CHAPTER IV

RESULTS AND DISCUSSION

1. Antioxidative activity of fermented soybean broths incubated with *Aspergillus* strains

Antioxidative activity of fermented soybean broths ranged between 0.21 and 0.48 mg trolox/mL sample (TEAC/mL sample) (Figure 9). *Aspergillus* inoculation resulted in stronger antioxidative activity than non-inoculated soybean broth (control). Among 33 strains, the fermented soybean broth incubated with *A. oryzae* BCC 3088 possessed the highest antioxidative activity (0.48 TEAC/mL sample), followed by *A. terricola* BCC 3026 (0.46 TEAC/mL sample), *A. ornatus* BCC 3101 (0.45 TEAC/mL sample) and *A. oryzae* BCC 3083 (0.44 TEAC/mL sample), respectively. Antioxidative activity of fermented soybean products inoculated with filamentous fungi such as *Aspergillus* and *Rhizopus* was significantly higher than non-inoculated soybean (Berghofer et al., 1998 and Santiago et al., 1992). This phenomenon is in accordance with that observed on miso, natto, temph, and fermented red bean and further demonstrates that the antioxidative activities of soybean can be enhanced through fermentation with a certain microorganism (Chung et al., 2002). The activity of antioxidants (compounds) corresponds to the number of hydrogens available for donation by hydroxyl groups. The radical scavenging effects of fermented soybean might be due to the hydroxyl groups in the antioxidant extract (Yen et al., 2003).

Therefore, the enhanced effect on antioxidative activity of fermented soybean broths incubated with *Aspergillus* varied with the starter microorganisms.

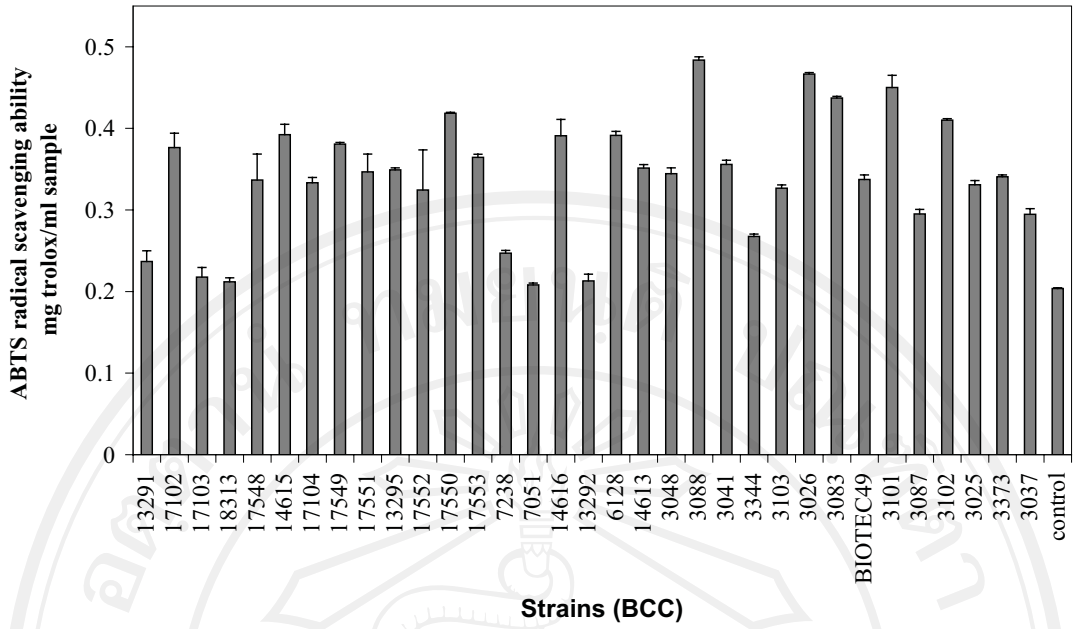

Figure 9. Antioxidative activity by scavenging effect on ABTS radical ability assay of fermented soybean broths with 33 strains of *Aspergillus*. Each fungal number represented in Table 9

2. β-glucosidase activity of fermented soybean broths

The fermented soybean broths incubated with *Aspergillus* which showed high antioxidative activity were selected for β-glucosidase activity assay. As shown in Figure 10, the specific activity of β-glucosidase activity of fermented soybean broths ranged between 0.01 and 0.31 unit/mL sample. The β-glucosidase activity of the 6 fermented soybean broths was higher than that of the non-inoculated soybean broth (control). The fermented soybean broths incubated with *A. oryzae* BCC 3088 showed the highest β-glucosidase activity, and followed by *A. terricola* BCC 3026, *A. ornatus* BCC 3101, *A. oryzae* BCC 3083, *A. sojae* BCC 3037 and *A. oryzae* BCC Biotec 49, respectively.

β-glucosidase catalyzed the hydrolytic cleavage of β-glycosidic linkages of low molecular mass glycosides and also is the key enzyme in the enzymatic release of

aromatic compounds from glucosidic precursors found in fruits and fermented products (Gueguen et al., 1996; Christine et al., 1998). *Aspergillus* strains are known for their ability to produce β-glucosidase with significantly higher yields than the other species. Esaki et al. (1999) reported that β-glucosidase produced from *A. saitoi* in the fermented soybean extract gradually hydrolyzed the glucoside isoflavones into aglycone isoflavones. It was suggested that the catalytic action of β-glucosidase during fermentation liberated aglycones of isoflavone glucosides resulted in the increased antioxidative activities (Esaki et al., 1994). Hence, the higher antioxidative activities observed with these fermented soybean broths, as shown in Figure 9 could thus be related to their high β-glucosidase activity.

3. β-glucosidase activity of fermented soybean in solid state

The 6 selected of *Aspergillus* strains in fermented soybean broths which showed high antioxidative activities and β-glucosidase activity were choosed for β-glucosidase activity assay and antioxidative activities assay of fermented soybean in solid state. The β-glucosidase activity of soybean fermented with 6 selected of *Aspergillus* strains for different periods were investigated. The enzyme activity was determined by using an extraction of each fermented soybean. As shown in Figure 11, the specific activity of β-glucosidase activity of fermented soybean ranged between 0.34 and 6.87 unit/g fermented soybeans. The β-glucosidase activity of all fermented soybean was higher than that of the soybean without inoculation (control). Additionally, the fermented soybean incubated with *A. oryzae* BCC 3088 at the fourth day showed the highest β-glucosidase activity.

