IV. RESULT AND DISCUSSION

4.1 PRELIMINARY RESEARCH

4.1.1 Chemical Composition

Table 1 summarizes the analytical results for the chemical composition of pea flour, rice flour, and sticky rice flour involving moisture content, ash content, protein, and crude fat content. Pea flour, rice flour, and sticky rice flour possessed moisture content of 7.73 %, 11.30 %, and 9.77 %, respectively. The moisture content of pea flour was in range (7.7-9.1 %) with those reported by other authors (Naguleswaran and Vasanthan, 2010; Chung et al., 2008), whereas for rice flours ranged from 6.10-12.52 % (Dias et al., 2010; Tavares, 2010; Liu et al., 2006; Chun and Yoo, 2004) and moisture content of 10.05 % for sticky rice flour was reported by Zhu et al., 2010. The difference in moisture content among them was mainly due to the difference in manufacturing.

In terms of ash content, pea flour (2.86 %) was the highest followed by rice flour (0.38 %) and sticky rice flour (0.17 %), respectively. Range of ash content for pea flours (2.24 -3.73 %), rice flours (0.40-0.72 %), and sticky rice flour (0.16-0.29 %) were reported by Dias et al. (2010); Naguleswaran and Vasanthan (2010); Petitot et al. (2010); Singh et al. (2010); Sung et al. (2008); Maninder et al. (2007); Latha et al. (2002); Lumdubwong and Seib (2000).

Table 1. Chemical composition of raw materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Crude fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea flour</td>
<td>7.68</td>
<td>2.83</td>
<td>21.84</td>
<td>0.80</td>
</tr>
<tr>
<td>Rice flour (RF)</td>
<td>11.21</td>
<td>0.39</td>
<td>7.21</td>
<td>0.41</td>
</tr>
<tr>
<td>Sticky rice flour (SRF)</td>
<td>10.37</td>
<td>0.18</td>
<td>6.12</td>
<td>0.29</td>
</tr>
</tbody>
</table>

The reported crude fat content of pea flours were 0.80-0.99 % (Naguleswaran and Vasanthan, 2010), which were near to the crude fat content determined in this study. However the crude fat content of rice flour (0.41 %) and sticky rice flour (0.29 %) were markedly lower than those reported by Dias et al. (2010) and Latha et al. (2002), for rice flour (0.87-0.90 %) and Sung et al. (2008), for sticky rice flour (0.45-0.94 %). The flours might be previously defatted (Maninder et al., 2007).

Protein content of 21.84 %, 7.20 %, and 6.12 % were observed during analysis for pea flour, rice flour, and sticky rice flour, respectively. Other results in protein content analysis by another authors were 21.4-26.8 % for pea flour, 6.93-8.11 % for rice flour, and 6.35-8.85 % for sticky rice flour (Naguleswaran and Vasanthan, 2010; Petitot et al., 2010; Tavares et al., 2010; Chung et al., 2008; Sung et al., 2008; Maninder et al., 2007; Chun and Yoo, 2004). Protein contents in legume grains range from 17% to 40 %, contrasting with 7-13 % of cereals, and being equal to the proteins contents of meats (18-25 %) (Genovese and Lajolo, 2001 in Costa et al., 2006). Hence, legume seeds including pea, are of prime importance in human nutrition due to their high protein content and are better known as a rich source of protein rather than rice (Singh, et al., 2004).
4.1.2 Apparent Amylose Content

Amylose has been considered to be the important factor influencing physicochemical properties of starch such as thermal properties, gelatinization, pasting behavior, textural properties, and susceptibility to enzyme attack (Copeland et al., 2009; Sajilata et al., 2006; Champagne, 1996), and thus it was becoming important to measure the amylose content of the samples.

Many of methods in determining amylose content have been developed such as differential scanning calorimetric method, lectin-binding method, and size exclusion chromatography method. Instead of those methods, iodine-binding procedure has been commonly used in amylose content determination. This method is based on the ability of iodine to form a helical inclusion complex with amylose molecules (Chen and Bergman, 2007).

In general, variations in amylose content are mostly affected by genetic and environmental factors (Hu et al., 2010). Table 2 comprises the result of apparent amylose content analysis. Pea flour, rice flour, and sticky rice flour were different in apparent amylose content. Pea flour contained 16.01% of amylose, and the closely same result (15.9%) was reported by Chung et al. (2008), whereas rice flour and sticky rice flour were 31.07% and 4.22%, respectively.

Table 2. Apparent amylose content of raw materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Apparent amylose content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea flour</td>
<td>16.01</td>
</tr>
<tr>
<td>Sticky rice flour (SRF)</td>
<td>4.22</td>
</tr>
<tr>
<td>Rice flour (RF)</td>
<td>31.07</td>
</tr>
</tbody>
</table>

Juliano (1992) classified rice cultivars into five categories according to their amylose content, include waxy (1-2 g/100 g), very low (2-12 g/100 g), low (12-20 g/100 g), intermediate (20-25 %) and high (25-33 g/100 g) amylose content. Based on this criteria, in this study, rice flour (31.07%) was categorized as intermediate amylose type, while sticky rice flour (4.22%) was very low amylose type. In other way, Chen et al. (2007) categorized rice with amylose content less than 5 % as glutinous which is similar with the term of sticky (Sasaki et al., 2009).

4.1.3 Granular Morphology

Starch granules morphology under 500x magnification of pea flour, rice flour, sticky rice flour, are presented on Figure 6. The starch granules were observed to be oval and irregular in pea flour with size range of 11-44 μm (Figure 6a). Most of the granules of pulse starches are oval, although spherical, round, elliptical and irregularly shaped granules are also found with granular size ranged from 8.0-50 μm (Hoover et al., 2010; Zhou et al., 2004). Protein bodies were also found on the surface of pea granules. This result is in accordance with that reported by Naguleswaran and Vasanthan (2010).

