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Preliminary Investigation on the Total Phenolic Content and Antioxidant Activity of Pineapple Wastes via Microwave-Assisted Extraction at Fixed Microwave Power

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Pineapple contains many bioactive compounds and nutraceutical values which are good for human health. Processing pineapple in industries leaves a lot of wastes which can cause serious environmental problems. Pineapple waste is a by-product of the pineapple processing industry and it consists of residual pulp, peels and skin. Studies on the content of phenolic and the antioxidant activities in wastes of fruits have been emerging for the past years due to the concern on sustainable environment. In this study, the phenolic compound was extracted from pineapple wastes (namely skin) by using MAE (Microwave-Assisted Extraction). The aims of the present paper are to determine the phenolic compound and antioxidant activity of pineapple waste and to find the optimum condition at 250 W microwave power by using MAE with the temperature varied at 30 °C, 60 °C, 90 °C and 120 °C. The phenolic content was measured by using the Folin-Ciocalteu reagent. For the antioxidant activity, the analysis was done by using DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay. The optimum condition for the extraction was found at 30 °C using deionised water as a solvent. At this optimum condition, the value of the phenolic content was achieved at the highest value, 206.46 mg GAE/g dry weight whereas the EC50, DPPH value was obtained at the lowest value, 13.65 mg/mL. From this research, it is proved that the pineapple skin is one of the good sources of antioxidant phenolic and serves as the best candidate to be a potential source of natural antioxidant for food and nutraceutical application.

1. Introduction

Pineapple, Ananascomosus L., Merr. is an important tropical fruit, that is consumed in many parts of the world as fresh fruit, juice, jam, jelly and dried product. The pineapple has a high nutritive value and is a rich source of vitamins A, B and C and several minerals such as calcium, phosphorus and iron (Mhatre et al., 2009). Even though there are some reports on the antioxidant activities of pineapple in relation to other fruits, they deal with only one or two parameters and not in detail or do not suggest possible components/mechanisms (Gardner et al., 2000). Mainly, pineapple contains of water, carbohydrates, sugars and carotene, beta. It also contains low amounts of protein, fat, ash and fiber. The antioxidants, namely flavonoids, vitamin A and C are among the constituents in the pineapple. The oxidative damage such as that caused by free radicals and chelating metals can be reduced by these types of antioxidants. It also has the enzyme complex protease called as bromelain. Bromelain contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium (Gardner et al., 2000).

Nowadays, people are concerned about the processed pineapples consumed worldwide. Processing pineapple industries are trying out or using new technologies to retain the nutritional quality of the pineapple fruit. This is to meet the demand of consumers who want healthy, nutritious and natural products. The wastes from the pineapples due to the process can cause environmental pollution problems if not utilised. For instance, many edible tropical fruits are processed into natural and concentrated juices, jellies, pulp and extracts back in Brazil. In these processes, de Oliviera et al. (2009) reported that seeds, peels and other parts are routinely discarded and eventually causing environmental problems. Recently, there are investigations or studies carried out in