β-glucosidase activity gradually increased with fermentation, especially after 2d, which was the stage of sporulation. This enzyme has a high specificity for

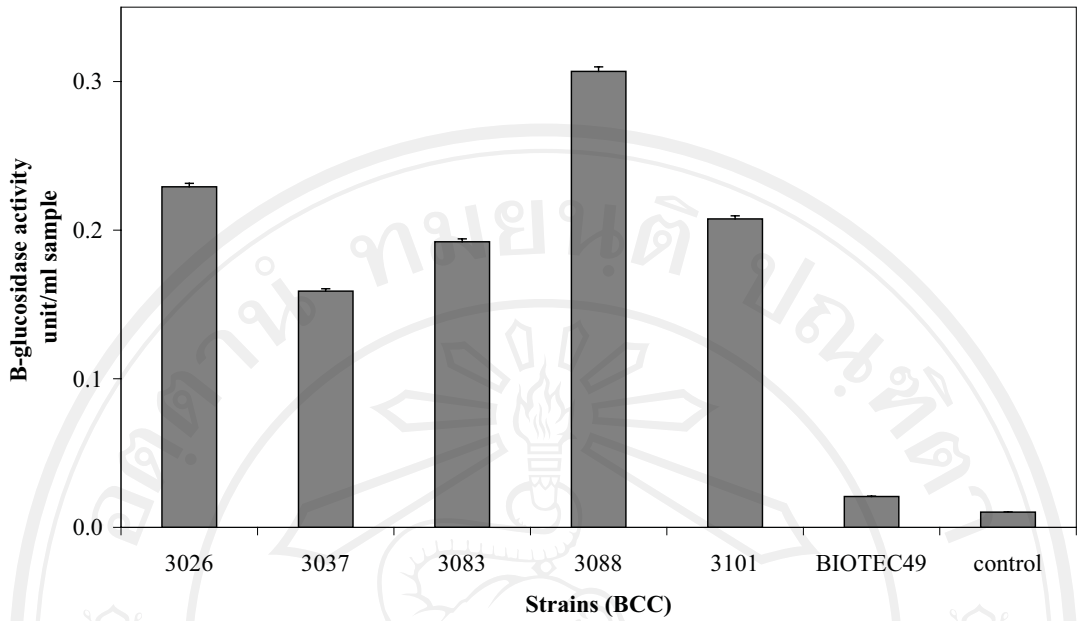

Figure 10. β-glucosidase activity of fermented soybean broths with 6 strains of *Aspergillus*

isoflavones (Hsieh et al., 2001). Murakami et al. (1984) reported that the β-glucosidase from filamentous fungi during fermentation liberated the isoflavones in soybeans resulted in the increased antioxidative activity in miso and tempeh, while it was also reported that a significant increase in the formation of a water-soluble antioxidative fraction, not the free aglycone, lead to the enhanced antioxidative activity of natto. β-glucosidase produced from the *A. saitoi* fermentation were gradually converted glucosides into aglycones, potent antioxidative substances (Esaki et al., 1994). Therefore, the high β-glucosidase enzyme activity in fermented soybeans producing from filamentous fungi was related to antioxidative activity as well.

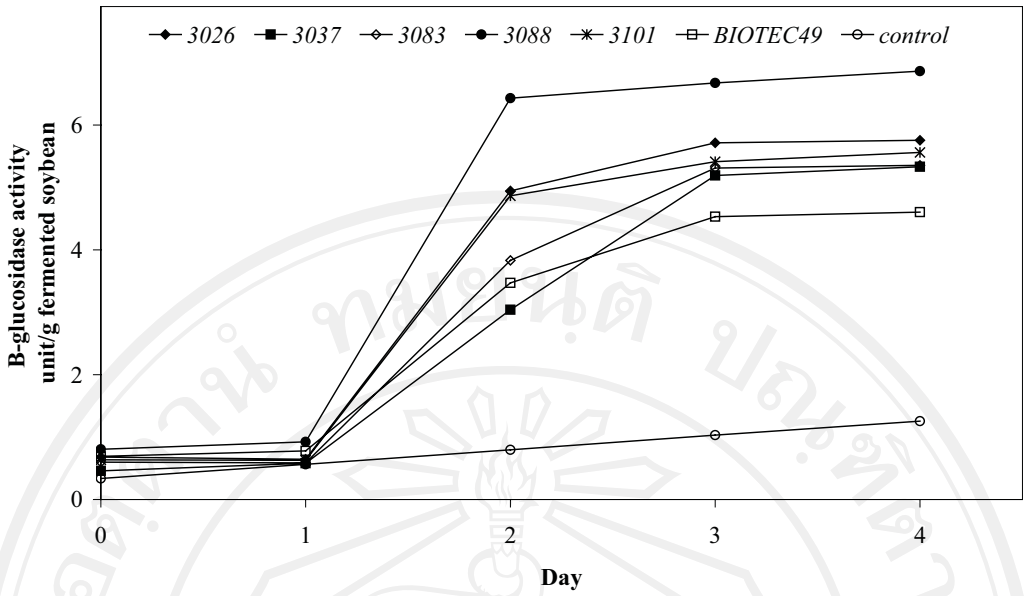

Figure 11. β-glucosidase activity of fermented soybean with 6 strains of *Aspergillus* during 4-day fermentation

4. Antioxidative activities of fermented soybean in solid state

The scavenging effect on ABTS radical ability assay of fermented soybean with 6 selected of *Aspergillus* strains for different periods were reported as mg trolox/g fermented soybean (TEAC/g fermented soybean). The results of the antioxidative activity were shown in Figure 12. The antioxidative activity ranged between 0.46 and 1.59 mg trolox/g fermented soybean. Soybean fermented with 6 selected of *Aspergillus* strains exhibited stronger antioxidative activity than that of the soybean without inoculation (control). Furthermore, the fermented soybeans incubated with *A. oryzae* BCC 3088 at the fourth day possessed the highest antioxidative activity (1.59 TEAC /g fermented soybean) among the 6 others selected of fermented soybean.

The FRAP assay is a method for assessing antioxidative activity reducing ferric to ferrous ion. The samples reduce d Fe^{3+}/tripyridyltriazine complex, present ed in stoichiometric excess, to the blue colored ferrous complex form with an increase in

absorbance at 593 n m. The reducing capacity of fermented soybeans with 6 selected of *Aspergillus s*trains for different periods were reported as mg $FeSO_4$/g fermented soybean. The results of the antioxidative capacity were shown in Figure 13. FRAP and ABTS assay profiles of fermented soybean showed the same tendency for antioxidative capacity. The antioxidative activity ranged between 0.097 and 0.650 mg $FeSO_4$ /g fermented soybean. The fermented soybean incubated with *A. oryzae* BCC 3088 at the fourth day possessed the highest antioxidative activity (0.65 mg $FeSO_4$ /g fermented soybean) among the 6 others selected of fermented soybean. In conclusion, the result of ferric reducing ability power (FRAP) assay was correlated with that of ABTS radical scavenging activity assay.

