Proximate Composition and Determination of Epigallocatechin Gallate Content in Melon Manis Terengganu

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Abstract
Melon Manis Terengganu (MMT), Cucumis melo L., is a newly developed variety of melon introduced specifically for Terengganu. MMT has been claimed to have a high antioxidant value. Epigallocatechin gallate (EGCG) has become one of the leading naturally derived polyphenols studied for its potential health benefits. In the present study, the proximate composition and EGCG content of MMT were determined and compared among the fruit parts (flesh, seeds, and peels). The powdered samples have undergone proximate analyses followed by the determination of EGCG concentration using high performance liquid chromatography (HPLC). The result revealed that MMT seed has highest protein (27.99 ± 0.36%), fat (28.79 ± 0.32%) and crude fibre (31.64 ± 1.25%) contents as compared to peel and flesh. MMT peel contained the highest carbohydrate (67.48 ± 0.37%) as compared to flesh and seed. The EGCG concentrations of MMT seed, flesh and peel were significantly different (p < 0.05) among one another with MMT peel as the highest EGCG concentration (0.042 ± 0.003 mg/mL). Therefore, MMT peel has the potential to consider as new sources of natural antioxidants for food and nutraceutical products as it contained the highest EGCG concentration as compared to seed and flesh.

1. Introduction

In Malaysia, there are three types of melons known as watermelon (Citrullus lanatus), rockmelon (Cucumis melo L. var. Cantalupensis) and honeydew (Cucumis melo L. var. Inodorus). These melons are widely cultivated and easily obtained in Peninsular Malaysia, such as Johor, Kedah, Kelantan, Pahang, and Terengganu (Masde & Mohd, 2016). Melon Manis Terengganu (MMT) is a newly developed variety of melon introduced specifically for Terengganu. Cucumis melo L. has been claimed to have high nutritional value such as Vitamin C, fibre, and antioxidants (Silva et al., 2020). Furthermore, MMT can be processed into various products such as ice cream, jelly, juice, and jam (Athirah et al., 2018).

MMT is one of the rockmelon (Cucumis melo L.) species under the Cucurbitaceae family, a moderately large family of flowering plants (Muhamad et al., 2018). According to Ajuru and Okoli (2013), the cucurbitaceae family is an outstanding family of dicotyledons. They have large leaves, creeping or climbing stems with simple or branched tendrils, fleshy fruits with leathery exocarp, numerous seeds and a woody rootstock. These plants are widely produced and consumed in the tropical parts of the world, such as Europe, Asia, and Africa (Mallek-Ayadi et al., 2018).

The increased consumption of fruits causes an increment in the volume of waste generated, especially peels and seeds. The production of melon (Cucumis melo L.) has increased in recent years and has high economic value. However, the parts of fruits such as peels and seeds are wasted and known as by-products. These inedible parts are discarded during processing and consumption (Rolim et al., 2018). In fact, instead of fruit flesh, the by-products (peels and seeds) from different fruits can be essential sources of valuable nutrients such as carbohydrates, fibre and protein due to their proximate composition.

The phenolic compounds have been associated with several potential positive health benefits due to their antioxidant activities and free radical-scavenging abilities (Menon &
Ramana Rao, 2012). Morais et al. (2017) proved that melon (Cucumis melo L.) contained phenolic and flavonoid compounds in all fruit parts (flesh, peels, and seeds). According to Ravindranath et al. (2021), Cucumis melo is rich in phenolic compounds. Meanwhile, according to another study by Ahmed et al. (2021), Cucumis melo is rich in carotenoid, phenolic and flavonoid compounds and high in antioxidant value (Silva et al., 2021). Even though the Cucumis melo is rich in bioactive compounds, several studies on the bioactive compounds seemed to reveal a lack of focus on the epigallocatechin gallate (EGCG) content in the melon (Cucumis melo L.). EGCG is Epigallocatechin-3-gallate (EGCG), a polyphenol mainly found in tea leaves and has received significant attention due to its protective role in the prevention of the diseases (Chowdhury et al., 2016). Several studies found that EGCG has several benefits in fighting chronic diseases such as cancer, diabetes, heart diseases, and obesity (Granja et al., 2017). Thus, this study focused on the proximate composition and EGCG content of mature MMT (Cucumis melo L.) fruit parts (flesh, peels, and seeds). Hopefully, this finding will increase these values to be used as food ingredients in the future.

