# CHAPTER 4
# RESULTS

## 4.1 Screening of β-glucanase producing fungi

### 4.1.1 β-glucanase production

In this study, 11 strains of fungi from BIOTEC Culture Collection (BIOTEC, NSTDA, Thailand) were chosen for the screening of β-glucanase. Screening for β-glucanase activities was done by adding the cultured supernatant or enzyme extract derived from the cultivation in wheat bran soybean medium.

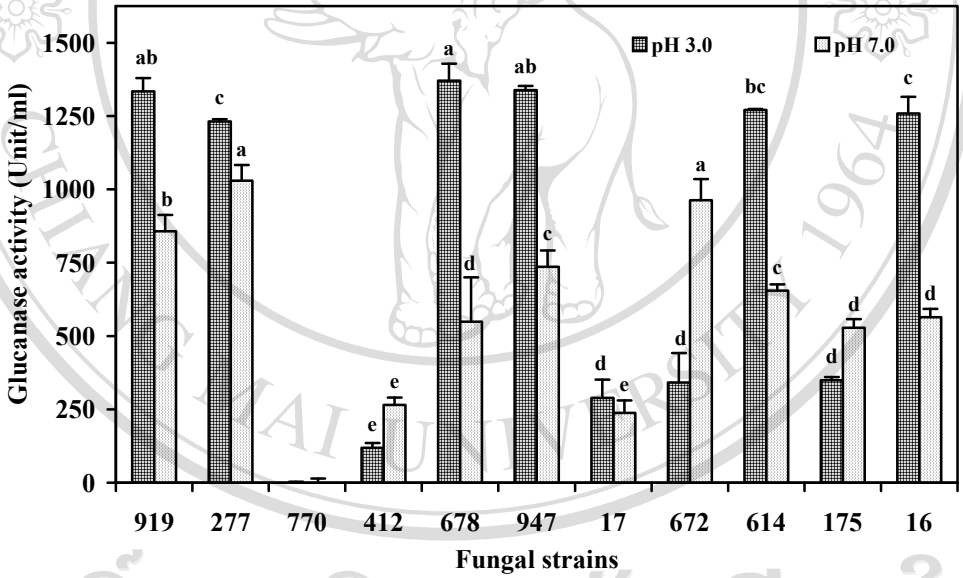


**Figure 4.1** β-glucanase productions at pH 3.0 and 7.0 of 11 fungal strains. (Means within the same parameter with different letter are significantly different at P< 0.05)



The results showed that 10 of the strains produced β-glucanase except *Nectria* sp. KPFC 770 (Figure 4.1). The highest activity of β-glucanase at pH 3.0 was obtained from *Pennicillium* sp. KPFC 678, followed by *Aspergillus* sp. KPFC 947, *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 614, *Aspergillus niger* KPFC 16 and

*Aspergillus* sp. KPFC 277 with the values of 1371, 1338, 1334, 1271, 1258 and 1231 U/ml, respectively. The high specific activity of β-glucanase a pH 7.0 were obtained from *Aspergillus* sp. KPFC 277, followed by *Nodolisporium gregarium* KPFC 672, *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 614 with values of 1030, 963, 857, 736, 655 and 564 U/ml, respectively.

Based on the activity at both pHs, *Aspergillus* sp. KPFC 947, *Aspergillus niger* KPFC 919, *Aspergillus niger* KPFC 16, *Aspergillus* sp. KPFC 614, *Aspergillus* sp. KPFC 277 and *Pennicillium* sp. KPFC 678 were selected for further studies.

### 4.1.2 Evaluation of safety

#### 4.1.2.1 Cytotoxicity test

The extracted samples of microorganism cultured in wheat bran soybean medium were tested with target cell (baby hamster kidney (BHK) and human hepatocyte cell line (HepG2)) as shown in Table 4.1.

**Table 4.1** The percentage of survival of baby hamster kidney (BHK) and human hepatocyte cell line (HepG2) with extracted samples of microorganism

| | Cell survival (%) | | | |
|---|---|---|---|---|
| | BHK | | HepG2 | |
| Dilution (mg/ml) | 0.1 | 0.01 | 0.1 | 0.01 |
| 16 | 98.4ab ± 0.7 | 100.5a ± 0.7 | 97.4a ± 3.6 | 91b ± 1.4 |
| 277 | 97.4ab ± 3.7 | 98.5a ± 2.2 | 86.2b ± 0.7 | 89bc ± 0.0 |
| 614 | 94.7ab ± 1.5 | 97.9a ± 0.0 | 83.2b ± 2.2 | 85.5c ± 0.7 |
| 678 | 90.5b ± 1.5 | 98.5a ± 0.7 | 79.1b ± 5.1 | 80.5d ± 3.5 |
| 919 | 102.1a ± 3.0 | 100.5a ± 0.7 | 102.0a ± 0.0 | 100a ± 0.0 |
| 947 | 76.8c ± 8.9 | 85.6b ± 4.4 | 97.4a ± 2.2 | 99a ± 1.4 |

Mean values with different letter (a,b,c,d) in the same column indicate significant differences (P< 0.05)



From table 4.1, the ranges of toxicity into 3 grouped into 3 categories, 1) non-toxic with % survival more than 90%, 2) slightly toxic with % survival between 50-90%, and 3) highly toxic with % survival less than 50%. Slightly toxicity was found

in BHK(21)C13 and HepG2 cell lines. These results indicated the slight effect on digestive tissues (BHK(21)C13 and HepG2). It was expected that this mortal influence would possibly be reduced when small amount of β-glucanase from six microorganisms was mixed with large amount of pig diet.

In summary, the results of the tests show that the samples were less toxic to the baby hamster kidney cell line and the human liver hepatocyte cell line. Thus, six microorganisms should be safe for animals.

#### 4.1.2.2 Aflatoxin test

The amount of aflatoxin (Table 4.2) in extracted sample from *Aspergillus niger* KPFC 16, *Aspergillus* sp. KPFC 277, *Aspergillus* sp. KPFC 614, *Pennicillium* sp. KPFC 678, *Aspergillus niger* KPFC 919 and *Aspergillus* sp. KPFC 947 were 3.10, 3.55, 3.84, 2.05, 2.44 and 4.14 ppb, respectively. U.S. Food and Drug Administration (FDA) set an action level for aflatoxins at 20 ppb for all foods including animal feeds (U.S. Food and Drug Administration, 1994). Thus, aflatoxin found in this study (2.03-4.14 ppb) is much less than the allowable amount.

#### 4.1.2.3 Ochratoxin test

The ochratoxin level in extracted sample (Table 4.3) from *Aspergillus niger* KPFC 16, *Aspergillus* sp. KPFC 277, *Aspergillus* sp. KPFC 614, *Pennicillium* sp. KPFC 678, *Aspergillus niger* KPFC 919 and *Aspergillus* sp. KPFC 947 were 24.65, 14.76, 12.00, 10.04, 20.14 and 12.78 ppb, respectively. The current maximum permitted level for orchratoxin in cereals and cereal-based food is between 5 and 50 ppb (Boutrif and Canet 1998). Thus, the ochratoxin level of 6 microorganism is much less than the allowable level (50 ppb).

**Table 4.2** Level of aflatoxin in extracted samples by ELISA test kit

| Microorganisms | Level of Aflatoxin (ppb) |
|---|---|
| *Aspergillus niger* KPFC 16 | 3.10 |
| *Aspergillus* sp. KPFC 277 | 3.55 |
| *Aspergillus* sp. KPFC 614 | 3.84 |
| *Pennicillium* sp. KPFC 678 | 2.05 |
| *Aspergillus niger* KPFC 919 | 2.44 |
| *Aspergillus* sp. KPFC 947 | 4.14 |

**Table 4.3** Level of ochratoxin in extracted samples by ELISA test kit

| Microorganisms | Level of Ochratoxin (ppb) |
|---|---|
| *Aspergillus niger* KPFC 16 | 24.65 |
| *Aspergillus* sp. KPFC 277 | 14.76 |
| *Aspergillus* sp. KPFC 614 | 12.00 |
| *Pennicillium* sp. KPFC 678 | 10.04 |
| *Aspergillus niger* KPFC 919 | 20.14 |
| *Aspergillus* sp. KPFC 947 | 12.78 |

According to the results, the selected strains for next study would be *Aspergillus* sp. KPFC 277, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 919 because these 3 microorganisms showed high β-glucanase activities at both pH 3.0 and 7.0 and contain aflatoxin and ochratoxin which are less than the allowable amounts. *Pennicillium* sp. KPFC 678, *Aspergillus* sp. KPFC 614 and *Aspergillus niger* KPFC 16 showed high β-glucanase at only pH 3.0. There are advantages of the favorable selection the microorganisms which are active at pH 7.0 than 3.0. These enzymes were expected to be active in small intestine which is extremely long and in neutral pH.

