Bioactive compounds and antioxidant activities of sprout soybean fermented with *Irpex lacteus* mycelia

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Received: 3 April 2017 / Revised: 8 August 2017 / Accepted: 29 August 2017 / Published online: 1 December 2017
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Abstract To enhance the biological activities of sprout soybean, beans were treated with steaming (SS), germinating (GS), or roasting (RS) prior to fermentation with *Irpex lacteus* mycelia for 20 days. The total phenolic, flavonoid, isoflavone, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of each fermented bean preparation were examined every 5 days for 20 days. The total phenolic content of SS, GS, and RS preparations was 9.61, 10.23, and 10.46 mg/g, respectively, after 15 days of fermentation. These concentrations were approximately 4–5 folds higher compared to initial levels. The total flavonoid content was 8–9 folds higher than initial levels. The isoflavone content was highest in the RS sample (6.84 mg/g). The DPPH radical scavenging activity of beans fermented with *I. lacteus* mycelia was increased 2–8 folds after 20 days of fermentation. These results indicate that antioxidant activity components were increased by fermentation of *I. lacteus* mycelia irrespective of soybean treatments.

Keywords *Irpex lacteus* mycelia · Sprout soybean · Germinating · Roasting · Isoflavone

Introduction

Soybeans (*Glycine max* L.) contain bioactive components such as high-quality proteins, fats, fibers, oligosaccharides, linolenic acid, saponin, lecithin, and isoflavones [1]. These bioactive components can prevent stroke and heart disease as well as various life style diseases [2–4]. Isoflavones are a main bioactive component of soybeans and have similar effects to estrogen which is effective in preventing osteoporosis and relieving menopausal symptoms. Soybeans have also been reported to help prevent prostate cancer in men as well as breast and uterine cancer in women [5].

Sprout soybean, a microcarpa [6], contains more isoflavones than other beans [7, 8]. Isoflavones exist in two forms, glycosides and aglycones. The absorption rates and bioactivity of isoflavones are higher in aglycones than glycosides. Therefore, it is important to convert isoflavones to forms with higher bioavailability [9]. Accordingly, many studies have aimed to convert isoflavones to their aglycone forms and to increase bioactive isoflavone content [10, 11]. The approaches used in these studies have included fermenting soybeans with *Cheonggukjang* or lactic acid bacteria after germinating or roasting [12, 13]. More recently, microorganism fermentation, heating, enzymatic reactions, salting, and germination processes have been applied to soybeans to increase their aglycone content [14, 15].

Mushroom mycelia have been studied for their potential food applications because they are less toxic and contain more bioactive components than their fruiting bodies [16]. For example, mushroom basidiomycetes are used to generate different fermented food such as bread, cheese, and alcoholic drinks [17, 18]. Moreover, liquid incubation using mushroom mycelia is actively studied as a method for producing pharmaceutical active components [19, 20]. In another approach, applications of fluid mushroom

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basidiomycetes on solid cultures of plants such as ginseng, Siberian ginseng, and rice were also studied to improve their effectiveness of these cultures [21, 22]. *Irpex lacteus*, a white-rot fungus [23] that usually grows on oak trees, has an extensive biological degradation capacity. Thus, this fungus can be used in environmental purification applications such as dye decolorization, breakdown of explosives, and decomposition of bisphenol A, endocrine disrupters and industrial waste [24]. *I. lacteus* has also been reported to show antibacterial and antifungal activity [23] and to contain nematicidal chemicals such as 5-(pentyl)-2-furalaldehyde, 5-(4-pentyl)-2-furalaldehyde and methyl-3- ρ -anisoxypionide, as well as other enzymes of interest such as carboxymethylcellulase, chitinase, cellulase, semicellulase, and acidic protease [24]. Kobayashi et al. [25] isolated a proteinase from *I. lacteus* and used it as a rennet substitute to produce Gouda cheese [26].

In this study, to enhance the antioxidant activities of sprout soybean, soybeans were first pretreated by steaming, germinating, or roasting and then fermented with *I. lacteus* mycelia. Finally, their bioactive content was analyzed.

Materials and method

Sample collection and preparation

Sprout soybeans, which were harvested near Jeonju, Korea, 2014, were provided by the Ongoul-farm Cooperative (Jeonju, Korea). Mycelia of the *I. lacteus* KCTC 26201 strain were transfer-cultured successively on potato dextrose agar (PDA, Difco, Sparks, MD, USA) at 25 °C for 5 days and stored in a refrigerator at 4 °C for further use. Sprout soybeans were dried in a heater controlled at 50 °C for 48 h, ground to powder with a grinder (HR 2860, Philips Type, Hong Kong, China), and stored at 4 °C.

