Abstract

Mangosteen (Garcinia mangostana L.) is one of the most popular fruits which has been widely used medicinally. The major constituents are mostly found in the pericarp particularly, xanthones, which are tricyclic isoprenylated polyphenols. Xanthones have been reported for its anti-oxidant, anti-inflammatory, anti-bacterial, anti-proliferative, proapoptotic and anti-carcinogenic activities. In this study, the mangosteen pericarps in various maturity levels were investigated using various characterization techniques such as PSA (Particle Size Analyzer), Scanning Electron Microscope (SEM), X-ray Diffraction (XRD), and X-ray Fluorescence (XRF). The results revealed the characteristic of mangosteen at various maturity levels.

Keywords: Mangosteen, Particle Size, SEM, Xanthone, X-ray Diffraction, XRD

1. Introduction

Plants and their fruits have been used since ancient time as supplement and medicine to treat diseases. Approximately two-third of world population uses plant based products as their main medicine. Garcinia mangostana L. (Clusiaceae), commonly known as mangosteen, have been used as a traditional medicine in Southeastern Asia for the treatment of diarrhea, dysentery, inflammation, ulcers and wound healing. Mangosteen is cultivated mainly in Indonesia, Malaysia, Philipina and Thailand. Medicinal properties of G. mangostana are associated with its bioactive compounds, xanthone. Xanthone is a tricyclic isoprenylated polyphenol. Previous investigation shows that xanthone are antioxidant, anti-bacterial, anti-proliferative, proapoptotic, anti-carcinogen and anti-inflammatory properties.

Xanthone can be found in every part of mangosteen, mainly in fruit pericarp. Mangosteen contains 17% of pericarp, which contains 62.05% of water, 0.63% of fat, 0.71% of protein, 1.17% of total sugar and 35.61% of carbohydrate. Xanthone content of mangosteen pericarp is about 108 to 124 mg per 100 g. The ripeness level significantly affects the xanthone content of mangosteen. In this study, we investigated the physical and chemical properties of mangosteen pericarps depending on the maturity level (Table 1), using various characterization techniques such as PSA (Particle Size Analyzer), Scanning Electron Microscope (SEM), X-ray Diffraction (XRD),
and X-ray Fluorescence (XRF). The study aims to get insight into the microstructure of mangosteen pericarp in accordance to their maturity level and xanthone content. Furthermore, it can give insight to maximize the yield of xanthones.

**Table 1.** Maturity level classification of mangosteen

| Maturity Level | Properties |
|----------------|------------|
| Level 1        | Green yellowish, high mucus content. The inner part of fruit cannot be separated from the flesh. |
| Level 2        | Yellow reddish with distribution of red colored fleck. Lower mucus content. The inner part of the fruit still cannot be separated from flesh. |
| Level 3        | Red brownish. Mucus still exists. The inner part of fruit can be separated from the flesh. |
| Level 4        | The color becomes red purple. The mucus is lesser. The inner part of fruit can be separated from the flesh and the fruit can be consumed. |
| Level 5        | Purple reddish. Fruit starts to ripe. The mucus disappears, and the fruit can be easily separated. |
| Level 6        | Purple blackish. The fruit is riped and can be eaten immediately. |

**2. Methods**

**2.1 Extraction**

The extraction of mangosteen pericarp was performed following maceration method. Mangosteen pericarp was obtained from Bandung, West Java. Mangosteen was air dried until the water content was about 5% prior to crushing in an electrical blender. Briefly 700 g of mangosteen pericarp mass was macerated using 96% ethanol (1 : 5) for 5 days and stirred every 3 hours. The filtrate was dried using evaporator and freeze dried to obtain the powder. The powder was stored in desiccators until used.

**2.2 Characterization**

The mangosteen extract in various maturity levels were subjected to microstructure characterization using PSA, SEM, XRD and XRF. For SEM analysis, the mangosteen extract was poured homogenously onto the carbon double tip on the SEM sample holder stub. For the elemental composition in SEM, an energy dispersive X-ray (EDX) detector attached on SEM (SEM JEOL JSEM2650LV) was used. For XRD analysis Imperial with Cu source was used. The XRD results were analyzed using Ritzfeld Refinement to derive information about the phase and crystals may occur.

**3. Results**

PSA measurement of the mangosteen extract in various maturity levels is presented in Figure 1 and Table 2. Particle of mangosteen powder at level 5 exhibited the smallest size, whereas those at level 3 exhibited the highest size.