### 4.1.3 Resistance to pH, heat treatment and proteolytic enzyme

#### 4.1.3.1 Resistance to pH and proteolytic enzyme

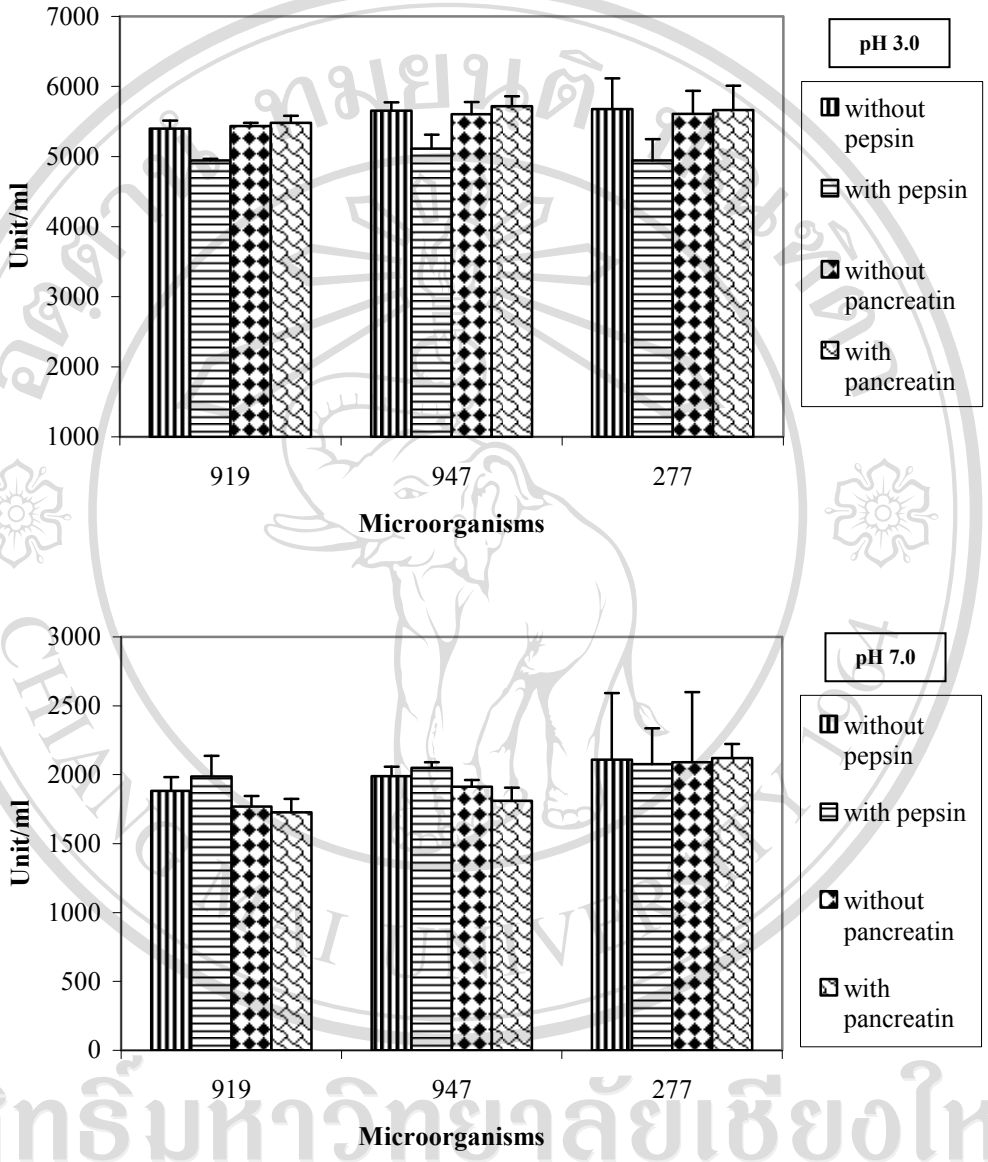


**Figure 4.2** Stability of β-glucanase produced from *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 at low pH with pepsin and neutral pH with pancreatin. Assay enzyme activity at pH 3.0 and 7.0

In order to confirm the efficacy of a β-glucanase preparation in a practical mash diet, the stability of the enzyme was tested *in vitro*, using conditions similar to those of the gastrointestinal tract of animal.

Figure 4.2 shows that when exposed to pH 3.0 with pepsin, the relative stability of β–glucanase exposure to pH 3.0 *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were 91.6, 90.4 and 87.1 % (assay at pH 3.0), respectively, and 105.6, 103.1 and 98.5 % (assay at pH 7.0), respectively.

Figure 4.2 also shows the relative stability of β-glucanase following exposure to pH 7 with pancreatin of *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 which were 100.8, 102.0 and 100.9 % (at pH 3.0), respectively, and 97.6, 94.7 and 101.4 % (at pH 7.0), respectively.

**4.1.3.2 Resistance to heat**

The thermal stability at 75 °C of β-glucanase produced by *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 after incubation at 75 °C for 2 and 5 min is illustrated in Figure 4.3. The β-glucanase activity of all selected strains decreased with the increasing exposure time at 75 °C. After incubation for 2 min, the β-glucanase residual activity at pH 3.0 of *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were 53.6, 76.6 and 46.5 %, respectively and decreased to 52.2, 71.1 and 34.5 %, respectively after being incubated for 5 min. The β-glucanase residual activity at pH 7.0 of *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were 62.1, 58.8 and 49.3 %, respectively and decreased to 62.1, 56.1 and 44.6 %, respectively after being incubated for 5 min.

Therefore, it was likely that β-glucanase from *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 could be used under pelleting conditions (less than 1 min) with a few loss of β-glucanase activity. It can be concluded that the crude β-glucanase from *Aspergillus* sp. KPFC 919, Aspergillus sp. KPFC 947 and *Aspergillus* sp. KPFC 277 was thermally stable.

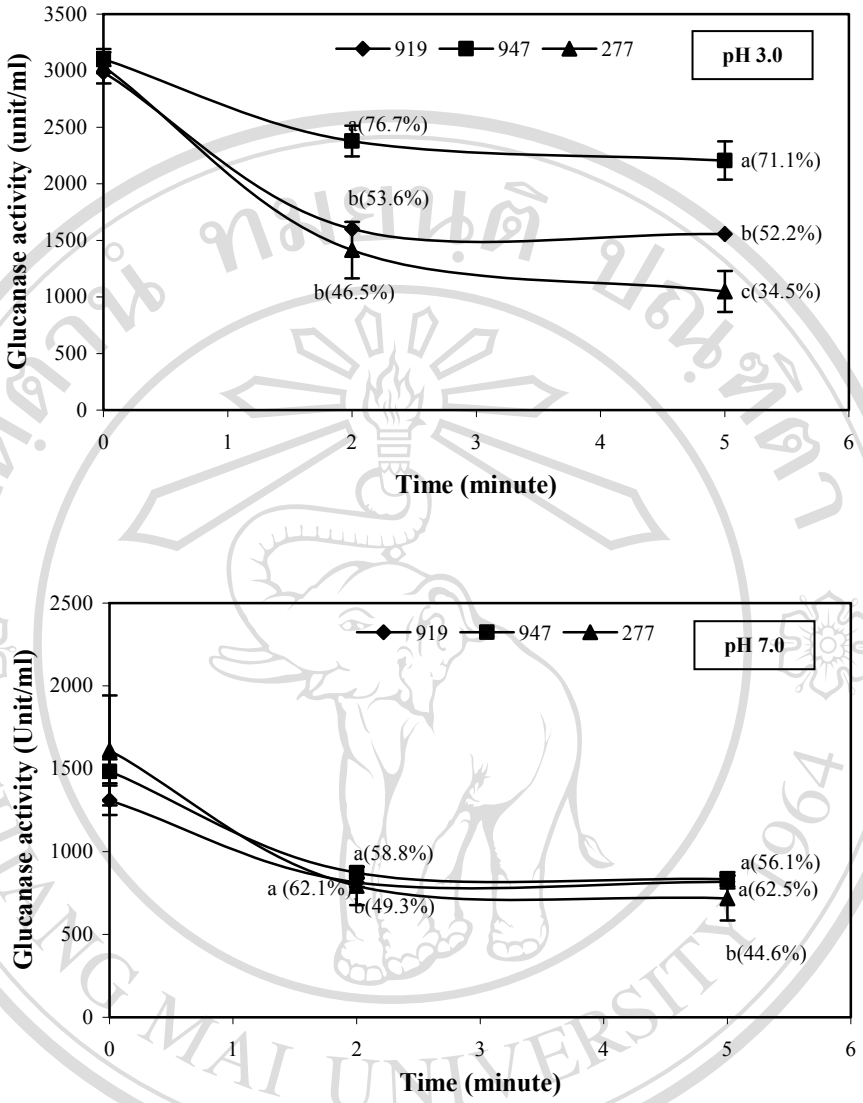


**Figure 4.3** Thermal stability of β-glucanase produced by *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 after incubation at 75 °C for 2 and 5 min. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different P< 0.05).

