Comparisons of antioxidant activities of two varieties of pumpkin (Cucurbita moschata and Cucurbita maxima) extracts

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Abstract. Pumpkin is a plant that is widely found in the territory of Indonesia. This study has evaluated the antioxidant activity, morphological, physicochemical and chemical properties of three components of C. moschata and C. maxima (the pulp, seed, and rinds). The C. moschata and C. maxima extracts were obtained by the maceration method using ethanol as the solvent. The higher antioxidant activity measured by DPPH assays was found in the rinds of C. maxima as of 33.8% of radical scavenging activity, followed by the pulp of C. maxima and seed of C. moschata as of 29.6% and 25.4% respectively. The higher carbohydrate content was established in the pulp of C. moschata with 78.6% and pulp of C. maxima with 69.5%. On the other hand, the seed of C. moschata and C. maxima had higher amounts of fats with 28.5% and 10.4%, respectively, while proteins were 19.2% and 12.3%, respectively. The C. moschata pulp, seed, and rinds also had been analysed using Scanning Electron Microscopy (SEM) for their morphological characteristic, The Fourier transform infrared spectroscopy (FTIR) for their functional groups and Color Reader for their color diversity. The result of this study showed that different part of C. moschata and C. maxima has good antioxidant activity and proximate composition so that appear to be promising comestibles for technological exploitation.

1. Introduction

Free radicals can cause oxidative stress and dangerous to the human body [1]. For that reason, antioxidants are necessary, because it can slow down the oxidation process. However, synthetic antioxidants have negative impact if it accumulates in the body such as cause cancer and other chronic diseases. Meanwhile, antioxidants from nature such as from plants became an interesting topic of research in recent years. Several extracts and phytochemicals have been shown to have an antioxidant ability [2]. Many antioxidants have been isolated from plants such as spices, vegetables, grain, herbs, fruits, roots, and leaves [3].

Pumpkin is a plant that is cultivated and widely found in Indonesia. There is an increasing interest for many variety uses of pumpkin because of its high nutritional value, digestive effects and good sensory characteristics [4,5]. The pulp of pumpkin is usually used in soups, pies, and bakery while the seeds are used as a snack and pharmaceutical. Therefore, pumpkin has high potential application for food, cosmetic and pharmaceutical industries [6]. Due to the good acceptance and industrialization of the derived products from pumpkins, more scientific information about the chemical and
physicochemical properties of this fruit is important. The purpose of this study was to compare the antioxidant activities of pumpkin seed, pulp, and rinds and also to study morphology and functional groups of the pumpkin seed, pulp and rinds flour. This work is important in the development of new products from pumpkin.

2. Material and Methods

2.1. Materials and General Instrumentations
The pumpkin was collected from Yogyakarta and Central Java Province, Indonesia. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, methanol, and ethanol were obtained from Sigma-Aldrich (Japan). Wako (Japan). The equipment used in this study was glassware, digital balance, Elisa Reader, SEM, FTIR, oven, and desiccator.

2.2. Preparation of ethanol extracts
The pumpkin was cut into pieces, peeled the rinds and separated the seeds. After drying process using oven for 3 days at 50 °C, it was made into flour. The ethanol extracts were obtained by macerated the pulp, seeds, and rinds flour with ethanol (1:8 w/w) for 3 days at room temperature.

2.3. Characterization of pumpkin flour morphology and functional group
SEM of the surface of dried pumpkin flour was performed using a Hitachi SU-70 instrument operating at 4 kV. FTIR analysis of the pumpkin flour was performed on a wavelength from 500 cm⁻¹ to 4000 cm⁻¹. The proximate analysis procedure uses method that refers to SNI 01-2891-1992 [7].

2.4. Colour test
Colour instrumental parameters were determined using colour reader (CR 20 Color Reader Konica Minolta). Results were expressed in L*, a* and b*, with L (how light or dark the sample is), ranging from black (0) to white (100), a* ranging from green (-60) to red (60) and b* ranging from blue (-60) to yellow (+60). Colour instrumental analyses were performed in three replicates.

