# Characteristic of *Garcinia Mangostana's* Pericarp Prepared by Mechanical Milling

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Abstract: *Garcinia mangostana*, commonly known as mangosteen is a tropical fruit that grows in Asian region. Nano Mangosteen's Pericarp have successfully been made by ball milling method with variations in milling time (30, 90, 150, 210 minutes), and non milling as a comparison. Mangosteen pericarp was dried in an oven at 70 ˚C for 12 hours, then continued by grinding and sieving using mesh 80, then milled. The effect of variations in milling time on the functional groups of nano mangosteen pericarp, also the correlation on total phenolic content and antioxidant activity in previous research were investigated. The morphology of the mangosteen pericarp shows that the grain size of mangosteen pericarp is getting finer along with increasing milling time. It can provide a clear reason for explaining the increasing solubility value of the samples. The zeta potential data shows that after being milled the mangosteen pericarp becomes unstable, thus it is easy to agglomerate. It was obtained that the total phenolic content and the antioxidant activity increased followed by longer milling times. The FTIR analysis indicated that the enhancement in total phenolic content is not due to transformation in functional groups of phenolic compounds.

## 1 INTRODUCTION

Free radicals are atoms or molecules that have unpaired electron. The unpaired electron cause free radicals to be very reactive then take electron from other compounds such as proteins, lipids, carbohydrates, and DNA to neutralize themselves (Liochev, 2013). The negative effects of free radicals on the body can be prevented by compounds called antioxidants. Antioxidants have the ability to give electron, bind, and end free radical chain reactions (Halliwell, 2012). Antioxidants are electron-giving compounds (donor electrons), which are able to inactive the development of oxidation reactions by preventing radical formation.

Antioxidants are compound that can slow or prevent the oxidation process. This compound can significantly slow down or inhibit the oxidation of substances that are easily oxidized even in low concentrations. Based on (Buck, 1991) sources of antioxidants are divided into two, namely natural antioxidants and synthetic antioxidants. Synthetic antioxidants are antioxidants obtained from the synthesis of chemical reactions and are produced for commercial purposes. Examples of synthetic antioxidants include Butyl Hydroxy Anisol (BHA), Butyl Hydroxy Toluene (BHT), Propyl galate, Tert-Butyl Hydroxy Quinon (TBHQ), Tocopherol and others. Natural antioxidants can generally be obtained from phenolic compounds or plant polyphenols which can be in the form of alkaloids,



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flavonoids, saponins, quinones, tannins, sterids/ triterpenoids (Gordon, 1994). Natural antioxidants are generally more desirable than synthetic antioxidants. Many natural antioxidants are plants, vegetables and fruits (Winarsi, 2007). Among fruits that contain a lot of antioxidant compounds are mangosteen fruit, especially on the mangosteen pericarp. Based on phytochemical research, mangosteen pericarp contains phenolic antioxidant compounds. These phenolic compounds are xanthones, anthocyanins, tannins, epikatekin and other phenolic acid compounds (Zadernowski *et al.,* 2009).

One of the problems that occur in the utilization of mangosteen pericarp waste, that is the active compound, the source of natural antioxidants tends to be less practical, has instability to the color, low solubility, and bioavailability that still tends to be low. Currently, nanotechnology is developed in food and drug products that can accelerate the rate of release of compounds of active compound, increase solubility and increase absorption in the body. (Ningsih *et al.,* 2017).

In the previous study, it was observed the effect the time of milling of mangosteen pericarp powder with a variation of time 0, 30, 90, 150 and 210 minutes using ballmill on its effect on antioxidant activity and total phenolic. It was reported that the particle size of mangosteen pericarp powder affects levels of total phenol and antioxidant activity seen from $IC_{50}$ values. Mangosteen pericarp with the smallest particle size has the largest total phenol content and the smallest $IC_{50}$. The largest total phenol content indicates the content of polyphenol compounds in samples with a large amount of content. $IC_{50}$ values describe the total antioxidants needed to capture free radicals as much as 50%. The results of Daratika *et al.,* 2018 reported that there was an increase in total phenol content along with an increase in milling time.

