Soybean Meal Fermented by *Aspergillus awamori* Increases the Cytochrome P-450 Content of the Liver Microsomes of Mice

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The effect of soybean meal fermented by *Aspergillus awamori* on the acute lethality of acetaldehyde, pentobarbital sleeping time, and cytochrome P-450 content of the hepatic microsomes was studied in mice. Most of the daidzin and genistin in soybean meal (SBM) were converted into the respective aglycones, daidzein and genistein, by fermentation. In experiment 1, mice were fed isonitrogenic test diets with one of the following five protein sources for 28 d: casein, SBM, fermented and hot-air-dried SBM (FSBM-HD), fermented and freeze-dried SBM (FSBM-FD), or methanol-extracted FSBM-FD (FSMB-FD-R). The acute lethality of acetaldehyde in mice fed the FSBM-FD diet was significantly lower than that in mice fed the SBM, FSBM-HD, or FSBM-FD-R diet. In experiments 2 and 3, mice were fed isonitrogenic test diets with one of the following four protein sources for 28 d: casein, SBM, FSBM-FD, and FSBM-FD-R. The pentobarbital sleeping time was significantly shorter and the cytochrome P-450 content was significantly higher in the mice fed the FSBM-FD diet than the respective value in mice fed the other test diets. In experiment 4, mice were fed one of eight diets which contained different levels of aglycone obtained by varying the proportion of FSBM-FD and FSBM-FD-R, for 28 d. The cytochrome P-450 content in hepatic microsomes increased as the dietary level of isoflavonoid aglycones increased, but there was a saturation phenomenon. These results suggest that soy isoflavonoid aglycones are more potent inducers of cytochrome P-450 than isoflavonoid glycosides.

Keywords: *Soybean; fermentation; isoflavonoids; P-450; mice*

Dietary constituents modulate the level of drug-metabolizing enzymes (Guengerich, 1984). Modulation of drug-metabolizing enzymes may be important in terms of human health since these enzymes can both activate and inactivate a wide range of xenobiotics. Drug-metabolizing enzymes have been divided into two groups: phase I enzymes, which include P-450s and MFOs and catalyze oxidative reactions, and phase II enzymes, which catalyze conjugative and/or other oxidative reactions.

Soybeans have been utilized for a long time as a nutritive food. There are many fermented products using soybeans, especially in Asian countries. These fermented soybean products include miso (fermented soybean paste), natto (Japanese fermented soybeans), tempe (traditional Indonesian fermented soybean), and others. Soybeans contain a variety of biologically active compounds, including isoflavonoids. Soybeans are a rich source of isoflavonoids. Fermented products such as miso and tempe contain a significantly higher amount of isoflavonoid aglycones than the respective glycosides. In these products, the 7-D-glucoside of isoflavonoids may be hydrolyzed by β-glucosidase produced by the bacteria responsible for the fermentation (Iitoi et al., 1992). Esaki et al. (1998), suggested that daidzin and genistin in soybeans are converted into the respective aglycones, daidzein and genistein, by β-glucosidase produced during *Aspergillus saitoi* fermentation. We found that *Aspergillus awamori*, which is utilized for manufacturing "awamori" (millet spirits) in the Okinawa area in Japan, converted daidzin and genistin in soybeans into the corresponding aglycones, daidzein and genistein. Isoflavonoid glycosides such as genistin and daidzin are metabolized by intestinal microflora and converted to their corresponding aglycones before absorption (Manach et al., 1996, Yasuda et al., 1996). The isoflavonoid aglycones, genistein and daidzein, are absorbed directly in the intestines (Slavin et al., 1998). It appears that the aglycone forms are more bioavailable than the glycoside forms (Hutchins et al., 1995). Sariaslani and Kunz (1986) found that genistein-induced cytochrome P-450 in *Streptomyces griseus*. However, information on the effect of isoflavonoids on drug-metabolizing enzymes is limited.

In this study, the effect of unfermented or fermented soybeans on the acute toxicity of acetaldehyde, pentobarbital-induced sleeping time, and induction of hepatic cytochrome P-450 was assessed in mice.

