Extraction conditions and antioxidant activities of the extract of pineapple peel by ultrasonic

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Abstract. In the present work, a ultrasonic-assistant method was used for the extraction of antioxidants (total phenol and total flavonid) in pineapple peel. The experimental parameters, including extraction time, the ratio of solution to peel and the concentration of solvent on the contents of total phenol and total flavonid were investigated. Orthogonal designs were also used to test the results of single experiments. Based on the contents of total phenol and total flavonid, the optimal ultrasonic conditions for extraction were taken as follows: extracting 65 min with 75% ethanol solution by ultrasonic, and the solution to peel ratio was 10:1. The antioxidant activities of the extract were also investigated. The research here indicated that pineapple was rich in natural antioxidants and possessed excellent antioxidant activities. It also provided application for the waste produced in pineapple processing.

1. Introduction
Pineapple (Ananas comosus [L.] Merril) is one of the most popular tropical fruits widely cultivated in Thailand, The Philippines, China, Brazil, and India (Zhang et al, 2012). This fruit is widely processed into juice and canned products in addition to direct consumption.

The researches about pineapple were mostly focused on the flesh, which is rich in nutrients such as vitamins, minerals, and phenolic phytochemicals (Alotham et al, 2009). Besides, pineapple is very well appreciated by its aroma. Over 400 compounds have been identified among different cultivar of fresh and processed pineapple products (Takeoka et al, 1991; Brat et al, 2004; Lamikanra & Richard 2004; Elss et al, 2005; Tokitomo et al, 2005; Pino, 2013; Montero-Calderón et al, 2010).

However, there was few reports about the peel of pineapple, especially the antioxidants and antioxidant activities. In the process of making juice, canned food and other kinds of products, tons of peel waste will be produced. Most of the peel was used as additive, fertilizer or directly discarded. This is totally a waste of natural resources.

In this paper, the antioxidants, including total phenol and total flavonid were extracted with a ultrasonic-assistant method from pineapple peel waste. The ultrasonic experimental parameters, including extraction time, the concentration of solvent and the ratio of solution to peel on the extraction were investigated. The optimal conditions for extracting antioxidants in pineapple peel were achieved and the antioxidant activities of the corresponding extract were investigated.

2. Materials and methods

2.1. Materials and reagents
Bali cultivar pineapples were collected just from the fruits planted in South Subtropical Crop Research Institute. Peel was separated from mature fruits and stored directly in vacuum-packaged polyethylene pouches at -20 °C until required for analysis. Rutin, gallic acid, 2,2'-diphenyl-2-picrylhydrazyl (DPPH) and ABTS radicals were received from Sigma-Aldrich Co. All the other regents were of analytical grade and used without treatment.

2.2. Experiment design

2.2.1 Extraction time
For extraction time assay, 5.00 g fresh peel was extracted by ultrasonic with 50 ml of 70% ethanol for different time (15, 30, 45, 60 and 75 min).

2.2.2 Ethanol concentration
To determine the effect of concentration, 50 ml of ethanol solutions (50, 60, 70, 80, and 90%) were mixed with 5.00 g fresh peel and ultrasonic for 30 min.

2.2.3 Ratio of solution to peel
The solution concentration, extraction time were set at 70%, 30 min when determining the effect of ratio of ethanol solution to the mass of peel. The ratios of 5:1, 10:1, 15:1 and 20:1 were used in this assay.

2.3. Determination of total phenol and total flavonoid
The extract was resolved in methanol to make a stock solution of 10 mg/mL. The total phenol content (TPC) was determined by using the FC assay described before (Du et al, 2014). The calibration curve was prepared by using a standard solution of gallic acid and the results were expressed as milligram gallic acid equivalent (GAE Equiv)/g Fresh weight (FW). Total flavonoid content was determined based on the method described by Kim et al (2002). The total flavonoid content was calculated by using a calibration curve of rutin standard and expressed as mg rutin equivalent (RT Equiv)/g FW.

