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## Cover Page Footnote

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# Effect of Mechanical Milling on the Total Phenolic Content and Antioxidant Activity of *Garcinia mangostana* Pericarp

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## Abstract

This study aimed to identify the effect of mechanical milling on the total phenolic content and antioxidant activity of mangosteen pericarp. Mangosteen pericarp was milled under different milling times (30, 90, 150, and 210 min). The particle morphology before and after milling was observed by scanning electron microscopy (SEM), and the average particle size was obtained from SEM images and analyzed statistically. The antioxidant activity was measured through the 2,2-diphenyl-1-picrylhydrazyl method. The total phenolic content for the non-milling sample was $14.52 \times 10^4$ µg GAE/g sample, and the highest total phenol content was $17.44 \times 10^4$ µg GAE/g sample for the sample milled for 210 min. The $IC_{50}$ value decreased for samples with milling 150 and 210 min, which showed strong antioxidant activity, whereas the value of gallic acid equivalent increased. SEM observations showed the presence of agglomeration in the morphology of mangosteen pericarp samples. The average particle size of the mangosteen pericarp decreased as the milling time increased (up to 4499 nm for samples milled for 210 min). Therefore, mechanical milling had a significant effect on the phenolic content and antioxidant activity, which indicated an increase in the bioavailability of mangosteen pericarp.

*Keywords: mangosteen pericarp, mechanical milling, antioxidant activity, phenolic content*

## Introduction

*Garcinia mangostana*, famously known as mangosteen, is a fruit that grows in tropical regions of Asia, such as Thailand, Malaysia, India, the Philippines, and Indonesia [1]. Mangosteen fruits are round in shape and consist of an outer pericarp, inner pericarp, aril, and cap. Mangosteen pericarp has a dark purple to red-purple color, but its aril is white. Parts that can be eaten directly from mangosteen fruit are only around 34% of the aril. Consequently, large amounts (around 66%) of the mangosteen pericarp are discarded as waste. However, the mangosteen pericarp is rich in phenolic compounds, such as xanthones, anthocyanins, proanthocyanidins, tannins, and phenolic acid [2]. Phenolic hydroxyl groups are useful in hydrogen donors, in which hydrogen-donating antioxidants react to reactive oxygen and reactive nitrogen species in a termination reaction [3]. Therefore, phenolic compounds in mangosteen pericarp have been extensively evaluated as a good source of natural antioxidants.

A previous report showed that a xanthone from mangosteen pericarp extracted with n-hexane, benzene, acetone, and 70% MeOH [4]. Meanwhile, α-mangostin of mangosteen pericarp was extracted separately in water and 50% ethanol. In general, the extraction process used to obtain the active compound in organic materials will leave liquid waste. This liquid waste has a negative impact on the environment because it contains organic

substances that make the liquid waste have a high chemical oxygen demand [4–5]. Moreover, liquid waste that is directly discharged into water decreases the dissolved oxygen level in that water, which causes a disrupted aquatic ecosystem that harms the biota in the water. An increase in the number of industries and production activities will cause serious problems. Therefore, a direct method to process organic materials is needed as an alternative to reduce waste. To date, studies on mangosteen pericarp that is processed directly without an extraction process are limited. Consequently, in this study, mangosteen pericarp was directly processed without any extraction and developed to be a functional drink that is practical and efficacious due to its small particles.

The particle size used in this study was reduced through a top-down method, namely, ball milling. A previous study [6] reported significant differences in the composition and concentration of phenolic acids in eight durum wheat samples observed between kernel parts (starchy endosperm, aleurone layer, and pericarp) due to the milling process. Therefore, this study aimed to understand the effect of the mechanical milling process on the total phenolic content and antioxidant activity of mangosteen pericarp.