According to (Garcia's, 1999) study, the relative ability of flavonoids from olive leaves to absorb radical cations using the ABTS + method is influenced by the presence of different functional groups, especially in the number and position of free hydroxyl groups in their structure. This is possible because of changes in functional groups along with changes in particle size. FTIR testing is carried out to analyze whether there is a change in functional groups in the organic compounds of mangosteen pericarp powder. This research are in accordance with the result that there was a positive correlation between flavanoid content and particle size in rice accession (Shen, 2009). There is also a study in (Luthria, 2008), that the value of total phenol content extracted from parsley leaves is obtained when the particle size of the extraction results is getting smaller.

In addition, it was reported that agglomeration in the morphology of mangosteen pericarp powder samples. According (Darusman, 2014) study, there was clumping or agglomeration of pure GMP (Glimepirid) drug particles which caused GMP to be hydrophobic so it was difficult to dissolve in water. Therefore, in this paper we will discuss potential zeta in each sample to find out its relation to solubility. The greater the potential zeta value, the better the stability of the solution will be to reject aggregation (Sari *et al.,* 2013). The purpose of this study was to characterize mangosteen pericarp samples including zeta analysis of potential mangosteen peel nano powder which might be influenced by the process when milling and identify the increase in the value of total phenol content with the hypothesis influenced by changes in functional groups.

# 2 MATERIAL AND METHODS

The raw material for grade A mangosteen is taken from Purwakarta, West Java. The material used consists of water, potassium bromide (KBr), Ethyl Alcohol. Whereas the tools used consist High Energy Milling Machine- Ellipse 3 Dimension (HEM-E3D), Bruker Tensor 37 FTIR Spectroscopy, Delsa Nano C-Particle Size Analyzer, and FEI Quanta 650-Scanning Electron Microscopy.

## 2.1 Preparation of Mangosteen Pericarp

Preparation of mangosteen pericarp powder samples was carried out in according with previous studies. In Daratika's *et al,.* research 2018, the raw material for mangosteen fruit is obtained from Purwakarta, Indonesia plantations. The pericarp of the mangosteen is boiled at a temperature of 200-250 °C for 15 minutes. Then the soaking process is done by using cold water to accelerate the cooling of the sample. Furthermore, the mangosteen pericarp slashed into small sizes around 50 mm in size. Then the mangosteen pericarp is dried at 65 °C. According to Afifah and Niwat, 2015 drying samples at temperatures below 75 °C can protect the damage of polyphenols. The mangosteen pericarp is then milled and sifted on 80 mesh. The process of removing the mangosteen powder particles in this study is the top

down method using the HEM-Ellipse 3 Dimension machine. Variations in milling time were observed as parameters to determine the relationship between milling time and particle size.

## 2.2 Characterization of Mangosteen Pericarp

Mangosteen pericarp powder was characterized by particle size and potential zeta using Delsa Nano C-Particle Size Analyzer. The sample was dissolved with ethyl alcohol solvent and measured at temperature 25 °C with refractive index 1.3611, liquid viscosity 1.1015 cPoise and scattering intensity 8238 cps. While potential zeta is analyzed to determine the nature of nanoparticle surface loads and distribution potential particle samples. The sample was dissolved in a water solvent then 0.9 mL of the sample was measured under conditions of temperature 24.8 °C, refractive index 1.3328, viscosity 0.8919 and dielectric constant 78.4.

SEM testing is also done to find out information about surface topography, composition, and other characteristics such as electrical conductivity. Tests are carried out using Scanning Electron Microscopy-FEI Quanta 650. To determine the solubility, the method of Al-Kahtani and Hassan (1990) was applied. By10 grams of sample and 100 mL of distilled water was put into a beaker glass. Rotate using magnetic bar at a speed of 200 rpm at room temperature 25 ° C (Jittanit, 2011).

## 2.3 Fourier Transform Infrared Spectroscopy- Bruker Tensor 37

Making pellets is done by entering 200 mg KBr into the mortar and mixing with 2 mg of sample. Mix until homogeneous and done quickly. The homogeneous mixture then made pellet with a mini hand press tool and pay attention to the process. The resulting pellets are stored in a dry place.