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order to make these wastes become benefits. Pitakere (2009) claimed that the pineapple wastes from these processing industries can be utilised to produce methane, animal feed, phenolics and bromelain.
Based on the report, many degenerative human diseases including cancer, cardio and cerebro-vascular diseases have been recognised as being a possible consequence of free radical damage to lipids, proteins and nucleic acids (Devasagayam et al., 2004). A possible way to fight these diseases is to improve or increase our body's antioxidant defences. For instance, with the high intake of fruits and vegetables daily, it can help to reduce the risk of such degenerative diseases (Bajpai et al., 2009). Fruits also help to improve health in other ways. For example, the pineapple juice can be consumed to alleviate sore throat and seasickness. In general, Mendiola et al. (2008) reported that the functional bioactivity of a plant extract depends upon the presence of compounds such as polyphenols, carotenoids and chlorophyll. Higdon and Frei (2003) summarised that the plants, which contribute in this area primarily due to their excellent antioxidant activity of polyphenolic compounds. Flavonoids, mainly present as colouring pigments in plants also function as potent antioxidants at various levels. Some studies showed that flavonoids could protect membrane lipid from oxidation (Terao et al., 1994). Phenolics are the compounds which are carbon-based that present in many plants. Due to their wide-ranging ecological effects from the organism to ecosystem level, the phenolics become one of the general interests (Appel, 1993). The phenolics are perhaps most noted for their ability to bind to proteins in vitro, forming soluble and insoluble complexes. These phenolic-proteins interactions are thought to be, in part, responsible for the putative function of phenolics as plant defense compounds. Studies of phytochemicals, phenolic compounds and antioxidant properties in waste parts e.g. rind, peel and seed of various fruits have been of increased interests for the past years because of the concerns on sustainability and renewable sources. Among the fruit wastes studied are guava leaf (Tachakittirungrod et al., 2007), peels of mangosteen, rambutan and pomegranate (Okonogi et al., 2007) and mango seed kernel (Maisuthisakul and Gordon, 2009). The antioxidant capacity and phenol content of three tropical fruits pulps, namely, honey pineapple, banana and Thai seedless guava were studied in the previous research extracted by using different solvent. Three solvent systems were used (methanol, ethanol and acetone) at three different concentrations (50 %, 70 % and 90 %) and with 100 % distilled water. The antioxidant capacity of the fruit extracts was evaluated using a ferric reducing/antioxidant power assay and the free radical-scavenging capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays. The efficiency of the solvents used to extract phenols from the three fruits varied considerably. The polyphenol content of Thai seedless guava was 123 to 191 gallic acid equivalents/100 g (GAE/100 g), that of pisang mas was 24.4 to 72.2 GAE/100 g, and that of honey pineapple was 34.7 to 54.7 GAE/100 g. Alothman et al. (2009) investigated that the high phenol content was significantly correlated with high antioxidant capacity. A research has been conducted by Amzad Hossain and Mizanur Rahman (2010) on the extraction of phenolic compound from pineapple by using mixing method with the magnetic stirrer. It was found that the phenolic content was present in the methanol extract in a substantial amount compared to ethyl acetate extract and water extract. This decreasing order is similar to the antioxidant activity in the extracts mentioned. Recently, the phenolic compound is extracted by using MAE (Microwave-Assisted Extraction). This technique has been done on bean, pine seed, and longan peels. A study summarised by Glanzer et al. (1986) showed that microwave-assisted extraction has many advantages; such as shorter time, less solvent, higher extraction rate and better product with lower cost. To date, there is no research done on pineapple waste by using this method. The scopes of work of this research are to determine the phenolic compound and antioxidant activity on pineapple waste and to find the optimum condition at 250 W microwave power by using MAE. It is expected that the pineapple waste serve as the most potential waste fruit in phenolic content.

2. Materials and Methods

2.1 Material

The pineapples were purchased from wet market at Seksyen 6, Shah Alam. The type of the pineapple used in this research was Josepine. This is because of the quality of Josepine pineapple will maintain even when the pineapple is fully ripened (BawangGoreng, 2011). The skin of the pineapple (waste) was peeled for the sample preparation. Ethanol 95 % (Hmbg Malaysia), FolinCiocalteu's Phenols Reagent (Merck GERMANY), Sodium Carbonate Anhydrous (Systerm Malaysia) and Gallic Acid (R&M, UK) were purchased from Laupik Solution, Petaling Jaya, Selangor. DPPH was purchased from Sigma Aldrich (M) Sdn Bhd. All the reagents and chemicals used were of analytical grade.

2.2 Sample preparation

The samples (skin) obtained were washed with distilled water and then frozen within 24 h in a chest-freezer at -19 °C. After 24 h in the freezer, the samples were washed again with distilled water and dried in the oven at 60 °C for 48 h. The dried samples were then ground using the Cutter Mill at the size of 0.5 mm. Each sample was

weight at 5 g each before the extraction process. The sample preparation was done in Faculty of Chemical Engineering, UiTM Shah Alam, Selangor.