The scanning electron micrographs for rice flour in Figure 6b shows the presence of mainly polygonal clusters having size in the range of 2.8-14 μm/individual granules. The individual granules, in the case of rice starch, develop into compact spherical bundles or clusters, known as compound granules, which fill most of the central space within the endosperm cells (Singh et al. 2003). In addition to normal granules, round starch granules were also found. The other granules were in round
shape which was possibly the granules of cassava starch as it was round granules of 5-35 μm diameter (Collado and Corke, 2003).

Figure 6. Granular morphology of pea flour (a), rice flour (b), sticky rice flour (c).

Sticky rice flour (Figure 6c) possessed average granule size of 4.3-17.1 μm/individual granules and exhibited the same morphological characteristics with that of rice flour, but the round shape granules were absent. Morphological properties of starch were reported to have a relationship with other physicochemical properties as well as starch digestibility which will be discussed later (Singh et al., 2006; Sajilata et al., 2006).

4.1.4 Thermal Properties

Thermal properties of pea flour, rice flour, and sticky rice flour, involving the gelatinization onset temperature ($T_o$), peak temperature ($T_p$), conclusion temperature ($T_c$), and enthalpy ($\Delta H$) were observed in this study. The thermograms are shown in Figure 7 and their details of transition temperatures and enthalpies associated with starch gelatinization are summarized in Table 3.

Pea flour, rice flour, and sticky rice flour were different in the transition temperatures ($T_o$, $T_p$, and $T_c$). Pea flour had a gelatinization temperature range ($T_o$-$T_c$) from 69.19 °C to 77.11 °C with endothermic enthalpy of 3.29 J/g. Experimental result for gelatinization temperature was in range of the result from another author, however the result for endothermic enthalpy was slightly lower. A wider gelatinization temperature range (61.9-81.3 °C) of pea flour was reported by Chung et al. (2008) with enthalpy of 4.8 J/g. Rice flour possessed the highest transition temperatures (72.45-81.10 °C) with endothermic enthalpy of 3.94 J/g, whereas the DSC thermogram of sticky rice flour was in accordance with the report of Hagenimana et al. (2005). Onset temperature ($T_o$), peak temperature
(Tp), and conclusion temperature (Tc) of sticky rice flour were 58.54 °C, 65.65 °C, and 74.50 °C, respectively, while literatures were 60.34 °C, 65.56 °C, and 73.01 °C.

These results established those already reported by Sodhi and Singh (2003) who observed the morphological and thermal properties of rice starch from different cultivars. The differences in To,Tp, and Tc in starches was attributed to differences in amylose content and granular structure. High amylose starches were reported to exhibit higher transition temperature (Jane et al., 1999 in Hagenimana et al., 2005). Rice flour with higher amylose content (31.07 %) compared to pea flour and sticky rice flour possessed higher transition temperatures. In addition to amylose factor, larger To, Tp, and Tc values was also attributed to the compact nature of small starch granules (Krueger et al., 1987 in Sodhi and Singh, 2003). The previous scanning electron micrograph showed that rice flour had a smaller and compact granules compared to those of pea granules.

In term of the extent of enthalpy, more energy is required to initiate melting in the absence of amylose (Krueger et al., 1987 in Sodhi and Singh, 2003). This established that sticky rice flour with the lowest amount of amylose (4.22 %) possessed higher enthalpy value (10.43 J/g) compared to those of rice flour and pea flour.

Further investigation on the DSC thermogram showed that two endothermic peaks were observed in the rice flour. This reflected the melting of two starch types present in the sample. It was in accordance with the results of scanning electron micrograph (SEM) in that polygonal granules of rice starch were mixed thoroughly with the round granules of cassava starch. Based on DSC results, it was postulated that the first peak corresponded to the melting of cassava starch which appeared at lower temperature (62.40-70.37 °C) with the endothermic enthalpy of 1.56 J/g. The second peak at higher temperature (72.45-81.10 °C) with the endothermic enthalpy of 3.94 J/g corresponded to that of rice flour. Results of this study were in agreement with those reported by Sasanatayart (2010) in that substitution of cassava flour for rice flour resulted in two endothermic peaks during gelatinization.
Table 3. Thermal properties of pea flour, sticky rice flour, and rice flour

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak</th>
<th>(T_o) (°C)</th>
<th>(T_p) (°C)</th>
<th>(T_c) (°C)</th>
<th>(\Delta H) (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea flour</td>
<td>1</td>
<td>69.19</td>
<td>73.23</td>
<td>77.11</td>
<td>3.29</td>
</tr>
<tr>
<td>Sticky rice flour (SRF)</td>
<td>1</td>
<td>58.54</td>
<td>65.65</td>
<td>74.50</td>
<td>10.43</td>
</tr>
<tr>
<td>Rice flour (RF)</td>
<td>1</td>
<td>62.40</td>
<td>66.87</td>
<td>70.37</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72.45</td>
<td>76.28</td>
<td>81.10</td>
<td>3.94</td>
</tr>
</tbody>
</table>

Corresponding mixed rice flours, therefore, three types of starch had been mixed (rice flour, sticky rice flour and cassava flour). However, there were only two endothermic peaks observed. The possible explanation was the peak of sticky rice flour and cassava flour merged into another, resulting in a broad single peak (65.84-72.75 °C). Hagenimana et al. (2005) reported the similar result and described that this phenomenon was usually occurred when two or more different flours were mixed.

4.1.5 Pasting Properties

The terms gelatinization and pasting have often been applied to all changes that occur when starch is heated in water. However, gelatinization includes the early changes and pasting includes later changes. Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually total disruption of the granules. Simply stated, starch paste can be described as a two phase system composed of a dispersed phase of swollen granules and a continuous phase of leached amylose (Collado and Corke, 2003).

