# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Optimization of soaking water and soaking time for the extraction of black glutinous rice milk

In the first section of the study, black glutinous rice powder with the biggest particle size of 595 µm was soaked in distilled water using a ratio of 1:2.5, 1:5 or 1:10 for rice and water, respectively, with a soaking time of either 30, 60 or 120 min. After the soaking period, the rice was extracted with α-amylase and amyloglucosidase enzymes and filtered with cleaned white clothes to produce black glutinous rice milk. The physical properties of the rice milk after the extraction process are shown in Table 4.1.

Collected data clearly displayed that the amount of soaking water was significantly affected viscosity and colour values of L* and a* of the rice milk ($p \leq 0.05$). The viscosity of black glutinous rice milk was significantly reduced with increasing amount of soaking water ($p \leq 0.05$) (Table 4.1). Higher amount of soaking water was also significantly reduced the lightness (L* value) and redness (positive a* value) of the rice milk ($p \leq 0.05$). The colour of cereals was affected by several factors, including concentration of light, acid-base of rice, species, harvesting time and amount of pigment (Brouillard and Delaporte, 1977; Abdel-Aal et al., 2006). The positive b* value (yellow color direction) of the rice milk was not significantly affected by the amount of soaking water ($p > 0.05$). Wongkhalaung and Boonyaratanakornkit (2000) reported colour values of rice milk of 90.25, -0.89 and 2.86 for L*, a* and b* values, respectively. Although the reported study used a water ratio of 1:2.5 for rice and water, respectively, it utilised Jasmine rice (*Khao Dok Mali* 105) as a raw material. A different rice type used as a raw material in the present study significantly affected the colour value results.

**Table 4.1** Physical properties of black glutinous rice milk affected by ratios of rice and water and soaking times.

| The ratio of rice and water | Soaking time (min) | Physical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | Viscosity (cP) | Color | | |
| | | | L* | a* | b*$^{ns}$ |
| 1:2.5 | 30 | 2.77±0.10$^{a}$ | 16.46±2.15$^{a}$ | 2.19±0.65$^{a}$ | 0.94±0.30 |
| | 60 | 2.82±0.23$^{a}$ | 15.38±0.91$^{ab}$ | 2.08±0.40$^{b}$ | 0.87±0.17 |
| | 120 | 2.95±0.17$^{a}$ | 13.40±1.20$^{c}$ | 1.73±0.20$^{abc}$ | 1.10±0.23 |
| 1:5 | 30 | 1.46±0.06$^{c}$ | 13.24±0.08$^{c}$ | 1.62±0.18$^{bc}$ | 1.10±0.03 |
| | 60 | 1.53±0.16$^{bc}$ | 14.31±1.08$^{bc}$ | 1.54±0.11$^{bc}$ | 1.04±0.10 |
| | 120 | 1.74±0.11$^{b}$ | 13.02±0.20$^{c}$ | 1.37±0.06$^{c}$ | 1.20±0.05 |
| 1:10 | 30 | 1.42±0.08$^{c}$ | 14.39±0.23$^{bc}$ | 1.57±0.27$^{bc}$ | 1.08±0.05 |
| | 60 | 1.42±0.07$^{c}$ | 14.35±0.59$^{bc}$ | 1.27±0.17$^{c}$ | 1.08±0.10 |
| | 120 | 1.43±0.07$^{c}$ | 14.57±0.47$^{bc}$ | 1.27±0.05$^{c}$ | 1.02±0.10 |

Data was mean ± standard deviation from three replicate experiments.

$^{a-c}$ Different letters within a column were significantly different at $p \leq 0.05$.

$^{ns}$ Not significantly different.

For the soaking time, this parameter did not significantly affect the physical quality of the black glutinous rice milk, except for the L* value at the ratio of 1:2.5 for rice and water, respectively. At this soaking ratio, higher soaking time significantly reduced the L* value of the rice milk (Table 4.1) ($p \leq 0.05$). Wongkhalaung and Boonyaratanakornkit (2000) reported colour values of rice milk. The lightness (L*) of the milk was lower with less greenish (a*) and yellowish (b*) than those of cow's milk.

The chemical properties of black glutinous rice milk after enzymes extraction can be seen in Tables 4.2 and 4.3. The ratio of soaking water significantly influenced most of the chemical characteristics of the rice milk, including pH, moisture content, fat, carbohydrate, total sugar, reducing sugar, total soluble solid, antioxidant properties, such as phenolic and anthocyanin, and the content of phytic acid ($p \leq 0.05$). For the pH values, significant reduction of pH was found as higher amount of distilled water was used to soak the rice powder. This could be partly affected by the pH of distilled water used in this experiment, which was 6.11 ± 0.11. Although distilled water should have a neutral pH of 7, the water will normally have a pH value of 5.2 to 6.0. This is due to exposure of the water to open air, causing some dissolution of carbon dioxide. Different buffering effects of other contaminants in the water cause variation in the pH value (Rona, 1996). Haas (1992) and Rona (1996) reported that pH of distilled water can be as low as 4.0, although it will be more common for the water to have pH values of 5.0 to 6.5.

At higher amount of soaking water, the moisture content of the rice milk significantly increased, while the fat, carbohydrate, total sugar, reducing sugar, total soluble solid, phenolic and anthocyanin contents were significantly reduced. Reduction in the seven chemical properties of the rice milk was due to a dilution factor at higher amount of distilled water used. Finding of fat and carbohydrate in the present study using the lowest soaking ratio was almost similar to the report of Wongkhalaung and Boonyaratanakornkit (2000) for rice-based yoghurt. The paper stated that the rice-based yoghurt from Jasmine rice had fat and carbohydrate contents of 2.67 and 25.1%, respectively.