## Materials and Methods

**Preparation of mangosteen pericarp.** Mangosteen pericarp was obtained from Purwakarta, Indonesia. The fruits obtained without physical or insect damage were washed, and the aril and pericarp of the mangosteen were separated. Subsequently, the mangosteen pericarp was boiled at 200 °C – 250 °C for 20 min to eliminate the tannin compounds from the pericarp samples. The boiled pericarp of mangosteen was cooled to room temperature through soaking with ice water to speed up the cooling process. The boiled pericarp of mangosteen was cut into a small size (about 0.5 $cm^2$) and dried at 65 °C for 12 h. Mangosteen powder was obtained by pulverizing the dried mangosteen and sieving it at 80 mesh. Further pulverization of mangosteen pericarp was performed by high-energy ball mill machines (HEM-E3D). The total milling time was 210 min. However, milling was stopped at 30, 60, 90, and 150 min to obtain the samples for analysis. The powder of mangosteen samples was physically tested for moisture content, solid content, and flow properties. The water content was measured by calculating the amount of water in the sample using the iodometric titration method. About 1 mg of the sample was transferred into a glass beaker. Testing was carried out with three repetitions for each sample. The solid content was measured by adding 0.5 g of the sample to an aluminum cup, and the drying shrinkage levels were measured. The results showed the maximum limit of the compounds lost in the drying process, and it was expressed as a percentage. The results were used to calculate the total solids in the sample. Flow properties were determined by a flow tester. About 5 g of the sample was placed in the funnel by covering the bottom and testing the flow time by measuring the powder cone height and surface diameter. Flow properties were determined by the tangent value, which signifies the nature of flow.

**Total phenolic content.** The total phenolic content was determined based on the research of Ghafar *et al*. [7]. This method applies colorimetric reactions measured using a Ultraviolet-Visible (UV-VIS) Spectrophotometer, which requires a substance reference, to measure the concentration of total phenolic content of hydroxyl groups in plant extract [8]. Polyphenol in plant extracts reacts with Folin–Ciocalteu reagent (specific redox reagents) to form a blue complex in a color that can be quantified [9]. Phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin–Ciocalteu reagent. About 10 mg of mangosteen pericarp samples that have been milled in specific time variations was dissolved in 1 mL of methanol p.a. Each sample was sonicated for 10 min and centrifuged at 10,000 rpm and 25 °C for 15 min. Three replications of each sample were prepared with a standard solution of gallic acid. The samples were diluted to a concentration of 200 ppm. Subsequently, 200 µL samples were mixed with 750 µL of Folin–Ciocalteu and left at room temperature for 5 min. Sodium carbonate (6%) was added, mixed at room temperature, and maintained for 60 min. The absorbance of the samples was measured at the wavelength 725 nm. The results were expressed as milligrams of gallic acid equivalent per gram of dry sample (mg GAE/g sample). The concentration of gallic acid used in this measurement was 10, 20, 30, 40, 50, and 70 ppm, and the absorbance was measured at 725 nm.

**Antioxidant activity.** The antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a radical. First, 10 mg of sample was dissolved into 1 mL of 80% ethanol, sonicated, and centrifuged for 15 min. The supernatant was dissolved in methanol p.a as a master sample to be diluted into 10 different concentrations (25, 50, 75, 100, 125, 150, 200, 250, 300, and 350 ppm) with three replications to obtain a functional regression. About 50 µL of every diluted sample was obtained and added with 1 mL of DPPH. Furthermore, the samples were incubated for 30 min and stored in the dark. The absorbance of the samples was measured at 517 nm. Ascorbic acid was used as a positive control at concentrations of 5, 10, 25, 50, and 75 ppm. The negative control contained only the DPPH solution, while the blank contained 80% ethanol. The scavenging activity of mangosteen pericarp samples was tested following the method described by Ghafar *et al*. [7]. The scavenging effect was determined by the ratio of decreased DPPH absorption against the absorption of DPPH solution (negative control) using Eq. (1).

$$Scavenging\ (\%) = \frac{Abs_{(control)} - Abs_{(sample)}}{Abs_{(control)}} \times 100 \quad (1)$$

A plotted graph was made to understand the relationship of scavenging against the concentration of each sample. From the linear equation, the inhibition concentration ($IC_{50}$) of the sample was calculated [7]. The value of $IC_{50}$ describes the total antioxidant needed to reduce the free radicals in DPPH as much as 50%.