MATERIALS AND METHODS

Preparation of Fermented Soybean Meal. Fermented soybean meals (FSBM) were prepared from commercially available, defatted soy bean meals (SBM, Nikko Seiyu Co. Ltd., Tokyo, Japan). SBM were steamed at 100 °C for 90 min. Aliquots (100 g) of the steamed soybean meals were cooled and mixed with 0.1 g of rice flour and then were inoculated with *A. awamori* (Hishiroku Co., Kyoto, Japan). The rate of inoculation was 8×10^7 spores per g of steamed soybean meal. Incubation was then carried out at 33−38 °C for 48 h. Following the first incubation, water was added to bring the product to 50% content, and this was further incubated at 45 °C for 41 h. The resulting fermented soybean meals were dried

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Table 1. Composition (g/kg) of Test Diets (Experiments 1−3)[a]

component	group				
	casein[b] diet	SBM[c] diet	FSBM-HD[d] diet	FSBM-FD[e] diet	FSBM-FD-R[f] diet
casein	200	−	−	−	−
SBM	−	334	−	−	−
FSBM-HD	−	−	334	−	−
FSBM-FD	−	−	−	329	−
FSBM-FD-R	−	−	−	−	382
mineral mixture[g]	35	35	35	35	35
vitamin mixture[g]	10	10	10	10	10
corn oil	50	50	50	50	50
sucrose	200	200	200	200	200
corn starch	505	371	371	376	323

[a] The SBM, FSBM-HD, FSBM-FD, and FSBM-FD-R diets were isonitrogenous with the casein (200 g casein/kg) diet. SBM, FSBM-HD, FSBM-FD, and FSBM-FD-R contained, respectively, 85.3, 85.4, 86.7, and 74.7 g nitrogen/kg. [b] Purchased from New Zealand Dairy Board, Willington, New Zealand. Casein contained 142.2 g nitrogen/kg. [c] SBM, soy bean meal. [d] FSBM-HD, fermented and hot air-dried soy bean meal. [e] FSBM-FD, fermented and freeze-dried soy bean meal. [f] FSBM-FD-R, FSBM-FD extracted with 800 mL methanol/L. [g] Based on AIN-76 (1977). Vitamin mixture used here contained 200 g choline bitartrate/kg.

by hot air (FSBM-HD) or freeze-dried (FSBM-FD). The SBM, FSBM-HD, and FSBM-FD were ground to a powder with a hammer mill (40-mesh, Model 2, Sogo Sangyo, Tokyo, Japan). To make FSBM-FD-R, FSBM-FD was refluxed for 1 h with 800 mL methanol/L (5 mL/g flour) to extract isoflavonoids and then filtered through a filter (no. 2; Advantic Toyo, Tokyo, Japan). The residues were soaked in absolute ethanol, filtered and then freeze-dried (FSBM-FD-R). Both processes were repeated two times.

Determination of Isoflavonoid Content. The amount of daidzin, genistin, daidzein, and genistein in SBM, FSBM-HD, FSBM-FD, and FSBM-FD-R were measured by a HPLC according to the method of Kitada et al. (1986). Briefly, 2 g of SBM, FSBM-HD, FSBM-FD, or FSBM-FD-R was refluxed for 1 h with 800 mL methanol/L (10 mL/g flour) in a boiling water bath. The extract was centrifuged for 20 min at 1500*g* and 5 °C. The supernatant was obtained and evaporated to dryness using a rotary evaporator (Model RE52B, Yamato, Tokyo, Japan). After evaporation, the dried extract was dissolved in 5 mL of 800 mL methanol/L. An aliquot was filtered through a membrane filter (cellulose acetate, pore size 0.45 mm; DISMIC-13cp; Toyo Roshi, Tokyo, Japan) before analysis by HPLC (Model 655, Hitachi, Tokyo, Japan). Separation of the isoflavonoids was performed on a reverse-phase column (Li-Chrosord RP-18, 5 mm, 25 cm long × 4.6 mm i.d.; Merck Co., Inc., Whitehouse Station, NJ) using a mixture of 2.5% acetic acid:acetonitrile (5:2, v/v). The flow rate was 1 mL/min. Daidzin, genistin, daidzein, and genistein were monitored by UV absorbance at 250 nm with the use of a spectrophotometer (Model L-7400, Hitachi, Tokyo, Japan).