The amount of total phenolic content, total flavonoid content and antioxidant activity (as $EC_{50}$ or the amount of antioxidant required to reduce the initial DPPH (1,1'-diphenyl-2-picryl-hyrazyl) concentration by 50%) in brown black glutinous rice (*Khao Niaw Dum*) was reported to be 60.60 ± 0.85 mg GAE (gallic acid equivalent)/100 g, 42.96 ± 0.38 mg GAE/100 g and 11.39 ± 5.35 g/ml, respectively (Vichapong et al., 2010). The reported phenolic figure was much higher than the phenolic compound foud

**Table 4.2** Chemical properties of black glutinous rice milk affected by ratios of rice and water and soaking times.

| The ratio of rice and water | Soaking time (min) | Chemical properties of black glutinous rice milk | | | | | |
|---|---|---|---|---|---|---|---|
| | | pH | Moisture content (%) | Protein content (%)[ns] | Fat content (%) | Ash content (%)[ns] | Carbohydrate content (%) |
| 1:2.5 | 30 | 5.57±0.18[ab] | 77.76±2.71[c] | 0.27±0.11 | 1.38±0.13[a] | 0.30±0.15 | 20.29±2.81[b] |
| | 60 | 5.55±0.04[abc] | 74.11±3.45[d] | 0.30±0.02 | 1.25±0.11[a] | 0.28±0.14 | 24.05±3.70[b] |
| | 120 | 5.58±0.04[a] | 74.69±1.07[cd] | 0.24±0.06 | 1.41±0.12[a] | 0.63±0.33 | 23.03±0.82[ab] |
| 1:5 | 30 | 5.40±0.10[cd] | 86.63±1.97[b] | 0.11±0.06 | 0.84±0.12[b] | 0.47±0.05 | 11.95±2.11[c] |
| | 60 | 5.38±0.06[cd] | 87.29±1.03[b] | 0.12±0.04 | 0.74±0.08[b] | 0.64±0.20 | 11.20±0.94[c] |
| | 120 | 5.41±0.02[bcd] | 85.81±0.74[b] | 0.19±0.02 | 0.75±0.06[b] | 0.59±0.22 | 12.66±0.61[c] |
| 1:10 | 30 | 5.19±0.03[e] | 93.05±0.72[a] | 0.16±0.05 | 0.28±0.05[c] | 0.64±0.16 | 5.88±0.87[d] |
| | 60 | 5.14±0.13[e] | 92.58±0.74[a] | 0.26±0.32 | 0.32±0.11[c] | 0.40±0.17 | 6.45±1.20[d] |
| | 120 | 5.12±0.06[e] | 92.04±1.55[a] | 0.09±0.01 | 0.26±0.05[c] | 0.50±0.03 | 6.11±1.56[d] |

Data was mean ± standard deviation from three replicate experiments.

[a-e] Different letters within a column were significantly different at $p \leq 0.05$.

[ns] Not significantly different.

**Table 4.2** Cont.

| The ratio of rice and water | Soaking time (min) | Chemical properties of black glutinous rice milk | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total sugar (%) | Reducing sugar (mg/ml) | Total soluble solid (°Brix) | Phenolic content (μg/ml) | Anthocyanin content (μg/g) | Phytic acid content (μg/g) |
| 1:2.5 | 30 | 18.27±0.56[a] | 141.9±0.51[b] | 26.4±0.80[a] | 111.1±0.10[a] | 459.7±0.06[b] | 603.7±5.13[a] |
| | 60 | 18.99±1.63[a] | 152.6±1.14[a] | 27.4±2.36[a] | 104.2±0.25[b] | 416.0±0.06[c] | 528.7±5.13[c] |
| | 120 | 18.51±0.82[a] | 143.0±0.46[b] | 26.7±1.18[a] | 115.0±0.15 [c] | 501.1±0.06[a] | 378.0±4.58[f] |
| 1:5 | 30 | 8.89±1.70[c] | 67.9±11.90[d] | 12.8±2.45[c] | 50.5±0.45[g] | 389.4±5.28[e] | 386.7±9.61[f] |
| | 60 | 9.91±0.78[bc] | 80.1±2.25[c] | 14.3±1.13[bc] | 61.4±0.31[e] | 364.2±0.06[f] | 420.3±16.2[e] |
| | 120 | 10.79±0.56[b] | 86.2±0.55[c] | 15.6±0.81[b] | 68.4±0.26[d] | 401.8±0.10[d] | 501.3±2.08[d] |
| 1:10 | 30 | 6.23±0.01[d] | 48.7±0.21[ef] | 9.0±0.00[d] | 51.2±0.40[f] | 274.9±0.06[h] | 295.7±2.52[g] |
| | 60 | 6.93±0.02[d] | 54.3±0.17[e] | 10.0±0.00[d] | 44.3±0.72[h] | 298.1±0.06[g] | 241.3±7.64[h] |
| | 120 | 5.81±0.74[d] | 45.3±6.15[f] | 8.3±1.15[d] | 37.3±0.53[i] | 297.0±0.06[g] | 558.7±4.51[b] |

Data was mean ± standard deviation from three replicate experiments.

[a-h] Different letters within a column were significantly different at $p \leq 0.05$.

**Table 4.3** Chemical properties of black glutinous rice milk affected by ratios of rice and water and soaking times based on dry basis.

| The ratio of rice and water | Soaking time (min) | Chemical properties of black glutinous rice milk | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total sugar (%) | Reducing sugar (mg/ml) | Total soluble solid (°Brix) | Phenolic content (µg/ml) | Anthocyanin content (µg/g) | Phytic acid content (µg/g) |
| 1:2.5 | 30 | 45.7±1.40$^{e}$ | 354.8±1.28$^{ef}$ | 65.9±2.01$^{ef}$ | 277.8±0.25$^{g}$ | 1149.2±0.14$^{f}$ | 1509.2±12.8$^{f}$ |
| | 60 | 46.9±3.12$^{de}$ | 381.5±2.84$^{def}$ | 68.5±5.89$^{ef}$ | 260.6±0.63$^{h}$ | 1040.1±0.14$^{g}$ | 1322.7±12.8$^{g}$ |
| | 120 | 46.3±2.04$^{de}$ | 357.1±1.15$^{ef}$ | 66.8±2.95$^{ef}$ | 287.4±0.38$^{f}$ | 1252.9±0.14$^{e}$ | 945.0±11.5$^{i}$ |
| 1:5 | 30 | 44.5±8.90$^{e}$ | 339.7±59.5$^{f}$ | 64.2±12.3$^{f}$ | 252.7±2.25$^{i}$ | 1047.0±26.4$^{g}$ | 1933.3±48.1$^{e}$ |
| | 60 | 49.6±3.92$^{de}$ | 400.5±11.3$^{de}$ | 71.5±5.63$^{ef}$ | 307.2±1.53$^{e}$ | 1820.8±0.29$^{d}$ | 2101.7±80.8$^{d}$ |
| | 120 | 54.0±2.82$^{cd}$ | 430.8±2.75$^{cd}$ | 77.8±4.07$^{cd}$ | 342.0±1.32$^{d}$ | 2009.0±0.50$^{c}$ | 1206.7±10.4$^{h}$ |
| 1:10 | 30 | 62.3±0.12$^{ab}$ | 487.3±2.08$^{b}$ | 90.0±0.00$^{ab}$ | 512.3±4.04$^{a}$ | 2749.3±0.58$^{b}$ | 2957.7±25.2$^{b}$ |
| | 60 | 69.3±0.15$^{a}$ | 543.0±1.73$^{a}$ | 100.0±0.00$^{a}$ | 443.0±5.29$^{b}$ | 2981.3±0.58$^{a}$ | 2413.3±76.4$^{c}$ |
| | 120 | 58.1±7.45$^{bc}$ | 453.0±61.5$^{bc}$ | 83.3±11.5$^{bc}$ | 373.0±5.29$^{c}$ | 2970.3±0.58$^{a}$ | 5587.7±45.1$^{a}$ |

Data was mean ± standard deviation from three replicate experiments.

