Pineapple peel wastes as a potential source of antioxidant compounds

V Saraswaty 1*, C Risdian 1, I Primadona 1, R Andriyani 1, D G S Andayani 1 and T Mozef 2

1 Research Unit for Clean Technology, Indonesian Institute of Sciences (LIPI)
Jalan Cisitu 21/154 D, Bandung 40135, Indonesia
2 Research Centre for Chemistry, Indonesian Institute of Sciences, Kawasan Puspiptek Serpong,
Tangerang Selatan, Banten, Indonesia, 15314
*Email: vsaraswaty@gmail.com

Abstract. Indonesia is a large pineapple (Ananas comosus) producing country. Food industries in Indonesia processed this fruit for new products and further resulted wastes of which cause an environmental problems. Approximately, one pineapple fruit total weight is 400 gr of which 60 g is of peel wastes. In order to reduce such pineapple peel wastes (PPW), processing to a valuable product using an environmentally friendly technique is indispensable. PPW contained phenolic compound, ferulic acid, and vitamin A and C as antioxidant. This study aimed to PPW using ethanol and water as well as to analyze its chemical properties. Both dried and fresh PPW were extracted using mixtures of ethanol and water with various concentrations ranging from 15 to 95% (v/v) at room temperature for 24 h. The chemical properties, such as antioxidant activity, total phenolic content (Gallic acid equivalent/GAE), and total sugar content were determined. The results showed that the range of Inhibition Concentration (IC)50 value as antioxidant activity of extracts from dried and fresh PPW were in the range of 0.8±0.05 to 1.3±0.09 mg.mL⁻¹ and 0.25±0.01 to 0.59±0.01 mg.mL⁻¹, respectively, with the highest antioxidant activity was in water extract. The highest of total phenolic content of 0.9 mg.g⁻¹ GAE, was also found in water extract.

1. Introduction
Pineapple (Ananas comosus) is one of the main agricultural commodities from Subang, West Java, Indonesia. The city produces over 135,000 tons of pineapple in the year of 2014 [1]. As the impact of pineapple food industries, pineapple peel wastes (PPW) are the important issue of waste management of which urgently to be overcame. PPW is therefore converted into highly valuable product, since contains considerable content of antioxidant property, sugar, phenolic compound, high fiber, and protein. PPW also provides high potential bromelain enzyme as functional material [2-4].

Generally, PPW has been used for animal feed or land fertilizer [5], as reducing agent or antioxidant, and substrate for production of bio-ethanol [6-7]. Antioxidant or reducing agent plays an important role both in health sector and chemical industry. Unfortunately, chemically synthesis antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are not safe for health. It is found that at high dose both BHA and BHT induced tumor growth [8]. Therefore we aimed to explore PPW as a potential of natural antioxidant. One of interesting pineapple applications as reducing agent is in biosynthesis of metal nanoparticles. It is found that biosynthesis of metal nanoparticles can be carried out by aqueous pineapple extract [9-10]. The work had successfully
produced silver and cooper nanoparticles. Chlorogenic acid and ferulic acid in aqueous pineapple extract is considered having an important role as reducing agent or antioxidant [9].

Study of antioxidant activity showed that methanol was the best organic solvent for pineapple extract [2,11]. However, methanol is a toxic organic solvent which caused several health problems [12]. Mixtures of ethanol and water are an environmentally preferable solvent [13]. Study on the pineapple as antioxidant by ethanol was considerable against DPPH radical and achieved at concentrations of 50 and 70% v/v, and is considerable against DPPH radical [11]. The study aimed to investigate the effect of water-ethanol at various concentrations for PPW as antioxidant activity.

2. Experimental

2.1. Material

PPW was collected from UKM Alam Sari, Subang, West Java Indonesia. PPW was washed under tap water and dried in a blower oven at 55°C for 48 hr. The dried PPW was grinded mechanically and further used for extraction. Meanwhile for fresh material, PPW was cut into small pieces, then directly macerated in organic solvent. Chemical reagents sulphuric acid, anthrone, methanol, Na_2CO_3, Follin Ciocalteu are pro analysis grade from Merck, meanwhile DPPH radical, ascorbic acid, gallic acid are from SIGMA-ALDRICH, and ethanol is technical grade from Bratachem.

2.2. Method

2.2.1. Extraction

Ten g of fresh and dried PPW were macerated in 100 mL of ethanol:water at various concentration (0:100, 15:85, 35:65, 55:45, 75:25 and 100:0 for 24 hr at ambient temperature). The filtrates were evaporated under rotary vacuum evaporator (Heidolph, Germany). The yield of extraction was determined as percentage of concentrated extract compare to weight of sample before extraction.