**Table 2.** Particle size of mangosteen pericarp extract as measured by PSA

| Mangosteen level | Size (nm)       |
|------------------|-----------------|
| 1                | 81.8 ± 21.1     |
| 2                | 72.6 ± 20.4     |
| 3                | 1244.1 ± 341.0  |
| 4                | 1112.2 ± 256.5  |
| 5                | 57.0 ± 14.5     |
| 6                | 199.2 ± 58.9    |

Data are presented in Mean ± Standard Deviation

Figure 2 shows the SEM image of the microstructure of mangosteen pericarp extract at the maturity levels 1-6 which generally showed granular and plate like structure composing of large coagulates. Sample of maturity levels 3 and 6 have shown clearly smaller powder grains than those of the sample of maturity levels 1, 2, 4 and 5. It was distinguished by the contrast deficiency by the sample with fine grain size. It is almost impossible to measure single grain on the sample 1, 2, 4 and 5 because the sample forms large block of particle agglomerations.
Figure 1. Particle size distribution of Mangosteen pericarp; a. level 1; b. level 2; c. level 3; d. level 4; e. level 5; f. level 6.
Figure 2. SEM SE images of mangosteen pericarp samples at maturity level and their corresponding EDX spectrum, a. level 1; b. level 2 c. level 3; d. level 4; e. level 5; f. level 6.
The corresponding EDX spectrums are also represented in Figure 2 on the right side revealing the elemental composition of the samples. Mainly the samples contain C, O, Cl and K. The mangosteen pericarp level 1 revealed the elemental content C, O, Cl, K and Si about 64.20%, 34.52%, 0.22%, 0.72% and 0.33% respectively. Mangosteen pericarp level 2 was crystalline in nature containing Al 0.1%, whilst Si was not detected. Level of C, O, Cl and K about 65.32%, 33.96%, 0.17% and 0.45% respectively. Mangosteen pericarp level 3 showed globular form, softer than mangosteen pericarp level 1 and 2 which was about 5 µm, as shown in EDX results. Level of C, O, Cl and K about 61.38%, 36.49%, 0.48% and 1.65% respectively. Mangosteen pericarp level 4 has granule size of 20 µm, crystalline and its level of C, O, Cl and K was 65.42%, 34.20%, 0.10% and 0.28%. Mangosteen pericarp level 5 showed granules form and also combined form. Its granule were between 5-50 µm, crystalline and mainly composed by C, O, Cl and K with 61.18%, 37.94%; 0.19% and 0.69% respectively. Mangosteen peri-carp level 6 showed granules form and also combined form. Granule was 10 µm, crystalline. Main components of Mangosteen pericarp level 6 were C, O, Cl, and K with 62.41%, 36.67%; 0.23% and 0.68% respectively.

Figure 3. XRD analysis of mangosteen pericarp extract, a. Mangosteen pericarp level 1; b. Mangosteen pericarp level 2; c. Mangosteen pericarp level 3; d. Mangosteen pericarp level 4; e. Mangosteen pericarp level 5; f. Mangosteen pericarp level 6.
Table 3. Elemental concentration of mangosteen pericarp extract analyzed with X-ray fluorescence (%)

| Elements        | Concentrations mangosteen pericarp level (%) |
|-----------------|----------------------------------------------|
| Potassium (K)   | 50.96 50.59 50.38 50.21 51.22 52.03         |
| Chloride (Cl)   | 12.20 11.18 12.83 9.61 11.90 13.45           |
| Calcium (Ca)    | 4.92  8.10 1.48  8.56  5.90  4.67            |
| Phosphor (P)    | 1.78  1.68 1.67  1.50  1.39  1.75            |
| Sulfur (S)      | 1.01  1.25 2.06  1.25  1.01  2.20            |
| Sodium (Na)     | 1.37  1.27 1.08  1.67  1.77  1.22            |
| Cadmium (Cd)    | 1.50  0.77 1.89  0.91  1.18  1.11            |
| Silver (Ag)     | 1.33  2.01 3.20  1.31  1.52  –               |
| Iron (Fe)       | 0.924 0.836 0.821 1.05 1.986 0.920           |
| Zinc (Zn)       | 0.879 0.386 0.257 0.482 0.712 0.988          |
| Molybdenum (Mo) | 0.712 – 0.854 0.678 – 0.575                  |
| Rubidium (Rb)   | 0.75  0.873 0.88  0.985 1.18  0.523          |
| Magnesium (Mg)  | 0.410 0.425 0.290 0.315 0.392 0.431          |
| Silicon (Si)    | 0.295 0.294 0.249 0.280 0.569 0.401          |
| Zirconium (Zr)  | 0.239 0.209 0.125 0.219 0.167 0.249          |
| Argon (Ar)      | 0.291 0.254 0.339 0.246 0.203 0.203          |
| Manganese (Mn)  | 0.218 0.218 0.156 0.453 0.195 0.293          |
| Titanium (Ti)   | 0.115 0.115 – – 0.089 0.504                  |
| Barium (Ba)     | – – 0.80 – 0.84 –                           |
| Gold (Au)       | – – 0.74 – – –                             |

The XRD patterns of mangosteen pericarp extract are shown in Figure 3. As shown in XRD analysis, mangosteen maturity 1, 2 and 3 were amorphous. Although sample 2 was previously seen as crystalline, it was mainly amorphous based on XRD results. Lowest peak in samples 4, 5 and 6 indicates carbon crystal and indicates high xanthone concentration.