2. Materials and methods

2.1 Sample preparation

The mature MMT with the average weight (650 g – 700 g), length (11 cm – 13 cm), diameter (10 cm – 12 cm) and sugar content (8 °Brix – 9 °Brix) was collected from the MMT plantation at Kampung Telaga Papan, Setiu, Terengganu. The mature MMT was washed under running tap water to remove any impurities, dust or foreign substances and separated into: flesh, peels, and seeds. A stainless-steel grinder. The sample powder was then sieved through 500 μm mesh and stored in an airtight container in the cabinet dryer at 50°C for 72 hours, the peel, seeds and sliced flesh were soaked in 0.2% sodium metabisulphite (to preserve content (8 °Brix – 9 °Brix) was collected from the MMT plantation at Kampung Telaga Papan, Setiu, Terengganu. The mature MMT was washed under running tap water to remove any impurities, dust or foreign substances and separated into: flesh, peels, and seeds. A stainless-steel grinder. The sample powder was then sieved through 500 μm mesh and stored in an airtight container in the cabinet dryer at 50°C for 72 hours, the peel, seeds and sliced flesh were soaked in 0.2% sodium metabisulphite (to preserve protective role in the prevention of the diseases (Chowdhury et al., 2016). Several studies found that EGCG has several benefits in fighting chronic diseases such as cancer, diabetes, heart diseases, and obesity (Granja et al., 2017). Thus, this study focused on the proximate composition and EGCG content of mature MMT (Cucumis melo L.) fruit parts (flesh, peels, and seeds). Hopefully, this finding will increase these values to be used as food ingredients in the future.

2.2 Proximate analyses

The proximate analyses were conducted according to the Association of Official Analytical Chemists (AOAC). The proximate analyses of MMT flesh, seeds and peel samples included moisture content, protein, fat, ash, crude fibre, carbohydrates and contents. The analyses of each sample were performed in triplicate.

2.2.1 Determination of moisture content

The sample’s moisture content was determined through the Association of Official Analytical Chemists (AOAC) Official Method 977.11-1980. Empty crucibles were pre-dried in the oven (Memmert, Schwabach, Germany) at a temperature of 105°C for 4 hours and then cooled at room temperature in a desiccator before being weighed (W1). Approximately 0.5 g of sample (W2) was spread evenly in the crucible, then transferred to a muffle furnace (550°C, 6 hours) and incinerated until it was free of black carbon particles. The weight of crucibles with lids and residues (W3) was taken after cooled in the desiccator. The following equation was used to compute the percentage of moisture content:

\[
% \text{Moisture} = \left( \frac{W2-W3}{W2-W1} \right) \times 100\%
\]

Where:

- \( W1 = \) Weight of empty crucible (g)
- \( W2 = \) Weight of sample (g)
- \( W3 = \) Weight of crucible + dried sample (g)

2.2.2 Ash content determination

Ash content was carried out based on AOAC Official Method 923.03-1923. A muffle furnace was used to pre-ignite (550°C, 3 hours) empty crucibles and their lids. Then, they were cooled in a desicator and weighed (W1). Approximately 0.5 g of sample (W2) was inserted into the crucible, then transferred to a muffle furnace (550°C, 6 hours) and incinerated until it was free of black carbon particles. The weight of crucibles with lids and residues (W3) was taken after cooled in the desicator. The following equation was used to compute the percentage of ash content:

\[
% \text{Ash} = \left( \frac{W3-W1}{W2} \right) \times 100\%
\]

Where:

- \( W1 = \) Weight of empty crucible (g)
- \( W2 = \) Weight of sample (g)
- \( W3 = \) Weight of crucible + ash (g)

2.2.3 Crude protein content determination

The nitrogen content was determined using AOAC Official Method 954.04. Approximately 0.5 g of sample was digested with 15 mL concentrated sulphuric acid (400°C) using a block digester (Gerhardt, Germany) with the presence of Kjeltabs, which acted as a catalyst in the reaction mixture. The digestion process ended as the sample colour changed from black to coral blue. A blank (without sample) was prepared. The digested mixture was transferred to a distillation unit (Gerhardt, Germany) and neutralised using sodium hydroxide. The ammonia liberated was collected in 25 mL of 2% boric acid containing 5 drops of protein indicator solution (methyl red- bromocresol green indicator). The distillate was titrated using 0.1 M of hydrochloric acid. The endpoint was noted as the boric acid solution changed from green to pink. The protein conversion factor (6.25) was used based on the assumption that the protein contains 16% nitrogen (Tomé, Cordella, Dib, & Péron, 2019). The percentage of crude protein content was calculated using the following equation:

\[
% \text{Nitrogen} = \left( \frac{0.1x (A-B) \times 14,007 \times 1000}{W} \right) / (\text{Weight of sample} \times 1000)
\]

% crude protein = % of Nitrogen x Protein Factor

Where:

- \( N = \) normality of HCl used (mol/L)
- \( A = \) volume of HCl used to titrate sample (L)
- \( B = \) volume of HCl used to titrate blank (L)
- \( 14,007 = \) molecular weight of nitrogen (kg/mol)
- \( W = \) weight of sample (g)
- \( F = \) protein conversion factor

2.2.4 Crude fat content determination

The crude fat content determination was based on AOAC Official Method 960.39-1960. Approximately 0.5 g (W1) of the

% Moisture = (W2-W3)/(W2-W1) × 100%

Where:

- \( W1 = \) Weight of empty crucible (g)
- \( W2 = \) Weight of sample (g)
- \( W3 = \) Weight of crucible + dried sample (g)
2.3 Extraction of phenolic compounds

According to the procedure described by Wissam et al. (2012), samples were extracted with deionised water following the automated fat extraction system (Gerhardt, Germany). After the extraction process was completed for 72 hours, the supernatant was collected by centrifugation at 2000 rpm for 10 minutes at 25°C (Mitchell et al., 2017) and filtered through Whatman filter paper No. 1 to obtain a clear solution. Then, 10 ml of deionised water was added to the solid residue and extracted twice for 20 minutes in a thermostatic water bath shaker at 50°C. The supernatants were pooled and freeze-dried using the pilot freeze dryer VirTisTM SP Scientific at -40°C for 72 hours.

2.4 Reversed-phase high performance liquid chromatography (HPLC) analysis

The samples were filtered through a polypropylene filter unit (0.45 μm) before being injected for reversed-phase HPLC analysis as described by Pasini et al. (2019), with acetonitrile:acetic acid (98:2 v/v) (Merck, Darmstadt Germany) as mobile phase A and methanol:water:acetic acid (95:3:2 v/v/v) (Merck, Darmstadt Germany) as mobile phase B. The conditions were held at 100% B for 7 minutes before returning to 7% B (starting condition) over 6 minutes. The flow rate is 0.8 ml/min. Shim-pack GIS HILIC column (250 x 4.6 mm) (Shimadzu, Japan) was used in this analysis. The column was allowed to re-equilibrate in 93% solvent A for 10 minutes before the next injection. The temperature of the column was maintained at 35°C. The injection volume was 1 μL. The separation was monitored by UV-Vis at 280 nm. Peak identification will be performed by comparing retention time and UV-Vis spectra. HPLC analysis was performed in triplicate (analysis for each sample is repeated three times).