Rapid visco analyzer (RVA) has been widely used to examine the pasting characteristics of starches. The main parameters that would be presented on the RVA curve are pasting temperature, peak viscosity, breakdown viscosity, setback viscosity, and final viscosity. The pasting profiles of pea flour, rice flour, and sticky rice flour have been observed by RVA and are shown in Figure 8. Their corresponding pasting parameters are summarized in Appendix 6.

![Figure 8. RVA curve for pea flour, rice flour, and sticky rice flour](image-url)
At the initial step, the viscosity increased rapidly with the increase of temperature as the granule swells. The increase in viscosity with temperature might be attributed to the removal of water from the exuded amylose by the granules as they swell (Ghiasi et al., 1982 in Sandhu and Singh, 2007). Pasting temperature reflects temperature at the onset of rise in viscosity and the higher pasting temperature indicates the higher resistance towards swelling (Sandhu and Singh, 2007). Sasaki and Matsuki (1998) in Zavareze (2010) reported the inverse correlation between amylose content and swelling power. The swelling power of the low-amylose starches is higher than that of the medium- and high-amylose starches, and consequently shorter time is needed to reach peak viscosity (Batey and Curtin, 2000 in Ragee and Abdel-Aal, 2006).

In this study, pea flour and rice flour possessed high pasting temperature that indicated resistance towards swelling due to high amylose content, whereas sticky rice flour with lowest amylose content had a markedly lower pasting temperature (67.28 °C). Pea flour which was lower in amylose content than rice flour was not significantly different in pasting temperature with that of rice flour (82.27 °C and 80.88 °C, respectively). This explained that amylose was not the only factor influencing pasting characteristic of starch. Contribution of other components such as protein and fibre to the pasting properties cannot be ruled out (Batey and Curtin, 2000 in Ragee and Abdel-Aal, 2006).

The peak viscosity was reached when granules swelling have been balanced with the granules broken by stirring. Results showed that the time needed to reach peak viscosity varied significantly among samples. Sticky rice flour with lowest amylose, and thus high swelling power, had a shorter peak time (3.64 min). Pea flour reached the maximum viscosity slightly earlier (5.62 min) than did rice flour (6.24 min).

Peak viscosity of pea flour, rice flour, and sticky rice flour were 54.3 RVU, 277.3 RVU, and 388.5 RVU, respectively. The low peak viscosity of pea flour might be due to the lower content of starch in pea flour compared to rice flour and sticky rice flour (Batey and Curtin, 2000 in Ragee and Abdel-Aal, 2006). The amount of starch fractions (total starch, non-resistant starch, and resistant starch) are presented on the Table 9 in the discussion on in vitro starch digestibility of pea cake and rice cake. In addition, more starch granules with a high swelling capacity which means low in amylose result in a higher peak (Singh et al., 2010b).

During the holding period of the viscosity test, the material slurries were subjected to high temperature and mechanical shear stress which further disrupt starch granules in the grains, resulting in amylose leaching out and alignment. This period was commonly associated with a breakdown in viscosity. Sticky rice flour, exhibited a markedly high breakdown viscosity (185.9 RVU) than did of rice flour (37.7 RVU) and pea flour (5.5 RVU). High values of breakdown are associated with high peak viscosities, which in turn, are related to the degree of swelling of the starch granules during heating. Conversely, low values of breakdown indicate high paste stability, and may have good potential as a food ingredient for food exposed to heat treatment at high temperature and mechanical stirring (Ragee and Abdel-Aal, 2006).

During cooling, re-association between starch molecules, especially amylose, will result in the formation of a gel structure and, therefore, viscosity will increase to a final viscosity. Final viscosity indicates the ability of the starch to form a viscous paste (Sandhu and Singh, 2007). This phase is commonly described as the setback region and is related to retrogradation and reordering of starch molecules. The low setback values indicate low rate of starch retrogradation and syneresis (Ragee and Abdel-Aal, 2006). Setback viscosity of pea flour, sticky rice flour, and rice flour were 18.3, 51.7, 213.1 RVU, whereas the final viscosity 67.1, 254.3, 452.7, respectively. It has been reported that
among flours, the order of peak and final viscosity is: cereal (except corn flour) > tuber > legume (Liu et al., 2006).

4.2 EXPERIMENT I

4.2.1 Apparent Amylose Content

Variation in the ratio of rice flour and sticky rice flour (100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10) was aimed to vary the amylose content through samples. It was hoped that the effect of variation in amylose content on physicochemical properties and enzymatic digestibility would be observed.

The results of apparent amylose content analysis for the mixture of rice flour and sticky rice flour could be seen on the following chart (Figure 9). Variation in the ratio of rice flour and sticky rice flour (100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10) was aimed to vary the amylose content through samples. It was hoped that the effect of variation in amylose content on physicochemical properties and enzymatic digestibility would be observed.

Figure 9. Amylose content of mixture between rice flour and sticky rice flour

Through ratios, the trend was clearly seen that amylose content decreased as the proportion of sticky rice flour increased, and they were all statistically different (p<0.05) in the amount of amylose content. The most extreme addition of sticky rice flour (10 %) resulted in the lowest amylose content (27.48 %) of the mixture. Conversely, the absence of sticky rice flour (ratio 100:0) resulted in the highest level of amylose (31.07 %).