However, activities retained by *Aspergillus* sp. KPFC 277 preparation exhibited the high enzyme activity at pH 3.0 and highest enzyme activity at pH 7.0 and temperature 40 °C as in the gastrointestinal tract of an animal, non-toxic to animal cells (a baby hamster kidney cell line and a human liver hepatocyte cell line) and contained aflatoxin (3.55 ppb) and ochratoxin (14.76 ppb) which were less than the allowable amounts. Furthermore, it was stable to gastrointestinal tract condition, stable at temperatures up to 75 °C for at least 15-30 sec in feed pelleting process. Therefore, *Aspergillus* sp. KPFC 277 was the best microorganism and subsequently chosen for the next investigations.

**4.2 Optimization of crude β-glucanase production**

**4.2.1 Effect of carbon source on β-glucanase production**

Figure 4.4, the highest β-glucanase production of 87,266.67 and 30,166.67 Unit/g medium at both pH 3.0 and 7.0 were achieved by cultivation in rice bran (solvent extract). These were followed by wheat bran (72,467 and 26,000 U/g at pH 3.0 and 7.0, respectively), rice bran (59,867 and 22,000 U/g at pH 3.0 and 7.0, respectively), corn (48,400 and 20,367 U/g at pH 3.0 and 7.0, respectively) and broken rice (41833 and 16100 U/g at pH 3.0 and 7.0, respectively). Because rice bran (solvent extract) is cheap material and generally used in local farm feed, the β-glucanase production was then carried out by using rice bran (solvent extract) as carbon source.

**4.2.2 Effect of carbon sources to nitrogen source ratio on β-glucanase production**

Soybean meal is a main source of protein and generally used in local farm feed. By the rule of thumb, enzyme which is produced from soybean meal media is best-matching for soybean meal digestion. So, β-glucanase production was then carried out by using soybean meal as nitrogen source. The attempt to develop a suitable medium formula that improves the β-glucanase productivity was carried out. *Aspergillus* sp. KPFC 277 was grown on various ratios of rice bran (solvent extract, C source) and soybean meal (N source) at ratios of 50 : 50, 60 : 40, 70 : 30, 80 : 20, 90 :10 and 100: 0. The media were mixed with distilled water at ratio of 1: 1.5, the cultures were inoculated with $10^5$ spores/g medium and incubated 30°C for 120 h.

The optimum ratio of rice bran (solvent extract) and soybean meal was observed at 100 % rice bran (solvent extract) (Figure 4.5). The culture medium had to contain 100% rice bran (solvent extract) which gave the β-glucanase activities of 154,303 and 52587 U/g at pH 3.0 and 7.0, respectively. From the results, rice bran (solvent extract) was selected as a raw material for the production of β-glucanase by *Aspergillus* sp. KPFC 277. This result showed that rice bran (solvent extract) has sufficient nitrogen source for β-glucanase production by *Aspergillus* sp. KPFC 277. The addition of soybean meal to medium did not enhance β-glucanase production.

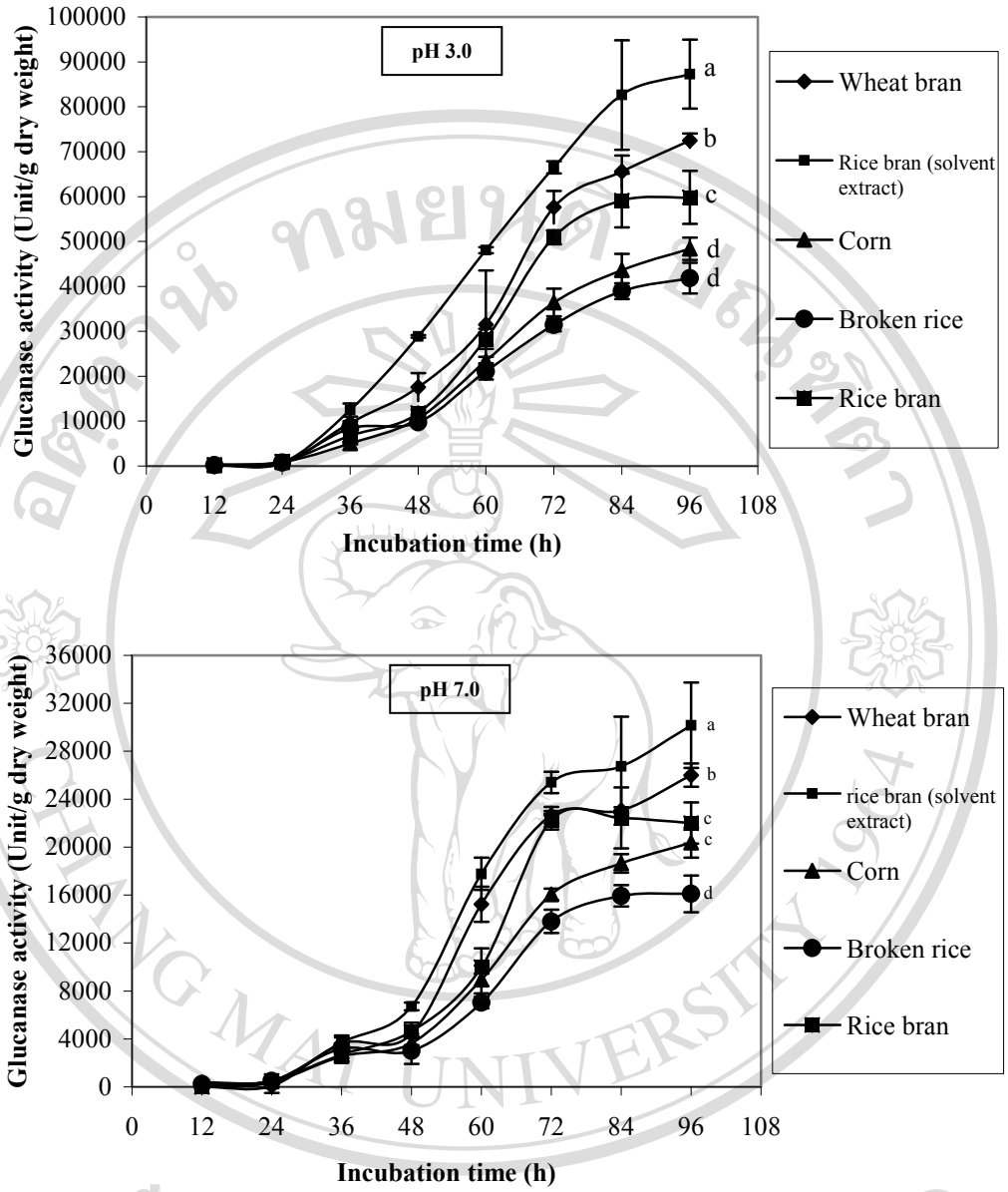


**Figure 4.4** Effects of carbon source on β-glucanase production by *Aspergillus* sp. KPFC 277 in solid-state fermentation. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different P< 0.05)