2.5. DPPH assay
Several concentrations of C. moschata extracts were dissolved in methanol and reacted with DPPH (1.01 mM) at room temperature. It was left to stand in the dark condition for 30 min. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer [8]. The scavenging activity was calculated using equation 1:

\[
\text{DPPH scavenging activity (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where A₀ is absorbance of the control and A₁ is absorbance of the sample. The DPPH radical scavenging assays were carried out in three replicates.

3. Results and Discussion

3.1. Proximate compositions of pumpkin flour
Proximate compositions of pumpkin flour (pulp, seed, and rind) of two varieties from C. moschata and C. maxima are shown in Table 1. The differences in pumpkins composition probably depend on several factors such as soil fertility, the variety, stage of maturity, and climate [9].

| Samples          | Water (%) | Ash (%) | Fat (%)  | Protein (%) | Carbohydrate (%) |
|------------------|-----------|---------|----------|-------------|------------------|
| Seeds (C. moschata) | 7.67±0.05 | 5.18±0.04 | 28.49±0.4 | 19.23±0.06 | 39.51±0.5 |
| Rind (C. moschata)  | 9.64±0.08 | 4.95±0.07 | 2.44±0.07 | 8.05±0.04  | 75.04±0.8 |
| Pulp (C. moschata) | 13.01±0.09| 4.32±0.05 | 0.16±0.01 | 3.99±0.06  | 78.64±0.9 |
| Seeds (C. maxima)  | 8.15±0.08 | 5.4±0.08  | 10.42±0.04| 12.33±0.08 | 63.70±0.7 |
| Rind (C. maxima)   | 9.96±0.05 | 10.3±0.06 | 5.59±0.04 | 12.84±0.06 | 61.25±0.6 |
| Pulp (C. maxima)   | 16.77±0.05| 6.68±0.04 | 2.14±0.03 | 4.90±0.06  | 69.51±0.7 |
The data indicated that the seeds of *C. moschata*, the seeds of *C. maxima*, and the rinds of *C. maxima* contained a higher percentage of protein, fat, and ash as of 19.23±0.06, 12.33±0.08, and 12.84±0.06 (for protein), respectively; 28.49±0.4, 10.42±0.04, and 5.59±0.04 (for fat), respectively; and 5.18±0.04, 5.4±0.08, and 10.3±0.06 (for ash), respectively. In the case of carbohydrate content, the pulp of *C. moschata* and the pulp of *C. maxima* had the highest percentage as of 78.64±0.9 and 69.51±0.7, respectively. These results were in agreement with the literature [6]. It was also reported that because of its low water activity, pumpkin flour has long shelf life and quality [10]. Another study was discovered that there was no gluten was found in pumpkin flour [6].

### 3.2. Morphology structure of pumpkins flour

Figure 1 shows the SEM image of the surface of the *C. moschata* flour.

![SEM images of the surface morphological structure of pumpkins flour](image)

*Figure 1. SEM images of the surface morphological structure of pumpkins flour: a. seed of *C. moschata* b. pulp of *C. moschata* c. rind of *C. moschata* d. seed of *C. maxima* e. pulp of *C. maxima* and f. rind of *C. maxima**

The SEM of seed, pulp, and rind of pumpkins flour are shown in Fig. 2a–f. The micrographs of the pumpkins flour show that the surface is rough and compact. The granule size of pumpkins flour is...
ranged from 2.5–12 nm. Its compact structure might be affected by protein-lipid complexes [11].

3.3. Antioxidant activity

The antioxidant activity of the extract of *C. moschata* and *C. maxima* was evaluated using DPPH assay.

| Samples          | DPPH scavenging activity (%) at 800 µg/ml |
|------------------|-------------------------------------------|
| Seed (*C. moschata*) | 25.42±1.5                              |
| Rind (*C. moschata*) | 21.58±1.2                               |
| Pulp (*C. moschata*) | 23.85±1.1                               |
| Seed (*C. maxima*) | 17.99±0.8                                |
| Rind (*C. maxima*) | 33.80±2.1                                |
| Pulp (*C. maxima*) | 29.60±1.8                                |

