CHAPTER IV

RESULTS AND DISCUSSION

The experiment was focused on the effect of carrier agent and core to coating ratio towards the encapsulation of *Annona muricata* Linn. tea extract. The samples are subjected to various analysis, such as antioxidant activity, encapsulation efficiency, powder recovery and particle size of the encapsulated form of *Annona muricata* Linn. tea extract.

4.1 Preparation of the Microcapsules

The core (soursop leaves tea extract) and the coating (maltodextrin, whey protein isolate, and maltodextrin-whey protein isolate) were prepared and analyzed for its moisture content, yield, total phenolic content, and antioxidant activity. The result of the core and coating material analysis can be seen in Table 4.1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Total Phenolic Content (mgGAE/g Sample)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soursop Leaves Tea</td>
<td>1.990</td>
<td>299.728</td>
<td>104.819</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>-</td>
<td>0</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>WPI</td>
<td>-</td>
<td>0</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Malto-whey</td>
<td>-</td>
<td>0</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

From the data shown in Table 4.1, it is concluded that the only material that contributes to the total phenolic content and antioxidant activity for the feed solution and microcapsules are the core material, which is soursop leaves tea extract. The moisture data content for both core and coating material will be used
to determine the amount material needed to produce microcapsule with the desired core to coating ratio.

4.2 Microencapsulation by Spray Drying

The mixture of both core and coating material with the desired ratio was diluted in distilled water into 1 L of volume. The feed solution then was undergone ultrasonication process for 15 minutes. Various amount of feed solution was taken for the feed solution analysis, and the remaining solution was spray dried with 160 °C, aspirator 100 %, pump 35 %, and flow rate of 40 mm. The microcapsules are subjected to the analysis of powder recovery, total phenolic content, encapsulation efficiency, antioxidant activity, and particle size analysis.

4.2.1 Moisture Content and Powder Recovery

The soursop leaves tea extract, carrier agent (maltodextrin, whey protein isolate, and maltodextrin-whey protein isolate), and encapsulated form of soursop leaves tea extract are subjected to moisture content analysis. The moisture content of the soursop leaves tea extract, carrier agent and encapsulated form of soursop leaves tea extract can be seen in Appendix A. The moisture content data of microcapsules will be used to calculate the amount of powder recovery, and also the amount of samples needed for antioxidant activity, total phenolic content, and encapsulation efficiency analysis.

The encapsulated soursop leaves tea extract are subjected to powder recovery analysis. Powder recovery is defined as the amount of the recovered products, determined by the ratio between the dry weight of the total recovered product and dry weight of the feed that are initially fed into the system. The
results and calculation of the powder recovery can be seen in Appendix B. Figure 4.1 shows the powder recovery of the microcapsules.

Based on Figure 4.1 above, it is inferred that core to coating ratio affecting the powder recovery of the microcapsules significantly \( (p \leq 0.05) \). Figure 4.1 shows a trend in which all microcapsules with 1:20 core to coating ratio has higher powder recovery percentage compared to microcapsules with 1:10 core to coating ratio. This result is supported by research done by Kwak et al. (2002) which stated that the powder recovery of lactase microcapsules is directly proportional to the increases in core to coating ratio and also supported by a research done by Quek et al. (2007) which stated that higher addition of carrier agents towards watermelon juice are improving the powder recovery of the watermelon juice powder. Research done by Ahmadi et al. (2015) also supports the results of this experiment in which increasing the concentration of carrier agents will increase the powder recovery of cornelian cherry juice powder. This results also supported by Fang and Bhandari (2010), which found that microcapsules with 1 : 20 had higher powder recovery due to the addition of carrier agent such as whey proteins will modified the surface properties of the
particles thus reduce the stickiness between the particle and dryer wall which in turn will increase the powder recovery of the powder.

The effect of carrier agent towards the powder recovery of the microcapsules is shown in Figure 4.2 below.

