African Journal of Agricultural Research Vol. 6(4), pp. 785-789, 18 February, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.169
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

8-Hydroxygenistein formation of soybean fermented with *Aspergillus oryzae* BCC 3088

Chirtchai Punjaisee[1], Chaiyavat Chaiyasut[1]*, Sunee Chansakaow[1], Somsak Tharata[2], Wonnop Visessanguan[3] and Suchart Punjaisee[4]

[1]Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.
[2]Central Laboratory (Thailand) Co. Ltd., Thailand.
[3]National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani, Thailand.
[4]Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.

Accepted 18 January, 2011

The isoflavone content of soybean was studied by incubated Thai native soybean (*Glycine max* (L) Merr, SJ.2) with *Aspergillus oryzae* BCC 3088. The changes of the isoflavone analogue content were analysed by HPLC. Mass spectrometry was used to confirm the presence of substances in fermented soybean with the authentic standards. After fermentation, total glycosides content of soybean fermented with *A. oryzae* BCC 3088 decreased but the proportion of aglycone markedly increased. As for the increases of isoflavone aglycone during the fermentation, the proportion of aglycones in total isoflavones was markedly higher in soybeans fermented with *A. oryzae* BCC 3088 than was those from uninoculated soybean. Furthermore, *8*-hydroxygenistein (*8*-OHG) was found at the fourth day of fermentation. Therefore, fermentation of soybean with *A. oryzae* BCC 3088 results in higher levels of isoflavone aglycones and their corresponding, 8-OHG, which may enhance more health benefits than unprocessed soybean.

Key words: *Aspergillus oryzae*, fermented soybean, isoflavone, 8-hydroxygenistein.

INTRODUCTION

Soybean is popularly known as a healthy food in many Asian countries and is mostly consumed as soymilk, tofu, and especially fermented products such as miso, tempeh, and natto (Hayashi et al., 1995). In recent years, the soybean isoflavones have been intensive investigated due to their possible role in prevention certain hormone-dependent and other diseases, including preventing certain forms of cancer, as well as reducing the risk of cardiovascular diseases (Anderson, 1995) and function by acting as estrogens (Cassidy, 1996).

The fungi, especially *Aspergillus* strains used in traditional manufacturing of fermented foods are safe since those microbes have been eaten by people over a long term of years (Barbesgaard et al., 1992). Two major isoflavones found in soybean are genistin and daidzin which are the glycoside conjugates of genistein, daidzein, respectively. Isoflavone glycoside could be hydrolyzed to the respective free isoflavone aglycone by fungal beta-glucosidase during soybean fermentation (Chou et al., 2002). Furthermore, the biotransformation of isoflavones has also been of interest, because the bioactivity of the compounds dramatically alters with the structure.

Moreover, the use of daidzein and genistein during the fermentation of Japanese soybean has yielded *O*-hydroxyisoflavones, a potent antioxidant (Esaki et al., 1999). In this study, we investigated 8-hydroxygenistein formation during fermentation of soybean with *A. oryzae* BCC 3088 which was isolated from koji in our previous report.

MARERIALS AND METHODS

Preparation of fermented soybean

Soybean (*Glycine max* (L) Merr; SJ.2) were obtained from Limsakdakun Co.Ltd., Chiang Mai, Thailand. Whole soybean was washed, soaked in water for 12 h and then autoclaved at 121°C for

*Corresponding author. E-mail: chaiyavat@gmail.com. Tel: +66-53-944340. Fax: +66-53-894163.

Table 1. Transformation of isoflavone content of soybean fermented with and without inoculation of *A. oryzae* BCC 3088.

Amount (ppm)	Soybean with no inoculation		Soybean inoculated with *A. oryzae* BCC 3088	
	Day0	Day4	Day0	Day4
Daidzin	249.8 ± 0.045^{cA}	142.1 ± 0.032^{dC}	198.3 ± 0.026^{cB}	61.6 ± 0.002^{gD}
Genistin	248.1 ± 0.041^{cA}	166.6 ± 0.044^{cB}	176.6 ± 0.011^{dB}	68.3 ± 0.004^{fC}
Daidzein	14.0 ± 0.005^{dC}	81.0 ± 0.001^{fB}	12.5 ± 0.00^{fD}	141.6 ± 0.002^{cA}
Genistein	21.3 ± 0.003^{dC}	40.3 ± 0.001^{gB}	12.3 ± 0.002^{fD}	110.0 ± 0.018^{eA}
8-hydroxygenistein	ND	ND	ND	0.08 ± 0.001^{h}
Total glucosides	497.9 ± 0.087^{bA}	308.7 ± 0.076^{bC}	374.9 ± 0.011^{bB}	129.9 ± 0.006^{dD}
Total aglycone	35.3 ± 0.001^{dC}	121.3 ± 0.003^{eB}	24.8 ± 0.001^{eD}	251.6 ± 0.016^{bA}
Total isoflavone	533.2 ± 0.088^{aA}	430.0 ± 0.079^{aB}	399.7 ± 0.010^{aC}	381.5 ± 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.