The increased reducing power observed may be due to the formation of reductants that could react with free radicals to stabilize and terminate radical chain reactions during fermentation. In addition, the intracellular antioxidants, peptides of the starter organism and their hydrogen-donating ability, may also contribute to this increased reducing ability (Yang et al., 2000). The soybean fermented with filamentous fungi containing abundance of β-glucosidase enzyme possessed enhanced antioxidative activities in various model systems (Chia-Hung et al., 2006). The antioxidative activity of fermented soybean products such as miso, tempeh and natto, inoculated with *Aspergillus oryzae*, *Rhizopus oligosporum* and *Bacillus natto*, respectively, was significantly higher than in non-inoculated steamed soybean (Berghofer et al., 1998). Moreover, they were more stable against lipid peroxidation too (Wang et al., 2004). The fermented soybean with high antioxidative activity was associated with β-glucosidase activity since daidzin and genistin found in soybean were converted to daidzein and genistein, the more potent antioxidant, respectively by

β-glucosidase enzyme. Consequently, the high β-glucosidase activity was related to the high antioxidative activity (Matsura et al., 1995).

Yang (2000), reported that fermented soybean exhibited excellent reducing power. Fermented soybean might produce certain metabolites with superior reducing power during fermentation. The antioxidant activity has been reported to be concomitant with the development of reducing power. Okuda et al. (1983) reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. Furthermore, reductones, such as ascorbic acid, can react directly with peroxides and also with certain precursors and thereby, prevent peroxide formation (Shimada et al., 1992). The reducing power of fermented soybean might be due to its electron-donating ability. Therefore, fermented soybean might contain reductones formed during fermentation, which could react with free radicals to stabilize and terminate radical chain reactions. However, this pattern was not observed in this research.

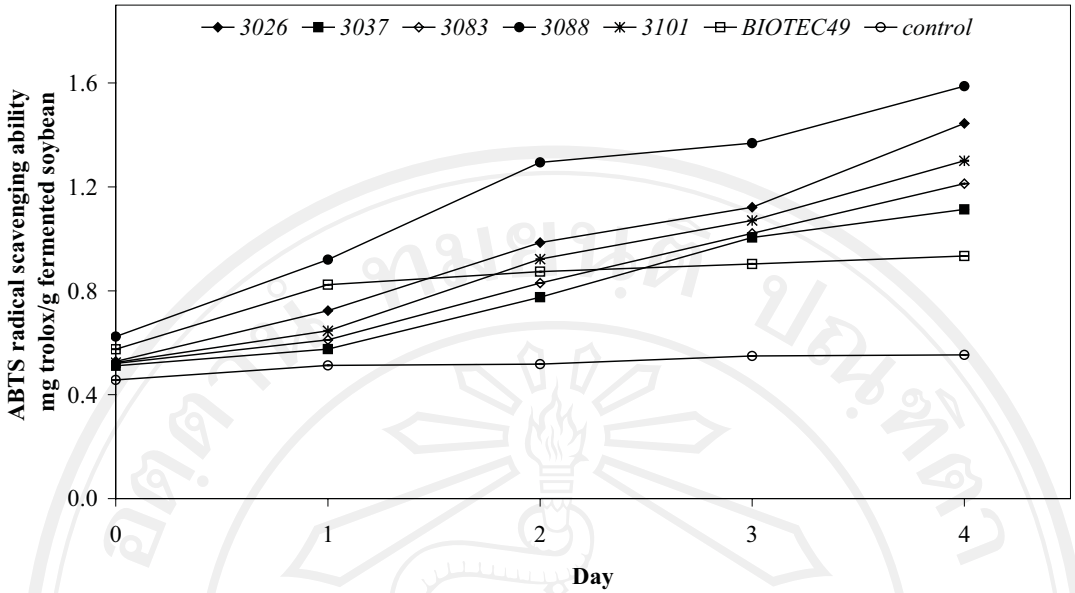

Figure 12. Antioxidative activity by scavenging effect on ABTS radical ability assay of fermented soybean in solid state with 6 strains of *Aspergillus* during 4-day fermentation

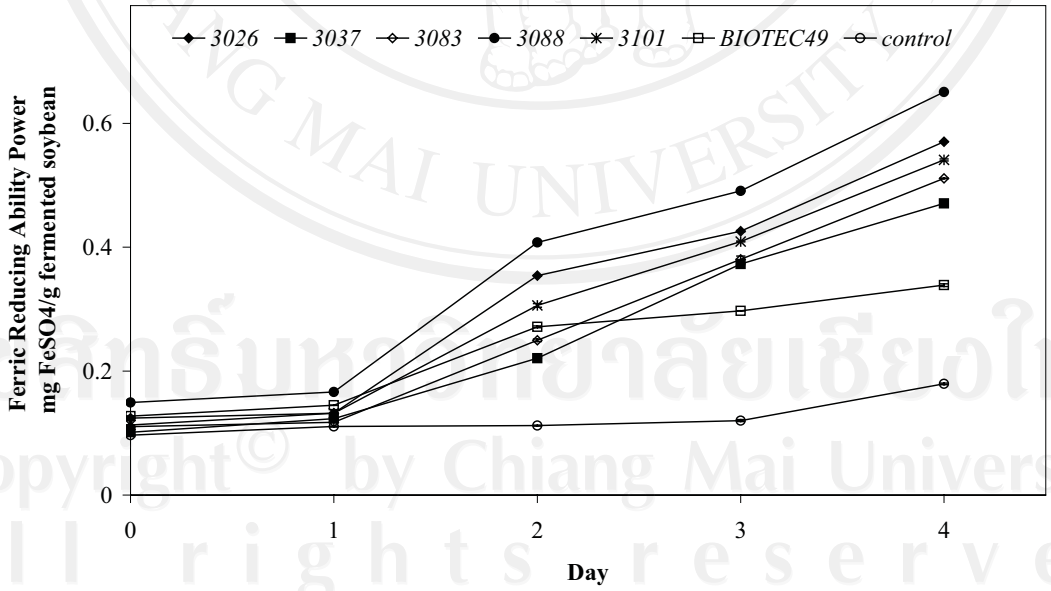

Figure 13. Antioxidative activity by ferric reducing ability power assay (FRAP) of fermented soybean in solid state with 6 strains of *Aspergillus* during 4-day fermentation

5. Total phenolic contents assay

Phenolics are ubiquitous secondary metabolites in plants comprising a large group of biologically active ingredients. (Deostri, 2000). Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinigenic, as well as ability to modify the gene expression. (Nakamura et al., 2003; Tapiero et al., 2002). Their activities in vitro and in vivo are related to a number of hydroxyl functional groups in their structures. The total phenolic contents of the methanol extract from fermented soybean using standard curve of gallic acid (R^2 = 0.9996) was reported as mg gallic acid equivalents (GAE)/g fermented soybean, shown in Figure 14. The fermented soybean incubated with *A. oryzae* BCC 3088 contained the highest amount of total phenolic contents (398.83 mg GAE/g fermented soybean) at the fourth day of fermentation in comparison with the 4 selected of *Aspergillus* strains. The total phenolic content of all fermented soybean were higher than that of the non inoculated soybean.