$^{a-i}$ Different letters within a column were significantly different at $p \leq 0.05$.

$^{ns}$ Not significantly different.

in this study. This could be due to preparation of the black glutinous rice milk by the addition of distilled water and heating during the enzymatic processes that might significantly reduce the amount of phenolic content in the final rice milk.

A study by Tananuwong and Tewaruth (2010) on black glutinous rice flour showed that the flour had total monomeric anthocyanins between 298 and 342 μg/g flour, depending on the solvent pH and total extraction time. Higher amount of anthocyanin found in this study, particularly for the soaking ratios of 1:2.5 and 1:5, could be affected by the application of enzymes, different methods to measure the anthocyanin content and different variety of black glutinous rice. The previous paper used black glutinous rice cv. Kam Doi Saked, while this study utilised black sticky rice from Doi Kham, Chiang Mai (The Royal Foundation, Thailand).

For the phytic acid content, it was affected both by the amount of soaking water and the soaking time (Table 4.2). At a rice and water ratio of 1:2.5, the phytic acid was significantly decreased as the soaking time increased ($p \leq 0.05$), while at higher soaking water ratios, the phytic acid was found to be the highest after soaking the rice powder for 120 min. The cereal flours showed values of phytic acid ranged between 3–4 mg/g for soft wheats, 9 mg/g for hard wheat and 22 mg/g for whole wheat. Corn, millet and sorghum flours reported a mean of 10 mg/g and oat, rice, rye and barley between 4 and 7 mg/g. Wheat brans had wide ranges (25–58 mg/g) (García-Estepa et al., 1999).

The factor of soaking time was also affected the amount of reducing sugar, phenolic and anthocyanin contents (Table 4.2). There was not any specific trend regarding the effect of soaking time on these components. The effect of soaking time was related to the soaking ratio. Both the soaking ratio and soaking time did not significantly affect the contents of protein and ash of the black glutinous rice milk ($p > 0.05$). For fibre, it was only analysed for some black glutinous rice milk samples. The amount was very low in the range of 0.013±0.003%. Table 4.3 displayed some chemical at black glutinous rice milk based on dry basis. From this table, it could be seen that higher amounts of total sugar, reducing sugar, total soluble solid, phenolic and anthocyanin contents were found in the soaking water ratio of 1: 10.

## 4.2 Optimum extraction condition of α-amylase to produce black glutinous rice milk

Based on the data in the section 4.2, the soaking ratio that was further investigated was 1:5 for rice powder and distilled water, respectively. This ratio was selected due to the amount of antioxidant compounds that were still more than half of the lowest soaking water ratio of 1:2.5. Although the soaking ratio of 1:2.5 significantly produced the highest amount of total sugar, reducing sugar and antioxidant components, the yield of the rice milk at this ratio was low, while the highest soaking ratio of 1:10 produced too high dilution of some rice milk components, such as fat and carbohydrate contents with the amount of antioxidant compounds was significantly lower than the soaking ratio of 1:5. The soaking time that was used in this section was 2 h because of higher viscosity, total sugar, reducing sugar, total soluble solid, phenolic content and anthocyanin content, compared to the other soaking times.

In this section, the optimum time and temperature of α-amylase extraction at pH 6.5 was investigated. The time of extraction was varied between 30 and 120 min with heating temperatures at the range of 60 to 80ºC. Naz (2002) stated that the optimum temperature for enzyme activities varied, for example α-amylase from *Bacillus subtilis* had an optimum temperature at 70-72ºC, α-amylase from *Bacillus licheniformis* had a temperature of 90ºC, while the application of α-amylase in the starch processing using temperatures of more than 105ºC.

The physical properties of the black glutinous rice milk are displayed in Table 4.4. The results showed that the time and temperature of α-amylase extraction significantly affected the viscosity and b* value of the rice milk ($p \leq 0.05$). A significant higher rice milk viscosity was found as higher extraction temperature was applied ($p \leq$

**Table 4.4** Physical properties of black glutinous rice milk affected by different extraction conditions of α-amylase.

| Temperature (°C) | Time (min) | Physical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | Viscosity (cP) | Color | | |
| | | | L* $^{ns}$ | a* $^{ns}$ | b* |
| 60 | 30 | 1.82±0.27$^{e**)}$ | 21.89±0.15 | 5.76±0.32 | 2.79±0.03$^{c}$ |
| | 60 | 2.10±0.46$^{de}$ | 22.17±0.52 | 5.21±0.86 | 2.95±0.06$^{c}$ |
| | 120 | 2.81±0.55$^{cd}$ | 21.44±0.40 | 4.60±0.26 | 3.10±0.45$^{bc}$ |
| 80 | 30 | 3.25±0.24$^{bc}$ | 21.39±0.10 | 6.47±0.25 | 3.09±0.19$^{bc}$ |
| | 60 | 3.48±0.14$^{bc}$ | 20.25±0.56 | 5.79±1.45 | 3.56±0.20$^{a}$ |
| | 120 | 3.96±0.27$^{b}$ | 21.12±0.15 | 5.79±0.87 | 3.61±0.25$^{a}$ |
| 90 | 30 | 5.96±0.71$^{a}$ | 22.00±1.33 | 5.60±0.44 | 2.83±0.18$^{c}$ |
| | 60 | 6.06±0.65$^{a}$ | 21.54±0.35 | 6.61±0.36 | 3.38±0.22$^{ab}$ |
| | 120 | 6.03±0.35$^{a}$ | 21.47±0.35 | 6.19±0.69 | 3.42±0.12$^{ab}$ |

Data was mean ± standard deviation from three replicate experiments.

$^{a-e}$ Different letters within a column were significantly different at $p \leq 0.05$.

$^{ns}$ Not significantly different.

0.05), while higher b* value (more yellow colour intensity) was obtained as longer extraction time was carried out. The result of the viscosity could be due to evaporation that might occur at higher heating temperature. The loss of water through evaporation during heating could increase the milk viscosity (Evans and Morriss, 1988). There was also a possibility from gelatinization of small particle sizes of rice grains at higher extraction temperatures. Longer extraction time did not significantly influence the viscosity of the rice milk ($p$ > 0.05). Different time and temperature of α-amylase extraction did not significantly influence L* and a* values of the rice milk ($p$ > 0.05).