30 min. After cooling, the cooked soybean was inoculated with the spore suspension of *A. oryzae* BCC 3088 at the level of 1×10^6 spore per gram of cooked soybean. The samples were then incubated at 30°C. Samples were collected at 24 h interval up to 4 days. The samples were powderized in blender (Model BBL550XL, Hawii, USA) under liquid nitrogen and frozen at -20°C immediately until use.

HPLC and LC-MS analysis for isoflavone compositions

Isoflavones content of fermented soybean were determined by high performance liquid chromatography (HPLC). Fermented soybean powder (1 g) was extracted with 5 mL of methanol with shaking at 60 RPM in a water bath for 12 h at 37°C (Lowri et al., 1998). The fermented soybean extracts were recovered by centrifugation using a centrifuge (Beckman model JE 25) at 12,000 xg at 4°C for 15 min. The resulting extract was filtered through a 0.45 µm membrane (Millipore Co., Bedford, Mass., U.S.A.) and then analyzed by HPLC. The HPLC analyses were carried out on Hewlett-Packard HP 1100 series equipped with autosampler, UV-visible detector (254 nm), and HP ChemStation Software. SupelcosilTMLC-18 (15 cm × 4.6 mm i.d., 5 µm) reversed-phase column (Supelco, USA) was run with a gradient solvent system initiated with 90% of solvent A (H_2O: methanol: acetic acid, 88: 10: 2, v/v) and 10% solvent B (methanol: acetic acid, 98: 2. v/v) to 100% solvent A in 20 min. The flow rate was set at 1.0 mL/min. The column temperature was controlled at 25°C.

Quantitative data for daidzin, daidzein, genistin, genistein, 8-hydroxydaidzein (8-OHD) and 8-hydroxygenistein (8-OHG) were obtained from comparison with known standards. Mass spectrometry was used to confirm the presence of substances in fermented soybean with the authentic standards. MS analysis was carried out on a Hewlett-Packard Model LC/MSD SL, USA. The capillary voltage of 4000V (positive) and 3500V (negative) was used with this analysis. The flow rate of N_2 was set at 13 L/min at 350°C on 50 psi of nebulizer pressure. The scan range of mass spectral was 100 to 500 m/z in API-ES mode.

Statistical analysis

All experiments were run in triplicate determinations. Analysis of variance (ANOVA) and mean comparison were performed by Duncan's multiple range test. Analysis was carried out using SPSS 11.0 for windows (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

The isoflavone content of fermented soybean was determined by HPLC method. The changes of daidzin, genistin, daidzein and genistein contents during fermentation were shown in Table 1 and Figure 1. After fermentation, total glucosides content of soybean fermented with *A. oryzae* BCC 3088 decreased, but the proportion of aglycones in total isoflavone markedly increased (Table 1 and Figure 1). As for the increase 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. From our previous report, *A. oryzae* BCC 3088 could produce β-glucosidase enzyme. These results are corresponded to Chia (2006); the fermented soybean incubated with filamentous fungi gave the highest amount of isoflavone aglycones content resulting in the highest antioxidative activities since β-glucosidase hydrolyzed daidzin and genistin and then released daidzein and genistein, the potent antioxidative substances, during fermentation.

Furthermore, the other isoflavone derivative, 8-hydroxygenistein (8-OHG) was found at the fourth day of fermentation (Figure 1) observed by HPLC analysis with Rt = 11.75 min. Conformation of this observation was supported by the mass spectral analysis. The similarities of mass fragmentation of mass spectral data compared with the authentic standard (Figure 2) allowed us to deduce this compound to be 8-OHG. According to Esaki (1999), the conversion of genistein into 8-OHG is presumed to have been responsible by the hydroxylase produced by *A. saitoi* fermentation during the stage of sporulation. Therefore, we could assume that *A.oryzae* BCC 3088 was able to produce hydroxylase enzyme during fermentation and then, hydroxylated the genistein into 8-OHG. In deliberation of the potent antioxidative substances, the formation of o-dihydroxyisoflavones especially 8-OHG are considered to be responsible for