![Figure 4.2 Effect of Carrier Agents towards Microcapsules Powder Recovery](image)

Note: Different notation indicates the significant difference (p ≤ 0.05)

The statistical analysis shows that types of carrier agents does not affect powder recovery of the microcapsules significantly (p ≥ 0.05). This results shows that all carrier agents roughly gives the same amount of powder recovery. Ozdemir and Cevik (2007) reported that the highest powder recovery for microencapsulation was obtained from whey protein. The high powder recovery is presumed to be a result of their efficient emulsifying capacity, especially in the presence of carbohydrates that will further enhance the powder recovery of microcapsules. However this experiment prove otherwise, the difference might be due to the different of core material used, as in this experiment the core material used were soursop leaves tea extracts.

4.2.2 Total Phenolic Content and Encapsulation Efficiency

The encapsulated soursop leaves tea extract are subjected to total phenolic content and encapsulation efficiency analysis. Encapsulation efficiency is defined
as the effectiveness of microcapsules to retain the phenolic content of the samples. This analysis was done based on two factor, which is carrier agent (maltodextrin, whey protein isolate, malto-whey) and core to coating ratio (1:10 and 1:20). Total phenolic content of feed solution and microcapsules are measured to calculate the encapsulation efficiency of microcapsules. The data and calculation regarding the total phenolic content of feed solution and microcapsules can be seen in Appendix D while the encapsulation efficiency data can be seen in Appendix E. Table 4.2 shows the encapsulation efficiency of the encapsulated soursop leaves tea extract.

<table>
<thead>
<tr>
<th>Carrier Agents</th>
<th>Core to Coating Ratio 1:10</th>
<th>Core to Coating Ratio 1:20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>55.477±3.337&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.187±3.526&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whey Protein Isolate</td>
<td>64.573±0.683&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.950±0.681&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malto-whey</td>
<td>71.393±3.988&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>74.330±0.381&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Different notation indicates the significant difference (p≤0.05)

From Table 4.2 above, it is inferred that all the encapsulation efficiency of the microcapsules are below 100%, meaning that the amount of the total phenolic content of the feed solution are decreased due to high temperature in spray drying. The results of this analysis are supported by Chaovanalikit <i>et al.</i> (2012), who stated that the increment of heat can decrease the total phenolic content of the samples, and also research done by Krishnaiah <i>et al.</i> (2012) also stated that high temperature in spray dryer cause some of the bioactive components are volatilized thus loss during the process. Therefore, it can be concluded that high temperature during spray drying resulted in lower total phenolic content of the soursop leaves tea extract microcapsules compared to the feed solution.

The encapsulation efficiency data were also subjected into statistical analysis by using Two Way ANOVA. The statistical analysis regarding the encapsulation efficiency of microcapsules are shown in Appendix F.
analysis in Appendix F shows that there are interaction between both factors. Table 4. shows the effect of both core to coating ratio and types of carrier agents

From Table 4.2, it is inferred that increasing core to coating ratio resulted in higher microcapsules encapsulation efficiency. All microcapsules with 1:20 core to coating ratio has higher efficiency compared to 1:10 microcapsules. This was also supported by research done by Bhusari and Kumar (2014), which indicates that retention of phenolic content was increased with increase in carrier agent addition rate which indicates that total phenolic contents were well encapsulated at higher carrier agent addition rates. Higher encapsulation efficiency means that the microcapsules are able to bind more phenolic content compared to the microcapsules that has lower encapsulation efficiency. Higher efficiency results are expected for microcapsules with 1:20 core to coating ratio since higher coating material resulted in higher encapsulation efficiency. Thus, this results are accordance with research done by Cilek et al. (2012) stated that microcapsules having core to coating ratio of 1:20 has higher encapsulation efficiency than microcapsules with core to coating ratio of 1:10 since higher amount of coating material resulted in higher amount of core entrapped in the microcapsules.