**Characterization of mangosteen pericarp.** The particle morphology of mangosteen pericarp before and after mechanical milling was observed by scanning electron microscopy (SEM; FEI Quanta 650). The water content of the mangosteen pericarp was analyzed using a Karl Fischer Moisture Titrator. All samples were tested for flow properties using flow tester tools. The total phenolic content and antioxidant activity were measured by Thermo Spectronic Helios Alpha 9423 UVA 1002E UV-VIS Doublebeam Spectrophotometer.

## Results and Discussion

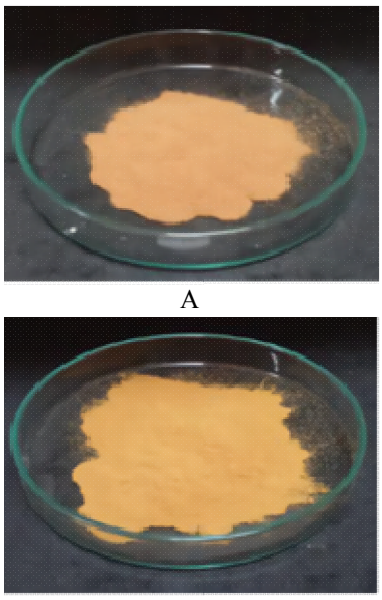
**Chemical properties of mangosteen pericarp.** In this study, the initial stages of preparation of the samples began with boiling the mangosteen pericarp in the water. This stage was used to separate tannins from mangosteen pericarp. Yokozawa *et al*. reported that tannins have more potential than flavonoids because almost all the tannins demonstrate significant scavenging action in low concentrations [10]. Subsequently, the mangosteen pericarp was dried at 70 °C for 12 h to maintain the polyphenol content of the mangosteen pericarp on the basis of the study of Satong-aun *et al*. [11], who reported that the drying temperature of 65 °C yields a high content of α-mangostin.

Physical and chemical data, such as particle analysis, flow properties, moisture, solid content, solubility value, total phenolic content, and antioxidant activity, from mangosteen pericarp samples are presented in Table 1. The moisture content of the mangosteen pericarp dried at 70 °C for 12 h had an average value of 5%–7%. The difference in moisture content between samples before and after milling was not significant. The moisture content of mangosteen pericarp powder for all treatments attained the Indonesian National Standard (SNI) 01-4320-1996 [12], in which the standard water content in instant powdered drinks is 3%–7%. Meanwhile, the substantial content value was related to the value of drying losses. This test revealed how many compounds in mangosteen pericarp were lost or volatile during the drying process. The drying loss data of mangosteen pericarp met the standards of the Health Department of the Republic of Indonesia [13], which was < 11.00. The flow properties of all parameters were at an angle of 25°–45°, which indicated that the natural powder could flow easily.

Mangosteen pericarp samples were prepared under different ball milling times to determine the treatment effect on the total phenolic content and antioxidant activity compared with the sample of mangosteen pericarp without milling. In this study, the different ball milling times were 30, 90, 150, and 210 min. Physically, both mangosteen pericarp samples, not milled and milled, had the same color, but mangosteen pericarp powder samples that were milled for 30, 90, 150, and 210 min showed a smoother texture than those that were not milled (Figure 1).

The total phenolic content for non-milling sample was $14.52 \times 10^4$ µg GAE/g sample, whereas the total phenolic contents for samples that were milled for 30, 90, 150, and 210 min were $14.06 \times 10^4$, $13.33 \times 10^4$, $14.23 \times 10^4$, and $17.44 \times 10^4$ µg GAE/g, respectively. The total phenolic content of mangosteen pericarp increased when the milling time exceeded 90 min. The phenolic substances or polyphenols contain numerous varieties of compounds, such as simple flavonoids, phenolic acids, complex flavonoids, and colored anthocyanins [14]. This result was probably due to the transformation of the functional group of phenolic compounds that altered the structure of the samples.

Table 1 also shows that mechanical milling decreased the particle size of mangosteen pericarp, and the smallest particle size was obtained for the milling time of 210 min, i.e., 4499 nm. Indeed, the particle size before and after milling significantly differed, showing the effectiveness of the milling process to decrease the particle size of mangosteen pericarp. However, the shape of mangosteen pericarp was similar before and after milling, as shown in Figure 2.