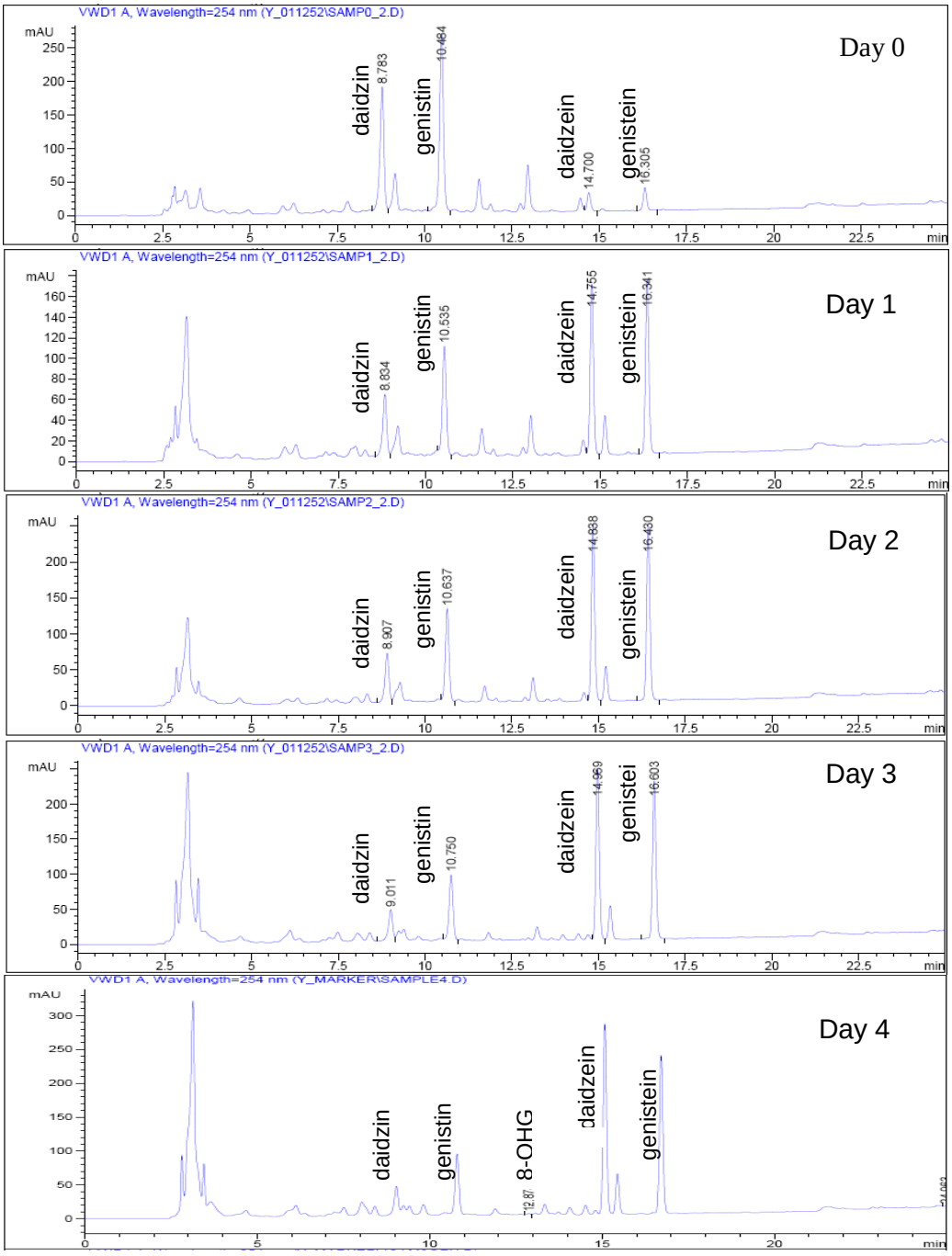

Figure 1. HPLC chromatograms of fermented soybean extract inoculated with *Aspergillus oryzae* BCC 3088 during fermentation.

the overall increased antioxidant activities. In our previous report, the antioxidative activity of fermented soybean markedly increased during fermentation. This should be explained by the formation of 8-OHG, the potent antioxidative substance. Esaki (1999) reported that the antioxidant activity of isoflavone substances in liposome system ranged from the lower to the higher activity as following: daidzein, genistein, 8-OHD and 8-OHG. Therefore, in this experiment, the conversion of isoflavone glucosides into aglycones and the formation of 8-OHG in soybean during fermentation are considered to be responsible for the overall increased antioxidant properties of our fermented soybean in our previous report. The formation process and mechanism of