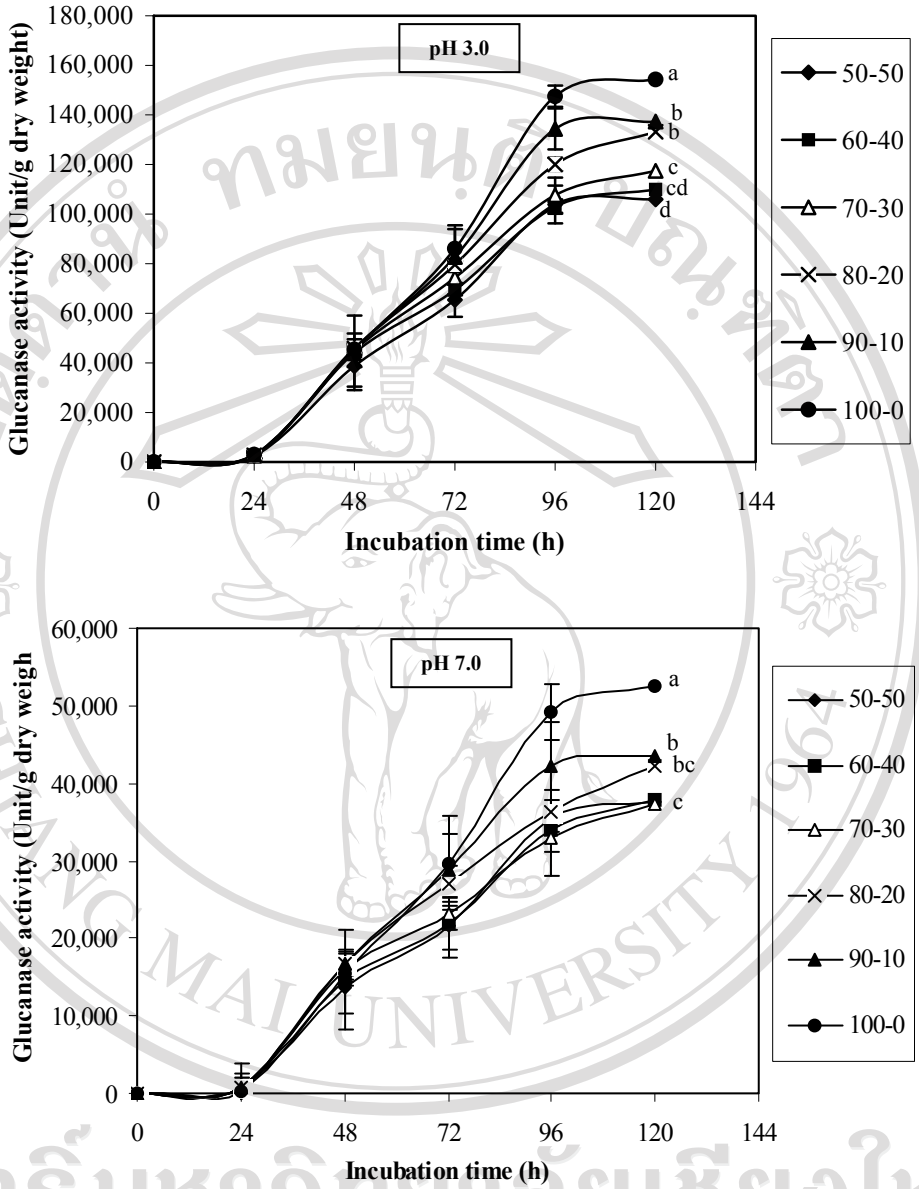


**Figure 4.5** Effect of carbon source to nitrogen source ratio on β-glucanase production by *Aspergillus* sp. KPFC 277 in solid-state fermentation. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different $P < 0.05$)

**4.2.3 Effect of media additives on β-glucanase production**

Optimized medium from 4.2.2 was added with mineral ($K_2HPO_4$ 2 %, $MgSO_4.7\ H_2O$ 0.4 %, $CaCl_2.2H_2O$ 0.2% (w/v)) 0.5 ml: 1g medium, Urea ( 1 g/kg medium) and Ami (10 ml/ kg medium). The media were mixed with distilled water at ratio of 1:1.5, the culture was inoculated with $10^5$ spores/g medium and incubated 30°C for 144 h.

Figure 4.6 showed that the addition of other nitrogen source and mineral did not increase the production of β-glucanase compared with control (added only distilled water).

**4.2.4 Effects of initial moisture content of medium on β-glucanase production**

The optimized medium from 4.2.3 and distilled water were mixed at the ratio of 1:1.0, 1:1.5, 1:2.0, 1:2.5 and 1:3.0. The culture were inoculated with $10^5$ spores/g medium and incubated 30°C for 120 h.

The effect of initial moisture contents of the medium on β-glucanase activity is depicted in Figure 4.7. Maximum enzyme production was observed at the ratio of medium to moisture of 1:1.5 (290,840 and 88,110 U/g at pH 3.0 and 7.0, respectively). The β-glucanase activity at pH 3.0 and 7.0 increased with increasing moisture levels up to 1:1.5. At higher and lower moisture level than 1:1.5, the yields tended to be reduced. The results indicated a relationship between moisture level and β-glucanase production.

In this study, the ratio of medium to moisture of 1:1.5 was the most suitable initial moisture content of the substrate for the β-glucanase production by *Aspergillus* sp. KPFC 277, and was subsequently applied in the next experiments.

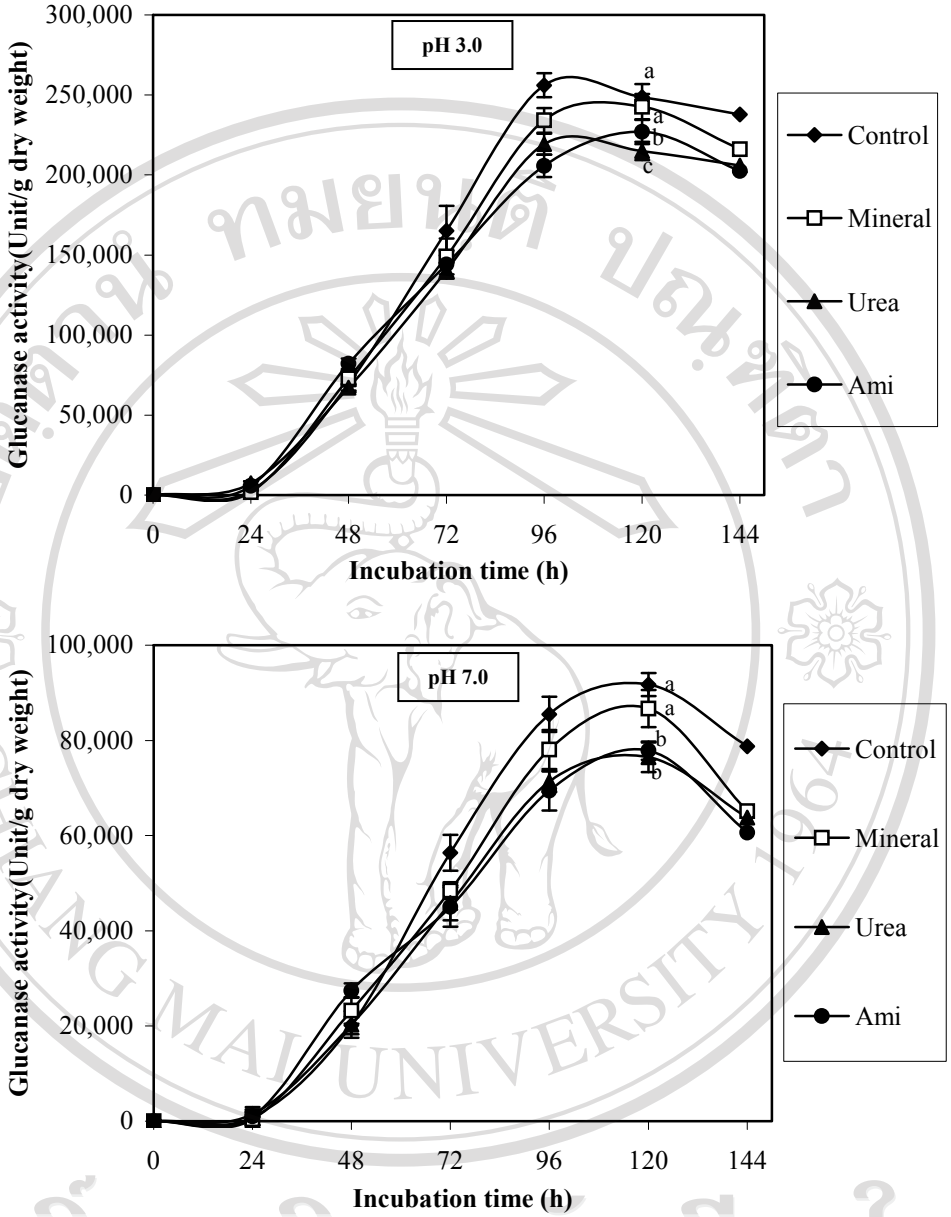


**Figure 4.6** Effect of media additives on β-glucanase production by *Aspergillus* sp. KPFC 277 in solid-state fermentation. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different $P< 0.05$)

**4.2.5 Effect of inoculum size and time course profiles on β-glucanase production**

To achieve the optimum inoculum size of *Aspergillus* sp. KPFC 277 for β-glucanase production, the inoculum was added to medium from ~ $1 \times 10^3$ to ~ $1 \times 10^6$ spores/g medium. The culivations were carried out at the optimum conditions obtained from 4.2.4.

