Antioxidant Activities from Two Varieties of Pear Peel Extracts using DPPH and CUPRAC Methods

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Abstract. Pear peels more often than not are considered nonbeneficial, therefore they generally end up discarded aside from being consumed along with the fruit. In this research, the antioxidant capacity of the peel of two most popular pears consumed in Indonesia, namely European Pear (EP) and Asian Pear (AP) is being identified and measured. For the results of DPPH antioxidant activity method, Asian pear peel extract dissolved in ethanol (IC₅₀ 50.72) has the highest antioxidant contents than European pear peel extracted in methanol 80% (67.95). For the results of CUPRAC antioxidant activity method, European pear extract dissolved in methanol 80% showed higher antioxidant activity with the value of 2.51 and even the highest activity in an incubated condition with the value of 1.54. From the measurements, it can be understood that the peel extract of Asian pears has a higher antioxidant capacity when dissolved at a normal temperature. However, in an incubated temperature extraction environment, peel extract of European pears shows the highest antioxidant capacity, with the existence of slow-reacting antioxidants in the peel of European Pears, could be the potential cause. From this research, it can be concluded that pear peels are a source of beneficial antioxidants, and the method of extraction of antioxidants from pear peel would determine the extractable useful antioxidants: In the case of European Pear peel, an incubation temperature measurement is more desired.

1. Introduction
Pear (Pyrus spp) fruit is popular among consumers due to its high nutritive value, good taste, and low caloric level. It has a low content of protein and lipids and is rich in sugars such as fructose, sorbitol, and sucrose. It has been found that pears contain 12.4% sugars, 0.5% protein, 0.3% lipids and 2.8% fiber [1]. Apart from their richness in macronutrients, pears also possess other nutritional components such as vitamins and antioxidants that are important as health-beneficial compounds [2]. Pear is a recommendable substitute for diabetics and the obese; moreover, its dietary fiber together with phenolics helps reduce the risk of cardiovascular diseases [3], [4]. The high content of vitamins C and E which have the antioxidant capacity in pear can help to protect the human body against free radicals [5].

Vegetables and fruits are good sources of phenolic compounds, which is an important bioactive compound with strong antioxidant activity. Compared to the pulp extracts, peel extracts had a higher total soluble phenolic content (TPC) and related antioxidant capacity. Aqueous pulp extracts had high α-amylase inhibitory activities with no correlation to phenolic content, while peel ethanolic extracts had the highest α-glucosidase inhibitory activity with positive correlation to its total phenolic content [6].
Some antioxidant methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (Cupric Ion Reducing Antioxidant Capacity) and FRAP (Ferric Reducing Antioxidant Power) were used to test the antioxidant activity of food, vegetables and fruits [7], [8]. The difference between the two methods is CUPRAC method measures the ability of antioxidants in reducing Cu²⁺-neocuproine into Cu⁺-neocuproine which happens in pH 7 and having the maximum absorbance in 450 nm wavelength, while DPPH indirectly measures antioxidant capabilities through the tested material’s reaction against free radicals produced in the testing method. Antioxidant from phenolic compounds which can be found in plants including flavonoid compounds, cynamate acid, coumarins, tocopherol, carotenoid, and organic polyfunctional acid [9].

In this research, we intend to identify phenolic and flavonoid contents in pear peel extract and study antioxidant activities of different polar solvent of pear peel extract with two different methods (DPPH and CUPRAC).

2. Material and Methods

2.1 Materials
DPPH (2,2-diphenyl-1-picrylhydrazyl), neocuproine, gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (USA), cupric chloride, European (Pyrus communis) and Asian (Pyrus pyrifolia) pear peels, ethanol, methanol 80%, aquadest, and all other reagents were analytical grades.

2.2 Preparation of sample
Peels of two varieties of pear were collected from Caringin Traditional Market in Bandung, West Java. European pear (Pyrus communis L.) abbreviated as EP (figure 1a), imported from Australia and Asian pear (Pyrus pyrifolia) as sample AP (figure 1b), imported from China. They were thoroughly washed with tap water, wet sortation, cut, dried, and ground into powder.

2.3 Extraction
Fifty grams of powdered sample was extracted by maceration using different polar solvents at room temperature for 24 hours. The methanol 80% (MET), aquadest (AQ), and ethanol (ET) extracts were repeated three times and excess of the solvent distilled-off in a vacuum rotary evaporator at 50°C. The obtained semisolid extracts were quantitatively transferred to the extraction solvent and preserved at 8°C until used for further experiments.

2.4 Total extraction yields
Total extraction both of pear peels were calculated by the following equation:

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\text{Yield (\%)} = \frac{\text{Amount of sample after drying}}{\text{Amount of used from extraction}} \times 100
\]

2.5 Total phenolic content (TPC)
Measurement of Total Phenolic Content (TPC) was done using Folin-Ciocalteu method which was adapted from Pourmorad [10], in which the absorbance was read at 765 nm wavelength. In this research, an analysis was done in triplicates for each available extract. To obtain a standard curve, a standard solution of gallic acid with a concentration of 30-180 µg/ml was used. The value of TPC is reported as total gallic acid equivalent per 1 g extract (mg GAE/g).