For the chemical characteristics of the black glutinous rice milk affected by different α-amylase extraction conditions, the collected data is presented in Table 4.5. The results clearly showed that the extraction time and temperature significantly affected all of the chemical properties of the rice milk, including pH, moisture content, total sugar, reducing sugar, total soluble solid, antioxidant components (phenolic and anthocyanin contents) and phytic acid ($p \leq 0.05$). The pH of rice milk was more affected by the extraction time, in which longer extraction time significantly produced lower pH values. Saipang et al. (2010) reported that soaking red-jasmine rice in various condition affected pH ($p \leq 0.05$). As the soaking temperature increased from 30 to 40ºC, the pH of soaking rice-water was decreased ($p \leq 0.05$). In this study at the same extraction time, higher pH values were observed at the highest extraction temperature of 90ºC.

There was not any specific trend for the moisture content of the black glutinous rice milk processed at different times and temperatures of α-amylase extraction (Table 4.5). The highest moisture content was found at the extraction temperature of 60ºC for a particular extraction time. Lower moisture contents at higher extraction temperature could be due to evaporation at higher temperature (Evans and Morriss, 1988). As the water lost during heating, the moisture content of rice milk was decreased.

For total sugar, reducing sugar and total soluble solid, the results in Table 4.5 showed that these components were found to be the highest in the black glutinous rice milk extracted at 80ºC for 60 min.

The phenolic compound was more affected by the α-amylase extraction time compared to the extraction temperature (Table 4.5). An extraction time of 120 min produced the highest phenolic compound in the rice milk samples that were extracted at 80 and 90ºC. The phenolic content was reported to be correlated with the grain size. Yafang et al. (2011) studied the length of rice samples ranged from 3.28 mm (PF36) to

**Table 4.5** Chemical properties of black glutinous rice milk affected by different extraction conditions of α-amylase.

| Temperature (°C) | Time (min) | Chemical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | pH | Moisture content (%) | Total sugar (%) | Reducing sugar (mg/ml) |
| 60 | 30 | 6.35±0.01[abc] | 91.51±1.20[ab] | 5.98±0.83[e] | 45.73± 4.56[cd] |
| | 60 | 6.33±0.02[abc] | 92.92±2.95[a] | 5.31±1.11[e] | 40.47±13.33[d] |
| | 120 | 6.16±0.02[d] | 87.57±0.70[bc] | 9.96±0.32[d] | 72.03± 2.47[b] |
| 80 | 30 | 6.37±0.09[ab] | 84.32±3.94[cd] | 11.92±1.98[bcd] | 65.03±10.51[b] |
| | 60 | 6.24±0.01[bcd] | 77.02±0.75[e] | 15.90±2.21[a] | 98.80±15.85[a] |
| | 120 | 6.22±0.01[cd] | 81.89±1.56[d] | 13.98±1.18[ab] | 92.10± 8.75[a] |
| 90 | 30 | 6.43±0.19[a] | 85.02±4.87[cd] | 12.83±1.30[bc] | 67.40±13.76[b] |
| | 60 | 6.34±0.10[abc] | 83.31±1.38[cd] | 12.02±1.00[bcd] | 58.47± 2.03[bc] |
| | 120 | 6.26±0.01[bcd] | 84.97±0.34[cd] | 10.96±0.20[cd] | 59.13± 0.80[bc] |

Data was mean ± standard deviation from three replicate experiments.

[a-e] Different letters within a column were significantly different at $p \leq 0.05$.

**Table 4.5** Cont.

| Temperature (°C) | Time (min) | Chemical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | Total soluble solid (°Brix) | Phenolic content (µg/ml) | Anthocyanin content (µg/g) | Phytic acid content (µg/g) |
| 60 | 30 | 8.63±1.21[e] | 117.6±0.32[d] | 283.4± 3.92[c] | 496.0± 6.24[a] |
| | 60 | 7.67±1.59[e] | 123.8±0.25[ab] | 249.2± 7.41[d] | 385.7± 5.86[b] |
| | 120 | 14.37±0.46[d] | 122.9±0.35[bc] | 156.1±21.01[f] | 313.7± 8.62[d] |
| 80 | 30 | 17.20±2.72[bcd] | 122.0±0.21[c] | 340.1±14.96[b] | 354.3±27.21[c] |
| | 60 | 22.93±3.19[a] | 123.5±0.98[ab] | 286.3±12.34[c] | 310.7± 1.53[d] |
| | 120 | 20.17±1.70[ab] | 124.1±0.15[ab] | 281.5±13.71[c] | 251.3±17.95[e] |
| 90 | 30 | 18.50±1.88[bc] | 111.6±1.80[e] | 336.9± 3.72[b] | 390.0± 7.00[b] |
| | 60 | 17.37±1.42[bcd] | 101.0±0.56[f] | 366.1± 9.56[a] | 259.3± 5.51[e] |
| | 120 | 15.83±0.31[cd] | 124.4±0.74[a] | 221.8±12.46[e] | 198.7±24.01[f] |

Data was mean ± standard deviation from three replicate experiments.

[a-f] Different letters within a column were significantly different at $p \leq 0.05$.

7.55 mm (BP603), the grain width ranged from 2.26 mm (BP620) to 3.24 mm (KA005), and the grain thickness ranged from 1.72 mm (BP608) to 2.28 mm (KA005). The smallest grain of PF36 and BP305 had phenolic contents of 100.7 and 78.7 mg GAE/100 g, respectively, and the rice BP033 with large grains had the lowest phenolic content (42.57 mg GAE/100 g). The phenolic finding was contradicted with the anthocyanin contents that were lower as the extraction time longer. The component of phytic acid was also noticed to be significantly higher at lower extraction time for each extraction temperature ($p \leq 0.05$). The highest phytic acid content was discovered in the black glutinuous rice milk extracted at 60ºC for 30 min. Brouillard and Delaporte (1977) and Abdel-Aal et al. (2006) mentioned that heat processing affected the antioxidant content, especially anthocyanin and phenolic compounds. Kong and Lee (2010) that studied about Heugjinjubyeo rice reported that high levels of anthocyanins were found in bran milling fractions (10.7 ± 0.03 mg/g sample), and endosperm fractions contained low concentrations of total anthocyanins(0.17 ± 0.01 mg/g sample). The highest amount of phytic acid was found in the bran fraction (227 ± 1.35 mg/g sample) and the lowest amount was determined in the endosperm milling (7.38 ± 1.19 mg/g sample).