4.2.2 Granular Morphology

Starch granules morphology of mixture between rice flour and sticky rice flour in the ratios of 100:0, 97.5:2.5, 95:5, 92.5:7.5, and 90:10 was observed under 500x magnification of scanning electron. As observed in the preliminary research, rice flour showed the presence of mainly polygonal clusters. The other granules were in round shape which was possibly the granules of cassava starch as it was round granules of 5-35 μm diameter (Collado and Corke, 2003). On the other hand, sticky rice flour exhibited only polygonal shape with no presence of round shape granules. Granular morphology of mixed rice flours are presented on Figure 10.
Figure 10. Granular morphology of mixture between rice flour and sticky rice flour in the ratios of 100:0 (a), 97.5:2.5 (b), 95:5 (c), 92.5:7.5 (d), 90:10 (e)

In the mixture of rice flour and sticky rice flour, round shape which was suspected to be cassava granules were also found in all of ratios that means sticky rice flour and rice flour were well mixed. Morphological properties of starch were reported to have a relationship with other physicochemical properties as well as starch digestibility which will be discussed later (Singh et al., 2006; Sajilata et al., 2006).
4.2.3 Thermal Properties

Results of thermal properties analysis established the scanning electron micrograph, especially for rice flour that two types of granule were observed. The thermogram is presented on Figure 11 and the extracted values are presented on Table 4. Corresponding mixed rice flours, therefore, three types of starch had been mixed (rice flour, sticky rice flour and cassava flour). However, there were only two endothermic peaks observed (Figure 11). The possible explanation was that the peak of sticky rice flour and cassava flour merged into another, resulting in a board single peak (65.84-72.75 °C). Hagenimana et al. (2005) reported the similar result and described that this phenomenon was usually occurred when two or more different flours were mixed. The similar second peaks (73.28-81.00 °C) were also observed in all of mixed flour with different ratios.

![Figure 11. DSC thermogram of the mixture between rice flour and sticky rice flour](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF:SRF (100:0)</td>
<td>1</td>
<td>62.40$^c$</td>
<td>66.87$^c$</td>
<td>70.37$^c$</td>
<td>1.56$^c$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72.45$^b$</td>
<td>76.28$^b$</td>
<td>81.10$^b$</td>
<td>3.94$^a$</td>
</tr>
<tr>
<td>RF:SRF (97.5:2.5)</td>
<td>1</td>
<td>66.30$^d$</td>
<td>69.96$^b$</td>
<td>72.75$^b$</td>
<td>0.72$^d$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.38$^a$</td>
<td>76.37$^a$</td>
<td>80.66$^a$</td>
<td>3.03$^b$</td>
</tr>
<tr>
<td>RF:SRF (95:5)</td>
<td>1</td>
<td>66.99$^c$</td>
<td>69.94$^b$</td>
<td>72.37$^b$</td>
<td>0.61$^d$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.36$^a$</td>
<td>76.32$^a$</td>
<td>80.55$^a$</td>
<td>3.29$^b$</td>
</tr>
<tr>
<td>RF:SRF (92.5:7.5)</td>
<td>1</td>
<td>65.84$^d$</td>
<td>69.80$^b$</td>
<td>72.52$^b$</td>
<td>0.85$^d$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.45$^a$</td>
<td>76.46$^a$</td>
<td>81.00$^a$</td>
<td>3.27$^b$</td>
</tr>
<tr>
<td>RF:SRF (90:10)</td>
<td>1</td>
<td>66.58$^{cd}$</td>
<td>69.85$^b$</td>
<td>72.23$^b$</td>
<td>0.69$^d$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>73.28$^a$</td>
<td>76.43$^a$</td>
<td>80.60$^a$</td>
<td>3.33$^b$</td>
</tr>
</tbody>
</table>
4.2.4 Pasting Properties

Addition of sticky rice flour to the rice flour decreased pasting temperature and peak time, but increased peak and breakdown viscosity, however the decrement or increment were not significant (Figure 12). The presence of cassava in the rice flour might affect the pasting properties of rice flour and its corresponding mixtures with sticky rice flour. Cassava was reported to increase breakdown, but decrease final and setback viscosity (Sasanatayart, 2010).

Figure 12. Pasting properties of the mixture of rice flour and sticky rice flour

From the other point of view, it was reported that the higher the viscosity of flour, the lower resistant starch content in the flour (Liu et al., 2006). The relationship between physicochemical properties and starch digestibility will be deeply discussed in the part 4.3.3 focusing on the in vitro starch digestibility of pea cake and rice cake.

4.3 Experiment II

4.3.1 Textural Properties of Rice Cake and Pea Cake

Commercial Thai traditional pea cake and rice cake were obtained from a local market. Trials were then conducted to obtain pea cake and rice cake with nearly same characteristics from the commercial one. Subjectively, both commercial and experimental cakes were same in the appearance (Figure 13 and Figure 14). Textural properties such as hardness and adhesiveness were the main parameter in comparing commercial cake and experimental cake objectively using texture analyzer. Desired characteristic was obtained from 20% of solid content, cooked for 25 minutes at 95 °C, and cold set at room temperature for 6 hours.
Texture is one of important sensory properties of food products. Instrumental texture profile analysis (TPA) has been used for many years for the measurement of food textural properties (Sanderson, 1990 in Lau et al., 2000). The test consists of compressing a bite-size piece of food two times in a reciprocating motion that imitates the action of the jaw and extracting from the resulting
force-time curve a number of textural parameters such as hardness, cohesiveness, springiness (elasticity), and adhesiveness, and into the secondary (or derived) parameters of fracturability (brittleness), chewiness and gumminess. The textural parameters obtained from TPA force/deformation curves have been reported to be well correlated with sensory evaluation (Szczesniak, 2002).

Hardness has been defined as the force necessary to attain a given deformation or the peak force during the first compression cycle (Lau et al., 2000). The hardness values of pea cake (267.94 g) and rice cake (152.89 g) resulted from experiment were not significantly different with those of commercial pea cake (260.16 g) and rice cake (158.39 g) as presented in Table 5. Through ratios, the trend seemed to be the higher addition of sticky rice flour, the lower the hardness value. Increment in the portion of sticky rice flour gave a decrement of amylose content. The extent of the amylose gel network and deformability of swollen granules were the main factors contributing to gel strength. Apparent amylose content has been reported to be positively related to the gel hardness. Starches that exhibit harder gels tend to have higher amylose content (Mua and Jackson, 1997 in Sandhu and Singh, 2007).