From Table 1, it can be observed that the scavenging activity of pumpkin flour extract (seed, rind, and pulp) ranged from 17.99 to 33.80% at concentration extract of 800 µg/ml. Ascorbic acid as a positive control in this experiment exhibited an activity of 89.31% at concentration of 200 µg/ml. Among the pumpkins flour extracts, the rind of *C. maxima* shows the highest scavenging activity of 33.80% at concentration extract of 800 µg/ml. It was followed by the pulp of *C. maxima*, seed of *C. moschata*, pulp of *C. moschata*, rind of *C. moschata*, and seed of *C. moschata* as of 29.60%, 25.42%, 23.85%, and 21.58% at concentration extract of 800 µg/ml, respectively. The extract of pumpkins have differences activities in DPPH assay possibly related to the differences compounds in the extract.

Several active compounds were detected on the pumpkin such as morin and catechin [12]. Pumpkin seeds are considered to be wastes, although in several countries they are consumed at a domestic scale [13]. Phenolic compounds and tocopherols which are rich of proteins, antioxidants and lipid were obtained in the seeds of pumpkin [14]. It is usually used in traditional medicine due to its antidiabetic, anti-bacterial, and antineoplastic activities [15]. A study also showed that protein isolated from pumpkin seed exhibited anti-peroxidative properties [16]. In particular, another study showed that diet consumption of pumpkin seed prevents the start of prostate testosterone-induced hyperplasia [17].

3.4. FTIR spectroscopy

FTIR spectroscopy is a technique for evaluating the functional groups as part of a compound in chemical analysis. In the food industries, it was also used as a monitoring quality control because of its rapid screening and quantification of chemical components in samples. Figure 2 shows the spectra of pumpkins flour (pulp, rind, seed) of *C. moschata* and *C. maxima*.

![Figure 2. The FTIR spectra of (a) *C. moschata* and (b) *C. maxima*.](image-url)
As can be seen in Figure 2, the FTIR spectra of pumpkins flour of seed, pulp, and rind of *C. moschata* (a) and *C. maxima* (b) were evaluated in frequency of 4000–500 cm\(^{-1}\). The range of frequency at 2950–2850 cm\(^{-1}\) is related to CH absorptions. Generally, a frequency at 3450–3250 cm\(^{-1}\) is hydroxyl groups (O-H) from carbohydrate or other compounds such as carboxyl acid and ketone. The absorption around 3120–3030 cm\(^{-1}\) is associated with (N-H) groups from amide. The bands at 1105–1010 cm\(^{-1}\) is associated with lipids. These spectra were in accordance with the literature [6].

### 3.5. Colour test

Table 3 shows the result of colour analysis of seed, pulp, and rind of *C. moschata* flour.

| Samples          | L*   | a*    | b*    |
|------------------|------|-------|-------|
| Seeds (C. moschata) | 43.5±0.2 | 10.6±0.2 | 29.0±0.1 |
| Rind (C. moschata)   | 67.8±0.5 | 4.9±0.3  | 22.9±0.3  |
| Pulp (C. moschata)    | 75.1±0.6 | 6.8±0.4  | 31.9±0.8  |
| Seeds (C. maxima)    | 66.7±0.7 | 5.5±0.2  | 35.9±0.9  |
| Rind (C. maxima)     | 61.1±0.7 | 17.0±0.6 | 44.6±1.0  |
| Pulp (C. maxima)     | 74.3±0.4 | 9.8±0.6  | 43.0±1.3  |

Highest b* values are related with the yellowish colour of the *C. moschata* flour. The b* values range from 22.9 to 44.6. The *C. maxima* pumpkin have higher b* values than the *C. moschata* pumpkin. The a* values are correlated with the reddish colour of the pumpkin flour. The a* values range from 4.9 to 17.0. The L* are related with the lightness, in this study the L* values range from 43.5 to 75.1.

### 4. Conclusion

In conclusion, the antioxidant activities of *C. maxima* were higher than those of *C. moschata*. This study showed that both of *C. maxima* and *C. moschata* flour (pulp, rind, and seed) is potential comestible that rich of antioxidants with DPPH radical scavenging activity in the range of 17.99 to 33.80% at concentration of 800 µg/ml.

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