Analysis of proximate sprout soybean components

Proximate sprout soybean components were analyzed by AOAC [27]. Moisture content was measured via the high pressure-heat drying method, ash content via the dry ashing method using a Muffle's furnace at 550 °C, crude protein via the Kjeldahl nitrogen determination method, and crude fat via Soxhlet method using diethyl ether.

Preparation of starter cultures

Soybean broth (5% = 5 g in 100 mL distilled water) was sterilized at 121 °C for 15 min. Activated mushroom mycelia were inoculated on PDA (potato dextrose agar) five or six times with a cork borer (5 mm) and cultured at 25 °C with shaking at 120 rpm for 5 days. To culture

liquid spawn, 10 mL of the culture fluid was inoculated onto soybean liquid medium and cultured at 25 °C with shaking at 120 rpm for 5 days.

Preparation of steaming beans, germinated beans, and roasted beans

Sprout soybeans were immersed in water for 10 h after washing with tap water. Excess water was drained and removed with a sifter for 1 h (moisture content 54.27–57.50%). The moist beans were steamed after sterilizing in an autoclave at 121 °C for 15 min [28]. To prepare germinated sprout soybeans, the beans were washed with tap water and watering every 3 h (moisture content, 61.28–61.79%). The germinated beans were used for media after sterilizing [10]. To prepare roasted sprout soybeans, the beans were roasted on an Electric Kettle (Dahwa Precision, Seongnam, Korea) at 120 °C for 30 min (moisture content, 4.10–4.25%) after washing, immersing, and removal of excess water. Roasted beans were used for media after sterilizing [11, 12].

Cultivation of mushroom mycelia

To culture mushroom mycelia, an aliquot of liquid spawn (10%, v/w) was inoculated onto the steamed, germinated, or roasted beans. Next, the mushroom mycelia cultured on each bean type were sampled, freeze-dried, pulverized with a grinder (HR 2860, Philips Type, Hong Kong, China), and passed through a sieve with 425- μ m mesh (Laboratory Test Sieve, Endecotts Ltd., London, UK). The resultant samples were used in subsequent assays.

Preparation of extracts

The mushroom mycelia powder (1 g) was resuspended in 20 mL of methanol (50%) and extracted in a shaking incubator (37 °C, 150 rpm) for 5 h [28]. The extract was analyzed for total phenolic content, total flavonoid content, isoflavone content, and DPPH radical scavenging ability.

Qualification and quantification of phenols, flavonoids, and isoflavones

Total phenolic compounds were measured using the modified Folin–Ciocalteu method. The absorbance of gallic acid, the assay standard, was measured at 765 nm using a spectrometer (UV-1601, Shimadzu, Kyoto, Japan) [29]. Total flavonoid contents were measured by the method described by the Korea Health Supplements Association [30]. Briefly, the absorbance of the liquid layer was measured at 415 nm using a spectrometer (UV-1601). Total flavonoid content (mg/g) was analyzed based on the

quercetin calibration curve. Isoflavone content was analyzed by high performance liquid chromatography (Waters 2695, alliance, Milford, MA, USA) with a YMC-Triart C18 column (4.6 × 250 mm, 5 µm, Kyoto, Japan) [31]. For quantitative analysis, the absorbance of each sample at 254 nm was measured by a photodiode array detector (WatersTM 996, Milford, MA, USA).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

DPPH radical scavenging ability was measured by the modified Blois method [32]. Briefly, 0.5 mL of extract was added to 0.2 mM DPPH (2 mL) and mixed by vortexing, after which the absorbance of the solution was measured at 517 nm. The DPPH radical scavenging ability was calculated according to the equation below. All experiments were conducted three times; data are presented as average. The radical scavenging ability of vitamin C (0.1 mM) was measured as a control group.

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \left(\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right) \right] \times 100$$

Statistical analysis procedure

All of the results were analyzed statistically by ANOVA. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, ver 12.0. SPSS Inc., Chicago, IL, USA). Significant differences were determined by Duncan's multiple range test ($P < 0.05$).

Results and discussion

Proximate components of sprout soybeans

Sprout soybeans contained moisture (10.16–14.09%), ash (4.5–5.3%), crude protein (35.3–43.1%), crude fat (13.5–16.5%), and carbohydrates (15.5–20.2%). The average weight of 100 sprout soybean grains used for this study was 6 g, classifying the soybeans as extreme-microcarpa.