2.6 Total flavonoid content (TFC)
Measurement of Total Flavonoid Content (TFC) was done using an adapted method from Chang et al [11]. The absorbance was read at 415 nm wavelength, with the analysis done in triplicates for each extract. To obtain a standard curve, the standard solution of quercetin with a concentration of 20-120 µg/ml was used. The value of TFX is reported as total quercetin equivalent per 1 g extract (mg QE/g).

2.7 IC\textsubscript{50} of DPPH scavenging activity
DPPH solution for this research was prepared according to Blois [12] with minor modification. The reaction to obtain a calibration curve was initiated using various concentrations of each extract that was pipetted into DPPH solution of 50 µg/ml (1:1). After 30 minutes of incubation, the absorbance can be read at 515 nm wavelength by using a spectrophotometer UV-Vis Infinite M200 Pro Multimode Reader of Tecan origin. For this test, methanol was used as a blank, DPPH solution of 50 µg/ml was used as a control, and ascorbic acid was used as standard.

2.8 EC\textsubscript{50} of CUPRAC capacity
The preparation of CUPRAC solution was adopted from Apak et al. [13]. In normal sample measurement, the CUPRAC solution was prepared in ammonium acetate buffer with pH 7. To initiate the reaction for obtaining a calibration curve, various concentration of each extract was pipetted into CUPRAC 50 µg/ml (1:1). After 30 minutes of incubation, the absorbance was read at 450 nm wavelength using a spectrophotometer UV-Vis Infinite M200 Pro Multimode Reader from Tecan. Ammonium acetate buffer was used as a blank, CUPRAC 50 µg/ml was used as a control and ascorbic acid as standard.

3. Result and Discussion
3.1 Total extraction yields of pear peels
In European pear peels (EP), total extraction yields from methanol 80% (MET), ethanol (ET), and aquadest (AQ) were 39.92%, 55.54%, and 30.95% respectively. In Asian pear peels (AP) total extraction yields were 46.38%, 62.94%, 37.40% respectively (Table 1). In both EP and AP, the highest extract yields were observed in ethanol extract and higher in the Asian pear peels, however, it cannot be concluded that higher yield equals higher antioxidation capacity.

| Sample | Total extraction yields (%) |
|--------|-----------------------------|
|        | MET | ET | AQ |
| EP     | 39.92 | 55.54 | 30.95 |
| AP     | 46.38 | 62.94 | 37.40 |

3.2 Total Phenolic Contents (TPC)
TPC among the various extracts were reported in term of gallic acid equivalent using the standard curve equation \( y = 0.0061x - 0.0144 \), \( R^2 = 0.9945 \). The total phenolic contents in EP peels were 96.35±2.75, 73.75±4.47, and 68.49±4.99 mg GAE/g from the 80% methanol, ethanol, and aquadest extracts, respectively. Statistically significant lower yields were observed in aquadest extracts as compared to ethanol and 80% methanol extracts, while the latter two showed significant differences too. In the case of AP peels, the contents from 80% methanol, ethanol, and aquadest extracts were 86.01±1.64, 79.22±1.89, and 65.47±4.18 mg GAE/g, respectively. Statistical significance was similar to the GP peel
samples. The total phenolic were maximized in 80% methanol extracts of the EP peels, and ethanol extracts of the AP peels. The total phenolic contents were higher in the EP peels than in AP peels.

3.3 Total Flavonoids Contents (TFC)

TFC among the various extracts were expressed in term of quercetin equivalent using the standard curve equation $y = 0.6444 \times + 7.7263$, $R^2 = 0.9928$. The total flavonoid contents in EP peels from 80% methanol, ethanol, and aquadest were 57.87±4.23, 68.72±5.34, and 46.69±3.81 mg QE/g, respectively. In the case of AP peels, the observed contents were 38.17±4.74, 47.05±3.32, and 35.12±4.91 mg QE/g, respectively. Both EP and AP peel was observed in ethanol extracts as the highest content of flavonoid. In EP peels, a significantly higher flavonoid contents were observed in aquadest extracts as compared to those from 80% methanol and ethanol extracts. No such difference was observed in the AP peels.

Flavonoid had higher antioxidant activity than phenolic acid. Flavonoid which had -OH in ortho C-3’, C-4’, -OH in C3, oxo function in C-4, double bond at C-2 and C-3 would give higher antioxidant capacity. The -OH with ortho position in C-3’-C-4’ had the highest influence on the antioxidant activity of flavonoid [14].

3.4 $IC_{50}$ of DPPH free radical scavenging activity

$IC_{50}$ of DPPH free radical scavenging activity of ascorbic acid was 2.05±0.001 µg/ml, and DPPH free radical scavenging activity of EP from 80% methanol, ethanol and aquadest were, respectively, 67.95±4.95, 82.77±5.71, and 97.02±3.89 µg/ml, with 80% methanol being the lowest of DPPH scavenging activity. $IC_{50}$ of DPPH free radical scavenging activity of AP were 59.89±2.77, 50.72±1.65, and 67.42±3.27 µg/ml for the same order of extracts. The ethanol solvent showed the lowest of DPPH scavenging activity. Comparison with ascorbic acid (2.05 µg/ml), statistically significant results for $IC_{50}$ of DPPH free radical scavenging activity were observed among the extracts both in EP and AP peels.