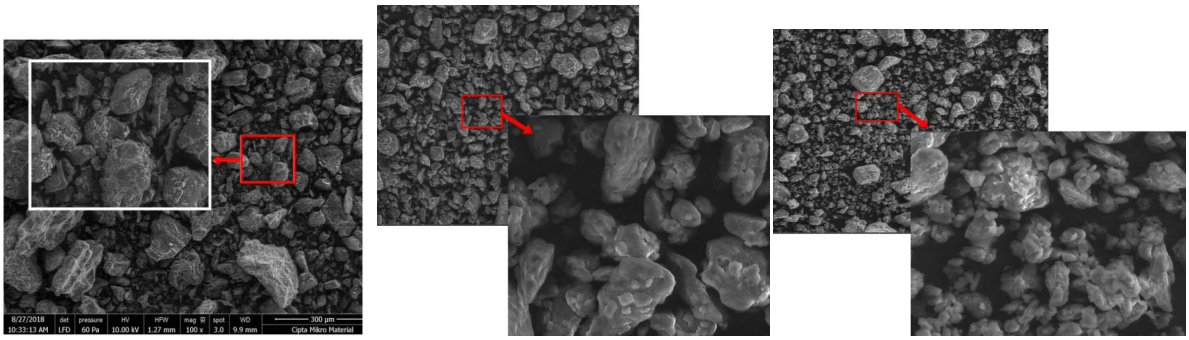
# 3 RESULT AND DISCUSSION

The morphology of the mangosteen pericarp samples measured using Scanning Electron Microscopy (SEM) can be seen in Fig. 1 and 2 indicating that the grain size getting finer along with increasing holding time of milling. Daratika *et al.,* (2018) reported that non milling mangosteen pericarp (escaped mesh 80) with a particle size of 438 nm had grain sizes around 82,142 nm, milled for 90 minutes with a particle size of 257 nm had grain sizes around 21,613 nm, and milled for 210 minutes with a particle size of 205 nm had grain sizes around 1,855 nm.

Naturally, the process of milling on a material in order to produce nanoparticles has two consequences, namely fracture and agglomeration. The first possible condition that occur when milling is fracture of the particle as a result of a sufficiently high stress field inside the particle which buildup during the impact between the media (Knieke C., 2012). The second possible condition is agglomeration, when the particle size are below 1 μm, the particles tend to agglomerate because of Browns's motion increased and smaller interparticle distances. Both of them enhancing the collision rate of the particles (Knieke C., 2012).

In mangosteen pericarp samples before milling (Fig. 1) and mangosteen pericarp samples which were milled 90 minutes and 210 minutes (Fig.2) there was a difference that the morphology of the mangosteen pericarp samples before milling did not occur between small particles and larger particles. Whereas in the mangosteen pericarp sample after milling there is a clumping or collision between smaller particles and larger particles. This indicates that there is agglomeration. Particle agglomeration can be interpreted as forming a collection of particles in solution and is one of the mechanisms that causes destabilization of colloids. There are several things that cause agglomeration, including the smaller particle size.

In DLVO theory, (Derjaguin and Landan, 1941), the agglomeration and stability of particle dispersion are determined by the sum of the attractive and repulsive force between individual particle. The attraction between particle is due to the van der Waals force. The interaction of the electrical double layer surrounding each particles is called electrostatic repulsive force. This theory indicate that agglomeration is related to the potential zeta value. The potential zeta value is used to characterize the characteristic of particle surface charge associated with nanoparticle electostatic interactions (Couvreur *et al.,* 2002). Electrostatic interactions are resistive repulsive forces between particles that affect stability in the suspension so that they can prevent particle aggregation.



(a) (b) (c)

Figure 1: The morphologies of mangosteen pericarp (non milling) mag 250x and 500x (a); milling 90 minutes (b); milling 210 minutes (mag. 250x and 1500x) (c).

Reflect the electrical potential of particle and is influenced by the composition of the particle and the medium in which it is dispersed (Singh *et al.,* 2009). The greater the electrostatic ability of a charge, the more stable it is rejecting aggregation. Conversely the smaller the electrostatic ability, the weaker it is to resist aggregation.