Animals and Diets. This study was approved by the Laboratory Animal Care Committee of Ehime University. The mice were maintained in accordance with the guidelines for the care and use of laboratory animals of Ehime University.

Male std:ddy mice aged 4 weeks (Japan SLC, Hamamatsu, Japan) with an initial weight of approximately 20 g were used in the experiments. The mice were housed in cages (three or four mice/cage) and were kept at 23 ± 1 °C with a 12-h light: dark cycle (light, 0700−1900 h). The mice were acclimated by feeding a commercial solid diet (MF, Oriental Yeast Co., Osaka, Japan) for 7 d. Then, the mice were divided into groups and allowed free access to the test diet and water. Each test diet contained the same level of protein.

Experiment 1: This experiment involved five groups of 20 mice each. The mice were fed one of the following five test diets for 28 d: casein, SBM, FSBM-HD, FSBM-FD, or FSBM-FD-R diet. The composition of each test diet is shown in Table 1. On d 28, acetaldehyde (11 mmol per 100 g of body weight; >99%, Merck Co., Inc., Whitehouse Station, NJ) was injected into the peritoneal cavity at 1000 h. The number of surviving animals 24 h after injection of acetaldehyde was counted.

Table 2. Composition (g/kg) of Test Diets (Experiment 4)

component	dietary group							
	I	II	III	IV	V	VI	VII	VIII
FSBM-FD[a]	−	47	94	141	188	235	282	329
FSBM-FD-R[b]	378	324	270	216	162	108	54	−
mineral mixture[c]	35	35	35	35	35	35	35	35
vitamin mixture[c]	10	10	10	10	10	10	10	10
corn oil	50	50	50	50	50	50	50	50
sucrose	200	200	200	200	200	200	200	200
corn starch	327	334	341	348	355	362	369	376
aglycone[d] (mg/kg)	33	101	168	235	302	370	437	505
protein (g/kg)	177	177	177	177	178	178	178	178

[a,b] See notes *e* and *f* to Table 1. [c] Based on AIN-76 (1977). Vitamin mixture used here contained 200 g of choline bitartrate/kg. [d] Sum of daidzein and genistein.

Experiment 2: This experiment involved four groups of nine mice each. The mice were fed one of the following four test diets for 28 d: casein, SBM, FSBM-FD, or FSBM-FD-R diet. The composition of each test diet is shown in Table 1. On d 28, a pentobarbital sodium solution (7.5 mg per 100 g of body weight; 50 mg/mL, Nembutal, Abbot Laboratories, North Chicago, IL) was injected intraperitoneally at 1000 h, and the length of sleeping time was measured. The pentobarbital sodium solution was diluted to 15 mg/mL with distilled water before injection.

Experiment 3: This experiment involved four groups of eight mice each. The mice were fed one of the following four test diets for 28 d: casein, SBM, FSBM-FD, or FSBM-FD-R diet. The composition of each test diet is shown in Table 1. On d 28, mice were killed by decapitation at 1000 h within a 30-min period. The liver was removed and homogenized with the ice-cold buffer described below. The homogenates were used for measurement of the amount of cytochrome P-450.

Experiment 4: This experiment involved eight groups of eight mice each. Mice were fed one of eight test diets which contained eight different levels of aglycone obtained by varying the proportion of FSBM-FD and FSBM-FD-R, for 28 d. The eight test diets were similar except for the source of protein, and the amount of corn starch and aglycone (Table 2). The protein source was FSBM-FD and/or FSBM-FD-R. On d 28, the mice were sacrificed by decapitation at 1000 h within a 30-min period. The liver was removed and homogenized with the ice-cold buffer described below. The homogenates were used for measurement of the amount of cytochrome P-450 in the hepatic microsomes.