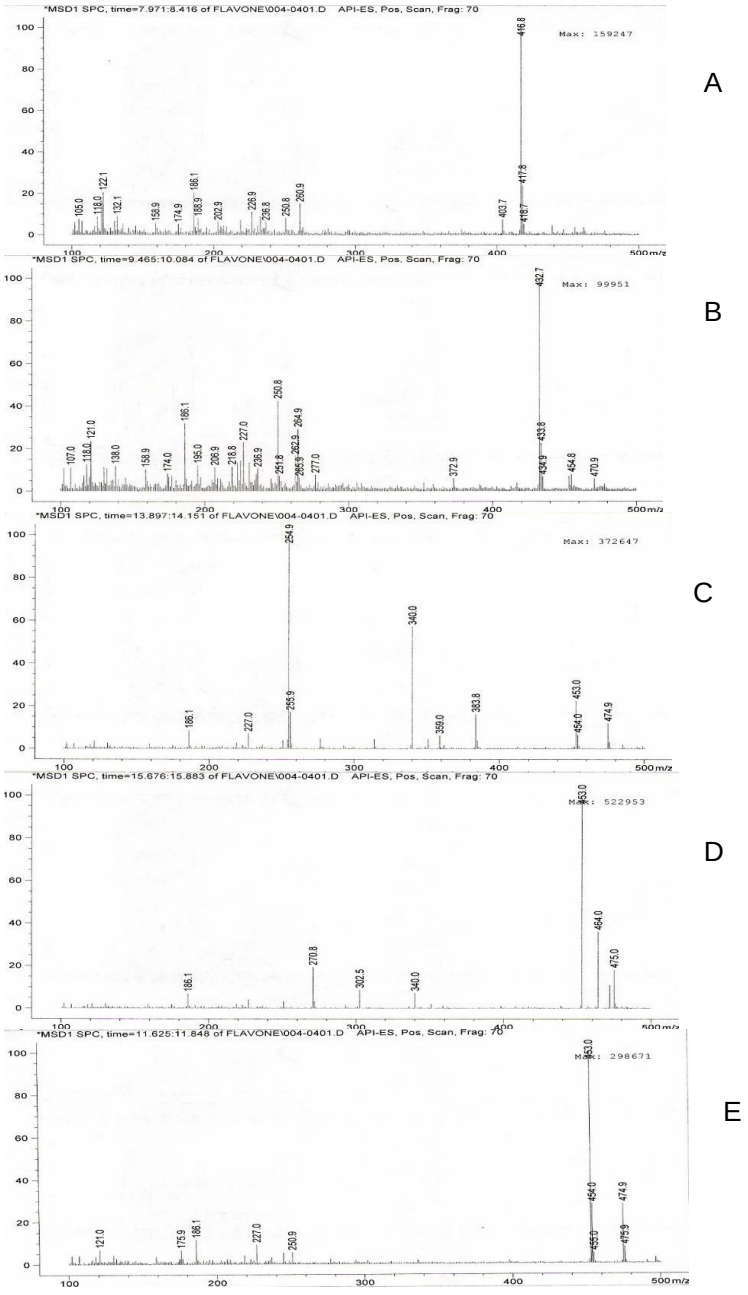

Figure 2. Mass spectral analysis of isoflavones at the fourth day fermentation of soybean fermented with *Aspergillus oryzae* BCC 3088. A: daidzin, B: genistin, C: daidzein, D: genistein, E: 8-OHG.

isoflavones and their derivative during soybean fermentation with *A. oryzae* BCC 3088 will be further investigated.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Pharmacy, Chiang Mai University, a grant from Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA) and supported by Graduate School, Chiang Mai University.

REFERENCES

Anderson JW, Johnstone BM, Cook-Newell ME (1995). Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. New Eng. J. Med., 333: 276-282.

Barbesgaard P, Heldt-Hansen HP, Diderichsen B (1992). On the safety of *Aspergillus oryzae*. A review. Appl. Microbiol. Biotechnol., 36: 569-572.

Cassidy A (1996). Physiological effects of phyto-oestrogens in relation to cancer and other human health risks. Proc. Nutr. Soc., 55: 399-417.

Chou ST, Chang CT, Chao WW, Chung YC (2002). Evaluation of antioxidative and mutagenic properties of 50% ethanolic extract from red bean fermented with *Aspergillus oryzae*. J. Agric. Food Chem., 65: 1463-1469.

Chia HL, Yi TT, Cheng CC (2006). Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. Food Microbiol., 23: 628-633.

Esaki H, Kawakishi S, Morimitsu Y, Osawa T (1999). New potent antioxidative 0-Dihydroxyisoflavones in fermented Japanese soybean products. Biosci. Biotechnol. Biochem., 63(9): 1637-1639.

Hayashi K, Suzuki K, Kawaguchi M, Nakajima T, Suzuki T, Murata M, Nakamura T (1995). Isolation of an antioxidant from *Penicillium roquefortii* IFO 5956. Biosci. Biotechnol. Biochem., 59: 319-320.

Lowri C, Michelle S, Marion K, Stephen B (1998). Chemical modification of isoflavones in soyfoods during cooking and processing. Am. Soc. Clin. Nutr., 68(suppl): 1486S-1491S.