Figure 4.8 shows the effects of inoculum size on the enzyme production. The maximal β-glucanase production (297,880 and 108,790 U/g at pH 3.0 and 7.0, respectively) was obtained at the inoculum size of ~ $1 \times 10^4$ spores/g medium but further increases of inoculum resulted in decreases in enzyme yields. The inoculum concentrations of less than ~ $1 \times 10^4$ spores/g medium were not adequate to allow good growth of the cultures, as well as enzyme production.

As illustrated in Figure 4.8, the β-glucanase production increased with the increased incubation time. The hightest enzyme production (297,880 and 108,790 U/g medium at pH 3.0 and 7.0, respectively) was observed at 96 h of incubation. A prolonged incubation time did not improve the enzyme production. Proteolytic enzymes concomitantly produced by fungi may cause cell lysis or destroy β-glucanase enzyme (Thacker and Baas, 1996).

Consequently, the most suitable incubation period for the β-glucanase production by *Aspergillus* sp. KPFC 277 in the solid-state fermentation (SSF) was 96 h. This was subsequently applied in the enzyme production studies.

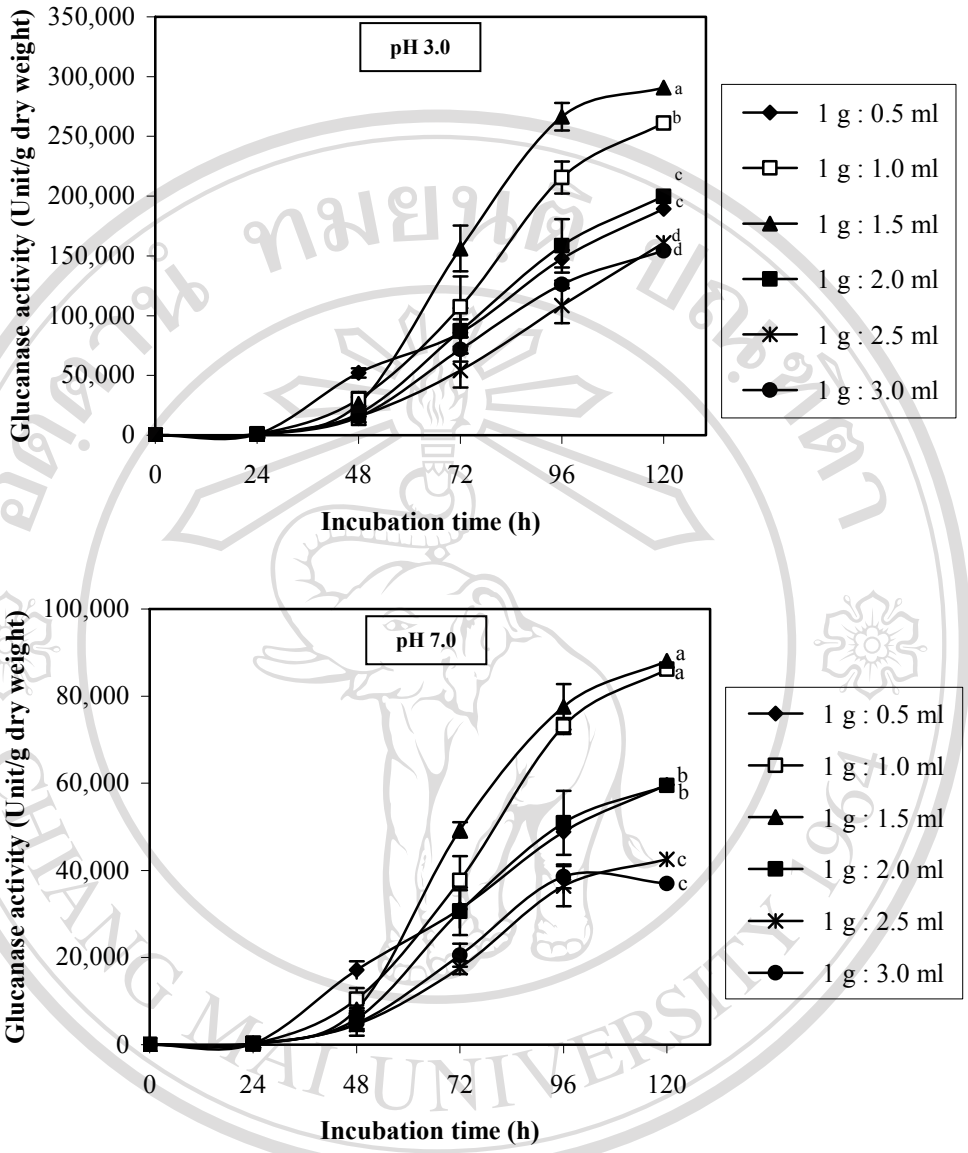


**Figure 4.7** Effect of medium to moisture ratio on β-glucanase production by *Aspergillus* sp. KPFC 277 in solid-state fermentation. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different $P< 0.05$)

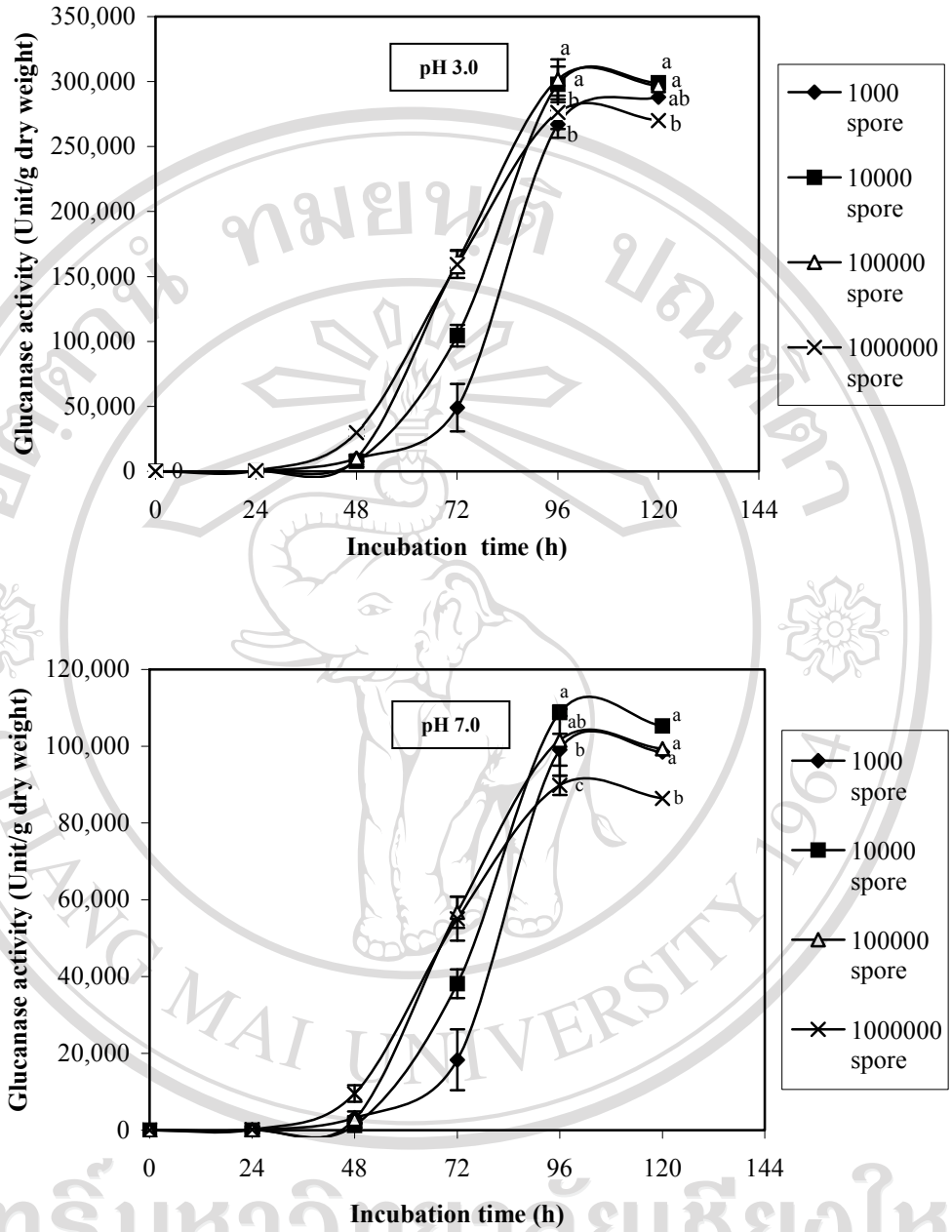


**Figure 4.8** Effect of inoculum size on β-glucanase production by *Aspergillus* sp. KPFC 277 in solid-state fermentation. Activity was assayed at pH 3.0 and 7.0, 40 °C. (Means within the same parameter with different letter are significantly different $P< 0.05$)