Table 5. Textural properties of rice cake and pea cake

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (g)</th>
<th>Adhesiveness (g.s)</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Gumminess</th>
<th>Chewiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially prepared pea cake</td>
<td>260.16</td>
<td>-66.39</td>
<td>0.95</td>
<td>0.94</td>
<td>245.28</td>
<td>232.69</td>
</tr>
<tr>
<td>Pea cake from experiment</td>
<td>267.94</td>
<td>-48.76</td>
<td>0.96</td>
<td>0.90</td>
<td>240.82</td>
<td>231.97</td>
</tr>
<tr>
<td>Commercially prepared rice cake</td>
<td>158.39</td>
<td>-42.81</td>
<td>0.92</td>
<td>0.93</td>
<td>147.78</td>
<td>136.06</td>
</tr>
<tr>
<td>Rice cake (100:0)</td>
<td>152.89</td>
<td>-53.85</td>
<td>0.93</td>
<td>0.84</td>
<td>127.97</td>
<td>119.18</td>
</tr>
<tr>
<td>Rice cake (97.5:2.5)</td>
<td>123.54</td>
<td>-70.97</td>
<td>0.91</td>
<td>0.86</td>
<td>106.32</td>
<td>96.45</td>
</tr>
<tr>
<td>Rice cake (95:5)</td>
<td>115.06</td>
<td>-79.52</td>
<td>0.88</td>
<td>0.85</td>
<td>97.67</td>
<td>85.98</td>
</tr>
<tr>
<td>Rice cake (92.5:7.5)</td>
<td>96.86</td>
<td>-80.79</td>
<td>0.88</td>
<td>0.83</td>
<td>80.62</td>
<td>71.04</td>
</tr>
<tr>
<td>Rice cake (90:10)</td>
<td>69.73</td>
<td>-85.83</td>
<td>0.82</td>
<td>0.87</td>
<td>60.50</td>
<td>49.78</td>
</tr>
</tbody>
</table>

Fracturability, brittleness, crunchiness, and crumbliness which are a similar concept, can be defined as the force with which a material fractures, or the first significant break during the first compression cycle divided by the original sample height then reported as a percentage, and thus a small brittleness value indicates a more brittle gel (Szczesniak, 2002). Brittle foods are a product of high degree of hardness and low degree of cohesiveness, and are never adhesive (Rosenthal, 1999). It has been understood that not all products will possess fracturability as occurred in this study. This was mainly due to the fairly high value of adhesiveness through all samples.
Adhesiveness is defined as the negative force area for the first bite and represents the work required to overcome the attractive forces between the surface of a food and the surface of other materials with which the food comes into contact (Szczesniak, 2002). For materials with a high adhesiveness and low cohesiveness, when tested, part of the sample is likely to adhere to the probe on the upward stroke. The adhesiveness values obtained from both commercial pea cake and rice cake were comparable with the experimental one. Commercial pea cake and rice cake had adhesiveness values of -66.39 g.s and -42.81 g.s, whereas the value obtained from experiment were -48.76 g.s and -53.85 g.s, respectively. In comparison with hardness through ratios, the opposite trend was exhibited. As the portion of sticky rice increased, the adhesiveness value increased. The increment of sticky rice portion in the cake contributed to the higher amylopection molecules which responsible to the adhesiveness of food products.

Cohesiveness is the extent to which a material can be deformed before it ruptures obtained from the ratio of the area under the first and second compression (Szczesniak, 2002). The comparable results were obtained for commercial cake and experimental cake. Cohesiveness values of commercial and experimental pea cake were 0.94 and 0.90, whereas for rice cakes were 0.93 and 0.84, respectively. Addition of sticky rice flour did not affect much the cohesiveness value. Through ratios between rice flour and sticky rice flour in the cakes (100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10), the values were 0.84, 0.86, 0.85, 0.83, 0.87.

In term of springiness or originally called elasticity, commercial and experimental cakes were not significantly different, both pea cake and rice cake. The values were 0.95 and 0.96 (for pea cakes, respectively), 0.92 and 0.93 (for rice cakes, respectively). Addition of sticky rice flour to the cake was lowering the springiness. Following the order ratios, the values decreased (0.93, 0.91, 0.88, 0.82). Springiness has been defined as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed, or is the distance the sample was compressed during the second compression to the peak force, divided by the initial sample height (Szczesniak, 2002).

Hardness, adhesiveness, cohesiveness, and springiness are the primary parameters, whereas chewiness and gumminess are secondary (or derived) parameters. Gumminess is the energy required to disintegrate a semi-solid food to a state ready for swallowing. It is derived by multiply hardness and cohesiveness (Szczesniak, 2002; Rosenthal 1999). Results showed that commercial pea cake (245.28) was not significantly different with that of pea cake (240.82) resulted from trials in term of gumminess. However, the gumminess of experimental rice cake (127.97) was markedly lower than that of commercial rice cake (147.78).

Chewiness is defined as the product of gumminess x springiness (which equals hardness x cohesiveness x springiness) and is therefore influenced by the change of any one of these parameters. Chewiness, tenderness and toughness are measured in terms of the energy required to masticate a solid food. They are the characteristics most difficult to measure precisely, because mastication involves compressing, shearing, piercing, grinding, tearing and cutting, along with adequate lubrication by saliva at body temperatures (Szczesniak, 2002). The values of chewiness for the commercial and experimental pea cake were 232.69 and 231.97 which were almost similar, whereas for rice cake 136.06 and 119.18, respectively. Through ratios, as the portion of sticky rice increased, the chewiness got smaller.

