	2.222	960	0.02
Methanol	90	1.971	834.5	0.07
	70	2.161	929.5	0.01
	50	1.975	836.5	0.01
Distilled Water		2.056	877	0.07

Appendix B.2 : Effect of solvent ratio on phenolic content

Types of Solvent	Water Ratio	Absorbance	Concentration (mg/L)	Standard deviation
Acetone	90	0.637	356.5	0.00
	70	1.827	951.5	0.00
	50	1.616	846	0.05
Methanol	90	0.8	438	0.15
	70	1.409	742.5	0.17
	50	0.876	476	0.14
Distilled Water		0.402	239	0.00

APPENDICES C

EFFECT OF TIME OF EXTRACTION

Appendix C.1: Effect of extraction time on antioxidant activity

Extraction Time (min)	Absorbance	Concentration (μmol/g)	Standard deviation
1	2.244	971	0.10
30	2.319	1008.5	0.07
60	2.377	1037.5	0.06
90	2.537	1117.5	0.04
120	2.638	1168	0.09

Appendix C.2: Effect of extraction time on phenolic content

Extraction Time (min)	Absorbance	Concentration (mg/L)	Standard deviation
1	1.365	720.5	0.00777
30	1.397	736.5	0.00907
60	1.178	627	0.0396
90	1.298	687	0.001
120	1.513	794.5	0.00231

APPENDICES D

EFFECT OF TEMPERATURE OF EXTRACTION

Appendix D.1: Effect of temperature of extraction on antioxidant activity

Temperature of Extraction (oC)	Absorbance	Concentration (μmol/g)	Standard deviation
40	2.13367	915.833	0.03
50	2.39	1044	0.04
55	2.42467	1061.33	0.03
60	2.205	951.5	0.09

Appendix D.2: Effect of temperature of extraction on phenolic content

temperature of extraction (oC)	Absorbance	Concentration (μmol/g)	Standard deviation
40	0.31	191.333	0.01
50	0.46	267.167	0.05
55	0.51	294.833	0.02
60	0.50	286.667	0.05
40	0.31	191.333	0.01

APPENDICES E

MINERALS PROFILE IN BANANA PEEL

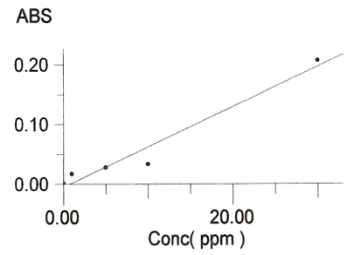
Table of Each Element 11/19/2010 10:51 AM

Analysis Mode : Flame/Manual
Analysis Name : Standard analisys
Comment :
Description : To determine Potassium(K), Sodium(Na) and Magnesium(Mg) in Fruits peel

Meas. Date : 11/19/2010 10:50 AM
Element : Mg STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0015	0.0176
STD2		1.00	0.00	0.0164	0.0221
STD3		5.00	0.00	0.0271	0.0260
STD4		10.00	0.00	0.0325	0.0287
STD5		30.00	0.00	0.2064	1.6388

Coefficient : K3=--
K2=--
K1=6.730292E-003
K0=-5.138687E-003
Corr.Coef. : 0.9781



UNK-001		65.34	0.00	0.4346	1.3750

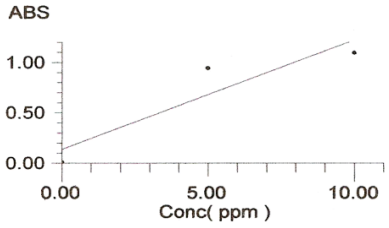
Table of Each Element 11/25/2010 3:45 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard analisys
Comment :
Description : Determination of Sodium (Na) and calcium(Ca) in fruits peel.

Meas. Date : 11/25/2010 3:45 PM
Element : Na STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0083	-0.0050
STD2		5.00	0.00	0.9412	0.1439
STD3		10.00	0.00	1.0920	0.2745

Coefficient : K3=--
K2=--
K1=1.083700E-001
K0=1.386500E-001
Corr.Coef. : 0.9231



UNK-001	Guava Peel	2.04	0.00	0.3592	0.0354
UNK-002	Banana Peel	0.85	0.00	0.2311	0.0150

Table of Each Element 11/25/2010 3:37 PM

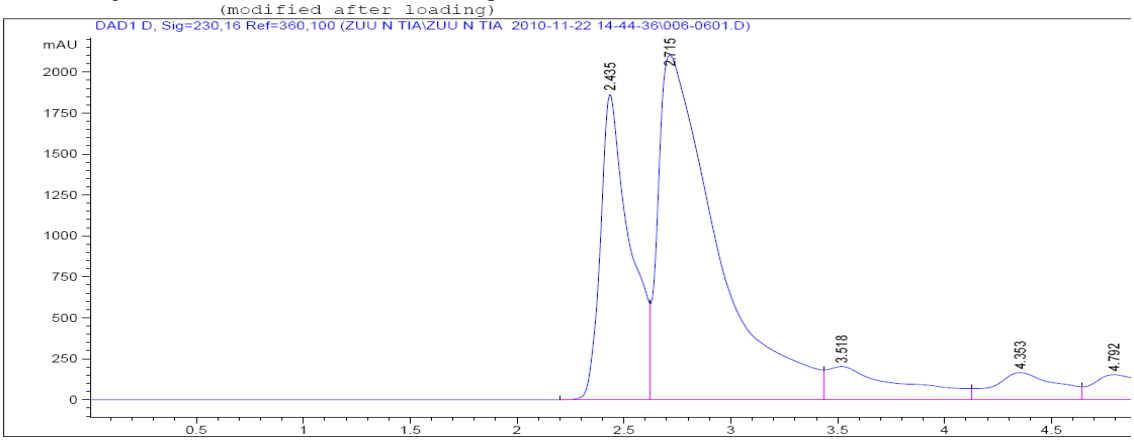
Analysis Mode : Flame/Manual
Analysis Name : Standard analisys
Comment :
Description : Determination of Sodium (Na) and calcium(Ca) in fruits peel.

Appendix E.2: Calcium (Ca) profile in banana peel

APPENDICES F

ASCORBIC ACID CONTENT IN BANANA PEEL

```
==========================================================================
Acq. Operator    : Emine                              Seq. Line :   6
Acq. Instrument : Instrument 1                        Location : Vial 6
Injection Date  : 11/22/2010 3:22:55 PM                  Inj :   1
                                                    Inj Volume : 10 µl
Acq. Method      : C:\CHEM32\1\DATA\ZUU N TIA\ZUU N TIA  2010-11-22 14-44-36\ZUU N TIA.M
Last changed     : 11/22/2010 1:14:22 PM by Emine
Analysis Method : C:\CHEM32\1\DATA\ZUU N TIA\ZUU N TIA  2010-11-22 14-44-36\ZUU N TIA.M
Last changed     : 11/22/2010 4:06:25 PM by Emine
                   (modified after loading)
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      11/22/2010 4:08:13 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 D, Sig=230,16 Ref=360,100

Peak RetTime  Type    Width     Area      Area     Name
  #   [min]           [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   2.435 BV      0.1318 1.78741e4   26.1677 ?
   2   2.715 VV      0.2880 4.00124e4   58.5781 ?
   3   3.518 VV      0.3176 4935.53516   7.2256 ?
   4   3.883         0.0000    0.00000   0.0000
   5   4.353 VV      0.2818 3424.62354   5.0136 ?
   6   4.792 VV      0.1947 2059.35742   3.0149 ?

Totals :                   6.83061e4
```

Appendix F.1: Ascorbic Acid content in banana peel