**4.3 Preservation of crude β-glucanase as dry powder**

The objective of the study was to develop a preservation process for the crude β-glucanase produced by *Aspergillus* sp. KPFC 277. The crude β-glucanase was mixed with various carriers (rice flour, glutinous rice flour and tapioca flour) at the ratio cultured diet (g) to carrier (g) 1:1. Then, the mixtures were dried at 40°C for 6 h in the incubator. After drying, the mixtures were subsequently ground with blender. The β-glucanase activity in the mixed sample was measured before drying and after grinding. The best carrier were judged from the enzyme product (powder form) which expressed the highest activity after drying.

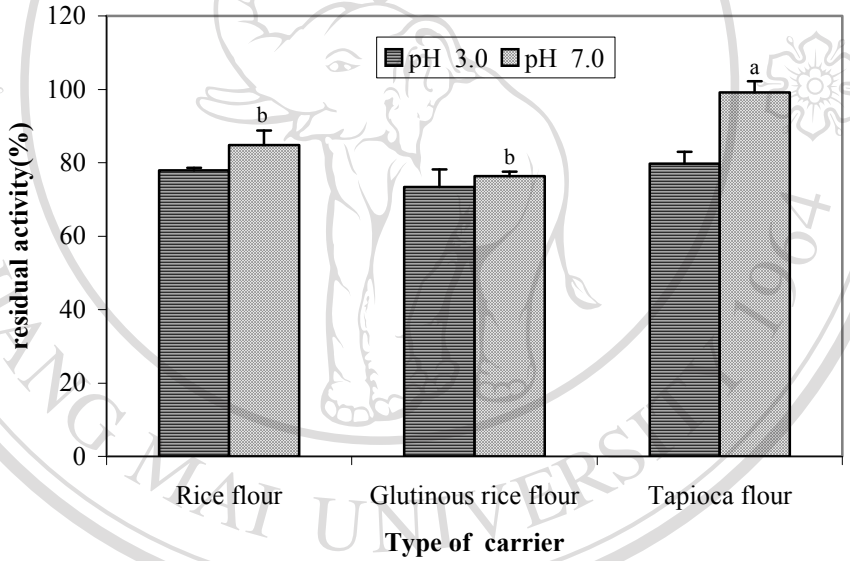


**Figure 4.9** Effect of carriers on activity of β-glucanase produced by *Aspergillus* sp. KPFC 277. (Means within the same parameter with different letter are significantly different P< 0.05)



Tapioca flour was suitable for drying process (Figure 4.9) which gave the percentage of 80 and 99 % at pH 3.0 and 7.0, respectively. This was followed by rice flour (78 and 85% at pH 3.0 and 7.0, respectively) and glutinous rice flour (73 and 76 % at pH 3.0 and 7.0, respectively). The price of tapioca flour (18 Baht/kg) was lower than rice flour (19 Baht/kg) and glutinous rice flour (20 Baht/kg). So tapioca flour was chosen for making enzyme in form of powder.

**4.4 Shelf-life of crude β-glucanase powder**

The objective of this study was to determine the effects of temperature, time and oxidation on β-glucanase activity during storage of crude β-glucanase powder. The effect of air exposure was carried out by comparing β-glucanase activity of products which were between the freshly-opened product and opened product. After, grinding, the product was sealed in an aluminum bag, and kept at 4°C, 30°C and 45°C. The β-glucanase activity of products were measured at 4, 8 and 12 weeks after storage. The effect of oxidation was carried out by comparing between the freshly-opened product and opened product at any time.

As illustrated in Figure 4.10, activity of enzymes stored for 12 weeks under refrigerated condition (4-7 °C) had lower effect than stored under room temperature (30 °C) and car temperature (45 °C). Storage under refrigeration reduced β-glucanase activity by 16-20% (close) and 11-18% reduction (open), while that stored at 30°C showed 27-30% (close) and 23-26% reduction (open) and while that stored at 45°C showed 26-33% (close) and 27-30% reduction (open).

The results showed that the crude β-glucanase produced by *Aspergillus* sp. KPFC 277 was relatively stable during the storage time of 12 weeks. Besides, air exposure had no effect on this product but high temperature had a negative effect, keeping in refrigerator helps avoid unexpected drawbacks.

**4.5 Other enzyme activity from *Aspergillus* sp. KPFC 277**

In addition to β-glucanase, crude β-glucanase from *Aspergillus* sp. KPFC 277 was assayed for the activity of amylase, cellulase, xylanase and protease compared with imported enzyme.



The results from this study showed that in addition to β-glucanase, *Aspergillus* sp. KPFC 277 also produced amylase, cellulase, xylanase and protease. The activities of amylase, cellulase, xylanase and protease are summarized in Figure 4.11. At pH 3.0, KPFC 277 showed activities of β-glucanase, amylase, cellulase, xylanase and protease; 245300, 26250, 118580, 18161 and 24512 U/g, respectively. At pH 7.0, KPFC 277 showed activities of β-glucanase, amylase, cellulase, xylanase and protease; 79970, 6630, 66990, 80197 and 2122 U/g, respectively.

For imported enzyme, at pH 3.0, showed activities of β-glucanase, cellulase, and xylanase; 140193, 57403 and 6256 U/g, respectively. But it had no activities of amylase and protease. At pH 7.0, imported enzyme showed activities of β-glucanase, amylase, cellulase and xylanase; 89873, 21990, 55816 and 35 U/g, respectively but it had no activities of protease.