## 4.3 Optimum extraction condition of amyloglucosidase to produce black glutinous rice milk

The results of the section 4.2 suggested that the optimum condition to extract black glutinous rice by enzyme α-amylase was at a heating temperature of 90ºC for 30 min. At this extraction condition, the contents of total sugar, reducing sugar and anthocyanin were the 2$^{nd}$ highest, while the amount of phenolic was moderate. The viscosity of the rice milk was also the highest when the rice milk was extracted at 90ºC for 30 min. In this section, the optimum amyloglucosidase extraction condition at pH 5.0 was figured out by studying extraction temperatures of 50, 55 and 60ºC with an extraction time of either 90, 180 or 360 min. Naz (2002) had stated that amyloglucosidase enzyme was stable at a temperature range of 40 to 65ºC, with an optimum temperature between 58 and 65ºC.

**Table 4.6** Physical propertie of black glutinous rice milk affected by different extraction conditions of amyloglucosidase.

| **Temperature (°C)** | **Time (min)** | **Physical properties of black glutinous rice milk** | | | |
|---|---|---|---|---|---|
| | | **Viscosity (cP)** | **Color** | | |
| | | | **L*** | **a*** | **b*** $^{ns}$ |
| 50 | 90 | $0.67\pm0.20^{f}$ | $19.93\pm0.60^{bc}$ | $4.82\pm0.57^{b}$ | 1.59±0.58 |
| | 180 | $1.48\pm0.44^{e}$ | $19.49\pm0.78^{c}$ | $5.17\pm0.46^{b}$ | 1.46±0.26 |
| | 360 | $1.50\pm0.12^{e}$ | $19.97\pm0.72^{bc}$ | $5.22\pm0.24^{b}$ | 1.86±0.15 |
| 55 | 90 | $1.41\pm0.09^{e}$ | $20.13\pm0.37^{bc}$ | $5.28\pm0.35^{b}$ | 1.83±0.16 |
| | 180 | $2.05\pm0.31^{cd}$ | $20.56\pm0.75^{bc}$ | $5.16\pm0.10^{b}$ | 1.64±0.28 |
| | 360 | $1.64\pm0.14^{de}$ | $20.17\pm0.66^{bc}$ | $5.63\pm0.19^{b}$ | 1.85±0.09 |
| 60 | 90 | $2.28\pm0.37^{c}$ | $21.56\pm0.46^{ab}$ | $6.63\pm1.20^{a}$ | 1.66±0.36 |
| | 180 | $4.11\pm0.13^{b}$ | $21.05\pm1.40^{abc}$ | $5.78\pm0.38^{ab}$ | 1.62±0.20 |
| | 360 | $4.97\pm0.35^{a}$ | $22.60\pm1.48^{a}$ | $6.71\pm0.49^{a}$ | 2.14±0.36 |

Data was mean ± standard deviation from three replicate experiments.

$^{a-f}$ Different letters within a column were significantly different at $p \leq 0.05$.

$^{ns}$ Not significantly different.

Applying different times and temperatures of amyloglucosidase enzyme significantly affected the viscosity, L* and a* values of the black glutinous rice milk ($p \leq 0.05$) (Table 4.6). The finding of the viscosity in this section was similar to the previous section that at higher extraction temperature, the viscosity of the rice milk was increased, which could be due to evaporation during heating (Evans and Morriss, 1988) and gelatinization of smaller sizes of rice particles.

For the L* and a* values, the highest values (whitest and highest redness colour direction) were found after the rice was extracted at 60ºC. Park et al. (2005) reported L* and a* values of the saccharified-rice (8ºBrix) were 37.64 and -0.52, respectively. The reported study used 0.15% α-amylase and 0.038% amyloglucosidase at heating condition of 60ºC. No significant effect of the extraction time was observed on the L* and a* values ($p > 0.05$). Different time and temperature of extractions also did not significantly influence the b* value.

The chemical properties of the black glutinous rice milk affected by different times and temperatures of amyloglucosidase extraction can be seen in Table 4.7. No significant effect of the enzyme extraction conditions on the pH and moisture content of the rice milk could be noticed ($p > 0.05$). Lower heating regimes applied in the 2$^{nd}$ enzyme extraction might reduce water evaporation from the rice milk, causing comparable moisture contents between different rice milk samples.

The extraction conditions of amyloglucosidase on the black glutinous rice, however, significantly affected the total sugar, reducing sugar, total soluble solid and antioxidant compounds of the rice milk (Table 4.7). Longer extraction time of the rice at a particular extraction temperature produced higher total sugar and total soluble solids in the final milk. The reducing sugar of the milk was more affected by the extraction temperature. Higher extraction temperature yielded higher reducing sugar in the rice milk, except for the extraction time of 3 h. Wongkhalaung and Boonyaratanakornkit (2000) that studied rice saccharified in closed container at 55ºC for 24 h showed that reducing sugar before adding amyloglucosidase was 9.36% and after adding the enzyme for about 20-21 h, it reached 23.8%.

For the antioxidant components, amyloglucosidase extraction time affected the amounts of phenolic and anthocyanin in the rice milk (Table 4.7). Longer extraction time significantly generated more phenolic contents in the rice milk ($p \leq 0.05$), except for the extraction temperature of 55ºC. At this temperature, the highest phenolic content was

**Table 4.7** Chemical properties of black glutinous rice milk affected by different extraction conditions of amyloglucosidase.

| Temperature (°C) | Time (min) | Chemical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | pH [ns] | Moisture (%) [ns] | Total sugar (%) | Reducing sugar (mg/ml) |
| 50 | 90 | 4.96±0.10 | 87.91±3.02 | 9.17±0.59[c] | 124.03±0.32[f] |
| | 180 | 4.79±0.10 | 85.45±0.73 | 9.17±0.73[c] | 127.63±0.21[c] |
| | 360 | 4.91±0.24 | 85.01±1.29 | 10.03±0.77[bc] | 123.17±0.25[f] |
| 55 | 90 | 4.86±0.31 | 86.93±2.27 | 9.79±0.15[bc] | 125.07±0.76[e] |
| | 180 | 4.90±0.16 | 85.54±0.28 | 9.75±0.11[bc] | 123.27±0.31[f] |
| | 360 | 4.77±0.10 | 84.62±0.39 | 10.52±0.04[ab] | 127.00±0.10[cd] |
| 60 | 90 | 5.20±0.16 | 86.71±1.47 | 9.15±1.11[c] | 130.97±1.21[b] |
| | 180 | 4.94±0.27 | 86.19±0.26 | 9.54±0.42[bc] | 126.60±0.10[d] |
| | 360 | 5.23±0.12 | 83.89±1.44 | 11.20±0.49[a] | 136.00±0.26[a] |

Data was mean ± standard deviation from three replicate experiments.

[a-e] Different letters within a column were significantly different at $p \leq 0.05$.

[ns] Not significantly different.