In plants, phenolics are usually found in conjugated forms through hydroxyl groups with sugar as glycosides (Robbins, 1980). Concentrations of phenolic compound were reported to increase in soybean (McCue and Shetty, 2003), fava bean (Vattem and Shetty, 2002) and cranberry pomace (Randhir et al., 2004) after fermentation. The increased total phenolic content in soybean after fermentation is consistent with findings reported by other investigators (Vattem and Shetty, 2002; Randhir et al., 2004). These investigators suggest that β-glucosidase, produced by fungi, catalyse the release of aglycones from the bean substrate and thereby increases their phenolic content. The extracts of soybean koji fermented with *Aspergillus oryzae* and *Aspergillus awamori*, contain the highest amount of total phenolic compound among the various soybean koji extracts tested (Chia et al, 2006).

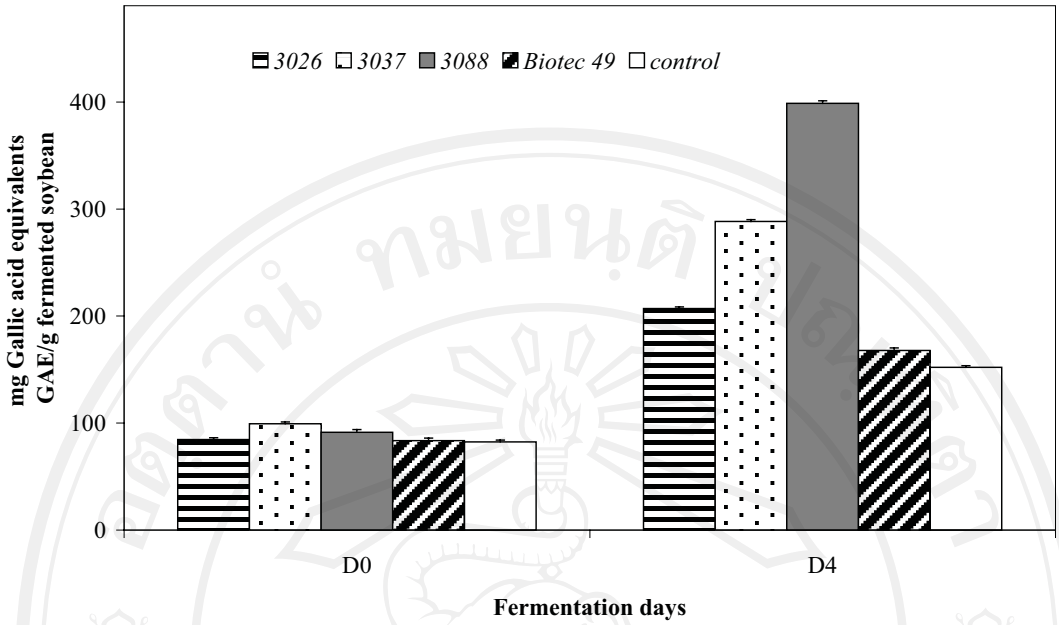

Figure 14. Total phenolic content of fermented soybean in solid state with 4 strains of *Aspergillus* at day0 and day4 after fermentation

Phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and metal-chelating ability (Shahidi et al., 1992; McCue and Shetty, 2003). Chia-Hung (2006) observed that the extracts of *Aspergillus oryzae* and *Aspergillus awamori*, soybean koji, contain the highest amount of total phenolic compound among the various soybean koji extracts tested. This observation further demonstrates that total phenolic content changes during the fermentation process. The higher antioxidative activities observed with these soybean koji extracts could thus relate to their high total phenolic content.

6. Total Flavonoid contents assay

Flavonoids represent the most common and widely distributed group of plant phenolics. Some research has demonstrated that many phenolics, including flavonoids and phenolic acids, have antioxidant capacities that are much stronger than those of vitamin C and E (Francesco et al., 1997; Liegeois et al., 2000). The total flavonoid contents of the methanol extract from fermented soybean using standard curve of catechin (R^2 = 0.9994) was reported as mg catechin equivalents (CE)/g fermented soybean, shown in Figure 15. The highest total flavonoid contents were observed in fermented soybean incubated with *A. oryzae* BCC 3088 (451.83 mg CE/g fermented soybean) at the fourth day of fermentation in comparison with the the 4 selected of *Aspergillus* strains. The total flavonoid contents of fermented soybean at day 4 were significantly higher than that of day 0. Summarily, the result of total flavonoid contents assay was correlated with that of total phenolic contents assay. The soybean fermented with *Aspergillus oryzae* BCC 3088 which showed higher total phenolic contents, resulting in higher total flavonoid contents too.

Flavonoids represent the most common and widely distributed group of water soluble polyphenolic molecules which have the diphenylpropane (C_6-C_3-C_6) skeleton. The flavonoids exhibit a wide range of antioxidative effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal ion chelators. Therefore, an increase in total flavonoid contents were positively correlated with the increased capacity in reducing power and DPPH radical-scavenging, the antioxidant activity in linoleic acid/water emulsion system and inhibition for lipid peroxidation by thiobarbituric acid reactive substances (Romero et al., 2004). The flavonoid family comprises 15 classes of compounds in which isoflavones are the predominant flavonoid in soybean seeds (Ho et al., 2002). Therefore, a larger increase

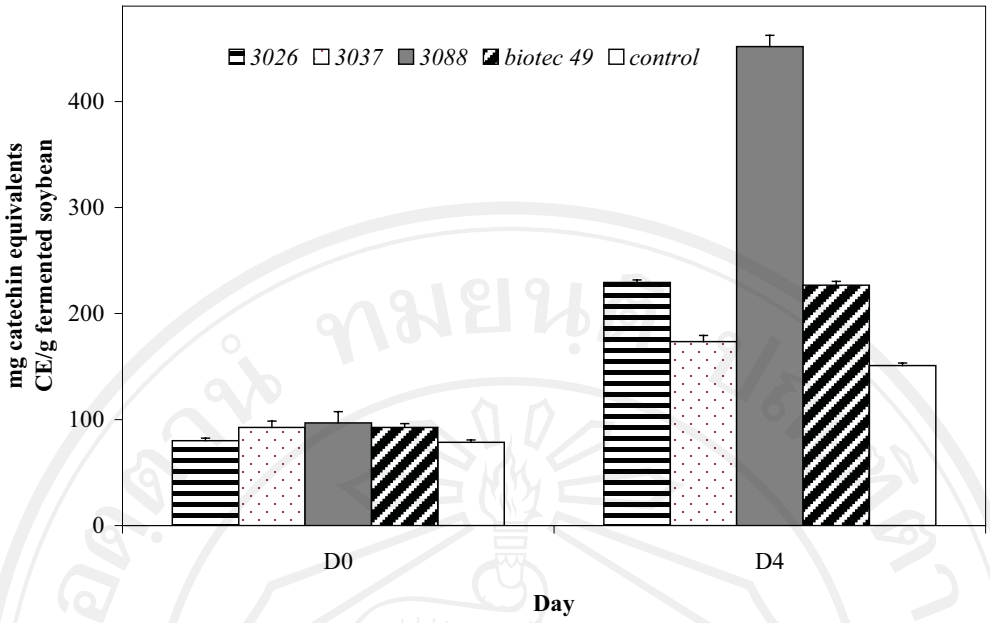

Figure 15. Total flavonoid content of fermented soybean in solid state with 4 strains of *Aspergillus* at day0 and day4 fermentation

in total flavonoid contents was attributed to the changes in isoflavone composition exerted by the activity of fungi during fermentation.