A

**Figure 1. Samples of Mangosteen Pericarp: (A) Not Milled and (B) Milled for 90 min**

**Table 1. Physical and Chemical Properties of Mangosteen Pericarp**

| Physical and Chemical Properties | Before Milling | After Milling | | | |
|---|---|---|---|---|---|
| | | 30 Min | 90 Min | 150 Min | 210 Min |
| Particle Size [SEM] (nm) | 21377 ± 17.68 | 10387 ± 4.535 | 6435 ± 2.928 | 5219 ± 2.232 | 4499 ± 1.478 |
| Total Phenolic Content ($\times 10^4$ µg GAE/g sample) | 14.52 | 14.06 | 13.33 | 14.23 | 17.44 |
| Antioxidant Activity ($IC_{50}$ µg/mL) | 478 | 326.47 | 308.76 | 300.94 | 254.84 |
| Moisture Content (%) | 5.55 ± 0.66 | 6.68 ± 0.03 | 5.70 ± 0.04 | 7.31 ± 0.03 | 5.80 ± 0.32 |
| Solid Content (%) | 91.21 ± 0.62 | 93.11 ± 0.84 | 92.75 ± 1.11 | 93.34 ± 0.15 | 93.33 ± 0.31 |
| Flow Properties ($^o$) | 14.35 ± 0.75 | 12.86 ± 2.93 | 28.95 ± 3.37 | 8.69 ± 0.93 | 22.72 ± 3.87 |
| Solubility Value ($\times 10^{-2}$ g/mL) | 2.49 ± 0.01 | 1.89 ± 0.01 | 2.15 ± 0.01 | 2.18 ± 0.01 | 2.15 ± 0.01 |

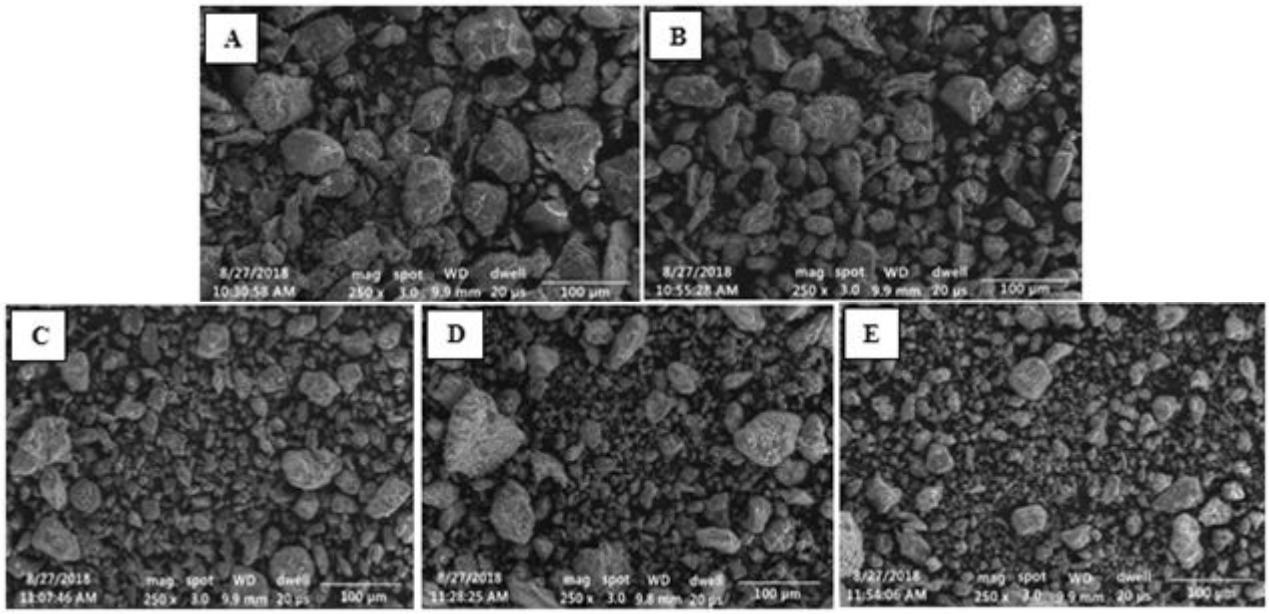



**Figure 2. SEM Images of Mangosteen Pericarp (Magnification, 250×): A. Mangosteen Pericarp not Milled; B. Mangosteen Pericarp Milled for 30 min; C. Mangosteen Pericarp Milled for 90 min; D. Mangosteen Pericarp Milled for 150 min; and E. Mangosteen Pericarp Milled for 210 min**