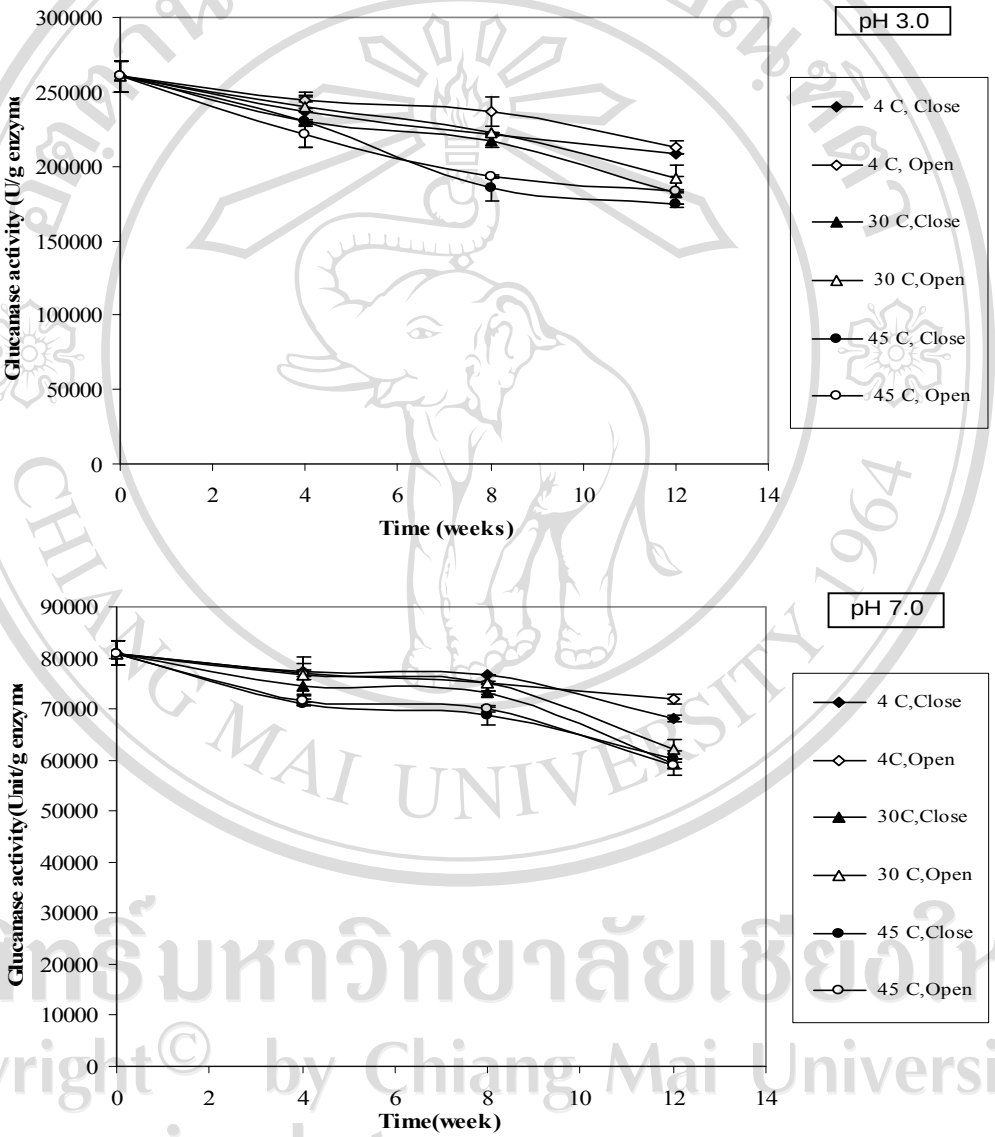


**Figure 4.10** Effect of temperature, time and oxidation on β-glucanase activity of crude enzyme from *Aspergillus* sp. KPFC 277 at pH 3.0 and 7.0 during storage 0-12 week at Refrigerator (4°C), Room temperature (25°C) and car temperature (45°C)

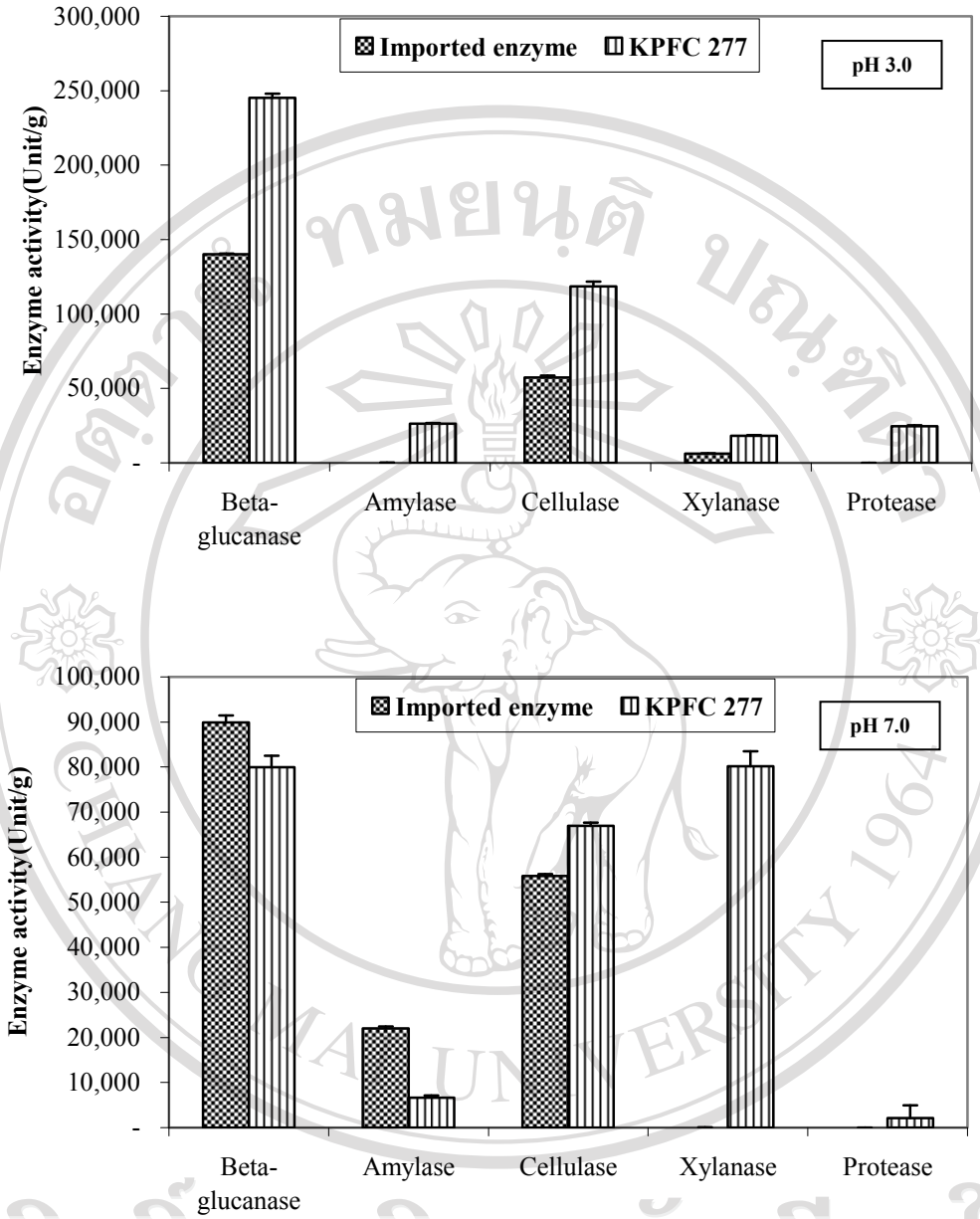


**Figure 4.11** Enzyme activity (Unit/g enzyme) at pH 3.0 and 7.0 by *Aspergillus* sp. KPFC 277 in crude β-glucanase KPFC 277 product and imported enzyme (**β-glucanase,** one unit of amylase is defined as the amount of enzyme that release 1 μg glucose/ml/min under the assay condition; **Amylase,** one unit of amylase is defined as the amount of enzyme that release 1 μg glucose/ml/min under the assay condition; **Cellulase,** one unit of amylase is defined as the amount of enzyme that release 1 μg glucose/ml/min under the assay condition; **Xylanase,** one unit of xylanase is defined as the amount of enzyme that release 1 μg xylose/ml/min under the assay condition. **Protease**, one unit of protease is defined as the amount of enzyme that release 1 ηg tyrosine/ml/min under the assay condition)

### 4.6 Testing of enzyme quality by *in vitro* digestibility

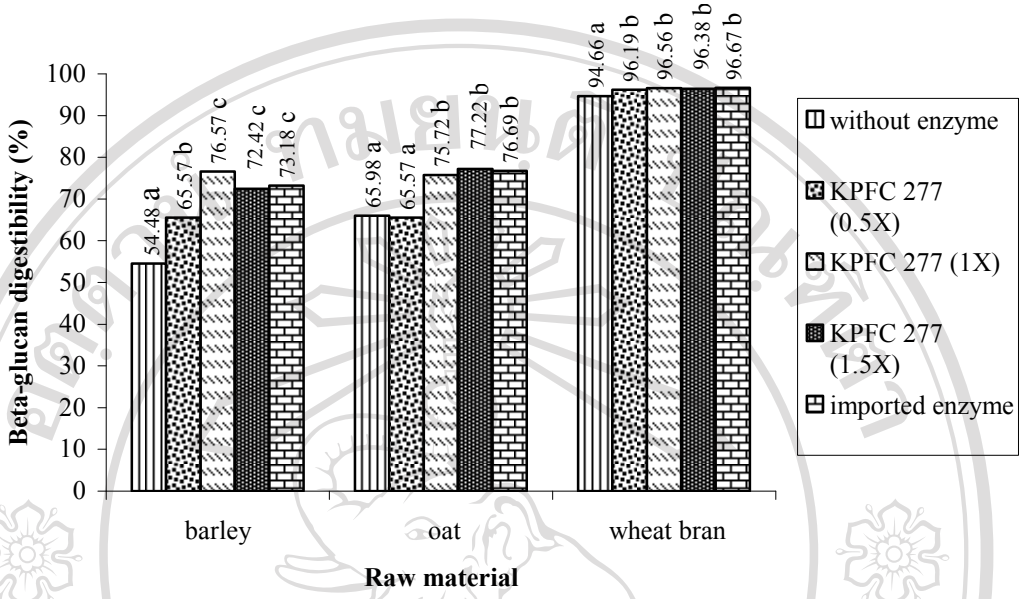


**Figure 4.12** *In vitro* digestibility of β-glucan in barley, oat and wheat bran. enzyme 277 (0.5x, 1x, 1.5x) = crude β-glucanase from *Aspergillus* sp. KPFC 277 (at the 0.5 times, 1.0 times and 1.5 times β-glucanase activity of imported enzyme). (Mean values with different letter (a,b,c) in the same parameter indicate significant differents P< 0.05)

The *in vitro* digestibility is the simulation study of the digestion processes in the stomach and small intestine with pepsin, followed by pancreatin. The comparison of *in vitro* digestibility of β-glucan in barley, oat and wheat bran supplemented with and without enzymes is shown in Figure 4.12. The *in vitro* digestibility of enzymes raw materials was significantly higher than without enzyme diet (P< 0.05). Supplemented with enzyme 277 1.0x or 1.5x exhibited better than 0.5x (P< 0.05), when compared at level of enzyme 277 1.0x or 1.5x with imported enzyme was not significantly different (P>0.05). From the results, supplemented crude β-glucanase from *Aspergillus* sp. KPFC 277 at the same β-glucanase activity to imported enzyme was selected for testing effect of enzyme on production performance in pigs.