2.5 Standard solution preparation

Stock solutions containing the standard substances were prepared and diluted to appropriate concentrations ranging from 0.2 – 1 μg/mL with the same solvent. Briefly, the calibration was achieved using the standard substance epigallocatechin gallate (EGCG) (Merck, Darmstadt, Germany). The standard calibration curve of peak area (y) against concentration (x) was plotted. A linear regression method was used to identify the slope and correlation coefficient of the linear regression equation.

2.6 Statistical analysis

Data obtained from all the proximate analyses and the concentration of EGCG were analysed using Analysis of Variance (ANOVA) of IBM SPSS for Windows Version 21.0 software. Statistical analysis was used to test whether there was a significant difference between samples based on the result of each analysis at a 95% confidence level. Post hoc Tukey’s multiple comparison test was used to determine which sample was significantly different from other samples in the analysis.

3. Results and discussion

Table 1: The proximate composition of different fruit parts (seed, flesh, and peel) of mature MMT samples

Data are reported as means ± SD with experiments performed in triplicate. Mean values in the same row with different
superscripts are significantly different (p < 0.05)

### 3.1 Proximate composition of MMT

The proximate composition of different fruit parts (seed, flesh, and peel) of mature MMT samples is presented in Table 1. The moisture, ash, protein, carbohydrate, and crude fibre analyses showed significant differences (p < 0.05) among different MMT fruit parts (seed, flesh, and peel). However, the result revealed that MMT flesh and peel have no significant difference in fat content (p < 0.05), whereas they were significantly different from the MMT seed sample (p < 0.05).

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Seed</th>
<th>MMT Samples</th>
<th>Flesh</th>
<th>Peel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisture</strong></td>
<td>5.83±</td>
<td>19.21±</td>
<td>9.49±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.34</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Ash</strong></td>
<td>4.98±</td>
<td>14.33±</td>
<td>8.24±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.12</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>27.99±</td>
<td>17.31±</td>
<td>13.31±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.36</td>
<td>0.45</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>28.79±</td>
<td>1.37±</td>
<td>1.49±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.34</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>32.40±</td>
<td>47.98±</td>
<td>67.48±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>0.25</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td><strong>Crude Fibre</strong></td>
<td>31.64±</td>
<td>14.66±</td>
<td>27.88±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>0.38</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

Among the fruit parts, MMT seed (28.79 ± 0.32%) showed the fat content, which was significantly higher than those of flesh (1.37 ± 0.34%) and peel (1.49 ± 0.35%). Yanty et al. (2007) reported that seeds from other varieties of melon also contained high amounts of fat. The fat content of the MMT seed was higher than that of honeydew melon seed (25.00%) (Yanty et al., 2007) but lower than that of Cucumis melo L. hybrid AF-522 seed (30.83%) (De Mello et al., 2000), Cucumis melo L. var. Saccharinus seed (32.30%) (De Mello et al., 2001) and muskmelon seed (37.16%) (Mohra, Pasricha, & Gupta, 2015). Mallek-Ayadi et al. (2018) concluded that melon seed is a rich source of oil which can be used in industrial applications. In contrast, the fat contents of MMT flesh and peel were significantly lower as compared to a seed.

In comparison, the carbohydrate content of MMT peel was the highest, followed by flesh and seed. Mallek-Ayadi et al. (2017) found that melon (Maazoun variety) peel contained 69.77% of carbohydrates which was slightly higher than that of MMT peel (67.48 ± 0.37%). The carbohydrate content of the MMT seed (32.40 ± 0.26%) was higher than that of MMT melon seed (29.96%) (Mallek-Ayadi et al., 2018). Koubala et al. (2016) reported that the total carbohydrate content of muskmelon (Cucumis melo L. var. Tbibis) was more abundant in the peel than in mesocarp and endocarp throughout the fruit maturation. The abundance of carbohydrates in peels may be attributed to an increase in the synthesis of cell wall polysaccharides which consist of cellulose, pectin, and hemicelluloses (Wang et al., 2021).