2.4. DPPH and ABTS radical scavenging activity test
The DPPH free radical scavenging activity of the extract was performed by measuring the decrease in absorbance of DPPH solution at 517 nm in the presence of the extracts by the method proposed by Liyana-Pathirana et al (2005) with minor changes. The solution of 0.2 mM was prepared by dissolving DPPH in methanol. For the evaluation of free radical scavenging activity, 1 ml of DPPH was added into 1 ml of the extract and reference solution. The mixture was then allowed to stand at room temperature for 30 min in dark before the absorbance at 517 nm was read. The control was prepared as above without extract. The antioxidant activity could be expressed as the following equation:

\[
\text{Scavenging activity=} \frac{(A_0 - A_s)}{A_0} \times 100\%
\]

where \(A_0\) and \(A_s\) were the absorbance at 517 nm of the control and sample solution, respectively.

The ABTS assay was evaluated on the procedure described by Re et al (1999). A solution consisting of 7 mM ABTS and 2.4 mM potassium persulfate (1:1 v/v) was reacted in dark for 12 h at room temperature before used. Then the solution was diluted by methanol to obtain an absorbance value of 0.7 at 734 nm. For the scavenging of ABTS, 50 µl of the extract and reference solution was added to 4 mL of the solution above. Methanol was used as control. After reaction for 10 min, the absorbance was measured at 734 nm. The free radical scavenging capability was calculated by the equation:

\[
\text{ABTS scavenging activity=} \frac{(A_c - A_s)}{A_c} \times 100\%
\]

where \(A_c\) and \(A_s\) were the absorbance at 734 nm of the control and sample solution, respectively.
3. Results and discussion
The use of sections to divide the text of the paper is optional and left as a decision for the author. Where the author wishes to divide the paper into sections the formatting shown in table 2 should be used.

3.1. Effect of ultrasonic time on the contents of total phenol and total flavonid

![Graph showing the effect of ultrasonic time on the contents of total phenol and total flavonid.](image)

Figure 1 The effect of ultrasonic time on the contents of total phenol and total flavonid.

3.2. Effect of ethanol concentration on total antioxidants
The contents of total phenol were increased when the concentration of ethanol was between 50 to 70 (Figure 2). However, the contents of total flavonid increased from 50 to 80% ethanol. At higher concentration, the dissolution of impurity like pigments increased, which would in turn decrease the connection of antioxidants with solution (Moon & Choi 2011). Besides, higher concentration would lead to the leakage of antioxidants. This explained why the contents of total phenol and total flavonid possessed such trends with the change of ethanol concentration.
3.3. Effect of the ratio of solution to peel
The results of ratio of solution to peel indicated that volume of ethanol showed little on the contents of total phenol and total flavonid (not shown here). So the ratio was fixed at 10.

3.4. Orthogonal designs
The results of single experiments were further validated by orthogonal designs. The optimal concentration and time for the extraction of total phenol and total flavonid were tested to be 75% ethanol, 60 min and 75% ethanol, 65 min, respectively. The extraction of antioxidants from pineapple peel by ultrasonic was chosen as 75% ethanol, 60 min and a ratio of 10 for solution to peel on the basis of the results of orthogonal designs. The contents of total phenol and total flavonid were 1.254 mg GAE /g (FW) and 0.55 mg RT/g (FW) under these conditions.

3.5. DPPH and ABTS radical scavenging abilities of the extract of pineapple peel
Ascorbic acid (Vc) was used as reference in radical scavenging ability evaluation at a concentration of 0.1 mg/mL.
Figure 3. DPPH scavenging abilities of pineapple peel extract and Vc.

The DPPH scavenging ability of the pineapple peel extract and Vc was given in Figure 3. The results indicated that the free DPPH radical scavenging percentage of the extract (47%) was almost half of that of Vc (91%). However, the ability of ABTS scavenging ability of pineapple peel extract (97%) was higher than that of Vc (92.8%, Figure 4). This indicated that the pineapple peel extract possessed excellent antioxidant abilities.

Figure 4. ABTS scavenging abilities of pineapple peel extract and Vc.

4. Conclusions
The extraction of antioxidants (total phenol and total flavonoid) in pineapple peel was studied in this research. The experimental parameters, including extraction time, the ratio of solution to peel and the concentration of solvent for the contents of total phenol and total flavonoid were investigated both in single experiments and orthogonal designs by a ultrasonic-assistant method. The results showed that the optimization conditions were as follows: extracting 60 min by ultrasonic with 75% ethanol
solution, and the ratio of solution to peel was 10:1. The antioxidant activities of the extract were also evaluated and results showed the extract of pineapple peel possessed excellent antioxidant abilities. The research here showed that pineapple peel could be used as natural sources of antioxidants and it also provided new application for the waste produced by pineapple processing.

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