Table 1 shows the solubility time of the mangosteen pericarp milled in a certain time variation (30, 90, 150, and 210 minutes) and non milling as a comparison. Along with the addition of milling time in the mangosteen pericarp samples dissolved in certain water with a temperature of 25 ºC. It can be understood because the longer milling time of mangosteen pericarp samples has a smaller grain size, also followed by the smaller particle size. Solubility of a substance will increase along with the reduces particle size of substance.

Table 1: Solubility time and zeta potential of mangosteen pericarp samples.

| Sample | Measurement Method | |
|---|---|---|
| | Solubility Time (s) | Zeta Potential (mV) |
| Before milling | 312.67±9.50 | 118.96 |
| After milling | | |
| 30 minutes | 196.33±2.08 | -18.03 |
| 90 minutes | 58.03±3.50 | -16.56 |
| 150 minutes | 44.73±1.95 | -20.3 |
| 210 minutes | 33.4±2.19 | -14.08 |
| 30 minutes | 196.33±2.08 | -18.03 |

Zeta potential is a parameter of electrical charge between particles in colloids. The magnitude of zeta potential provides information about stabilization of the samples Patel *et al.,* (2011) informing about the guidelines for classifying nanoparticles dispersions with zeta potential value (±) 0 – (±) 10 mV are very unstable, (±) 10 – (±) 20 mV are relatively stable, (±) 20 – (±) 30 mV are quite stable, and > (±) 30 mV are very stable. The higher zeta potential value, the more it will prevent flocculation. Zeta potential of mangosteen pericarp samples can be observed in Table 2. Non-milling mangosteen pericarp have a very high zeta potential value, which shows the repulsive forces of the particle are stronger, then the samples have a high stability to resist aggregation. While, for mangosteen pericarp samples that are milled at a certain time variation (30, 90, 150, and 210 minutes) have a potential zeta value of less than 30 mV. The low potential zeta represents the attractive forces between the dispersion particles exceeding the repulsive forces.This causes these particles to be easy to agglomerate, further causing flocculation.

In the previous work, the correlation between total phenolic content and antioxidant activity against particle size of *Garcinia mangostana's* Pericarp has obtained that non milling sample with a particle size had total phenolic content around 14.52 x 104 µg GAE/g samples, while the sample which is milled for 210 minutes having the highest total phenolic content (17.44 x 104 µg GAE/g samples) accompanied by the lowest $IC_{50}$ value (254.84 µg/ml) that shows strong antioxidant activity (Daratika *et al.,* 2018). It can be observed that by applying milling to reduce the particle size of the sample produces a different total phenolic content. In the initial hypothesis, it is caused by the transformation of functional groups from phenolic compounds. A functional group is defined as a group of atoms joined in a specific manner, that gives the chemical properties of the organic compound and are the centers for chemical reactivity.