**Table 4.7** Cont.

| Temperature (°C) | Time (min) | Chemical properties of black glutinous rice milk | | | |
|---|---|---|---|---|---|
| | | Total soluble solid (°Brix) | Phenolic content (μg/ml) | Anthocyanin content (μg/g) | Phytic acid content (μg/g) |
| 60 | 90 | 13.13±0.92[c] | 53.6±1.76[f] | 172.0±12.50[f] | 315.3±42.10[b] |
| | 180 | 13.23±1.05[c] | 66.1±0.53[f] | 188.5± 8.44[f] | 238.7±14.84[c] |
| | 360 | 14.47±1.10[bc] | 67.8±0.70[ab] | 400.1±11.26[ab] | 538.7±35.57[a] |
| 80 | 90 | 14.13±0.21[bc] | 67.8±0.87[ab] | 411.9±18.07[a] | 236.7±10.97[c] |
| | 180 | 14.07±0.15[bc] | 89.9±1.06[a] | 397.4± 9.59[ab] | 317.7±17.62[b] |
| | 360 | 15.17±0.06[ab] | 69.0±0.86[c] | 371.2±16.47[c] | 240.7±16.62[c] |
| 90 | 90 | 13.20±1.60[c] | 60.8±0.93[bc] | 377.7±13.12[bc] | 526.0±28.62[a] |
| | 180 | 13.77±0.61[bc] | 74.9±0.91[d] | 328.4±16.01[d] | 323.7± 8.50[b] |
| | 360 | 16.17±0.70[a] | 78.5±0.83[e] | 294.0±11.46[e] | 237.3±15.63[c] |

Data was mean ± standard deviation from three replicate experiments.

[a-f] Different letters within a column were significantly different at $p \leq 0.05$.

found at the extraction time of 180 min. Prolonged extraction time also significantly produced more anthocyanin when an extraction temperature of 50ºC was applied ($p \leq 0.05$). At higher temperatures, higher amount of anthocyanin was significantly achieved at shorter extraction time. Abdel-Aal et al. (2006) reported total monomeric anthocyanin content of the crude extract, 288 mg cyanidin equivalent/g flour, was much lower than total anthocyanin content in black rice, 3276 mg/g grain.

Data of phytic acid showed that this component was affected both by the time and temperature of amyloglucosidase extraction (Table 4.7). The highest amount of phytic acid was obtained when the rice was processed by amyloglucosidase at 50ºC for 6 h or at 60ºC for 1.5 h. García-Estepa et al. (1999) reported that the phytic acid for oat brans was half of that of wheat bran (20 mg/g) and higher value (58 mg/g) than that for rice bran.

Since the next section would be utilised to produce fermented rice from the black glutinous rice milk, the number of microorganisms in the rice milk samples were established in this section. The numbers of total microorganisms and lactic acid bacteria are exhibited in Figures 4.1 and 4.2, respectively. The highest number of total microorganisms was uncovered in the milk samples extracted at the highest amyloglucosidase temperature of 60ºC (Table 4.1). Longer extraction time significantly reduced the amount of the total microorganisms. There was a possibility that part of the total microorganisms was *Bacillus* spp., since this genus had been reported to cause outbreaks from rice products (Adams and Moss, 2000) and was present in soil and water (Mc Clure, 2006). The *Bacillus* spp. can produce spores and some of them are thermophilic, including *B. coagulans*, *B. smithii* and *Geobacillus* (formerly *Bacillus*) *stearothermophilus* (Mc Clure, 2006).

Contradicted to the total microorganisms, the lactic acid bacteria were found to be the highest in the black glutinous rice milk extracted at 50ºC. This could be due to some species of the lactobacilli that had a maximum temperature of 50ºC, some even grew at 55ºC, even though most of the lactobacilli were generally mesophilic with the best grow temperatures between 20 and 40ºC (Schillinger et al., 2006). The detection of microorganisms in the rice milk indicated that the milk was not a sterile product, although it had passed some extraction processes and heating at 90ºC for 30 min to inactive the enzyme. However, the microbial number of lower than 30 cfu/ml should not cause a problem during rice fermentation, since the starter cultures should be added in the range of 6 to 7 log cycle at the beginning of the fermentation (Adams and Moss, 2000).

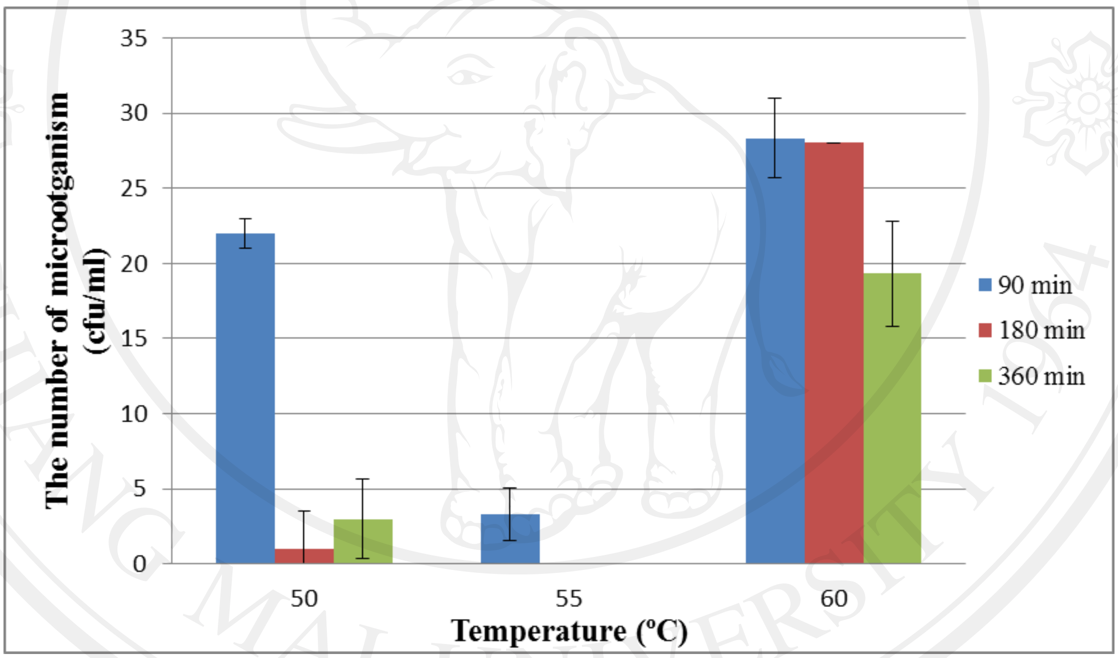


**Figure 4.1** The number of total microorganism (cfu/ml) in black glutinous rice milk affected by different extraction conditions of amyloglucosidase.