2.3 Microwave-assisted extraction

The extraction process was conducted at Faculty of Science, Universiti Putra Malaysia, Serdang, Selangor. The model of the MAE used was ETHOS SEL, purchased from Milestone Company, Italy. In this preliminary experiment, the power used was fixed at 250 W. The parameters varied were the temperature (30 °C, 60 °C, 90 °C and 120 °C) and the type of solvent used (100 % ethanol, 50 % ethanol in deionised water and 100 % deionised water). The duration of the experiment was 20 min and 10 % of stirrer rotation). After the extraction procedure, the sample obtained was centrifuged at 10,000 rpm, for 20 min and 27 °C. The extract obtained was then analysed for total phenolic content and antioxidant activity.

2.4 Total phenolic content

The total phenolic content was measured using the Folin-Ciocalteu method (Singleton et al., 1999). 1 mL of aqueous extract of pineapple skin was added to 10 mL Folin-Ciocalteu reagent, followed by addition of 4 mL of an aqueous 7.5 % solution of sodium carbonate. The mixture was stirred and incubated at 40 °C for one hour. The solution was then measured by UV-Spectrometer at 765 nm. A blank sample consisting solvent (only) for each of sample was used as reference. The results were expressed in milligram of Gallic Acid Equivalent (GAE)/g of dry sample of pineapple. All samples were analysed in duplicates. The calculation can be done as Eq(1):

$$T = C \times V/M \quad (1)$$

Where T is the total phenolic contentin mg gallic acid (GAE)/g dry sample, C is the concentration of gallic acid established from the calibration curve (mg/L), V is the volume of the extract solution (mL) and M is the weight of the extract (g).

2.5 Scavenging activity on DPPH Radical

The scavenging activity or antioxidant activity of the extract was analysed by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) (Blois, 1958). In this method, the extract in 95 % ethanol at different concentration (1 mg/mL, 2 mg/mL, 3 mg/mL, 5 mg/mL) was added to 8 mL 0.004 % w/v solution of DPPH in 95 % ethanol. The reaction mixture was incubated at 30 °C for 30 min in the dark. The scavenging activity on DPPH radical was determined by measuring the absorbance at 517 nm. The antioxidant activity was expressed as a percentage of scavenging activity on DPPH radical as shown in Eq(2):

$$S.A\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100\ \% \quad (2)$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample/extract.

2.6 Sample Statistical Analysis

All the experiments were conducted in duplicates and the mean was calculated using MS Excel Software.

3. Results and Discussion

3.1 Total phenolic content

Polyphenolic compounds are very important fruit constituents, by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals (de Oliviera et al., 2009). The total phenolic content was measured for all the samples by using three solvents (100 % ethanol, 50 % ethanol in deionised water and 100 % deionised water) and at four different temperatures (30 °C, 60 °C, 90 °C, 120 °C). Upon calculating the value of total phenolic content, the standard calibration curve should be prepared in advance.

From the standard calibration curve, the graph shows linearity for the gallic acid in the range of 50 - 500 mg/L, with a correlation coefficient (R^2) of 0.97. The results of total phenolic content were obtained from the standard calibration curve and shown in Table 1. Based on Table 1, the extraction at 30 °C with water as a solvent has the highest phenolic content, 206.46 mg GAE/g dw, followed by ethanol in deionised water (50 - 50); 173.94 mg GAE/g dw. At 90 °C of extraction using ethanol in deionised water (50 - 50), the value of total phenolic content obtained was 164.76 mg GAE/g dw and at the extraction of 30 °C using ethanol, the value of total phenolic content was 118.02 mg GAE/ g dw. As for the extraction at 120 °C, the values of total phenolic content were 94.98 mg GAE/ g dw and 99.42 mg GAE/ g dw for ethanol and deionised water. The trends in the reading of total phenolic content were high at the low temperature, namely 30 °C, and as the temperature increased, the

value of total phenolic content decreased. This is because when the temperature is increased, it leads to chemical and enzymatic decomposition. It will be the main mechanism causing the degradation of phenolic (Hung-Der et al., 2007).