DPPH can be characterized as a stable free radical, and it has a characteristic to be dissolved in methanol or ethanol, and its colors can be absorbed at a wavelength of 515-520 nm. Its color would change when the free radicals are scavenged by antioxidants [14]. Classification by Blois [12] revealed that a sample with $IC_{50}$ or $EC_{50} < 50$ µg/ml was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, while a weak antioxidant with $IC_{50}$ or $EC_{50} > 150$ µg/ml. Based on the value of $IC_{50}$ of DPPH scavenging activity it can be concluded that all of peel extracts of EP and AP can be categorized as strong antioxidants. Considerably higher scavenging activities were observed from 80% methanol extract and ethanol extracts as compared to the activities of ascorbic acid, a synthetic antioxidant.

3.5 $EC_{50}$ of CUPRAC capacity

With CUPRAC procedure, hydroxyl radicals (OH) can also be detected, and OH scavengers’ activity can also be measured. Among all reactive oxygen species (ROS), hydroxyl radical is the most reactive [15]. It can be stated that a sample has an antioxidant capacity in a CUPRAC assay if the corresponding sample has a lower reduction potential than the reduction potential of Cu (II)/Cu (I) which was 0.46V. When this happens, the sample can reduce Cu (II) to Cu (I) and the sample will be oxidized [16].
Table 2. EC$_{50}$ of CUPRAC with a different condition and solvent compared to ascorbic acid

| Sample | Solvent | EC$_{50}$ CUPRAC capacity (µg/mL) | Normal | Incubated | Ascorbic Acid |
|--------|---------|----------------------------------|--------|-----------|---------------|
| EP     | 80% methanol | 2.51±0.29 | 1.54±0.29 | 0.10±0.02 |
|        | ethanol    | 3.67±0.24 | 2.76±0.20 |           |
|        | aquadest   | 5.19±0.45 | 4.12±0.23 |           |
| AP     | 80% methanol | 3.05±0.19 | 2.58±0.09 |           |
|        | ethanol    | 4.20±0.08 | 3.35±0.06 |           |
|        | aquadest   | 5.99±0.12 | 5.13±0.12 |           |

The CUPRAC method was applied as two interrelated procedures, there are normal (N) and incubated (I). The capacity of antioxidants with the CUPRAC method obtained from normal and incubated solutions of EP and AP are listed in Table 2.

The effect of elevated temperature incubation (50°C for 20 min) in antioxidant capacity of EP and AP was found significantly influenced (p<0.05) between normal and incubated measurements. The EC$_{50}$ of CUPRAC capacity of 80% methanol, ethanol, and aquadest solvent increased from normal to incubated there are from 2.51 to 1.54 µg/ml, 3.67 to 2.76 µg/ml, and 5.19 to 4.12 µg/ml, respectively in EP and 3.05 to 2.58 µg/ml, 4.2 to 3.35 µg/ml, and 5.99 to 5.33 µg/ml, respectively in AP.

It is apparent that the highest CUPRAC capacity was obtained in 80% methanol solvent which EP is higher than AP after incubation. Incubation measurements are needed because some antioxidant compounds were assayed after incubation [after Cu(II)-Nc reagent addition] so as to enable complete oxidation such as naringin and naringenin. Slow reacting antioxidants needed elevated temperature incubation to complete their oxidation with the CUPRAC reagent [17]. Statistically, EC$_{50}$ of CUPRAC capacity for all the pear peel extracts are significantly different if compared to Ascorbic Acid (0.1 µg/ml) as the standard reference compound. Even if the highest result is still 10 times lower than ascorbic acid, the extracts can still be categorized as a strong antioxidant. Further studies are demanded to ascertain the free radical scavenging activities and to find out the optimum levels or combination of levels of extracting temperature and of solvent concentrations that give the highest activity through well-designed experiments.

### 4. Conclusion

In order to correctly assess the antioxidant activity of a sample, various methods that could give different results and perspective should be used in parallel. Based on the data above, it can be said that the CUPRAC and DPPH capacities of pear peels correlate strongly with their total phenolic and flavonoid contents which 80% methanol was a better solvent for extracting phenolic compound from pear peels. Phenolics and flavonoids from two varieties of pear peel extracted have exhibited a potent antioxidant activity as evaluated by the CUPRAC method as a more selective method of determining antioxidant compounds, especially for slow-reacting antioxidant compounds. Moreover, both of European and Asian pear peels have strong antioxidant activities with DPPH and CUPRAC methods.

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Acknowledgments
This work was done in Universitas Muhammadiyah Bandung, Bandung, Indonesia, and supported fully by a research grant from The Ministry of Research and Higher Education of Indonesia, 2019.