Total phenolic and flavonoid content

Polyphenolic compounds, which are secondary metabolites from plants, have been reported to have varied physiological activity in the human body. Therefore, efforts have been made to extract antioxidants from plants [33]. Mushroom mycelia (10%) were inoculated onto processed beans (steamed, germinated, or roasted) and cultures, after

which the total phenolic and flavonoids content of the beans was analyzed at 5-day intervals for 20 days (Figs. 1, 2).

Nonprocessed sprout soybeans comprised approximately 3 mg/g phenolic compounds; this content was not significantly different from the content of any processed bean type after freeze-drying ($P < 0.05$). After 15 days of fermentation, however, the total phenolic content increased from 2.71 to 9.61 mg/g in the steamed beans, 3.83 to 10.23 mg/g in the germinated beans, and 3.24 to 10.46 mg/g in the roasted beans. Thus, all processed beans showed approximately 4–5 times higher total phenolic content than their unprocessed counterparts. The phenolic content of steamed beans continued to increase until 20 days of fermentation. The phenolic content of steamed and roasted beans increased gradually until 15 days, after which the phenolic content of steamed beans decreased. These results are consistent with those of Joung et al. [21], who investigated the antioxidant activity of ginseng cultured with various types of mushroom mycelia (e.g., *Phellinus linteus*, *Ganoderma lucidum* and *Hericium erinaceum*). Joung et al. [21] showed that the phenolic content in ginseng cultured with mushroom mycelia were higher than those in raw ginseng. Our results are also similar to those of Shon [28]. In the study by Shon, beans were fermented with *Tricholoma matsutake* (pine mushroom) mycelia and examined for changes in bioactive content. Shon found that the bioactive activity was greatest after 9 days of fermentation; moreover, the bioactive content differed depending on the type of mycelia and culture medium. One explanation for this difference is that new phenolic compounds are produced due to the degradation of macromolecular compounds into low molecular weight phenolic compounds during fermentation; different mushrooms may possess distinct enzymes that lead to varying types of enzymatic hydrolysis [33].

The total flavonoid content, as determined based on quercetin quantitation, ranged from 0.32 to 0.47 mg/g, depending on the processing method. However, the flavonoid content of beans processed by different methods was not significantly different ($P < 0.05$). In contrast, after 15 days of fermentation, the total flavonoid content increased from 0.32 to 2.53 mg/g in the steamed beans, 0.38 to 2.64 mg/g in the germinated beans, and 0.47 to 3.41 mg/g in the roasted beans. Thus, the postfermentation flavonoid content was approximately 8–9 times higher than the prefermentation counterparts. Beans fermented with mushroom mycelia also showed a tendency toward increased total flavonoid content after 20 days of fermentation. Specifically, the flavonoid content increased from 1.04 to 3.10 mg/g in the steamed beans, 1.13 to 1.86 mg/g in the germinated beans, and 1.08 to 4.34 mg/g in the roasted beans compared with the control beans. Each of

Fig. 1 Changes in total phenolic content of sprout soybeans after fermentation with *Irpex lacteus* mycelia. Data are expressed as mean $\pm$ SD of triplicate experiments. *SS* steamed sprout soybeans, *GS* germinated sprout soybeans, *RS* roasted sprout soybeans. Mean values with different superscripts are significantly different at $P < 0.05$ by Duncan's multiple range test

Fig. 2 Changes in total flavonoid content of sprout soybeans after fermentation with *Irpex lacteus* mycelia. Data are expressed as mean $\pm$ SD of triplicate experiments. *SS* steamed sprout soybeans, *GS* germinated sprout soybeans, *RS* roasted sprout soybeans. Mean values with different superscripts are significantly different at $P < 0.05$ by Duncan's multiple range test

these values increased approximately 2- to 4-fold after fermentation. Thus, the data indicate that the total flavonoid content in all three types of processed bean increased after fermentation, consistent with the results obtained for the total phenolic content. These increases are presumably due to the enzymatic activity induced by mushroom mycelia and heat treatment.

Change of total isoflavones in beans fermented with mushroom mycelia

Table 1 shows the changes in isoflavones, including glycoside forms (daidzin, glycitin, and genistin) and aglycone forms (daidzein, glycitein, and genistein), according to the processing method for beans that were fermented with *I. lacteus* mycelia (10%) for 20 days. The total isoflavone

content (dry base) were 2.37 mg/g in steamed beans, 4.91 mg/g in germinated beans, and 6.85 mg/g in roasted beans, the latter of which was the highest. These values are higher than those described by Moon et al. [7], who reported 276.09 mg/100 g of isoflavones in sprout soybeans. This difference might be due to the different varieties of sprout soybeans used and/or the distinct growing conditions [7, 8].