According to Raji and Orelaja (2014), the crude fibre of any seed indicates the presence of a reasonable quantity of trapped water (bound) held by the hydrophilic polysaccharides of the fibre. The crude fibre contains indigestible materials, reducing constipation by increasing bowel movement (Dai & Chau, 2017). MMT seed showed the highest crude fibre content compared to peel and flesh. The crude fibre content of the MMT seed was slightly lower than that of golden melon (Cucumis melo L.) seed 33.94 ± 0.01% (Raji & Orelaja, 2014), but higher than that of MMT melon seed (25.32%) (Mallek-Ayadi et al., 2018), honeydew melon seed (23.30%) and Cucumis melo L. hybrid AF-522 seed (19.00%) (Yanty et al., 2007). The fibres content of muskmelon (Cucumis melo L. var. Tbibis) mesocarp (flesh) decreased throughout the fruit maturation, while that of the endocarp (seed) decreased at the early stage and increased at the entire stage of maturation (Koubala et al., 2016).

In general, the results showed that the moisture and ash contents of MMT flesh were the highest compared to seed and peel. According to Olayinka and Etejere (2018), this result was reasonable since plants in the Cucurbitaceae family have been known to have a high amount of water in their fruits. Olayinka and Etejere (2018) also found that the ash content was respectively high in the amount in the pulp than the rind of both watermelon and cucumber. Therefore, the pulp of both sample fruits could serve as a better source of roughages and minerals than the rind.

The moisture content of any food material represents a measure of the life span of the food. It suggests how long food material can be stored without becoming mouldy (Raji & Orelaja, 2014). The high protein content of the seed makes it suitable for the fortification of foods (Raji & Orelaja, 2014). Protein content in the seed is the highest compared to the other parts due to its essential amino acids, as reported by Siddique et al., (2015). Cucumis melo var. Tbibish seeds are considered to be good sources of protein and contain most of the essential amino acids in proportions similar to soybeans where the Cucumis melo var. Tbibish contains all of the essential amino acids, with isoleucine, threonine, lysine, histidine, methionine, and tryptophan being present in the seeds.
Furthermore, the MMT seed possessed the highest protein, fat and crude fibre contents compared to peel and flesh. This result was similar to that reported by Da Cunha et al. (2020), that the raw material extracted from melon seeds is an ideal alternative source of functional food to promote health due to the high content of protein (17.64%), lipids (30.43%), and fibre (30.43%). The result also revealed that MMT peel contained the highest carbohydrate as compared to flesh and seed, while the moisture and ash contents of MMT peel were higher than that of MMT seed. Mallek-Ayadi et al. (2017) have also concluded that melon peel could be a rich carbohydrate source.

3.2 EGCG content of MMT

In this study, the HILIC column was used to determine the concentration of EGCG in MMT seed, flesh, and peel. The retention time of EGCG in standard solution (0.4 µg/mL) was 18.099 minutes, as shown in Figure 1 (A). Based on Figure 1 (B), peak 18 represented the EGCG in MMT flesh and the retention time was 17.538 min. For MMT peel (Figure 1 (C)), peak 22 represented the EGCG with 18.599 minutes of retention time, whereas peak 17, shown in the chromatogram of MMT seed (Figure 1 (D)) with 17.560 minutes of retention time, represented the EGCG compound. The concentrations of EGCG in the MMT seed, flesh and peel extracts were determined through a linear EGCG standard calibration curve (concentrations ranging from 0.2 to 1.0 µg/mL, \( R^2 = 0.994 \)).

The EGCG concentrations in fruit parts (seed, flesh, and peel) of mature MMT samples are presented in Table 2. Among the mature MMT fruit parts, peel showed the highest EGCG concentration (0.042 ± 0.003 mg/mL) followed by flesh (0.022 ± 0.002 mg/mL) and seed (0.015 ± 0.003 mg/mL). The result revealed that mature MMT seed, flesh and peel were significantly different (\( p < 0.05 \)) in their EGCG concentrations.