2.2.2. Total sugar content

Total sugar content was determined according to anthrone method [19] with a slight modification. One g of anthrone was dissolved in 500 mL 72% v/v sulphuric acid. One mL of sample was put in a test tube and added by 5 mL of anthrone reagent. The reaction was incubated in a water bath at 80°C for 11 min. The absorbance was then measured at 630 nm by a UV-VIS Spectrophotometer (Hitachi, U2800) with glucose as standard. Total sugar content was calculated based on glucose standard curve. The analysis was performed triplicate.

2.2.3 Antioxidant activity

Free radical scavenging effect was performed according to the following protocol: 50 μL of samples at various concentrations (0.5, 1, 2, 4 and 8 mg.mL⁻¹) was put into 96-micro wells plate and added with 200 μL of 0.077 mM DPPH. The reaction was incubated at room temperature for 15 min. The absorbance was then measured at 520 nm by ELISA reader (Multiskan, BioRad). Percentage of inhibition was calculated by using the following formula:

\[
\% \text{ Inhibition} = \left(\frac{C - S}{C}\right) \times 100\%
\]

C is absorbance of DPPH, and S is absorbance of sample. Ascorbic acid was used as positive control. The procedure was performed triplicate [14].

2.2.4. Total phenolic content

Total phenolic content was determined spectrophotometrically according to Follin-Ciocaltelau method. Sample of 300 μL of at concentration of 1000 μg/mL was mixed with 1200 μL Folin Ciocalteau (10% v/v) and 1500 μL Na_2CO_3 (7.5% w/v). The reaction was incubated at 55°C for 15 min. Absorbance was then measured at λ 760 nm by UV-VIS Spectrophotometer (Hitachi, U2800). Gallic acid was used as
standard and total phenolic content was calculated based on gallic acid standard curve. The total of phenolic content was expressed as gallic acid equivalent (GAE). The procedure was performed triplicate [15].

3. Result and discussion

3.1 Yield of extraction

PPW is highly containing water of which will cause a rapid rotting process and significantly pollutes environment. Air contamination occurs due to putrid odor whereas land contamination due to microbial growth. Water content of PPW is shown in Table 1. It shows that water content of fresh PPW is higher than dried PPW. This results revealed that high water content of PPW was reduced through drying process.

Yield of PPW in various ethanol concentration is shown in Table 1. It shows that yield of dried PPW extraction is higher than fresh PPW. This is due to the effect of drying process in which significantly reduced water content. Moreover, water content in fresh PPW directly diluted ethanol concentration and increased sample weight. Meanwhile, effect of ethanol concentration on either dried or fresh PPW extraction is similar. This result indicated that compounds of PPW were majorly soluble in mixture of ethanol and water, in which 55% v/v of ethanol/water is the most appropriate solvent for extraction. This process of extraction followed the principle of like-dissolves-like [16]. Total sugar content determination is important since sugar plays important role in promotion of microbial growth as the carbon source. However, since microbial growth was not found, total sugar content in dried PPW was not evaluated. In addition, water content of dried PPW was less than 10% for which is not promote for microbial growth. Table 1 shows that PPW water extract of PPW reveals lower sugar content compared with ethanol extract. This is due to the sugar in PPW was more easily extracted by the mixture of ethanol-water rather than by water solely. Fresh PPW extract contains sugar so that easily promote the growth of microbes. Therefore, mixtures of ethanol and water has an advantage. In extraction process, presence of ethanol will reduce the growth of microbes due to that ethanol is an organic solvent of which exhibits a rapid and broad spectrum antimicrobial activity [17].

Table 1. Yield of extraction, antioxidant activity and total sugar content.

|       | Water content (% w/w) | [Ethanol] (% v/v) | Yield (% w/w) | Antioxidant activity (IC50, mg.mL-1) | Total Sugar* (% w/w) |
|-------|-----------------------|-------------------|---------------|-------------------------------------|---------------------|
| Fresh PPW | 85.5                  | 0                 | 5.16          | 0.2±0.009                           | 3.69                |
|        | 15                    | 6.48              | 0.4±0.004     | 7.51                                |
|        | 35                    | 6.57              | 0.4±0.003     | 7.16                                |
|        | 55                    | 6.94              | 0.5±0.020     | 7.40                                |
|        | 75                    | 5.70              | 0.6±0.040     | 6.23                                |
|        | 95                    | 4.18              | 0.6±0.001     | 5.65                                |
| Dried PPW | 5.43                  | 0                 | 24.67         | 0.8±0.050                           | n.d                 |
|        | 15                    | 30.95             | 1.3±0.090     |                                     |
|        | 35                    | 28.93             | 1.2±0.001     |                                     |
|        | 55                    | 45.38             | 0.9±0.070     |                                     |
|        | 75                    | 30.09             | 0.9±0.070     |                                     |
|        | 95                    | 30.37             | 1.2±0.110     |                                     |