Table 4.2 also shows that microcapsules that used malto-whey as the carrier agent with 1:20 core to coating ratio have the best encapsulation efficiency, followed by whey protein and maltodextrin respectively. Increasing the core to coating ratio leads to higher encapsulation efficiency for all microcapsules regardless of the types of carrier agents used. This results are in accordance with review done by Gharsalloui et al (2007) who stated that
carbohydrates (including maltodextrin) exhibit low viscosities at high solids contents and good solubility, but lack interfacial properties required for high encapsulation efficiency thus resulted in low encapsulation efficiency. While proteins such as whey protein isolate possess a high binding properties, thus the combination of maltodextrin and whey protein isolate as a coating or carrier agent give higher encapsulation efficiency compared to when maltodextrin is used alone as the sole carrier agent. This results are also supported with research done by Qilong et al (2013) who found that the addition of whey protein to maltodextrin microcapsules slightly enhances the encapsulation efficiency of honey microcapsules compared to using maltodextrin alone.

4.2.3 Antioxidant Activity

The soursop leaves tea extract and encapsulated soursop leaves tea extract were subjected to antioxidant analysis by DPPH radical scavenging method. This analysis was done based on two factors, which is carrier agent (maltodextrin, whey protein isolate, malto-whey) and core to coating ratio (1:10 and 1:20). Data regarding the effect of carrier agents and core to coating ratio towards antioxidant activity can be seen in Appendix G. Based on the statistical analysis of microcapsules antioxidant activity shown in Appendix H, it is inferred that there is significant interaction between both factors to the antioxidant activity of microcapsules (p≤0.05). Table 4.3 shows that increasing core to coating ratio is linear with the increment of microcapsules antioxidant activity. On the other hand, changing the carrier agent also gave significant effect towards the antioxidant activity of microcapsules, as the antioxidant activity of maltodextrin, whey protein isolate, and malto-whey microcapsules is significantly different with each other.
Table 4.3 Antioxidant Activity of Microcapsules (ppm)

<table>
<thead>
<tr>
<th>Carrier Agents</th>
<th>Core to Coating Ratio</th>
<th>1:10</th>
<th>1:20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>181.440±0.738</td>
<td>120.581±2.576</td>
<td></td>
</tr>
<tr>
<td>Whey Protein Isolate</td>
<td>150.071±4.996</td>
<td>139.756±0.268</td>
<td></td>
</tr>
<tr>
<td>Malto-whey</td>
<td>116.975±1.096</td>
<td>100.557±0.159</td>
<td></td>
</tr>
</tbody>
</table>

Note: Different notation indicates the significant difference (p≤0.05)

This results implied that increasing the core to coating ratio are significantly increased the antioxidant activity of microcapsules. Lower IC₅₀ value reflected higher antioxidant activity, thus all microcapsules with 1:20 core to coating ratio has higher antioxidant activity compared to microcapsules with 1:10 core to coating ratio. This is probably due to higher coating will resulted in better emulsifying capacity thus provide better antioxidant activity. Higher encapsulation efficiency means that the amount of the core retained in the microcapsules are higher compared to the one with lower encapsulation efficiency, thus resulting in better antioxidant activity. Therefore, it can be concluded that in this experiment, the amount of core to coating ratio are directly proportional with encapsulation efficiency and antioxidant activity of microcapsules (Calabrone et al., 2015; Cilek et al., 2012).

From Table 4.3 also, it is inferred that the combination of maltodextrin and whey protein isolate gives better antioxidant activity compared to other, with malto-whey with 1:20 core to coating ratio has the highest antioxidant activity. This result are coherent with review stated by Gharsalloui et al. (2007) who stated that Proteins such as whey protein isolate possess a high binding properties, while maltodextrin has a poor emulsifying abilities. Thus the combination of maltodextrin and whey protein isolate as a coating or carrier agent give higher antioxidant capacity compared to using maltodextrin or whey protein isolate alone as the sole carrier agent because it has better emulsifying and oxidative stabilities.
compared to whey protein isolate and maltodextrin (Qilong et al., 2013; Ozdemir and Cevik, 2007).