Measurement of Hepatic Cytochrome P-450. Fresh liver was homogenized in 6 vol of 0.1 M Tris/HCl buffer (pH 7.4) containing KCl (7.4 g/L) and EDTA (0.372 g/L) with a Potter-Elvehjem homogenizer (Asahi Techno Glass Co., Tokyo, Japan). The homogenate was centrifuged for 15 min (10000*g*) at 4 °C. The postmitochondrial supernatant was centrifuged for 90 min (105000*g*) at 4 °C. The microsomal pellets were suspended in 0.1 M Tris/HCl buffer (pH 7.4) containing glycerin (200 mL/L) and EDTA (0.372 g/L). Using this suspension, total cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato (1964). The amount of microsomal protein was determined according to the method of Lowry and Rosebrough (1951), with bovine serum albumin as the standard.

Statistical Analysis. For experiment 1, the statistical difference between groups was determined by χ-square analysis with Yates correction (Breillout et al., 1987). The results of the remaining experiments were tested for statistical significance by ANOVA and Duncan's multiple range test (Shibata, 1974). Differences were considered to be significant at $p < 0.05$. The relationship between the cytochrome P-450 content of hepatic microsomes and the dietary level of isoflavonoid aglycones was analyzed by nonlinear regression using CA-Cricket Graph III (Computer Associates International Inc., Islandia, NY).

Table 3. Isoflavonoid and Protein Content (mg/kg) of Soy Bean Meal (SBM), Fermented and Hot Air-Dried Soy Bean Meal (FSBM-HD), Fermented and Freeze-Dried Soy Bean Meal (FSBM-FD), and Residual FSBM-FD Extracted with 800 mL Methanol/L (FSBM-FD-R)

	SBM[a]	FSBM-HD[b]	FSBM-FD[c]	FSBM-FD-R[d]
daidzin	792	41	33	nd
genistin	1104	112	113	8
daidzein	80	552	632	52
genistein	96	615	704	49
total[e]	2072	1320	1482	109
aglycone/total (%)	8.5	88.4	90.1	92.3
protein[f] (g/kg)	533	534	542	467

[a−d] See notes *c*−*f* to Table 1. [e] Sum of daidzin, genistin, daidzein, and genistein. [f] Protein content was determined by the Kjeldahl method (Miller and Houghton, 1945) using a N-to-protein conversion factor of 6.25.

RESULTS

Isoflavonoid Content of SBM, FSBM-HD, FSBM-FD, and FSBM-FD-R. The isoflavonoid content of SBM, FSBM-HD, FSBM-FD, and FSBM-FD-R is shown in Table 3. SBM contained a higher amount of isoflavonoid glycosides than the respective aglycones. However, FSBM-HD and FSBM-FD contained a larger amount of isoflavonoid aglycones than the respective glycosides. The weight percentage of aglycone to total isoflavonoid in SBM, FSBM-HD, and FSBM-FD was 8.5, 88.4, and 90.1%, respectively. The isoflavonoid content of FSBM-HD and FSBM-FD was approximately 40% and 30% less, respectively, than that of SBM. The isoflavonoid content of FSBM-FD-R was only 7% of that of FSBM-FD. The protein content of FSBM-FD-R was less than that in SBM, FSBM-HD, and FSBM-FD.

Experiment 1: The results of the acute lethality of acetaldehyde are shown in Table 4. The acute lethality of acetaldehyde was significantly lower in mice fed the FSBM-FD diet than in mice fed the SBM, FSBM-HD, and FSBM-FD-R diets. However, the acute lethality of acetaldehyde did not differ significantly between the mice fed the casein diet and mice fed the FSBM-FD diet. The FSBM-HD diet caused diarrhea. The body mass gain in mice fed the FSBM-HD diet was significantly lower than that in mice fed the casein diet, but did not significantly differ from those fed the SBM, FSBM-FD, and FSBM-FD-R diets.

Experiment 2: The results of pentobarbital-induced sleeping time are shown in Table 5. The pentobarbital-induced sleeping time in mice fed the FSBM-FD diet was significantly shorter than that in mice fed the other test diets. However, the liver weight of the four groups of mice did not differ significantly.