The total isoflavone content of all processed sprout soybeans decreased during fermentation. Moreover, the results presented here indicate that the isoflavones were converted from glycoside forms into aglycone forms. In particular, glycoside forms were not detected after 10 days of fermentation. The total isoflavone content decreased from 5.99 to 4.08 mg/g in the steamed beans, 7.02 to 5.39 mg/g in the germinated beans, and 6.92 to 4.85 mg/g

Table 1 Changes in isoflavone content of sprout soybeans after fermentation with *Irpex lacteus* mycelia

Samples	Fermentation days	Isoflavone contents (mg/g)							
		Glycoside type			Aglycone type			Total aglycone	Total isoflavones
		Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein		
SS		1.62 ± 0.22	0.43 ± 0.07	1.59 ± 0.21	0.45 ± 0.05	ND	0.43 ± 0.06	0.89 ⁱ	4.52 ^d
	0	2.61 ± 0.01	0.61 ± 0.00	2.57 ± 0.02	0.86 ± 0.01	ND	0.67 ± 0.02	1.53 ^h	7.32 ^a
	5	ND	ND	0.09 ± 0.08	1.69 ± 0.35	0.39 ± 0.02	1.53 ± 0.28	3.61 ^d	3.69 ^e
	10	ND	ND	ND	2.37 ± 0.03	0.44 ± 0.01	2.65 ± 0.04	5.46 ^a	5.46 ^c
	15	ND	ND	ND	1.40 ± 0.04	0.41 ± 0.02	1.71 ± 0.03	3.52 ^d	3.52 ^e
	20	ND	ND	ND	1.47 ± 0.06	0.41 ± 0.02	1.18 ± 0.06	3.06 ^e	3.06 ^f
GS		1.47 ± 0.06	0.44 ± 0.05	1.53 ± 0.03	0.63 ± 0.02	0.09 ± 0.02	0.75 ± 0.02	1.47 ^h	4.91 ^d
	0	2.48 ± 0.01	0.64 ± 0.00	2.43 ± 0.01	0.83 ± 0.00	ND	0.63 ± 0.01	1.47 ^h	7.02 ^{ab}
	5	ND	ND	0.08 0.01	2.21 ± 0.01	0.45 ± 0.01	2.58 ± 0.02	5.23 ^a	5.31 ^c
	10	ND	ND	ND	2.33 ± 0.09	0.43 ± 0.01	2.68 ± 0.07	5.45 ^a	5.45 ^c
	15	ND	ND	ND	2.34 ± 0.02	0.41 ± 0.02	2.67 ± 0.00	5.41 ^a	5.41 ^c
	20	ND	ND	ND	2.28 ± 0.03	0.42 ± 0.01	2.69 ± 0.02	5.39 ^a	5.39 ^c
RS		2.11 ± 0.03	0.37 ± 0.01	2.30 ± 0.05	1.43 ± 0.00	ND	0.64 ± 0.01	2.06 ^g	6.84 ^b
	0	2.01 ± 0.00	0.36 ± 0.01	2.14 ± 0.01	1.54 ± 0.02	0.13 ± 0.01	0.70 ± 0.01	2.37 ^f	6.88 ^b
	5	0.15 ± 0.01	ND	0.34 ± 0.02	1.99 ± 0.07	0.33 ± 0.03	2.05 ± 0.03	4.36 ^c	4.85 ^d
	10	ND	ND	ND	2.31 ± 0.04	0.44 ± 0.01	2.69 ± 0.07	5.44 ^a	5.44 ^c
	15	ND	ND	ND	2.30 ± 0.02	0.40 ± 0.01	2.72 ± 0.06	5.43 ^a	5.43 ^c
	20	ND	ND	ND	2.26 ± 0.04	0.39 ± 0.01	2.07 ± 0.02	4.73 ^b	4.73 ^d

Data are expressed as mean ± SD of triplicate experiments

SS steaming sprout soybean, GS germinating sprout soybean, RS roasting sprout soybean, ND not detected

Mean values with different superscripts in the same column are significantly different at $P < 0.05$ by Duncan's multiple range test

in the roasted beans after 20 days of fermentation. Also, the isoflavones in the sprout soybeans changed from glycoside forms into aglycone forms after 10 days of fermentation.