Figure 3 shows the presence of agglomeration in the sample. Naturally, the process of milling on the material to produce nanoparticles has two consequences, namely, fracture and agglomeration. The first possible condition that occurs during milling is fracture of the particle as a result of a sufficiently high-stress field inside the particle, which builds up during impact between the media [15]. The second possible condition is agglomeration due to the small particle size (below 1 µm). The particles tend to agglomerate because of the increase in Brownian motion and the small interparticle distances. Both conditions enhanced the collision rate of the particles [15].

In this study, the total phenolic content and antioxidant activity were measured to determine the effects of variations in milling time. The phenolics present in fruits have received extensive attention because of their potential antioxidant activity. Polyphenols are the main compound in mangosteen pericarp extract and tea [16]. Figure 4 shows the curve of the gallic acid standard for the measurement of the total phenolic content. In the total phenolic content measurement, gallic acid was used as a positive control. The results were expressed in gallic acid equivalent (GAE).

Antioxidants have an "aromatic ring structure," which allows them to neutralize free radicals. The method used to determine the antioxidant activity of mangosteen pericarp was based on DPPH as source of free radical. The DPPH is a synthetic radical that does not disintegrate in water, methanol, and ethanol [17]. It is stable at room temperature and produces a violet solution in alcohol

[18]. Mangosteen pericarp samples were found to show radical scavenging activity against DPPH. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen [19]. The $IC_{50}$ value was obtained by plotting the scavenging activity on a curve, as shown in Figure 5. The $IC_{50}$ value of the mangosteen pericarp samples is shown in Table 1.