## 4.4 The effect of full fat milk powder addition on the production of fermented rice from black glutinous rice milk

From the previous section of amyloglucosidase extraction, it was selected that the rice milk would have the highest viscosity, total sugar, reducing sugar and total soluble solid with reasonable amounts of antioxidant compounds when the black glutinous rice was extracted with the enzyme at 60ºC for 360 min. The chosen amyloglucosidase condition and α-amylase extraction at 90ºC for 30 min were further employed in this section.

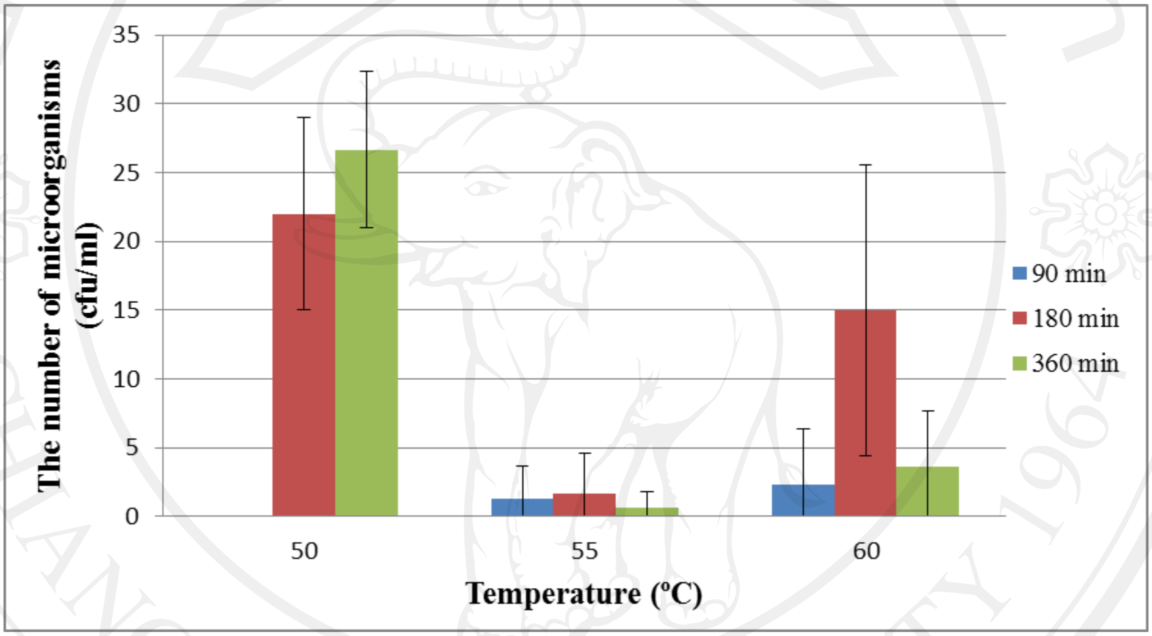


**Figure 4.2** The number of lactic acid bacteria (cfu/ml) in black glutinous rice milk affected by different extraction conditions of amyloglucosidase.

This section was dedicated to produce fermented rice from the black glutinous rice milk with an extra addition of full fat milk powder between 0 and 2% (w/v). An amount of 0.02% (w/v) of freeze dried cultures was supplemented in the warm rice milk (a temperature of about 40ºC) that had been pasteurised at 90ºC for 30 min and incubated at 43 ± 1ºC for about 14 h until the pH of the milk reached 4.2-4.4. Since the rice milk was adjusted to pH 5.0 for the amyloglucosidase extraction, before pasteurisation the pH of the milk was increased to around 6.0 using sterile 1 N NaOH.

The physical properties of fermented black glutinous rice showed that the addition of full fat milk powder significantly affected the viscosity, L* and b* values of the final product ($p \leq 0.05$) (Table 4.8). A supplementation of 2% full fat milk powder significantly produced the highest viscosity, whitest and more yellow colour intensity. It was reported that higher levels of total solids in the yoghurt mix produced greater viscosity/consistency of the end product (Tamime and Robinson, 1999). The effect of milk powder on the fermented rice colour could be due to the colour of the milk itself that had L*, a* and b* values of 85.12 ± 0.21, -5.01 ± 0.09 and 4.49 ± 0.11, respectively. The presence of full fat milk powder did not significantly affect the a* value ($p > 0.05$).

**Table 4.8** Physical properties of fermented black glutinous rice product affected by full fat milk powder addition.

| **Full fat milk powder addition (%)** | **Physical properties of black glutinous rice yoghurt** | | | |
|---|---|---|---|---|
| | **Viscosity** | **Color** | | |
| | **(cP)** | **L*** | **a*** $^{ns}$ | **b*** |
| 0 | 1.54±0.11$^{b}$ | 17.62±0.28$^{c}$ | 1.55± 0.20 | 1.98±0.07$^{ab}$ |
| 1 | 1.59±0.07$^{b}$ | 19.30±0.27$^{b}$ | 8.12±10.55 | 1.54±0.54$^{b}$ |
| 2 | 1.82±0.10$^{a}$ | 20.92±0.54$^{a}$ | 2.40± 0.31 | 2.42±0.10$^{a}$ |

Data was mean ± standard deviation from three replicate experiments.

$^{a-c}$ Different letters within a column were significantly different at $p \leq 0.05$.

$^{ns}$ Not significantly different.

**Table 4.9** Chemical properties of fermented black glutinous rice product affected by full fat milk powder addition.

| Full fat milk powder addition (%) | Chemical properties of black glutinous rice yoghurt | | | | |
|---|---|---|---|---|---|
| | **pH** [ns] | **Moisture (%)**[ns] | **Total sugar (%)** | **Reducing sugar (mg/ml)**[ns] | **Total acidity (%)**[ns] |
| 0 | 4.45±0.14 | 87.99±1.22 | 7.90±0.64[b] | 108.1±0.42 | 0.75±0.02 |
| 1 | 4.59±0.09 | 87.17±0.73 | 7.88±0.53[b] | 108.2±0.50 | 0.74±0.03 |
| 2 | 4.57±0.15 | 86.69±1.52 | 9.55±0.50[a] | 108.5±0.47 | 0.79±0.05 |

Data was mean ± standard deviation from three replicate experiments.

[a-b] Different letters within a column were significantly different at $p \leq 0.05$.

[ns] Not significantly different.