7. DPPH radical-scavenging activity assay

DPPH radical-scavenging activity of soybean fermented with *A. oryzae* BCC 3088 was higher than that of soybeans naturally fermented (Figure 16) in which the EC_{50} values were 13.0 mg/mL and 22.5 mg/mL, respectively. Scavenging of DPPH free radical is the basis of a common antioxidant assay based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH). As an odd electron becomes paired off in the presence of a hydrogen donor, the absorbance is decreased and the resulting decolorization is stoichiometric with respect to the number of electrons captured. The results show that the soybean fermented with *A. oryzae* BCC 3088 possibly contained

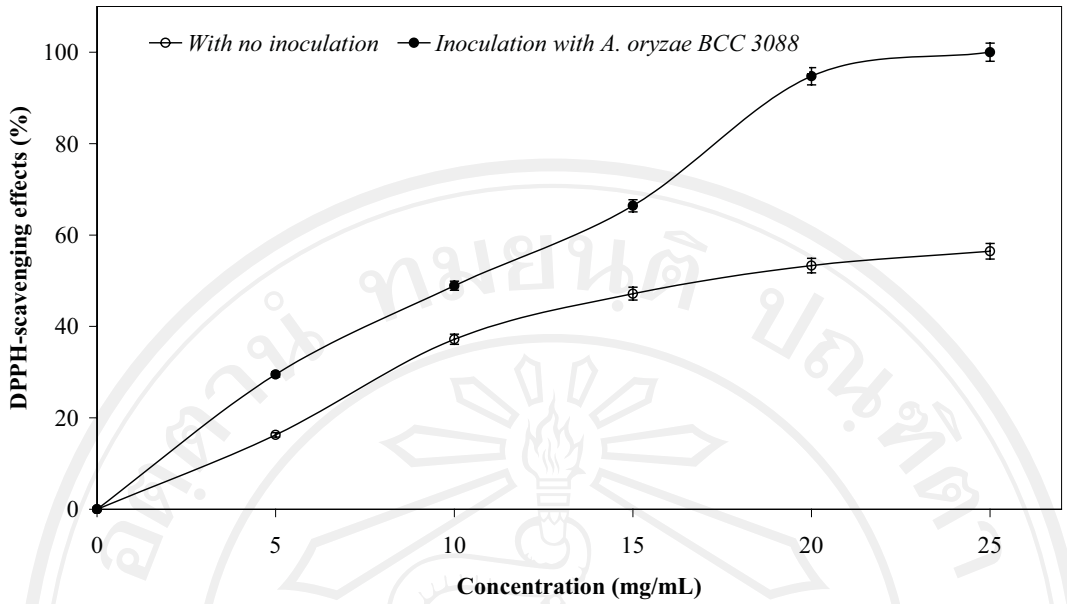

Figure 16. DPPH radical-scavenging activity of the methanol extract of soybean fermented with and without inoculation of *A. oryzae* BCC 3088 at the 4th day fermentation

substances that were more active as hydrogen donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. Among naturally occurring antioxidative components in soybean, antiradical activity was positively influenced by the phenolic compounds (Moktan et al, 2008; Bors et al., 1990). Along with previous reports on soybean koji fermented with other fungal strains, higher DPPH-scavenging effect would be a result of the ability of fungi to metabolize isoflavone precursors in soybean to their more active isoflavone forms (Chia et al., 2006, Esaki et al., 1997). From a total of 21 fungal strains from 9 different genera, only tested *Aspergillus* strains isolated from fermented soy foods, including five *A. oryzae* strains, one *A. sojae* strain, and one *A. tamarii* strain, were able to metabolize both daidzein and genistein to 8-hydroxydaidzein and 8-hydroxygenistein, respectively (Chang et al., 2007).

8. Lipid peroxidation assay

The soybean fermented with *A. oryzae* BCC 3088 exhibited stronger inhibitory activity against linoleic acid peroxidation than soybean naturally fermented (Figure 17), in which the EC_{50} values were 24.7 mg/mL and 45.5 mg/mL, respectively. The results conformed with Esaki (1997) that the fermented soybean incubated with *Aspergillus saitoi*, which has been utilized for manufacturing, had the most antioxidative activity against lipid peroxidation. Lipid peroxidation leads to rapid development of rancid and stales flavors, and is considered as a primary mechanism of quality deterioration in lipid foods and oils (Güntensperger et al., 1998). Corresponding with DPPH-scavenging effect, the fermented soybean with *A. oryzae* BCC 3088 was likely to contain substances that can function both as an antioxidant and as a free radical acceptor that can convert free radicals into harmless substances through an energy-decreasing procedure. This action is extensive and effective in eliminating free radicals ranging from the superoxide anion to H_20_2 to lipid peroxide free radical, as well as having an antioxidant effect on unsaturated fatty acids and lipids. Through different chemical mechanisms, including free radical quenching, electron transfer, radical addition, the phenolic compounds formed have been reported on their ability to suppress lipid peroxidation (Mathew and Abraham, 2006). Rather than their mere presence, a synergism of various phenolic compounds and/or other components present in the extract may also contribute to the total antioxidant activity (Shahidi et al., 1994).