Experiment 3: The results are shown in Table 6. The liver weight and microsomal protein content in the liver did not differ significantly among the four groups of mice. The cytochrome P-450 content of the hepatic microsomes of mice fed the SBM and FSBM-FD diets was significantly higher than that of mice fed the casein or FSBM-FD-R diet. On the other hand, the cytochrome P-450 content in the liver of mice fed the FSBM-FD diet was significantly higher than that of mice fed the SBM diet. When the relationship between cytochrome P-450 content and dietary level of isoflavonoid aglycones was calculated, the regression equation was found to be

$$Y = 0.078 + 0.350 \log X \quad (r^2 = 0.991) \qquad (1)$$

where Y represents the cytochrome P-450 content and X represents the level of isoflavonoid aglycones (mg/kg) in each test diet.

Experiment 4: The curve in Figure 1 shows the relationship between cytochrome P-450 content and dietary level of isoflavonoid aglycones. The equation of this "dose−response" curve was found to be

$$Y = 0.050 + 0.279 \log X \quad (r^2 = 0.912) \qquad (2)$$

where Y represents the cytochrome P-450 content and X represents the level of isoflavonoid aglycones (mg/kg) in each test diet. Figure 1 indicates a saturation phenomenon in hepatic cytochrome P-450 content with increasing dietary level of isoflavonoid aglycones.

DISCUSSION

Isoflavonoid glycosides are hydrolyzed to their aglycones by the action of β-glucosidase produced from *Rhizopus oligosporus*, which is used for the production of tempe (Ebata et al., 1972), and from *A. saitoi*, which is used for the production of "awamori" (millet spirits) (Esaki et al., 1998). In fermented soybean products such as miso, approximately 90% of the isoflavonoids are present in the unconjugated form (Coward et al., 1993). In this study, the level of isoflavonoid aglycones in FSBM-HD and FSBM-FD was significantly higher than that in SBM, which suggests that isoflavonoid glycosides were hydrolyzed to unconjugated aglycones during fermentation. On the other hand, the total content of isoflavonoid in FSBM-HD and FSBM-FD was approximately 60% and 70%, respectively, of that in SBM, which suggests that the fermentation process itself resulted in degradation of isoflavonoids, in addition to hydrolysis of the glycosides. Hutchins et al. (1995) showed that fermentation of soy decreases the isoflavonoid content of the product. Also, the C-ring of flavonoids, and most likely also that of isoflavonoids, is cleaved by human intestinal bacteria (Xu et al., 1994).

The protein content of FSBM-FD-R was less than that of FSBM-HD and FSBM-FD (Table 3). Fermentation increases the soluble nitrogen content of foods (Nout and Rombouts 1990). Therefore, the lower protein content in FSBM-FD-R compared with that in FSBM-HD and FSBM-FD may have resulted from a loss of soluble nitrogen compounds during refluxing with hot 800 mL methanol/L for extracting isoflavonoids.

It has been thought that the oxidation of acetaldehyde to acetic acid is catalyzed by acetaldehyde dehydrogenase located in the mitochondria or cytosol of cells in the liver (Koivula and Koivusalo, 1975; Tottmar et al., 1973; Tsutsumi et al., 1988). However, it was recently demonstrated that hepatic microsomal aldehyde oxygenase catalyzes the oxidation of an aldehyde to its corresponding carboxylic acid and that this involves cytochrome P-450 (Watanabe et al., 1991; Telelius et al., 1991). Also, cytochrome P-450 (CYP)2E1 is an essential enzyme in the microsomal acetaldehyde-oxidizing system (MAOS) (Kunitoh et al., 1996; Kishimoto et al., 1997). Drug metabolism, particularly phase I oxidation, mainly involves microsomal cytochrome P-450. Information on the effect of isoflavonoid on drug-metabolizing enzymes is limited. However, Sariaslani and Kunz (1986) reported that soy flour and genistein induce cytochrome P-450 in *S. griseus*. Iitoi et al. (1992) have shown that various kinds of miso (soybean paste) tend to increase the level of cytochrome P-450 activity in rats. The isoflavonoid content of FSBM-FD-R was only 7%