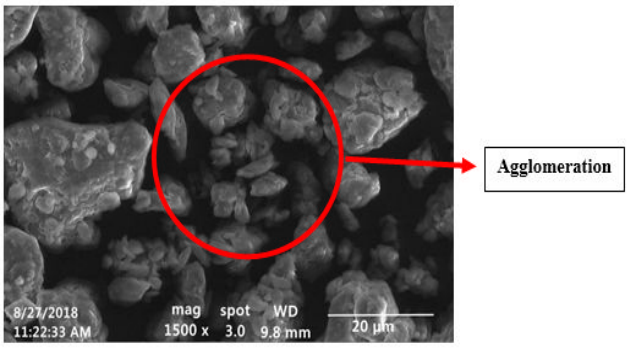



**Figure 3. Morphologies of Mangosteen Pericarp Milled for 150 min**

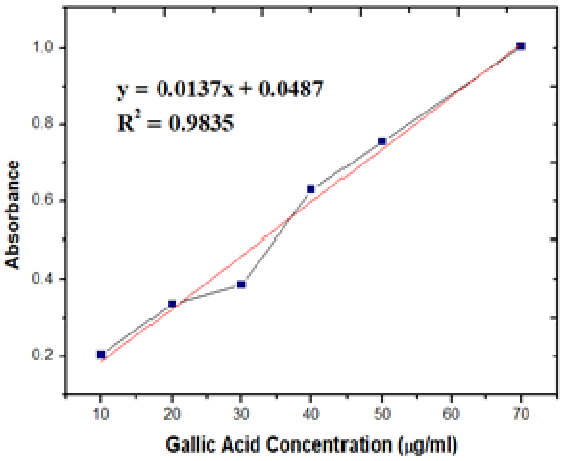



**Figure 4. Standard Curve of Gallic Acid**

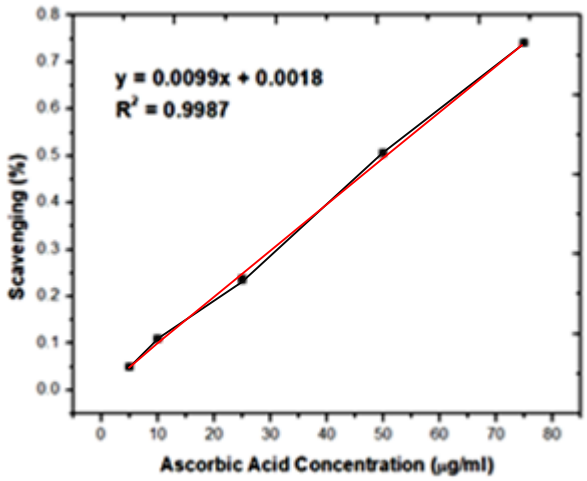
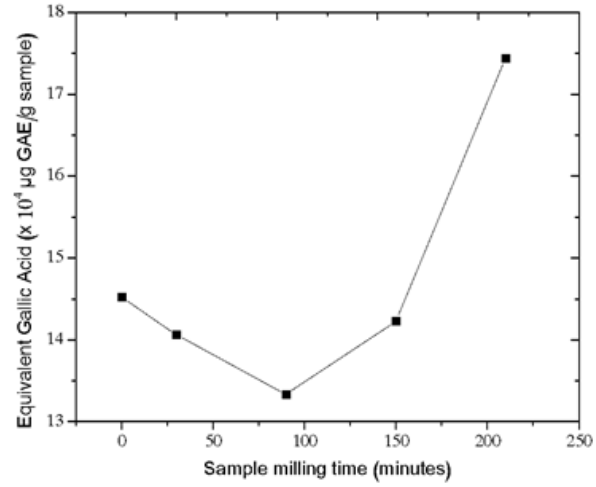
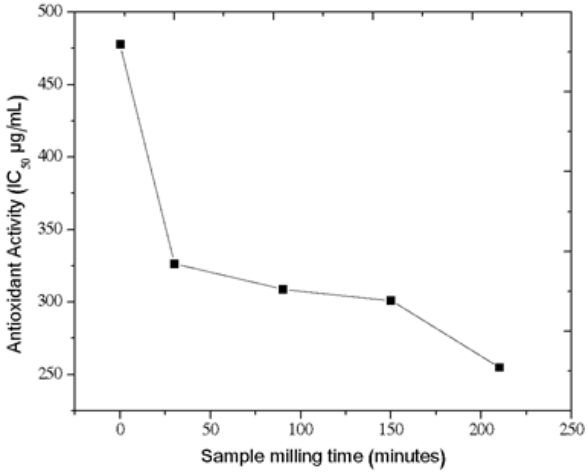



**Figure 5. Scavenging Activity Curve of Ascorbic Acid**




A




B

**Figure 6. Curve of Gallic Acid Equivalent (A) and $IC_{50}$ (B) from Mangosteen Pericarp Samples**

A low $IC_{50}$ value suggested strong antioxidant activity, and vice versa. Ascorbic acid has an $IC_{50}$ value of 50.32 µg/mL.

Figure 6 shows the relationship between gallic acid equivalent as a total phenolic content parameter and $IC_{50}$ as an antioxidant activity parameter of mangosteen pericarp samples. The $IC_{50}$ values of samples milled for 150 and 210 min decreased, which showed strong antioxidant activities, whereas the values of GAEs increased. This result showed a positive correlation between the total phenolic content and antioxidant activity, which was consistent with the existing theory. However, no correlation was found between the two parameters for the non-milling mangosteen pericarp samples and samples milled for 30 and 90 min. These findings might be due to the differences in the type of phenolic compounds and the amount of individual phenolic compound present in the samples.

## Conclussion

Results showed the presence of agglomeration in the milled mangosteen pericarp samples. The particle size decreased as the milling time increased. The smallest particle size was obtained in the mangosteen pericarp sample milled for 210 min with a size of 4499 nm. The milling time affected the total phenol content and the antioxidant activity of mangosteen pericarp samples. At high milling time (150 and 210 min), the total phenolic content increased with the increase in the milling time accompanied with a decrease in the $IC_{50}$ value, indicating strong antioxidant activity.

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