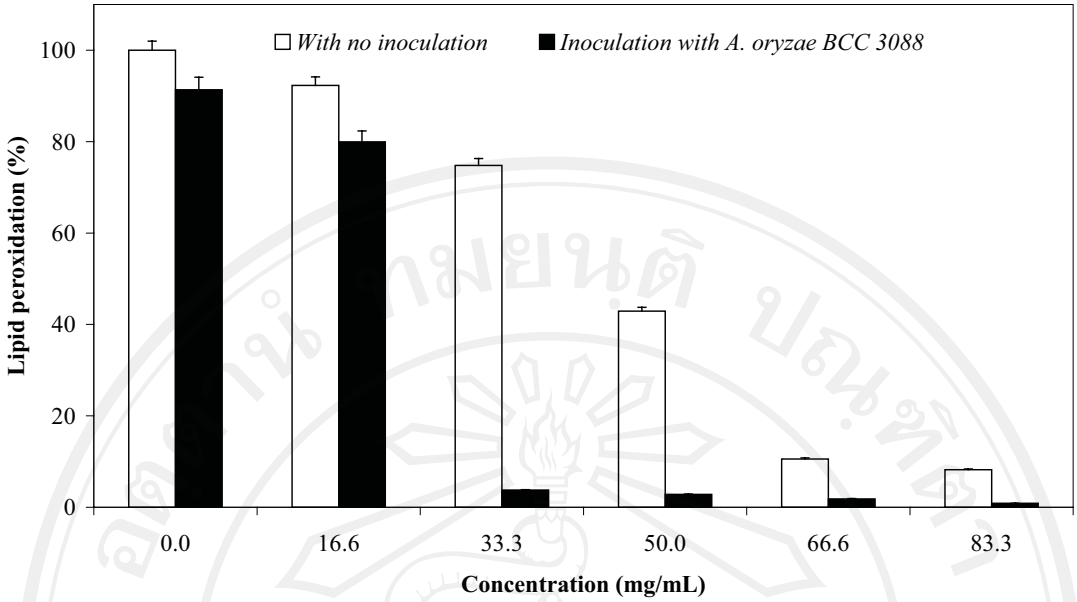

Figure 17. Comparison of the capacity of the methanol extracts of soybean fermented with and without inoculation of *A. oryzae* BCC 3088 to inhibit the production of thiobarbituric acid reactive substances (TBARS).

9. Plasmid Relaxation assay

Soybean fermented with *A. oryzae* BCC 3088 showed higher antioxidative activity in inhibiting DNA relaxation than those naturally fermented without inoculation at any concentrations tested (Figure 18). Based on band intensity, treatment of DNA with fermented soybean extract of *A. oryzae* BCC 3088 reduced the concentration of open or relaxed circular DNA in a dose-dependent manner. DNA strand breakage was induced in the presence of H_2O_2 and Fe^{2+}, while DNA in the presence of H_2O_2 or Fe^{2+} alone did not show significant strand breakage. Induced oxidative damage in DNA through Fenton reaction is thought to arise via a site-specific mechanism, i.e. involving the interaction of a transition metal ion with DNA

prior to its reaction with H_2O_2 to produce the damaged DNA species (Chevion, 1988).

					With no inoculation				**Inoculation with *A. oryzae* BCC 3088**			
Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
H_2O_2	-	+	-	+	+	+	+	+	+	+	+	+
Fe^{2+}	-	-	+	+	+	+	+	+	+	+	+	+
Extract (mg/mL)	-	-	-	-	**2.5**	**5.0**	**7.5**	**10**	**2.5**	**5.0**	**7.5**	**10**

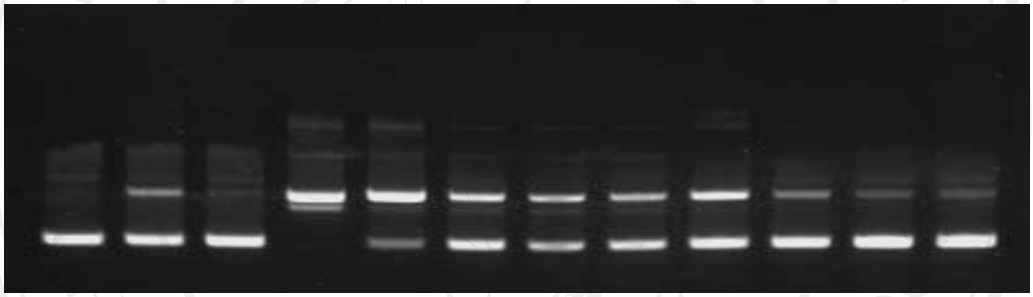

Figure 18. Inhibitory effect of the methanol extracts of soybean fermented with and without inoculation of *A. oryzae* BCC 3088 on plasmid DNA relaxation in Fenton reaction system. OC and SC represent open/relaxed circular and supercoiled forms of DNA, respectively

Apart from scavenging ability on hydroxyl radicals, this study implies that part of the antioxidant activity of the fermented soybean extract might arise from their iron chelating ability.

10. Protein oxidation inhibition assay

The dose-response inhibition of copper-induced oxidation protein oxidation of the methanol extracts from fermented soybean incubated with *A. oryzae* BCC 3088 and the soybean naturally fermented were shown in Figure 4. Based on the extent of fragmentation, the extract of fermented soybean inoculated with *A. oryzae* BCC 3088 showed higher antioxidative activity in inhibition than control group at any concentrations tested. Oxidants such as hydrogen peroxide (H_2O_2) and various kinds of ROS are implicated in mediating a wide array of damage to proteins (Shacter, 2000). Collectively, these ROS can lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation (Berlett and Stadtman, 1997). Proteins highly influence the physical characteristics of foods and so oxidative changes of these biomolecules may have a significant effect on food integrity. According to Stagos et al. (2007), it is also speculated that phenolic compounds formed by the action of inoculated fungi may be a mechanism accounting for the protective activity of natural antioxidants against induced oxidation of proteins.

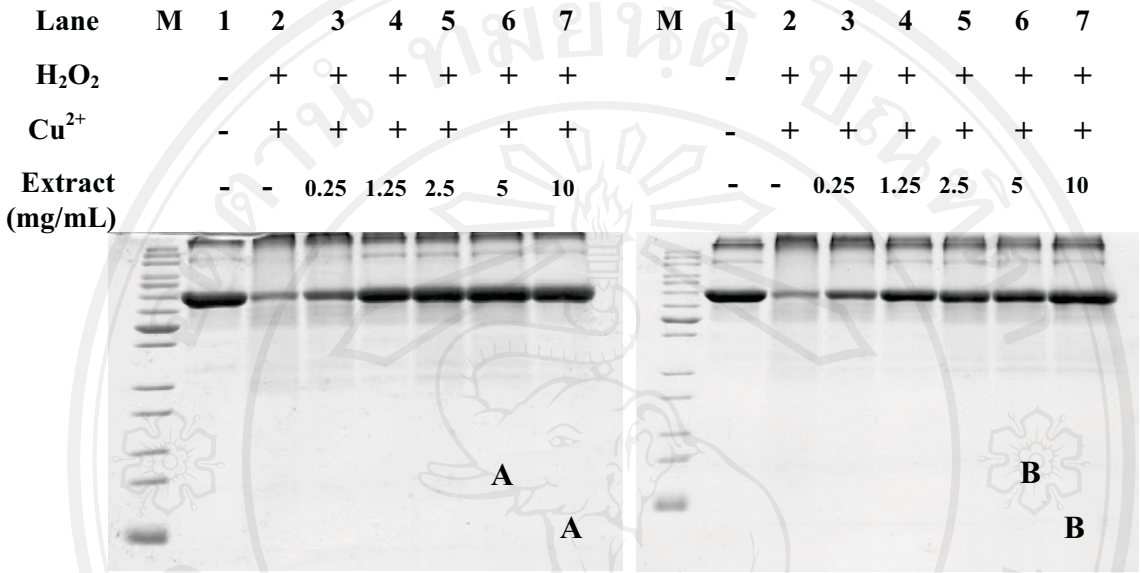

Figure 19. Protein oxidation inhibition assay of the methanol extracts of soybean fermented with (A) and without inoculation (B) of *A. oryzae* BCC 3088.