Table 1: Total phenolic content of the extract at the microwave power of 250 W, with different temperatures and types of solvent

Temperatures (°C)	Types of solvents	Phenolic content (mg GAE/g dw)
30	Ethanol	118.02
30	Water	206.46
60	Ethanol-Water (50 - 50)	173.94
90	Ethanol-Water (50 - 50)	164.76
120	Ethanol	94.98
120	Water	99.42

3.2 Scavenging activity on DPPH radical

Antioxidant properties, mainly radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological system (Gulcin, 2006). DPPH is a kind of stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997) which was widely used to investigate radical scavenging activity now for its advantage of ease and economical. Ashutosh Singh et al. (2011) reported that free electrons present in DPPH get paired off in presence of antioxidants and the absorption decreases as the result of extinction of DPPH's purple colour to yellow-coloured. The antioxidant power is indicated by the degree of discoloration (Yingming et al., 2008) which could be determined by measuring of a decrease in the absorbance at 517 nm.

Table 2: DPPH radical scavenging of the extract at the microwave power of 250 W, with different temperatures and types of solvent

Temperatures (°C)	Types of solvents	EC50 (mg/mL)
30	Ethanol	98.04
30	Water	13.65
60	Ethanol-Water (50 - 50)	19.14
90	Ethanol-Water (50 - 50)	21.15
120	Ethanol	30.92
120	Water	32.53

EC_{50} value, defined as the concentration of antioxidant required for 50 % scavenging of DPPH radicals, is a parameter widely used to measure antioxidant activity; which is smaller value of EC_{50} corresponds to a higher antioxidant activity of the plant extract (Maisuthisakul et al., 2007). The value of EC_{50} is tabulated in Table 2 below. Based on the Table 2, it was found that by operating the extraction at 30 °C using the deionised water as a solvent, the smallest value of EC_{50} was obtained, which is 13.65 mg/mL. For the temperature ranging from 60 °C to 120 °C, the value of EC_{50} increased except for the extraction at 30 °C using ethanol, the value of EC_{50} was very high. It indicates the sample contains lower the scavenging activity of the scavengers as more amount of the scavengers were required to achieve 50 % scavenging reaction and the scavengers are less effective.
Several studies have reported on the relationships between phenolic content and antioxidant activity. Some authors found a correlation between the phenolic content and the antioxidant activity, while others find no relationship between those parameters. It was reported that there is a strong relationship between the phenolic content and antioxidant activity in selected fruits, vegetables and grain products (Velioglu et al., 1998). No correlation found between antioxidant activity and phenolic in the study on some plant extracts containing phenolic compound (Kahkonen et al., 1999). In our present study, the phenolic content and antioxidant activity exhibit a good correlation. According to Amzad Hossain and Mizanur Rahman (2011), this is in line with a research reported that the tropical pineapple extract contains phenolic and the antioxidant activity with low EC_{50} value.

4. Conclusions

Microwave-Assisted Extraction (MAE) is well-known as one of the new extraction techniques that can reduce the extraction time, produce higher yield and has lower production cost compared to the conventional method, which is time-consuming extraction. In this research, the value of microwave power was fixed at 250 W. The

parameters varied were the temperature and the type of solvent used. Based on the result, a good correlation was found between phenolic contents and antioxidant activity at EC_{50}, DPPH value. The extraction at 30 °C using deionised water exhibits as the most optimum condition in operating the extraction process at the microwave power of 250 W. This is because at this parameter, the phenolic content achieved the highest value, 206.46 mg GAE/ g dw, whereas for EC_{50}, the DPPH value obtained has the lowest value, 13.65 mg/mL. The pineapple skin is one of the good sources of antioxidant phenolic. To date, we know that this is the first report that uses the MAE to extract the pineapple wastes, specifically. Further study needs to be carried out on the effect of the microwave power as well as the temperature and the type of solvent used.

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Reference

Alothman M., Bhat R., Karim A.A., 2009, Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents, Food Chemistry 115, 785–788.

Amzad Hossain M., Mizanur Rahman S.M., 2011, Total phenolic, flavanoids and antioxdiant activity of tropical fruit pineapple, Food Research International 44, 672-676.

Appel H.M., 1993, Phenolics in ecological interactions: the importance of oxidation, Chemical Ecology 19, 1521–1552.

Ashutosh Singh S., Kebba S., Stan K., Danielle J.D., Yvan G., Valerie O., Raghavan G.S.V., 2011, Microwave-Assisted Extraction of Phenolic Antioxidants from Potato Peels, Molecules 16, 2218-2232.