XRF is X-ray method similar to EDX, yet it measures smaller element above 11 atomic number. XRF was performed to confirm results obtained from EDX and XRD analysis. Results of XRF are shown in Table 3. Most of elements were in accordance to previous results analyzed with EDX and there were also several more elements found. Elements K, Cl, Ca, P, S, Na, Cd, Fe, Zn, Rb, Mg, Si, Zr, Ar and Mn were detected in all samples. Meanwhile, Ag, Mo and Ti were found in mangosteen pericarp level 1. Similarly, Ag and Ti were detected in mangosteen pericarp level 2; Ag, Mo, Ba and Au were detected in mangosteen pericarp level 3; Ag and Mo were detected in mangosteen pericarp level 4; Ag, Ti and Ba were detected in mangosteen pericarp level 5; Mo and Ti were detected in mangosteen pericarp level 6.

4. Discussion

Dehydration is an efficient alternative for fruit storage in which reduction of water is associated with the decline of chemical and enzymatic reactions that can damage foods. Gentle drying techniques, such as osmodehydration or lyophilisation (freeze drying), have been used as new alternative for the commercialization of tropical fruits to reduce the difficulties for its handling and transport and to acquire added value solids products. In this study, we evaluated microstructure of freeze dried mangosteen pericarp extract at various maturity levels. The structural studies of the fruit powders were done using PSA, SEM, XRD and XRF.

In the present study, particle size of the mangosteen pericarp extract was clearly heterogeneous. Particle of mangosteen pericarp extract at level 5 exhibited the
smallest size, whereas those at level 3 exhibited the highest size. Particle size distribution does not follow a typical ideal Gauss bell form. Additionally, it seems that the maturity level were not in correlation with the particle size. The particle size may depend on the processing method. This can be also originated from the coagulation of mangosteen particles.

SEM technique is a powerful tool for determining and observing the caking phenomenon on the powder surface. The corresponding EDX spectrums revealing the elemental composition of the samples, exhibited component C, O, Cl and K in samples. XRF is X-ray method similar to EDX, yet it measures smaller element above 11 atomic numbers. XRF was performed to confirm results obtained from EDX and XRD analysis. Most of elements were in accordance to previous results analyzed with EDX and there were also several more elements found.

Crystallization is essential for the stability of powdered juice and can be investigated through XRD analysis. Amorphous state is indicated with diffused and large peaks in which the molecules are disorderly displayed producing disperse bands, whereas crystalline materials yield sharp and defined peaks since they are presented in a highly ordered state\textsuperscript{18}. In this study, mangosteen pericarp level 1, 2 and 3 were amorphous, whilst mangosteen pericarp level 4, 5 and 6 indicates carbon crystal.

This present study, found that in mangosteen pericarp contain elements of K, Cl, Ca, P, S, Na, Cd, Fe, Zn, Rb, Mg, Si, Zr, Ar, and Mn. Other studies also mention that in the mangosteen fruit contain Na, K, Mg, Ca, Fe, Mn, Zn, and Cu\textsuperscript{19}.

Fe, Ca, Zn, Cu, and Mn are present in wound fluid, indicating that these elements at the right concentration can be helpful in the wound healing process. Fe concentrations decrease in wound fluids after a day or two days, because most of the Fe has been used in the wound healing process. Ca is also required in various physiological processes such as blood coagulation, neuromuscular excitability, cellular adhesiveness, maintenance structural integrity and functions of cell membranes\textsuperscript{20}.

Zn has a very important role in wound healing, Zn capable to enhance platelet activity and aggregation. Zn deficiency will increase the production of inflammatory cytokines and oxidative stress, obstruct PMN chemotaxis and compromises Neutrophil extracellular traps. Lack of Zn also disrupt monocytes adhesion. Zn plays a role in increasing T lymphocyte populations, and granulation tissue formation. Zn was shown to increase keratinocyte migration and also effective for in vivo angiogenesis\textsuperscript{21}.

Mn is firmly associated with protein synthesis and widely distributed in mammalian tissue\textsuperscript{20}.

5. Conclusion
Mangosteen pericarp with highest xanthone concentration has crystalline structure and contains iron, calcium, zinc, and manganese, the elements which in the correct concentration can give benefit for wound healing processes. These findings provide information regarding characteristics of mangosteen peel that can be further used for the optimization of xanthone content.

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7. Conflict of interest
Authors declare that there is no conflict of interest.

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Microstructural Characterization of the *Garcinia mangostana* Fruit at Different Maturity Level

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