The correlation between antioxidant activity and encapsulation efficiency of microcapsules can be seen in Appendix I. From Appendix I, it is shown that there is a significant correlation between antioxidant activity and encapsulation efficiency. In which higher encapsulation efficiency resulted in higher antioxidant activity and higher encapsulation efficiency means higher amount of phenolic component retained in the microcapsules. Recent research stated by Gavamukulya et al. (2014), extracted soursop leaves possess phenolic components that are affecting the antioxidant activity of the extract. Therefore, it is concluded that phenolics component are affecting the antioxidant activity, but other component such as alkaloids and acetogenins are also presents and affecting the antioxidant activity of the soursop leaves tea microcapsules.

Comparing the antioxidant activity of microcapsules and the soursop leaves tea extract which is shown in Table 4.1, it is inferred that the core retained in the microcapsules has lower antioxidant activity compared to the soursop leaves tea extract except for the Malto-whey 1:20 microcapsules in which has higher antioxidant activity compared the soursop leaves tea extract. The IC₅₀ value of the soursop leaves tea extract were 104.779 ppm while the antioxidant activity of the core extract retained in microcapsules are ranging from 100.557 ppm – 181.440 ppm. Therefore, it can be concluded that the % change in IC₅₀ were ranged from 104.199% - 57.749%.
The decreases of antioxidant activity in the core extract retained in the microcapsules might be due to the inability of those microcapsules to retain all the component that directly contributes to antioxidant activity of microcapsules such as phenolic component. However, it is to be noted that there are increase of the core extract antioxidant activity in the malto-whey 1:20 microcapsules. This result might be due to the structural changes happened during spray drying process which involves thermal operation. This result are in accordance with the research done by Adri and Wikanastri (2013), that the condition of heat-based drying process caused the active component in the soursop leaves tea to be increased while Uzlifah (2014) also mentioned that the prolonged boiling of the soursop leaves may cause increment in the antioxidant activity of the soursop leaves. Therefore, the increasing antioxidant activity of the malto-whey 1:20 microcapsules might be due to the structure changes occur to the core extract during the drying process.

4.2.4 Particle Size

The soursop leaves tea extract microcapsules are subjected to particle size analysis using electronic microscope with 100x magnification. The pictures of particle size of microcapsules are shown in Appendix J. The results of the particle size analysis can be seen in Appendix J while the statistical analysis for microcapsules particle size is shown in Appendix K. From Appendix K, it is inferred that there is significant interaction between both factors towards the particle size of the microcapsules. Table 4.4 shows the effect of core to coating ratio towards the particle size of microcapsules.
Table 4.4 Particle Size of Microcapsules (µm)

<table>
<thead>
<tr>
<th>Carrier Agents</th>
<th>Core to Coating Ratio</th>
<th>1:10</th>
<th>1:20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td></td>
<td>4.861±0.147&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.211±0.151&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whey Protein Isolate</td>
<td></td>
<td>3.511±0.180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.205±0.677&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malto-whey</td>
<td></td>
<td>3.183±0.201&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.967±0.150&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Different notation indicates the significant difference (p≤0.05)

From Table 4.4, it is inferred that microcapsules that 1:10 maltodextrin microcapsules has significantly bigger particle size compared to the other microcapsules. Therefore, it can be inferred that microcapsules with 1:20 core to coating ratio has smaller particle size compared to the 1:10 microcapsules. These results are in accordance with research done by Cilek et al. (2012) who found that sour cherry pomace with 1:20 microcapsules has slightly lower particle size compared to sour cherry pomace with 1:10 microcapsules due to samples having higher core to coating ratio had higher amount of coating material which led to well mixing and higher energy density compared to the one with lower core to coating ratio. As a result, microcapsules having 1:20 core to coating ratio had smaller particles compared to 1:10 microcapsules. Cilek et al. (2012) research results are also supported by research done by Poungchandang and Sertwasana (2010) who found that the particle size of ginger oil microcapsules are decreased with increased concentration of maltodextrin and liquid glucose as the carrier agent. This results are in accordance with research done by Pang et al. (2014) who found that spray dried Orthosiphon stamineus using maltodextrin as the carrier agent has higher mean particle size compared to the one using whey protein isolate as the carrier agent due to the difference in viscosity in which maltodextrin microcapsules has slightly higher viscosity compared to whey protein isolate.