Table 4. Acute Lethality of Acetaldehyde in Male std:ddy Mice after 28 d of Consuming the Casein, SBM, FSBM-HD, FSBM-FD, or FSBM-FD-R Diet[a] (Experiment 1)

	group				
	casein[b] diet	SBM[c] diet	FSBM-HD[d] diet	FSBM-FD[e] diet	FSBM-FD-R[f] diet
body weight gain (g/28 d)	25[h] (1)	22[g,h] (1)	20[g] (1)	23[g,h] (1)	23[g,h] (1)
no. of survivors/no. of mice tested	12/20	11/20*	9/20*	18/20	10/20*

[a] Values are the mean ($n = 20$), with SE in parentheses. Values not sharing a common letter were significantly different ($p < 0.05$). * $p < 0.05$ when compared to group FSBM-FD. [b] See note *b* to Table 1. [c–f] See notes *c*–*f* to Table 1.

Table 5. Pentobarbital Sleeping Time of Male std:ddy Mice after 28 d of Consuming the Casein, SBM, FSBM-FD, or FSBM-FD-R Diet[a] (Experiment 2)

	group			
	casein[b] diet	SBM[c] diet	FSBM-FD[d] diet	FSBM-FD-R[e] diet
sleeping time (min)	214** (31)	211** (38)	89* (9)	165** (14)
liver weight (g)	1.93 (0.10)	2.03 (0.08)	2.12 (0.11)	1.95 (0.09)

[a] Values are the mean ($n = 9$), with SE in parentheses. Values not sharing a common number of asterisks were significantly different ($p < 0.05$). [b] See note *b* to Table 1. [c–e] See notes *c*, *e*, and *f* to Table 1.

Table 6. Cytochrome P-450 Content of the Hepatic Microsomes of Male std:Ddy Mice after 28 d of Consuming the Casein, SBM, FSBM-FD or FSBM-FD-R Diet[a] (Experiment 3)

			cytochrome P450	
group	liver weight, g	microsomal protein, mg·g^{-1} liver	nmol·mg^{-1} protein	nmol·g^{-1} liver
casein[b] diet	2.07 (0.08)	23.0 (1.0)	0.42* (0.04)	9.7* (1.2)
SBM[c] diet	1.98 (0.08)	26.1 (2.1)	0.65** (0.03)	17.1** (1.8)
FSBM-FD[d] diet	1.99 (0.11)	25.2 (1.1)	0.92*** (0.05)	23.4*** (2.1)
FSBM-FD-R[e] diet	1.94 (0.09)	24.0 (1.7)	0.51*** (0.05)	12.3* (1.4)

[a] Values are the mean ($n = 8$), with SE in parentheses. Values not sharing a common number of asterisks were significantly different ($p < 0.05$). [b] See note *b* to Table 1. [c–e] See note *c*, *e*, and *f* to Table 1.

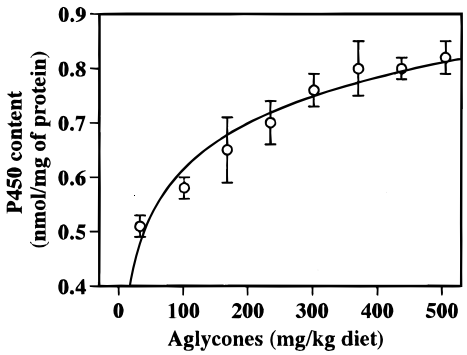

Figure 1. Relationship between the cytochrome P450 content of the hepatic microsomes and the dietary level of isoflavonoid aglycones. Mice were fed one of eight tests diets which contained different levels of aglycone obtained by varying the proportion of FSBM-FD and FSBM-FD-R, for 28 d. Values are means ± SEM, $n = 8$. The relationship can be described as $y = 0.050 + 0.279 \log x$ ($r^2 = 0.912$).

of that of FSBM-FD. The quantity of cytochrome P-450 in the liver of mice fed the FSBM-FD-R diet was significantly lower than that in mice fed the FSBM-FD diet ($p < 0.05$). Therefore, the higher acute lethality of acetaldehyde and the longer pentobarbital sleeping time in mice fed the FSMB-FD-R diet compared with mice fed the FSBM-FD diet might be caused by the lower induction of cytochrome P-450.