**4.7 β-glucan analysis**

Table 4.4 shows the average β-glucan contents of 14 cereal grains and 2 diets by McCleary and Codd (1991). As can be seen from Table 4.1, barley grains have the highest β-glucan (3.78%), followed by oat (3.2%) and wheat bran (2.01%).

**Table 4.4** β-glucan contents of raw materials and diets

| **Raw materials** | **β-glucan (%)** |
|---|---|
| Wheat bran | 2.017 ± 0.06 |
| Barley | 3.780 ± 0.35 |
| Oat | 3.203 ± 0.24 |
| Rye | 0.295 ± 0.00 |
| Casava pulp | 0.051 ± 0.00 |
| Rice bran | 0.084 ± 0.05 |
| Corn | 0.180 ± 0.00 |
| Soybean meal | 0.156 ± 0.01 |
| Casava meal | 0.226 ± 0.00 |
| Rice bran (solvent extract) | 0.239 ± 0.00 |
| Full fat soybean | 0.022 ± 0.01 |
| Canola meal | 0.049 ± 0.01 |
| Rape seed meal | 0.033 ± 0.02 |
| Sunflower meal | 0.193 ± 0.02 |
| Piglet diet 1 | 0.332 ± 0.08 |
| Piglet diet 2 | 0.309 ± 0.06 |

**4.8 Effects of enzyme on production performance in pigs**

Average daily feed intake (ADFI) and average daily gain (ADG) of the piglets were not significantly different among the different treatments ($p > 0.05$) (Table 4.5). However, at the same level of β-glucanase activity added, the piglet fed with diets containing KPFC 277 or imported enzyme tended to have a better production performance in terms of ADG and show significant difference in terms of feed conversion ratio (FCR) ($p < 0.05$) than the piglet fed with the without enzyme diets. Therefore, the piglets fed with KPFC 277 or imported diet tended to have the lower feed cost per gain (31.82 and 31.81 Baht/kg, respectively) without enzyme diet (34.48 Baht/kg). The results showed that the supplement of crude β-glucanase preparation of *Aspergillus* sp. KPFC 277 was of the same quality with imported enzyme. Table 4.6 shows that the addition of β-glucanase to diet does not affect to illness rate and mortality rate.

The overall results of these experiments indicated that passage through the stomach of the pig did not inactivate β-glucanase and provided a favorable indication that supplementation of piglet diets with β-glucanase definitely has the potential to improve pig performance.

**Table 4.5** Growth performance of piglets offered diets with and without enzymes.

| Parameter | Diet | | |
|---|---|---|---|
| | **Without enzyme** | **With imported enzyme** | **KPFC 277** |
| Average initial weight (kg) | 8.30 | 8.12 | 8.12 |
| Average final weight (kg) | 21.85 | 22.50 | 22.29 |
| Total weight gain (kg) | 13.55 | 14.38 | 13.94 |
| Total feed intake (kg) | 28.82 | 28.35 | 27.99 |
| **ADFI (kg/d)** | | | |
| Week 1 | 0.413 | 0.384 | 0.388 |
| Week 2 | 0.616 | 0.603 | 0.571 |
| Week 3 | 0.699 | 0.697 | 0.709 |
| Week 4 | 0.714 | 0.751 | 0.739 |
| Week 5 | 0.835 | 0.872 | 0.761 |
| Week 6 | 0.842 | 0.743 | 0.832 |
| **Week 1-6** | **0.686** | **0.675** | **0.674** |
| **ADG (kg/d)** | | | |
| Week 1 | 0.287 | 0.258 | 0.278 |
| Week 2 | 0.311 | 0.324 | 0.286 |
| Week 3 | 0.349 | 0.346 | 0.367 |
| Week 4 | 0.353 | 0.351 | 0.363 |
| Week 5 | 0.322 | 0.426 | 0.378 |
| Week 6 | 0.314 | 0.349 | 0.352 |
| **Week 1-6** | **0.323** | **0.342** | **0.337** |
| **FCR** | | | |
| Week 1 | 1.43 | 1.51 | 1.45 |
| Week 2 | 2.01 | 1.95 | 2.05 |
| Week 3 | 2.05 | 2.06 | 1.95 |
| Week 4 | 2.03 | 2.17 | 2.07 |
| Week 5 | 2.77 | 2.07 | 2.03 |
| Week 6 | 2.86 | 2.20 | 2.57 |
| **Week 1-6** | **2.14a** | **1.97b** | **2.00b** |
| **FCG (baht)** | **34.48** | **31.88** | **31.90** |

KPFC 277 = crude β-glucanase from *Aspergillus* sp. KPFC 277, ADFI = average daily feed intake, ADG = average daily gain, FCR = feed conversion ratio, FCG = feed cost per gain. (Mean values with different letter (a,b) in the same row indicate significant differents $P< 0.05$)

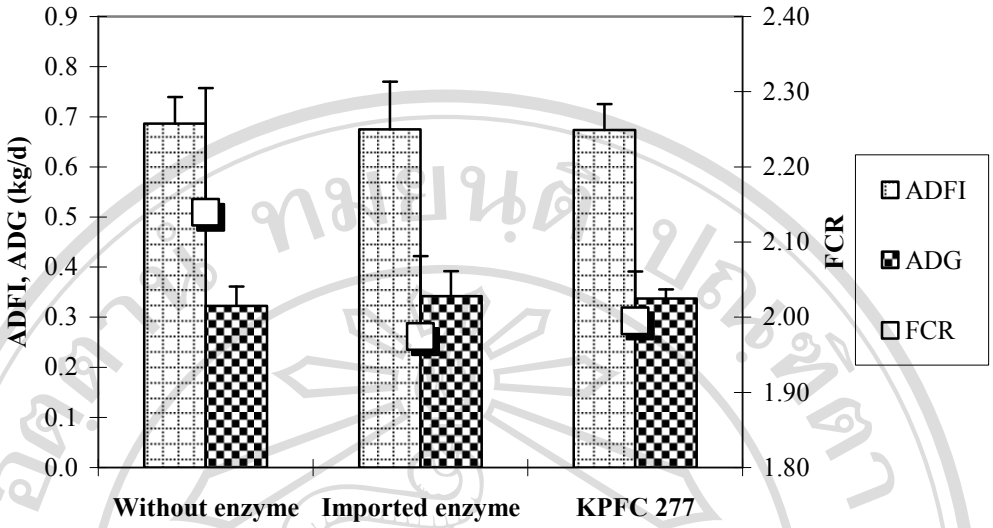


**Figure 4.13** Growth performance of piglets given diets with or without enzymes. (Mean values with different letter (a,b) in the same parameter indicate significant differences $P< 0.05$)
(ADFI = average daily feed intake, ADG = average daily gain, FCR = feed conversion ratio)

**Table 4.6** Illness rate and mortality rate of piglets offered diet with or without enzymes

| **Parameter** | **Diet** | | |
|---|---|---|---|
| | **Without enzyme** | **With imported enzyme** | **KPFC 277** |
| **Illness rate (%)** | | | |
| Week 1 | 11.9 | 14.3 | 15.5 |
| Week 2 | 9.5 | 4.8 | 6.0 |
| Week 3 | 1.2 | 2.4 | 2.4 |
| Week 4 | 2.4 | 3.6 | 1.2 |
| Week 5 | 1.2 | 0 | 1.2 |
| Week 6 | 3.6 | 2.4 | 0 |
| Week 1-6 | 5.0 | 4.6 | 4.4 |
| **Mortality rate (%)** | | | |
| Week 1-6 | 1.2 | 1.2 | 1.2 |

KPFC 277; crude β-glucanase powder produced by *Aspergillus* sp. KPFC 277.