Among textural parameters, hardness and adhesiveness have been considered as the major parameter in comparing texture properties of cakes (Yu et al., 2010). Figure 15 shows the relationships between amylose content and textural parameters (hardness and adhesiveness). Positive correlation ($R^2 = 0.895$) was found between amylose content and hardness. Conversely, negative correlation ($R^2 = 0.899$) was appeared between amylose content and adhesiveness.
4.3.2 Color Properties

Color has been considered as an essential property of food, characterizing food identity and quality. Food color is analyzed either by direct inspection (sensorial analyses) or by instrumental methods. The instrumental color measurements eliminate interindividual errors and are more reproducible (Socaciu and Diehl, 2009). The most commonly used technique for evaluating the color of food is colorimetry by the measurement of CIE tristimulus XYZ values, which are transformed mathematically to a coordinate system that describes color, such as Hunter Lab and CIE L*a*b*. In the agricultural and food industries, the most popular numerical color-space system is the L*a*b*, which is also referred to as the CIELAB system (CIE, 2000 in Socaciu and Diehl, 2009).

L* value indicates the brightness of sample ranged from 0 (black) to 100 (white). a* value indicates a mixture colors of red and green. The +a* value indicates red color with range of 0-100, whereas –a* value indicates green color with range of 0-(-80). b* value indicates a combination of yellow and blue. +b* range for 0-70 indicates yellowness while –b* range for 0-(-70) for blueness (Francis, 1996).

Experimental results in Table 6 showed that pea cake and rice cake were different in term of L*, a*, and b* values. Pea cake possessed L* value of 64.41, a* value of -1.33, and b* value of 13.57, whereas rice cake 57.42, -2.98, and 2.68, respectively. Higher L* value indicated that pea cake were lighter in color than that of rice cake, whereas higher b* value indicated that pea cake more yellow compared with rice cake. This was mainly due to the origin of the pea flour that was yellow in color. The reported L*, a*, b* values for pea flour from different cultivars were ranged from 43.6 to 67.1; 2.3 to 6.2; and 5.8 to 17.4, respectively (Singh et al., 2010b).

Ratio of rice flour and sticky rice flour in the cakes (100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10) did not give an ordered color properties. Addition of sticky rice flour to rice flour slightly affected color of the corresponding cakes. However, trend of color changes were not in line with the mixing ratio of flour. Relationship between amylose content in the raw materials and color properties of corresponding cakes remained unclear in this study. Further investigations are highly needed to reveal the effect of amylose content to the color properties of pea cake and rice cake.
4.3.3 In vitro Starch Digestibility of Pea Cake and Rice Cake

Starch digestion is a highly important metabolic response and the rate and extent of starch digestibility and absorption are nutritionally important. It can be determined both in vivo and in vitro. The digestibility of starch measured in vivo is a time-consuming, expensive process that requires many human subjects with specific attributes. Hence, investigation of in vitro digestibility as a replacement for the in vivo method is an increasingly researched topic (Frei et al., 2003). Consequently, in vitro procedures were recently developed in attempt to mimic human digestion (Englyst et al., 1992). In such procedures, pancreatic α-amylase digestion is frequently used, followed by the measurement of released glucose (Sasaki et al., 2009).

Differences in starch digestibility have been ascribed to various factors, including the botanical source, amylose/amylopectin ratio, processing condition, other components such as protein, lipid, α-amylase inhibitors, and anti-nutrients (Singh et al., 2010a).

Table 7 summarizes the amount of starch fractions (total starch, non-resistant starch, and resistant starch) of pea cake and rice cake prepared from the mixture between rice flour and sticky rice flour (ratio range of 100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10). In this study, the amount of resistant starch was apparently higher in pea cake than in rice cake, and through ratios, the higher the portion of sticky rice in the cake, the higher the resistant starch amount observed.

Table 7. Starch fractions of pea cake and rice cake

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-resistant starch (%TS)</th>
<th>Resistant starch (%TS)</th>
<th>Total starch (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea cake</td>
<td>90.38</td>
<td>9.62</td>
<td>41.35</td>
</tr>
<tr>
<td>Rice cake (100:0)</td>
<td>99.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice cake (97.5:2.5)</td>
<td>99.48&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice cake (95:5)</td>
<td>99.55&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>77.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice cake (92.5:7.5)</td>
<td>99.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>80.52&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice cake (90:10)</td>
<td>96.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

Amylose content has been considered as an important factor influencing RS content in starch. (Lehmann & Robin, 2007). Starch with higher amylose content is known to have less susceptibility to
enzymatic hydrolysis (Sasaki et al., 2009). Rice cakes vary in amylose content followed this order as rice cake (100:0) was the highest in resistant starch content compared with those of other rice cakes (97.5:2.5, 95:5, 92.5:7.5, 90:10).

Figure 16 explains the relationship between amylose content and starch fractions. Starch fractions (resistant and non-resistant) indicate the digestibility of starch. As the amylose content increased, resistant starch increased ($R^2=0.911$) and digestibility decreased, or on the other hand, as the amylose content decreased, non-resistant starch increased ($R^2=0.911$) and digestibility increased.

The results of the present study exhibited the opposite trend for the comparison of pea cake and rice cakes in general. Pea cake prepared from pea flour was higher in amount of resistant starch. This revealed that the amount of RS did not solely depend on amylose content.

Differences in hydrolysis mechanism of $\alpha$-amylase were also taking part in explaining a higher digestibility of cereal starches than that of leguminous starches. The diffusion of the $\alpha$-amylase into the substrate is considered as an important step of hydrolysis. Previously, hydrolysis of starch was considered as starting from the surface of the granule. However, it was found that native cereal starches such as corn and sorghum contain peripheral pores and channels, which enable $\alpha$-amylase penetration, resulting in an inside-out hydrolysis mechanism (Lehmann and Robin, 2007).