Isoflavone composition

The primary isoflavones in soybean were daidzein, genistein and their respective ß-glycosides, daidzin and genistin, especially, the isoflavone derivative, 8-OHG, which are known to be the antioxidative components (Table 10). Most of the soy products have a total isoflavone concentration of 1-3 mg/g in which the isoflavones appear mostly as the glycoside conjugates (Esaki et al., 1999). After fermentation, total glucosides content of soybeans fermented with *A. oryzae* BCC 3088 decreased about to 2.9 fold, but the proportion of aglycone in total isoflavone fermented with and without *A. oryzae* BCC 3088 markedly increased about 10 and 3.4

folds, respectively (Table 10). Aglycone concentration was remarkably higher in soybean fermented with *A. oryzae* BCC 3088 whereas glycoside concentration decreased significantly after 4 days of fermentation (Table 10). As for the increases of aglycone isoflavone during the fermentation, the proportion of aglycones in total isoflavones was markedly higher in soybean fermented with *A. oryzae* BCC 3088 than was those from uninoculated soybean. Furthermore, the other isoflavone derivative, 8-hydroxygenistein (8-OHG) was found in the fourth day of fermentation (Fig.20). The mass spectral analysis was used to confirm the isoflavone substances in soybean fermented with *A. oryzae* BCC 3088 at day4 by comparing the spectra data with known authentic standards (Table 11, Fig. 21). The liberation of lipophilic aglycones of isoflavone glucosides such as daidzein and genistein by the catalytic action of β-glucosidase during fermentation resulted in the increased aglycone isoflavones and antioxidative activity of miso and tempeh (Esaki et al., 1994). The aglycones are present in the soybean grains in small quantities, varying from 1% to 3% of the total isoflavones (Góes-Favoni, et al., 2010). According to Murphy et al. (2002), a higher content of aglycones might be a result from the action of β-glucosidase (β-d-glycoside glycohydrolase, EC 3.2.1.21), endogenous in soy (Carrão-Panizzi et al., 2004; Matsura et al., 1995) and the associated β-glucosidase of the fermenting microbes, which promote the hydrolysis of the β-glucoside conjugates, converting them to aglycones.

In consideration of the possible radical scavenging activity of fermented soybean, isoflavone aglycones and the formation of o-dihydroxyisoflavones especially 8-OHG are considered to be responsible for the overall increased antioxidant properties. Being respectively liberated from genistin by β-glucosidase, 8-OHG was formed from genistein by microbial hydroxylation (Esaki et al., 1999).

These isoflavones exhibited significantly stronger antioxidative activity than daidzein and genistein in both oil and lipid/aqueous systems.

Table 10. Isoflavone content of soybean fermented with and without inoculation of *A. oryzae* BCC 3088.

Amount (mg/ 100g sample)	With no inoculation		Soybean inoculated with *A. oryzae* BCC 3088	
	Day0	Day4	Day0	Day4
Daidzin	149.9 ± 0.045^{cA}	85.3 ± 0.032^{dC}	119.0 ± 0.026^{cB}	37.0 ± 0.002^{gD}
Genistin	148.9 ± 0.041^{cA}	100.1 ± 0.044^{cB}	106.0 ± 0.011^{dB}	41.0 ± 0.004^{fC}
Daidzein	8.4 ± 0.005^{dC}	48.6 ± 0.001^{fB}	7.5 ± 0.00^{fD}	85.0 ± 0.002^{cA}
Genistein	12.8 ± 0.003^{dC}	24.2 ± 0.001^{gB}	7.4 ± 0.002^{fD}	66.0 ± 0.018^{eA}
8-hydroxygenistein	ND	ND	ND	0.048 ± 0.001^{h}
Total glucosides	298.8 ± 0.087^{bA}	185.4 ± 0.076^{bC}	225.2 ± 0.011^{bB}	78.0 ± 0.006^{dD}
Total aglycone	21.2 ± 0.001^{dC}	72.8 ± 0.003^{eB}	14.9 ± 0.001^{eD}	150.4 ± 0.016^{bA}
Total isoflavone	320.0 ± 0.088^{aA}	258.2 ± 0.079^{aB}	239.9 ± 0.010^{aC}	228.3 ± 0.010^{aC}

Means with different small letters in the same column and capital letters in the same row indicated significant differences ($p < 0.05$) between treatments.
ND: Not detectable

Table 11. Mass spectrometry analysis of isoflavone, A: Authentic standard of isoflavones, B: Soybean fermented with *Aspergillus oryzae* BCC 3088 at 4th day fermentation.

Authentic standard of isoflavones		Mass analysis
Retention time(min)	Compound name	Ion mass (m/z)
8.14	Daidzin	418,417,186,154,157
9.80	Genistin	455,434,433,186,157
11.68	8-OHG	476,475,454,453,287,251,227
13.98	Daidzein	476,475,454,453,351,340,277,256,255
15.55	Genistein	475,464,454,453,340,293,272,271,186

(A)

Soybean fermented with *Aspergillus oryzae* BCC 3088		Mass analysis
Retention time(min)	Compound name	Ion mass (m/z)
8.15	Daidzin	419,418,417,186,159
9.81	Genistin	471,455,435,434,433,186
11.75	8-OHG	476,475,455,454,453,251,227
14.01	Daidzein	475,454,453,340,256,255
15.58	Genistein	475,464,453,340,217,186

(B)