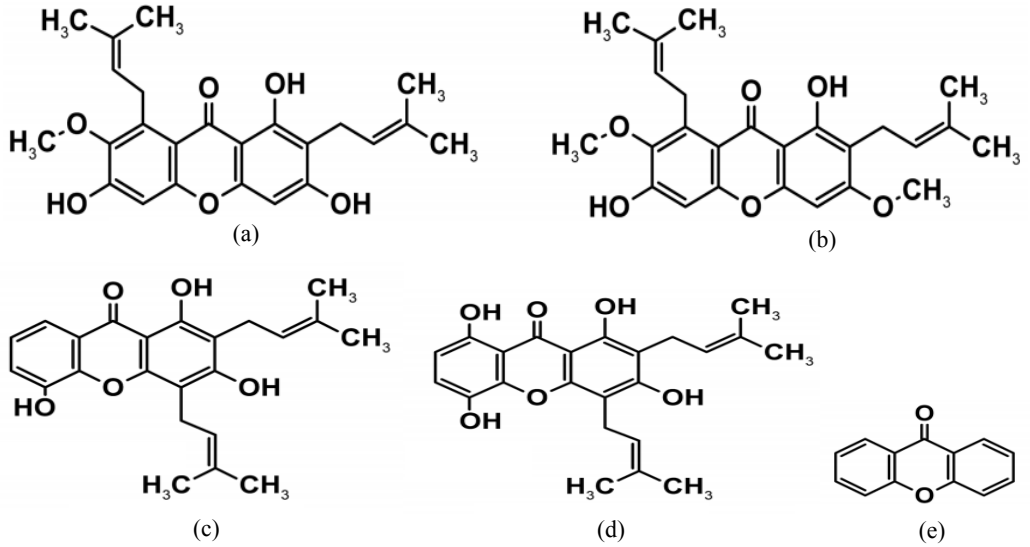



Figure 2: Chemical structure of xanthones and their derivatives: α-mangostin (a); β-mangostin (b); gartanin (c); 8-desoxygartanin (d); xanthone (e) (Walker E. B., 2007).

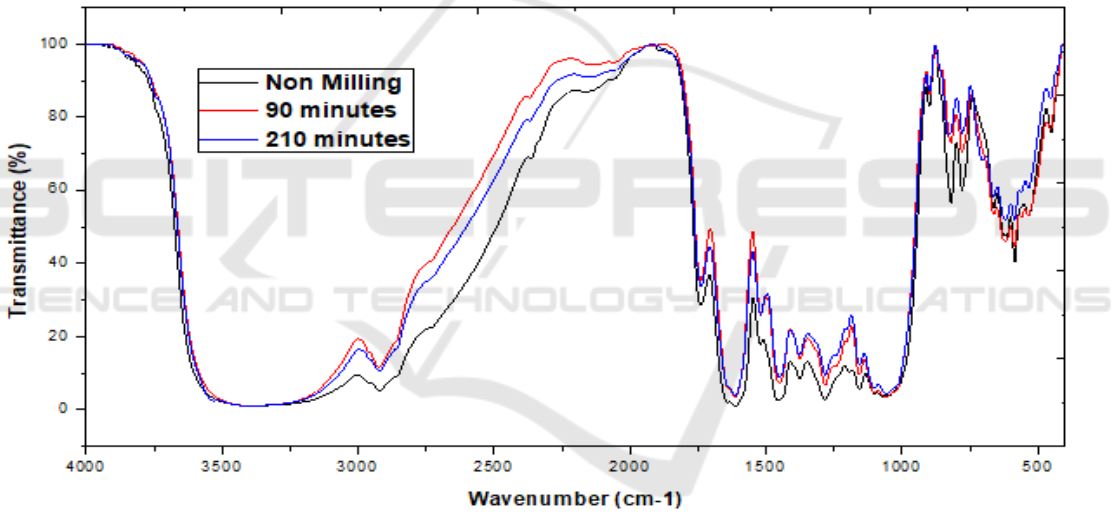



Figure 3: FTIR spectra of mangosteen pericarp.

In this study, Fourier-transform infrared spectroscopy (FTIR) measurement accomplished to obtain the functional group of mangosteen pericarp. The FTIR spectra were collected in transmission mode and covering the spectral range from 400 to 4000 cm-1 using Bruker Tensor 37 FTIR spectrophotometer. The band intensities in different regions of the spectrum for non-milling mangosteen pericarp as a control and milled samples (90 and 210 minutes) were analyzed and are shown in Figure 3. Naturally, mangosteen fruit is a rich source of phenolic compound such as xanthones, proanthocyanidins, anthocyanins, and phenolic acids (Naczk *et al.,* 2011). Mangosteen pericarp contains of α-, β-, γ- mangostin, 8-deoxygartanin, mangostinone a and b, gartanin, garcinone b and mangostanol (Muchtaridi *et al.,* 2016), (Jung *et al.,* 2006) reported that mangosteen pericarp conceived xanthones as the major phenolic, with the chemical structures can be observed in Figure 2.

The analysis using FTIR spectrophotometer shows the presence of C-H, C=C aromatic, C-O eter, O-H fenol. This functional group is appropriate with functional groups in compounds xanthone, and their derivatives with the structure as in Figure 2. Xanthones is a cyclic polyphenol ketone compound with the chemical formula $C_{13}H_8O_2$, basic structure xanthones consists of three benzene with one benzene in the middle which is a ketone.