The particle size of the starch and the surface area to starch ratio also play an important role for the hydrolysis (Colonna et al., 1992). A smaller granule size shows higher enzymatic susceptibility. This corresponded with the results of SEM. Pea starch granules which had lower enzyme susceptibility were bigger in average size compared with rice starch granules.

In addition, legumes contain much more protein and anti-nutrients than cereals have, which may affect the starch digestibility (Singh et al., 2010a). Interactions of starch with fiber, protein, lipid, and other food components can prevent effective diffusion and adsorption of the enzyme (Colonna et al., 1992).

Results of chemical analysis of raw materials showed that pea flour was the highest in both protein and lipid content compared with those of rice flour and sticky rice flour. Scanning electron
micrograph for pea flour also clearly showed the presence of protein bodies on the surface of granules which might act as a barrier towards starch digestibility (Hamaker and Bugusu, 2003 in Singh et al., 2010a). Higher lipid content in pea flour increased the possibility of amylose-lipid complex formation that lead to enzymatic resistance (Sing et al., 2010a). The high concentration of anti-nutrients such as phytic acid, lectins, enzyme inhibitors in legumes may also play a role in lowering starch digestibility. Phytic acid is the most important phosphate reserve compound in many plants. The phytic acid may affect the starch digestibility through interaction with amylase protein and/or binding with salivary minerals such as calcium which is known to catalyze amylase activity (Sing et al., 2010a).

In this study, pea and rice cake were simply cooked and cold set. The retrograded amylose formed during cooling of the gelatinized starch has been classified as RS type 3, most moist-heated foods therefore contain some RS3 (Haralampu, 2000). Singh et al. (2010) stated that processed legumes considerably contain significant amount of RS3 in comparison to other cereal products, irrespective of the processing treatment.

4.4 EXPERIMENT III

4.4.1 In Vitro Starch Digestibility

In the experiment part II, cold setting time was prolonged and temperature was modified. Cold setting conditions were varied (6 hours, room temperature; 6 hours, refrigeration temperature; 24 hours, refrigeration temperature) to observe the effect on starch digestibility, especially resistant starch amount among samples. The samples were pea cake, rice cake prepared from solely rice flour, and rice cake prepared from mixture of rice flour and sticky rice flour in the ratio of 90:10 which represented the extreme addition of sticky rice flour from the experiment part I.

Pea cake was significantly higher (p<0.05) in resistant starch amount than those of all rice cake ratios as can be seen on Figure 17. Both rice cake and rice cake (90:10) exhibited an increment in the amount of resistant starch during cold setting. The increment was from 0.54 % (6h, room temperature) to 0.73 % (6h, 4 °C) for rice cake. Continued storage at 4 °C for 24 hours gave a higher resistant starch amount (0.95 %), whereas for the ratio of 90:10, amount of resistant starch were 0.34% (6h, room temperature), 0.45 % (6h, 4 °C), and 0.56 % (24h, 4 °C). These findings were all in agreement with those reported by other authors that resistant starch amount increased during storage (ranged from 0 to 72 h) in refrigeration temperature (Islas-Hernandez, 2006; Sajilata et al., 2006). This was attributed to the retrogradation of amylose (Kumari et al., 2007).

Pea cake exhibited the opposite trend from rice cakes (Figure 17). Longer storage in refrigeration temperature lowered the amount of resistant starch in pea cake. The decrement was from 9.62 % (6h, room temperature) to 7.46 % (6h, 4 °C). Continued storage at 4 °C for 24 hours gave a lower resistant starch amount (6.92 %). This result established the previous report from Sajilata et al. (2006) that there was no significant increase in resistant starch content of stored legumes, even lesser resistant starch content on storage in comparison with fresh samples. The decrease in resistant starch with prolonged storage, especially in legumes, could also be due to the formation of starch–protein or starch–lipid complexes, in conjunction with the depletion of water needed for recrystallization. Availability of water is essential in the formation of resistant starch, as it is involved in recrystallization of amylose (Rabe and Sievert, 1992 in Niba, 2003).
4.4.2 Textural Properties

Influence of cold setting conditions to the textural properties of pea cake and rice cake were also investigated. The changes of textural properties involved hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were summarized in Figure 18.

In this study, longer storage in refrigeration temperature significantly increased hardness, adhesiveness, gumminess, and chewiness value of all samples. Hardness value of 267.94 g was obtained from pea cake after cold setting at room temperature for 6 hours. The same sample stored at refrigeration temperature gave a higher hardness value of 521.71 g. Continued storage at refrigeration temperature for 24 hours leaded to the further increment of hardness value (851.16 g). Rice cake and rice cake prepared from mixed sticky and rice flours (90:10) followed the same order of pea cake during cold setting. The increments of hardness values were 152.89 g, 197.78 g, 269.35 g, respectively (for rice cake), and 69.73 g, 78.63 g, 121.90 g, respectively (for rice cake 90:10). The increment gel firmness is mainly caused by retrogradation of starch gels, which is associated with the syneresis of water and crystallization of amylopectin, leading to harder gels (Miles et al., 1985 in Sandhu and Singh, 2007). This was confirmed by the research work done by Ji et al. (2007) who studied the staling of cake prepared from rice flour and sticky rice flour.