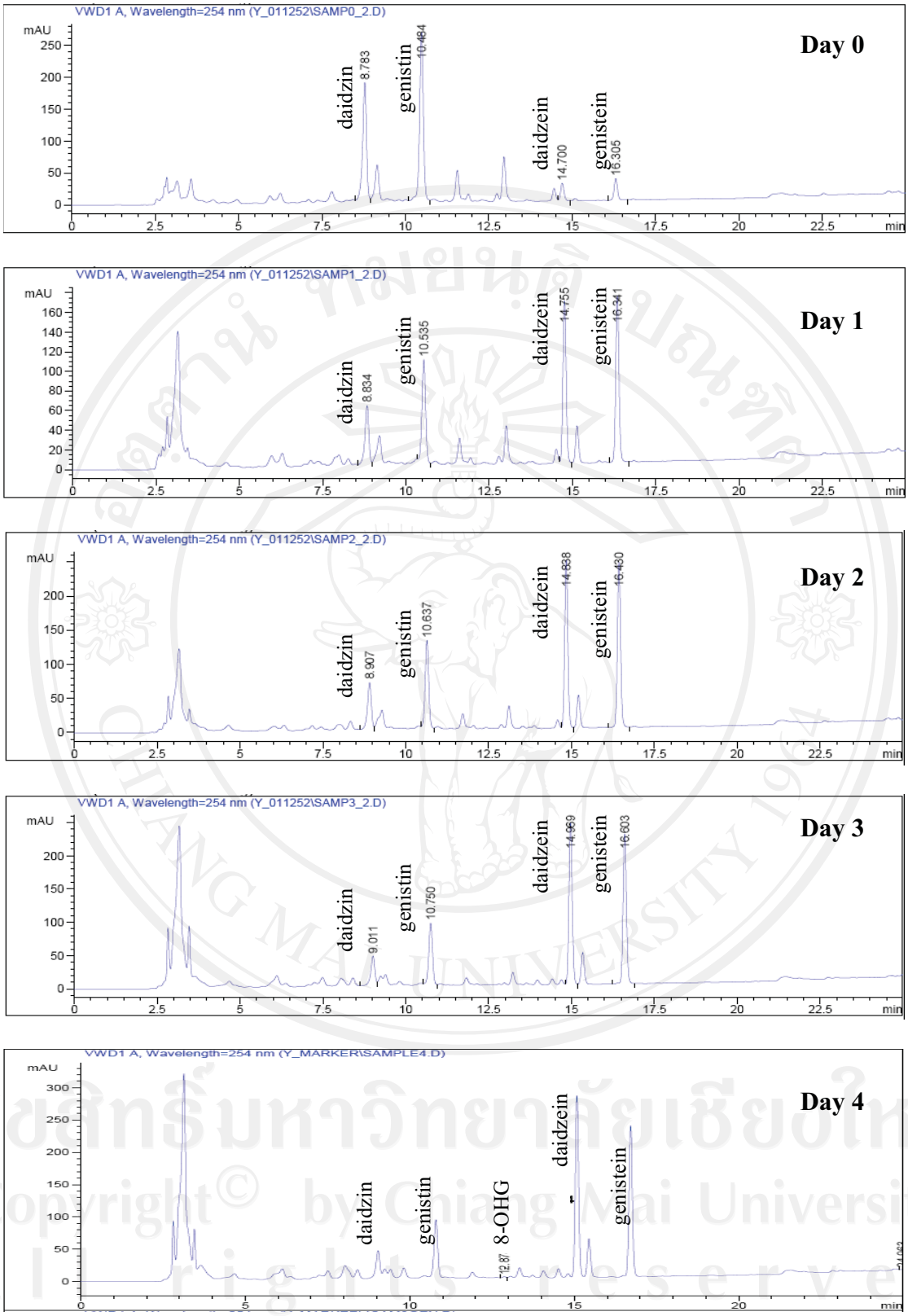

Figure 20. HPLC chromatograms of fermented soybean extract inoculated with *Aspergillus oryzae* BCC 3088 during fermentation.

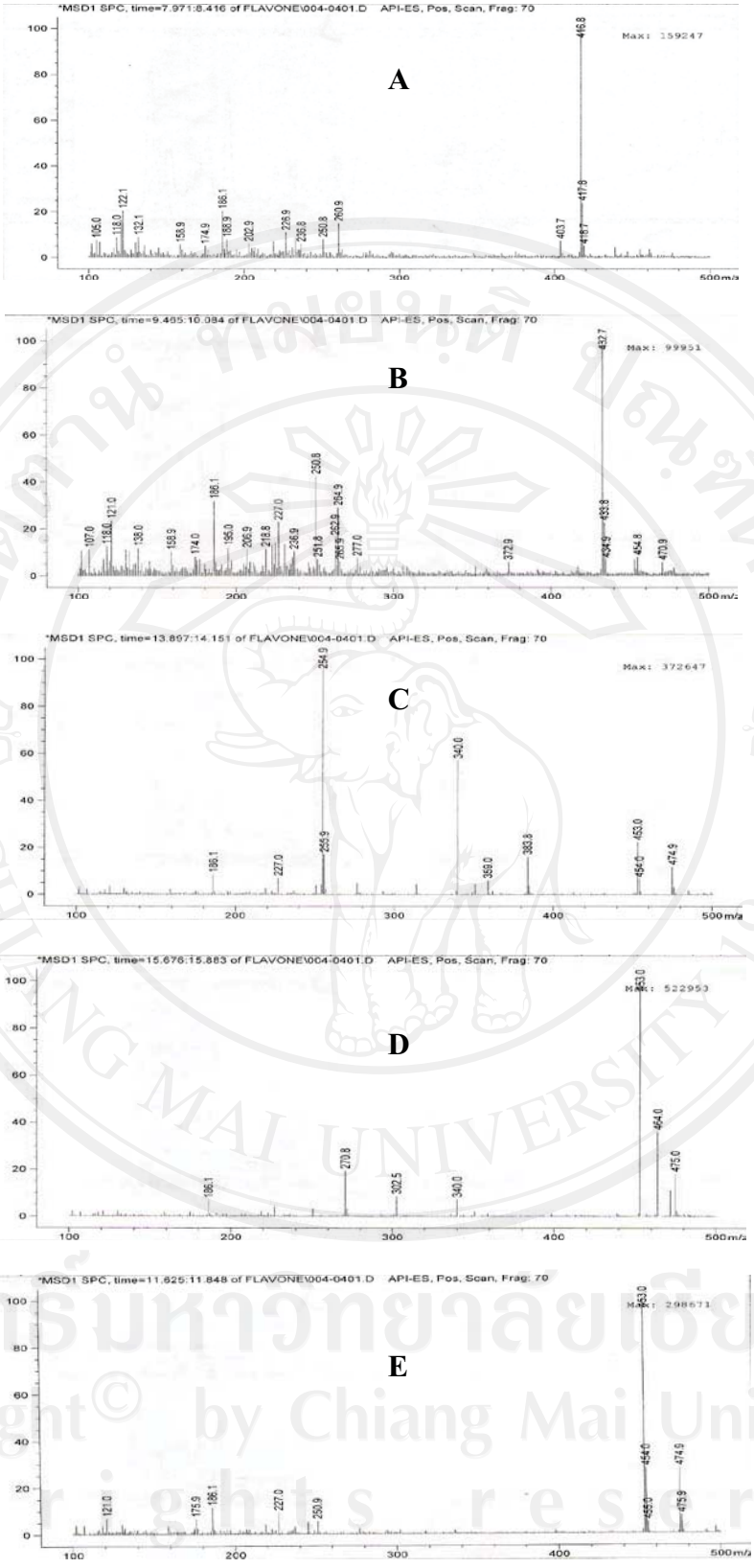

Figure 21. Mass spectral analysis of isoflavones in the fourth day fermentation of soybean fermented with *Aspergillus oryzae* BCC 3088. A: daidzin, B: genistin, C: daidzein, D: genistein, E: 8-OHG.