Bajpai V.K., Yoon J.I., Kang S.C., 2009, Antioxidant and antidermatophytic activities of essential oil and extracts of Metasequoiaglyptostroboides Miki ex Hu, Food and Chemical Toxicology 47, 1355–1361.

BawangGoreng, 2011, Pineapple Nutrition <laman-seri.blogspot.com/2011/11/khasiat-nenas.html> accessed 20.06.2010 (In Malay).

Blois M.S., 1958, Antioxidant Determinations by the Use of A Stable Free Radical, Nature 26, 1199-1200.

de Oliviera A.C., Valentim I.B., Silva C.A., Bechara E.J.H., de Barros M.P., Mano C.M., Goulart M.O.F., 2009, Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues, Food Chemistry 115, 469-475.

Devasagayam T.P.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S.S., Lele R.D., 2004, Free radicals and antioxidants in human health: Current status and future prospects, Association of Physicians of India 52, 794–804.

Gardner P.T., White T.A.C., McPhail D.B., Duthie G.G., 2000, The relative contribution of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices, Food Chemistry 68, 471–474.

Glanzer K., Sanglo A., Valko K., 1986, Microwave extraction-a novel sample preparation method for chromatography, Chromatography 371, 299-306.

Gulcin I., 2006, Antioxidant and antiradical activities of L-Carnitine, Life Sciences 78, 803-811.

Higdon J.V., Frei B., 2003, Tea catechins and polyphenols: health effects, metabolism and antioxidant functions, Critical Reviews in Food Science and Nutrition 43, 89–143.

Hung-Der J., Ku-Sheng H., Chuan-Liang H., Sheng-Hsien L., Min-Sheng S., 2007, Principal Phenolic Pythochemicals and Antioxidant Activities of Three Chinese Medicinal Plants, Food Chemistry 103, 749-756.

Kahkonen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M., 1999, Antioxidant activity of plant extracts containing phenolic compound, Agricultural and Food Chemistry 47, 3954-3962.

Maisuthisakul P., Gordon M.H., 2009, Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product, Food Chemistry 117, 332–341.

Maisuthisakul P., Suttajit M., Pongsawatmanit R., 2007, Assessment of phenolic content and free radical-scavenging capacity for some Thai indigeneous plants, Food Chemistry 72 (2), 145-171.

Mendiola J.A., Marin F.R., Senorans F.J., Reglere G., Martin P.J., Cifuentes A., Ibanez E., 2008, Profiling of different bioactive compounds in functional drinks by high-performance liquid chromatography, Chromatography A 1188, 234–241.

Mhatre M., Tilak-Jain J., De S., Devasagayam T.P.A., 2009, Evaluation of the antioxidant activity of non-transformed and transformed pineapple: A comparative study, Food and Chemical Toxicology 47, 2696–2702.

Okonogi S., Duangrat C., Anuchpreeda S., Tachakittirungrod S., Chowwanapoonpohn S., 2007, Comparison of antioxidant capacities and cytotoxicities of certain fruit peels, Food Chemistry 103, 839–846.
Pitakere M., 2009, Pineapples <www.foodscience.wikispaces.com/Pineapple> accessed 16.08.2010.
Singleton V.L., Orthofer R., Lamuela-Raventos R.M., 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent/methods, Enzymology 299, 152-178.
Soares J.R., Dins T.C.P., Cunha A.P., Ameida L.M., 1997, Antioxidant activity for some extracts of thymus zygis, Free Radical Research 26, 469-478.
Tachakittirungrod S., Okonogi S., Chowwanapoonpohn S., 2007, Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract, Food Chemistry 103, 381–388.
Terao J., Piskula M., Yao Q., 1994, Protective effect of epicatechin, epicatechingallate, and quercetin on lipid peroxidation in phospholipid bilayers, Biochemistry and Biophysics 308, 278–284.
Velioglu Y.S., Mazza G., Gao L., Oomah B.D., 1998, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, Agricultural and Food Chemistry 46, 4113-4117.
Yingming P., Kai W., Siqin H., Hengshan W., Xiaomei M., Chunhuan H., Xiaowen J., Jie Z., Fujuan H., 2008, Antioxidant activity of microwave-assisted extract of longan (DimocarpusLongan Lour), Food Chemistry106, 1264-1270.