Relating the particle size data towards other parameter, it is inferred that particle size of microcapsules are probably affecting the antioxidant activity and
encapsulation efficiency of microcapsules. Appendix J shows the correlation between particle size, encapsulation efficiency, and antioxidant activity of microcapsules.

Table 4.5 Particle Size Correlation

<table>
<thead>
<tr>
<th>Types of Correlation</th>
<th>Pearson Correlation</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size and Efficiency</td>
<td>-0.968</td>
<td>0.001</td>
</tr>
<tr>
<td>Particle Size and IC₅₀</td>
<td>0.928</td>
<td>0.008</td>
</tr>
</tbody>
</table>

From Table 4.5 above, it can be inferred that particle size of microcapsules are correlate significantly to the antioxidant activity and encapsulation efficiency of microcapsules (p ≤ 0.05). From the pearson correlation value, it is shown that particle size and encapsulation efficiency has negative value while particle size and antioxidant activity gives positive value. However, it is to be noted that the higher IC₅₀ values of microcapsules resulted in lower antioxidant activity. Thus, it is concluded that the particle size of microcapsules are inversely proportional with the encapsulation efficiency and antioxidant activity of microcapsules. This result is in accordance with research done by Cilek et al. (2012), who stated that bigger particle size resulted in lower encapsulation efficiency and antioxidant activity of sour cherry pomace microcapsules. Another research stated by Bunghez et al. (2012) also reported that particles having smaller size were more efficient in antioxidant activity compared to particles with larger size due to smaller particles size having larger surface area compared to the one with larger size. Therefore, smaller particle size of microcapsules is preferred because smaller particle size provides better antioxidant activity

4.2.5 Liquid Chromatography –Mass Spectrometry (LC-MS)

In order to determine structure changes due to encapsulation, the core extract and microcapsules are subjected to LC-MS analysis. The LC-MS graphs
are shown in Appendix L. Table 4.6 shows the LC retention time for both core extract and microcapsules.

Table 4.6 LC Retention Time (minutes)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Core Extract</th>
<th>Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.46</td>
<td>2.46</td>
<td>4.05</td>
</tr>
<tr>
<td>4</td>
<td>4.05</td>
<td>4.71</td>
</tr>
<tr>
<td>4.7</td>
<td>4.71</td>
<td>7.18</td>
</tr>
<tr>
<td>6.2</td>
<td>7.18</td>
<td>12.12</td>
</tr>
<tr>
<td>11.2</td>
<td>12.12</td>
<td></td>
</tr>
</tbody>
</table>

From Table 4.6 above, it is inferred that microcapsules has 2 similar retention times with core extract which is 4 and 4.7. This shows that the microcapsules are successfully retained the component from the core extract. The mass Spectrometry was done to be able to determine the component retained in microcapsules. The results of the Mass Spectrometry analysis for both retention times are shown in Figure 4.3 and Figure 4.4 respectively.
Retention Time 4

(a) Core Extract

(b) Microcapsules

Figure 4.3 Mass Spectrometry of core extract and microcapsules
Retention Time 4.7

(a) Core Extract

![Mass Spectrometry of core extract](image1)

(b) Microcapsules

![Mass Spectrometry of microcapsules](image2)

Figure 4.4 Mass Spectrometry of core extract and microcapsules

From Figure 4.3 and 4.4, it is inferred that the component molecular weight found in the microcapsules are similar with the core extract. This means that the microcapsules were able to retain the core extract component efficiently. Acetogenin derivatives such as murisolin, annoglaxin, and bulatacin has a various molecular weight, ranging around 500-650 g/mol. Therefore, from the molecular mass shown in both Figures, it is inferred that the microcapsules were able to retained the acetogenin derivatives such as Annonacin, Bulatacin, Solamin, Squasmodatin B and Murisolin which is also found in the core extract. These
components probably contributed to the antioxidant activity of microcapsules, as
the antioxidant activity of malto-whey microcapsules are not significantly
different with the antioxidant activity of the soursop leaves extract as shown in
Appendix M.