Full Length Research Paper

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

Chiritchai Punjaisee1, Chaiyavat Chaiyasut1*, Sunee Chansakaow1, Somsak Tharata2, Wonnop Visessanguan3 and Suchart Punjaisee4

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

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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 popular 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.

MATERIALS 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...
oybean fermented with and without inoculation of A. oryzae BCC 3088, the cooked soybean

30 min. After cooling, the cooked soybean was inoculated with the spore suspension of A. oryzae BCC 3088 at the level of 1x10^⁶ 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, Hawai, 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 50 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. Supelcosil™ TMLC-18 (15 cm x 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 (H₂O: 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, daidzein and genistein contents in fermented soybean were obtained by 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 N₂ 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.

**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 isoflavones markedly increased (Table 1 and Figure 1). As for the increase of aglycone isoflavones 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-di-hydroxyisoflavones especially 8-OHG are considered to be responsible for

<table>
<thead>
<tr>
<th>Amount (ppm)</th>
<th>Soybean with no inoculation</th>
<th>Soybean inoculated with A. oryzae BCC 3088</th>
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<tbody>
<tr>
<td></td>
<td>Day0</td>
<td>Day4</td>
</tr>
<tr>
<td>Daidzin</td>
<td>249.8 ± 0.045&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>142.1 ± 0.032&lt;sup&gt;DC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genistin</td>
<td>248.1 ± 0.041&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>166.6 ± 0.044&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>Daidzein</td>
<td>14.0 ± 0.005&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>81.0 ± 0.001&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genistein</td>
<td>21.3 ± 0.003&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>40.3 ± 0.001&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>8-hydroxygenistein</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total glucosides</td>
<td>497.9 ± 0.087&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>308.7 ± 0.076&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total aglycone</td>
<td>35.3 ± 0.001&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>121.3 ± 0.003&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total isoflavone</td>
<td>533.2 ± 0.088&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>430.0 ± 0.079&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
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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.
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.

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REFERENCES