![Figure 1: HPLC chromatograms of EGCG in standard solution (0.4 µg/mL) (A), MMT flesh (B), MMT peel (C) and MMT seed (D)](image)

### Table 2: The EGCG concentrations in fruit parts (seed, flesh, and peel) of mature MMT samples

<table>
<thead>
<tr>
<th>MMT Samples</th>
<th>EGCG Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>0.015± ± 0.003</td>
</tr>
<tr>
<td>Flesh</td>
<td>0.022± ± 0.002</td>
</tr>
<tr>
<td>Peel</td>
<td>0.042± ± 0.003</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD with experiments performed in triplicate. Mean values in the same column with different

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21
superscripts are significantly different (p < 0.05)

The result showed that all the fruit parts of mature MMT contained the amount of EGCG concentration. EGCG is the major catechin abundant in green tea and several fruits, including mature pomegranate (*Punica granatum* Linn.) fruits (Dey et al., 2015). In addition, Chem and Indies (2010) reported that the amount and types of phenolics might change during the growth and maturity of bitter melon. These changes could affect the antioxidant activity of the extracted phenolics. Besides, Deng et al. (2012) found that different fruit residues (peel and seed) had various antioxidant potency, and the variation was considerable. They also revealed that catechin was one of the main bioactive compounds widely found in these residues.

The MMT peel showed the highest EGCG concentration compared to flesh and seed. This finding followed those of Morais et al. (2015), which evaluated the seven tropical fruit parts (pulp, seed, and peel). Moreover, the results showed that the highest phenolic contents were found in peels compared to pulps and seeds. Rolim et al. (2018) found that the melon (*Cucumis melo* L.) peel extracts showed a higher total phenols compound than the melon seed extracts with 110.7 ± 15.03 mg gallic acid equivalent per 100 gram (mg GAE/100 g) and 75.2 ± 7.39 mg (GAE/100 g) respectively. They also revealed that catechin concentrations in the melon peel aqueous extracts were higher (4.10 μg/mL) than that of the melon seed aqueous extracts (1.77 μg/mL). The melon peel is more exposed to environmental stress conditions, which may have caused higher phenolic contents than in the seeds (Rolim et al., 2018).

According to Chel-Guerrero et al. (2022), the peels of tropical fruits are a potential source of various bioactive compounds, such as flavonoids, polyphenols, terpenoids, carotenoids and alkaloids. In addition, Ayala-Zavala et al. (2011) also reported that the contents of functional compounds in different tissues of exotic tropical fruits are primarily in peels and seeds and a lesser extent, in the pulps. However, the results showed that MMT seed possessed the lowest EGCG concentration compared to peel and flesh. This finding is in agreement with a previous study by Ibrahim and El-Masry (2016) that revealed the melon (*Cucumis melo* L.) fruits of *Inodorus* cv. *Manis Terengganu 1*). The result showed that all the fruit parts of mature MMT contained the amount of EGCG concentration, while the MMT peel contains the highest carbohydrate compared to flesh and seed. Among the mature MMT fruit parts, peel showed the highest EGCG concentration. Therefore, besides MMT flesh, the MMT by-products (peel and seed) can be considered good sources for the development of novel functional foods due to their nutritional value. Moreover, MMT peel has the potential to be considered a new source of natural antioxidants for food and nutraceutical products as it contained the highest EGCG concentration as compared to seed and flesh.

4. Conclusion

The MMT seed has the highest protein, fat and crude fibre contents compared to peel and flesh, while the MMT peel contains the highest carbohydrate compared to flesh and seed. Among the mature MMT fruit parts, peel showed the highest EGCG concentration. Therefore, besides MMT flesh, the MMT by-products (peel and seed) can be considered good sources for the development of novel functional foods due to their nutritional value. Moreover, MMT peel has the potential to be considered a new source of natural antioxidants for food and nutraceutical products as it contained the highest EGCG concentration as compared to seed and flesh.

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