n.d = not determined, PPW : Pineapple peel wastes, *based on dry extract.
3.2 Antioxidant activity of PPW
Antioxidant activity of PPW extract in various ethanol concentrations was evaluated. The lower the IC$_{50}$ value the more the active extract. Table 1 shows, the lowest IC$_{50}$ value of antioxidant activity was found in water extract, both for fresh and dried PPW. These results indicated that water extract contains polar compound as phenolic compound and serves as antioxidant. Moreover, the increase of total sugar content in mixture of ethanol:water affected the decrease of PPW antioxidant activity. On the other hand, IC$_{50}$ value of fresh PPW was smaller than dried PPW. This result indicated that drying of PPW at 50°C affected antioxidant activity in which significantly increased IC$_{50}$ value. The lower antioxidant activity in dried extract may due to bioactive compound degradation which is sensitive to heat. Other antioxidant activity of different variety of pineapples have been carried out of which IC$_{50}$ value of antioxidant activity (DPPH free radical scavenger) were in the range of 12.4 – 29.0 mg.mL$^{-1}$ [11]. It was found that current IC$_{50}$ of PPW extract was low both for fresh and dried PPW (Table 1). This result indicated that currently, PPW shows as high potential source of antioxidant for DPPH free radical scavenger.

3.3 Total phenolic content of PPW
Phenolic compounds has strong correlation with antioxidant activity. Therefore, total of phenolic content from both dried and fresh PPW was evaluated. The results are shown in Figure 1. It shows that in ethanol 75% fresh PPW contained higher total phenolic content than dried PPW. We assumed, the drying process of PPW would degrade compounds and significantly affect the antioxidant activity.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Total Phenolic content/GAE (% w/w) of PPW extract at various ethanol concentrations in water.

From Figure 1 it shows that the highest total phenolic content of fresh PPW was found in water extract (0% ethanol), whereas for dried PPW was of 75% v/v ethanol extract. The results of total phenolic content in dried PPW almost the same with selected Malaysian pineapple [18].
The different of total phenolic content from fresh and dried PPW was due to heat processing. Fresh PPW was not processed and phenolic compound has yet decomposed. Whereas total phenolic content in water extract is the highest among other. This due to water extract of PPW contain polar compound of which more soluble in water than in aqueous ethanol.

From Figure 1 shows that PPW extract contained 540 – 1260 mg GAE/100 g PPW, nearly about 10 times higher than previous study was of 34.7-54.7 [18] and 22.7-53.8 mg GAE/100 g fruit [11].

4. Conclusion
PPW from Subang, West Java, has succesfully been converted into a potential source of antioxidant. Ethanol/Water of 55% (v/v) provided high yield of antioxidant and avoided microbial contamination.

Acknowledgement
Author thanks to Research Unit for Clean Technology, Indonesian Institute of Sciences, for support this research under Riset Mandiri 2016 and Ms. Rossy Choerun Nissa for her technical help on this research.

References
[1] BPS 2015 Subang in Figure BPS Kabupaten Kota Subang hal. 76
[2] Hossain M A and Rahman M M S 2011 Food Res. Int. 44 672-6
[3] Ramalingan C, Srinath R and Islam M M 2012 Elixir Food Scie. 45 7822-26
[4] Ketnawa S, Cha iwut P and Rawdkuen S 2011 Food Bioprod. Process. 90 385-91
[5] Makinde O A, Odeyinka S M and Ayandiran S K 2011 Livest. Res. Rural Dev. 23
[6] Pornpunyapat J, Chotigeat W and Chetpattananondh P 2014 Adv. Mat. Res. 875-7 242–5
[7] Erukainure O L, Ajiboye J A, Okafo O Y, Kosok R S B, Owolab O F O and Adenekan I S O 2012 J. Food Biochem. 36 643–7
[8] Kahl R and Kappus H 1993 Z. Lebensm. Unters. Forsch., 196 329–38
[9] Ahmad N and Sharma S 2012 Green Sustain.Chem. 2 141-7
[10] Ranjitham A M, Ranjani G S, and Caroling G 2015 Int.J. Pharm Tech Res. 8 750–69
[11] Yusri A and Siow L F 2014 J. Food Stud. 3 40
[12] Kraut J A and Kurtz I 2008 Clinical J. the American Soc. of Nephrology. 3 208–25
[13] Capello C, Fischer U, and Hungerbühler K 2007 Green Chem. 9 927–34
[14] Saraswaty V, Risdian C, Budiwati T A and Tjandrawati M, 2013 Pros. Teknol. Untuk Mendukung Pembang. Nas. 1 196–200
[15] Saraswaty V, Risdian C, Lelono R A A and Mozef T 2015 Oxid. Antioxid. Med. Sci. 4 97
[16] Chew K K, Khoo M Z, Ng S Y, Thoo Y Y, Aida W M W, and Ho C W 2011 Int. Food Res. J. 18 1427–35
[17] Mcdonnell G and Russell A D 1999 Clinical Microbiol. Rev. 12 147–79
[18] Alothman M, Bhat R, and Karim A A 2009 Food Chem. 115 785-8
[19] Yemm E W and Willis A J 1954 Biochem J. 57 508-14