**Table 4.9** Cont.

| **Full fat milk powder addition (%)** | **Chemical properties of black glutinous rice yoghurt** | | | |
|---|---|---|---|---|
| | **Total soluble solid (°Brix)** | **Phenolic content (μg/ml)[ns]** | **Anthocyanin content (μg/g)[ns]** | **Phytic acid content (μg/g)** |
| 0 | 12.03±0.68[ab] | 67.87±5.86 | 142.43±4.41 | 407.00±13.75[a] |
| 1 | 12.80±0.44[ab] | 71.70±5.67 | 143.80±7.70 | 279.70±26.28[b] |
| 2 | 13.90±0.72[a] | 74.27±9.00 | 143.93±1.61 | 270.33± 6.66[b] |

Data was mean ± standard deviation from three replicate experiments.

[a-b] Different letters within a column were significantly different at $p \leq 0.05$.

[ns] Not significantly different.

Since the fermented black glutinous rice had a very low viscosity compared to the normal yoghurt from cow's milk acidified with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (a viscosity of 30.43 x $10^3$ mPa.s (Donkor et al., 2007)), the analyses of syneresis and water holding capacity could not be carried out for the fermented black glutinous rice in this study.

Several chemical characteristics of the fermented black glutinous rice are displayed in Table 4.9. An addition of full fat milk powder did not significantly affect the pH, moisture content, reducing sugar and total acidity of the black glutinous rice yoghurt ($p > 0.05$). Similar pH values of different treatments of the rice yoghurt were mainly due to different fermentation times. Since the pH of the yoghurt was needed to reach pH values around 4.4 - 4.6, different incubation times of 22, 15 and 11 h were carried out for the rice yoghurt containing 0, 1 and 2% full fat milk powder respectively. However the full fat milk powder significantly influenced the total sugar and total soluble solid of the fermented rice product ($p \leq 0.05$). At 2% full fat milk powder, the total sugar and total soluble solid of the yoghurt were found to be the highest. This could be attributed to the presence of lactose in the full fat milk powder. Walstra et al. (2006) wrote that the amount of lactose in the skim milk powder was 51% (w/w).

Data of phytic acid (Table 4.9) showed this component was significantly reduced with the presence of full fat milk powder ($p \leq 0.05$). The presence of the milk powder supported the growth of starter culture (Figure 4.3) that might reduce the amount of phytic acid in the fermented rice. The regular intake of probiotic preparation may represent a cheap and safe tool in order to convert a diet with a low potential for bioavailability of trace minerals and proteins, such as the vegetarian diet, into a diet with a high bioavailability potential. The benefit of such an approach would not be restricted to vegetarians (Famularo et al., 2005).

For the presence of starter cultures, *S. thermophilus* and *L. bulgaricus*, in the fermented black glutinous rice, the number of these organisms is presented in Figure 4.3.

In general, the number of the cultures in the final product was within 4 to 5 log cycles. This number was much lower than the normal yoghurt product from cow's milk, which should be presented at higher than 8 log cycle (Wirjantoro, 2011). Lower number of the cultures in this study could be attributed to different nutritional composition between rice and cow milks.

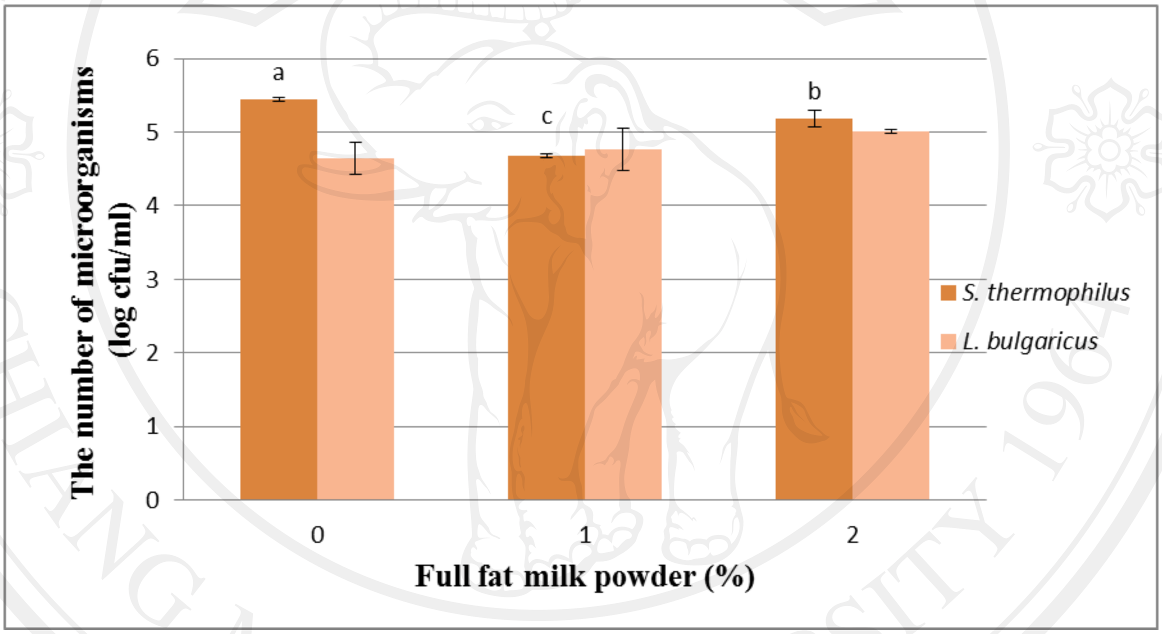


**Figure 4.3** The number of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (log cfu/ml) in fermented black glutinous rice milk product.

The presence full fat milk powder in the black glutinous rice milk was significantly affected the number of *S. thermophilus* (Figure 4.3) ($p \leq 0.05$). The highest number of the streptococci was determined in the fermented rice without supplementation of full fat milk powder. This could be contributed to the longer fermentation time of the fermented rice. Tamime and Robinson (2000) stated that the growth of *S. thermophilus* demanded the presence of amino acids and peptide. Since the rice milk had a low protein content (Table 4.2), the growth of the streptococci in the rice milk could be a slow process. On the other hand, when the full fat milk powder was added into the rice milk, the growth of the streptococci would be faster with the presence of the extra nutrient. However, when a certain acidity was reached due to the production of lactic acid by the starter culture, the number of the streptococci would be affected too (Walstra et al., 2006).

The number of *L. bulgaricus* was not significantly influenced by the presence of full fat milk powder ($p > 0.05$). The amount of lactobacilli of 4.64 ± 0.22, 4.76 ± 0.28 and 5.01 ± 0.09 log cfu/ml was discovered in the fermented rice products with 0, 1 and 2% full fat milk powder, respectively. The data indicated that the lactobacilli grew better at higher addition amount of full fat milk powder. To have a better growth of *L. bulgaricus* in the yoghurt production, the microorganism needs formic acid, production of $CO_2$, pyruvate and $HCO_3$ (Tamime and Robinson, 2000; Walstra et al., 2006).