The levels of aglycones rose from 0.89 to 5.46 mg/g in the steamed beans, 1.47 to 5.45 mg/g in the germinated beans, and 2.06 to 5.44 mg/g in the roasted beans after 10 days of fermentation. When the soybean was fermented using mushroom mycelia, the conversion to aglycone form isoflavones was confirmed through fermentation of about 10 days. Thus, the conversion rate rose 2.5–7.0 times after fermentation. Sprout soybean has been reported to be rich in isoflavones [8] compared to other beans, especially after 24 h of germination [10]. Moreover, the isoflavone content in roasted *Rhynchosia nulubilis* was higher than that in nonroasted beans [11]. This tendency is consistent with the results presented by Jeong et al. [15] that glycoside isoflavones in *Cheonggukjang* dropped by one-third and aglycone isoflavones increased by around 50-fold after 45 h of fermentation, resulting in approximately half of the total original isoflavone content after fermentation. Shon [28] reported that the total isoflavone content were decreased in steamed and germinated beans after

fermentation with *Tricholoma matsutake* mycelia, however, the isoflavone content was highest on the after 9 days of fermentation.

In all processed sprout soybeans fermented with mushroom mycelia, it took around 10 days for glycoside isoflavones to be converted to bioactive aglycone isoflavones. Moreover, on the after 10 days of fermentation, all processed sprout soybeans had 5.45–5.46 mg/g of aglycone isoflavones (Table 1, Fig. 3). Accordingly, these data indicate that high aglycone isoflavone yields can be obtained from fermented beans when sprout soybeans (already rich in isoflavones) are heat-treated and fermented with mushroom mycelia.

DPPH radical scavenging ability

To measure the antioxidant activity in fermented sprout soybeans, the beans underwent different types of processing (steaming, germination, roasting) followed by fermentation with mushroom mycelial fluid (10%) for 20 days. The ability of the soybeans to scavenge DPPH radicals was assessed every five days during this time (Fig. 4). The DPPH radical scavenging ability of all

Fig. 3 HPLC chromatogram changes of roasted sprout soybeans fermented with *Irpex lacteus* mycelia for 20 days.

(A) Chromatogram after 0 day fermentation.

(B) Chromatogram after 5 days fermentation.

(C) Chromatogram after 10 days fermentation.

(D) Chromatogram after 15 days fermentation.

(E) Chromatogram after 20 days fermentation

Fig. 4 Changes in DPPH radical scavenging activity of sprout soybeans after fermentation with *Irpex lacteus* mycelia. Data are expressed as mean $\pm$ SD of triplicate experiments. *SS* steamed sprout soybeans, *GS* germinated sprout soybeans, *RS* roasted sprout soybeans. Mean values with different superscripts are significantly different at $P < 0.05$ by Duncan's multiple range test

processed sprout soybeans increased after fermentation. Roasted beans showed the highest increase (6.64–53.79%), followed by germinated beans (16.50–45.64%) and steamed beans (41.43–84.66%). Therefore, the DPPH radical scavenging ability of beans increased about 2- to 8-fold after fermentation. This finding is consistent with the results of Cho and Joo [34] and Kim et al. [35], both of whom showed that DPPH radical scavenging ability is correlated with phenolic content. Also, Shon [28] reported that the DPPH radical scavenging ability of germinated beans and steamed beans fermented with *Tricholoma matsutake* mycelia tended to increase sharply in the early stage of fermentation. Kim et al. [35] studied the effect of temperature on small black soybean antioxidant activity. Kim and colleagues found that beans roasted at 250 °C for

30 min showed the greatest phenolic content and the greatest DPPH radical scavenging ability (around 58% greater than control beans). There was no significant difference in the antioxidant activity of the processing method. However, the data presented here indicate that fermenting steamed, germinated, or roasted sprout soybeans with *I. lacteus* mycelia greatly increased their DPPH radical scavenging ability. The correlation coefficient between the amount of bioactive compounds and the antioxidant activity is shown in the Table 2. The correlation coefficient between DPPH radical scavenging ability and total phenolic content in all soybean fermented by *I. lacteus* mycelia was relatively high (0.6190–0.9888), and a relatively low correlation with isoflavone content (0.3673–0.7054). A likely explanation is that heat-treating

Table 2 Correlation coefficients between DPPH radical scavenging activity, total phenol content, total flavonoid content, and isoflavone content of the sprout soybean fermented with *Irpex lacteus* mycelia

R^2	SS			GS			RS		
	Total phenol	Total flavonoid	Isoflavone	Total phenol	Total flavonoid	Isoflavone	Total phenol	Total flavonoid	Isoflavone
DPPH radical scavenging activity	0.6190	0.3592	0.3940	0.9888	0.70538	0.5680	0.7576	0.5091	0.3673

SS steamed sprout soybeans, GS germinated sprout soybeans, RS roasted sprout soybeans

beans alters their physicochemical properties, resulting in increased levels of different bioactive compounds [9, 35].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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