Table 2: The functional groups of mangosteen pericarp.

| Wavenumber (cm-1) | | | Bond | | | Functional Group |
|---|---|---|---|---|---|---|
| 0' | 90' | 210' | 0' | 90' | 210' | |
| 780, 45 | 780,61 | 780,11 | C-H | C-H | C-H | Aromatic ring |
| 817,58 | 816.76 | 830.79 | C-H | C-H | C-H | |
| 899,78 | 898.54 | 898.51 | C-H | C-H | C-H | |
| 1073,82 | 1053.20 | 1053.87 | C-O | C-O | C-O | Eter |
| 1156,42 | 1156.50 | 1156.62 | C-O | C-O | C-O | |
| 1281,6 | 1282.29 | 1282.03 | C-O | C-O | C-O | |
| 1375,12 | 1374.97 | 1376.73 | C-H | C-H | C-H | Alkana |
| 1453,33 | 1451.53 | 1450.67 | C-H | C-H | C-H | |
| 1505 | 1518.83 | 1518.88 | C=C | C=C | C=C | Aromatik ring |
| 1610,97 | 1612.02 | 1612.58 | C=C | C=C | C=C | Alkena |
| 1740.14 | 1738.86 | 1739.18 | C=O | C=O | C=O | Keton |
| 2922.69 | 2925.99 | 2923.37 | C-H | C-H | C-H | Alkana |
| 3396.31 | 3397.68 | 3396.73 | O-H | O-H | O-H | Phenol |

Almost all derivative molecules of xanthones have a phenolic group in consequence xanthones often called as polyphenols. Thus, it can be seen that these compounds are polyphenols compounds contained in the mangosteen pericarp (Muchtaridi *et al.,* 2016) and have antioxidant activity. The results of FTIR data analysis showed that the functional groups of the samples (non milling and milled with variations of time 90 and 210 minutes) did not indicate a significant difference in their spectra. Therefore, it can be seen that there is no functional group transformation in the mangosteen pericarp samples. It can be understood by reason because there is no chemical treatment that causes changes in functional groups.

(Zhou *et al.,* 2004) have reported that micro-milled aleurone of Switzerland red wheat extracted with 50% acetone showed significantly higher sovent-extractable phenolic content compared to untreated counterpart, which each individual phenolic compound is examined increased for different extents. Recent work carried out (Rosa *et al.,* 2013) reported that wheat bran's antioxidant activity was linearly correlated with specific surface area, the medium particle size, and the proportion particles smaller than 50 μm in diameter. Particle-size reduction processes using ball milling, nano-ball milling, and ultra-fine grinding have been shown increase the accessibility of phenolic compounds to extraction solvents (Wang *et al.,* 2014). The basic principles of engineering nanoparticle materials is by utilizing the influence of particle size, the effect of surface area, and the interaction between nanoparticles and other materials.

In the previous study, it was found that the increase in milling time on mangosteen pericarp sample produces higher total phenolic content accompanied by stronger antioxidant activity (Daratika *et al.,* 2018). The fact obtained from the FTIR analysis stated that the difference values of total phenolic content that are milled with a certain time variation is not caused by the transformation of functional groups so that there are changes in the type and amount of phenolic compounds which present in the samples. However, there are physical processes that occur when reducing particle size. The small size of nanoparticles has a greater comparison between surface area and volume when compared to similar particle with the larger size. Consequently, there will be more atoms on the surface of the nanoparticle material that come in direct contact with material, causing this nanoparticle to be more reactive. For this reason, an increase in antioxidant activity and total phenol content is caused by the better exposition of phenolic compounds when samples milled, thereby improving the accessibility of hydrogen to bind free radicals from DPPH as synthetic free radicals to form complex antioxidants that are stable.

# 4 CONCLUSIONS

Mechanical treatment of milling results in finer particles and smaller zeta potential than samples without milling. However, the milling treatment did not change the functional group of mangosteen

pericarp. Characterization using SEM shows that the mangosteen pericarp that is milled in a longer time have a finer grains which causes agglomeration. This has an effect on increasing the solubility time along with the small particle size. This has an effect on increasing the solubility time along with the small particle size. Besides the smaller particle size also affects the total phenol content and antioxidant activity as in previous studies.

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