Adhesiveness also increased during cold setting. The increasing values for pea cake were -48.76 g.s, -79.43 g.s, -96.68 g.s, respectively, whereas for rice cake and rice cake (90:10) were -53.85 g.s, -82.92 g.s, -100.30 g.s, and -85.83 g.s, -90.09 g.s, -114.20 g.s, respectively. These results exhibited an opposite trend with that reported by Yu et al. (2009) in Yu et al. (2010), of which amylose retrogradation contributed to the decreasing adhesiveness during storage.
Figure 18. Textural properties (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness) of pea cake, rice cake, and rice cake prepared from ratio of rice flour: sticky rice flour (90:10) at various cold setting conditions.
The existing possible explanation is reported by Hoseney (1998). As the temperature goes down, starch chains become less energetic, hydrogen bonds become stronger, giving a firmer gel. However, the starch chains have a tendency to interact strongly each other, thereby force the water out of system. This resulted in moist surface of the cakes. Subsequently, the cakes became more adhesive. As the gel got firmer, consequently, the energy required to masticate a solid food to a state ready for swallowing (chewiness) and the energy required to disintegrate a semi-solid food to a state ready for swallowing (gumminess) were getting higher during cold setting and storage. Through cold setting conditions (6h, room temperature; 6h, 4°C; 24h, 4°C), gumminess gradually increased from 240.82, 483.82, to 744.52; 127.97, 178.06, to 250.85; and 60.40, 51.50, to 104.08, for pea cake, rice cake, and rice cake (90:10), respectively. Chewiness increased as well as gumminess, ranged from 231.97, 464.40, to 713.77; 119.18, 163.70, to 232.64; and 49.78, 43.58, to 91.22, for pea cake, rice cake, and rice cake (90:10), respectively.

In term of springiness and cohesiveness, there was no orderly trend through all samples after cold setting. The springiness values of pea cake (0.96) were same through all of cold setting conditions. Rice cake behaved the similar trend with pea cake. Through cold setting conditions (6h, room temperature; 6h, 4°C; 24h, 4°C), there was no significant increment or decrement. There was an increment from 0.82 to 0.85, and finally 0.88 for rice cake (90:10). Summarized cohesiveness values through all of cold setting conditions were 0.90, 0.93, 0.87 for pea cake; 0.84, 0.90, 0.93 for rice cake; and 0.87, 0.66, 0.85 for rice cake prepared from the mixture of rice flour and sticky rice flour at the mixing ratio of 90:10.

Texture is a multi-parameter sensory attribute, and thus the changes in textural properties are definitely complex mechanism and even are not well understood. Therefore, further investigations on the textural properties and their influencing factors should be carried on (He and Hoseney, 1990 in Ji et al., 2007).

4.4.3 Color Properties

Color measurements serve as quality index of raw and processed food for use in quality control, determination of conformity of food quality into a claimed specification, and analysis of quality change as a result of food processing and storage (Socaciu and Diehl, 2009). Color properties involved \(L^*, a^*, b^*\) values of pea cake, rice cake, and rice cake prepared from mixed flour were investigated through three condition of cold settings (6h, room temperature; 6h, 4°C; 24h, 4°C).

The lightness of pea cake was found to slightly increase (64.41 to 65.53) after storage the refrigeration temperature for 6 hours (Figure 19). Longer storage at refrigeration temperature (24h, 4°C) gave a significant lower \(L^*\) value (61.22). The same trend was exhibited by rice cake. The increment of \(L^*\) was from 57.42 to 58.83, and longer storage resulted in lower value (57.27). On the other hand, rice cake prepared from mixing ratio of 90:10 between rice flour and sticky rice flour behaved differently. There was a linear increment through cold setting conditions (56.11, 56.61, 56.66).

In term of greenness or redness (± \(a^*\) value), pea cake behaved the same trend in which there was an increment from -1.33 to -1.38, then decreased to -1.09 (Figure 20). Inversely, both rice cake and rice cake (90:10) behaved disorderly compared to that of pea cake. The \(a^*\) value of them through cold setting conditions were -2.98, -3.31, -3.31; and -3.04, -3.20, -3.11, respectively.

The yellowness (\(b^*\) value) of pea cake increased from 13.57 to 15.05 after storage at 4°C for 6 hours, however the value then decreased to 10.27 (Figure 21). The similar order (increased then decreased) was also exhibited by rice cake. The value raised from 2.68 to 4.25 then decreased to 3.92.
Rice cake (90:10) possessed its own order through cold setting conditions. Linear increment in $b^*$ value was exhibited (2.24, 3.50, 3.63).

Figure 19. $L^*$ value of pea cake, rice cake, and rice cake prepared from ratio of rice flour : sticky rice flour (90:10) at various cold setting conditions

Figure 20. $a^*$ value of pea cake, rice cake, and rice cake prepared from ratio of rice flour : sticky rice flour (90:10) at various cold setting conditions

Figure 21. $b^*$ value of pea cake, rice cake, and rice cake prepared from ratio of rice flour : sticky rice flour (90:10) at various cold setting conditions
The most possible explanation on color properties in starch-based product was that the changes were mainly due to the retrogradation of amylose molecules. Longer storage gave more interaction between starch chains, and eventually the formation of crystals. As a result, gel became opaque, more rigid and rubbery (Hoseney, 1998). This established the reports that at the early stage of retrogradation process, turbidity development was occurred (Karim et al., 2000). Furthermore, Sandhu and Singh (2007) reported that turbidity values of starch suspensions increased progressively during storage of starch gels at 4°C. Turbidity development in starches during storage has been attributed to the interaction of several factors, such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain length, intra or interbonding, lipid and cross-linking substitution (Jacobson et al., 1997 in Sandhu and Singh, 2007).

Relationship between color and other properties was reported to be a complex thing. The key problem that prevents accurate and reproducible color measurements is that most foods have non-uniform surfaces and heterogenic composition, with profound effects on light and color reflection and perception (Socaciu and Diehl, 2009). In addition, study on color properties of starch-based product and its influencing factors have been received little emphasis, and thus further study is highly required.