Soy isoflavonoid aglycones are absorbed directly in the intestines (Slavin et al., 1998). However, isoflavonoid glycosides are metabolized by intestinal microflora and converted to their corresponding aglycones before absorption (Manach et al., 1996; Yasuda et al., 1996). Therefore, it appears that aglycone forms are more easily absorbed than glycoside forms (Hutchins et al., 1995). Sariaslani and Kunz (1986) reported that feeding genistein induces a larger and more rapid cytochrome P-450 induction than feeding soy flour. The amount of cytochrome P-450 in the liver of mice fed the SBM diet was significantly lower than that of mice fed the FSBM-FD diet. The level of cytochrome P-450 in the liver may depend on the amount of isoflavonoids absorbed. The low level of cytochrome P-450 in the liver of mice fed the SBM diet may have been due to the very low level of dietary aglycones and may have led to the higher acute lethality of acetaldehyde and the longer pentobarbital sleeping time than in mice fed the FSBM-FD diet.

The acute lethality of acetaldehyde in mice fed the FSBM-HD diet was significantly higher than that in mice fed the FSBM-FD diet. However, the isoflavonoid content, aglycone/total ratio, and protein content of FSBM-HD and FSBM-FD were nearly the same. The color of FSBM-FD was light brown, but that of FSBM-HD was dark brown. FSBM-HD caused diarrhea, but FSBM-FD did not. Rats fed 5% and 10% Maillard reaction products developed severe diarrhea, and the body weight of animals fed 10% Maillard reaction products was significantly lower than that of rats fed a control isocaloric diet (O'Brien and Walker, 1988). FSBM-HD was dried by hot air, which can lead to the formation and development of Maillard reaction products. The food protein value can be adversely affected by these reactions, and, in particular, lysine may be converted to nonbioavailable N-substituted lysine or blocked lysine. The body mass gain in mice fed the FSBM-HD diet was lower than that in mice fed the FSBM-FD diet. The lower body mass gain would have been caused by lower food intake and/or lower digestibility of proteins and/or energy sources. We did not measure food intake. In rodents, caloric restriction decreased the induction of cytochrome P-450 (Hart et al., 1996). In mice that were fed diets with varying content of proteins, the reduction in hepatic total cytochrome P-450 was correlated with the reduction in

dietary protein content (Czygan et al., 1974; Adekunle et al., 1977). Thus, the level of cytochrome P-450 in mice fed the FSBM-HD diet may have been lower than that in mice fed the FSBM-FD diet, although we did not measure the amount of cytochrome P-450 in the liver of mice fed the FSBM-HD diet. These may explain the higher acute lethality of acetaldehyde in mice fed the FSBM-HD diet than in mice fed the FSBM-FD diet.

As shown in Figure 1, the inductive effect of isoflavonoid aglycones on cytochrome P-450 was found to be dose-dependent and showed a saturation phenomenon. On the other hand, Helsby et al. (1997) reported that intraperitoneal administration of each of the isoflavonoids equol and genistein at 0.440 mg/kg per d for 4 d did not induce the expression of any of the cytochrome P-450 isomers, CYP1A2, CYP2E1, or CYP 3A1 in mice. Although we did not measure food intake, it seems that the difference between our results and their results is due to a difference in the quantity of isoflavonoid intake.

In summary, most of the daidzin and genistin in SBM were converted into their respective aglycones, daidzein and genistein, upon *A. awamori* fermentation. The acute lethality of acetaldehyde was significantly reduced, and the pentobarbital sleeping time was significantly shorter in mice fed FSBM-FD than in mice fed SBM. This study also suggest that soy isoflavonoid aglycones may be potent inducers of cytochrome P-450. However, soybean contains various chemical compounds other than isoflavonoids. Therefore, chemical compounds other than isoflavonoids may involve in the increase of cytochrome P-450 content of the liver microsomes. It is necessary to investigate whether genistein or daidzein itself increases or not the cytochrome P-450 content of the liver microsomes.

ABBREVIATIONS USED

FSBM-FD, fermented and freeze-dried SBM; FSBM-FD-R, FSBM-FD extracted with 800 mL methanol/L; FSBM-HD, fermented and hot air-dried SBM; SBM, defatted soy bean meal.

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